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The Organisers of Prion 2007 note that the opinions and information presented in the abstracts are those of the authors.
# Programme at a Glance

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<td>Session 7 - Hot Topics</td>
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<td>12.20</td>
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S1.1
Prion Protein Aggregation, Amplification and Inhibition
Caughey, Byron
Rocky Mountain Labs, NIAID, NIH, LPVD, USA

In TSE/prion diseases, normal prion protein (PrPC) undergoes a conformational conversion into oligomeric forms that are associated with infectivity (e.g., PrPSc). Comparisons of a range of PrPSc-containing particles revealed that the most infectious particles per unit protein are non-fibrillar oligomers of ~600 kDa rather than larger amyloid fibrils. Attempts to detect PrPSc with ultrasensitive PMCA-like amplification reactions using recombinant E. coli-expressed PrPC as a substrate will be presented. Recent progress in our attempts to reduce the risks posed by TSE diseases will also be discussed. The apparent infectivity of contaminated samples can be decreased by orders of magnitude by mixing with cyclic tetrapyrroles or non-CpG phosphorothioate oligonucleotides. Prophylactic treatments of rodents with these compounds quadrupled their survival times after high-dose intraperitoneal inoculations with scrapie. Direct intracerebral treatments with a mixture of a porphyrin and pentosan polysulfate beginning weeks after high-dose intracerebral inoculations of scrapie substantially increased survival times over treatments with either compound alone. Thus, such treatments show efficacy against established brain infections. Mechanistic studies suggest a common mechanism of action for several of the most effective classes of anti-TSE compounds. This mechanism involves inhibition of conversion by binding to PrPC, causing it to cluster and be internalized from the cell surface. Potential physiological roles of PrPC binding to natural homologs of prophylactic conversion inhibitors such as porphyrins (e.g. hemin), oligonucleotides, and sulfated glycosaminoglycans will be considered.

S2.1
Induction of Cerebral β-Amyloidosis in Transgenic Mice
Jucker, Mathias1; Walker, Lars2
1Hertie Institute of Clinical Brain Research, Department of Cellular Neurology, Germany; 2Yerkes National Primate Research Center, USA

The misfolding and aggregation of specific proteins is well-established in the pathogenesis of Alzheimer’s disease, but little is known about how protein aggregation is initiated in vivo. We show that intracerebral injection of highly dilute, amyloid-β (Aβ)-containing human or APP23 transgenic (tg) mouse brain extract can induce cerebral β-amyloidosis and associated pathology in APP23 tg mice in a time- and concentration-dependent manner. By injecting extracts from APPPS1 tg mice into APP23 hosts and vice versa, our results suggest the occurrence of polymorphic Aβ species with varying biological activities, reminiscent of prion strains. Formic acid treatment, but not boiling, abolished the Aβ-inducing activity of the extract. Moreover, β-amyloid induction was effectively blocked when brain extracts were Aβ-immunodepleted, or by Aβ-immunization of the host. Notably, intracerebral injections of synthetic Aβ preparations in concentrations similar to brain extract levels, as well as cell culture-derived Aβ, did not yield significant seeding activity, suggesting that β-amyloid induction is dependent on a conformation of Aβ that is generated in the in vivo environment. Our results demonstrate that cerebral Aβ-amyloidosis can be induced by intracerebral Aβ-rich brain extract, and that induction is governed by intrinsic properties of both agent and host. The biochemical nature of the seeding agent and whether peripheral administration of the Aβ-rich brain extract can induce cerebral β-amyloidosis is the focus of ongoing experiments.

S3.1
Cellular Pathology of Prion Diseases
Jeffrey, Martin
Veterinary Laboratories Agency Lasswade, Penicuik, UK

Immunohistochemistry of scrapie and other prion disease infected tissues shows that disease associated accumulations of the prion protein (PrP) occurs in a range of morphological types which differs according to the species, strain, tissue and cell type. It is postulated that such accumulations in brain are the cause of neurological disease and that different tertiary or quaternary structures of infectious isoforms of prion protein provide the information necessary to code for strain properties. We have determined the cellular pathology of PrP accumulation using immunogold electron microscopy for several experimental strains of murine scrapie, and for naturally occurring ovine scrapie, BSE of cattle and FSE of cats.

In sheep scrapie brains PrP accumulation corresponded ultrastructurally with abnormal clathrin and ubiquitin mediated endocytosis, increased number of endolysosomes, microfolding of plasma-membranes, extracellular PrP release and intercellular PrP transfer between neurons and, or, glia. When different sheep or murine scrapie strains are compared, the same accumulation types of PrP and associated sub-cellular lesions are present in all strains examined although in different proportions. Similarly natural BSE and FSE show different proportions of same PrP types and lesions.

Scrapie infected lymphoid tissues show cell membrane changes of follicular dendritic cells (FDCs) and macrophages. PrP accumulation is associated with increased retention of immune complexes on FDCs and with internalised cell membrane PrP via an abnormal endocytosis mechanism in macrophages morphologically similar to that seen in neurons. By contrast the cell membrane internalisation of macrophage PrP is via a non-clathrin mediated mechanism.

We conclude that:

a) The main toxic effects of PrP are seen at the cell membrane and are not primarily due to its accumulation in the cytoplasm.

b) The trafficking pathways of PrP depend on strain and cell type.

c) A single prion strain provokes several different pathological PrP membrane-protein-interactions implying individual prion strains consist of, or result in, accumulation of a variety of PrP forms rather than a single entity. Potentially, different strains may be encoded by different proportions of the same PrP isoforms.

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Rocky Mountain Labs, NIAID, NIH, LPVD, USA

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Ten years after the identification of vCJD, there are a number of continuing scientific uncertainties that have implications for risk assessment and the formulation of public health policy. Critical unknowns include the prevalence of human infection with BSE or vCJD, the species-barrier between bovines and humans, the risk from cumulative low level exposures and whether or not there will be secondary transmission through routes other than blood transfusion. Evidence is available in relation to some of these issues but policy decisions often have to be formulated on the basis of uncertainty and mathematical modelling of risk has influenced a number of recommended measures to protect public health. Descriptive data on the evolution of vCJD outbreaks in the UK and other countries underpins risk analyses, but there remains uncertainty about the future numbers of cases and there is a possibility of sub-clinical infection, perhaps related to genetic determinants of disease expression.

Specific questions of relevance to public health include:

1. What is the incubation period of vCJD?
2. What is the population prevalence of infection with BSE/vCJD?
3. Why is there a mismatch between current prevalence estimates and the observed number of vCJD cases?
4. Will there be further outbreaks of vCJD related to variation in PRNP genotype?
5. Will there be other routes of secondary transmission?
6. Will atypical animal TSEs have an impact on human health?

These questions will be critically reviewed as background and introduction to the subsequent presentations in the ‘Epidemiology, Risk Assessment and Transmission’ session.
In vitro Amplification and Detection of Variant Creutzfeldt-Jakob Disease PrPSc

FC1.1

NMR and Fluorescence Structural Studies of an Anti-Prion Protein scFv Fragment


1University of Bayreuth, Department of Biopolymers, Germany; 2Heinrich-Heine-University, Institute of Neuropathology, Germany; 3Friedrich-Löffler Institute, Institute of Immunology, Germany

Background: The conversion of cellular prion protein, PrPc, into its scrapie isoform PrPSc is a key event in prion diseases. Preventing this structural transition is a promising route to therapy. We have chosen PrPc as a target for intervention by means of antibody fragments. In particular, helix 1 of PrP (PrPc-H1) has previously been shown to be a suitable target for a potential antibody-therapy (Korth, 1997). Single chain variable fragments (scFv) from a hybridoma cell line W226 generated through immunization of PrPko mice with purified PrPc were shown to bind to PrPc with sub-nanomolar affinity and to exhibit strong antiprion activity.

Aims: We aim to structurally characterize this novel scFvW226 in its complex with PrPc in order to understand the molecular basis of prion propagation inhibition by helix 1 targeting antibodies.

Methods: The antibody epitope has been mapped by fluorescence spectroscopy using alanine-scanning. The solution structure of the isolated antibody fragment as well as of its complex with PrPc-H1 is studied by means of high-field TROSY-NMR spectroscopy.

Results: Our fluorescence studies revealed that three residues in PrPc-H1 make essential contributions to binding. Surprisingly, the residues involved in this high-affinity interaction form a very polar, charged binding surface for scFvW226. NMR studies of the free scFvW226 and of the bound form with PrPc-H1 show clear differences in the spectra allowing to allocate the binding region on the antibody part.

Discussion: The structural description of the binding interface of this therapeutically active antibody-fragment with its epitope provides first insight into the underlying principles of the high-affinity binding to PrPc. Ultimately, knowledge of the high-resolution structure of these two protein bodies builds the foundation for the rational design of novel anti-prion drugs.

Efficient in Vitro Conversion of CWD PrP-RES by Serial Protein Misfolding Cyclic Amplification


Institutes: 1College of Veterinary Medicine and Biomedical Sciences, Colorado State University; Department of Microbiology, Immunology & Pathology, USA; 2Dartmouth Medical School, Department of Biochemistry, USA; 3University of Kentucky, Department of Molecular Biology and Genetics, USA

Purpose: Chronic wasting disease (CWD) of cervids is a transmissible spongiform encephalopathy (TSE) of increasing prevalence in North America. Infectious CWD prions have been demonstrated in saliva and blood of deer by bioassay; however, detection of PrP(Sc) in these fluids has not been possible using conventional in vitro assays. To enhance CWD PrP(Sc) detection and study potential trans-species infection, we have employed both non-denaturing amplification (Supattapone et al.) and protein-misfolding cyclic amplification (PMCA)(Soto et al.) to convert and amplify cervid (and other species) PrP(Sc) to CWD PrPRES in vitro.

Materials/method: PrP(Sc) from brain tissue of cervids and other species was converted to protease-resistant PrP(Res) by spiking normal brain homogenates (NBH) (10% w/v) with CWD-positive deer brain homogenate followed by incubation at 37C with 40 sec sonic pulses delivered every 30 min over a period of 48 hours. Serial PMCA was accomplished by transfer of aliquots of each reaction mixture into fresh NBH followed by re-amplification. CWD PrP(Res) was detected by immunoblotting after protease K digestion of control and test samples.

Results: Using NBH from cervid PrP 1536 transgenic mice (Browning et al.) in serial PMCA, we have succeeded in amplifying CWD PrP(Res) over 6.5 x 10^7-fold after six rounds. Surprisingly, PMCA using white-tailed deer NBH resulted in just ~10-fold increases in PrP(Res) per round, a vexingly low yield given the efficient transmission of CWD in nature. That Tg(cerPrP)1536 NBH contains ~4-fold greater concentration of PrPC(WD) compared with deer NBH may provide at least a partial explanation for this difference. Finally, in a series of trans-species PrPC(WD) amplification experiments, we have documented reasonably efficient PrP(Res) amplification using NBH from ferrets, a species shown to be susceptible to CWD infection in vivo.

Conclusion: We report (to our knowledge for the first time) efficient CWD PrP(Res) amplification in vitro. We are currently applying this methodology to detection of PrPC(WD) in body fluids and excreta from infected animals as well as to probe potential susceptibility of non-cervids to CWD infection.
The idea of infectious proteins implies that protein conformations can be generated in vitro and de novo without prior contact to existing PrPSc templates. It has been reported that in vitro generated synthetic prions with a deleted N-terminus (PrP89-231) were infectious in a transgenic mouse model overexpressing the same N-terminally deleted PrP construct (PrP89-231). Here, we set out to demonstrate infectivity of an E. coli-expressed recombinant mouse PrP with a mutation corresponding to a human genetic PrP mutation leading to an expanded octarepeat domain. We had previously shown that the expanded OR domain had particular affinity for PrPSc and an inherent multimerization tendency. Recombinant mouse PrP and mouse PrP with 10 additional octarepeats (moPrP14OR) were expressed in Escherichia coli and purified via IMAC columns. Refolding was done with different protocols. Recombinant proteins were passed into mouse PrP overexpressing tg20 mice. We report on cases coming down with prion disease after inoculation with moPrP14OR but not wild-type moPrP in the second passage after >140 days, demonstrating a species barrier-like effect with shortening of incubation time to 60 days on the third passage. MoPrP14OR is thus able to induce a self-perpetuating conversion to PrPSc, although with poor initial efficiency. Part of this inefficient prion initiation maybe due to a species barrier-like effect due to non-homologous sequences of donor and host PrP species. We will discuss how the biophysical characteristics of expanded OR could initiate and template this process.

**FC1.5** Synthetic Prions from Recombinant Mouse Prion Protein with an Expanded Octarepeat Domain

Korth, Cl; Leivied, P; Stitz, L

Heinrich Heine University Duesseldorf, Neuropathology, Germany; *Friedrich-Loeffler Institute, Germany

Institute, Germany

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Recombinant mouse PrP and mouse PrP with 10 additional octarepeats (moPrP14OR) were expressed in Escherichia coli and purified via IMAC columns. Refolding was done with different protocols. Recombinant proteins were passed into mouse PrP overexpressing tg20 mice.

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**FC2.1** Discrimination of Prion Strains by the Cell Panel Assay

Mahal, BP; Demczyck, CA; Ercan, D; Weissmann, C

Scripps Florida, Infectology, USA

Cell lines can be highly heterogeneous with respect to their susceptibility to different prion strains. For example, N2a-PK1 cells can propagate the strains RML and 22L but not Me7 and 87V. The sister clone N2a-R33 can propagate 22L but not RML. We set out to establish a cell-based assay to discriminate between prion strains in cell culture. Subclones of various cell lines were exposed to different prion strains and assayed by the Scrapie Cell Assay (SCA) (Köhnen et al. PNAS; 100: 11666-71; 2003). Clones showing high susceptibility to one or more of the strains were isolated and subjected to further rounds of subcloning and selection. Four cell lines showing different responses to the various strains were assembled into a panel. The panel was exposed to a diluted series of strains and the Response Index (RI) was determined for each strain. The RI on the different cell lines revealed a distinct strain signature allowing us to differentiate between prion strains 22L, RML, Me7 and the mouse-adapted BSE strain 301C. Using the panel assay, we found that in some cases propagation of prions in cell culture, for example 22L in R33, leads to a change of their characteristic signature.

The different characteristics after passage in cell culture may be a result of a change in the strain or due to a transient modification imparted by the cells, such as the glycosylation pattern of the PrP or some associated cell component. The modified prions have been inoculated into mice and will be assayed on the cell panel to see whether the modified characteristics are maintained or revert back to that of the original strain.

**FC2.2** In Vitro Prion Propagation of Prion Strains

Morales, R; Castilla, J; Saal, P; Soto, C

University of Texas Medical Branch, Neurology, USA

Prions are unconventional infectious agents responsible for transmissible spongiform encephalopathies. It has been extensively described that the misfolded form of prion protein (termed PrP*) represents the major, or probably the only component of the infectious agent. It is well established that the prion infectious agent, like conventional micro-organisms, exhibit strain variation. Prion strains can be differentiated by their particular in vivo and in vitro characteristics, including clinical signs, differences in incubation period, pattern of brain lesions and biochemical properties of PrP*.

Understanding how a single protein can provide the diversity to sustain the strain phenomenon has been a challenge for the prion hypothesis. We have described that PrP* can be propagated indefinitely in vitro to generate infectious material using the protein misfolding cyclic amplification (PMCA) technology. Here we demonstrate that prion strains characteristics can be replicated in vitro by PMCA. Our results show in vitro amplification of various established mouse strains including RML, ME7, 301C, 139A, and 79A. PrP* associated to all these strains was in vitro propagated using the same PrP* substrate and the newly generated misfolded protein exhibit the typical properties of the parental strain. Moreover, wild type mice inoculated intracranially with in vitro generated PrP* from each strain developed clinical signs, brain lesion pattern and biochemical characteristics identical to the animals inoculated with brain infectious material. Interestingly, incubation times and thus the specific infectivity (infectivity per unit of PrP*) was the same for in vitro and in vivo generated infectious material in each strain. In vitro replication of prion strains has been expanded to sincP7 polymorphic mice models as well as crossing species barriers (interspecies transmission). The idea of infectious proteins implies that protein conformations can be generated in vitro and de novo without prior contact to existing PrPSc templates. It has been reported that in vitro generated synthetic prions with a deleted N-terminus (PrP89-231) were infectious in a transgenic mouse model overexpressing the same N-terminally deleted PrP construct (PrP89-231). Here, we set out to demonstrate infectivity of an E. coli-expressed recombinant mouse PrP construct with a mutation corresponding to a human genetic PrP mutation leading to an expanded octarepeat domain. We had previously shown that the expanded OR domain had particular affinity for PrPSc and an inherent multimerization tendency.

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**FC2.3** Sporadic CJD Strain Classification by Transmission to Human Transgenic Mice

Bishop, M; Barron, R; McConnell, P; Head, M; Ironside, J; Will, R; Manson, J

1UK National CJD Surveillance Unit, UK; 2Roslin Institute / Neuropathogenesis Unit, UK

Background: The publication by Parchi et al (Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects, Ann Neurol, 1999: 46 (2), 224-33) is widely used to group sporadic Creutzfeldt-Jakob disease (sCJD) cases into six different subtypes.

Aims/Objectives: Our goal was to determine whether these sCJD groups would show distinctive transmission properties to laboratory mice and therefore be described as sCJD strains, or whether the PrP-Sc type or codon 129 genotype showed dominance in the transmission results.

Methods: CNS material was prepared from typical cases of 'Parchi' types and inoculated into transgenic mice expressing human prion protein with genotype variation at codon 129.

Results: Examination of the transmission properties (incubation period, lesion profile, and immunocytochemical and/or biochemical detection of PrP-Sc) has shown:

1) Six types of sCJD (MM1, MV1, VV1, MM2, MV2, VV2) give different phenotypes on transmission to transgenic mice.

2) Data suggest that PrP-Sc type has a more dominant effect on the lesion profile than codon 129 genotype.

3) Material containing a dominance of type 2 Valine/PrP-Sc is the most efficient at transmission to transgenic mice.

Conclusion: This sCJD transmission dataset will subsequently allow for classification of novel atypical sCJD cases.

This work is partly funded by the EU TRARM NeuroPrion project.
**Oral Abstracts**

**FC2.4**

**Prion Strain Interference in the Central Nervous System**

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**Background:** Prion strains can interfere with each other. Infection of a host with a slowly replicating prion strain (i.e. blocking strain) prior to superinfection with a quickly replicating strain can extend the incubation period, or completely block the superinfecting strain from causing disease. The ability of the blocking strain to interfere with or completely block the superinfecting strain is dependent on the interval between inoculation of the prion strains.

**Objectives:**
1. Determine if replication of the blocking strain is required for interference to occur.
2. Investigate the effect of the blocking strain on the replication of the superinfecting prion strain.
3. Identify the location of strain interference in the central nervous system.

**Methods:** Hamsters were infected in the sciatic nerve with the long incubation period drowsy (DY) transmissible min encephalopathy (TME) agent 60, 90 or 120 days prior to sciatic nerve superinfection of hamsters with the short incubation period hyper (HY) TME agent. Western blot and immunohistochemistry were used to determine the abundance and distribution of HY and DY PrPSc.

**Results:** Increasing the interval between TME agent inoculations resulted in an extension of the HY TME incubation period or a complete block of the HY TME agent to cause disease. The ability of the DY TME agent to extend the incubation period of HY TME corresponded with detection of DY PrPSc in the lumbar spinal cord. The increase in the incubation period of HY TME or inability of HY TME to cause disease in the co-infected animals corresponded with a reduction in the abundance of HY PrPSc in the lumbar spinal cord. When the two strains were not directed to the same populations of neurons within the lumbar spinal cord, interference between HY and DY TME did not occur.

**Discussion:** This data suggests that DY TME agent replication not only interferes with the ability of the HY TME agent to cause disease, but also interferes with HY TME agent replication. The ability of the DY TME agent to interfere with replication of the HY TME agent is dependent on infection of a common population of neurons. Therefore, a critical parameter for prion strain interference is not if two prion strains are inoculated at the same time or separately, but when and where prion replication occurs.

**FC2.5**

**Extraneural Replication of Ruminant and Human Prions in Transgenic Mouse Models**

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**The ability to infect lymphoreticular organs is a long recognized feature of TSE agents, and there is evidence that extraneural replication can play a pivotal role in the pathogenesis of the infection.**

We have studied the lymphopitohem of sheep scrapie. BSE and CJD infectious sources in transgenic mice expressing either ovine/VRG (tg338) or human129 prion protein (tg650) by examining the accumulation of PrPSc and infectivity in their spleen. It was found that tg338 mice intracerebrally inoculated with a class of scrapie primary isolates common in the field accumulated prions with clearly distinct strain properties in the spleen and brain tissues, based on their PrPres molecular profile and biological properties on further passing. Such a dual distribution was altered upon inoculation by peripheral route. Biological cloning allowed the individualisation of two strain components with fairly different tropisms. One of which, designated Lan19K, appeared to be essentially devoid of lymphopitohem, similar to that found for Nor98 atypical scrapie propagated on the same mice. In contrast, another group of scrapie isolates promoted PrPres accumulation at equivalent levels in the spleen and in the brain. These data indicate that the lymphoinvasive potential of natural scrapie strains is quite variable, and that this criterion could be useful as an aid to further investigate their diversity in mouse bioassay. Similar strain-related discrepancies were observed following transmission of classical and atypical BSE agents.

**Transmission of human TSE isolates to tg650 mice revealed a prominent infection of lymphoid tissue by vCJD agent while only minimal involvement was observed in the case of sCJD agent, thus faithfully reproducing the situation reported in infected humans. Intracerebral inoculation of a reference vCJD sample led to the propagation of prions with either vCJD or vCJD-like strain properties in the brain, but invariably vCJD-like in the spleen. Remarkably, following inoculation by peripheral route, vCJD agent failed to produce an efficient neuroinvasion leading to a clinically silent, sustained extraneural infection along the whole mouse life. Collectively these observations show that prions with different strain properties can propagate concomitantly in the nervous and lymphoid tissues, and point to further complexity in the interactions that natural prion agents can establish with the infected host.**

**FC2.6**

**Elements Modulating Transmission Barrier and Virulence in BSE-Derived Prions**

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**Background:** BSE is considered a promiscuous strain because have been able to infect a wide range of species. Nevertheless, the transmission barrier found for BSE infection in other species is in a broad range from low to high susceptibility depending on the host. When prion replication barrier is crossed both biochemical and biological prion strain properties can change as an adaptation to the new species. However, the elements modulating these changes are not well known.

**Objectives:** This work is addressed to analyse the putative elements modulating the biological, biochemical and histopathological properties of BSE after crossing the transmission barrier in different species.

**Methods:** A collection of BSE-derived prions having the same strain origin (BSE inoculum) but harbouring different PrP amino acid sequences has been generated by intracerebral inoculation in four different mouse lines expressing PrP from different species (bovine, murine, porcine and ovine). These BSE-derived prions allow analysing those properties associated to the prion strain versus those depending on the PrP amino acid sequence. Biochemical, histopathological and biological properties of the new generated BSE-derived prions have been analysed in comparison to both the original BSE inoculum and others non-related prion strains.

**Results:** The different BSE-derived prions inoculated in BoPrP-Tg110 showed lack of transmission barrier, independently of the PrP amino acid sequence of the inoculum (a marked transmission barrier is present for non-BSE prion strains). BSE-prions generated after passage in mice expressing ARQ-ovine PrP increase its virulence (survival time is shortened). By contrast, the passage of BSE strain in mice expressing murine-PrP decreases its virulence (survival time is lengthened). In both cases, the new biological properties are maintained after subpassage in BoPrP-Tg110.

**Discussion:** These results indicate that BSE transmission barrier is modulated by strain (corresponding depending) properties rather than by PrP amino acid sequence. Interestingly, passage of BSE strain in host expressing other species PrP modifies its biological properties altering the survival time when analysed in BoPrP-Tg110 mice. These alterations are due to elements included in the PrP amino acid sequence but not to other species-specific factors. These elements are under consideration.

**FC2.7**

**Accelerated Studies of Interspecies Prion Transmission Using Combined Transgenic and PMCA Technologies**

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**The threats to humans and livestock from interspecies prion transmission are difficult to assess because the factors controlling this process remain uncertain. The origin and potential for further interspecies transmission by cervid prions are of particular concern due to the evident endemic region of Northern Colorado and Southern Wyoming, chronic wasting disease (CWD) is a contagious prion disease of wild animals. Transgenic mouse models have been valuable for understanding the interplay between PrP primary structure and prion strains in determining interspecies prion transmission. The development of PMCA prompted us to explore the feasibility of accelerating these studies by combining in vitro prion amplification with transgenic approaches. Here we show using PMCA and Tg(CerPrP)1536 mice that PMCA-amplified cervid prions retain the biological and biochemical properties of naturally occurring CWD prions. We also show that PMCA can be used to overcome the barrier to prion transmission that normally exists between rodents and cervids. We induced the production of novel RML-derived cervid prions by amplifying CerPrP isolated from Tg(CerPrP) mouse brains with RML following several rounds of PMCA amplification. Surprisingly, these RML-cervid prions had accelerated transmission properties in Tg(CerPrP)1536 mice compared to CWD and RML prions. The biochemical strain properties of the newly derived CerPrPSc were markedly different to the novel constituting CWD prions. In contrast, production of similar RML-derived cervid prions required several hundred days in Tg(CerPrP) mice. Our studies demonstrate that the combination of transgenic and PMCA technologies is a powerful means of generating novel prion strains and for accelerating the study of interspecies prion transmission.**

**加速物种间普利奥病毒感染的结合转基因和PMCA技术**
Strain-specific properties of prions are believed to be encoded by the conformation of misfolded prion protein, PrP\textsuperscript{\alpha}. We and others have developed cell lines that are susceptible to some prions strains but not to others. The mechanism by which cells distinguish between prion strains is unknown. It has been proposed that the key issue is whether or not the PrP\textsuperscript{\alpha} of the host can readily assume the conformation of the incoming PrP\textsuperscript{\beta}. We have isolated several murine cell lines of different origin, in particular CAD5 and PK1, which can be efficiently and persistently infected by both RML and 22L prions. Infectivity is measured by the Scnoglo Cell Assay (SCCA), a sensitive, rapid and quantitative procedure carried out in cell culture. Exposure of PK1 cells to low levels of an inhibitor, SB3110, renders them resistant to infection by RML, but not by 22L prions, while CAD5 cells remain completely susceptible to both prion strains when exposed to even a tenfold higher dose of the inhibitor. Surprisingly, while SB3110 inhibits infection of PK1 cells by RML, it does not inhibit RML replication when administered once infection has been established: Chronically RML-infected PK1 cells propagate and secrete infectious prions for weeks in the presence of SB3110 at concentrations that are inhibitory to de novo infection. Our findings demonstrate that establishment of persistent prion infection proceeds in at least two distinct phases, the first comprising adsorption and probably some transport processes, which may differ for at least some prion strains and are differentially subject to inhibition by SB3110, and a second phase, namely replication, which is not affected by the inhibitor.

Investigating the Subcellular Location of the Infectious “Prion” and its Relationship to PrPres in a Cell Culture Model of Prion Disease

Methods: Nycodenz gradients were used to perform density gradient fractionation of cell lysates of prion infected cells. The location of PrP\textsuperscript{\alpha} and subcellular organelles within the fractions were determined by western blot or dot blot analysis. Fractions were used to infect recipient cells. Various factors influencing efficiency of transmission were evaluated using a cell blot method and quantitation of PrP\textsuperscript{\alpha} produced by recipient cells.

Results: The most infectious fractions were not always “raft” associated, and did not correlate with those containing the highest levels of PrP\textsuperscript{\alpha}. Further, other co-factors not associated or bound to the infectious “prion”, have a relatively minor influence on infection efficiency.

Conclusions: Our results provide evidence to support the contention that although PrP\textsuperscript{\alpha} may constitute an important component of the infectious unit, other closely related PrP species (PrP\textsuperscript{\beta}), and/or co-factors associated with the infectious entity, are also likely to be determinants of transmission efficiency.

Dynamics and Distribution of Infectivity in Sheep Blood

Expression of the PrP\textsuperscript{\alpha} protein is essential for transmissible spongiform encephalopathy (TSE) or prion diseases to occur, but the underlying mechanism of infection remains unresolved. PrP\textsuperscript{\alpha} is differentially glycosylated giving rise to \(\alpha\)-, \(\beta\)- and \(\gamma\)-glycosylated species but the significance of this variation in glycosylation is unknown. To address the hypothesis that glycosylation of host PrP\textsuperscript{\alpha} is a major factor influencing TSE infection, we have inoculated our gene-targeted transgenic mice which possess restricted N-linked glycosylation of PrP\textsuperscript{\alpha} with three TSE strains. We have demonstrated that glycosylation of host PrP is not necessary to allow transmission of TSE infectivity to a new host. The requirement of each PrP\textsuperscript{\alpha} glycosylation site has been dissected further and has revealed that different TSE strains have dramatically different requirements for each of the glycosylation sites of host PrP and moreover we show that the host PrP has a major role in determining the glycosylation state of de novo generated PrP\textsuperscript{\beta}.
FC3.4  
The Role of the Neuromuscular Junction in Prion Agent Infection of Muscle Cells: A Model for Transynaptic Spread of the Prion Agent  
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Skeletal muscle is one site of infection in prion diseases but the pathway and mechanism involved in infection has not been elucidated. In this study we used in vivo and in vitro models to investigate the role of the neuromuscular junction (NMJ) in entry of the prion agent into muscle cells. Direct unilateral inoculation of the HY strain of the transmissible mink encephalopathy agent into the hypoglossal nerve resulted in initial deposition of PrPSc in the nerves and skeletal muscle cells of the tongue. At the earliest time point of muscle cell infection, the distribution of PrPSc was found to be in a restricted, focal pattern within 60% to 90% of the PrPSc-positive cells and this pattern co-localized with markers for the NMJ in 47% to 89% of the PrPSc-positive cells. At clinical disease, PrPSc was widely distributed throughout the cytoplasm and the focal PrPSc pattern and co-localization with the NMJ was found in less than 7% of the PrPSc-positive muscle cells. These findings strongly suggest that upon initial entry of the prion agent into muscle cells that PrPSc is present at the NMJ and suggests that agent entry is via transynaptic spread from the motor nerve terminal.

To investigate the mechanism of prion infection of muscle cells in vitro, C2C12 cells (a myoblast cell line that can undergo differentiation into myotubes) were incubated with a scrapie brain homogenate or co-cultured with non-neuronal cell lines infected with 22L scrapie, but neither treatment resulted in infection of C2C12 cells. However, co-culture of C2C12 cells with 22L scrapie-infected neuronal cell lines, N2a or GT1 cells, resulted in establishment of scrapie infection in myoblasts and myotubes. Co-culture with 22L scrapie neuronal cell lines in which myogenic differentiation was blocked did not result in infection of C2C12 myoblasts. These findings indicate that transfer of scrapie infection to muscle cells in vitro requires a neuronal cell and a differentiated muscle cell, which suggests that a NMJ between these cell types is necessary for transsynaptic spread of scrapie infection. Studies that promote or inhibit NMJ formation in nerve-muscle co-cultures and these effects on scrapie infection of muscle cells will be presented.

FC3.5  
Granulomas Induce Prion Replication Competence Independent of Follicular Dendritic Cells  
Heikenwälder, M1; Margalith, I2; Julius, C2; Polymenidou, M2; Haybäck, J2; Marcel, A2; Schwarz, P2; Kummer, M1; Aguzzi, A1  
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Prions colonize organs of the central nervous and the immune system, both in animals and humans. It was recently demonstrated that chronic lymphofollicular inflammations alter the tropism of prions, thereby transforming organs previously believed to be devoid of prions (e.g. pancreas, kidney, liver, mammary gland) into sites of prion accumulation 1,2. Additionally, other conditions of inflammation or lymphoproliferative disorders (e.g. inclusion body myositis, lymphoma) were demonstrated to accumulate abundant PrPSc 3,4. Here we investigated whether granuloma, extremely common in ruminants and other species, induce ectopic prion accumulation and replication. Granulomas were induced subcutaneously in wild-type, PrPC overexpressing and Prnp/o mice prior to intraperitoneal prion administration. Induced granuloma contained prominent immunohistochemical and molecular hallmarks similar to those found in humans. In contrast to spleens and granulomas of peripherally infected Prnp/o mice, wild-type mice showed PrPSc and prion infectivity in spleens and granulomas investigated at various time points post prion inoculation (50 or 90dpi). Skin homogenates isolated form the identical animals were shown to be devoid of prion infectivity. Histoblot analysis identified the presence of PrPSc in granulomatous nodules of wild-type but not PrPoa/o mice. Here, we describe replication of prion infectivity in the absence of mature FDCs and identify the compartment responsible for prion replication in granulomas. These data indicate that granulomas can be sites of prion infectivity and point to a novel mechanism of peripheral prion replication.

FC3.6  
Role of the GALT in Scrapie Agent Neuroinvasion from the Intestine  
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Following oral exposure some transmissible spongiform encephalopathy (TSE) agents accumulate first upon follicular dendritic cells (FDCs) in the gut-associated lymphoid tissues (GALT). Studies in mice have shown that this accumulation is obligatory for the efficient delivery of the TSE agent to the brain. However, which GALT are crucial for disease pathogenesis is uncertain. Mice deficient in specific GALT components were used here to determine their separate involvement in scrapie agent neuroinvasion from the intestine. In the combined absence of the GALT and FDCs (LTß -/- mice), mice that lacked both Peyers patches (PPs) and remaining lymphoid tissues, mice that lacked both PPs and FDCs (LTß -/- mice) or PPs alone (WT>LTß -/- mice) did not restore disease susceptibility. We have also shown that isolated lymphoid follicles (ILFs) are important novel sites of TSE agent accumulation in the intestine. Mice that lacked PPs but contained numerous FDC-containing mature ILFs succumbed to scrapie at similar times to control mice. Although early scrapie agent accumulation also occurs within the MLNs, their presence in WT-LTß -/- mice did not restore disease susceptibility. We have also shown that isolated lymphoid follicles (ILFs) are important novel sites of TSE agent accumulation in the intestine. Mice that lacked PPs but contained numerous FDC-containing mature ILFs succumbed to scrapie at similar times to control mice. Since the formation and maturation status of ILFs is inducible and influenced by the gut flora, our data suggest that such factors could dramatically affect susceptibility to orally acquired TSE agents. In conclusion, these data demonstrate that following oral exposure scrapie agent accumulation upon FDCs within lymphoid tissue within the intestine itself is critically required for efficient neuroinvasion.

FC3.7  
The Immune System Facilitates BSE Transmission into Mice  
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TSEs (prion diseases) can present as sporadic, familial or acquired. The major host determinant of incubation period is the PrP gene, with PrP expression critical for disease. vCJD arose from food-borne BSE, and BSE transmits unusually efficiently to laboratory mice, making this a manipulable model for defining host elements involved in the transmission of TSE agents between species. We have previously demonstrated that cross species transmission does not depend on the degree of homology between donor and recipient PrP. We have also shown that there are large and consistent incubation period differences in mouse strains carrying the same Prnp genotype and that the incubation period of the shortest strain can be prolonged by splenectomy prior to infection. In addition, we have reported that SCID mice are not susceptible to BSE infection even after intracerebral inoculation. We now report on further attempts to define the role of the immune system in the transmission of cattle BSE to mice including attempts to reconstitute susceptibility in immunodeficient mice by engraftment with immunocompetent bone marrow. We conclude that the transmission of bovine BSE requires the presence of functional follicular dendritic cells in the recipient mice. Funding BBSRC and DEFRA
**FC3.8**

**Immunotherapeutic Effect of Anti-PrP Monoclonal Antibodies on TSE Mouse Models: Pharmacodynamic and Pharmacokinetic Analysis**


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Prion diseases are transmissible neurodegenerative disorders for which no curative or palliative therapeutic exists. Passive immunization with antibodies directed against the cellular form of the prion protein (PrPc) has been shown to delay the onset of disease after peripheral contamination. However, mechanisms and parameters determining their in vivo efficacy remain unknown. Here, we have characterized the main lines of the pharmacokinetic properties of anti-PrP antibodies in different prion mouse models expressing various levels of PrPc (Prnp0/0, C57BL/6, Tg20 and Tg338 mice) in relation with therapeutic effect, as assessed by incubation duration and delay in PrPSc accumulation process. We have shown that treatment efficacy after peripheral inoculation is correlated to the ability of antibodies to form stable and long-lasting complexes with endogenous plasmatic PrPc, reminiscent of a PrP-Fc2-like molecule. Pharmacokinetic investigations of these circulating PrPc-anti-PrP antibody complexes allowed us to substantially optimize passive immunization efficacy in various mouse models of prion diseases. We also identified that in vivo treatment with anti-PrP antibodies specifically induced a dramatic increase in total plasmatic PrPc (up to 100 fold), proportional to therapeutic efficacy. This increase was demonstrated to be linked to a mobilisation of PrPc from peripheral tissues: this phenomenon was accompanied by a modification of PrP metabolism and PrP expression level in key cellular target of the prion peripheral replication. This event could partly explain the therapeutic action of anti-PrP antibodies. Finally, our work allowed us to define better the main characteristics of anti-PrP mAbs associated with therapeutic efficacy and factors determining the issue of the treatment. This study will be a useful guide in designing optimized therapy for prion diseases.

**FC4.1**

**A Novel Sorting Nexin (SNX33) Interferes with Prion Infection by Modulation of PrPc Shedding**

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The cellular prion protein (PrPc) is a glycosylphosphatidylinositol (GPI)-anchored protein trafficking in the secretory and endocytic pathway and localized mainly at the plasma membrane. Intramembrane cleavage and shedding of PrPc in the middle, the extreme C-terminal part or within the GPI-anchor, have been implicated in PrP processing. These mechanisms modulate conversion of PrPc into its pathogenic isoform PrPSc, implicated in the pathogenesis and transmission of prion diseases, by reducing the substrate for prion conversion. This provides similarities with the well-characterised processing of the Alzheimer precursor protein APP. Sorting nexins are a family of proteins with important functions in protein trafficking. Here, we investigated the role of the newly described sorting nexin-33 (SNX33) in the trafficking and processing of PrPc. We found that over-expression of SNX33 in neuronal and non-neuronal cell lines results in increased shedding of full-length PrPc from the plasma membrane and modulates the rate of PrPc endocytosis. This was paralleled by reduction of PrPSc formation in persistently infected cells and of de novo infection. Using deletion mutants we demonstrate that production of PrP-fragment N1 is not influenced by SNX33. Our data provide new insights into the cellular mechanisms of PrPc shedding and show how this can affect prion conversion.

**FC4.2**

**Cellular Prion Protein Overexpression Promotes Prion-like Conformations in Transgenic Mouse and Transfected Cells**

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PrPSc, an abnormal misfolded isoform of the cellular prion protein, PrPc, is a pathognomonic marker of prion disease. Diagnosis of prion infections is based largely on the detection of PrPSc, which is typically partially resistant to proteinase K (PK) digestion, but may also be present to varying degrees in a PK sensitive form. Our laboratory has developed three different PrPSc-reactive engineered antibodies, displaying PK sequence grafts composed of amino acids 19-33, 89-112 and 136-158. These IgG reagents have been shown to bind robustly to PrPSc before or after PK treatment, but do not recognize PrPc. In this study, we have sought to evaluate the effect of PrP overexpression upon its conformation. PK conformation in the brains of transgenic mice, and in transfected cultured cells, has been examined both immunologically, using the grafted IgGs 19-33, 89-112 and 136-158, and with the use of sucrose velocity gradients. Firstly, four transgenic mouse lines overexpressing mouse PrP (tg20), hamster (tg7), human (tg603) and ovine (tg338) PrP between 4–fold to 8–fold over wild-type levels, were studied using an immunoprecipitation assay. PK sensitive forms of PrP reacting with all three of the motif-grafted antibodies were clearly immunoprecipitated from brain homogenates prepared from each of these mouse lines, but not from wild type mice. This phenomenon was found to be independent of the age of the animals (within a 3-month to 15-month age range). Similarly, a significant quantity of PrPSc-like molecules was also recovered from lysates of transfected RK13 cells overexpressing mouse PrP following immunoprecipitation with IgGs 19-33, 89-112 and 136-158. Subsequently, PrP from the supernatant of tg20 mice was allowed to sediment through a sucrose gradient. These experiments indicated that, in contrast to equivalently prepared wild type brain samples, a subpopulation of PrP in the tg20 tissues can be observed in higher sucrose fractions, partially overlapping those from which PrPSc can be recovered from prion-infected brains. These findings indicate that a proportion of the PrP population encountered in tg20 brain tissue likely exists in a polymeric state. These data collectively indicate that PrPSc-like molecular forms, as characterized by antibody reactivity and separation profiles, can be detected in healthy non-prion infected transgenic mice overexpressing wild-type PrPc as well as in transfected cells also overexpressing PrPc.

**FC4.3**

**Transmission of AA-amyloidosis: Similarities with Prion Disorders**

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The systemic amyloidoses are characterized by widely spread amyloid deposits that can affect virtually every organ in the body. The precursor protein, which varies between different forms is produced at one or several locations, circulates with the plasma and is finally deposited as fibrils in the target organs by mechanisms yet to be determined. In one of the more common forms, systemic AA-amyloidosis, the substrate protein serum AA (SAA) is an acute phase reactant, with significant production only when certain proinflammatory signal substances are upregulated. A persistently high plasma concentration of SAA is a prerequisite for AA-amyloidosis to develop. AA-amyloidosis can easily be induced in many strains of mice by an inflammatory challenge, typically after a long lag phase. This phase is dramatically shortened by administration of amyloid fibrils extracted from an amyloidotic mouse, given intravenously, intra-nasally or given in the drinking water. The fibrillar extract is very potent, active down to pg of protein and facilitates amyloid formation even when given several months before an inflammation is induced. Also amyloid-like fibrils, produced in vitro from synthetic peptides have a clear effect, supporting the idea that the active principle is the misfolded and aggregated protein. AA-amyloidosis occurs in many avian and mammalian species. AA-fibrils from some, but not all species seed murine amyloidosis, showing a species barrier. AA-amyloidosis occurs in species, used as human food and may therefore be a risk factor. Consequently, AA-amyloidosis has similarity with prionoses, differing by the need of an upregulated production of the substrate SAA.
Disease-associated Prion Protein Oligomers Inhibit the 26S Proteasome

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Background: The mechanism of cell death in prion disease is unknown but is associated with the production of a misfolded conformer of the prion protein. Our previous work has suggested a clear role for ubiquitin proteasome system (UPS) dysfunction in the pathogenesis of prion disease (Kristiansen et al., 2005, JBC). Degradation of intracellular proteins via the UPS is a complex and tightly regulated process that plays a critical role in cellular processes. The 26S proteasome consists of a 20S proteolytic core (14 α-subunits and 14 β-subunits) complexed at one or both ends with a 19S regulatory complex. The proteolytic activity of the 20S proteasome resides in its two inner β rings.

Aims: This study aimed to examine further the nature of the PrP species responsible for neurotoxicity, to evaluate the role of the UPS in prion disease pathogenesis, and to investigate whether there was a direct mechanistic link between the two.

Methods: Proteasome GFP-reporter substrates, fluorogenic peptides, and a novel activity probe for the proteasome β-subunits were used to demonstrate the effect of disease-associated prion protein in pure 26S proteasome and cell lines. To assess whether UPS impairment also occurred in prion-mediated neurodegeneration in vivo, we prior-infected a transgenic mouse model that allows the functional status of the UPS to be monitored (Lindsten et al., 2003, Nat. Biotech.).

Results: We report that disease-associated prion protein specifically inhibits the key proteolytic β-subunits of 26S proteasome. This was demonstrated both in pure 26S proteasome and 3 different cell lines. By challenge with recombinant prion and other amyloidogenic proteins, we demonstrate that only the prion protein in a non-native β-sheet conformation inhibits the 26S proteasome at stoichiometric concentrations. Pre-incubation with an antibody specific for aggregation intermediates abrogates this inhibition, consistent with an oligomeric species mediating this effect. Prion infection caused specific inhibition of the UPS in our GFP-proteasome reporter transgenic mice together with accumulation of ubiquitin deposits. To the best of our knowledge, this is the first reported in vivo evidence for functional proteasome impairment in a neurodegenerative disease characterized by protein aggregation.

Conclusion: Together, these data suggest a mechanism for intracellular neurotoxicity mediated by oligomers of misfolded prion protein.

Induction of Cellular Autophagy Reduces Prions

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The tyrosin kinase inhibitor imatinib can significantly reduce PrPSc and infectious prions in prion-infected cells, by increasing lysosomal clearance for prions (Ertmer et al., 2004). Imatinib treatment of prion-infected mice showed that this anti-prion effect is found also in vivo to a certain extent (Yun et al., 2007). Recently, we have reported that imatinib causes a general induction of cellular autophagy (Ertmer et al., 2007). Here, we provide experimental evidence that induction of autophagy is indeed responsible for reducing cellular levels of PrPSc. Application of inhibitors of autophagy in parallel with imatinib antagonised the anti-prion effect of imatinib. On the other hand, the mTOR inhibitor rapamycin which is an inducer of autophagy reduced PrPSc similar to imatinib. Of note, autophagy inhibitors alone increased PrPSc. Our studies also shed light on the reciprocal adapting of prions to cells and of cells to prions and which positive or negative role autophagy plays in this scenario. It is conceivable that a certain level of autophagy is beneficial for prion propagation, whereas too much results in total cellular clearance, eventually accompanied by apoptotic cell death. Overall, we demonstrate that inducers of autophagy have the potential to interfere with prion propagation in infected cells. Future studies have to show whether induction of autophagy can be used as a novel experimental avenue for therapy against prion diseases.

Revertant Subclones Derived from Highly Susceptible Cells as a Tool for the Identification of Susceptibility Factors

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Polymorphisms in the prion protein gene are well-known susceptibility factors of prion diseases. However, significant differences in incubation times for scrapie in mice with the same genotype indicate a role of prion protein-independent factors. To identify genetic factors that render cells susceptible to prions we subdivided the highly susceptible neuroblastoma clone N2aPK1 to isolate revertant clones. Three out of seven hundred clones (R2, R5 and R7) showed approximately a hundred-fold reduced rates of prion propagation as compared to the parental clone. This strategy for the isolation of resistant cells keeps epigenetic variations at a minimum as evidenced by the similarities of gene expression profiles between N2aPK1 subclones as compared to clones independently derived from neuroblastoma cells. To determine the impact of prion protein expression levels on susceptibility we transfected revertant clones with the mouse prion protein and pooled stably expressing clones after 10 days of antibiotic selection. Remarkably, enhanced levels of the prion protein (PrPc) as determined by PrPc surface expression levels were not associated with increased rates of prion propagation in revertant as well as in susceptible cells arguing that factors other than the prion protein are required for the cellular propagation of prions.

Hsp70 Prevents PrP Misfolding and Protects Drosophila Neurons against PrP Neurotoxicity

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Prion diseases are a group of lethal neurodegenerative disorders affecting humans and animals and are associated to PrPSc deposition in the brain. It is clear that misfolding and conversion of the normal prion protein (PrPc) into the pathogenic PrPSc is a key event leading to rapid spongiform neurodegeneration and death. Unfortunately, a major gap exists in our understanding of how the conformational conversion of PrP occurs and how it ultimately kills neurons. Recent in vitro studies suggest that molecular chaperones may be key factors mediating PrP misfolding. However, the functional relevance of this finding is unknown since chaperone activity has not been manipulated in animal models of prion diseases. To gain insight into the in vivo role of Hsp70 in PrP misfolding, we created a Drosophila model of sporadic prion conversion. In transgenic flies, wild type PrP from hamster accumulates in membranous structures associated to the Golgi and the secretory machinery, as well as in the cellular membrane. PrP-expressing flies display axonal degeneration and neuronal cell loss associated to progressive PrP insolubility and fibrillar deposition of PrP. Fly-produced PrP exhibits conformational features consistent with PrPSc from infected hamsters, as evidenced by guanidine denaturation and immunoreactivity to PrPSc conformational antibodies. When human Hsp70 was expressed in our PrP flies, we found that Hsp70 colocalizes with PrP aggregates and prevents misfolding. Strikingly, overexpression of human Hsp70 also protects against PrP-dependent neurodegeneration in the fly brain. Thus, we propose that Hsp70 and other molecular chaperones can potentially alleviate PrP neurotoxicity in prion diseases. Finally, our data indicate that Drosophila is an ideal system to genetically dissect fundamental, unknown aspects of PrP-associated pathology.
FC4.1.1
Transmission Results in Squirrel Monkeys Inoculated with Human sCJD, vCJD, and GSS Blood Specimens: the Baxter Study
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Background: Rodent and sheep models of Transmissible Spongiform Encephalopathy (TSE) have documented blood infectivity in both the pre-clinical and clinical phases of disease. Results in a (presumably more appropriate) non-human primate model have not been reported.

Objective: To determine if blood components (red cells, white cells, platelets, and plasma) from various forms of human TSE are infectious.

Methods: Blood components were inoculated intra-cerebrally (0.1 ml) and intravenously (0.5 ml) into squirrel monkeys from 2 patients with sporadic Creutzfeldt-Jakob disease (sCJD) and 3 patients with variant Creutzfeldt-Jakob disease (vCJD).

Additional monkeys were inoculated with buffy coat or plasma samples from chimpanzees infected with either sCJD or Gerstmann-Sträussler-Scheinker disease (GSS). Animals were monitored for a period of 5 years, and all dying or sacrificed animals had post-mortem neuropathological examinations and Western blots to determine the presence or absence of the misfolded ‘prion’ protein (PrP\textsuperscript{Sc}).

Results: No transmissions occurred in any of the animals inoculated with blood components from patients with sporadic or variant CJD. All donor chimpanzees (sCJD and GSS) became symptomatic within 6 weeks of their pre-clinical phase plasmapheresis, several months earlier than the expected onset of illness. One monkey inoculated with purified leukocytes from a clinical GSS chimpanzee developed disease after 36 months.

Conclusion: No infectivity was found in small volumes of blood components from 4 patients with sporadic CJD and 3 patients with variant CJD. However, a single transmission from a chimpanzee-passaged strain of GSS shows that infectivity may be present in leukocytes, and the ‘shock’ of general anaesthesia and plasmapheresis appears to have triggered the onset of illness in pre-clinical donor chimpanzees.

FC5.1.2
Interim Transmission Results in Cynomolgus Macaques Inoculated with BSE and vCJD Blood Specimens
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BSE and vCJD transmitted to cynomolgus macaques reproduce many features of human vCJD, including clinical symptoms, neuropathological hallmarks of vCJD, PrP\textsuperscript{Sc} electrophoretical pattern and, most importantly, the wide distribution of infectivity in peripheral organs. The latter characteristic distinguishes vCJD from sCJD in both humans and cynomolgus macaques, and prompted us to use this non-human primate model for further investigations of vCJD and its risk for human health. The occurrence of four vCJD infections in humans transfused with blood from patients who later developed vCJD has raised concern about blood transfusion safety in countries with vCJD.

In this collaborative European study, we investigated the infectivity of blood components and whole blood administered by intracerebral (ic) and intravenous (iv) routes. Buffy-coat and whole blood was inoculated by ic and iv route, respectively, from two vCJD patients and from two clinical vCJD-inoculated macaques. Transfusions were also performed from whole blood and blood leucodepleted according to hospital practice standards from two clinical BSE inoculated macaques.

Blood infectivity during the preclinical phase is being examined in orally infected macaques. Whole blood was collected and transfused from one such animal two years after oral challenge, whereas buffy-coat and plasma from two animals at 2 and 4.5 years post-challenge, respectively, have been inoculated by the ic route.

This is an ongoing study in which recipient animals continue to be observed at various times post-inoculation. So far, we have had one positive transmission in one animal transfused 65 months earlier with 40 ml of whole blood from a vCJD macaque (the characteristics of the disease in this animal will be shown in a separate poster by E. Comoy). This positive transmission reproduces transmission of vCJD in humans, with an incubation of 5.5 years compatible with incubation periods observed in humans.
FC5.3

Assessing the Risk of vCJD Transmission by Dentistry; Distribution of Infectivity in Oral Tissues of VM Mice after Simulated Oral Feeding of BSE-301V

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The clinical phenotype of bovine spongiform encephalopathy has been extensively reported in early accounts of the disorder. Following the introduction of statutory active surveillance, almost all BSE cases have been diagnosed on a pathological/molecular basis, in a pre-symptomatic clinical stage. In recent years, the active surveillance system has uncovered atypical BSE cases, which are characterized by distinct conformers of the PrPSc, named high-type (BSE-H) and low-type (BSE-L), whose clinicopathological phenotypes remain unknown. We recently reported two Italian atypical cases with a PrPSc type similar to BSE-L, pathologically characterized by PrP amyloid plaques. Experimental transmission to TgBov mice has recently disclosed that BASE is caused by a distinct prion strain which is extremely virulent. A major limitation of transmission studies to mice is the lack of reliable information on clinical phenotype of BASE in its natural host. In the present study, we experimentally infected Fresian/Holstein and Alpine/Brown cattle with Italian BSE and BASE isolates by i.c. route. BASE infected cattle showed survival times significantly shorter than BSE, a finding more readily evident in Fresian/Holstein and in keeping with previous observations in TgBov mice. Clinically, BSE-infected cattle developed a disease phenotype highly comparable with that described in field BSE cases and in experimentally challenged cattle. On the contrary, BASE-inoculated cattle developed an amyotrophic disorder accompanied by mental dullness.

The molecular and neuropathological profiles, including PrP deposition pattern, closely matched those observed in the original cases. This study further confirms that BASE is caused by a distinct prion isolate and discloses a novel disease phenotype in cattle, closely resembling the phenotype previously reported in scrapie-inoculated cattle and in some subtypes of inherited and sporadic Creutzfeldt-Jakob disease.
Chronic wasting disease (CWD) is the prion disease widespread in cervids (white-tailed deer, mule deer, elk, and moose). The presence of significant prion infectivity reported in the muscles of CWD-affected animals in the United States and elsewhere may have been exposed to CWD. In order to determine whether the CWD prion, like the bovine spongiform encephalopathy, is transmissible from cervids to humans, cervidized transgenic mice (Tg12) and humanized transgenic mice (Tg1 and Tg40) were created, which express the elk prion protein (PrP) and human PrP in a mouse PrP-null background respectively. The cervidized Tg12 mice intracerebrally inoculated with CWD isolates from elk, mule deer, and white-tailed deer became infected with relatively short average incubation times (118±6 days for elk 1, 142±7 days for elk 2, 187±18 days for mule deer, and 180±3 days for white-tailed deer). The humanized Tg40 mice and Tg1 mice similarly inoculated with human sCJDMM1 became infected with an average incubation time of 263±13 days for Tg40 mice and 268±5 days for Tg1 mice. In contrast, all of the humanized transgenic mice intracerebrally inoculated with CWD isolates from the three cervid species failed to develop the hallmarks of CWD throughout their natural lifespan. Since the host range of a prion strain could change after adaptation in another species and CWD has been transmitted to cattle and sheep after experimental inoculation, humanized and cervidized Tg mice were also inoculated with cattle- or sheep-adapted CWD, but the mice failed to become infected so far. Our data point to the existence of a significant species barrier between CWD and humans, which may strongly limit the human transmissibility of CWD. Supported by NINDS NS052319, NIA AG14359, CDC UR8/CCU515004 and Charles S. Britton Fund. The first three authors contributed equally.

**FC5.7**

Precipitation of Prion Protein Facilitates Formation and Removal of High Molecular Aggregates

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Background: Investigational studies demonstrating the prion reduction capacity of the manufacturing procedures of biologicals are performed by spiking product intermediates with prion material and running the manufacturing process at lab scale. Under certain conditions the total clearance of two combined manufacturing steps did not approximate to the sum of the individual steps, as from the heterogenous spike one fraction was preferentially removed by both steps.

Aims/Objectives: Studies were performed to evaluate the modification of a subfraction not removable by the manufacturing steps in a way that it could be removed by standard methods inherent in the manufacturing procedures for biologicals.

Methods: Prion protein, usually spiked to intermediates of biologicals, was processed and fractionated in low and high molecular prion aggregates. The low molecular aggregates were precipitated by different means and the precipitate collected by centrifugation or filtration. The precipitate was then solubilised and the protein solution subjected to process steps, differentiating subfractions of prion protein aggregates.

Results: Process conditions resulting in the modification of low molecular prion aggregates to high molecular prion aggregates will be presented.

Conclusion: The heterogeneity of prion spike material can be overcome by implementing further process steps in the manufacturing procedure of biologicals resulting in the removal of all subfractions of prion material. This allows for further improvement of the prion reduction capacity of the manufacturing process.
FC7.2
Enzymatic Degradation of PrPSc Fails to Dis-Infect Bovine BSE Brain Homogenates
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Prions are considered to be dependent on their protein component for infectivity. A previous study with keratinase demonstrated degradation of PrPSc from infected bovine and ovine brain stem homogenates after a unique heat pretreatment procedure. This discovery offered the possibility of a mild option for removal of prion infectivity (Langeveld et al., 2003, J. Infect. Dis., 188:1782-1789). Using these methods, the level of infectivity reduction of bovine prions was tested in transgenic Tgbov XV mice that are susceptible for bovine BSE over a range of 7 orders of magnitude. To assure the degradation of PrPSc different antibodies with specific epitopes distributed over bovine PrP such as SAF32, 12B2, 9A2, 6H4, 94B4, and F99/97.6.1 were used for detection after SDS-PAGE and Western blotting. The infectivity of the untreated inoculum and keratinase digested PrPSc was compared using two different wild-type vole genotypes. The de novo generation of infectious prions from bank voles (Clethrionomys glareolus) and human PrP such as SAF32, 12B2, 9A2, 6H4, 94B4, and F99/97.6.1 were used for detection. Using these methods, the level of infectivity reduction of bovine prions was tested in transgenic Tgbov XV mice that were susceptible for bovine BSE over a range of 7 orders of magnitude. To assure the degradation of PrPSc different antibodies with specific epitopes distributed over bovine PrP such as SAF32, 12B2, 9A2, 6H4, 94B4, and F99/97.6.1 were used for detection after SDS-PAGE and Western blotting. The infectivity of the untreated inoculum and keratinase digested PrPSc was compared using two different wild-type vole genotypes. The de novo generation of infectious prions from bank voles (Clethrionomys glareolus) and human PrP such as SAF32, 12B2, 9A2, 6H4, 94B4, and F99/97.6.1 were used for detection. However, there are no reported cases of spontaneously-occurring prion diseases in humans and maybe in other species, i.e. atypical bovine spongiform encephalopathy (BSE) in European and USA cattle and atypical scrapie cases in sheep suggest that spontaneous prion diseases may happen infrequently but ubiquitously. Our findings support the existence of prions in normal human brains that may be involved in the pathogenesis of prion diseases. (Richard Bessen and Witold K. Surewicz provided scrapie-infected brain tissues and recombinant human PrP. Supported by the CJD Foundation, Steris Co., NIH AG14359 and AG08702, CDCUR8/CCU515004, and the Britton Fund).

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FC7.3
An Increase in PrP*20 Precedes Prion Accumulation
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Prion diseases are characterized by the accumulation in the brain and other organs of proteinase K (PK)-resistant prion protein PrPSc. Although it is known that PrPSc is derived from a PK-sensitive cellular PrP (PrPC) via a transition of alpha-helix into beta-sheets, the mechanism of the conversion of PrPC into PrPSc is poorly understood. The seeding model proposes that a small amount of PrPSc or PrPSc precursor (PrP*) is present in the normal brain in reversible equilibrium with PrPC. When precursor monomeric PrP* molecules form a highly ordered nucleus, PrP* can be converted to PrPSc polymers. Two key elements are required by the seeding model: 1) the presence of a small amount of endogenous PrPSc or PrP* in the uninfected brain; 2) the formation of PrPSc-derived oligomers. However, the presence of the endogenous PrPSc-like species in normal brains, let alone its propensity to form aggregates and convert PrPC, has remained elusive. We have obtained the first evidence that normal human and animal brains contain a novel PrP species called PrP*20. PrP*20 is detergent-insoluble and PK-resistant. Furthermore, it forms aggregates of size similar to those of PrPSc present in prion diseases. PrP*20 is also present in wild type neuroblastoma cells and it is affected by the presence of pathogenic mutations. In addition, PrP*20 was found to be increased in the absence of PrP27-30 in the brain biopsy from a case that then showed prominent PrP27-30 and classical CJD at autopsy suggesting that an increase in the level of PrP*20 characterizes the early stages of prion diseases. Our findings support the existence of prions in normal human brains that may be involved in the pathogenesis of prion diseases. (Richard Bessen and Witold K. Surewicz provided scrapie-infected brain tissues and recombinant human PrP. Supported by the CJD Foundation, Steris Co., NIH AG14359 and AG08702, CDCUR8/CCU515004, and the Britton Fund).

FC7.4
De Novo Generation of Prions in a Cell-free System
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Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative disorders affecting both humans and animals. There is no available treatment or therapy for these fatal diseases. The infectious agent associated with TSEs (termed prion) appears to be composed uniquely of a protein, which is a conformationally-modified version (PrP*) of the cellular prion protein (PrPC). The disease is propagated by the conversion of host PrP* into PrPSc induced by small quantities of PrPSc. Interestingly, prions occur in the form of different strains that show distinct biological and physicochemical properties. TSEs can have diverse origins, including genetic, sporadic (putatively spontaneous) and infectious. The occurrence of sporadic cases of prion diseases in humans and maybe in other species, i.e. atypical bovine spongiform encephalopathy (BSE) in European and USA cattle and atypical scrapie cases in sheep suggest that spontaneous prion diseases may happen infrequently but ubiquitously. However, there are no reported cases of spontaneously-occurring prion disease in experimental wild-type rodent models. We have used a novel technique, Protein Misfolding Cyclic Amplification (PMCA) to rapidly propagate prions in the test tube, using normal brain homogenate as substrate. Prions propagated in vitro are infectious in vivo and maintain their prion strain specificity. PMCA has been used to efficiently amplify a variety of prion strains from mouse, hamster, bank vole, deer, cattle, sheep and human. Therefore, to mimic spontaneous generation of infectivity in vitro becomes one of the most important challenges in the prion field. We show here, for the first time, the de novo generation of infectious prions from bank voles (Clethrionomys glareolus) starting with non-infectious brain homogenates. Several biochemically different prion strains were generated using two different wild-type vole genotypes. The de novo in vitro generated PrPSc was highly infectious after its inoculation in bank voles. We show an extensive characterization of this “spontaneous” phenomenon.
Background: The clinical role of the polymorphism M129V in the human PrP gene is well documented. MV129 heterozygous individuals appear to be protected from prion infection. Also most cases of sporadic CJD afflict individuals homozygous either for MM or VV. Differences in codon 129 genotype give rise to differences in phenotype regarding plaque distribution in the CNS as well as clinical symptoms. Despite this clinical knowledge, little is known about the molecular background to this phenomenon.

Aims: In this study we wanted to elucidate the molecular mechanism of PrP misfolding in vitro by investigating aggregation, fibrillation kinetics and seeding propensity of different 129-mutants to discover differences on the molecular level. The variants used in this initial study were M129A, M129V, M129L, M129M, M129W and M129P. Mutants were chosen to vary hydrophobicity, stereochemistry and secondary structure preference.

Methods: After recombinant expression and purification of recombinant human PrP90-231 mutants, aggregation was initiated by subjecting the protein to agitation under physiological conditions regarding pH, temperature and buffer salt. PrP aggregation kinetics was followed by measuring sample turbidity and the kinetics of conversion into amyloid-like fibrils was followed by Thioflavin T (ThT) fluorescence. Results and Conclusion: Under the conditions explored so far all mutants aggregated within minutes followed by conversion into amyloid-like fibrils within a few hours as determined by ThT fluorescence. The amyloid-like conversion followed stochastic nucleation for the mutants as revealed by variations of the lag-phase from chemically identical samples. Hydrophobicity at position 129 (M129, M129V, M129L, M129M, M129W) did not appear to be rate determining for the fibril conversion whereas the M129P mutant appeared to show faster conversion than ThT positive fibrils.

Background: The human prion diseases are characterized by depositions of amyloid plaque from misfolded prion protein (HuPrP) in various regions of the brain depending on disease. Aggregation and amyloidogenesis of HuPrP is hence strongly correlated with prion disease.

Aims: In this work we investigated if it was possible to induce amyloid fibril formation of recombinant HuPrP90-231 starting from native folded protein under physiological salt, pH and temperature.

Results: Intense shaking of the folded protein under these native conditions induced irreversible conversion of native HuPrP90-231 into aggregates composed of amyloid-like fibrils of HuPrP90-231 within hours. Formed aggregates were positive using thioflavin T (ThT) fluorescence and showed Congo red birefringence. Fibrillar morphology was verified by transmission electron microscopy where solid state conversion of amorphous aggregates into amyloid-like structures was observed. Under these conditions the kinetic profile of aggregation of HuPrP90-231 showed that within minutes the protein assembled into large amorphous non-thioflavinophilic aggregates which after >2 converted into amyloid-like fibrils. The amyloid fibrillization kinetics followed a traditional sigmoidal trajectory with a lag phase and an equilibrium phase. Interestingly, the lag phase of the ThT assay showed unusual variability indicating a stochastic onset of fibril conversion. That the lag phase was due to nucleation was evident because seeding with preformed HuPrP90-231 fibrils shortened the lag-phase.

Conclusion: Amyloid formation of HuPrP90-231 can be achieved starting from the native prion protein under genetic conditions without addition of denaturant or altered pH. The process is catalyzed by addition of preformed amyloid seeds. It is plausible that amyloid seeding reflect the mechanism of transmissibility of prion diseases. Elucidating the mechanism of PrP aggregation and amyloidogenesis

Background: The in vitro technique PMCA (Protein Misfolding Cyclic Amplification) uses bursts of ultrasound to drive the conversion of PrP C to PrPSc in cell free brain cytosol, as well as trafficking mutants, namely N-terminally substituted PrP and disulfide bridge-less PrP. By 18 months of age, mice expressing the mouse equivalent codon responsible for familial fatal insomnia develop a severe neurodegenerative disease detectable by classic histological methods as well as in vivo methods including behavioral assays and magnetic resonance imaging. Lesions are widespread. Surprisingly, these mice show resistance to infection by three strains of prions. We therefore conclude that this mutation can cause disease spontaneously, independent of infection by an exogenous agent. Details of initial transmission experiments of spontaneous prion disease will be presented.

Background: The in vitro technique PMCA (Protein Misfolding Cyclic Amplification) uses bursts of ultrasonic sound to drive the conversion of PrP C to PrPSc in cell free brain homogenates (Soto et al. FEBS Letters 2005, Nishina et al. Biochemistry 2006). Although the in vitro produced PrPSc has been shown to retain infectivity, little is known about the mechanism of PrPSc amplification.

Objective: To elucidate the mechanism of in vitro PrP C to PrPSc conversion, by investigating the composition of the rate limiting step in the conversion process.

Methods: The fidelity of the Sc237 strain characteristics were monitored over multiple rounds of PMCA amplification, using Western blots, conformation-dependent immunoassay (CDI), and bioassay. We also assess the effects of changing reactant concentrations upon the kinetics of amplification.

Results: We show that Syrian hamster Sc237 prions retain their strain characteristics after many rounds of PMCA, using Western blots, conformation-dependent immunoassay (CDI), and bioassay. We also assess the effects of changing reactant concentrations upon the kinetics of amplification.

Conclusion: The results suggest that a well-defined mechanism for PrP C to PrPSc conversion is operating during the cycles of high energy sonication. Although PMCA in its current form is not able to provide appreciable kinetic information, we show that the rate limiting step of the amplification is sensitive to the composition of the amplification mixture.
It has been suggested that the trafficking of PrPc plays an essential role in its conversion to PrPSc. Thus, it has been reported that surface expression of PrPc is important for scrapie production; it has also been demonstrated that this conversion event requires that the protein enter the endocytic pathway. However, the precise subcellular compartment in which the prion conversion event occurs has not been identified. Several lines of evidence suggest that the late endosomal/lysosomal compartment is a likely location for scrapie production since in some cell lines PrPSc has been found to accumulate in late endosomes. Conversely, other studies implicate the Golgi as a candidate for scrapie accumulation. Despite these observations, none of these organelles has been demonstrated to be directly involved in the conversion of PrPc to PrPSc. Using different approaches we perturbed trafficking between different endocytic compartments and analyzed their impact on PrPSc production. Interestingly, a significant amount of PrPSc is localized in endosomal recycling compartment. Our results suggest that neither early nor late endosomes represent major sites of PrPSc production but implicate the endosomal recycling compartment in scrapie production.

Discussion: Our findings imply that accessibility of solvent to the prion disulfide is an important feature for scrapie production. The prion protein undergoes a transition from a disulfide bond that is inaccessible to solvent to a high energy configuration that is accessible to solvent. Accessibility of solvent to the disulfide bond is governed by the positioning of three aromatic residues in the vicinity of the bond: Y162, F176, and Y216. We hypothesized that replacing these aromatic residues with alanine, a neutral amino acid, would increase the rate of cleavage of the disulfide and conversion to the scrapie structure. The C179-C214 disulfide bond was found to exist predominantly in either a low energy configuration that is inaccessible to solvent or a high energy configuration that is accessible to solvent. There is a considerable body of circumstantial evidence that the bond is cleaved and then reforms when the protein transitions from the normal to the scrapie structure. The mechanism of cleavage of the bond is unknown.

Results: Replacement of Y162, F176, and Y216 with alanine decreased the stability of the purified proteins. We found that: i) peptide 89-103 exists in solution at all pHs as monomer only; ii) peptide 141-152 exists as dimer only; iii) peptide 106-126 exhibits both dimer and monomer species with a loss of monomer occurring at pH 9.0. The neurotoxic peptide 106-126 is the most sensitive to solution conditions that may be an early event in the refolding of the prion protein into the abnormal form.
**P01.09**

**HSP90 Family Proteins Modify the Conformation of Copper-Loaded Prion Protein in a Nucleotide-Dependent Manner**

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The quality control system prevents proteins from misfolding/aggregating states, and its dysfunction is apparently involved in the development of prion diseases. The causative agent of prion diseases is suggested to be a disease isoform of prion protein (PrP\(\text{Sc}\)), which is produced by a conformational change of a cellular prion protein (PrP\(\text{C}\)), but the mechanism of this process is yet unknown. To find cellular components modifying the conformation of PrP\(\text{Sc}\), we constructed an assay system using a-helix-rich, 3F4-tagged recombinant prion protein (rPrP\(\text{Sc}\)) as a substrate. This assay system is composed of two reactions: 1) denaturation of the rPrP with candidate subcellular fractions, 2) subsequent digestion with a low concentration of trypsin which can degrade tryptic-sensitive sites of the rPrP. Consequently, we purified the activity by three chromatography steps and identified it as heat shock protein 90 (Hsp90) by TOF/MS/MS. Hsp90 is a 90-kDa chaperone protein abundantly expressed in the cytosolic and nuclear compartments. In the in vitro assay, no nucleosides such as ATP were necessary for Hsp90-assisted conformational modification of the rPrP. In the presence of 0.1 mM copper, however, rPrP was converted into a conformation that was more resistant to the trypsin digestion, of which the Hsp90-assisted conformational modification required ATP or ADP, but not AMP. Non-hydrolyzable ATP analogues, ATP-γS and AMP-PNP also enabled Hsp90 to modify the conformation of copper-loaded rPrP, suggesting that the hydrolysis of ATP is not required for the reaction in the presence of copper. In addition, Grp94, an endoplasmic-resident homologue of Hsp90, also modified the conformation of the copper-loaded rPrP in the presence of ATP or ADP. These results suggest that Hsp90 family proteins may be related to the conformational modification of copper-loaded PrP\(\text{Sc}\) in a nucleotide-dependent manner.

**P01.10**

**Cell-Density-Dependent Fluctuation of PrP\(\text{res}\) in Prion-Infected Neuro2a Cells**

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Cells persistently infected with prion are widely used for researches in prion diseases such as studying the mechanism of prion propagation and screening anti-prion compounds. Protease-resistant PrP, designated PrP\(\text{res}\), is usually detected in cells infected with prion, and the detection of PrP\(\text{res}\) is often used as an indicator of the existence of prion. The amount of PrP\(\text{res}\) in prion-infected cells is expected to fluctuate depending on culture conditions. Therefore, we investigated the fluctuation of PrP\(\text{res}\) and total PrP in Neuro2a (N2a) cells infected with prion. N2a sublines, ScN2a and N2aI, and N2aII, mouse PrPC overexpressing N2a subline, were used. These sublines persistently infected with prion, and N2aII, mouse PrPC overexpressing N2a subline, were seeded at various cell numbers, and cells were harvested every 24 hr for the detection of total PrP and PrP\(\text{res}\). The amount of PrP\(\text{res}\)-peptide showed time-dependent increase, especially the level of PrP\(\text{res}\) markedly increased when cells grew to sub-confluent and then confluent. Total PrP levels in ScN2a3 and ScN2a5 also showed cell-density-dependent increase. Compared with an immediately before passage, the PrP\(\text{res}\)-peptide level was obviously low at 24 hr after passage. Further investigation revealed that the decrease of PrP\(\text{res}\)-peptide level within 24 hr after passage was not due to the digestion of PrP\(\text{res}\)-peptide at the passage, but the level of PrP\(\text{res}\) decreased rapidly within 24 hours after seeding the cells. These results suggested that PrP\(\text{res}\)-peptide levels in ScN2a3 and ScN2a5 were fluctuated by physiological conditions determined by cell density and/or cell growth. To investigate the effect of cell density on PrP\(\text{res}\)-peptide formation, ScN2a3 and ScN2a5 were co-cultured with N2aI subline that is resistant to prion challenge. The PrP\(\text{res}\)-peptide levels in ScN2a3 and ScN2a5 increased in proportion to the decrease of co-cultured N2aI cell numbers. In contrast, condition medium obtained from N2a3 and N2aI sublines did not have major effect on the level of PrP\(\text{res}\)-peptide in the corresponding ScN2a sublines. Taken together, these results suggest that certain cellular microenvironment associated with cell density, possibly direct contact between cells, will be involved in the PrP\(\text{res}\)-peptide formation in ScN2a cells.

**P01.11**

**Screening for Endogenous Factors Involved in the Formation of Protease Resistant Prion Protein**

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It is suggested that endogenous factors are involved in the formation of PrP\(\text{Sc}\)/res or the conformational change from PrP\(\text{C}\) to PrP\(\text{Sc}\)/res. In order to identify the factors, we used RNAi technique in persistently prion-infected neuroblastoma cells and knocked down genes of the proteins on the cell membrane. Modification in PrP\(\text{Sc}\)/res formation in this assay was studied by immunoblotting, and at the same time, reduction in mRNA expression levels was confirmed by quantitative RT-PCR. So far, more than 200 genes have been screened and the data of protein “G” is shown here as a representative.

The protein “G” is a component of a certain receptor in neuronal cells. PrP\(\text{Sc}\)/res formation levels were reduced in the knockdown experiment with either vector-driven short-hairpin RNAs or synthetic ones targeting each different sequence in the gene. On the other hand, PrP\(\text{C}\) expression levels were increased. Further, treatment of the cells with a selective antagonist for the receptor composed of “G” exhibited the similar results to the knockdown study, and treatment with a potent agonist for the receptor showed the opposite results to the knockdown study. These findings suggest that the receptor composed of “G” might be involved in the PrP\(\text{Sc}\)/res formation or the PrP\(\text{C}\) conformational change, and could be a new target for anti-prion drugs.

**P01.12**

**Evaluation of The Diagnostic Sensitivity of Approved Rapid Tests for TSE Active Surveillance in Clinically-Healthy Sheep from Scrapie Affected Flocks**

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TSE active surveillance in EU in sheep and goats is based since 2006 on a screening procedure using TSE rapid tests specific for ovine and caprine animals, approved according to the Commission Regulation (EC) 233/2006. The amendment to EC 898/2001 follows the Institute of Reference and Material Measurements evaluation performed on 9 rapid tests for small ruminants, in which diagnostic sensitivity and specificity were assessed; furthermore the analytical sensitivity, defined as the lowest concentration of pathological prion protein (PrPSc), obtained in a dilution series of clinical, confirmed cases of classical scrapie and that can be distinguished from background noise, was calculated. Aim of the current study was to evaluate the diagnostic sensitivity of 3 of the 8 approved TSE rapid tests in order to verify, in field conditions, their suitability for the detection of affected animals in the early preclinical stage of scrapie. The study was carried out starting from 716 asymptomatic Sarda breed of sheep with various genotype, aged over 18 months and belonging to 16 flocks in which, previously, classical scrapie cases were confirmed. Those flocks were known to have a quite high mean prevalence of preclinical-scrapie infection (over 10%) thus providing an adequate number of scrapie cases. The medulla oblongata was collected from each sheep in the framework of “stamping out” actions, which were carried out in accordance to the European regulations. The samples were submitted to an appropriate post-mortem protocol and then divided into 5 aliquots to be available for examination by confirmatory Western Blotting (WB) and by three rapid tests (Test Bio-Rad TeSeE Sheep/Goat, IDEXX HerdChek BSE-Scrapie Antigen Test Kit EIA, Test Prionics Check Western Small Ruminant) and for molecular characterization. To date, a first group of 49 positive samples, confirmed through confirmatory WB, were available for sensitivity evaluation. A total of 47 samples resulted positive at Biorad test (sensitivity 95.92 % with 95% confidence interval 86.02-99.50), 48 at IDEXX (97.96, 95% CI 89.15-99.95) and 47 at Prionics (95.92, 95%, CI 86-99.50), with 46 samples being positive for all the three tests. Our preliminary findings show that all three tests are valid tools for scrapie diagnosis in clinically-healthy sheep displaying a low amount of PrPSc, with minor differences in sensitivity among them. Using such accurate tests, an estimate of scrapie prevalence can be obtained.
Protein Misfolding

**P01.13**

**Conformation, Self-Association and Ligand Binding Behaviour of The Expanded Octarepeat Domain of The Prion Protein**

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Insertion of additional repeats into the PrP gene has been genetically linked to familial Creutzfeldt-Jakob disease (CJD). Consequently, expansion of the N-terminal octarepeat (OR) domain from 4 (wild-type) up to 13 consecutive repeats appears to make PrPC prone to prion conversion. The OR domain itself is however redundant for the propagation of pre-existing infectivity and transgenic mice expressing mutant PrP with extra repeats develop neurological disease but no prion infectivity. Hence, we assumed that expanded OR domains can initiate the formation of new prions by capturing at PrP molecules in such a way as to facilitate conversion or through the enhanced recruitment of host factors. Recently, we have shown that the expanded OR domain alone is a highly selective ligand for a subpopulation of PrPSc from infected hamster brain. In addition, we showed how the expanded but not the wild-type domains self-associate, forming well-defined multimeric complexes. Both features are strictly pH-dependent, occurring only at physiological pH. We then compared the conformation of the expanded to that of the wild-type OR domain. For this purpose, we expressed 4-, 8-, 10- and 13-OR fragments as N-terminal fusions to the protein G B1 domain (7 kDa), since the fragments alone are poorly soluble at pH >7. Using CD spectroscopy, we found that the conformation of the 13-OR fragment did not differ significantly from that of the wild-type version. Both fragments displayed a CD profile that suggests a polyproline helix II-like conformation, setting it apart from a non-structured state. Furthermore, the self-association of the expanded OR domain was found to depend exclusively on hydrophobic interactions, showing a distinct dissociation threshold at 0.2% sarcosyl, as determined by dynamic light scattering. Thus, we present the first detailed characterization of the wild-type and mutant OR domains at physiological pH, showing it to have an extended but non-random conformation.

**P01.14**

**PrP forms Intermolecular β-Sheets when Anchored on Raft-like Lipid Membranes**

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Whereas most studies on the structure of the prion protein were carried out on recombinant prion protein in solution, in vivo PrPc is anchored on the outer surface of the cell membrane. Therefore, we studied the structure and the interaction of posttranslationally fully modified PrPc anchored on raft-like lipid membranes. PrPc was prepared from PrPc overexpressing CHO-cells and purified as described recently (Ellink, K. and Riesner, D. 2004 in Methods and Tools in Biosciences and Medicine: Techniques in Prion Research (S. Lehmann, ed.) 4-10). Studies of the thermodynamics and kinetics of the anchoring process of PrPc into the membrane have shown that depending upon the saturation of the membrane the concentration of PrPc free in solution but in equilibrium with membrane binding is between 10-10 and 10-8M (Ellink, et al., 2007, Biol. Chem. 388, 79). We extended these studies by analysing the structure of membrane-bound PrPc applying FT-IR in the attenuated total reflection mode. The spectra exhibited a constant portion, i.e. which is independent upon the binding process and independent upon the concentration of PrPc, and a variable portion, i.e. showing up upon binding and increasing with concentration. After deconvolution of the spectra in different secondary structure components as described recently (Oellesch and Gerwert, submitted) it could be concluded from the constant portion that the globular structure of PrPc as known from NMR-analysis remains unchanged after binding to the membrane. However, the variable portion of the spectra showed, that the segment of the structure, which is flexible in solution, is involved in forming intermolecular β strands between PrPc-molecules on the membrane. One might suggest, that β-sheet rich aggregates which contribute to the structure of infectious PrPSc are pre-formed already in form of PrPc-aggregates anchored on the membrane.

**P01.15**

**Quantitative Mass Spectrometry Analysis of the Pathological PrP Allotypes Present in the Brain of gCJD Affected Individuals**

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Transmissible spongiform encephalopathies (TSEs) are neurodegenerative disorders characterized by the accumulation, principally in the central nervous system (CNS), of the abnormal form (PrPSc) of a host encoded protein, the cellular prion protein (PrPc). In humans some forms of TSEs have a genetic etiology, as they are associated to mutations in the PrP gene: thus it is extremely important to elucidate the role played by the altered residue in the pathological conversion of the protein.

Aim of our work was to deduce the involvement of the mutated residue in the molecular mechanisms underlying genetic TSE pathogenesis by means of a quantitative mass spectrometry analysis of the PrPSc allotypes accumulated in the brains of genetic Creutzfeldt-Jakob disease (gCJD) affected individuals. We have integrated our previous data on the gCJD patient carrying the R208H mutation with those regarding V210I gCJD patients in order to verify if diverse mutations regulate in integrated our previous data on the gCJD patient carrying the R208H mutation with those regarding V210I gCJD patients in order to verify if diverse mutations regulate in

**P01.16**

**Seeded Fibrillization of Recombinant Bovine Prion with BSE-prions**

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Conversion of the cellular isofrom of the prion protein (PrPC) to the infectious isofrom of PrP (PrPSc) plays a key role in the development of prion diseases. To simulate the structural transition in vitro we developed a solution system of well balanced concentrations of SDS and NaCl. Under these conditions an intermediate, partially denatured state of PrP is soluble for weeks but forms fibrils afterward (Leffers et al. 2005, Stöhr et al. manuscript submitted ). In the present work we show for the first time the conversion of bov recPrP (25-241) into amyloid as assayed by Thioflavin T (ThT)-fluorescence-assay. Furthermore we extended the study in respect to seeded fibrillogenesis of bov recPrP. This study is based on a seeded-conversion assay as established in our group using SHa recPrP and hamster scrapie prions (Weinmann et al. manuscript in preparation). In the present study minute amounts of purified bovine BSE-prions prepared without PK-digestion serve as effective seeds for the conversion of bov recPrP (25-241) into an fibrillar, amyloid state as proven by ThT-fluorescence-assay. This conversion is specific for the seed, since it occurs within hours, whereas bov recPrP in the absence of seeds is converted into fibres only after weeks of incubation. The system is similar to the seeded-fibrilization of the Soto-group (Castilla et al. 2005) but clearly different in two aspects, first that it is purely in vitro i.e. without any cellular compounds and second it avoids incubation-sonification cycles by strong homogenous stirring.
P01.17
Systematic Development of New Compounds for the Causal Therapy of Prion Diseases based on Molecular High-throughput Screening

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We developed a high-throughput screening assay based on the SIFT (Scanning for Intensely Fluorescent Targets) technique for the identification of drugs, which interfere with the interaction between PrP²⁰ and PrP²⁴ at the molecular level. Screening a library of 10,000 drug-like compounds yielded 80 active compounds, which were confirmed by dose-response curves with half-maximal inhibitory effects ranging from 0.3 to 60 µM. Among these, six compounds displayed an inhibitory effect on PrPSc propagation in scrapie-infected N2a cells. Four of these candidate drugs shared a N-benzylidene-benzohydrazide (NBB) core structure, which therefore represents a new lead structure for the development of therapeutics against prion diseases. Molecular SIFT screening of a second library of 10,000 drug-like compounds in combination with a systematic primary cellulture-screening of the same compound libraries, and molecular screening of newly generated focused compound libraries resulted in the identification of further potential lead structures with favourable medical chemistry profiles. Selected compounds including NBBs were also tested in vivo and could be shown to significantly prolong survival times of Scrapie-infected mice when applied i.p. for 14 days late in the incubation period.

P01.18
Scrapie Prion Strains have Distinct Conformational Stability and Sedimentation Properties

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Much remains to be learned about the physical relationship between infectivity and aggregated PrP, and to which extent it varies according to the prion strain. Transmission of natural sheep scrapie and BSE infectious sources to transgenic mice overexpressing the VRQ allele of ovine PrP (tg338 line) has allowed us to establish a panel of biologically cloned strains that have been shown to produce stable and clearly distinct phenotypes based on the electrophoretic profile of PrPSc, its regional distribution in the brain, and the incubation time. We have sought to further compare these strains on the basis of their physicochemical features.

We first studied the individual conformational stability of four strains by measuring the resistance to denaturation by guanidine-HCl (GdnHCl). At variance with that recently reported for rodent-adapted strains, we failed to observe a straight correlation between [GdnHCl]1/2 values and incubation times.

We next compared the sedimentation properties of abnormal PrP and infectivity of each strain by velocity gradient centrifugation, by using detergent-solubilised, infected brain homogenate instead of PrPres-enriched material. Gradient fractions were analysed for PrPC, PrPSc and PrPres content by ELISA and western blotting, and for infectivity by bioassay in tg338 mice. The detergent treatment conditions were optimised so as to separate most of the PrPSc from the bulk of PrPres. PrPres was found to sediment in the middle region of the gradient, however the position and shape of the peak noticeably differed among the strains, reflecting PrPSc aggregates of different sizes. Examining the distribution of infectivity revealed even more striking differences. Thus, 99% of the infectivity was separated from about 90% of PrPres in the case of a fast strain, whereas in the case of BSEov agent the peaks of PrPres and infectivity were largely overlapping. From these results it was concluded that the relationship between infectivity and misfolded PrP multimers is not univocal, suggesting that the size of the most infectious particles may substantially differ among prions strains.

P01.19
In Vitro Analysis of the Molecular Basis of Strain Variation

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The prion hypothesis states that the infectious agent is predominantly composed of misfolded PrP. One of the challenges to this hypothesis is the existence of prion strains. Different strains of the infectious agent are thought to arise from different conformations of PrP⁰ that can be faithfully passed onto PrP⁰ during conversion. To account for the molecular basis behind strains, it has been suggested that there is the participation of strain specific co-factors that aid in the refolding process during conversion. This may also be aided by the preferential targeting of different TSE strains to specific brain regions and potentially different cell types. This project aims to investigate the possible involvement of accessory molecules in strain definition. Potential accessory molecules will be sourced from TSE susceptible cell lines and analysed using the cell free conversion assay. Alternatively, scrape associated fibrils of mouse scrapie can be analysed by mass spectrometry to identify co-factors that are required by individual prion strains for efficient conversion of PrP⁰ to PrP⁰. In vitro refolding assays will form the basis for establishing an assay that promotes refolding of PrP⁰ with the aid of strain specific co-factors, into a β-sheet rich isoform that mimics PrP⁰ from different TSE strains, without seeding with exogenous PrP⁰. We will present an update on progress towards achieving our aims.

P01.20
Molecular Dynamics Simulations of Prion Protein Variants

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At the Neuropathogenesis Unit, we make use of a variety of techniques to investigate the effect of mutations and polymorphisms in the gene encoding the prion protein, including the use of transgenic mice and cell free misfolding assays. However, understanding of the molecular mechanisms responsible for the phenotype associated with individual mutations requires knowledge of the intricacies of protein folding and misfolding at an atomic level. As a result of concomitant improvements in computer technology and in force field approximations of biomolecules, molecular modelling is becoming an increasingly useful tool to investigate protein folding dynamics.

We have recently published work that demonstrates the significant protective effect of a mutation at codon 168 (Pro to Leu) in ovine PrP in ovine PrP, based on both in vivo and in vitro data. The molecular effect of this mutation may be to stabilise PrP⁰, destabilise PrPSc and aid in the refolding process during conversion. This may also be aided by the preferential targeting of different TSE strains to specific brain regions and potentially different cell types. This project aims to investigate the possible involvement of accessory molecules in strain definition. Potential accessory molecules will be sourced from TSE susceptible cell lines and analysed using the cell free conversion assay. Alternatively, scrape associated fibrils of mouse scrapie can be analysed by mass spectrometry to identify co-factors that are required by individual prion strains for efficient conversion of PrP⁰ to PrP⁰. In vitro refolding assays will form the basis for establishing an assay that promotes refolding of PrP⁰ with the aid of strain specific co-factors, into a β-sheet rich isoform that mimics PrP⁰ from different TSE strains, without seeding with exogenous PrP⁰. We will present an update on progress towards achieving our aims.
Mouse PrP(1-28) Peptide Reduces PrPSc Levels in a Scrapie Infected Mouse Hypothalamic Cell Line

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Cell penetrating peptides (CPPs) are a heterogeneous group of short peptides capable of penetrating cellular membranes and translocate linked macromolecules into a variety of cells. Heparan sulfate proteoglycans (HSPGs) have been proposed to be implicated in the uptake mechanism of certain types of CPPs. Evidence suggests a foremost endocytic, lipid raft-dependent form of uptake although alternative, competitive and parallel entry mechanisms may also exist. The N-terminal (1-28) part of the mouse prion protein (mPrP(1-28)) has CPP-like properties and it may interact with HSPGs. In addition, HSPGs are considered to be involved in conversion of the cellular prion protein (PrPC) to the misfolded and pathogenic isoform of the prion protein (PrPSc). This led us to investigate whether presence of CPPs may interfere with prion infection by competitive binding to the cellular HSPGs involved in PrPSc propagation. Healthy (GT1-1) and scrapie-infected (ScGT1-1) mouse neuronal hypothalamic cell lines were cultured in the presence of different CPPs across a range of concentrations and time spans. Cell extracts were analyzed by Western blot for relative levels of PrP(1-28) (GT1-1) or PrPSc (ScGT1-1) compared to untreated control cultures. Treatment with mPrP(1-28) reduced PrPSc levels in ScGT1-1 cells, but did not affect the PrP(1-28) levels in GT1-1. This effect is sequence specific since treatments with other CPPs or PrP peptides tested (transportan-10, penetratin, pol-y-L-arginine, mPrP(23-50) or bovine PrP(1-30)) resulted in no reduction of PrP(1-28) or PrPSc levels in ScGT1-1 cell cultures. Taken together, these results may provide important clues to the process of PrPSc conversion.

The Prion Protein 3F4 Epitope Revisited: Characterization of the Minimal Epitope and its use as a Molecular Tag

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The commonly used monoclonal antibody (Mab) 3F4, has for nearly two decades contributed significantly to our understanding of the normal cell biology of the prion protein (PrP), as well as the disease related abnormalities occurring in prion diseases. The 3F4 antibody binds to human, hamster and feline PrP with high affinity, requiring two Met residues at positions corresponding to 109 and 112 in the human PrP Species, that lack one of the Met residues, like cattle and sheep, or both, like rat and mouse, do not react with the 3F4 antibody. Previous observations have led to the commonly accepted notion that the 3F4 epitope consists of a tetra-peptide Met-Lys-His-Met, encompassing residues 109-112 in human PrP. Here, we have characterized the epitope by studying its binding to synthetic peptides and by analysis of mutated ovine PrP::GFP constructs expressed in the murine neuroblastoma cell line N2a. We found the 3F4 epitope to consist of the hepta-peptide Lys-Thr-Asn-Met-Lys-His-Met (106-112), where the Lys 109 is critically important for 3F4 binding. We also demonstrate that the hepta-peptide constituting the minimal 3F4 epitope can be used as a discrete, moveable high-affinity tag, which might be useful in applications beyond prion research.

Fibrillization of the Prion Protein at a Membrane Surface

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Prion diseases or transmissible spongiform encephalopathies are fatal neurodegenerative disorders that are characterised by the misfolding of the cellular prion protein, PrPc, into the insoluble β-sheet rich isoform, PrPSc. Evidence suggests that cellular membranes may provide the denaturing environment that promotes protein-misfolding, leading to subsequent oligomerization and fibrillation of the prion protein. The aim of this study is to characterise prion conversion and fibrillation induced by model membranes in vitro.

The interaction of the recombinant prion protein with membranes that contain varying percentages of negatively charged and zwitterionic lipids has been characterised. Binding of PrP(90-231) to lipid vesicles is monitored by the blue shift in tryptophan fluorescence as the tryptophan residues move from a polar solution environment to a hydrophobic membrane environment. The results show that prion interaction with membranes is dependent on the concentration of negatively charged lipids within the membrane.

Structural analyses, through circular dichroism, show that on membrane binding the prion protein undergoes a structural conversion that results in increased β-sheet structure. An isodichroic point at 188 nm suggests that this is a stable two state conformational transition.

Negative stain electron microscopy was employed to characterise the morphology of aggregates formed on the membrane surface. This reveals that a high percentage of negatively charged lipid within the membrane induce the formation of amorphous aggregates, whilst lower percentages of negatively charged lipid are more likely to promote ordered aggregation.

The study highlights the cellular conditions that may promote the formation of ordered membrane-associated prion fibrils.

How Do Glycosaminoglycans Promote the Propagation of Prions?

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Background: It is recognised that ancillary factors may contribute to the pathogenesis and transmission of prion diseases. Of particular interest have been polyanions, including nucleic acids and glycosaminoglycans (GAGs). However the exact nature of this interaction is poorly understood.

Aim: To elucidate the molecular basis of the GAG prion protein (PrP) interaction.

Methods: We are using a cell free model of protease resistant PrP (PrPSc) formation to elucidate the role of GAGs in prion disease. This model, the conversion activity assay (CAA), can be used to generate PrPSc from brain homogenate or cell lysate derived PrP and is driven by a PrPSc seed derived from prion infected tissue. The CAA enables the study of the role of GAGs in the formation of PrPSc through degradation/inhibition of endogenous GAGs or disruption of the GAG/PrP interaction. To further characterise the GAG PrP interaction we have modified GAG expression in a rabbit kidney cell line (RK13) expressing exogenous mouse PrP (moRK13). This system is also being used to express mutant forms of PrPSc to further investigate the molecular basis of GAG mediated PrPSc formation.

Results: Conversion activity in the CAA is disrupted by conditions that disrupt the electrostatic interaction typically associated with a GAG protein interaction. These conditions also disrupt the ability of brain and cell lysate derived PrP to bind a purified source of GAGs. Depletion of endogenous GAGs present in brain homogenates used as a source of PrP in the CAA implicates GAGs in PrPSc formation. The molecular basis of this effect is being further investigated using lysates of moRK13 cells in which GAG expression has been modified.

Discussion: GAG analogs and polyanions have shown great potential as anti-prion therapeutics. In this study we are seeking to elucidate the molecular basis of this interaction with the aim of developing effective and targeted therapeutics.
Protein Misfolding

P01.25
Immunization Against PrP and Fusion PrP Recombinant Peptides
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Immunization against prions has been attempted in several animal models. However, the low immunogenicity of this molecule in wild type (wt) animals has hampered the attempts for active immunization. In this study we aimed to evaluate the immunogenicity of various forms of mouse recombinant PrP (mrPrP) in wt mice and their possible protective role in an animal model of transmissible spongiform encephalopathies (TSEs).

Murine PrP was cloned and expressed either alone or fused to the heat shock protein DnaK, a bacterial homologue of human HSP70. The recombinant proteins were administered to groups of mice (c57bl/6); the first group received purified mrPrP solubilized in urea, the second purified mrPrP inclusion bodies, solubilized in a mild detergent solution. The third group received the mrPrP-DnaK fusion, whereas a mix of solubilized mrPrP and recombinant DnaK were administered to the fourth group. A group that received the adjuvant only and a group which was not immunized were also included. The immunization scheme consisted in priming and two boosts, with 14 days intervals. 10 days after the second boost, the anti-mrPrP titer was estimated by ELISA and Western blotting (WB). The mice were then challenged intraperitoneally with the RML scrapie strain and observed for the appearance of clinical symptoms. When the mice reached terminal stage they were sacrificed and their tissues prepared for immunohistochemistry and WB. On at least two mice of each group splenic lymphocytes were harvested and lymphocyte proliferation assays, as well as RT-PCR analysis of the expression of Il-2, Il-4, Il-6 and Ifn-g genes in the cultured lymphocytes in the presence of mrPrP were performed.

Data from the ELISA and WB analysis indicate that only mice that received the mix of mrPrP and DnaK mounted a measurable immune response against mrPrP. Anti-serum from one of these mice also recognized PrP\(^{\text{c}}\) in whole murine brain homogenates, but not purified PrP\(^{\text{c}}\). However mice that received the solubilized purified inclusion bodies succumbed to disease later than the other mice. Results form the cell proliferation assay and the RT-PCR analysis indicate the elicitation of cell mediated immune response.

Our data are also indicative that protection against TSEs does not necessarily correlate with the stimulation of production of anti-PrP antibodies and that the administration of mrPrP inclusion bodies could prove an effective immunization procedure.

P01.26
Phenotypic Heterogeneity of Cellular Prion Proteins in Human, Sheep, Cattle and the Mouse
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Prion diseases are neurodegenerative disorders affecting both humans and animals. During pathogenesis the host encoded cellular prion protein (PrP\(^{\text{c}}\)) is converted into its infectious isoform (PrP\(^{\text{sc}}\)) which accumulates mainly in favoured brain regions. PrP\(^{\text{sc}}\) is partial resistant to proteases, insoluble in detergent solutions and highly resistant to inactivation procedures. Many different PrP\(^{\text{sc}}\) glycoform banding patterns have been identified; however, it is not known why and which PrP\(^{\text{sc}}\) glycoforms selectively accumulate in favoured brain regions. Little attention has been given so far to the differentiation of PrP\(^{\text{sc}}\); however, these proteins are the “substrates” for development of prion diseases. Thus it is conceivable that some PrP\(^{\text{sc}}\) subspecies and conformations which are differentially and posttranslationally modified may interact more or less efficiently with PrP\(^{\text{c}}\), and different protein isoforms will be formed during conversion.

With a set of antibodies we therefore typed the PrP\(^{\text{c}}\) glycoform banding patterns deriving from different brains and regions by densitometry. We found heterogeneous PrP\(^{\text{c}}\) phenotypes. One pattern is characterized by high signal intensity of the di glycosylated isoform using antibodies binding to the N-terminal region whereas the other exhibits high intensity for proteins consisting of non glycosylated full length and glycosylated truncated PrP\(^{\text{c}}\) isoforms when using antibodies recognizing the C-terminal region. PrP\(^{\text{c}}\) are modified by attachment of various N-linked glycans which may influence protein solubility. Different PrP\(^{\text{c}}\) protein profiles derived from one sample were identified on the basis of solubility by differential centrifugation. Taken together, we found marked variations in the processing of PrP\(^{\text{c}}\) which may lead to interregional differences in the glycoform composition of PrP\(^{\text{c}}\).

P01.27
A Novel Resistance-linked Ovine PrP Variant and Its Equivalent Mouse Variant Modulate the in Vitro Cell-Free Conversion of rPrP to PrPres
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Prior diseases are associated with the conversion of the normal cellular prion protein, PrPC, to the abnormal disease associated PrPSc. This conversion can be mimicked in vitro using the cell-free conversion assay. This assay can be modified to use bacterial recombinant PrP as a substrate and mimic the in vivo transmission characteristics of rodent scrapie. Here we demonstrate that the assay replicates the ovine polymorphism barriers of scrapie transmission. In addition, the recently identified ovine PrP variant ARL168Q, which is associated with increased survival of sheep to experimental BSE, modulates the cell-free conversion of ovine recombinant PrP to PrPres by 3 different types of PrPSc, reducing conversion efficiencies to levels similar to the ovine resistance-associated ARR variant. Also, the equivalent variant in mice (L164A) is resistant to conversion by 87V scrapie. Together these results suggest a significant role for this position and/or amino acid in conversion.

P01.28
Withdrawn
**PO1.29**

**Ovine and Bovine Species/polyorphism-specificity of the Protein Misfolding Cyclic Amplification (PMCA)**

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Polymorphisms in the prion protein (host and donor PrP genotype) are known to influence TSE susceptibility and transmissibility in vivo.

These modulating effects on the underlying molecular conversion of prion proteins can be readily assessed in vitro.

The so-called VRQ allelic variant of sheep PrP is generally associated with high susceptibility to scrapie.

The ARR allelic variant is in general considered to encode lowered susceptibility for scrapie, although this might be questioned for atypical and/or sheep derived BSE.

In vitro tests assessing the (potential) species/polymorphism barriers support these findings on the molecular level and they can measure virtually every species barrier.

In the past we used a Gdn-based conversion system to measure the effect of species- and polymorphism-barriers on prion protein conversion efficiencies.

These studies revealed a clear correlation between barriers observed in vivo and the corresponding in vitro conversion efficiencies.

Here we describe the use of the PMCA technique to measure conversions efficiencies between ovine and bovine allelic variants.

The protein misfolding cyclic amplification (PMCA) is a technique allowing the in vitro generation of protease-resistant prion protein (PrPRes) using only a small seed of disease-associated prion protein (PrPd) to convert normal cellular prion protein (PrPc) in brain homogenates in contrast to Gdn-based systems.

Several incubation and sonication steps allows the diluted positive starting material to be amplified, detectable by Western blot analysis but also by bioassays.

After fine-tuning (for specificity) the PMCA conditions to ovine and bovine derived materials, we were able to measure species/polymorphism-barriers as determined before using Gdn-based conversion reactions.

Conversion specificity ("transmission profiles"), reproducibility, (in)stability of glycoprofiles, as well as the surprising "transmission profile" of sheep derived BSE, will be discussed.

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**PO1.30**

**Use of a Travelling Wave Based Ion Mobility Mass Spectrometry Approach to Resolve Prion Proteins of Varying Conformations**

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Protein misfolding diseases have been proposed to be initiated by a change in protein shape. A new mass spectrometry technique (termed ion mobility) has recently been developed that is able to yield information regarding molecular shape. This may be able to provide an insight into the conformational changes of prion proteins by measuring the change in cross section of the protein on misfolding. Measurement of misfolded protein would aid diagnosis and promote understanding of the disease propagation.

Ion mobility mass spectrometry measures how quickly a gaseous ion moves through a drift cell that is filled with a buffer gas under high pressure with the influence of a weak electric field. More compact ions with a small collision cross section will drift more quickly than extended ions. A travelling wave based ion mobility mass spectrometry approach is utilised to measure the change in cross section of the prion protein on misfolding.

All experiments were carried out on a Synapt HDMS System (Waters, Manchester, UK) which has a hybrid quadrupole/ion mobility/orthogonal acceleration time-of-flight (oa-TOF) geometry. Ions are accumulated in a trap travelling wave (T-Wave) device and periodically released into the ion mobility separation (IMS) T-Wave where they separate according to their mobility. The ions are then propelled through a transfer T-Wave to the oa-TOF for mass analysis. Ion arrival time distributions are recorded by synchronization of the oa-TOF with the gated release of ions into the IMS T-Wave.

Recombinant prion samples of Syrian hamster prion protein, SHaPPrP23-231) and SHaPPrP80-231), were expressed in both predominately alpha-helix and misfolded predominately beta-sheet forms. Circular dichroism experiments were carried out in order to confirm the nature of the secondary structure of the prion proteins. Estimations of the rotationally averaged cross sections of the proteins were made by reference to standards of known cross section. The ability of the approach to differentiate the normal cellular prion protein from the experimentally misfolded prion protein is demonstrated.

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**PO1.31**

**Effects and Binding of Quinacrine, Chlorpromazine, and 9-Aminoacridine on the Human Prion Protein - A Molecular Dynamics Study**

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Human prion diseases are invariably fatal with currently no practical means for diagnosis or effective treatment. Over the years a number of transmissible spongiform encephalopathy (TSE) drug candidates have been proposed, chosen mainly from a large pool of compounds whose use in the treatment of some other diseases has been established, along with a knowledge of the safe clinical doses of administration [1]. However, what is almost completely lacking at the moment is the fundamental understanding of the effects of TSE drug candidates on the different forms of prion proteins at the atomic level. Yet, as is the case for many other diseases, it is just this information that holds the key to understanding how the pertaining drugs function and how more efficient drugs can be further designed.

We use molecular dynamics computer simulations to study, at the atomic level, the interaction of a human prion protein (hPrPc) precursor (residues 125 - 228 at pH 7.0) [2] with TSE drug candidates. In this initial study we have considered the interaction of hPrPc with quinacrine (QA) and chlorpromazine (CPZ), which have been used for decades to treat malaria and schizophrenia, respectively, and are known to inhibit the conversion of PrPc to the disease-related form PrPSc [3]. We identify the binding sites of these compounds on the hPrPc, along with possible changes in the structure and dynamics of the protein. The modelling is further complemented by comparing the results obtained with QA and CPZ to those obtained with 9-aminoacridine. The latter is a compound related to QA but which, on the other hand, is known not to inhibit the conversion of PrPc to PrPSc [3].


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**PO1.32**

**Disulphide Tethers Give New Insight into The Prion Protein Conformational Change**

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Prion diseases are neurodegenerative diseases that effect people and animals. Prion hypothesis states that infectious agent is composed of protein only and does not contain nucleic acids as do conventional pathogens. In the course of the disease the the native cellular prion protein in the presence of the infectious prion converts from the mainly alpha helical into the insoluble beta sheet amyloid form called PrPSc. The high resolution structure of PrPSc is not known, so it is very difficult to propose the mechanism of conformational change. The knowledge on this structural transformation is important for an understanding of the etiology of the disease as well for the design of possible therapies. Our approach to map the structure of the converted PrP (prion protein) was to restrict the different structural segments of PrP by covalent links, preventing the structural transition of the defined segments of the structure. We have selected the sites of introduced disulfides based on the three dimensional structure of mouse prion protein. It is expected that introduction of additional disulfides would stabilize the structure and also make it more rigid, but its influence on the replication should be position dependent. We have introduced two types of disulfides - short-range mutants, placed in the three surface loops between the elements of the regular secondary structure and long-range mutants, with disulfide bridges enclosing several secondary structure elements. We confirmed correct folding of the mutants by circular dichroism and nuclear magnetic resonance spectra of the isolated proteins. Most short range mutants show comparable thermodynamic stability to the wild-type, while long range and some short-range mutants are significantly more stable than the wild-type protein. We also followed in vitro fibrillation of mutants. We discovered that there is no correlation between the protein stability and structural conversion, which allows us to map the structural segments of PrP structure important for the conformational change. Our model provides structural information for the improvement of the model of conformational transition.
findings suggest a mechanism where cationic domains of PrPC may play a role in the propagation through a variety of prion transmission studies. Taken together, these two positively charged domains have been reported to be important for prion affected binding. Binding occurs through a conformational domain that is only present in PrPSc from PrPC.

The peptide:PrPSc interaction was not sequence specific, but amino acid composition important, but not exclusive, role in the interaction between the peptides and PrPSc. Neither hydrophobic nor polar interactions appear to correlate with binding activity. The peptide:PrPSc interaction was not sequence specific, but amino acid composition affected binding. Binding occurs through a conformational domain that is only present in PrPSc, is species independent, and is not affected by proteinase K digestion. These two positively charged domains have been reported to be important for prion propagation through a variety of prion transmission studies. Taken together, these findings suggest a mechanism where cationic domains of PrPSc may play a role in the recruitment of PrP to PrPSc.

**P01.35**

**Characterization of Human PrP-derived Peptides that Discriminate Full-length PrPSc from PrPC**

Perez, D; Lau, A; Yam, A; Wang, X; Gao, C; Timoteo, G; Chien, D; Wu, P; Nowaritz, USA

Upon our initial discovery that PrP-derived peptides were capable of capturing the pathogenic prion protein (PrPSc), we have been interested in how these peptides interact with PrPSc. After screening peptides from the entire human PrP sequence, we found two peptides (PrP19-30 and PrP100-111) capable of binding full-length PrPSc in plasma, with greater than 3000-fold binding specificity to PrPSc over the normal prion protein (PrPC). To detect captured PrPSc, we have developed a highly sensitive sandwich ELISA with a limit of detection of 4 attomoles (0.5 pg/mL) of human rPrP.

With these sensitive tools, we have been able to develop a method to detect PrPSc using a gas chromatography-mass spectrometry (GC-MS) platform. This method has allowed us to identify several PrPSc-specific peptides, which may be useful in the development of new diagnostic assays for prion diseases.

**P01.36**

**Mimicking Pathological Conversion of PrP to PrPSc In Vitro**

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The pivotal event in the initiation of prion disease is the conversion of normal cellular prion protein (PrPC) into PrPSc that is believed to be made up entirely of miss-folded PrP molecules which become tightly aggregated. The consensus of scientific opinion is that only PrP molecules are involved in this process. We have discovered an alternative prion protein ("prionin") that is encoded and expressed from within the normal PrP transcription unit in all species of PrP genes. Prionins are small (39 amino acids in hamsters to 102 in mouse) proteins that are expressed in a prion disease-specific manner and they are transmissible between species. In this study we show that prionins can convert PrP to proteinase K-resistant conformers in vitro. Nanogram quantities of a "converting factor" (cf) are sufficient. From the amino acid sequence of the PrPC induced to PrPSc, we have designed a nanogram quantity of a "converting factor" (cf) that can react with a proteinase K-resistant core as well as truncated recombinant human, cattle and murine PrP (in PrPSc) to a 22-25 kDa proteinase K-resistant conformer. Conversion started immediately upon addition of cf to the various PrP preparations and proceeded with time during incubation with gentle shaking (2.5x100 min-1) at 4°C, ambient temperature and 37°C. No infectious seeding material was added to any of the incubations. Conversion was observed in animals containing larger number of amyloid plaques. Inoculation of tg2576 mice showed incubation periods similar to the original infectious material, suggesting that strains characteristics are maintained. In vitro data showed cross-seeding aggregation between PrP and Abβ. Our findings suggest an interaction between Alzheimer’s and prion pathologies, indicating that one protein misfolding process may be an important risk factor for the development of a second perhaps more prevalent disease.
The difference to the binding site found by Y2H most likely is due to the use of peptide fragments by including them into their native tertiary context lead to binding. Surface defined by helices 1 and 3 of PrP. CSP studies of full length PrP and the binding site suggested from Y2H-assays. In particular, binding takes place on the PrP and LRP do not interact in solution. However, weak binding could be detected by measuring PRE. These data indicate binding in a region similar, but not identical to PrP-LRP interaction surface are elucidated by means of solution NMR-spectroscopy. In particular, we apply a combination of paramagnetic relaxation enhancement (PRE) and chemical shift perturbation (CSP) to identify residues responsible for binding.

Results: We show that the peptides corresponding to the isolated binding domains of PrP and LRP do not interact in solution. However, weak binding could be detected using spin-labelled peptides mimicking the binding domain of LRP and full length PrP by measuring PRE. These data indicate binding in a region similar, but not identical to the binding site suggested from Y2H-assays. In particular, binding takes place on the surface defined by helices 1 and 3 of PrP. CSP studies of full length PrP and the extracellular domain of LRP confirm these findings.

Discussion: Due to the size of the complex of both proteins (~47 kDa) we started from isolated binding domains, which did not interact. Presumably, this is due to the lack of stable conformers responsible for binding. Conformational stabilization of the isolated peptide fragments by including them into their native tertiary context lead to binding. The difference to the binding site found by Y2H most likely is due to the use of peptide fragments only, which may lead to substantial loss of conformational and tertiary information. Knowledge of such binding interfaces at high resolution builds the foundation for the rational design of novel anti-prion drugs.
**P01.41**
Can Dendrimers Block the Formation of Fibrils?
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Background: Dendrimers are a new class of polymers with a well-defined molecular structure. They adopt a spherical shape and are prepared in a step-wise manner from branched monomer units. The more layers that are attached, the higher the so-called generation of dendrimer is. Dendrimers are promising materials in many biomedical applications such as transfection of DNA and carrying drugs. Recently, it has been shown that dendrimers have their own potentially therapeutic activity against prion diseases [1]. The potency of amino-terminated dendrimers in eliminating PrPSc from Sc229 cells was proved. It was suggested that the presence of amino surface groups that increases with generation was crucial for purging activity. These promising results encouraged studies on other types of dendrimers. Solassol et al. successfully tested phosphorus containing cationic dendrimers for anti-prion activity [2].

Objectives: We used an alternative method to cell-based assays to screen the anti-prion dendrimers. Terminated prion peptides in the absence of cellular factors were exposed to destabilizing factors to mimic the conditions that lead to creation of fibrils.

Methods: The accumulation of amyloids was monitored by changes in the fluorescence of thioflavine T, which is sensitive to the presence of amyloid fibrils. These studies were accompanied by Fourier transformed infrared spectroscopy to check the impact of dendrimers on the secondary structure of peptides and by electron microscopy to observe morphology of fibrils.

Results: It has been shown that the higher the dendrimer generation, the larger the degree of inhibition of the amyloid aggregation process and the more effective dendrimers are in disrupting the already existing fibrils.

Discussion: The mechanisms of inhibiting amyloidogenesis by dendrimers were formed [3, 4]. It has been postulated that dendrimers can interact with peptide monomers, block fibril ends and break existing fibrils.


**P01.42**
Probing the Prion Protein at the Cell Surface with FLIM and FRET Measurements
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The prion protein (PrP) is being expressed in N2a-BOS cells fused to CFP (cyan fluorescent variant of GFP) and YFP (yellow variant of GFP). PrP in mammals is GPI-anchored (glycosylphosphatidylinositol) to the membrane and to have the protein attached to the membrane the signal to GPI-anchor was cloned downstream to the FRET pair to attach the fusion protein to the cell surface. A clone selection protocol was optimized in order to obtain stable N2a clones expressing the fusion proteins. Cells were cultured on glass cover-slips to allow fluorescence images to be recorded. The fusion proteins at the cell surface were detected through the intrinsic fluorescence of the FP and the integrity of the fusion will be verified by western-blotting. Fluorescence lifetime imaging microscopy (FLIM) will be used to probe PrP at the cell surface. FLIM will be combined with fluorescence resonance energy transfer (FRET) measurements for the pair CFP-YFP. The combination of FLIM and FRET provides high spatial (nanometer) and temporal (nanosecond) resolution and is expected to give insight into conformational changes of PrP and to detect PrP-PrP interactions at the cell surface.

**P01.43**
Time Course of the Development of PrP Aggregates in a Mouse Model of Prion Disease
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Background: Abnormal prion protein (PrP) has a property of partial protease resistance, which is one of the grounds for the diagnosis of transmissible spongiform encephalopathies (TSEs), i.e. prion diseases. However, recent studies have revealed that protease-sensitive abnormal PrP (PrPSc) also exists considerably in the TSE brains, and PrP oligomers have more intense infectivity and neurotoxicity than highly aggregated fibrils. Accordingly, protease-resistant PrP (PrPSc or PrPres) might account for only a part of prions, which prompted us to establish another method for the detection of abnormal PrP.

Objectives: We designed the simplified gel filtration method to detect PrP aggregates and/or oligomers, and applied it to the study on a TSE mouse model to examine the time-course development of abnormal PrP.

Methods: Gel filtration spin columns, CHROMA SPIN (Clontech, USA), were used for size-exclusion fractionation of PrP without protease digestion. Serially corrected brain samples of NZW mice inoculated with the Fukuoka-1 strain intracranially were processed to whole brain homogenates in the buffer with detergents. Gel-fractionated samples were applied to western blotting to detect PrP in each molecular mass range and the densities were measured. Conventional protease-digestion assay was also performed to detect PrPres in the same samples.

Results: Mice inoculated with scrapie agent were died at around 4.0 months post inoculation. Spongiform change and abnormal PrP deposition detected by immunohistochemistry were apparently observed from 3.0 months post inoculation particularly in the thalamus. PrPres was drastically increased from 3.5 months, whereas the increase of PrP aggregates detected by the gel filtration spin column method became apparent from 3.0 months.

Discussion: Gel filtration assay under certain conditions with detergents is useful for the detection of abnormal, detergent-insoluble PrP aggregates. Application of spin columns provides the benefit of simple and safety handling in the closed system. In our study, the development of PrP aggregates preceded PrPres multiplication in the TSE mouse model. Because this assay does not include protease digestion process, the detected PrP aggregates in the early disease stage would be sPrPSc molecules. Protease-sensitive PrP aggregates may affect the pathological process in TSEs, thus we would have need to investigate the other properties of abnormal PrP than the protease resistance.

**P01.44**
Prolonged Incubation Time and Differential Processing of PrP-CWD in Experimentally Infected Rocky Mountain Elk Lacking the 132M Prnp Allele
O’Pourte, K.; Spraker, T.; Greer, J.; Gidewicz, T.; Hamir, A.

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Background: The relationship between host Prnp genotype, susceptibility, and incubation time varies among the ruminant prion diseases, including chronic wasting disease (CWD). The Rocky Mountain elk Prnp gene encodes a nonsynonymous mutation at codon 132, resulting in substitution of leucine (L) for methionine (M). A polymorphism at the corresponding site (codon 129) is associated with varying patterns of susceptibility in human prion diseases. We have previously established the predisposition to CWD in homozygous 132MM elk in captivity and the approximate doubling of incubation time in 132LM elk following oral challenge.

Objective: We have now defined the prolonged incubation time in 132LL elk to be approximately triple that of MM132 elk. To examine potential mechanisms for the prolonged incubation period, PrP-CWD from elk of the short (132MM), intermediate (132LM) and long (132LL) incubation were characterized. Methods: The protease resistant core of PrP-CWD from brain of experimentally infected elk of each of the three genotypes was examined by Western blot analysis using amino-terminus (ab P4) and carboxyl-terminus (ab F99/97) antibodies, with and without protease K (PK) digestion, and with and without deglycosylation.

Results: Western blot analysis and antibody epitope mapping of PK-digested PrP-CWD demonstrated a reduction in the apparent molecular weight and loss of the antibody P4 epitope in the PK-resistant core of PrP-CWD from 132LL elk and a significant increase in the ratio of deglycosylated to glycosylated isoforms of PrP-CWD in these samples.

Discussion: Differential glycosylation and altered folding of PrP-CWD in 132LL elk suggest that intracellular processing of PrP-CWD may be one of the mechanisms associated with the prolonged incubation period associated with the polymorphism.
PO1.45
Cholesterol Analogs Modulate Lipids and Hamper Prions in Cultured Cells and Retard Scrapie in Mice
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In prion-infected cells, the formation of the scrapie prion protein PrP\textsuperscript{Sc} from its normal precursor PrP\textsuperscript{C} seems to be controlled by lipid rafts, but the mechanisms remain obscure. Lipid rafts are lateral, partially ordered assemblies of cholesterol and of sphingolipids within cellular membranes. Because natural cholesterol analogs vary in their propensity to form ordered lipid domains in vitro, they may provide a way to modulate rafts in vivo. We report that several sterols modified rafts and their lipids and reduced prions in chronically infected Scn2a and ScgT1 cells. Cholesterol sulfate, a poor domain former, and dehydroepiandrosterone (DHEA) both decreased PrP\textsuperscript{Sc} but modulate rafts in vivo. We report that several sterols modified rafts and their lipids and reduced prions in chronically infected Scn2a and ScgT1 cells. Cholesterol sulfate, a poor domain former, and dehydroepiandrosterone (DHEA) both decreased PrP\textsuperscript{Sc} but modulate rafts in vivo.

PO1.46
Spontaneous Fibrillation of Rec.Bovine PrP(102-233) in Vitro
Kalnov, S; Grigorov, V; Pokidishev, A; Tissebov, V; Balandina, M; Gibadulin, P; Verkhovsky, O
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The conformational conversion of the normal cellular isoform of the prion protein, PrP\textsuperscript{C}, into an abnormal pathological isoform, PrP\textsuperscript{Sc}, underlies a group of fatal neurodegenerative disorders known as transmissible spongiform encephalopathies (TSE) or prion diseases. In the present work we developed conversion protocols for generating amyloid fibrils from bovine rPrP encompassing residues 102-240. The morphological features and partial resistance to PK-digestion of fibrils have been demonstrated. RecPrP was expressed in Esherica coli system and purified using nickel-nitrilotriacetic acid metal-affinity chromatography (Qiagen). Aliquots withdrawn during time-course of incubation after each 24 hour. The kinetic of fibrils formation was monitored by electron microscopy (EM).

PO1.47
Quantifying the Species Barrier in Chronic Wasting Disease by a Novel in vitro Conversion Assay
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1University of British Columbia, Brain Research Centre, Canada; 2Public Health Agency of Canada, National Microbiology Laboratory, Canada; 3Animal Diseases Research Institute, Canada Food Inspection Agency, National Reference Laboratory for Scrapie and CWD, Canada; 4Ontario Cancer Institute and Department of Medical Biophysics, University of Toronto, Canada

Background: Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy that can affect North American cervids (deer, elk, and moose). Although the risk of CWD crossing the species barrier and causing human disease is still unknown, however, definite bovine spongiform encephalopathy (BSE) transmission to humans as variant CJD (vCJD), it would seem prudent to limit the exposure of humans to CWD.

Aim: In view of the fact that BSE can be readily transmitted to non-bovid species, it is important to establish the species susceptibility range of CWD.

Methods: In vitro conversion system was performed by incubation of prions with normal brain homogenates as described before, and protease K (PK) resistant PrP was determined by immunoblotting with 84H monoclonal prion antibody.

Results: Our results demonstrate that PrP\textsuperscript{Sc} from cervids (including moose) can be efficiently converted to a protease-resistant form by incubation with elk CWD prions, presumably due to sequence and structural similarities between these species. Interestingly, hamster shows a high conversion ratio by PrP\textsuperscript{DEN}. Moreover, partial denaturation of substrate PrP\textsuperscript{C} can apparently overcome the structural barriers between more distant species.

Conclusions: Our work correctly predicted the transmission of CWD to a wild mouse. We find a species barrier for prion protein conversion between cervids and other species, however, this barrier might be overcome if the PrP\textsuperscript{C} substrate has been partially denatured in a cellular environment. Such an environment might also promote CWD transmission to non-cervid species, including humans.

PO1.48
Novel Application Specific Monoclonal Antibodies Targeting Human Prion Protein
Jones, W; Head, MW; Connolly, JG; Fanquhar, CF; MacGregor, IR
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Background: Monoclonal antibodies (mAbs) raised against human prion protein (PrP) may prove to be valuable reagents in the detection, treatment and general understanding of human prion diseases, however to date no such antibodies are available.

Aim/Objective(s): To develop a method for the purification, under native conditions, of cellular prion protein (PrP\textsuperscript{C}) from human platelets and to use this material as an immunogen to raise a panel of mAbs targeting human PrP.

Methods: PrP\textsuperscript{C} was purified from human platelet lysates using a combination of cation exchange and Cu\textsuperscript{2+} affinity chromatography. This purified PrP\textsuperscript{C} was used to immunize PrP null mice, spleen cells from the immunised mice were fused to SP2/0 myeloma cells and stable hybridomas, secreting anti-PrP mAbs, were single cell cloned. The mAbs produced were characterized by isotype, epitope mapping; binding to both native/denatured platelet PrP and native/denatured a-helical/ß-sheet recombinant mouse PrP; immunoprecipitation of PrP\textsuperscript{C}, disease associated PrP (PrP\textsuperscript{D}) and its corresponding prion protein K resistant core (PrP\textsuperscript{C}73-30) from neurological control/vCJD brain homogenates.

Results: Following immunization with purified platelet PrP\textsuperscript{C}, all mice developed a strong, predominantly IgG isotype, anti-PrP immune response. From four separate fusions a total of twelve stable hybridoma cell lines secreting anti-HuPrP mAbs were identified and single cell cloned. A number of these mAbs displayed novel PrP binding properties, cross-reactivity with mouse PrP, differential binding to native/denatured PrP and PrP\textsuperscript{D}/PrP\textsuperscript{C}/73-30 selectivity in immunoprecipitation experiments.

Conclusions: A panel of 12 mAbs raised against native human platelet PrP\textsuperscript{C} has been produced. Initial characterisation suggests that some of these antibodies may prove to be useful reagents not only in the detection, treatment and understanding of human prion diseases but in TSE research more generally.
Protein Misfolding

P01.49
Size Matters
Weber, D1; Geise, A; Pliening, N; Mitteregger, G; Reznicek, L; Thormig, A; Beeckes, M; Kreutzschmar, H1
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Background: In serial transmission protein misfolding cyclic amplification (sPMCA) experiments, newly formed misfolded and proteinase K-resistant PrP (PrPres) catalysed the structural conversion of cellular prion protein (PrPC) as efficiently as PrPSc from brain of scrapie (263K)-infected hamsters confirming an autocatalytic misfolding cascade. However, the fact, that PrPres generated in vitro was associated with ten times less infectivity than an equivalent quantity of brain-derived PrPSc costs doubt on the "protein-only" hypothesis of prion propagation and has supported theories invoking additional molecular species of infectious PrP or further agent-associated factors.

Aims: We reasoned that apart from conformational differences and potential cofactors, the specific biological infectivity of PrPres preparations should also depend on the size distribution of PrPres aggregates which in turn should affect the number of infectious units per given amount of PrPres.

Results/Discussion: Using biosassays of infectivity, we could clearly demonstrate that ultrasonic treatment breaks up aggregates of misfolded PrP into smaller units and that sonication-induced fragmentation of prion aggregates is associated with a pronounced prolongation of incubation times by reducing aggregate stability and facilitating clearance from the brain. In contrast, when coupled to NC-particles, PrPres generated in vitro by sPMCA induced clinical disease in wild-type hamsters as efficient as PrPSc-derived from brains of diseased animals. Notably, if biological clearance was evaded by using N2a cells infected with differentially sonicated scrapie brain homogenates, the propagation of misfolded prion protein followed a bell-shaped curve. Applying the serial PMCA approach to a M15oxime antibody we achieved a 100,000-fold total amplification of hamster PrPres concurrently to a 2.514 (373,000-fold) dilution of the initial PrPSc.

Conclusion: By combining sPMCA with prion delivery on carrier particles we could resolve the apparent discrepancy between the amount of PrPres and infectivity which we could relate to differences in the size distribution of PrP aggregates and consecutive differences in regard to biological clearance.

P01.50
Copper(2) Inhibits in Vitro Conformational Conversion of Ovine Prion Protein Triggered by Low pH
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Background: One of the critical events in the pathogenesis of transmissible spongiform encephalopathies (prion diseases) is the conversion of a cellular prion protein (PrPC) into a protease-resistant, β-sheet rich isoform (PrPSc). Under certain conditions, prion protein (PrP) undergoes a conformational change from PrPC to PrPSc.

Aim: To gain insight into the conformational conversion of ovine prion protein PrP23-256(OvPrP23-256) at various pH and/or in the presence of copper (2) chloride (CuCl2).

Methods: Secondary structure of recombinant OvPrP23-256 with scrapie susceptible ARD genotype was analyzed by circular dichroism (CD) spectrum.

Results: In consistent with other previous studies, acidic pH triggers the formation of protease-resistant and β-sheet rich isoform of OvPrP23-256. Copper treatment of OvPrP23-256 at moderate acidic condition pH 5.0-6.0 as well as physiological condition (pH 7.4) also makes OvPrP23-256 adopt protease-resistant and β-sheet rich conformation. However, at lower pH condition (2.0-4.5) with copper treatment, OvPrP23-256 exhibited a more α-helix content with low β-sheet.

Discussion and Conclusion: Together, the study demonstrated that Cu2+ can drastically modulate conformational conversion triggered by acidic pH condition in OvPrP23-256, which possesses protease resistance and β-sheet rich phenotype. These results provide new insight into the prion protein conformational conversion process.

P01.51
Mathematical Modeling for Prion Propagation
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Prions are proteinaceous agents that cause the transmissible spongiform encephalopathies (TSE), affecting both animals and humans. Prions consist largely, if not entirely, of a misfolded, host-encoded glycoprotein designated PrP (prion protein). There are several theories that model prion replication mainly focusing on conversion of the normal cellular prion protein (PrPC) to a misfolded pathogenic form (PrPSc) followed by aggregated fibril formation. Here we consider the nucleated polymerization model and simulate molecular reactions by modifying previously reported estimations for reaction rates (Rubenstein et al., Biophysical Chemistry, 2006) to investigate dynamic behavior of this model in a neuronal cell. Gillespie-Direct discrete event simulator has been applied on a prion propagation model based on ordinary differential equations.

PrP is an abundant protein in neuronal cells, and in the present model initial PrPSc concentration was modified to 50,000 molecules per cell, according to our previous observations (Cashman et al, Cell 1990; Cashman, unpublished). Monte Carlo simulation of the nucleated polymerization model result shows a rapid decrease of PrPSc to 600 molecules in the first two days and a smooth slope to steady state after 70 days. Experimental data from our lab demonstrates a decrease of cell surface prion protein immunoreactivity, consistent with this modeling prediction (Griffin et al., Adv. Protein Chem. 2001). By adjusting the model parameters and retaining the same slope, PrPSc concentration increased and PrPSc aggregates formed much earlier than previously reported.

Half lives are around 4 hours for PrPC and more than two days for PrPSc. Experimental data from our lab demonstrates a decrease of cell surface prion protein immunoreactivity, consistent with this modeling prediction (Griffin et al., Adv. Protein Chem. 2001). By adjusting the model parameters and retaining the same slope, PrPSc concentration increased and PrPSc aggregates formed much earlier than previously reported. Here, as part of an ongoing project, we present a mathematical modeling, that covers both experimental and simulation approaches in prion disease research.

P01.52
Identification of a Mild-Denatured Monomer as the Neurotoxic Isoform of Human PrP 90-231 (hPrP 90-231)
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Background: The identification of the PrP conformer responsible for the neurotoxic events during prion disease is critical for developing effective therapeutic strategies against prion diseases. Because of its high tendency to aggregate, the major difficulties in understanding the cytotoxic structure of the PrP are largely due to its heterogeneous nature which often consists of a mixture of monomers, fibrils and non-fibrillar oligomeric species. Previous studies indicated that either soluble oligomeric species or amyloid fibrils, generated from the full-length PrP and a variety of its fragments, are cytotoxic. However, in spite of numerous investigations, to date, the relationship between toxicity and structural state of the protein still remains uncertain.

Objective: The aim of the present study was the identification of the cytotoxic conformations of hPrP90-231, as well as the understanding of the molecular mechanisms by which it may cause neuronal death.

Result: We demonstrate that a mild-denatured monomer of the human PrP fragment 90-231 (hPrP90-231, incubated for 1h at 53°C), represents the unique toxic PrP species for SH-SY5Y neuroblastoma cells. When thermal treatment is further prolonged (3 or 5 days of incubation at 37°C), the protein becomes structured in macro-aggregates and fibrils. Their addition to the culture medium does not determine reduction of cellular viability, similarly to what observed with the native hPrP90-231. Thus, neither the native state nor macro-aggregates but only the mild-denatured monomers possess cytotoxic effects. Using spectroscopic and immunocytochemical techniques we demonstrate that the toxic conformer is characterized by a higher exposure of hydrophobic regions that favors the cellular uptake of the protein and the formation of insoluble PrP aggregates that trigger pro-apoptotic "cell death" signals.

Conclusion: Our results indicate that the toxic conformers of hPrP90-231 are constituted by monomers and/or small oligomers characterized by a high content of β structures and by an increased exposure of hydrophobic regions.
Molecular Dissection of SecPrP and NtmPrP Forms in Cell Membranes

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Efficient prion infection requires as first step the recognition of the precursor PrPc from specific membrane domains of the cell surface. This population of PrP is extraordinarily complex involving both PrPc and PrPd forms and their processing products, under different ligand-bound states. To decipher this complexity in molecular terms we have generated major-form expressing cells using PrP mutants which in vitro generate mainly either the PrPc or the PrPd forms. Floatation assays using cold Bri26 extracts show that whereas PrPc distributes preferentially at the lowest density fractions of a nycodenz gradient, whereas PrPd shows a more irregular distribution. From these preliminary findings highlighting an urgent public health need for a blood test capable of identifying people incubating vCJD.

Methods: Serial dilutions of vCJD brain (seed) in non-CJD brain (substrate) were prepared and either frozen or subjected to 48 cycles of PMCA, each cycle consisting of a burst of sonication followed by a 30 min. incubation at 37°C. These samples were then analysed by CDI and dissociation-enhanced lanthanide fluoroimmunoassay (DELFIa), using Europium-labelled 3F4 as the anti-PrP detection antibody.

Results: Without PMCA, CDI detected vCJD PrPSc to a dilution limit of 10^{-10} equivalent to 10 ng vCJD brain tissue. Treatment of the samples with PMCA increased sensitivity by 100-fold. A similar increase in sensitivity was observed when healthy human platelet homogenate was used as a substrate for PMCA. CDI obviated the problem of high background observed when Western blotting was used to detect PrPSc amplified using platelet homogenates.

Discussion: We have shown that CDI can be combined with PMCA to detect very low levels of PrPSc. As the PMCA-CDI technique is not dependent on PrPC digestion, this population of PrP has the potential to detect proteinase K resistant forms of PrPSc. This method can utilize platelets as a readily available source of human PrPC for PMCA and both assays are conducted in a 96 well format. We are currently optimising PMCA-CDI for detecting PrPSc in vCJD peripheral tissues and blood.

Characterization of the Interaction between Prion Protein and Heparin

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Interaction between prion protein and endogenous glycosaminoglycans on the cell surface is proposed to play a key role in the infection and transmission of the prion, since sulfated glycosaminoglycans such as heparin and pentasulfated polysulphate (PPS) exert anti-prion activities by competitively inhibiting the interaction. However, interaction between prion protein and sulfated glycan is not fully evaluated. In this report, interaction between prion protein and heparin as a representative sulfated glycan was investigated to clarify which structural unit in heparin is responsible for the binding of prion protein, and which region of prion protein is responsible for the binding.

Heparin is heterogeneous in composition and chain length, but there are a few known methods to destruct the partial structure of heparin specifically. Comparison of the anti-prion activities of the modified heparin prepared by two different methods revealed that a certain disaccharide unit is essential to the potency for inhibiting PrPres formation in prion-infected cells. Then, “Sugar Chip” was prepared by immobilizing the disaccharide unit on a sensor chip to investigate the interaction with prion protein using surface plasmon resonance (SPR) technique. Because recombinant prion protein, murine (PrP23-251), exhibited a significant binding profile on the Sugar Chip, truncated domains of the recombinant prion protein were tested for their binding to the Sugar Chip. It was found that N-terminal domain of prion protein, 23-89, was necessary for the interaction with the disaccharide unit in heparin. Finally, SPR analysis revealed that the interaction between the N-terminal domain of prion protein and the disaccharide unit was competitively inhibited by PPS. These results suggest that the SPR-based assay system composed of the prion protein and the Sugar Chip might be utilized as either a facile anti-prion chemical screening method or a research tool to investigate the mechanism of anti-prion chemicals.

Electro-elution, A Novel Method to Remove Transmissible Spongiform Encephalopathy-associated PrP\textsubscript{Sc} from Stainless Steel Surgical Instruments

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Transmissible Spongiform Encephalopathy (TSE) infectivity is unusual in being resistant to autoclaving and the most commonly used chemical disinfectants. The iatrogenic transmission of TSEs has been demonstrated via surgical instruments, and concerns have arisen about the efficacy of the conventional decontamination techniques used to reprocess reusable instruments. We have developed the novel cleaning process of electro-elution that utilizes an electrical current through an electrolytic buffer to effectively remove protein contamination from the surface of stainless steel.

Stainless steel discs, manufactured as surgical instruments, were contaminated with murine passaged scrapie (ME7) brain homogenate. The discs were subjected to a range of conditions to determine the ability of electro-elution to remove the deposits. To determine whether there was any residual PrP\textsubscript{Sc} remaining on the disc after electro-elution Direct Blot was used. Western Blot was used to determine whether there was any PrP\textsubscript{Sc} in the electrolyte solution after the electro-elution process.

Initial experiments removed ME7 brain homogenate from the surface of the stainless steel disc in 12 volts in 0.5% sodium carbonate electrolyte buffer. These experiments tested up to 5mg equivalent of brain material, with the sensitivity of the Direct Blot being 3.1pg. Examination by Western Blot of the electrolyte buffer after electro-elution has shown no detectable PrP\textsubscript{Sc} present, which suggests the PrP\textsubscript{Sc} molecule is undergoing some form of degradation. Our investigation into the process of electro-elution has shown it can effectively remove dried on protein contamination from the surface of surgical instrument grade stainless steel without any visible signs of damage. The simplicity of the electro-elution process means that it could be used in conjunction with current sterilization techniques to recycle high risk surgical instruments without incurring large financial penalties. Additional research utilizing bioassay techniques is required to determine whether the electro-elution process does indeed have the capacity to destroy the infectious agent that causes the TSE disease.
Protein Misfolding

PO1.57
Influence of the Cellular Membrane on Prion Protein Infection Mechanism
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The conversion of the cellular isoform of the prion protein (PrPc) into the disease-associated PrPSc plays a crucial role in development of prion diseases. Within its cellular pathway PrPc undergoes several posttranslational modifications, i.e. attachment of two N-linked glycans and a glycosylphosphatidylinositol (GPI) anchor to its C-terminus. With help of this anchor prion protein is linked to special membrane domains on the exterior cell surface, called rafts. There are several hints leading to a hypothesis, that the conversion process might take place either at the membrane surface or in its close proximity (1, 2). In order to study the influence of the membrane environment on the structural transition process we purified posttranslationally modified PrPc from transgenic CHO-cells (3, 4). The purification method consists of two chromatographic steps namely: the copper immobilized metal-chelate affinity chromatography that is followed by a highly specific immunopurification step, where different monomolar and recombinant antibodies were tested. The purified, soluble CHO-PrPc was then inserted into model membranes bound on a chip surface and the thermodynamics and kinetics of the insertion were analyzed quantitatively (5). Next we studied the interaction with an exogenously added second component i.e. PrP aggregates in 3-sheet rich structure. These experiments were performed using a Biacore device, which utilizes the surface plasmon resonance (SPR) technique. We could observe a different behavior of the aggregated protein depending on the presence of PrPc anchored in the lipid bilayer. These differences were significant and showed no or little binding when the aggregated PrP particles were injected over an empty lipid bilayer in comparison with a very strong, disrupting effect on the membrane-bound PrPc.


PO1.58
High Resolution TSE Strainotyping by LC-MS/MS - Distinctions Between Ovine BSE, Classical Scapie and Experimental Scrapie Isolates
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Background: As new forms of TSE continue to be identified, their differential diagnosis becomes increasingly important. Biochemical screening tests can differentiate TSEs, making use of differing structural properties of PrPSc. BSE and classical scrapie can be distinguished by Western blotting (WB): protease K (PK) digested PrPSc (PrP27-30) reveals a characteristic shift of the unglycosylated band to lower molecular weight, attributed to a significant difference in PK cleavage sites. Determining the N-terminal amino acid profile (N-TAAP) of PrP27-30 in detail, utilizing a high resolution method such as mass spectrometry (MS), would also allow differentiation between TSEs where differences in PK cleavage sites are more subtle.

Aims: The PMCA methodology was used to the amplification of PrPSc present in blood samples from RML infected mice culled at various time point throughout the incubation period for disease. Also, the coupling of PMCA to the amplification of PrPSc captured by immunoprecipitation from large volumes of blood and subsequent detection by high sensitivity ELISA.

Methods: Blood samples were diluted into substrate brain homogenate and subjected to PMCA. Serial PMCA (sPMCA) involved repeated rounds of amplification and re-dilution into fresh substrate. PrPSc recovered from immunoprecipitation of RML brain homogenate spiked into whole blood was also subjected to sPMCA. All samples were analysed by Western blotting and ELISA.

Results: After sPMCA, blood samples from RML infected mice showed amplification of PK resistant material to a level detectable by standard PK digestion and forms fibrils. In a cell-free essay the conversion reaction can be imitated by incubating highly purified PrPc molecules together with PrPSc seeds in an appropriate conversion buffer. Under these conditions we can show, that PrPSc itself can induce the conversion of PrPc into a partially protease K resistant form, termed PrPres. Newly converted PrPres can be detected by antibodies that discriminate between PrPres and PrPSc. Bacterially expressed PrPc was used as substrate and mouse passaged scrapie and BSE strains/isolates as seeds. Even differences in the molecular masses of PrPres (after PK treatment) of mouse passaged BSE and scrapie strains (Me7, 22A, 87V, BSE/Bl6) are transmitted to the newly formed PrPres fragments which illustrates the efficiency of PrPSc to transduce its specific conformation even in this cell-free environment. Double incubation of a mouse passaged scrapie and BSE strain/isolates resulted in two PrPres fragments which were distinct in their molecular masses. Moreover, the yield of newly converted PrPc following coinoculation, was higher in comparison to the individual incubation reactions indicating a synergistic interaction between the two prion strains respectively. In another set of experiments it was shown that even single or few amino acid substitutions in the prion protein have a major effect on its convertibility. The cell-free assay can therefore also be used to elucidate the influence of amino acid polymorphisms on the PrP conversion process.

PO1.59
Discrimination between Prion-infected and Normal Blood Samples by PMCA
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Background: Diagnosis of prion disease from blood samples will require the detection of minute quantities of PrPSc. Protein Misfolding Cyclic Amplification (PMCA) is a technique which can amplify small amounts of seed PrPSc to a level detectable by conventional methods. Application of PMCA to the testing of blood samples may enhance the ability to detect PrPSc in blood and allow ante-mortem detection of prion disease.

Aims: PMCA methodology was used to the amplification of PrPSc present in blood samples from RML infected mice culled at various time point throughout the incubation period for disease. Also, the coupling of PMCA to the amplification of PrPSc captured by immunoprecipitation from large volumes of blood and subsequent detection by high sensitivity ELISA.

Methods: Blood samples were diluted into substrate brain homogenate and subjected to PMCA. Serial PMCA (sPMCA) involved repeated rounds of amplification and re-dilution into fresh substrate. PrPSc recovered from immunoprecipitation of RML brain homogenate spiked into whole blood was also subjected to sPMCA. All samples were analysed by Western blotting and ELISA.

Results: After sPMCA, blood samples from RML infected mice showed amplification of PK resistant to levels readily detectable by western blotting and standard ELISA. Detectable levels of PK resistant material were detected following PMCA of material recovered by immunoprecipitation of RML spiked into 8ml of whole blood.

Discussion: Serial PMCA performed on small volumes of whole blood samples (1ml) gave amplification of PK resistant material to a level detectable by standard immunomassays. Amplification of PrPSc immunoprecipitated from a large volume of whole blood suggest that PMCA can provide a valuable amplification step for the determination of prion infection in combination with immunopurification and high sensitivity ELISA.

PO1.60
In-Vitro Studies on the Molecular Mechanisms of the Abnormal Prion Protein Formation
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Prion diseases are characterised by the conversion of cellular prion protein, PrPc into its pathogenic form, termed PrPSc. Compared to PrPc, PrPSc carries a partial resistance to proteinase K and forms fibrils. In a cell-free assay the conversion reaction can be imitated by incubating highly purified PrPc molecules together with PrPSc seeds in an appropriate conversion buffer. Under these conditions we can show, that PrPSc itself can induce the conversion of PrPc into a partially protease K resistant form, termed PrPres. Newly converted PrPres can be detected by antibodies that discriminate between PrPres and PrPSc. Bacterially expressed PrPc was used as substrate and mouse passaged scrapie and BSE strains/isolates as seeds. Even differences in the molecular masses of PrPres (after PK treatment) of mouse passaged BSE and scrapie strains (Me7, 22A, 87V, BSE/Bl6) are transmitted to the newly formed PrPres fragments which illustrates the efficiency of PrPSc to transduce its specific conformation even in this cell-free environment. Double incubation of a mouse passaged scrapie and BSE strain/isolates resulted in two PrPres fragments which were distinct in their molecular masses. Moreover, the yield of newly converted PrPc following coinoculation, was higher in comparison to the individual incubation reactions indicating a synergistic interaction between the two prion strains respectively. In another set of experiments it was shown that even single or few amino acid substitutions in the prion protein have a major effect on its convertibility. The cell-free assay can therefore also be used to elucidate the influence of amino acid polymorphisms on the PrP conversion process.
**P01.61**

**Prion Protein Paralogue Doppel Interacts with α2-Macroglobulin: A Plausible Mechanism for Doppel-Mediated Neurodegeneration?**


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Toward understanding the functions of the cellular prion protein (PrPc) and its paralogue doppel (Dpl) in the central nervous system, we fused the Fc region of human immunoglobulin to the C-termini of these proteins. Using PrP-Fc and Dpl-Fc, we identified the restricted expression of binding partners for these molecules in the granule cell layer of the cerebellum in both wild-type and PrP-deficient mice (Legname et al., 2002). Here, we describe the identification of rat α1 inhibitor-3 (α1I-3), a plasma protease inhibitor, as an interacting partner of Dpl. Together with α1I-3, Dpl also interacts with the mouse and human homologues α2-macroglobulin (A2M) but additional studies argue that PrP does not react with A2M directly. Moreover, PrP and Dpl seem to bind strongly to each other, as demonstrated by both ELISA and Biacore studies. Based on these findings, we propose a novel paradigm in which ectopic expression of Dpl induces neurodegeneration in mice through the withdrawal of a natural inhibitor of metallo-proteases such as A2M from the extracellular matrix. While A2M has been implicated as a modifier in Alzheimer’s disease (Saunders and Tanzi, 2003), it remains to be determined if it has a similar role in the prion diseases.

**References**


**P01.62**

**A Search for Auxiliary Proteins in Prion Replication**


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Background: Prion diseases in humans and animals are caused by conversion of a normally folded, nonpathogenic isoform (PrP0) of the prion protein to a misfolded, pathogenic isoform (PrPSc) that is transmissible. Genetic experiments have shown that the conversion reaction involves as-yet unidentified protein cofactors. The ultimate proof of the identity of a conversion cofactor, or protein X, is the substantial decrease in susceptibility, or even resistance, to prion infection in animals in which protein X expression has been ablated. Alternatively, transgenic overexpression of protein X should lead to an accelerated onset of prion disease. Many proteins that are upregulated in prion disease or relevant to other neurodegenerative disorders, such as Alzheimer’s disease (AD), have been proposed as possible modulators of the prion incubation time; however, the identity of protein X remains elusive.

Aim: In our search for protein X, we analyzed the effect of 21 genes in a large number of mouse models on the incubation time after infection with prions. Among these genes associated with AD, inflammation, signaling, protein expression, cycling, and maintenance.

Methods: Mice in which the gene of interest was ablated or transgenically overexpressed were inoculated with mouse-adapted prions and the times until onset of neurologic symptoms were measured.

Results: Most genes tested here did not significantly affect incubation times in the respective mouse model after infection with prions. However, we identified the Interleukin 1 receptor, type I (Ilt1r1) as a significant modifier of prion incubation time, with Ilt1r1 knockout mice living 13% longer than wild-type mice.

Conclusion: Although Ilt1r1 showed a statistically significant influence on the incubation time, incubation times in prion-inoculated Ilt1r1-/- mice were only minimally longer than in inoculated wild-type mice. Thus, making it unlikely that Ilt1r1 is protein X. While some of the genes tested here may have a role in the normal function of PrPc, this work clearly shows that many genes formerly implicated as potential auxiliary proteins in prion replication have no discernable effect on prion disease. Thus, we conclude that they are unlikely to be protein X.

**P01.63**

**Free Energy Landscape Analysis of Prion Protein from Human, Bovine, Swine, Canine, Feline, Mouse, and Elk**

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Transmissible spongiform encephalopathies (SEs) and prion disease are neurodegenerative disease caused by structure transformation of cellular prion (PrPc) to abnormal isoform (PrPsc). The most important theme in prion diseases is the conformational transition of PrPc to PrPsc. PrPc and PrPsc, and chemical difference between PrPc and PrPsc, is still unknown. It appears 2-Macroglobulin: A Plausible Mechanism for Doppel-Mediated Neurodegeneration? toward understanding the functions of the cellular prion protein (PrPc) and its paralogue doppel (Dpl) in the central nervous system, we fused the Fc region of human immunoglobulin to the C-termini of these proteins. Using PrP-Fc and Dpl-Fc, we identified the restricted expression of binding partners for these molecules in the granule cell layer of the cerebellum in both wild-type and PrP-deficient mice (Legname et al., 2002). Here, we describe the identification of rat α1 inhibitor-3 (α1I-3), a plasma protease inhibitor, as an interacting partner of Dpl. Together with α1I-3, Dpl also interacts with the mouse and human homologues α2-macroglobulin (A2M) but additional studies argue that PrP does not react with A2M directly. Moreover, PrP and Dpl seem to bind strongly to each other, as demonstrated by both ELISA and Biacore studies. Based on these findings, we propose a novel paradigm in which ectopic expression of Dpl induces neurodegeneration in mice through the withdrawal of a natural inhibitor of metallo-proteases such as A2M from the extracellular matrix. While A2M has been implicated as a modifier in Alzheimer’s disease (Saunders and Tanzi, 2003), it remains to be determined if it has a similar role in the prion diseases.

**References**


**P01.64**

**Role of Oligomers in the Neurotoxicity of Gerstmann-Sträusler-Scheinker Disease Amyloid Protein**


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In prion diseases, PrP-amyloid is found in the brain of patients suffering from Gerstmann-Sträusler-Scheinker disease (GSS). PrP cerebral amyloid angiopathy, variant CJD, and kuru. In familial GSS the amyloid plaques are mainly composed of a prion protein fragment encompassing residues 81-122-145-146. We previously showed that a synthetic peptide spanning residues 82-146 readily forms fibrils and has the physicochemical features accounting for the massive amyloid deposition of PrP in GSS. Moreover, PrP82-146 was found to be toxic to cortical neurons. Therefore, we have focussed our attention on the first stages of PrP82-146 fibrillation. In particular, by using a photo-induced-cross-linking technique, we have demonstrated that this peptide forms oligomeric species constituted mostly by dimers and trimers and, at a lower extent, by larger structures up to 15-mers. Electron microscopy analysis revealed that PrP82-146 aggregation is a step-wise process starting with formation of oligomers and protofibrils, which combine to produce the amyloid fibrils. FTIR analysis showed that monomers are mainly unstructured and form low order aggregates after 7 days. At this stage, the FT-IR bands at 1623.2 cm-1 and at 1698.8 cm-1 suggested that the early aggregates were characterized by an antiparallel β-sheet intermolecular interaction. After 10 days, the presence of the band at 1626.1 cm-1 suggested assembly into mature fibrils. Cellular toxicity of PrP82-146 paralleled the fibrillization process, since the biological effects were evident after the commencement of the structural rearrangement. In particular, the presence of oligomers was a committed step for the toxic effect since the addition of an anti-oligomer antibody abolished all biological effects. Stable soluble oligomers were also toxic to cells and noteworthy with an inverse correlation with their size. In conclusion, in GSS prefibrillar oligomers may play a role in the neurodegenerative process.
**P01.65**

A Different Metabolic Isoform of PrPSc in Species Other then Mice

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Nonglycosylated and monoglycosylated forms of mouse PrPsc, but not those from bovine, ovine or human brain samples, can be recognized by IP2C, a new aPrP mAb. Contrarily to its specificity for mouse PrPsc, IP2C reacts willingly with all the corresponding recombinant PrPs. Deglycosylation of diverse brain samples did not confer to IP2C affinity to non-reacting forms of PrP; suggesting its activity requires a free asparagine, as opposed to the aspartic acid resulting from deglycosylation of N-linked sugars by PNGase. Immuno blotting of brain samples and recombinant rodent PrP in the absence of ltre significantly increased IP2C reactivity, while reduction and alkyl cation of the disulfide bond almost abolish its activity, indicating the IP2C epitope, in addition to a free asparagine, comprises an intact disulfide bond between residues C179 and C214. Consistent with this possibility, PEPscan studies fail to identify a linear epitope for IP2C in the PrP sequence. We hypothesize that in species higher then mice, the lower bands of PrPsc may comprise mostly deglycosylated as opposed to non-glycosylated forms, resulting from the activity of cellular PNGases, thereby presenting aspartic acid at residue 180 instead of asparagine. Ongoing sequencing experiments will determine if indeed this is the case.

**P01.66**

Proximal Ligation-based Detection of Biomarkers of Protein Folding Disorders

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The development of sensitive and specific techniques for protein detection could provide opportunities for early detection and therapeutic intervention in protein folding disorders, for instance Prions- and Alzheimer’s diseases. We aim to develop extremely sensitive and specific molecular tools for quantitative measurement of soluble protein oligomers at very low concentration, using proximity ligation assay.

Proximity ligation assay (PLA) is a recently developed method in which specific proteins are analyzed by converting detection reactions to DNA sequences. In this method target molecules are recognized by two or more proximity probes that are prepared by attaching DNA strands to affinity binders. When a pair of such probes bind to a common target molecule, the free ends of the probes are brought in proximity and can be hybridized to a connector oligonucleotide, allowing the ends to be joined by enzymatic DNA ligation. The ligation products are then amplified by PCR and distinguished from unreacted probes.

Here, we present the application of PLA for sensitive identification of oligomers and aggregated prion proteins, and Ab peptide. The abnormal prion proteins can be detected and distinguished from normal proteins by PLA without requirement of protein K treatment. Soluble Ab oligomers, which have been shown to mediate neurotoxicity, may be used as a candidate biomarker for diagnosis of Alzheimer’s disease at early stages.

The combination of efficient PCR amplification and the use of two or more binding reagents provide very high sensitivity and specificity of detection, surpassing the conventional protein detection methods. Furthermore, the proximity ligation technique can be carried out as in the homogenous assay – requiring only small amount of materials to be tested –, or in a heterogeneous format in which the target molecules to be detected are immobilized onto 96-well microplates, while using unreacted probes, the remaining unreacted materials are washed away. Proximity ligation can, therefore, provide a powerful molecular tool for detection and study of the biology of protein folding disorders.

**P01.67**

Metal Ion Coordination to Oligomeric Intermediates on the Pathway of Oxidative Prion Protein Aggregation

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Transmissible spongiform encephalopathies (TSE), a group of fatal neurodegenerative disorders, are apparently caused by a posttranslational conversion of the cellular prion protein (PrPc) into an abnormal pathological isoform (PrPsc). This process is characterized by dramatic changes in the secondary and tertiary structure of PrP, resulting in the formation of highly β-sheeted and insoluble aggregates. Consequently, the enlightenment of the mechanisms of aggregate formation as well as the structural analysis of intermediate oligomeric states will pave the way to understand the molecular principles of prion disease, enabling the design of therapeutic strategies. Taking into account that oxidative stress has been proposed to be a pivotal event in prion diseases, are apparently caused by a posttranslational conversion of the cellular prion protein (PrPc), we show for the first time that the PrPsc editing affects prion propagation remains to be established.

**P01.68**

Properties of an Anti-mouse Prion Protein-Specific Single-chain Antibody

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The abnormal isoform of prion proteins is believed to be the agent responsible for Transmissible Spongiform Encephalopathies (TSE), such as human sporadic Creutzfeldt-Jakob disease. These nervous system disorders are closely associated with conformational alterations of the host-encoded cellular prion proteins (PrPc) into their pathological form, PrPSc. Experiments in mice have shown that anti-PrP antibodies can interfere with prion replication. Single-chain antibodies (ScFv) are fully functional molecules consisting of the variable regions of antibody heavy and light chains and can be efficiently expressed in a number of systems including bacteria and plants. The objective of this study was to generate ScFvs with high affinity and specificity for mouse PrP and to perform large scale expression and purification of these ScFv antibodies for subsequent experiments. RNA samples from hybridoma cells were prepared and cDNA synthesized using First-Strand CDNA Synthesis Kit (GE Healthcare). ScFvs specific for prion protein were isolated using phage-display. This ScFv was subsequently expressed in a bacterial system, purified to homogeneity and characterized by ELISA. After 3 rounds of panning against the N-terminal region of the mouse PrPc, 10 individual ScFv clones recognizing the peptide epitope were identified and sequenced. The ScFv consisted of 249 amino acids and sequence variations were found in both the complementarity determining regions (CDRs) and the frame regions. Clone pJB-M02-02 produced the highest signal against the peptide epitope and was observed to possess properties similar to the original monoclonal antibody. The induction of the ScFv gene in E.coli resulted in significant accumulation of the ScFv within 3 hours. Although several protein induction conditions were tested, the ScFv was expressed exclusively in inclusion bodies. After purification of the ScFv from the insoluble protein fractions and a protein refolding protocol, the refolded ScFv was characterized by ELISA and found to be functional. We have isolated a ScFv using phage display techniques which recognizes the N-terminal part of the mouse PrP protein. We have expressed it in large quantities and purified it to homogeneity. The properties of the ScFv are similar to that of the original monoclonal antibody and provides us with a valuable tool to investigate the structure and function of PrP.
The starting point for understanding prion protein misfolding is the characterisation of normal genotypes in their native state. We have therefore expressed wild type mouse, hamster and human prion proteins, as well as 3F4-tagged mouse prion protein, in Xenopus oocytes. Oocytes were separately injected with RNAs encoding the various prion proteins and crude membrane fractions of the expressed proteins were extracted and assayed by Western Blotting using 3F4 and 6H4 monoclonal antibodies. All expressed proteins were recognised by both antibodies, except for wild type mouse prion protein which as expected was recognised by 3F4. The expressed prion proteins were similar in size and abundance (>100ng/oocyte) to prion protein extracted from human brain. Non-glycosylated, mono-glycosylated, di-glycosylated and additional, higher molecular glycoforms were expressed. The non-glycosylated forms were similar in size to recombinant human and hamster prion protein and all forms were proteinase K sensitive (50 µg/ml, 1 hour, 37 °C). Following treatment with PNGase F, the glycoforms reduced to a full length, non-glycosylated band although truncated forms of prion protein were also detected in some extracts. PIPLCL treatment of intact oocytes expressing 3F4-tagged mouse prion protein reduced the amount of protein detectable by Western blotting compared to a matched set of controls. This suggests that prion protein was present on the cell surface and had been liberated by cleavage at the GPI anchor. Supporting this interpretation, prion protein could be detected on the surface of intact live oocytes by placing them directly upon wetted PDVF membrane and then immunoblottting the imprint. Thus oocytes offer a very versatile system for producing large amounts of wild type and mutant glycosylated prion proteins from several species and for studying their folding and biochemical properties under native conditions.

**Protein Misfolding**

**P01.69** Comparison of Glycosylated Hamster, Mouse and Human Prion Protein Expressed in Xenopus Oocytes

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**P01.70** Ease of Unwinding of Helix-2 Correlates with Stability of Allelic Variants of Ovine Prion Protein

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Susceptibility to scrapie disease in sheep, the archetypal prion disease, correlates with polymorphisms within the ovine PrP gene. The VRQ and AL141RQ allelic variants are associated with classical scrapie, whilst the ARR, AF141Q and AHQ allelic variants are associated with atypical scrapie. Furthermore, PrPSc associated with atypical scrapie is less resistant to proteolytic digestion than its counterpart in classical scrapie. This may reflect an inherent difference in stability between allelic variants of ovine PrP associated with these different forms of scrapie. To address this, we have investigated the conformational stability and proteolytic susceptibility of allelic variants of ovine PrP associated with classical and atypical scrapie. We find that the melting temperature of ovine recombinant VRQ and AL141RQ PrP is higher than that of AF141Q, AHQ and ARR. In addition, monoclonal antibody studies show that the region around helix-1 of VRQ and AL141RQ is less accessible compared to other ovine PrP allelic variants. Our preliminary data suggest that proteolytic cleavage of VRQ and AL141RQ proceeds with different kinetics compared to allelic variants associated with atypical scrapie. Through the use of molecular dynamics simulations we have found that the resistance to proteolytic digestion correlates with the ease of unwinding of helix-2 and a concurrent conformational change of the helix-2 - helix-3 loop. These data reveal significant differences in the overall stability of different allelic variants of ovine PrP and consequently, have implications for the differences in stability of PrPSc associated with classical and atypical scrapie.

**P01.71** Functional Recombinant Single Chain Antibody Against PrP

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Prion diseases are characterized by the transformation of normal cellular PrP into the infectious PrPSc with aggregation-prone proteins. We were able to prevent prion disease after peripheral prion infection in mice expressing 3F4-tagged mouse prion protein, thus suggesting that the prion protein fragment containing the PrPSc determinant is essential for prion disease propagation. Furthermore, immunization of mice with prion protein fragments led to a strong polyclonal immune response, which recognized the prion protein in Western blotting and with ELISA but not with prion protein aggregate. The 3F4 scFv anti-prion antibody was isolated from a phage display library and produced in rabbit and mouse cells with the same specificity as the polyclonal immune response. The recombinant scFv was generated using a standard hybridoma technology. Amplified V L and V H fragments of this IgG1 were cloned into pET22b including c-terminal c-myc and His 6-tags for expression in E. coli. Following treatment with E. coli extract, the scFv was purified to homogeneity by Ni-NTA chromatography to a high level of purity (>98%), obtaining up to 15mg of purified scFv from 1L culture.

Binding of scFv-W226 to recombinant mouse PrP was verified by ELISA and thermostability assays revealed the scFv was stable at 37°C in serum for at least 28 days. Moreover, 50% ELISA reactivity was still present at 60°C indicating a particularly high thermostability. This high thermostability has been reported to correlate with in vivo half life time. Inhibition of prion propagation was analyzed in cultured mouse neuroblastoma cells infected with prion protein (Scn2a). The scFv was anti-prion active with an EC50 in the low nM range. Thus a high affinity anti-prion active recombinant scFv has been constructed, which is soluble, thermostable to a high grade and may pass the blood brain barrier. In vivo studies in mice infected with PrPSc are currently underway and results will be reported.

**P01.72** Fusion with Yeast Sup35p-NM Modulates Cytosolic PrP Aggregation in Mammalian Cells

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In mammalian prion diseases, an abnormally folded, aggregated form of the prion protein (PrPSc) appears to catalyze a conformational switch of its cellular isoform (PrPC) to an aggregated state. A similar prion-like phenomenon has been reported for the Saccharomyces cerevisiae translation termination factor Sup35p that can adopt a self-propagating conformation. We have compared aggregation propensities of chimeric proteins derived from the Sup35p prion domain NM and PrP in the cytosol of mammalian cells. Here we demonstrate that cytosolic Sup35p-NM and PrP display strikingly different aggregation behaviours, with Sup35p-NM remaining soluble and cytosolic PrP readily aggregating. Notably, fusion of domains NM and/or PrP with different N-terminal tags results in the aggregation of Sup35p-NM and PrP, indicating that the PrP aggregation propensity is strongly modulated frequency and size of the aggregates. NM-PrP chimera formed large aggregates reminiscent of coalescent NM aggregates found in yeast cells displaying the [PSI+] prion phenotype. In parallel, we investigated aggregation propensities of NM and NM-PrP in vitro. While NM readily self-assembled into fibrils, NM-PrP fibrillation depended on induction by agitation. Thus, our results provide new insights into protein aggregation behaviours, suggesting that aggregation of yeast and mammalian prion proteins in the cytosol of mammalian cells is strongly influenced by cellular conditions or factors that either inhibit or promote accumulation of these aggregation-prone proteins.
Protein Misfolding

P01.73
Evaluation of Plate Processing Instrumentation for Rapid TSE Assays
Debson, C; Navelkova, Z; Buchbauerova, J; Simmons, P; Campos, S; Garcia, D; Cook, J; Clarke, J; Heath, N; O’Brien, A; Stumpf, R
*Murex Biotech, UK; *Stani Venerinarni Ustav Olomuc, Czech Republic; *Enfer Testing, Ireland; *Laboratorio Regional de Sanidad y Produccion Animal, Spain; *Enfer Scientific, Naas, Ireland; *Abbott Diagnostics Division, USA

Automated ELISA plate handling has obvious and previously reported benefits with respect to laboratory throughput, bench space and labour intensity. This study assesses plate processing instrumentation with respect to pipetting capabilities and overall assay performance.

The Strattec 4PS instrument has been adapted by several companies for use as an automated analyser in the field of human blood testing and as such is, IVDV compliant, well established and ubiquitous. Enfer Scientific have adapted this machine for use as a plate processor for the ELISA part of the Enfer Version 3 rapid test for TSE. This Enfer adaptation is known as the en4lisa.

The Enfer TSE Version 3 test features coloured reagents which allows reagent addition verification to be performed on the en4lisa. This feature has been used in the study to assess pipetting precision throughout the individual steps of the assay. Additionally, assay performance has been assessed by means of, testing negative samples and repeatedly testing a blinded positive panel.

The study was performed under routine conditions in TSE testing laboratories in the Czech Republic, Ireland and Spain. The data show that the pipetting precision of the en4lisa is equivalent to, or better than, that normally achieved by manual users and the assay performance is consistent with the manual assay.

P01.74
Investigation of Prions Structure and Stability by NMR
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Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of fatal neurological diseases of humans and animals characterized by memory and cognitive disturbances. At the end of the twentieth century, the bovine spongiform encephalopathy (BSE), better known as mad cow disease, has been proved to be transmissible to humans. This brought a lot of concern for public health and a lot of questions for scientists. There are two major forms of prion protein: the native and non-infectious form (PrPC), which corresponds mainly to an alpha-helix structure, and the misfolded infectious form, corresponding to an assembly of beta-sheet forming amyloid fibrils (PrPSc). The solution structure of the non-infectious form of the mouse prion protein (121-231) was determined in 1996. Since then, about 30 structures of the globular portion of PrPC have been characterized for different organisms (mostly from mammals). However, only a few minor differences are noticeable from comparing one structure to another. The key to understanding prion formation may then not be hidden in the structure of PrPC, but more likely in a hypothetical transition state. Thus far, the structure to another. The key to understanding prion formation may then not be hidden in the structure of PrPC, but more likely in a hypothetical transition state. But how is the normal prion protein (PrPC) converted into an unfolding fibril state (PrPSc)? To identify the possible regions required/responsible for amyloid fibril formation, we are using NMR methods to characterize the stability of PrPC and the transition state between PrPC and PrPSc. We hope to get local structural information about the prion conversion, which seems to be the source of prion infectivity.

P01.75
A Replica-Exchange Molecular Dynamics Study of Prion Misfolding Pathways and B-rich Folds
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Prion diseases are believed to be associated with a rare misfold leading from the cellular, monomeric, soluble, beta-helical PrP\(^{\beta}\) isoform to a pathogenic, aggregated, insoluble, beta-rich PrP\(^{\beta}\)-rich isoform of unknown structure. The goal of the present simulations is to scan the conformational space available to the PrP monomer via extensive enhanced sampling provided by protein-energy based replica-exchange molecular dynamics. The misfolding free energy surface is characterized as a function of the radius of gyration and of the fraction of native contacts. 1.3% of the conformations sampled display an enhanced beta content (> 19 residues). A new clustering algorithm is applied to sort the structures of this pool in function of the topology of their beta-contacts. 7 major beta-folds are thus identified and analysed in regard to their simulation transition temperatures (folds exclusively present at low and/or high temperature) and abundance. Different in vitro misfolding experiments involving soluble PrP beta-oligomers as well as PrP\(^{\beta}\)-rich are reviewed in regard to their simulation transition temperatures (folds exclusively present at low temperature) and abundance. Different in-vitro misfolding experiments involving soluble PrP beta-oligomers as well as PrP\(^{\beta}\)-rich are reviewed in regard to their simulation transition temperatures (folds exclusively present at low temperature) and abundance. Different in-vitro misfolding experiments involving soluble PrP beta-oligomers as well as PrP\(^{\beta}\)-rich are reviewed in regard to their simulation transition temperatures (folds exclusively present at low temperature) and abundance. Different in-vitro misfolding experiments involving soluble PrP beta-oligomers as well as PrP\(^{\beta}\)-rich are reviewed in regard to their simulation transition temperatures (folds exclusively present at low temperature) and abundance. 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In recent years cases of transmissible spongiform encephalopathies (TSE) were identified in small ruminants (SR) that differed from classical scrapie in the molecular characteristics of the disease associated proteinase K-resistant part of the pathological prion protein (PrPsc). PrPsc and PrPres. In Switzerland, nine cases of SR TSE were identified in the years 2004-2005 by active and passive surveillance. In this study we analyzed the molecular and neuropathological phenotype of these cases in detail. One sheep was identified as classical scrapie, whereas six sheep as well as two goats were classified as atypical scrapie. The latter revealed an uniform PrPres electrophoretic mobility pattern distinct from classical scrapie and bovine spongiform encephalopathy regardless of the genotype, the species and the neuroanatomical structure. Remarkably different types of neuroanatomical PrPsc distribution were observed in atypical scrapie cases by both Western immunoblotting and immunohistochemistry. Our findings suggest that the phenotype variation in atypical scrapie is larger than expected and thus impact on disease surveillance strategies.

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Recent BSE classification of unusual isolates has improved awareness about additional types of bovine spongiform encephalopathy (BSE). This has led to distinction of H-type and L-type isolates that can be discriminated from common BSE (C-type). The latter revealed an uniform PrPres electrophoretic mobility pattern distinct from classical scrapie and bovine spongiform encephalopathy regardless of the genotype, the species and the neuroanatomical structure. Remarkably, different types of neuroanatomical PrPsc distribution were observed in atypical scrapie cases by both Western immunoblotting and immunohistochemistry. Our findings suggest that the phenotype variation in atypical scrapie is larger than expected and thus impacts on disease surveillance strategies.

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This first case of H-type BSE was detected in a retrospective and incomplete search truncated between amino acids 160-173. Banding patterns were distinct from those observed with classical BSE. These bands were observed with antibodies P4, L42 and 6H4. P4 and L42 also labelled a band of detected using antibodies Sha31 and 6H4 had a higher molecular mass (~20 kD) than using a modified Bio-Rad TeSeE Western blot and detected using a panel of PrPSc. Distinct prion strains have been identified that are operationally defined by differences in neuropathology when inoculated in experimental animals. Experimental evidence suggests that prion strain diversity is encoded by distinct conformational differences in PrPSc. As an example, the hyper (HY) and drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy (TME) have different clinical signs, incubation periods, neuropathology and the biochemical and structural properties of PrPSc.

Objective: Investigate if in vitro amplification of the HY and DY TME prion strains maintains the unique strain properties. To accomplish this we used the protein misfolding cyclic amplification (PMCA) technique.

Methods: The PMCA technique is designed to mimic, at an accelerated rate, some of the fundamental steps involved in PrPSc conversion in vivo. In brief, a small amount of PrPSc is incubated with excess PrPC to increase PrPSc abundance. The newly formed PrPSc is sonicated to fragment it into smaller units, which will catalyze the formation of new PrPSc. This incubation and sonication cycle is repeated several times. We used HY or DY infected hamster brain as the PrPSc source and uninfected hamster brain as the PrPC source.

Results: After several rounds of sonication and incubation cycles we were able to amplify both of the HY and DY TME strains. Based on biochemical studies, the amplified strains are not different from those obtained from previous in vivo studies.

Conclusions: These results show that different prion strains can be amplified in vitro, suggesting that the PMCA technique can mimic the conditions needed for strain-specific in vivo conversion of PrPC into PrPSc.
Reconstitution of N2a Cells to be Expressed Bovine PrP<sup>C</sup> and Suppressed Endogenous Mouse PrP<sup>C</sup> by Lentivector-mediated RNAi

Kang, S-H; Basset-Leobon, C; Lacroux, C; Peoch', K; Stressenberger, N; Langeveld, J; Head, M; Haue, J; Schecher, P; Delisle, MB; Andreotti, O; E Cole Nat Rev Microbiol 4, 765-775.

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Reference:

Beyond the PrPres Type 1/Type 2 dichotomy in Creutzfeldt-Jakob Disease

Cassard, H; Uro-Coste, E; Simon, S; Bilheu, JM; Perret-Laudet, A; Ironside, J; Haik, S; Basset-Leobon, C; Lacroux, C; Peoch', K; Stressenberger, N; Langeveld, J; Head, M; Haue, J; Schecher, P; Delisle, MB; Andreotti, O; E Cole Nat Rev Microbiol 4, 765-775.

Analysis of Bovine Prion Protein Gene Sequence Variation in Animals with Classical and Atypical BSE

Polak, MP; Larska, M; Rola, J; Zmudzinski, JF National Veterinary Research Institute, Department of Virology, Poland

B. Variation within prion protein gene sequence have major impact on the susceptibility to prion diseases in humans and sheep. However no major differences between healthy cattle and bovine spongiform encephalopathy (BSE) affected individuals were identified. Recent studies indicate that susceptibility to bovine spongiform encephalopathy is associated with 23-base pair (bp) and 12-bp indel sequences. Identification of atypical BSE in older cattle in several countries pointed at the possibility of spontaneous spread of this new form of prion disease due to possible mutations within prion gene (PRNP) sequence.

A.O. Therefore the aim of the study was to analyze and to compare prion protein gene sequences in animals showing classical and atypical BSE for any genetic traits differentiating both forms of the disease.

M. Analysis included: octapeptide-repeat polymorphism; sequence analysis of exon 3 region; deletion/insertion polymorphism within the promoter sequence (23-bp), intron 1 (12-bp) and 3 untranslated region – UTR (14-bp) of PRNP gene. R. No major differences were found as for the octapeptide-repeats. Most dominant genotype in both classical and atypical BSE involved 6/6 homoyzous animals. Sequence comparison within exon 3 region also showed no differences. Results from indel sequence analysis within three regions of PRNP gene were also quite uniform between both forms of BSE.

D. Therefore no genetic traits explaining the appearance of atypical BSE could be found. However, it is too early to reject the hypothesis that genetic makeup is not involved in atypical BSE. Further and more detailed studies including more cases of atypical BSE would be more reliable to draw such a conclusion.

Can Brain Samples with Low PrP<sup>Sc</sup> but High Infectivity be Assayed using Currently Available Diagnostic Kits?

King, D Roslin Institute (Edinburgh), Diagnostic Approaches to TSEs, UK

Background: The conversion of the cellular prion protein PrP<sup>C</sup> to an insoluble aggregated isoform PrP<sup>Sc</sup> is taken to be the key process in the pathogenesis of Transmissible Spongiform Encephalopathies (TSEs) which include Bovine Spongiform Encephalopathy (BSE), Scrapie in sheep, Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker disease (GSS) in humans. The isoform PrP<sup>Sc</sup> consequently forms the basis for most biochemical diagnostic tests in the identification of these diseases. However studies have shown that high titres of TSE infectivity can be present in brain tissue of animals which show clinical and vacuolar signs of TSE disease, but contain low or undetectable levels of PrP<sup>Sc</sup> (using standard techniques such as immunohistochemistry, or immunoblot of PK treated tissue extract). These data question whether PrP<sup>Sc</sup> is a reliable diagnostics marker.

Aims: Using one particular model system showing low PrP<sup>Sc</sup> but high infectivity we aim to determine whether such tissues will be detected by two leading diagnostic assays namely the TeSeE sheep/goat kit (Bio-Rad) and the IDEXX HerdChek BSE-Scrapie kit (IDEXX laboratories).

Methods: Kits were used following manufacturers instructions but were optimised to account for the small amounts of starting material (50 – 200mg of brain tissue).

Results: Initial studies demonstrated that the IDEXX kit had a higher sensitivity than the TeSeE kit. Both kits were able to detect control tissues from a wide range of strains such as ME7, 139A, 78W, and 79A. For 263K and GSS infected brain however some samples were interpreted as negative. Results were confirmed using an optimised immunoblot from the original starting samples.

Discussion: From the panel of control samples both assays demonstrated reliability where results were reproducible and obtained rapidly. However, certain samples containing extremely low levels of PrP<sup>Sc</sup> failed to be detected by both systems. Reliance on protease-resistant PrP<sup>Sc</sup> as a sole measure of infectivity is questionable as previous studies have shown that abnormal isoforms of PrP<sup>Sc</sup> and TSE infectivity do not correlate and therefore the relationship between PrP<sup>Sc</sup> and infectivity is unclear. The possibility of false positive or false negative results in PrP<sup>Sc</sup>-dependant assays could lead to misdiagnosis of disease.
**P02.17**

Clinical, Neuropathological and Biochemical Features of Sporadic and Variant Creutzfeldt-Jakob Disease in the Squirrel Monkey (Saimiri sciureus)

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Background: The use of experimental animal models in prion diseases has a long and successful history, providing valuable information on issues such as disease pathogenesis and agent strain. The squirrel monkey (Saimiri sciureus) has been shown to be highly susceptible to experimental challenge with human prion disease, yet little information is available on the phenotype of different human prion strains in this species.

Objectives: Investigations on the clinical, neuropathological and biochemical features of squirrel monkeys experimentally challenged with either sporadic or variant CJD were carried out to establish if strain characteristics are maintained after transmission in this model.

Materials and methods: Brain homogenates from sporadic or variant CJD patients were inoculated into the frontal cortex of squirrel monkeys. Animals were kept under constant clinical surveillance. At post-mortem, formalin fixed CNS tissue was taken for neuropathological and immunohistochemical analysis with frozen CNS tissue taken for the biochemical detection of PrPSc.

Results: Clinical presentation was similar in both sporadic and variant CJD challenged animals; however, clinical features were more severe in sporadic CJD challenged animals with a shorter disease duration. Neuropathological analysis showed two distinct patterns of spongiform change and PrP deposition, mirrored the neuropathological features in humans, although amyloid plaques were absent in both sporadic and variant challenged animals. Western blot analysis for PrPSc showed a single PrPSc isoform in animals challenged with variant CJD resembling that observed in human variant CJD. In all animals challenged with sporadic CJD a single PrPSc isoform resembling human sporadic CJD type 1 was observed.

Conclusion: Neuropathological and biochemical analysis have shown that sporadic and variant CJD can be distinguished in the squirrel monkey and that many of the strain characteristics that define these agents are conserved after transmission in this model.

**P02.18**

Molecular and Biological Strain Typing of Sporadic and Variant Creutzfeldt-Jakob Disease

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Background: The presence of distinct human prion strains has been inferred from the phenotypic heterogeneity observed in sporadic CJD. Classically, TSE strains are defined by their biological properties after transmission to inbred lines of mice, specifically by incubation period and pattern of vacuolar pathology in specified brain regions (the lesion profile). More recently, it has been proposed that PrPSc may act as the surrogate marker for agent strain in what has been commonly referred to as ‘molecular strain typing’.

Objectives: We aim to examine the relationship between PrPSc type and agent strain by transmission of different subtypes of sporadic CJD, as classified according to PRNP codon 129 genotype and PrPSc type, to wild-type mice and to compare these with transmission of variant CJD.

Materials and methods: Wild-type VM and II mice were experimentally challenged with brain homogenate from sporadic or variant CJD patients. At post-mortem, formalin fixed tissue was taken for pathological and immunohistochemical analysis and frozen CNS tissue taken for biochemical analysis.

Results: Preliminary results show the successful transmission of variant CJD and some subtypes of sporadic CJD to VM and RII mice. Sporadic CJD transmission occurs only from cases containing at least one methionine at PRNP codon 129. Within the sporadic CJD cases that did transmit, transmission occurred from cases with type 1 and type 2 PrPSc. However, the lesion profiles from sporadic CJD transmissions show a similar pathology in all cases and in both mouse strains. In transmissions from sporadic and variant CJD showing positive pathology, Western blot analysis of PrPSc in the CJD inoculum and the recipient mouse brain show conservation of PrPSc type across the single successful sCJD MV2 transmission in which a PrPSc type 1 was observed in the recipient mouse.

Conclusion: These transmission studies confirm the biochemical and biological differences between the agents responsible for sporadic CJD and variant CJD, but interestingly classical strain typing fails to confirm the differences in agent strain proposed to account for the pathological subtypes of sporadic CJD.

**P02.19**

Luminescent Conjugated Polymers – Conformation Sensitive Optical Probes for Staining and Characterization of Prion Strains

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Several questions still remain regarding the molecular mechanism of protein aggregation and the information encoded in the disease associated multiple conformations found in the prion protein. Some of these questions are particularly complex, including the so-called “strain phenomenon” which is believed to be associated with distinct tertiary and/or quaternary structure of the prion aggregates.

Here we report that histochemical LCP stains yield a simple and sensitive method for characterization of native prion aggregates having distinct biochemical and histopathological properties, indicative of specific prion strain isolates. LCPs reliably distinguished a variety of prion protein aggregates, including mouse-passaged prion strains of bovine spongiform encephalopathy (bSBE), sheep scrapie (mPSS), chronic wasting disease (mCWD) and the Rocky Mountain Laboratory (RML) prion strain. LCPs stained all amyloidogenic prion deposits identified by ThT and Congo red. In addition, an anionic LCP, PTAA, being bound to congophilic mCWD, mBSE or mPSS plaques emitted light with different colors. Hence, the emission from an individual LCP can be an anionic LCP, PTAA, being bound to congophilic mCWD, mBSE or mPSS plaques stained all amyloidogenic prion deposits identified by ThT and Congo red. In addition, an anionic LCP, PTAA, being bound to congophilic mCWD, mBSE or mPSS plaques emitted light with different colors. Hence, the emission from an individual LCP can be used to distinguish congophilic aggregates, formally classified as amyloid, arising in individual murine prion isolates. LCPs also stained diffuse non-congophilic prion aggregates and by using two LCPs with distinct ionic-side chain functionalities, a difference in the stainability pattern of the LCPs was observed. Each of the prion aggregates and by using two LCPs with distinct ionic-side chain functionalities, a difference in the stainability pattern of the LCPs was observed. Each of the prion isolates being used display unique signatures in a variety of biochemical and histopathological parameters, suggesting that each isolate represents a unique prion strain. Therefore, the photophysical phenomena revealed by the emission spectra of PTAA or the stainability difference seen for LCPs with distinct ionic side-chain functionalities are most likely strain specific. The technique is also demonstrated for natural prion diseases, such as sporadic CJD, BSE, atypical BSE (BASE), scrapie and CWD.

**P02.20**

Primary Isolation of the Scrapie Agent in RIII Mice: Identification of Sheep PrP Genotype-associated Profiles

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Background: Lesion profile and incubation period analysis are effective mouse bioassay parameters for transmissible spongiform encephalopathy (TSE) strain analysis. Using these methods, RIII (Prnp a) mice have proven useful in distinguishing BSE from scrapie at primary isolation: BSE presents a reproducible lesion profile with peaks at brain areas 1, 4 and 7 (dorsal medulla, hypothalamus and septal nuclei), while classical scrapie lesion profiles are believed to be more variable.

Aim: The aim of this study was to analyze a large number of scrapie isolates in RIII mice and explore trends in the resulting lesion profiles and incubation periods at primary isolation.

Methods: 62 positive scrapie field cases were collected from individual farms. A 10 % oesophageal homogenate was inoculated into RIII mice (20 mice per inoculum). Post mortem TSE diagnosis was confirmed histopathologically on H&E sections. Lesion profiles and incubation periods were assessed using standard methodology (Fraser and Dickinson, 1966).

Results: 31 inocula with 5 or more clinically and H&E positive mice were profiled. Profiles presented as 3 distinct subgroups. One subgroup comprising of 10 inocula showed consistent peaks at brain areas 1, 4 and 7. All inocula for this subgroup were derived from sheep of genotype ARQ/ARQ. The incubation period of disease in these mice was lower than other groups. Whilst this profile exhibits similarities to BSE in RIII mice at primary isolation, the profile described here was different in some respects, for example profiles were higher and a different shape. Also additional histopathology was not consistent with BSE. A second subgroup of 5 inocula gave an alternative 1-4-7 profile and derived from ARQ or VQG homozygotes. A third non 1-4-7 profile was associated with the V allele in 13 of 16 inocula.

Conclusion: Results demonstrate that in RIII mice discrete classical scrapie profiles can be distinguished on primary isolation in contrast to previous belief. It appears that these profiles are associated with the PrP genotype of the sheep donor. Of particular interest is the ARQ/ARQ-associated 1-4-7 profile, where caution should be taken to distinguish this profile from BSE. Further studies are required to assess the stability of this profile on sub-passage.
PrPSc was serially amplified through many rounds of PMCA to remove the initial mechanism of antigen presentation and induction of T cell response. This work is not of PrPc. Further analysis will be carried out to check a potential role of PrPc in the activation but the various DC function tested were not influenced by the presence or results indicate that the synthesis of PrPc by DC is clearly up-regulated by the cellular inflammation induce by irradiation, we have measured the accumulation of DC to consider their RedOx and their growth capacity. Furthermore, using a model of over express PrPc): no difference was observed between these DC when we co-localisation with specific markers of these membrane domains (cholera toxin). No As shown by confocal microscopy: PrPc was localized on lipids raft as revealed by its expression of PrPc at a similar level that was observed on mature DCs. Whatever the activation, PrPc expression was the same; this was confirmed by the quantification of PrPc RNA.

Dendritic cells (DCs) reside at the interface between innate and adaptive immunity, they are known for their antigen-presenting capacity and their role in primary specific immune responses. via pathogen recognition receptors, they detect and then internalize and process foreign molecules for antigen presentation, they participate also in the first steps of propagation of PrPSc in animals. As amplification of PrPSc requires the presence of PrPc, we investigated the ability of DC to synthesize PrPc. DCs were derived from bone marrow progenitors and PrPc expression was analyzed. Progenitors as well as immature cells did not synthesize PrPc whereas mature DCs expressed PrPc at their surface. DCs activation was induced by various molecules such as IfNg, anti CD40 or ligands of LTR, in each case, the activation was checked by the increase expression of Class II molecules and the secretion of various cytokines (IL12, IL6, TNF). DC activation induced also the expression of PrPc: at a similar level that was observed on mature DCs. Whatever the activation, PrPc expression was the same; this was confirmed by the quantification of PrPc RNA.

As shown by confocal microscopy: PrPc was localized on lipids raft as revealed by its co-localisation with specific markers of these membrane domains (cholera toxin). No PrPc was observed in the intracellular vesicles indicating the absence of internalization of PrPc even after crosslinking using anti-PrPc antibodies. As PrPc was previously shown to bind cupper and to interfere with the RedOx balance of the cells. We compared DC from various mouse strains (PrP-KO and Tg220 witch over express PrPc): no difference was observed between these DC when we considered their RedOx and their growth capacity. Furthermore, using a model of inflammation induce by irradiation, we have measured the accumulation of DC to specific localization, neither the speed of cell response, nor the amount of cells accumulated were influenced by the absence or the over expression of PrPc. All our results indicate that the synthesis of PrPc by DC is clearly up-regulated by the cellular activation but the various DC function tested were not influenced by the presence or not of PrPc. Further analysis will be carried out to check a potential role of PrPc in the mechanism of antigen presentation and induction of T cell response. This work is supported by the EC program FOOD-2004-TS.4.5.3 number 23144

Molecular Properties of BSE and BASE Agents following Denaturation Assessed by a Panel of Monoclonal Antibodies
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For a long time BSE has been thought to be caused by a unique prion strain (C-type) with stable and specific lesion profile, molecular features and glycoctype in which the glycosylated form of PrP was predominant. Despite, the occurrence of atypical BSE cases in a number of European countries has pointed out the existence of at least two further BSE strains with distinctive biochemical properties. In particular, in Italy two atypical BSE cases were found in more than 10 year old cattle which were characterized by a different type of disease associated PrP (L-Type) showing a lower molecular weight of the unglycosylated isoform, predominance of the monoglycosylated dominant and a peculiar deposition pattern in the brain compared to typical Italian BSE.

In order to gather further information on the molecular features of C-type and L-type PrP samples from BSE- and BASE-affected cattle underwent treatment with various concentrations of denaturing agents such as guanidine and urea whose use in conformation stability assays to accomplish molecular strain typing of various TSE agents has been reported.

Reactivity of denatured BSE and BASE samples was then assessed by means of a well characterized panel of monoclonal antibodies (MAbs) that can efficiently detect both strains and by the reference MAb 6H4. Preliminary results showed that 6M guanidine, followed by proteinase K (PK) digestion, completely abolished the reactivity of L-type with 6H4 and G-terminal MAb, while C-type PrP was still detectable though to a lower extent. After 4 and 8 M urea treatment both C-Type and L-Type PrP were detectable but a reduction in signal intensity was observed in samples treated with the highest concentration.

Taken together this data indicated that, as well known, C-Type and L-Type PrP are denatured by guanidine and urea but, interestingly, L-Type seemed to be less stable to high guanidine concentrations than C-Type. This suggests the existence different molecular properties between the BSE and BASE strains that may also account for their distinct biological properties recently highlighted in transmission experiments in mice.
The characteristics of scrapie strain circulating in small ruminants has not been extensively investigated. The aim of this study was the characterization of the clinical, pathological and biochemical aspects of a highly pathogenic isolate of natural scrapie in sheep called « TAC ». Transgenic mice overexpressing normal murine PrP (Tg20) have been inoculated intracerebrally with this isolate. This study has been performed in collaboration with the French scrapie surveillance network. This scrapie strain was first isolated from a lamb which has been probably infected by maternal transmission. This lamb developed fastly the disease after only 6 months of incubation (2 to 3 years normally). A histological study and a molecular characterization of the strain were performed on the infected mice brain and a strain typing was established. The inoculated mice exhibited a very short incubation period (100 ± 18 days) compared to other strains (i.e. 599 ± 120 days for O69 scrapie strain) clinical signs were not obvious, and mice died suddenly. The lesion profile identified is close to that of other scrapie strains profile and clearly different from the BSE one. The PrPsc molecular profiles have confirmed that the « TAC » isolate is a classical scrapie strain.

The normal physiological functions of the cellular prion protein (PrPc) are still elusive. This GPI-anchored protein exerts many functions, including roles in neuron proliferation, neuroprotection or redox homeostasis. However, there are conflicting data concerning its role in synaptic transmission. While several studies report that PrPc participates in NMDA-mediated neurotransmission, parallel studies describe normal behaviour of PrPc-deficient mice. However, abnormal axon connections have been described in the dentate gyrus of the hippocampi of PrPc-deficient mice similar to those observed in epilepsy. Indeed, a report indicates increased susceptibility to kainate (KA) in these mutant mice. Here we extend the observation of these studies by means of several histological and biochemical analyses of KA-treated mice. PrPc-deficient mice showed increased sensitivity to KA-induced seizures in vivo and in vitro in organotypic slices. In addition, we demonstrate that this sensitivity is cell specific since interference experiments to abolish PrPc expression increased susceptibility to KA in PrPc-expressing cells. Finally, we indicate a correlation of susceptibility to KA in cells lacking PrPc with the differential expression of GluR6 and GluR7 KA receptor subunits using real-time RT-PCR methods. Taken together, these results indicate that PrPc exerts a neuroprotective role against KA-induced neurotoxicity, probably by regulating the expression of KA receptor subunits.
P02.29
Selective Susceptibility of Porcine species to Prion Strains as Assayed in Transgenic Mice Expressing Porcine PrP

Background: There have been no reports of naturally occurring TSE in porcine species. Nevertheless, pigs are susceptible to BSE infection but showing a strong transmission barrier by parenteral route. Previous work in our group using transgenic mice expressing porcine PrP (PoPrP-Tg001) showed a strong transmission barrier for BSE by intracerebral route.

Objectives: The main aim of this work was to characterise the susceptibility of porcine species to different prion strains in transgenic mice expressing porcine PrP Tg mice.

Methods: A wide panel of inocula has been used as intracerebral inoculum in PoPrP-Tg001: BSE, RML, different human and sheep TSEs.

Results: From the different strains assayed, only the BSE prion strain has been able to be transmitted to mice. Remarkably, BSE after passage in sheep increased its transmission ability in PoPrP-Tg001 mice that is maintained after subpassage.

Discussion: Our results confirm that porcine species shows a strong transmission barrier to a wide range of prion strains but the risk of infection of BSE after passage in sheep need to be considered.

P02.30
Molecular Characterization of Atypical Scrapie Isolates in Goats from the Spanish Surveillance Programme

Objectives: The main aim of this work was to generate a model for examination of PrP. We characterized proteinase K resistant and sensitive PrP isolated from PoPrP-Tg001 mice over different time points.

Methods: A panel of recombinant poPrP- and control-worms were used for the generation of model mouse strains. The resulting mice were characterized by biochemical and physiological properties.

Results: The recombinant poPrP- and control-worms were characterized by biochemical and physiological properties. The resulting mice were characterized by biochemical and physiological properties.

Discussion: Our results confirm that porcine species shows a strong transmission barrier to a wide range of prion strains but the risk of infection of BSE after passage in sheep need to be considered.

P02.31
Caenorhabditis Elegans as a Heterologous Expression System for Human Prion Protein

Heterologous expression of prion protein is widely used for the analysis of its physiological and pathophysiological properties. Different cell culture systems and animal models like hamster or mice are used to examine PrP function and conditions that influence infection or infectiosity for several aims with the known advantages and disadvantages.

For further characterization of PrP function and interaction-proteins we established the nematode C. elegans, that has no endogenous PrP, as an expression system of human PrP. Although C. elegans is a simple eucaryotic organism, cellular mechanisms and biochemical pathways resemble in many points that of humans. Additionally the worms are easy to culture and to manipulate and have a short generation time.

The aim of this work was to generate a model for examination of PrP. We characterized PrP transformed C elegans strains by determination of life spans and motoric phenotypes and also by applying proteomic approaches (difference gel electrophoresis 2D-DIGE). As control-worms we generated a strain that was transformed with the empty pBY871 vector. Whereas the life spans and readouts of thrashing assay, radial locomotion assay and quantification of reversals and body bends don’t differ between PrP- and control-worms we found proteins that are differentially expressed in PrP- and control-worms compared to controls. We identified proteins that were either up or down regulated dependent on PrP expression, e.g translation elongation factor and an aconitase.

Next steps will be to express mutant PrP associated with neurodegeneration and/or infectiosity and characterize again the proteome and genome compared to PrPC. If there is a phenotypical change the generated model could be useful not only for the analysis of general PrP trafficking and turnover but also for the study of infection cofactors.

P02.32
Atypical Scrapie Field Cases Characterization in Spanish Ovine Breeds

In the present work, we report atypical cases found in Spain during the last years and since the first one detected in 2003 (Mayoral T et al. 2004). In 2006 at least 96 positive ovine cases have been detected, 65% classical cases and 35% atypical cases. These atypical cases show discordant results when screening, discriminatory and confirmatory methods are compared. Moreover, biochemical characteristics (resistance to the accumulated PrPsc to PK digestion, altered immunoblot profile and evidence of a lower band of PrPsc around 12-11 KDa known to be associated with atypical scrapie) from atypical cases are clearly different from classical ones.

Also genotyping analysis and neuroanatomical studies (distribution of PrPsc aggregates detected by immunohistochemistry and histopathology) show differences between both groups. All atypical cases were genotyped and distribution of polymorphisms found were ARQ/ARQ 18%, ARQ/AHQ 21%, ARR/ARR 29%, ARR/AHQ 14%, ARQ/ARQ 6% and ARR/ARR 12% quite different from classical cases where near 92% samples were ARQ/ARQ. Biochemical and histopathological studies in spanish atypical isolates and its comparison with typical cases and Nor98 strain will also be shown for better understanding of these cases.

P02.29
Selective Susceptibility of Porcine species to Prion Strains as Assayed in Transgenic Mice Expressing Porcine PrP

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P02.30
Molecular Characterization of Atypical Scrapie Isolates in Goats from the Spanish Surveillance Programme

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Laboratorio Central de Veterinaria, TSEs Department, Spain

P02.31
Caenorhabditis Elegans as a Heterologous Expression System for Human Prion Protein

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P02.32
Atypical Scrapie Field Cases Characterization in Spanish Ovine Breeds

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P02.33
Characterising TSE Strains by 2 Dimensional Immunoblotting
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Background: Experimental transmissible spongiform encephalopathy (TSE) strains are defined by stable forms of prion that, upon inoculation in animals, cause disease with unique incubation time and lesion profile. Various distinct scrapie strains have been isolated, however, until recent identification of two atypical phenotypes, evidence indicated that BSE in cattle was caused by a single strain. Traditional methods used for strain discrimination have relied on measurements of disease characteristics in mouse models. Biochemical strain typing approaches are largely based on the molecular characterisation of PrP by using 1D immunoblotting (IB). This approach has limitations however and 2D IB offers much promise for identifying strain specific differences in PrP which 1D IB cannot readily resolve.

Aims: To characterise PrP from mice infected with experimental TSEs by 2D IB with a view to distinguishing between ME7, 22A, 87V, 79A and 301V experimental strains and to investigate the potential to identify distinct TSE strains in sheep (natural and experimental disease).

Methods: 10% brain homogenates were analysed by 2D IB using 6H4 and P4 antibodies. Deglycosylation was carried out using PNGase F enzyme in an overnight incubation at 37°C.

Results: Experimental TSEs in mice models exhibited strain specific changes within the total PrP and PrP profiles. Analysis of natural and experimental TSE strains in sheep showed differences between the 2D profiles after deglycosylation, with ovine BSE and CH1641 cases exhibiting fewer PrP isoforms than ovine scrapie and SSBP-1. A greater range of basic glycosylated PrP isoforms were also detected in SSBP-1 than CH1641 profiles, indicative of a strain specific PK cleavage site in both cases.

Conclusion: These studies indicated that the high power of 2D IB to resolve PrP glycoforms and truncated products provides the possibility of distinguishing subtle multiple differences between strains which is not readily possible with 1D methods.

This work was funded by Defra, UK

P02.34
Diversity in the Biological and Chemical Properties of TSE Agent-Strains: Implications for TSE Agent Structure
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Under controlled conditions TSE agent strains produce a highly reproducible set of measurable biological properties, notably incubation periods and the distribution and intensity of neuropathological change. These biological properties require TSE infectious agents to be genetically independent of the host, but to replicate under strict host control. Phenotypic properties have been used to characterise a series of experimentally derived TSE strains whose properties can be further compared. Differences in the degree to which PrP is glycosylated and its migration on SDS-PAGE show phenotypic variation between TSE strains, although these properties are also influenced by host factors.

TSE agents also show strain-specific responses to inactivation with heat, high pH and SDS, suggesting coherent differences in the molecules which comprise the structure of the infective agents. Heat inactivation properties are primarily specified by TSE strain; although passage history in the host can have an effect. Differences in heat inactivation properties have allowed two passage lines of the TSE strain ME7 to be distinguished. The kinetics of the inactivation of TSE agents with heat suggest two different components must be involved in the structure. These findings have not been reconciled with models of TSE agents based solely on the conversion of the host protein PrP into an abnormal conformation that is proposed to be infectious. Much of the abnormal PrP can be solubilised into a non-sedimentable form, not associated with infectivity, suggesting that most PrP in the PK-resistant fraction is not intrinsically infectious, and that PrP associated with the structure of the infectious agent must form part of a sedimentable particle. Accordingly structural models of TSE agents are being developed to include a mechanism for maintaining and replicating host-independent genetic information and diversity in structure compatible with the differences in inactivation properties between TSE strains.

P02.35
Molecular Features of the Protease-resistant Prion Protein (PrPres) in H-type BSE
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Western blot analysis of PrPres accumulating in the brain of BSE-infected cattle have demonstrated 3 different molecular phenotypes regarding to the apparent molecular masses and glycosylation profiles of PrPres bands. We initially described isolates (H-type BSE) essentially characterized by lower PrPres molecular mass and decreased levels of the diglycosylated PrPres band, in contrast to the classical type of BSE. This type is also distinct from another BSE phenotype named L-type BSE, or also BASE (for Bovine Amyloid Spongiform Encephalopathy), mainly characterized by a lower representation of the diglycosylated PrPres bands as well as a lower PrPres molecular mass.

Retrospective molecular studies in France of all available BSE cases older than 8 years old and of part of the other cases identified since the beginning of the exhaustive surveillance of the disease in 2000/1 allowed to identify 7 H-type BSE cases, among 594 BSE cases that could be classified as classical, L- or H-type BSE.

By Western blot analysis of H-type PrPres, we described a remarkable specific feature with antibodies raised against the C-terminal region of PrP that demonstrated the existence of a more C-terminal cleaved form of PrPres (named PrPres2), in addition to the usual PrPres form (PrPres1). In the unglycosylated form, PrPres2 migrates at about 14 kDa, compared to 20 kDa for PrPres1. The proportion of the PrPres2 in cattle seems to be higher compared to the PrPres1. Furthermore another PK-resistant fragment at about 7 kDa was detected by some more N-terminal antibodies by stable presumed to be the result of cleavages of both N- and C-terminal parts of PrP. These singular features were maintained after transmission of the disease to C57Bl/6 mice.

The identification of these two additional PrPres fragments (PrPres2 and 7kDa band) reminds features reported respectively in sporadic Creutzfeld-Jakob disease and in Gerstmann-Sträussler-Scheinker (GSS) syndrome in humans.

P02.36
Investigation of Prion Decontamination from Different Chromatographic Gels by Sodium Hydroxide
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Sodium hydroxide (NaOH) is one of the recommended treatments for the decontamination of transmissible spongiform encephalopathies (TSE) agents. The World Health Organization recommends the use of 1N NaOH for 1 hour at room temperature for a complete inactivation of these agents. Such recommendations are based on the results of inactivation experiments that were performed in liquid phase on crude brain homogenates, and that did not include kinetics of inactivation. Few studies are available regarding the efficiency of NaOH treatments on different kind of surfaces and matrixes, including the chromatography gels which are used in the purification of plasma derived products. We have assessed the efficiency of prion decontamination by NaOH on two ion-exchange chromatography gels by using a screening method on a batch format (DEAE Toyopearl and CM-LS Tris-acryl gels). A bead suspension was loaded in a tube and put in contact with crude brain homogenate (0.2% w/v - 260K hamster strain of scrapie). The unbound fraction was discarded and the remaining beads were treated with different concentrations of NaOH under several conditions of time and temperature. The beads were rinsed and submitted to elution in NaCl 2M. The amount of PrP-sc was quantified by Western blot in both the elution solution and remaining beads suspension. The reduction factors (RF) were calculated relative to the level of PrP-sc detected in spiked beads that were treated with water instead of NaOH. The results showed efficient PrP-sc inactivation on a DEAE Toyopearl gel with 0.2M NaOH (RF > 4 log10 for the elution solution and RF > 2.5 log10 for the bead suspension) in a short time (18 minutes) at room temperature. Similarly, efficient PrP-sc inactivation was observed on a CM-LS Tris-acryl gel treated with 0.2M NaOH (RF > 3.5 log10 for the elution solution and RF > 2.5 log10 for the bead suspension) after 60 minutes at 4°C. Taken together, these results show that NaOH treatments are efficient in inactivating PrP-sc on DEAE toyopearl and CM-LS Tris-acryl chromatography gels, with no PrP-sc detected on treated beads. These experiments will be extented to other gels and should be completed by an infectivity assay. In conclusion, the chromatography gels treatment by NaOH, that is already applied in industrial conditions, contribute to the safety of plasma products regarding the theoretical risk of vCJD transmission.
P02.37
A Third Case of BASE in Italy
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Following the identification of two BASE (Bovine Amyloidotic Spongiform Encephalopathy) cases in Italy in 2004, other atypical BSE isolates have been identified worldwide (36 cases in total) and experimentally transmitted to different species. Recently a new case of atypical BSE in a fallen stock 15 year old cow of Piedmontese breed has been identified in Italy by the active surveillance system.

In order to well define the characteristics of this atypical BSE case, epidemiological, genetic and immunobiological studies have been conducted in our laboratories. Distribution and pathological prion protein (PrPres) features have been investigated in both neural and extraneural tissues.

Thirty-nine different tissues including central nervous system, viscera, skeletal muscles and lymphoreticular system were collected and examined by four different diagnostic methods: Western blot (WB), Immunohistochemistry (IH), Histoblot (HB) and IDEXX HerdCheck BSE Antigen Test Kit EIA. Furthermore PCR amplification of the PrP gene was performed and amplicon analysis was carried out by DNA sequencing. An epidemiological questionnaire was filled in.

Immunohistochemical characterization showed a molecular pattern of PrPres different from typical BSE and referable to BASE cases. At the moment the presence of PrPres was only detected in neural tissues. The genetic analysis revealed a wild-type genotype of the PrP gene carrying six copies of octapeptide repeats. Neither clinical signs nor consumption of meat feed were recorded.

Our findings confirmed the identification of a third case of BASE in Italy. Moreover the age of the animal and the non-use of meat feed strengthen the hypothesis of a sporadic origin of this neurological disorder. The analysis of such a great number of tissues will help in understanding the BASE pathogenesis under natural conditions.

P02.38
Involvement of the MEK1/2/ERK1/2 Signaling Pathway in Formation of Prions from Different Strains
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The MEK1/2-ERK1/2 signaling pathway plays a major role in cell proliferation and differentiation and is activated by growth factors, hormones and neurotransmitters. The cascade cooperates in the transmission of extracellular signals to their intracellular targets via sequential activation of protein kinases. We have previously shown that stimulation of the MEK1/2-ERK1/2 pathway leads to increased formation of PrPSc.

Furthermore, we have shown that inhibition of MEK1/2 using specific inhibitors (U0126, PD98059, SL327) clears gonadotropin-releasing hormone-secreting neuronal cells (GT1-1 cells), infected with the RML strain of scrapie, from PrPSc.

In order to examine if the activity of MEK1/2 and ERK1/2 is affected by a scrapie infection, we inoculated GT1-1 cells with brain homogenate from mice infected with the scrapie strain RML, 22L and Me7. Inoculation of GT1-1 cells with RML and 22L resulted in persistently infected cells, while inoculation with Me7 did not lead to accumulation of proteinase K resistant PrPSc. Cells were harvested every other passage after infection and the levels of activated (phosphorylated) MEK1/2 and ERK1/2 was analyzed by Western blot. Furthermore, we wanted to investigate if MEK1/2 inhibitors could be effective in curing GT1-1 cells infected with other scrapie strains and preliminary results indicate that the levels of PrPSc in GT-1 cells infected with 22L can be decreased by treatment with the MEK1/2 inhibitor U0126. In this project we will characterize the involvement of MEK1/2-ERK1/2 in formation of PrPSc from different scrapie strains and thus increase the basic knowledge about the metabolism of prions.

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P02.39
Regulation of Cathepsin Activity Affects Accumulation of PrPSc in ScGT1-1 Cells
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Background: During prion diseases, PrPSc accumulates as a partially processed protein, PrP 27-30 in infected cells. We have previously shown that inhibition of specific lysosomal proteases, cathepsin B and L, in GT1-1 cells infected with the RML strain of scrapie (ScGT1-1), increases the amount of PrPSc, indicating that these proteases are important for PrPSc degradation. Variations in the degradation rates and cleavage-mechanisms between different strains may affect the accumulation and distribution of the different strains in cells.

Objectives: We here ask the question whether cathepsins are involved in degradation of different prion strains and if the regulation of cathepsin activity can modify the cellular clearance of PrPSc.

Methods: We investigated if inhibition of specific cathepsins increases the amount of the prion strains RML, Me7 and 22L in the GT1-1 cells. Using Western immunoblotting we studied the effects on PrPSc in ScGT1-1 cells after serum starvation and reduction of glucose, as well as other treatment known to affect cathepsin activity in different cell types. Changes in cathepsin activities after these treatments were measured using cathepsin B- and L-specific fluorescent substrates.

Results: We have analyzed effects in ScGT1-1 cells after treatments affecting cathepsin activities and found that starvation for 48 hrs caused decreased levels of PrPSc from the RML strain. This decrease was blocked by co-incubation with specific inhibitors of cathepsin B (Z-Arg-OH) and cathepsin L (Z-Phe-Tyr-Aldehyde) indicating that these cathepsins are important for the starvation-induced reduction of PrPSc in the ScGT1-1 cells. We have also investigated the effect of specific cathepsin inhibitors on the levels of PrPSc from different prion strains during treatments decreasing the amount of PrPSc in the cells.

Conclusions: We conclude that manipulations of cathepsin activity in prion-infected cells can affect the amount of accumulated PrPSc in the cells, leading to altered balance between the formation and the degradation of PrPSc. The identification of mechanisms regulating the amount of PrPSc in a cell are important for elucidating the mechanisms involved in potential clearance of PrPSc from prion infected cells.

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P02.40
Small Ruminants with Transmissible Spongiform Encephalopathies Analyzed for Properties of PrPSc and PrPres
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Due to strain dependent variations disease associated PrP (PrPd) varies phenotypically. PrPd is a reliable marker for disease even if its appearance varies depending on epitope exposure, susceptibility to proteases, tissue variations and disease incubation time. There is a clear need to have methods available for efficiently detecting all variants. Therefore, antibodies covering several sites of the ovine PrP sequence are tested to find ways to detect in a single assay as many scrapie variants as possible. This was performed by Western blotting and ELISA on both digested and undigested brain stem homogenates, and under native and denatured conditions. A large set of TSE isolates from sheep including classical scrapie, experimental BSE and atypical cases were subjected to this study. More than 100 negative controls of which 30% with known ages were used to investigate the possible effects of variables like breed, genotype, age, herd and country of origin. Western blotting appeared to be a good tool to detect all types of TSEs although the Nor98 cases are suffering of too stringent conditions of proteinase K, confirming a risk when using such method of detection. A novel ELISA set-up without the use of proteinase K allowed detection of all types of TSEs. In a second stage, the same ELISA approach also could discriminate between the known types of TSE by applying appropriate antibodies, digestion with PK and denaturation. This study offers a unique test set up, where non-digested brain homogenates could be used for detection of TSEs. This observation might lead to applications for general TSE detection.

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P02.41

Identification of CH1641-like Isolates in Sheep during the Monitoring for BSE in Small Ruminants in France (2002-2006)
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Since 2002, the surveillance of TSE in small ruminants in the EU has been increased and routinely involved molecular discrimination of scrapie from a possible BSE infection. In this context, we developed a discriminatory BSE-scarpia Western Blot method that has been approved at the EU level. Between 2002 and 2006, 777 sheep were detected as positive for TSE during the active surveillance. 246 (31.7%) were classified as Nor98-like with the confirmatory Western blot. 29 samples (3.7%) could not be characterized. The 502 other samples showed a classical three bands pattern of PrPres (non Nor98-like) and were subsequently tested using our method. In addition, 192 TSE positive and non Nor98-like cases detected in TSE infected flocks were also analysed. Most of the classical isolates (497 (99.9%) index cases and 189 (98.4%) from TSE-infected flocks) had PrPres with a higher molecular weight of the unglycosylated PrPres than a cattle BSE control, as well as strong labelling with the N-terminal P4 antibody. Large variations of the molecular weights (1.2 kDa) were however observed among these samples, although these were consistently higher than the cattle BSE control. On the other hand, we found 5 (1%) index cases and 3 (1.6%) cases from TSE-affected flocks presenting patterns with some molecular similarities to ovine BSE (lower molecular weight than the BSE control and low signal with P4). However, with regard to the glycoform profiles, the molecular patterns presented more similarities to the CH1641 experimental isolate, since these samples all displayed lower proportions of the diglycosylated PrPres band. Three of these CH1641-like samples were transmitted to TgDoxPp4 ovine transgenic mice, that confirmed more similarities to CH1641 than to ovine BSE. These data suggest the presence of (a) some scrapie strain(s) producing unusual PrPres phenotype in sheep, that should be further investigated.

P02.42

Characterization of CWD Prion Strains Uding Transgenic Mice
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Although the distribution and host range of chronic wasting disease (CWD) is increasing, whether or not different CWD strains occur in various geographic locations or in different cervid species remains unclear. To address this we used cervidized transgenic mice (TgCerPrP(1536)) to analyze CWD prions isolated from mule deer, white-tailed deer and elk from various geographic locations in North America. Isolates were characterized on the basis of incubation times in TgCerPrP(1536) mice. In all cases serial transmissions were performed in TgCerPrP(1536) mice. The biochemical properties of CerPrPsc produced in the brains of diseased mice were assessed by western blotting and conformational stability assays. We also used histoblot analysis to obtain a global portrait of the neuroanatomical distribution of CerPrPsc in the brains of diseased TgCerPrP(1536) mice in conjunction with immunohistochemical staining and hematoxylin and eosin staining to more fully characterize neuropathology. The glycosylation ratios of CerPrPsc were similar for all samples analyzed with the diglycosylated species of CerPrPsc being chiefly represented. Additionally, the stability of CerPrPsc, assessed by treatment with increasing concentrations of guanidine hydrochloride, demonstrated that the various CWD isolates were associated with similar conformations of CerPrPsc. Consistent with these results, there was little variation in the mean incubation times of CWD samples originating from different locations or species. However, while most inoculated TgCerPrP(1536) mice developed prion disease around 200 days post-inoculation, occasionally the incubation time of CWD prions approached 300 days. The neuropathological profile of mice TgCerPrP(1536) mice with more rapid incubation times consisted of extensive CerPrPsc deposition in the corpus callosum and frequently the hippocampus with corresponding neuronal vacuolation. In contrast, TgCerPrP(1536) mice with longer incubation times profiles displayed an unsystematic pattern of CerPrPsc deposition and vacuolation with irregular large plaques scattered throughout the brain and no CerPrPsc accumulation in the corpus callosum. Our data suggest the existence of one prevailing CWD strain in the United States and Canada, and raise the possibility of a co-existing second strain in some cases.

P02.43

Preliminary Observations on the Experimental Transmission of Chronic Wasting Disease (CWD) from Elk and White-tailed Deer to Fallow Deer
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Background: Chronic wasting disease (CWD), a prion disease causing transmissible spongiform encephalopathy (TSE), was first identified in captive cervids in Colorado in 1967. CWD has been experimentally transmitted by intracerebral inoculation of brain material from infected brain material into a variety of domestic, wild and laboratory animal species. Among domestic animals, goat, sheep and cattle have been reported to develop experimental prion disease after intracerebral inoculation of brain material from infected brain material into a variety of domestic, wild and laboratory animal species. Although the distribution and host range of chronic wasting disease (CWD) is increasing, whether or not different CWD strains occur in various geographic locations or in different cervid species remains unclear. To address this we used cervidized transgenic mice (TgCerPrP(1536)) to analyze CWD prions isolated from mule deer, white-tailed deer and elk from various geographic locations in North America. Isolates were characterized on the basis of incubation times in TgCerPrP(1536) mice. In all cases serial transmissions were performed in TgCerPrP(1536) mice. The biochemical properties of CerPrPsc produced in the brains of diseased mice were assessed by western blotting and conformational stability assays. We also used histoblot analysis to obtain a global portrait of the neuroanatomical distribution of CerPrPsc in the brains of diseased TgCerPrP(1536) mice in conjunction with immunohistochemical staining and hematoxylin and eosin staining to more fully characterize neuropathology. The glycosylation ratios of CerPrPsc were similar for all samples analyzed with the diglycosylated species of CerPrPsc being chiefly represented. Additionally, the stability of CerPrPsc, assessed by treatment with increasing concentrations of guanidine hydrochloride, demonstrated that the various CWD isolates were associated with similar conformations of CerPrPsc. Consistent with these results, there was little variation in the mean incubation times of CWD samples originating from different locations or species. However, while most inoculated TgCerPrP(1536) mice developed prion disease around 200 days post-inoculation, occasionally the incubation time of CWD prions approached 300 days. The neuropathological profile of mice TgCerPrP(1536) mice with more rapid incubation times consisted of extensive CerPrPsc deposition in the corpus callosum and frequently the hippocampus with corresponding neuronal vacuolation. In contrast, TgCerPrP(1536) mice with longer incubation times profiles displayed an unsystematic pattern of CerPrPsc deposition and vacuolation with irregular large plaques scattered throughout the brain and no CerPrPsc accumulation in the corpus callosum. Our data suggest the existence of one prevailing CWD strain in the United States and Canada, and raise the possibility of a co-existing second strain in some cases.

P02.44

Discovery of New Prion-Like Factor in Saccharomyces Cerevisiae
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A new cytoplasmic genetic element has been found in budding yeast S. cerevisiae. This plurionic factor was revealed as a nonsense suppressor in a haploid yeast strain on a background of an unknown chromosomal mutation and substitution of yeast translation termination factor eRF3 with fusion protein Aß40-SUP35MC (Tsaponina et. al, 2005). The system was originally designed for analysis of human beta-amyloid peptide aggregation and the prion-like factor was proposed to affect the aggregation of Aß40-SUP35MCp. We designated this new factor as [Aß40] which stands for Amyloid Beta Aggregase.

Aß40 is inherited stably through cell generations and demonstrates dominant non-Mendelian inheritance in mice. It is curable by yeast antiprion agent GuHCl and as for all known yeast prions [Aß40] propagation depends on the chaperone Hsp104. [Aß40] like another yeast prion [PIN1] is curable by depletion and inactivation of HIS104 but not its overproduction. However, we have shown that [Aß40] is neither [IP5] nor [PIN1]. Screening of the determinant of [Aß40] is currently underway.

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Here, we show that PrP C is a component of a multimolecular complex within neuronal cells. Furthermore, we investigated the capability of MVs derived from prion-infected N2a neuronal cells are able to shed MVs. Electron microscopy and/or immunoblot analyses showed that the isolated MVs express both PrP C and the axonal membrane protein Gap-43. We further demonstrate that MVs from RML-infected N2a PK1 cells carry PrP Sc, as shown by the detection of Proteinase K-resistant PrP . In vitro infection experiments clearly showed that PrPSc-bearing MVs are capable of transmitting prion infectivity to recipient non-infected cell, which then replicate prions up to 30 passages after challenge.

Discussion: These observations suggest that PrPSc is internalised via the caveolin-mediated pathway, and causes an abnormal disease-related alteration in endoplasmic reticulum structure. In contrast to current dogma, this study shows that sheep scrapie is associated with cytopathology of germinal centres, which we attribute to abnormal antigen complex trapping by FDCs and abnormal endocytic events in TBMs. These sub-cellular alterations differ from those observed in scrapie-infected sheep brains, suggesting that different PrPd / cell membrane interactions occur in different cell types.

Expression of Cellular Prion Protein (PrPC) Affects Survival of Red Blood Cells in Mice

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Microvesicles (MVs) are submicron elements that are shed from the plasma membrane of most eukaryotic cells undergoing activation or apoptosis. The segregation of specific proteins is followed by blebbing of the membrane surface, leading to the formation of MVs and their release in the extracellular environment. The functional role of MVs is still largely unknown. However, a lot of evidence shows that they participate in a variety of intercellular adhesion processes and are able to induce cellular response(s). It has been previously shown that both PrP- and PrP+ MVs are present in cell culture supernatants in a secreted, exosome-associated form and that exosomes bearing PrP+ are infectious both in vitro and in vivo.

The aim of this study was to evaluate the presence of PrP+ in MVs from human plasma and the ability of neuronal cells to release MVs bearing PrP+; hypothesizing a possible role for these structures in the mechanism of PrP+ diffusion and prion neuroinvasion. Furthermore, we investigated the capability of MVs derived from prion-infected N2a PK1 cells in transmitting prion infection in vitro.

Here, we show that PrP+ is a component of a multimolecular complex within microvesicle microdomains in human plasma. Moreover, we demonstrate that neuroblastoma N2a PK1 cells release MVs in cell culture supernatants, indicating that neuronal cells are able to shed MVs. Electron microscopy and/or immunoblot analyses showed that the isolated MVs express both PrP+ and the axonal membrane protein Gap-43. We further demonstrate that MVs from RML-infected N2a PK1 cells carry PrP+, as shown by the detection of Proteinase K-resistant PrP+ in vitro infection experiments clearly showed that PrPSc-bearing MVs are capable of transmitting prion infectivity to recipient non-infected cell, which then replicate prions up to 30 passages after challenge.

Thus, release of microvesicles-associated PrP+ by infected cells, in addition to cell-cell contact, could be considered as an acellular mechanism underlying the spread of prions.

Analysis of Murine CNS Proteomes from PrP0/0, PrP+101L, PrP+G3 and WT Mice

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The prion protein (PrP) is fundamental to TSE disease biology and its conversion from the normal, cellular form (PrPC) to a detergent insoluble, protease resistant isoform (PrPSc) appears to be a pre-requisite for disease progression. Aggregates that accumulate in TSE disease are strongly immunopositive for PrPSc leading to the suggestion that PrPSc aggregation may be responsible for neurodegeneration via a ‘gain of function’ mechanism. However, in some TSE cases, extensive pathology can exist in the absence of detectable levels of PrPSc. The role of PrPSc in neuropathogenesis is therefore unclear and an alternate hypothesis suggests that the loss of PrPC function during disease progression could be responsible for neurodegeneration. We hypothesise that PrPC may function as a neuroprotective molecule and believe that mutations in the PrP+ gene could initiate pathological disease due to impaired functioning of PrPC. The normal biological role of PrP+ is still unclear and mice devoid of PrPC (PrP+0) were developed in order to address this point. These mice show subtle defects in synaptic transmission, mitochondrial function and circadian rhythm and an initial, collaborative, microarray based pilot study of wildtype (WT) versus PrP+0 mice uncovered several intriguing differences between them. Our work intends to confirm and build on this preliminary microarray data and aims to define more specifically the temporal molecular changes in PrP0/0 mice and establish whether mutant PrP+, with a reduced neuroprotective function, can invoke similar changes. Here we present data generated from the comparative proteomic analysis of mouse CNS tissue from WT, PrP+0/0, PrP+101L & PrP+G3 mice at 400 and/or 700 days of age. Soluble mouse brain proteins have been subjected to isoelectric focusing, separated by SDS PAGE and then silver stained. Gel images have been digitised and comparative analyses have been performed using Progenesis SameSpots analysis software. Using this approach we have identified several protein changes occurring in WT and PrP+0/0 brain tissue from 700-day old mice. Furthermore, we have expanded the study and begun to characterise the changes in protein expression that occur at 400days in transgenic mice that posses mutant PrP molecules (P101L and G3) as well as in WT and PrP+0/0 mice. Ultimately, we hope to gain an insight into the influence that PrPC can have in normal cellular function and begin to define a role for PrPC in neuroprotection during ageing.

Expression of Cellular Prion Protein (PrPC) Affects Survival of Red Blood Cells in Mice

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Documented transmissions of vCJD by blood transfusion underline the need of better insights into red blood cell prion biology. One of key unknown remains physiological function of PrPc. PrPc is expressed on CD34+ hematopoietic stem cells and its expression is regulated during blood cell differentiation. Human as well as mouse red blood cells (RBC) express approximately 200-2000 PrPc molecules / cell (Hladova et al., 2000). We analyzed the expression of PrPc in normal and regressing. Scrapie was associated with FDC dendrite hypertrophy and electron microscopy and/or immunoblot analyses showed that PrPSc was present in association with the cell membrane, non-coated pits and vesicles, and also with discrete, large and random endoplasmic reticulum networks, which co-localised with ubiquitin.

Taken together our study demonstrates that physiological role of PrPc expression on RBC may lay in facilitating their longer survival in circulation. (GACR 310/04/S4319, MSMT 0021620806).
The possibility that vCJD may be transmitted by blood transfusion is an important public health issue. The involvement of lymphoreticular tissues in the peripheral pathogenesis of vCJD raises concerns that infectivity may enter the bloodstream in association with recirculating lymphocytes. We have shown that sheep infected with bovine spongiform encephalopathy (BSE) can transmit vCJD in man, as the distribution of prPSc and/or infectivity in lymphoid tissues of sheep orally challenged with bovine clearly resembles that of vCJD patients. We have previously demonstrated that both bovine and natural scrapie infectivity can be transmitted via transfusion of whole blood and buffy coat, taken from both pre-clinical and clinical donors, to date. 3 out of 5 recipients of bovine-infected blood, taken from donors at a clinical time-point, have developed vCJD (approximate transmission rate of 60%). The equivalent transmission rate for recipients of bovine-infected blood taken from pre-clinical animals is approximately 40-45%. We are currently setting up a new project to investigate the distribution of bovine infectivity in whole blood and separated components (plasma, platelets and erythrocytes), and the effectiveness of human leukodepletion filters in removing infectivity. The methods used for collection of blood and separation and filtration of components will follow as closely as possible those routinely employed for human blood by transfusion services, and have initially been developed and tested on uninfected sheep blood in the laboratories of the study. A secondary aim of the project is to develop a bioassay for measurement of titres of infectivity in blood components, using transgenic mouse lines that over-express ovine prP. The aim of these experiments is to determine qualitative and quantitative data on the changes in infectivity in blood and its clinically relevant components with time, as well as assessing the effect of leukodepletion of such products and the potential for secondary transmission by blood transfusion.

Neuronal Loss and Prion Protein (PrP) Deposition in Sporadic Creutzfeldt-Jakob Disease

The mechanisms involved in neurodegeneration are still poorly understood in human prion diseases, although neuronal death is a central component of the neuropathological pattern. Neurone degeneration varies according to the forms of the prion diseases, although neuronal death is a central component of the neuropathological pattern. Neurodegeneration varies according to the forms of the prion diseases, although neuronal death is a central component of the neuropathological pattern. Neurodegeneration varies according to the forms of the prion diseases, although neuronal death is a central component of the neuropathological pattern. Neurodegeneration varies according to the forms of the prion diseases, although neuronal death is a central component of the neuropathological pattern.

Scrapie in ’plt’ Mice : Role of Dendritic Cell Migration in Prion Pathogenesis

The involvement of lymphoreticular tissues in the peripheral pathogenesis of vCJD raises concerns that infectivity may enter the bloodstream in association with recirculating lymphocytes. We have shown that sheep infected with bovine spongiform encephalopathy (BSE) can transmit vCJD in man, as the distribution of prPSc and/or infectivity in lymphoid tissues of sheep orally challenged with bovine clearly resembles that of vCJD patients. We have previously demonstrated that both bovine and natural scrapie infectivity can be transmitted via transfusion of whole blood and buffy coat, taken from both pre-clinical and clinical donors, to date. 3 out of 5 recipients of bovine-infected blood, taken from donors at a clinical time-point, have developed vCJD (approximate transmission rate of 60%). The equivalent transmission rate for recipients of bovine-infected blood taken from pre-clinical animals is approximately 40-45%. We are currently setting up a new project to investigate the distribution of bovine infectivity in whole blood and separated components (plasma, platelets and erythrocytes), and the effectiveness of human leukodepletion filters in removing infectivity. The methods used for collection of blood and separation and filtration of components will follow as closely as possible those routinely employed for human blood by transfusion services, and have initially been developed and tested on uninfected sheep blood in the laboratories of the study. A secondary aim of the project is to develop a bioassay for measurement of titres of infectivity in blood components, using transgenic mouse lines that over-express ovine prP. The aim of these experiments is to determine qualitative and quantitative data on the changes in infectivity in blood and its clinically relevant components with time, as well as assessing the effect of leukodepletion of such products and the potential for secondary transmission by blood transfusion.

Scrapie in ’plt’ Mice : Role of Dendritic Cell Migration in Prion Pathogenesis

We conclude that peripheral spreading of prions appears poorly dependent on cell migration, at least in the mouse model, and that dendritic cells are not involved in the peripheral spreading of scrapie in the mouse model.
The cellular prion protein (PrP) is a membrane-bound glycoprotein abundantly expressed in neurons and glial cells within the CNS. The scrapie prion protein (PrPSc) is a conformationally-altered isoform of PrP that is responsible for prion diseases, termed transmissible spongiform encephalopathies (TSE), a group of neurodegenerative diseases that affect a wide variety of mammal species, including humans. The presence of the cellular isoform of PrP is necessary for the establishment and further evolution of prion diseases and the physiological conditions where PrP is present seem to modulate the alterations in TSE brains.

In this work, we have examined the presence of PrP in GABAergic, glutamatergic, nitricergic, cholinergic, serotoninergic and orexinergic cells within the rat brain.

Our observations show that PrP is widely expressed in neurons that contain markers for inhibitory populations of cells throughout the rat brain. Within the cerebral cortex, the PrP-containing cells gives a rationale basis for the interpretation of the histopathological alterations in TSE and might help analyze some pathogenic process.

The coexistence of PrP with other non-GABAergic neurotransmitters in several cell types is in agreement with the imbalances reported in several forms of animal and human TSE.

Taken together, our data demonstrate that the investigation of the chemical nature of the PrP-sc-containing cells gives a rationale basis for the interpretation of the histopathological alterations in TSE and might help analyze some pathogenic mechanisms of PrPSc.
Potential of Cell Substrates used for Production of Biologicals to Propagate Transmissible Spongiform Encephalopathy (TSE) Agents
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1OBRR, CBER, Food and Drug Administration, Lab Bacterial Parasitic Unconventional Agents, USA; 2American Red Cross, J Holland Laboratory, USA

Background: TSE agents have contaminated a variety of products, including human-tissue-derived therapeutics and animal vaccines. Many biologicals are prepared in cell cultures. Although most cell cultures studied have resisted infection with TSE agents, a few have been successfully infected. Susceptibility of cultured cells to infection with TSE agents cannot be predicted from species or tissues of origin or the level of expression of prion protein (PrP).

Aims: We are investigating the susceptibility of several cell lines used or proposed for manufacture of various biologic products to propagate TSE agents.

Methods: We inoculated bacteria-free filtrates of three reference TSE agent inocula-brain suspensions containing the agents of BSE, variant Creutzfeldt-Jakob disease (vCJD) and sporadic CJD into several cell lines important or potentially important in the manufacture of biologic products.

Results: Cell lines studied to date include, Vero (green monkey), CHO (Chinese hamster), MDCX (canine), Rabli (rabbit), HEK 293 (human), and WI-38 (human diploid). We also studied lines of human-neuroblastoma-derived cells (SH-SY5Y), including lines engineered to overexpress mutations associated with familial TSEs. Cell-exposed to TSE agents were serially propagated for 30 passages and selected passages tested for appearance of TSE-associated PH (PrP^TSE) and for persistence of infectivity by intracerebral inoculation into TSE-susceptible transgenic mice and squirrel monkeys (BSE-exposed cells only). Normal cellular PrPc was demonstrated in all cell lines tested (except MDCX). No PrP^TSE was found in any exposed cells. Known susceptible cells exposed to a human TSE agent as positive control accumulated detectable PrP^TSE. No exposed cell line tested has transmitted TSE to mice or monkeys after more than a year of observation.

Conclusion: We have not yet found evidence that any candidate cell substrate exposed to three TSE agents, including BSE, has sustained or propagated infectivity. Methods with increased sensitivity for detecting PrP^TSE in cultures are under development, and additional bioassays in susceptible animals are planned.

Increased Citrullinated Proteins in The Brain of Scrapie-Infected Mice
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Prion diseases are neurodegenerative disorders by a pathogenic isoform of the prion protein (PrP^Sc). Although the key event in the pathology of prion diseases is thought to be the conversion of cellular prion protein (PrP^C) to the protease-resistant pathogenic form (PrP^Sc), factors that contribute to neurodegeneration in scrapie-infected animals are poorly understood. It can be suggested that there are possibilities of structural differences or differential post-translational modification between PrP^C and PrP^Sc. Citrullination is a post-translational modification process that arginine residue in protein convert to citrulline in a calcium(Ca^2+)-dependent condition by peptidylarginine deiminases (PADs). PAD2 is widely expressed type in various tissues and a unique expressed type in brain. It has been reported that the increased citrullinated proteins were observed in degenerative disorders. To elucidate the involvement of protein citrullination in prion diseases, we examined whether citrullinated proteins are produced in the brains of scrapie-infected mice. PAD2 immunoreactivity and its expression levels of both mRNA and protein were significantly increased in scrapie-infected brains and PAD2 was mainly detected in reactive astrocytes. Supporting this data, the activity of PAD2 in scrapie-infected brains was significantly increased compared to control brains. We have also found that citrullinated proteins of varied molecular weights were detected in both control and scrapie-infected brains and that the level of citrullinated proteins increased in the brains of scrapie-infected mice. This study suggests that PAD2 has an important role in the pathogenesis of prion diseases and that the citrullinated proteins may be involved in the pathogenesis of prion diseases.
Background: Immuno-therapeutic approaches in TSE face several pitfalls, most of which relate to a strong natural tolerance of the adaptive immune system. Experiments in the mouse revealed that peripheral T cells directed to identified MHC class II epitopes exist but are anergic, and that the B-cell primary repertoire is hardly permissive for efficient antibody responses to PrPc epitopes. Breaking CD4+ T-cell tolerance to PrP proves possible, but uncontrolled effector responses may induce deleterious autoimmune reactions.

Aim(s)/Objective(s): A promising alternative strategy is to design immunogens that could evoke humoral responses directed to cryptic PrP epitopes only accessible on PrPsc isoforms, by forcing B-cell cooperation with helper T-cells directed to exogenous (non-PrP) peptides.

Methods: Analyses on two distinct proposed PrPsc structural models termed ‘beta-helix’ (Govaerts et al., 2004) and ‘spiral’ (DeMarco et al., 2006) allowed us to identify epitopes buried in physiological PrPc and likely to be specifically exposed on scrapie-associated isoforms. Chimeric peptides coupling the potential PrPsc B-cell epitopes with MHC II (Ia-b) exogenous epitopes were designed using ab initio simulations (Derreumaux, 2000) with the requirement that the B-cell epitopes remain accessible to solvent. Specificity and intensity of antibody responses in immunised mice were analysed by ELISA and cytotoxicity methods.

Results: We will present results from a series of vaccine experiments in 139A infected C57BL/6 mice with different combinations of PrP peptide segments, helper T-cell epitopes and adjuvants. Two overlapping 8-mer and 9-mer peptides located between helices H1 and H2 of the PrPc structure were conjugated to two different exogenous Ia-b restricted epitopes.

Conclusion: In comparison with published trials, this structure-based, in silico strategy proves particularly efficient, even when applied more than 10 weeks after scrapie inoculation to mice. Conclusions will be proposed on how to optimise active immunotherapeutic treatments using conformational PrPsc epitopes.
P03.21
Pattern of Sheep Scrapie Spreading into the Brain
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It has been shown that an oral infection of hamsters with scrapie (263K) is followed by the spread of the infectious agent from the gastrointestinal tract along the splanchic nerves to the midthoracic spinal cord and along the vagus nerves to the obex region (McBride et al., J Viral 2001). The findings in highly scrapie susceptible sheep, raised at premises where scrapie had been occurring, are remarkably similar and indicate that classical scrapie might be transferred orally as well (van Keulen et al., Arch Viral Suppl 2000). The sequential investigation of the brains of orally infected hamsters shows further that PrPSc is spread from the dorsal motor nucleus of the vagus to thalamic nuclei and the frontal cortex (Schulz-Schaeffer et al., JIV, Int Conf Neuropathol 2000). In BSE and natural scrapie the dorsal motor nucleus of the vagus and the solitary tract nuclei are the first affected structures of the brain, too (Schulz-Schaeffer et al., Am J Pathol 2000; van Keulen et al., Arch Viral Suppl 2000).

However, in this study we analyse the pattern of PrPSc distribution in brains of naturally infected sheep (field cases) from the point where the brain stem is reached. For that purpose we use the PET blot technique (Schulz-Schaeffer et al., Arch Viral Suppl 2000) which allows a sensitive topographical detection of PrPSc and thereby a specific identification of the affected structures. As the animals have been culled in the course of scrapie eradication when a flock proved to have scrapie, different stages of infection are present and make it possible to view the spread of PrPSc in the brain. Our findings support the idea of an oral infection with classical scrapie between the individuals of a flock.

P03.22
Unaltered Neurodegeneration in Prion-infected Neurografts Devoid of BCL-2 or BAX
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Prion diseases or transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative disorders such as, Creutzfeldt-Jacob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and scrapie in sheep. It has been postulated that the disease-associated prion protein (PrPSc) is the infectious agent, which replicates by conversion of the cellular prion protein (PrPC) into a likeness of itself. Accumulation of PrPSc and neuropathological changes, such as spongiosis, gliosis and neuronal death are typical hallmarks of prion diseases, but the mechanisms how prions proceed to damage neurons are still unknown. Prion-induced neuronal cell death appears to be associated with apoptosis. Expression levels of the apoptotic proteins like BAX and BCL-2 are altered in hippocampal neurons of prion-infected hamsters. Recently, an anti-apoptotic function against BAX-induced apoptosis has been postulated for PrPSc in cultured cells and binding of PrPSc to the carboxy-terminal region of BCL-2 has been shown in a yeast two-hybrid system. To further characterize the role of BAX and BCL-2 in prion-induced neurodegeneration, we transplanted either BAX- or BCL-2-deficient neuroectodermal tissue into the brain of PrP null recipient mice. After long-term exposure to mouse RML prions, typical histopathological features of the disease, such as gliosis, spongiosis and PrPSc accumulation were found strictly restricted to the grafted tissues. Moreover, apoptosis was detectable in the brain grafts by staining for activated caspase-3 and TUNEL. Our results indicate that BAX and BCL-2 are not directly involved in the mechanisms leading to the neurodegeneration in prion diseases.

P03.23
Investigating the Effect of Conditional Expression of PrP Protein in the Intestine upon TSE Pathogenesis
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The host PrP protein is essential for the establishment of transmissible spongiform encephalopathy (TSE) diseases. Although PrP is expressed at high levels in the central nervous system, it is also expressed in a wide variety of peripheral cells. Oral injection of TSE infected material is thought to be the most common route in most natural forms of TSE disease; however, the details of how ‘infectivity’ crosses the gut epithelial barrier and enters the nervous system following oral exposure are not fully understood. Therefore the aims of this project are to investigate the requirement of PrP expression in intestinal epithelial cells and enteric neurones in oral infection. To achieve this we have used our conditional transgenic mouse lines (generated using Cre-LoxP techniques) that possess floxed PrP genes. By crossing these animals with transgenic mice possessing inducible intestinal epithelial cell specific or enteric neurone specific Cre recombinase we are able to generate mouse models with restricted PrP expression in these cell types. We have shown the induction of Cre recombinase activity in the intestinal epithelial cell mouse model to produce excellent recombination in the villi of the intestine. Studies are now underway to infect these animals orally with TSE agents to determine the effect of removal of PrP expression from these cells on TSE disease.

P03.24
In Vivo Identification of Functional Sub-Domains within Central Domain of the Cellular Prion Protein
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The physiological function of PrP in PrP disease remains elusive. Mice devoid of PrP develop normally but are resistant to scrapie; introduction of a PrP transgene restores susceptibility to the disease. Recently we were able to demonstrate that expression of PrP<sup>Cav1</sup>, a PrP variant lacking 40 central residues (94-134), induces a rapidly progressive, lethal phenotype with extensive central and peripheral myelin degeneration (Baumann et al. EMBO 2007). This phenotype could be dose-dependently rescued by coexpression of full length PrP. Here we extend our previous study by assessing the impact of the subdomains on the phenotype creating two novel transgenic mouse models - PrP<sup>Cav2</sup>, a PrP variant lacking 40 central residues (94-134) and PrP<sup>Cav3</sup>, a PrP variant lacking hydrophobic core residues 111-134. Like PrP<sup>Cav1</sup>, PrP<sup>Cav3</sup> expression causes a progressive, lethal phenotype with similar severe alterations in central and peripheral white matter, while PrP<sup>Cav2</sup> does not seem to provoke any obvious phenotype. PrP<sup>Cav1</sup> does not show any alteration in the post translational processing while both PrP<sup>Cav2</sup> and PrP<sup>Cav3</sup> prevent the formation of the carboxyterminal fragment C1. We therefore propose that the absence of a neuroprotective activity associated with C1 formation may be responsible for the observed phenotypes.
P03.25
Does the Erythroid Lineage have a Role in Prion Disease?
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Background: For many prion diseases there is an obligate expansion and dissemination of infectious material within the lymphoreticular system and particularly within the spleen. Although it is well established that blood can carry infectivity, there is confusion over which of the haematopoietic lineages can potentially harbour, transport and propagate the infectious agent. Our previous studies, identified a dramatic TSE-associated decrease in the expression of an erythroid-specific transcript, termed erythroid-associated factor (ERAF). This finding suggested a previously unrecognized role for cells of the erythroid lineage in the peripheral pathogenesis of TSEs.

Aims: We have undertaken both transcriptomic and proteomic approaches to further investigate the involvement of haematopoietic tissues in TSE diseases.

Methods: We have extended our original findings on the effect of TSEs on the erythroid lineage. Firstly, we measured the expression of a number of erythroid-specific transcripts in haematopoietic tissues of Control and Infected animals to determine whether ERAF was the only erythroid transcript affected. As a second approach, we compared protein expression patterns between control and infected samples. This approach has enabled us to explore the profiles of over 3000 proteins found in haematopoietic tissues.

Results: We report that the dramatic down regulation of several functionally-diverse erythroid genes is a common feature of all scrapie-infected mice studied. In contrast, expression of the same genes is largely unaltered in five virus models, with parallels to TSE pathogenesis. In addition we have identified a further 25 proteins from haematopoietic tissue which show differential expression as a result of infection.

The simplest explanation for this overall loss in erythroid expression is due to the loss of a cell type and flow cytometry has identified a precursor erythroid lineage that is decreased during prion infection.

Conclusion: Our data suggests that a number of different cellular processes are affected in the blood of infected animals. These findings suggest that a degree of "global down regulation" of erythroid gene expression is a consistent feature of murine scrapie. This loss of erythroid function is accompanied by a decrease in an erythroid precursor cell population. A prion disease-associated profile of erythroid gene/protein expression may prove a useful indicator for disease in peripheral tissue.

P03.26
Studies on Pathogenesis of Spanish Goat Scrapie Analyzing PRPSC Distribution by Immunohistochemistry
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Scrapie is a transmissible spongiform encephalopathy (TSE) that causes a neurodegenerative disorder in sheep and goats. Either in both species, an abnormal form of the prion protein (PrPsc) accumulates in nervous and lymphoid tissues during the pathogenesis of the disease. In addition, it has been described the accumulation of PrPsc in other barrier limiting tissues as kidney or endocrine functional organs as adrenal gland. In this study, histopathological and immunohistochemical analysis has been performed in different target tissues (central and peripheral nervous systems) and several organs well known as target for replication (lymphoreticular system) or not well determined their importance on goat scrapie pathogenesis (heart, skin, kidney, urinary bladder, lung, liver, alimentary tract, pancreas, mammary gland, muscle and endocrine tissues) of three natural scrapie affected goats. In most of the animals, PrPsc can be detected in the ileal Peyer path and mesenteric lymph nodes. Later on, it can be detected in gut-associated and peripheral lymphoid tissue. The results seem to show that PrPsc propagation and distribution pass through lymphoreticular system followed by axonal transport detecting PrPsc in peripheral and central nervous system. As a last stage, PrPsc can be detected as perivascular deposits in the central nervous system, suggesting a possible haematogenous dissemination of the disease. Other localizations were reticular and fasicular zones of the adrenal gland, suggesting either haematogenous or axonal propagation of PrPsc. We have also sequence the complete open reading frame of the PRNP gene, founding polymorphisms in 18, 138, 154 and 211 codons.

P03.27
Age of Onset and Death in Inherited Prion Diseases are Highly Heritable
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The common polymorphism at codon 129 of the prion protein gene (PRNP) is known to affect prion disease susceptibility and phenotype. Mouse quantitative trait loci (QTL) studies support the existence of multiple modifiers of incubation time unlinked to Prnp but evidence to support the existence of human prion disease modifiers has been lacking. We present the correlation of age at onset or death, expressed as a composite Z score, between first degree relatives in multiple large UK inherited prion disease families. The common polymorphism at codon 129 of the prion protein gene (PRNP) is known to affect prion disease susceptibility and phenotype.

The simplest explanation for this overall loss in erythroid expression is due to the loss of a cell type and flow cytometry has identified a precursor erythroid lineage that is decreased during prion infection.

Conclusion: Our data suggests that a number of different cellular processes are affected in the blood of infected animals. These findings suggest that a degree of "global down regulation" of erythroid gene expression is a consistent feature of murine scrapie. This loss of erythroid function is accompanied by a decrease in an erythroid precursor cell population. A prion disease-associated profile of erythroid gene/protein expression may prove a useful indicator for disease in peripheral tissue.

P03.28
Consequences of Copper and Manganese Imbalances on the Scrapie Progress
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Environmental exposure to some metals is thought to explain the occurrence of sporadic Transmissible Spongiform Encephalopathy (TSEs); however, the published results are not conclusive. The present study is focused on the assessment of the effect of copper depletion and/or manganese enhancement in diet on susceptibility to Scrapie as well as on its progress. A total of 118 prion protein deficient (PPrP0/0) and transgenic (Tg20) mice were divided into different groups according to metal altered diet. Animals were inoculated by intraperitoneal injection, also including matched controls in all groups. In addition to the incubation period, the severity of neuropathological changes was assessed in all animals. Infection was detected in 100% of Tg20 mice whereas no PPrP0/0 was observed in any PPrP0/0. Spongiosis in all of those receiving a copper depleted diet was the highest, but it was also found in non-infected animals. These observations suggest this diet as the main responsible for this lesion and for its worsening in the inoculated animals. The highest intensities of glial fibrillary acidic protein (GFAP) immunostaining were also associated with the copper depleted diet. Dietary manganese supplementation was demonstrated to have a negative effect on neuronal counts. In conclusion, this study demonstrates that certain environmental factors could aggravate the neurological Scrapie lesions, as reported with regard to other neurodegenerative diseases where some metalloenzymes plays a pivotal protector role against the oxidative stress.
Transmissible spongiform encephalopathies (TSEs) are infectious neurodegenerative diseases in which the causative agent is thought to be PrPSc, an aberrant isoform of the normal cellular prion protein, PrPC. While TSEs have been studied extensively in mammals, very little is known about TSE pathogenesis in fish. As fish farming is an important industry that provides high protein nutrition for humans, both the prospect of a prion disease developing in fish and the possibility of farmed fish being contaminated with infectious mammalian PrPSc are of major concern.

We have undertaken a study with sea bass to evaluate the possibility of transmission of TSEs to fish. Two groups of sea bass were force fed with scrapie-infected sheep or BSE-infected bovine brain homogenates, while similar control populations were fed with normal brain homogenates. Following this challenge the inoculated fish were studied for clinical and behavioral signs of disease on a daily basis. At regular times during the post-inoculation period fish from each challenge group were sacrificed and their tissues subjected to histopathological examination, immunohistochemical detection of residual mammalian PrP and western blot analysis for detection of both mammalian and fish PrPs. The results from the first three years of this study show no indication that prion disease has been transmitted to sea bass. To demonstrate this more rigorously, a small group of seabass were rechallenged with scrapie brain homogenate after 3 years and monitored for a further 6 months. As well, we have used a cell culture system to evaluate the potential for in vitro conversion of recombinant fish PrP molecules to protease-K resistant isoforms in scrapie-infected N2a cells.

We concluded that full length PrP is an essential component of the nervous system and can even partially substitute PrPC function. These results indicate an essential role of the hydrophobic core domain of PrP for the normal physiological function of PrP.

The possibility that young adults were exposed to greater levels of BSE by dietary preference has not been substantiated, suggesting that factors related to age, e.g. immunosenescence, may influence susceptibility to infection. Indeed, epidemiological evidence suggests that most cattle were infected with BSE as calves and studies of natural sheep scrapie suggest that lambs may be more susceptible to infection than adults. The capacity of the immune system to mediate immune responses to pathogens declines with age. A number of studies have shown that this decline in immune function is related to alterations in lymphocyte and follicular dendritic cell (FDC) function. As FDCs are critically involved in the pathogenesis of many TSEs age related effects on immune function may influence pathogenesis.

To examine age related influences on TSE infection we aged mice to approximately two thirds of their natural lifespan (600 days) and injected them peripherally (orally or intraperitoneally), or intracerebrally with the ME7 strain of scrapie. Studies in mice have shown that FDC function is impaired at this age. The intracerebrally injected aged mice developed both clinical and pathological signs of scrapie. None of the peripherally challenged aged mice developed clinical disease; however vascular degeneration in brain was detected in 33% of intraperitoneally and 42% of orally challenged mice. In addition, infectivity levels in lymphoid tissues from aged mice were substantially lower than those of the young mice. We suggest that this impaired pathogenesis coincides with reduced immune function and provides evidence that immunosenescence may influence TSE pathogenesis.
**P03.33**

**Immunohistochecmical Features of an H-Type Case of Atypical Bovine Spongiform Encephalopathy in Sweden**

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Until the date (May 2007), a single case of bovine spongiform encephalopathy (BSE) has been detected in Sweden. It was characterized in tissue homogenates as an unusual BSE case of the H-type based on the molecular features of the protease partially resistant core of the prion protein (PrPSc). These included higher molecular weight of the PrPSc bands, affinity to the N-terminus specific monoclonal antibodies (mAbs) 12B2 and P4, and peculiar banding pattern with mAb SAF 84 showing an additional band between 12.14 kDa. The immunohistochecmical features were studied on sections of medulla applying mAbs with coverages a broad range of the PrP sequence. The following antibodies were applied: F99/160.1, F99/97.6.1, P4, 12F10, 12B2, 94B4 and R145. All the mAbs resulted in immunostaining but the intensity of different disease associated PrP (PrPd) types varied with the different antibodies. The most frequent PrPd type was thin punctate deposition in the neuropil, sometimes coalescing into coarser granules. This affected the complete grey matter and was most intensive in the lateral aspects of the nucleus of the spinal tract of the trigeminal nerve, followed by the solitary tract nucleus. Perineuronal, and to lesser extent linear, PrPd deposition was also observed. The mAbs F99 and R145 produced the most severe intraneuronal and intramicroglial PrPd depositions, which were also observed, though at a lower level, with mAb 94B4. Accumulation of intraneuronal granules of PrPd was more evident in the large neurons of the reticular formation and of the cuneatus and olivary nuclei. This study describes the epitope recognition of the PrPd in the Swedish H-type case and provides some information of its cellular localization and distribution in the medulla. The authors acknowledge Linda Terry, Veterinary Laboratory Agency, UK, for providing the mAb R145.

**P03.35**

**The Cellular Prion Protein Prevent Autophagic Cell Death in Neuronal Cells**

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The cellular prion protein (PrPc) is abundantly expressed in neurons of the central nervous system. One of the known functions of the PrPc is anti-apoptotic roles preventing neuronal cell death. It has been reported that autophagy is activated during amino-acid or serum deprivation and various pathological situations. Autophagy is an intracellular bulk degradation system, which delivers cytoplasmic components to the lysosome/vacuole. To investigate whether PrPc is involved in an autophagic cell death pathway, we compared expression patterns of microtubule-associated protein 1 light chain-3 (LC3), an autophagy marker, in Prnp-deficient (Prnp-/-) neuronal cells to those with wild-type (WT) neuronal cells. The expression level of LC3-II, an autophagosomal membrane associated form, was increased in the Prnp-/- neuronal cells compared to WT neuronal cells under serum deprivation conditions, but not under normal growth medium conditions. In addition, under serum deprivation conditions, LC3-positive punctuated structures were co-localized with lysosomal membrane glycoprotein-2 (LAMP-2), indicating the presence of autophagolysosomes in Prnp-/- neuronal cells. These co-localizations were not observed from WT neuronal cells. Our data suggest that PrPc may play a pivotal role in preventing the autophagic cell death pathway in neuronal cells.

**P03.34**

**Detection of PrPSc in Plasma from Scrapie Sheep in the Preclinical Stages Using a Multimer Detection System-3D**

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MDS-3D was used to test 10 plasma samples from scrapie infected preclinical sheep in comparison with the normal controls. These sheep showed no symptoms and were naturally infected. The sheep had the most susceptible genotype of VRQ/VRQ. We used MDS-3D as previously reported, which is based on the multimeric expression of epitopes on aggregated disease-associated prion protein, in contrast to the cellular form that is monomer. These characteristics enable the MDS-3D to distinguish infectious samples of scrapie sheep in preclinical stage vs normal sheep. The sensitivity and rapidness of the MDS-3D system demonstrated that this assay could be used in an automated screening system for monitoring the human samples to ensure the safety of blood and blood products.

**P03.36**

**PrP Immunohistochemistry in Human Prion Diseases: from Antibody Screening to a Standardized fast Immunodiagnosis using Automation**

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Demonstration of pathological prion protein accumulation in the central nervous system is required to establish the diagnosis of transmissible spongiform encephalopathies (TSEs). In humans, this is frequently achieved using prion protein (PrP) immunohistochemistry in paraffin-embedded tissue, a technique that requires multiple epitope retrieval and denaturing pre-treatments. In addition to be time-consuming, this procedure induces tissue alterations that preclude accurate morphological examination. The aim of this study was to simplify the procedure of PrP immunohistochemistry in human tissue, together with increased sensitivity and specificity. We screened a panel of 50 monoclonal antibodies produced using various immunogens (human and ovine recombinant PrP, PrP peptides, denatured scrapie-associated fibrils from 263K-infected Syrian hamsters) and directed against different epitopes along the human PrP sequence. A panel of different forms of genetic, infectious and sporadic TSEs was assessed. The most efficient antibodies were then used in different simplified procedures and checked for their efficiency at 37°C. We identified a monoclonal antibody allowing a high specific and fast immunodiagnosis with very limited denaturing pre-treatments. A standardized and reliable fast immunostaining procedure was established using an automated diagnostic system (Lexes, Ventana Medical Systems) and allowed PrP detection in the central nervous system and in tonsil biopsies. It was evaluated in a series of 300 patients with a suspected diagnosis of TSEs and showed high sensitivity and specificity.
**P03.37**

Evaluation of Neuroinflammation Mechanisms in Co-cultures of Neurons, Astrocytes and Microglia from Newborn Hamsters

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Inflammatory events mediated by activation of microglia and astrocytes participate to the neuronal death occurring in TSE. Cell activation is accompanied by morphological changes, proliferation and release of neurotoxic mediators. Understanding microglia-astrocyte interactions and the mechanisms involved in the regulation of microglia activation and neurotoxicity are important steps toward the identification of treatments for neurodegenerative diseases. The hamster model is commonly used for in vivo studies of TSE pathogenesis, but these studies lack of direct evidence of the functional properties exerted by activated microglia. We developed an astrocyte-microglia co-culture system from neonatal hamster brains to study the molecular events related to glial activation. Moreover, we studied microglial induced apoptosis on hamster neurons in two different co-culture systems: microglia plated over neurons and microglia plated over a membrane well insert in the culture chamber of neurons, allowing medium exchange without physical contact of the two cell types. Astrocyte-microglia and neuron-microglia co-cultures were exposed to the neurotoxic, fibrillogenic HuPrP 106-126 peptide, using LPS and H2O2 as positive control and scrambled HuPrP 106-126 as negative control of glial activation. The presence of microglia in both the culture systems is necessary to stimulate, immediately after peptide inoculation, the production of mediators of oxidative stress (NO, ROS) and the mRNA expression of pro-inflammatory cytokines (IL-1beta and TNF-alfa by real-time RT PCR). These neurotoxic molecules are responsible for neuronal death even in the absence of a physical contact between neurons and microglia. Conversely to the rapid and high onset of IL-1beta and TNF-alfa, IL-10 expression is very low. IL-10 is a potent inhibitory factor in the CNS cytokine network involved in decreasing the expression of cytokine receptors as well as pro-inflammatory cytokine production by microglia.

**P03.38**

Genetic Susceptibility of Sheep to Experimental BSE Transmission

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Susceptibility to Transmissible Spongiform Encephalopathy has been shown to be genetically controlled by the PRNP gene in small ruminants, mouse and human. A genetic selection for the resistant AR136R154R171 allele is underway in sheep at a European scale. The influence of the PRNP gene on BSE transmission in sheep is strategic for the control of this disease. Experimental BSE inoculations were performed by intracerebral and oral routes in susceptible and resistant New Zealand TSE free sheep at various ages. Possibilities of horizontal and vertical transmission were tested using sentinel or pregnant ewes. Animals were sacrificed in kinetics or at time of clinical disease and their tissues tested by immunohistochemistry, immunohistochemical assays and inoculation into Rii1 and Tggbv mice.

The incubation period of ARR/ARR sheep inoculated intracerebrally was much longer (7/10 positives, 1383 days +/- 63) that the one of ARQ/ARQ sheep (9/9 positives, 477 days +/- 33). After oral inoculation at birth, PrPres deposits were detected from 4 months of age in lymphoid and nervous tissues of ARQ/ARQ lambs. Their incubation period was 19 months whereas sheep carrying the ARR allele are still alive at 46 months. However, one 10 months old ARR/ARR sheep had PrPres deposits and carried infectivity in its spleen. Two sentinel ARQ/ARQ ewes contracted the disease with an incubation period of 23 months evidencing the possibility of horizontal transmission of BSE in sheep flocks. Lymphoid and nervous PrPSc positive tissues transmitted the BSE strain in mice with minor modifications of their western blot and lesion profiles.

The results evidence the global stability of the BSE strain after two passages in sheep, its short incubation period in this species even after oral transmission and the relative resistance to TSEs of sheep carrying the ARR allele with the limitation of their possible carrier status.

**P03.39**

Prions in Peripheral Nervous System Tissues in Bovine Spongiform Encephalopathy (BSE)

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It has been shown that the abnormal isoform of prion protein (PrPSc) is not limited to the specified risk materials in bovine spongiform encephalopathy (BSE). We investigated PrPSc distribution by a highly sensitive western blotting (WB) in tissues from naturally and experimentally BSE-infected cattle. In natural BSE, confirmed by PrPSc detection at the medulla - obex - ox, PrPSc was detected in autonomic and somatic components of the peripheral nerve system (PNS), including certain cranial nerves. To determine whether parts of the PNS, other than those implicated directly in the hypothetical pathogenetic spread of BSE prions from the intestine to the central nervous system (CNS), become involved before or after the CNS is affected, we investigated PrPSc distribution by WB in dorsal root ganglia, stellate ganglion, phrenic, radial, and sciatic nerves and adrenal, relative to detection of PrPSc in CNS of cattle orally inoculated with BSE affected brain and culled sequentially. In experimental BSE-infected cattle, PrPSc was first detected in dorsal root ganglia concurrent with PrPSc in the CNS and only subsequently in the other tissues tested. PrPSc was confirmed in the adrenals of cattle that showed clinical signs. No PrPSc was detected in the PNS of BSE-negative cattle. This study shows that, with respect to dorsal root ganglia, a paravertebral sympathetic ganglion and the somatic nerves examined, PrPSc is detected inconsistently in the PNS during the disease course, coincident with, or after it accumulates in the CNS and is potentially widespread in PNS in natural cases at the time of diagnosis.

**P03.40**

Distribution of PrPCWD in the ObeX and Cranial Lymphoid Tissues of Chronic Wasting Disease Affected Elk(Cervus Elaphus Nelsoni) in Republic of Korea

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Chronic wasting disease has been recognized as an important prion disease in native North America deer and Rocky mountain elk. Chronic wasting disease was first detected in imported elk from Canada in 2001 in Republic of Korea. More cases were also found in elk which were reared in 2004 and 2005. Epidemiologically related 268 elk were subjected to diagnosis on Chronic wasting disease after the detection of index case in 2004. Medulla oblongata, at the level of oxeb, patatile tonsil and medalis retropharyngeal lymph node from them were examined using immunohistochemistry with a cocktail of two monoclonal antibodies (F9/98/160.1.5+fF9/97.6.1). Disease-specific prion protein (PrPScw) was detected in 12 of them. Clinical signs were not observed in any of these elk. On the basis of the neuroanatomy of the medulla oblongata, at the level of oxeb, deposits of PrPScw were observed mainly at the parasympathetic region of the dorsal motor nucleus of the vagus nerve(DMNW) which is the target area for the confirmatory diagnosis and the nucleus of the solitary tract. The lymphoid tissues were confirmed as positive if any of the lymphoid follicles was immuno-labelled. This results supports that the dorsal motor nucleus of the vagus nerve(DMNW) is the most useful target area for the confirmatory diagnosis.
**P03.41**

Amino Acid Polymorphisms of PrP Gene in Korean Native Goats (Capra Aegagrus Hircus)

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Scrapie is a fatal neurodegenerative disease of sheep and/or goats which belongs to transmissible spongiform encephalopathies. Scrapie has never been reported in Republic of Korea. Polymorphism in the amino acid sequence of prion protein (PrP) plays a significant role in susceptibility or resistance to scrapie following exposure. In Republic of Korea, 522,534 goats (mostly Korean native goats) and 1,202 sheep were reared according to the Ministry of Agriculture and Forestry data in 2005. Unlike most European countries, goats are more important small ruminants in Republic of Korea. In goats, PrP amino acid dimorphism at codon 142, 143, and 240 has been described. Only dimorphism at codon 142 was associated with an incubation period. However, another PrP variants containing only three instead of the usual five octapeptide repeats, with additional Tryptophan to Glycine substitution in codon 102 may also be associated with an increased incubation period of scrapie in goats. In this study we investigated the PrP polymorphisms in Korean native goats. This study has examined the PrP genotypes of 60 goats raised in Namwon branch station of National Livestock Research institute (NLRI). The genomic DNA was isolated from goat whole blood and the PrP gene including the whole open reading frame (ORF) 794bp was amplified using PCR. Generated PCR fragments of goat PrP gene was sequenced using an automated DNA sequencing device (model 377 DNA sequencing). We found the well-known polymorphism at codon 102, 143, 240 and the existence of non-silent polymorphism at codon 42, 138 in Korean native black goats. 15% of tested all goats had Arginine/Arginine at codon 143. It need further research to know whether these novel polymorphisms affect susceptibility to scrapie.

**P03.42**

Copper Binding and Redox Activity of the Infectious Isoform of the Prion Protein Monitored by Fluorescence and EPR Spectroscopy

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The structure of the infectious agent responsible for prion diseases has not been fully characterized, but evidence points to a β-rich conformer of the host-encoded prion protein. It undergoes a structural transition that has been associated with a gain of toxic function, inducing neuronal oxidative stress via production of free radicals. In particular, tyrosyl radical formation has been suggested to play a part in the redox cycle that leads to hydrogen peroxide production of copper-binding PrPβ [1]. Such radicals are usually only transiently stable and rapidly decay. The technique of spin trapping involves the formation of radical adducts which are more stable than the primary free radical and the identity of the trapped radical can be characterized by EPR spectroscopy. Our preliminary EPR spin trapping studies suggested the generation of carbon-centered radicals in full-length and C-terminal fragments of the α and β isoforms of recombinant moPrP. We have further used fluorogenic spin traps, whose fluorescence is altered upon trapping a radical, to enable a large number of mutant constructs to be analysed under a range of pH, buffer and copper loading conditions in a high-throughput assay. We have examined a variety of full length and truncated PrP constructs containing various single and double mutations to identify differences between the α and β conformers as a function of both pH and copper loading.


**P03.43**

Identification of Early Natural Scrapie-Specific Gene Expression Changes in Tonsil and Peyer’s Patches of Sheep Using a Sheep cDNA Microarray

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All currently used routine diagnostics for TSEs are thus far solely based on the detection of pathologically folded accumulated prion protein (PrP). The process of TSE agent-uptake, which most likely takes place in the gut via the ileal Peyer’s patches, is still poorly understood, as is the host response to the TSE agent itself. This research focuses on the identification of new biomarkers (other than PrP) that might be useful to extend current TSE diagnostics and/or allowing us to better understand the TSE micro-pathogenesis. We studied the host response by determining the gene-expression-profiles in the very early phase of a natural scrapie infection in sheep using own generated sheep cDNA arrays. We have used a self-generated sheep cDNA microarray containing about 7.4k unique features in triplicate. These features were derived from a large normalised EST library of tonsil and Peyer’s patches of sheep (non-infected and infected of different PrP genotypes). The unique features were determined by sequencing, clustering and assembling over 13,000 sequenced ESTs into 1,850 contigs and 2,594 singletons. To probe the sheep arrays we collected and mRNA preserved many different tissues that include tonsil, Peyer’s patches of ileum and jejenum, brain regions, blood, muscle regions and RLN. These materials were collected at different time points (0, 3, 8 and 35 weeks) of sheep having different PrP genotypes after natural exposure to scrapie or from scrapie-free sheep. Tissues used for array hybridisation were also checked by IHC for scrapie to be able to link the found expression differences to the kinetics of PrPSc deposition. Approximately 200 genes in total for the ileal Peyers-patch from VRQ/VRQ animals at the time points 0, 3, 8 and 32 weeks of age were significantly regulated (182 up- and 18 down-regulated). One of the 154 remaining genes was specific regulated in the 3 weeks time point. A selection of these genes are being confirmed by RT-PCR methods on a large selection of other tissues as well. The current status of the gene expression profiling will be presented in relation to PrP genotype, length of incubation, tissue, and PrPSc accumulation.

**P03.44**

Variant CJD can Lead to Silent Extraneural Infection and to Propagation of Distinct Prions in the Brain of Human PrP Transgenic Mice

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Much remains unknown about the evolution of the variant Creutzfeldt-Jakob disease (vCJD) epidemic due to uncertainty in ascertaining the prevalence of infection and because the disease might remain asymptomatic or produce an alternate, sporadic-like phenotype. To further model human-to-human vCJD prions spreading, we used a newly developed transgenic mouse model that overexpress human prion protein with methionine at codon 129, the only allele found so far in clinically vCJD-affected patients. These mice were infected with prions derived from variant and sporadic CJD cases by intracerebral or intraperitoneal routes, and were analysed for transmission efficiency and strain phenotype in brain and spleen tissues. After intracerebral challenge, all infected mice developed a typical clinical disease at around 150 days and 500 days for sCJD and vCJD primary isolates, respectively. Of note, one out of four inoculated vCJD cases consistently led to the propagation of both sporadic-like and variant-like CJD prions in the brain, while vCJD-type prions invariably accumulated in the spleen. After intraperitoneal challenge, an inefficient neuroinvasion was observed, resulting in a silent infection with life-long persistence of vCJD prion in the spleen.

Our findings have implications in terms of vCJD diagnosis and control. They highlight i) the potential of this agent to establish a subclinical infection in lymphoreticular tissues, thus increasing the level of concern about the risk for iatrogenic transmission; ii) the possibility that person-to-person transmission of vCJD might produce alternative disease phenotypes and that different prions might propagate in different tissues of the same individual.
To understand the functional role of cellular prion protein (PrP<sup>C</sup>) in the initiation and maintenance of prion disease within the host, it is important to obtain a more detailed understanding of PrP<sup>C</sup> transcription in tissues during the development of disease. Using an experimental model with oral infection, we examined the effect of scrapie and the accumulation of the scrapie related form of the prion protein (PrP<sup>Sc</sup>) on the expression level of PrP mRNA in the ileal Peyer’s patch of sheep. In the early phase of infection prior to PrP<sup>Sc</sup> accumulation, no effect on the PrP expression was detected. However, it was found that lambs with PrP genotypes associated with high susceptibility for scrapie generally had higher PrP mRNA levels than lambs with less susceptible genotypes. Further, in highly susceptible VRQ/VRQ sheep at a stage of disease with high accumulation of PrP<sup>Sc</sup>, real-time RT-PCR and microdissection were used to investigate levels of PrP mRNA in four different tissue compartments. An increased level of PrP mRNA was found in lymphoid follicles of infected sheep compared with controls indicating up-regulation of PrP expression in the follicles to compensate for the loss of PrP<sup>C</sup> converted to PrP<sup>Sc</sup>, or that PrP<sup>Sc</sup> accumulation directly or indirectly influences the PrP expression. Still, the PrP expression level in the follicles was low compared with the other compartments investigated suggesting that although increased PrP expression could contribute to PrP<sup>Sc</sup> accumulation, other factors are also important in the processes leading to accumulation of PrP<sup>Sc</sup> in the follicles.

**P03.45**
Increased PrP mRNA Expression in Lymphoid Follicles of the Ileal Peyer’s Patch of Sheep Experimentally Exposed to the Scrapie Agent

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One route to increased understanding of the neuropathogenesis of transmissible spongiform encephalopathies is to study how infection alters the pattern of CNS gene expression. We have previously taken this approach in a mouse model of scrapie and identified changes in neuronal and glial cell transcripts during preclinical and clinical disease (Brown et al., Biochem Biophys Res Comm, 334:86-95, 2005). To determine whether these or similar genes also show changes in human prion disease we have isolated RNA from autopsy human brain material from specimens of frontal cortex from vCJD brains held in the UK National CJD Surveillance Unit (Edinburgh) and similar material from the UK MRC Sudden Death Brain Bank (Edinburgh). RNA was extracted using Qiagen RNeasy Lipid Tissue Mini kits, followed by additional phenol-chloroform extractions to ensure removal of any remaining protein. RNA was analysed for integrity (RNA Integrity Number, RIN) and yield on an Agilent Bioanalyzer 2100 (RNA 6000 nano labchips). A total of 288 RNA samples were generated. Yield and integrity of the RNA did not correlate with age, sex, post-mortem interval or brain pH. RNA of sufficient quality (RIN > 4.5) for quantitative(q) RT-PCR and gene expression array analysis was recovered from 23% of the samples. Of these, 8 vCJD, 8 other neurological disease and 8 non-neurological disease samples were matched for sex and were closely matched for age (within 10 years). These samples are currently being analysed for gene expression changes using pathway-specific oligonucleotide microarrays (Superarray) including the human Alzheimer’s disease Oligo GEArray (which contains 114 pathway specific genes) and Q-PCR.

**P03.47**
Isolation, Characterisation and Gene Expression Analysis of RNA from Human Autopsy Brain Specimens from UK National CJD Surveillance Unit Tissue Bank

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Some authors have connected organophosphate compounds to Transmissible Spongiform Encephalopathy (TSE) susceptibility. Nevertheless, the actual role played by these compounds still remains unclear. The aim of the study presented was to assess the effect of oral exposure of dimethoate (DMT) on Scrapie susceptibility as well as on the progress of the disease by using a genetically modified murine model. A total of 48 lacking (PrP<sup>0/0</sup>) and 70 over-expressing PrP gene (Tg20) C57BL/6 mice were included in the present study. A portion of them was intraperitoneally (IP) inoculated, whilst the rest was maintained as non infected control. Specifically, they were inoculated in 78 cases and the remaining 40 were used as non-inoculated control mice. Exposure to DMT dissolved in drinking water since the beginning of the experiment in the corresponding cases (no control) was performed. The incubation period, spongiosis, PrP<sup>Sc</sup> deposits, glial over-expression (GFAP), neuronal loss (NeuN) and amyloid plaques were the variables which were assessed in all animals. According to the results provided, a treatment consisting of a daily 15 mg/Kg dose of DMT for 5 weeks did not show any effect on any of the variables assessed either on any of the transgenic lines probed. Despite more exhaustive studies for assessing different doses and organic compounds are required, this finding constitutes an empirical study that rules out the possibility that this compound may have a predisposing effect on TSEs.
**Pathology and Pathogenesis**

**P03.49**
**Selective Re-routing of Prion Protein to Proteasomes and Alteration of its Vesicular Secretion Prevent PrPSc Formation**

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Prion diseases are a class of human devastating disorders associated to the conversion of the cellular prion protein (PrPC) into the abnormal scrapie isoform (PrPSc), for which no effective therapeutic strategies exist. Intradobies represent promising therapeutic agents against conformational diseases in general, because of their virtually infinite capacity to specifically recognize all different conformations of a protein, even the pathological isoforms. Moreover, they have unique advantages since they can target the antigen in different intracellular compartments including extracellular milieu, are highly specific reagents and are very stable in mammalian cells, especially when expressed in the secretory compartment.

ER-retained anti-prion single-chain Fv fragments have been proved to be an effective tool for inhibition of PrPC trafficking to the cell surface and antagonizing PrPSc formation and infectivity (1, 2). In the present study, we have stably expressed the secreted version of the anti-prion BH4 intrabody (Sec-BH4) in order to compel PrPC outside the cells. In PC12 anti-prion expressing cells, we found that i) PrPC is forced to be degraded by proteasomes; ii) cell surface PrPC level markedly decreases; iii) PrPC is efficiently secreted but not found associated to exocytosed vesicles and iv) PrPSc accumulation is completely inhibited. Against the ER-retained version of the same intrabody, expression of Sec-BH4 scFv does not influence maturation, glycosylation state and does not induce misfolding of endogenous PrPC. Instead, Sec-BH4 scFv markedly alters the intracellular PrPSc stability (3). The finding that both the ER-retained and the secretory version of the BH4 intrabody block PrPSc formation by re-routing the intracellular traffic of prion and in different ways, indicates that the anti-prion intrabodies are not only a valuable tool to study the pathogenic mechanisms of prion diseases at molecular level but also an effective therapeutic strategy to inhibit scrapie infectivity.

1. Cardinale, A; Filesi, I; Vetrugno, V; Pochiari, M; Sy MS and Biocca S (2005) J. Biol. Chem.; 7; 280;685-94.

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**P03.50**
**Pathological Prion Protein in the Olfactory System of Sheep Affected by Natural Scrapie**

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The investigation of prion deposition in mucosal surfaces undergoing normal turnover is crucial to discover the possible infectivity of excreta and to evaluate their role in the horizontal transmission of prion diseases. Nasal mucosa infection by prions has recently been described in hamsters after intracerebral challenge.

The present study was focused to determine whether prion protein is present in the olfactory system (OS) of natural scrapie affected sheep, examining both the olfactory mucosa and its related brain areas.

Samples of nasal mucosa taken at the level of ethmoturbinates, ventral nasal concha and nasal septum from 24 natural scrapie affected sheep were examined by immunohistochemistry (IHC) and western blot (WB) for the presence of scrapie prion protein (PrPSc). OS related brain areas of the selected sheep (olfactory bulb, olfactory tract, frontal cortex, pyriform lobe and hippocampus) were analyzed too.

Twenty one samples of olfactory mucosa were positive by WB: IHC confirmed WB positive results in 10 cases. Prion spread was assessed both in peripheral and central OS of the examined sheep. PrPSc was mainly localized at the level of ethmoturbinates in the perineurium of olfactory nerve bundles, but it was also discovered in nasal lymphoid tissue, nasal mucus and in olfactory receptor neurons. PrPSc deposition in brain always occurred in olfactory bulb, less in the other areas. Submeningeal, subependymal and perivascular were the prevalent patterns detected.

Our results are consistent with OS involvement in natural scrapie infection. Moreover, the finding of PrPSc both in the brain areas directly in contact to the cerebrospinal fluid (CSF) and in the olfactory nerve perineurium bounding the subdural space extension that surrounds nerve rootlets, might be consistent with PrPSc presence in CSF, though this has never been assessed before. Further studies are in progress to substantiate CSF potential role to convey scrapie infectivity.

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**P03.51**
**Do Tonsilar FDCs Express PrPC in Sheep?**

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In 2007, two cases of natural scrapie were confirmed on sheep flocks in the south of Belgium. Always fatal, this endemic disease is associated with the accumulation of a pathogenic prion protein (PrPSc) in the lymphoreticular system prior to neuroinvasion. PrPSc has been demonstrated, their implication in natural scrapie must be confirmed.

Studies on the kinetics of the prion reveal that the oral route is the main route of scrapie infectivity. PrPSc is known to be transmitted to sheep through oral intake of infected material, and it is crucial to discover the possible infectivity of excreta and to evaluate their role in the horizontal transmission of prion diseases. Nasal mucosa infection by prions has recently been described in hamsters after intracerebral challenge.

In 2007, two cases of natural scrapie were confirmed on sheep flocks in the south of Belgium. Always fatal, this endemic disease is associated with the accumulation of a pathogenic prion protein (PrPSc) in the lymphoreticular system prior to neuroinvasion. PrPSc has been demonstrated, their implication in natural scrapie must be confirmed.

PrPSc deposition in brain always occurred in olfactory bulb, less in the other areas. Submeningeal, subependymal and perivascular were the prevalent patterns detected.

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**P03.52**
**Intact Hearts: A New Tool for Elucidating the Physiology of the Prion Protein**

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The elusive function of PrPα hampers the understanding of the molecular mechanism at the basis of prion diseases, and the development of suitable therapeutic protocols. Use of cell model systems, and genetically modified animals, have nevertheless suggested a number of potential roles for the protein, ranging from cell survival to differentiation. Because we now know that muscle is involved in PrPα pathophisiology, we have considered intact heart paradigms for the in situ study of the cell-protecting function of PrPα. Isolated muscle organs retain the cell native environment and are also more suitable to experimental designs than whole animals. Accordingly, by taking advantage of mice expressing different PrPα amounts (WT, KO and overexpressers), the protection of PrPC against cell oxidative injuries was investigated in isolated hearts subjected to ischemia/reperfusion protocols that involve oxidative stress. Our prediction was that hearts from adult PrPC-null mice manifest an overt phenotype after transient, or long-lasting, ischemia, resulting in exacerbation of heart oxidative injuries. Conversely, PrPα overexpressing mice should demonstrate a higher resistance over ROS production. Myocardial viability was assessed by lactic dehydrogenase (LDH) release into the coronary effluent, while quantification, by immunoblot assays, of myocardial damage was based on myofibrillar protein oxidation.

We found that in PrPα-KO hearts 30 min-reperfusion after 45 min of no-flow ischemia was associated with a larger LDH release, compared to hearts from WT mice. Conversely, hearts from overexpressors displayed a decreased susceptibility to reperfusion injury. The protection by PrPα over ROS damage was also evident from the myofibrillar oxidation pattern of the hearts isolated from the different animals. This data thus supports both the value of the in situ muscle paradigm and the role of PrPα against oxidative insults.

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**P03.48**
**Corona, C; Martucci, F; Iulini, B; Manea, B; Mazza, M; Pezzolato, M; Perazzini, A; Bona, MC; Acutis, PL; Caramelli, M; Casalone, C**

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Prion diseases are a class of human devastating disorders associated to the conversion of the cellular prion protein (PrPC) into the abnormal scrapie isoform (PrPSc), for which no effective therapeutic strategies exist. Intradobies represent promising therapeutic agents against conformational diseases in general, because of their virtually infinite capacity to specifically recognize all different conformations of a protein, even the pathological isoforms. Moreover, they have unique advantages since they can target the antigen in different intracellular compartments including extracellular milieu, are highly specific reagents and are very stable in mammalian cells, especially when expressed in the secretory compartment.

ER-retained anti-prion single-chain Fv fragments have been proved to be an effective tool for inhibition of PrPC trafficking to the cell surface and antagonizing PrPSc formation and infectivity (1, 2). In the present study, we have stably expressed the secreted version of the anti-prion BH4 intrabody (Sec-BH4) in order to compel PrPC outside the cells. In PC12 anti-prion expressing cells, we found that i) PrPC is forced to be degraded by proteasomes; ii) cell surface PrPC level markedly decreases; iii) PrPC is efficiently secreted but not found associated to exocytosed vesicles and iv) PrPSc accumulation is completely inhibited. Against the ER-retained version of the same intrabody, expression of Sec-BH4 scFv does not influence maturation, glycosylation state and does not induce misfolding of endogenous PrPC. Instead, Sec-BH4 scFv markedly alters the intracellular PrPSc stability (3). The finding that both the ER-retained and the secretory version of the BH4 intrabody block PrPSc formation by re-routing the intracellular traffic of prion and in different ways, indicates that the anti-prion intrabodies are not only a valuable tool to study the pathogenic mechanisms of prion diseases at molecular level but also an effective therapeutic strategy to inhibit scrapie infectivity.

1. Cardinale, A; Filesi, I; Vetrugno, V; Pochiari, M; Sy MS and Biocca S (2005) J. Biol. Chem.; 7; 280;685-94.
**Pathology and Pathogenesis**

**P03.53**
Scrapie EAE Co-induced Mice, Pathology and Second Generation Transmission

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When mice infected with scrapie were induced for EAE, an immune mediated model of CNS inflammation, the co-induced mice died from a distinct progressive neurological disease long before control mice succumbed to classical scrapie. Extensive pathological examinations suggest that the co-induced mice suffered from exacerbated inflammation, as seen by increased levels of demyelination, immune cell infiltrates, and gliosis in spinal cords. Interestingly, PrPSc depositions were found in demyelinated white matter areas, suggesting that in scrapie-EAE mice, activated and scrapie infected immune cells may infiltrate into the CNS. As opposed to white matter PrPSc, total brain PrPSc accumulation was similar in high titer experiments between dying co-induced mice and asymptomatic scrapie infected mice sacrificed at the same time. In low titer prion experiments, animals dying of the same co-induced syndrome sometimes presented high levels of brain PrPSc while others did not. Second generation titration experiments suggest that prion titers in the brains of the diverse co-induced mice were independent from their clinical status and related to their levels of PrPSc accumulation. We hypothesize that while inflammatory processes affecting the CNS may have severe clinical implications in subjects incubating prion diseases, such clinical insult did not result in increased infectious potential of the affected animal.

**P03.54**
Investigation into the Role of PrP<sup>C</sup> Cleavage in Intracellular Signalling and Disease Pathogenesis

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Background: The cellular prion protein (PrP<sup>C</sup>) is known to undergo two cleavage events (termed alpha and beta cleavage). These events are influenced by cellular factors such as copper and oxidative stress. The cell membrane is known to have an important role in the transduction of signals from the extracellular environment into the cell. This, combined with the association of the N-terminal PrP<sup>C</sup> cleavage fragments with intracellular signalling, may suggest that PrP<sup>C</sup> is involved in cellular survival responses by the activation of signal transduction cascades. The cleavage event that has been termed beta cleavage is associated with disease, and several disease associated mutations have been linked with altered cellular cleavage. The effect of this altered cleavage on the cell or the susceptibility to infection is unknown.

Aim(s)/Objective(s): This project aims to investigate the role of the N-terminal cleavage of PrP<sup>C</sup> in intracellular signalling, and establish if aberrant processing is involved in disease pathogenesis either by transduction of abnormal signals or by altering susceptibility to infection.

Methods: The aims have been addressed using a combination of cell culture methods, including cellular signalling and infectivity assays, and recombinant protein techniques.

Results: Perturbation of the membrane environment by agents that alter membrane fluidity has been shown to influence the cleavage profile of both human and mouse PrP<sup>C</sup> when expressed in the RK13 cell line. PrP<sup>C</sup> from different species is processed differentially both at the basal level and in response to insult. Investigation into these effects on cellular signalling pathways finds that cellular signalling proteins are altered, in both the amount of total protein and level of activation, differently for the mouse and human PrP<sup>C</sup>.

Discussion: The membrane environment is critical for the transduction of signals into the cell from external stimuli. The finding that modification of the membrane environment influences the cleavage profile of PrP<sup>C</sup> and affects cellular signalling proteins suggests the involvement of PrP<sup>C</sup> processing in signal transduction.

**P03.55**
Prion Infection Correlates with Hypersensitivity of P2X7 Nucleotide Receptor in Mouse Microglial Cells

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The pathological features of prion diseases are brain vacuolation, neuronal death, astrocytosis and microgliosis. The microglial activation follows PrP<sup>C</sup> accumulation and precedes neuronal cell loss in various rodent models of prion diseases. Activated microglia were found to colocalize with the spongiform change in the brain tissues of prion-infected animals, suggesting that these cells play an important role in the pathogenesis of prion diseases. The P2X7 receptor (P2X7R) is one of the major ATP-gated ion channels expressed in microglia, and transduces ATP-mediated signals to these cells to respond to physiological and pathological changes in the brain. ATP interacts with P2X7R and induces several cellular responses:

- Ca<sup>++</sup> influx, formation of large non-selective membrane pores, induction of microglial cell death, and release of mature interleukin-1β (IL-1β). The expression of P2X7R in microglia is upregulated in the rodent models of several neurodegenerative diseases, such as Alzheimer’s disease, indicating the possibility that the activation of P2X7R in microglia correlates with neuropathogenic processes in the brain. Recently, we have established mouse microglial cells persistently infected with various mouse-adapted prion strains. In this study, as an attempt to characterize cellular alterations in prion-infected microglial cells, we investigated P2X7R functions in these cells. Scrapie-infected microglial cells showed hypersensitivity of P2X7R, as demonstrated by an increase in the intracellular Ca<sup>++</sup> concentration, the formation of pores in the cell membrane, the induction of microglial cell death, and the release of mature IL-1β. Furthermore, we demonstrated upregulation of P2X7R mRNA in the brains of scrapie-infected mice. These results suggest that prion infection correlates with hypersensitivity of P2X7R in microglial cells.

**P03.56**
Tau Protein and Hyperphosphorylated Tau Protein in CSF for the Diagnosis of Sporadic Creutzfeldt-Jacob Disease

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Sporadic Creutzfeldt-Jacob disease (CJD) has an incidence of approximately 1/million/year in Europe and is the only form of CJD that has been described in Norway. Due to few specific pre-mortem diagnostic signs, it is difficult to separate CJD from rapidly developing Alzheimer’s disease (AD) and other rapidly developing neurological diseases. The total concentration of tau protein (tTau) in CSF has previously been found to separate patients with CJD from those with AD. An increased concentration of tTau is regarded to be an unspecific marker for degradation of neurons. The success of tTau as a marker for CJD depends on whether other neurological diseases have the same high rate of neuronal decay. As 181-hyperphosphorylated tau (P-Tau) is more specific for AD, one would expect the diagnostic specificity for CJD to rise by combining the two markers. The ratio of tTau/P-Tau has been described to separate CJD from other neurodegenerative diseases without overlap.

We compared the performance of tTau alone and tTau/P-Tau-ratio for the diagnosis of CJD.

CSF for routine analyses of the biological markers tTau and P-Tau were sent to our laboratory from several neurological departments in Norway from August 2005 to May 2007. All patients with tTau values >1200 nG/L (ordinarily used reference limit 450 nG/L) were selected; approximately 30 patients; this included all patients with definite and probable CJD.

Choosing cut-off values for tTau and tTau/P-Tau with 100% sensitivity for CJD, gave a higher specificity for tTau/P-Tau than for tTau alone. The predictive value of a positive test for tTau/P-Tau was also slightly higher than for tTau. The remaining (non-CJD) patients had AD or AD combined with vascular dementia (AD/VD), and a few patients had other rapidly progressing neurological diseases. As expected, tTau/P-Tau separated CJD better from AD, AD/VD than tTau. t-Tau and tTau/P-Tau are useful but non-specific markers for CJD.
**P03.57**

**Slovak Accumulation of Genetic Creutzfeldt-Jakob Disease with Exogenous Risk Factor**

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CJD specific mutation at the PRNP gene - E200K in slovak carriers is characterized by incomplete penetrance (59%). Therefore for clinical manifestation of the disease also other endo- or exogenous factors could not be excluded. Several experimental studies demonstrated that levels of Cu, Mn are probably essential for PrPc stability. Previously we demonstrated Mn/Cu disbalance in the soil and food chain in the slovak area of focal genetic CJD accumulation as a result of environmental Mn contamination by factories with ferroalloys production. The aim of study was to clarify the possible role of these metals in the development of clinical manifestation of the disease and their exogenous influence on the focal CJD accumulation in Slovakia. Mn, Cu levels in brain tissue were determined by flame atomic absorption spectrometry (18 genetic cases were divided into 2 subgroups in regard to their origin - 9 cases from CJD region, 9 out of CJD region, 18 sporadic cases, 18 negative controls).

Analyses demonstrate i) increased Mn levels in genetic “CJD region” and sporadic CJD in comparison to controls. Mn level in genetic “CJD region” cases is significantly higher in comparison to all other groups; ii) Cu status differences between groups are not statistically significant, decreased concentration has been found in genetic cases “out of CJD region”; iii) Mn/Cu ratios are increased in all CJD groups in comparison to controls. Metal ratios in genetic “CJD region” cases are significantly higher in comparison to sporadic cases and also to controls, but not to the other genetic subgroup.

Results support data demonstrating the involvement of investigated metals in the pathogenesis of CJD. More important than Mn/Cu absolute levels is the metal disbalance since it was detected in the CNS of both genetic subgroups as well as in sporadic cases. The reasons of Mn/Cu disturbance ratios are different: in genetic “CJD region” cases it is linked with the excessive environmental Mn level in the area, in genetic cases from the rest of Slovakia this disbalance is caused by decreased Cu level (at present of unknown reason). Our data indicate the environmental Mn/Cu disbalance as a possible exogenous CJD-risk factor which may in coincidence with verified endogenous (genetic) risk contribute to the focal accumulation of genetic CJD in Slovakia. Work was supported by Science and Technology Assistance Agency, contract No. APVT-21-019004.

**P03.58**

**Chemokine CXCL13 Expression in Scrapie-Infected Brain Tissue**

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The gliosis in scrapie-infected brain tissue is associated with an upregulation of cytokines and chemokines. One of the highly induced chemokines is CXCL13, in the periphery known to be a chemoattractant for B-cells. CXCL13 mRNA-levels are upregulated at early stages of the disease and show a further increase during disease progression. Immunohistochemical time-course studies confirmed increasing CXCL13 expression in the CNS during scrapie infection. Using a double-staining procedure with anti-GFAP and anti-CXCL13 antibodies we could show for the first time that CXCL13 is expressed by activated astrocytes in the CNS. To gain more insights into the functional biology of this chemokine in the CNS mice transgenic for CXCL13 were generated. To ensure astrocyte-specific CXCL13-expression, a GFAP promoter construct was used. CXCL13 transgenic mice showed a normal development, fertility, and morphology. To investigate the role of this chemokine in the pathogenesis of neurodegenerative amyloidosis, 6 weeks-old transgenic mice were infected intracerebrally with the scrapie strain 139A and characterized in comparison to similarly infected age- and sex-matched wild-type controls. Infected CXCL13 transgenic mice showed reduced survival times. Changes in gliosis and associated expression of cytokines, chemokines and other inflammatory factors will be analysed by immunohistochemistry and real-time PCR.

**P03.59**

**Vaccination with Peptide-Loaded Dendritic Cells Overcomes Self Tolerance and Confers Resistance to Scrapie**

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Prion diseases are fatal neurodegenerative disorders caused by the accumulation of an abnormal protein, PrPSc, an isomorph of a host-encoded protein (PrPc). Since PrPSc is seen as self by the patient immune system, strong tolerance must be overcome in order to design efficient immunoprophylactic and immunotherapeutical treatments. The aim of the present study was to find out whether immunisation with dendritic cells (DC) might raise T and B cell responses against PrP in wild type mice.

Bone-marrow derived dendritic cells (DCs) were generated in vitro from PrP-deficient or sufficient mice in the presence of GM-CSF for 7 days. On d7, LPS was added for DC maturation. On d8, DC were collected and loaded for 4 h with immunogenic prion or PrP constructs. To ensure astrocyte-specific CXCL13-expression, a GFAP promoter construct was used. CXCL13 transgenic mice showed a normal development, fertility, and morphology. To investigate the role of this chemokine in the pathogenesis of neurodegenerative amyloidosis, 6 weeks-old transgenic mice were infected intracerebrally with the scrapie strain 139A and characterized in comparison to similarly infected age- and sex-matched wild-type controls. Infected CXCL13 transgenic mice showed reduced survival times. Changes in gliosis and associated expression of cytokines, chemokines and other inflammatory factors will be analysed by immunohistochemistry and real-time PCR.

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**P03.60**

**In Vivo Detection of Thalamic Gliosis: A Patho-Radiologic Demonstration in Familial Fatal Insomnia**

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Increasing evidence supports the usefulness of brain MRI for the diagnosis of human prion diseases. From the neuroradiological point of view, fatal familial insomnia is probably the most challenging to diagnose because brain lesions are mostly confined to the thalamus. We report a patient with fatal familial insomnia and normal MRI images (including FLAIR sequence and diffusion-weighted imaging). Results of MR spectroscopy combined with the measurement of apparent diffusion coefficient of water in different brain areas were analyzed. Because the MRI study was performed only four days before patient death, we were able to compare radiological data with the lesions observed at the neuropathological level. The neuroradiological study showed, in the thalamus but not in the other brain regions studied, an increase of apparent diffusion coefficient of water and a metabolic pattern indicating of gliosis. These alterations closely correlated with neuropathological data showing an almost pure glialosis that was restricted to the thalamus. Considering MRI as a model of thalamic-restricted gliosis, this case demonstrates that multisquences MRI can detect prion-induced gliosis in vivo, as confirmed by a neuropathological examination performed only a few days after.
**P03.61**

**Single Treatment with RNAi against PrP is Neuroprotective and Prolongs Survival in Prion Diseased Mice**

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Background: We previously showed that knockout of adult neuronal PrP expression during neuroinvasive disease in mice reversed early pathology and behavioural changes and resulted in long-term survival.

Methods: Validating therapeutic approaches based on targeting host PrP.

Results: Knockdown needed to achieve effective therapy.

Discussion: The immune system plays a central role in prion diseases by providing reservoirs for prion accumulation and replication. Follicular dendritic cells (FDCs) efficiently replicate and accumulate prions in lymphoid compartments, presumably by using antigen-trapping molecules of the complement system. However, the exact mechanism how prions are captured and targeted to FDCs is still unknown. Mice deficient of the immune system to self PrP can be conferred by lymphocytes primed against the Prion Protein.

**P03.62**

**Detection of Proteinase K Resistant Prion Protein in Human Urine**

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Recent concern about the possible secondary spread of vCJD through blood transfusion and blood products has highlighted the need for a sensitive test for the identification of PrPTSE in clinical specimens collected in a non-invasive way. In addition, a more accurate estimate of the prevalence of pre-clinical vCJD in the population may be possible if there were a screening test that could be applied to easily available material such as urine. As a step towards this goal, the detection of putative PrPTSE in the urine of CJD patients has been improved, based on proteinase K digestion of samples and Western blotting. In its present form, the test uses electrophoresis and Western blotting, membranes are incubated with an anti-PrP antibody conjugated directly with horseradish peroxidase. The presence of PrP on the membrane is indicated by a colorimetric detection system. The results of this study demonstrate the presence of proteinase K resistant bands in urine from some sCJD and vCJD patients, but not in unaffected individuals.
Pathology and Pathogenesis

P03.65
Effectiveness of Phthaloacynine Tetrasulfonate Against Rodent-Adapted Human TSE Strains
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It became increasingly urgent the need of an effective therapy for the human transmissible spongiform encephalopathy (TSE) diseases especially after the evidence of the potential inter-human transmissibility of the variant CJD by blood. None of the many tested compounds for an anti-TSE activity had revealed a therapeutic effectiveness in the advanced stage of the pathology. However a cyclic tetrapyrrrole compound, phthaloacynine tetrasulfonate (PcTS) has recently demonstrated to be effective against 2 different rodent-adapted scrapie strains 263K and RML, suggesting that it might be potentially useful also against human TSE strains. We have tested the effect of PcTS on mouse-adapted human TSE strains isolated from variant CJD (mvCJD strain) and Gerstmann Sträussler syndrome (Kfu strain), and on rodent-adapted scrapie strains (mouse strain 139A and hamster strain 263K). Groups of 8-13 animals were intraperitoneally treated with 10 mg/Kg PcTS 3 times/week for 4 weeks starting immediately after the intraperitoneal infection with 10% brain homogenate of mvCJD, Kfu and 139A in C57BL mice and 263K in golden Syrian hamsters. PcTS treatment prolonged significantly the median survival time with respect to controls in mice inoculated with Kfu (351 vs 401, P<0.003), 139A (209 vs 235, P<0.0001) and mvCJD (271 vs 310, P<0.03). But, it did not significantly prolong the median survival time (113 vs 140, P=0.2) in hamsters inoculated with 263K scrapie strain. PcTS showed its highest effectiveness against the mouse-adapted human strains Kfu and vCJD. Since data in the literature and ours, demonstrated that the maximum anti-TSE activity of PcTS occurs at time of infection, we conclude that PcTS could be proposed as a potential candidate for an early treatment of human TSE diseases when a reliable preclinical test will be available.

P03.66
Central Nervous System Extracellular Matrix Changes in Scrapie Infected Mice
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The mature extracellular matrix (ECM) of the central nervous system mainly contains hyaluronic acid, chondroitin sulphate proteoglycans including the lecitan family, and glycoproteins such as tenasin-R. They are either dispersed in the neuropli or forming aggregates around neurons that are known as perineuronal nets (PNNs). In several neurodegenerative diseases it has been observed that remodelling of the ECM implies the modification of its components. In human transmissible spongiform encephalopathies (TSEs) loss of PNNs related to PrP deposition has been reported. Scrapie is a neurodegenerative disease belonging to the group of TSEs that affects small ruminants (sheep and goat).

We have histochemically evaluated the alterations of the PNNs and their components, tenasin-R, brevican, neurocan (two lecitanic) and hyaluronic acid, in a murine model of scrapie. CSTBLA mice were inoculated intraperitoneally with the Rocky Mountain Laboratory (RML) scrapie strain. Euthanasia was performed at 260 dpi, when evident clinical signs of the disease were manifest. A thorough neuropathological characterisation was performed by assessing histopathological changes, PrP deposition and astroglial and microglial reaction. ECM changes were studied parallel to GFAP immunostaining, as an indicator of the degree of lesion.

A dramatic loss of PNNs was observed, as well as of its components: tenasin-R, brevican and, to a lesser extent of hyaluronic acid. On the contrary, neurocan was increased in the neuropi. All these changes were observed in areas where GFAP immunostaining showed more reactive astrocytes, suggesting that the most relevant ECM alterations happen in areas with the major degree of astroglisis.

This study was financed by the project EET2002-05168-C04 of the Spanish Ministry of Science and Technology (MCyT).

P03.67
Murine Model of PrP-amyloid Formation without Spongiform Degeneration
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Background: Prion diseases or transmissible spongiform encephalopathies (TSE) are characterized by the accumulation of PrP-TSE, which serves as a diagnostic marker of disease, and its detection usually indicates the presence of TSE infectivity.

Aim: We studied the relationship between PrP-TSE and infectivity in transgenic (Tg) mice expressing mutant human PrP101L.

Methods: We performed bioassays in Tg 101L mice using brain extracts from 2 cases of GSS P102L having phenotypically different forms of disease.

Results: Brain extracts from a patient with PrP-amyloid plaques and spongiform degeneration transmitted a spongiform encephalopathy to all Tg 101L mice inoculated. Similar results were obtained in mice inoculated with those mouse brains. In contrast, only 1/22 mice developed any disease 622 days after inoculation with brain from a second patient having PrP-amyloid without spongiform degeneration. Surprisingly, many mice without disease nonetheless had PrP-amyloid plaques in their brains, perhaps reflecting subclinical TSE. PrP-TSE was found by immunoblot analysis in one of them. Tg 101L mice inoculated with brain homogenates from an asymptomatic Tg 101L mouse with amyloid plaques did not develop disease. Several of the Tg 101L passage mice also had PrP-amyloid plaques. We did not observe PrP-TSE in most brains tested, even after precipitation with NaPTA, digestions using less PK, or digestions with PK at 40C. We also detected no PrP-TSE by DELFAR or antigen capture enzyme immunoassays. Third passage experiments are underway.

Conclusion: PrP-amyloid accumulated in the brain of Tg mice inoculated with brain homogenate from a human prion disease without causing either clinical illness or spongiform degeneration. Mouse brains containing PrP-amyloid induced appearance of more PrP-amyloid when passaged in mice. These results suggest several possibilities: (i) very low levels of infectivity might be present in Tg mice with PrP-amyloid plaques, (ii) under some circumstance, PrP-amyloid might dissociate from infectivity, (iii) PrP-amyloid might sometimes sequester infectious particles into inert aggregates, or (iv) 101L-PrP-amyloid might aggregate and precipitate without acquiring other properties of a self-replicating TSE agent.

P03.68
A New Experimental Model for Studying Scrapie and Prion Disease in Sheep
Ulvdal, MJ; Baardsen, K; Brandlund, E; Meling, S; Ersdal, C; Espenes, A; Press, CMCL
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A new experimental sheep model for studying the pathogenesis and development of scrapie and prion disease has been developed at the Norwegian School of Veterinary Science (NVH), Norway. At NVH, an experimental sheep model involving oral dosing of 6-8 weeks old lambs has been used to investigate the pathogenesis of scrapie (Ulvdal et al. 2005, Ersdal et al. 2005). The need to follow animals for several years has made these studies laborious and time-consuming. This new experimental model involves oral dosing of lambs with infective brain tissue at birth, before the lambs receive colostrum from their mothers. In this pilot study, 13 lambs were inoculated orally with either scrapie infected brain or normal brain material of the same genotype. The lambs were then kept with their mothers in closed isolation facilities, observed by video surveillance, and euthanized when obvious and typical clinical symptoms of scrapie were observed. All lambs were of the homozygous V131M R144Q/144Q PrP genotype. Seroclinical symptoms appeared gradually from 3-4 months, and at euthanasia, 5 months after dosing, all lambs dosed with infectivity had typical symptoms. Various organs and tissues (six brain areas, the whole spinal cord, various ganglia, lymphoid tissues, adrenals and intestines) were examined by immunohistochemistry with relevant monoclonal or polyclonal anti PrP antibodies (Ersdal et al. 2003, 2005), western blot and gene expression. The results so far show that the model is extremely effective, quick and is well suited for studying pathogenesis. This new experimental approach represents an improved model for studying preclinical diagnosis of prion disease in live sheep (examination of blood, CSF, and biopsy-obtainable tissues).


**Pathology and Pathogenesis**

**P03.69**

**Infection of Metallothionein 1&2 Knockout Mice with RML Scrapie**

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Metallothioneine (MT) are heavy metal-binding, antioxidant proteins with relevant roles described in many pathological conditions affecting the central nervous system (CNS). Regarding prion diseases, a number of publications demonstrate an upregulation of MT-I+II in the brains of TSE affected cattle, humans and experimentally inoculated rodents. Since the prion protein also binds copper, and oxidative stress is one of the events presumably triggered by PrPsc deposition it seems plausible that MTs have a relevant role in the outcome of these neurodegenerative processes.

To gain knowledge of the role of MTs in TSE pathology, particularly that of MT-I+II, a transgenic MT-I+II knockout mouse model was intracerebrally inoculated with the mouse adapted Rocky Mountain Laboratory (RML) strain of scrapie; 129SvJ mice were used as controls (WT). Clinical signs were monitored and survival curves were drawn. Animals were humanely sacrificed when scored positive clinically. Brains were fixed following intracerebral perfusion with 4% formaldehyde, paraffin embedded, and processed for histological, histochemical and immunohistochemical evaluation.

The incubation period did not show significant differences between MT-I+II KO and WT mice, nor did the evolution of neurological signs. Upon neuropathological characterization of the mice brains moderate differences were observed regarding astroglial and microglial response, spongiosis score and PrPsc deposition, particularly in brain regions to which the studied strain showed a stronger tropism (i.e. hippocampus and thalamus). These differences might be a consequence of the lack of antioxidant protection usually given by MT-I+II in such a neuroinflammatory scenario in the CNS, and suggest that these MT isoforms afford a limited neuroprotection in this TSE model.

This study was financed by the Health Department of the Catalan Government (Departament de Salut, Generalitat de Catalunya).

**P03.70**

**Abnormal Prion Protein in the Retina of Rocky Mountain Elk (Cervus Elaphus Nelsoni)**

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Background: Chronic wasting disease (CWD), a transmissible spongiform encephalopathy, has been reported in captive and free-ranging mule deer (Odocoileus hemionus hemionus), white-tailed deer (Odocoileus virginianus) and Rocky Mountain elk (Cervus elaphus nelsoni). An abnormal isoform of a prion protein (PrPCWD) that has been associated with CWD has been reported in numerous internal organs other than the brain and lymphoid tissues including the retina of mule deer.

Objective: Investigate the possibility of PrPCWD in the retina of elk with CWD.

Methods: Eyes from nine elk (genotype at codon 132: 6MM, 1ML and 2LL) were collected and fixed in Davidson’s fixative, sectioned and immunostained stained with Anti-Prion 999 (Ventana Medical Systems, Inc. Tucson AZ) and P54. Slides were also stained with hematoxylin and eosin.

Results: Prion was only found in the retina, all other regions of the eye were free of PrPCWD. PrPCWD was found in 8 of 10 layers of the retina and the optic nerve. Patterns of PrPCWD observed in the MM and ML elk were different as compared to the LL elk. An occasional ganglion cell within the ganglion cell layer contained an intracytoplasmatic staining.

Conclusion: Elk in the later stages of CWD have an abundance of PrPCWD in retinal tissues and optic nerve. This lesion may affect vision in these elk. Genotypes did result in different patterns within the retina. The LL genotype at codon 132, which has a prolonged incubation period, had much less PrPCWD in the retina especially within the inner and outer plexiform layers. Also the LL elk seemed to have more intracytoplasmatic staining within ganglia cells as compared to the MM and ML elk.

**P03.71**

**Structure-Activity Relationship of Heparan Mimetics: New Outlines for the Modeling of Novel Anti-Prion Drugs Candidates**

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Prion diseases are long invariable fatal diseases for which a great number of therapeutics have been tested. Heparan mimetics (HM) are a family of polysulfated molecules that includes HM2602 and HM-CR36 that together with pentosan polysulfate, have been reported among the more efficient drugs used in experimental models of prion diseases. These sulfated polyanions are presumed to act as competitors of the heparan sulfates, natural co-receptors for PrP on the cell surface. The poly-alcoholic backbone of HM offers a wide range of substitution possibilities. Here, we report the syntheses of a library of HM of different molecular sizes, containing various sulfation and carboxylation levels, and bearing or not hydrophobic core substitutions. We report the first study regarding the relationship between these structural features and the compounds capacities to bind to PrPC and PrPSc in an ELISA type test, and to inhibit the replication of PrPSc in chronically infected cells (ScGt1-7). EDS50 determinations and effect comparisons at fixed doses in both models shown that i) molecules with a moderate sulfation level (substitution degree from 1 to 2) were more active than the highly sulfated (substitution degree superior to 1.4) and than the poorly sulfated (substitution degree < 1) ones, ii) that the presence of carboxylic groups decreases compounds efficacies, and that iii) the presence of hydrophobic moieties improves anti-PrP activities even for the poorly sulfated products. Among the tested hydrophobic cores, the phenylalanine methylester amide functionality showed the best activity, followed by the ethylhexylamide and the n-octylamide. The importance of all these factors including their type of interactions with PrPSc and PrPres will be discussed.

**P03.72**

**Pathological Prion Protein Deposits and Neurodegenerative Changes in Sporadic and Genetic Creutzfeldt-Jakob Disease Slovak Medical University**

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The problem, whether neurodegeneration in human prion diseases (PD) are caused by the lack of cellular prion protein (PrPc) or by the toxic effect of pathological prion protein (PrPres) is not solved. Both have support and could probably participate in the development of CNS lesions, but in individual PD their involvement may be different. It is proven that detectable levels of PrPres does not correlate with CNS lesions. The study compares PrPres demonstrated by antiPrP monoclonal antibodies using protease K treated cerebellar slides from sporadic and genetic Creutzfeldt-Jakob disease(CJD) cases. Morphology and intensity of PrPres immunoreactivity (IR) were compared in 25 sporadic and 25 CJD with mutation E200K (CJD-E200K). IR was evaluated as diffuse, patchy or focal. IR findings were correlated with severity of degenerative lesions, M129V polymorphism, age of patients and duration of the clinical stage. Besides of the stripe-like, coarse granular deposits in the cerebellar molecular layer, described previously in CJD-E200K patients, other striking difference between sporadic and genetic CJD was found. While sporadic cases showed predominantly diffuse synaptic IR with few granules in the molecular layer and diffuse/patchy granular deposits, with plaques in the granular layer in most of genetic cases IR had only patchy or focal character, synaptic IR was restricted to the deep molecular layer and deposits in the granular layer were less abundant. Besides the different IR pattern comparison revealed evidently more PrPres deposits in cerebella of sporadic comparing to genetic CJD-E200K cases. Despite of small amount of PrPres in the genetic CJD-E200K, there was no difference between CJD groups in the severity of spongiform changes in the molecular layer, loss of granular cells, reduced Purkinje cell or high number of axonal torpedoes. No correlation between the IR and the age of patients or duration of the disease was found. Because methionine homozygots predominated in both CJD groups, correlation with polymorphism M129V could not be done. Comparison of PrPres deposits in sporadic and genetic CJD showed in both groups similar neurodegenerative changes but distinct PrPres reactivity, indicating comparable neuronal loss, developing in parallel with different accumulation of pathological PrPres. Lack of correlation between IR and severity of lesions indicate that a neurodegeneration less dependent on PrPres deposits may be involved in pathogenesis of CJD-E200K.
**P03.73**

**Microarray Gene Expression of a Murine Transgenic Model of the Bovine Spongiform Encephalopathy**

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Microarray gene expression is a scientific laboratory tool that allows identifying genes with altered expression patterns in determined time points and circumstances and, consequently, gives information about the possible variation in the levels of the related proteins.

Many human and animal transmissible spongiform encephalopathies have been object of this gene expression analysis, being Scrapie the most studied animal TSE. Bovine spongiform encephalopathy is a neurodegenerative disease that produces fatal effects to the affected animal population. Even though the brain histopathological changes associated to the disease have been well described as well as many processes involved in its pathogenesis, there still are many blanks surrounding this disease. Despite the social and economical importance of BSE, as a zoonotic disease, no microarray gene expression studies have been yet published.

Since these techniques have never been applied to BSE, it was our objective to use them in a transgenic mice model in order to obtain new data of the disease as an orientation for further investigations on the pathogenesis of BSE.

Twenty transgenic mice were inoculated intracerebrally with an infective BSE homogenate and humanely sacrificed at different time points of the disease. Twenty age and time matched controls inoculated with healthy brain homogenate were also sacrificed. The brain of these mice was immediately collected and processed for RNA extraction. DNAs synthesized from the extracted mRNAs were hybridized with Gene Chip microarrays. The resulting data was filtered and statistically analyzed and further comparisons between the control and the infected group revealed changes throughout all the time course of the disease. Some of these results were tested by either RT-PCR or immunohistochemistry techniques using paraffin embedded brain samples.

The observed gene expression changes were indicative of an inflammatory reaction of the brain in advanced stages of the disease. The results also showed changes in neuronal metabolism and functionality from early stages of the encephalopathy. This study was financed by the project EET0202-05168-C04 of the Spanish Ministry of Science and Technology (MoCyT).

**P03.74**

**Involvement of the Heat Shock Cognate Protein 70 in Protection of Mouse Neuroblastoma Cells from Cytosolic Prion Protein-mediated Cytotoxicity**

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The molecular mechanisms of prion-mediated neurodegeneration are not yet fully understood. Recent in vitro and in vivo data indicated that neuronal death might be triggered by cytosolic accumulation of misfolded cellular prion protein (PrP\(^{\text{C}}\)) due to impairment of posttranslational degradation of misfolded PrP\(^{\text{C}}\) molecules. Approximately 10% of PrP\(^{\text{C}}\) is misfolded, recognized by the endoplasmic reticulum and retrotranslocated to the cytoplasm for degradation by the proteasome. However, contradicting results on cytosolic PPrP (Cy-PrP)-mediated neurotoxicity in cultured cells have been reported.

To investigate Cy-PrP-mediated effects in cultured cells, we transfected mouse neuroblastoma cells (N2A) with Cy-PrP and determined cytotoxicity using several assays. We found that Cy-PrP expression was not sufficient to trigger cytotoxicity in N2A cells and that Cy-PrP accumulated in fine foci and colocalized with the heat shock cognate protein 70 (Hsc 70).

Since molecular chaperones have been suggested to play an important protective role in PrP\(^{\text{C}}\)-mediated pathogenesis, our interest focused on the effects of a specific Hsc 70 siRNA-mediated knock-down on Cy-PrP-mediated cytotoxicity.

Our results will reveal if the Cy-PrP-mediated toxic phenotype can be rescued by knocking down the putative cytoprotective element.

**P03.75**

**FDDNP Labelling of Prion Amyloid Fibrils in Vitro**

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As presented in previous Prion meetings, a fluorescent probe FDDNP, whose 18F-labelled analogue has been used to detect ß-amyloid plaques and neurofibrillary tangles in Alzheimer disease patients1, labels prion plaques in vitro2. In this study, FDDNP PET scan has recently been used in Indiana kindred Gerstmann-Strausler-Scheinker (GSS) disease patients and presymptomatic subjects to view brain prion patholgy in vivo. In these studies, FDDNP has shown considerable potential for diagnostic use. However, ahead of use of FDDNP in clinical diagnosis, detailed biochemical studies of FDDNP interaction with prion protein are needed.

In the present study, TEM-verified hamster and mouse full-length recombinant prion protein (rPrP) fibrils of highly purified PrP\(^{\text{P}}\) were used in fluorescence FDDNP titration experiments, where FDDNP behaviour was compared to Thioflavin T (ThT). In a similar model, submicromolar range for K\(_d\) of FDDNP in recombinant human truncated PrP (rPrP) aggregates had been determined. Binding of FDDNP to fibrils was further characterized under fluorescent microscope. Radioassays of [\(^{18}\text{F}\)]FDDNP binding to full-length rPrP fibrils are planned.

FDDNP was found to bind to full-length rPrP fibrils avidly and comparably to ThT. FDDNP labelled fibril morphology can be studied under fluorescent microscope. Based on tissue labelling and earlier fluorometric determination of FDDNP K\(_d\) on truncated rPrP aggregates2, we expect to obtain binding constants of [\(^{18}\text{F}\)]FDDNP to full-length mouse and hamster PrP fibrils similar to the binding constants of [\(^{18}\text{F}\)]FDDNP to ß-amyloid fibrils.

These results may offer the basis for further development of FDDNP as a diagnostic tool in prion diseases.

3 Hafner Bratkovic et al, Prion 2005, Dusseldorf; poster.
4 Ghetti et al, Prion 2006; Turin; poster.

**P03.76**

**Bcl-2 Overexpression Rescues Cerebellar Degeneration in Prion-Deficient Mice that Overexpress Amino-Terminally Truncated Prion**

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PrP knockout mice that overexpress a amino-truncated form of PrP\(^{\text{C}}\) (sPrP) are atactic and display cerebellar cell loss and premature death. Studies on the molecular and intracellular events that trigger cell death in these mutants may contribute to elucidate the functions of PrP\(^{\text{C}}\) and to the design of treatments for prion disease. Here we examined the effects of Bcl-2 overexpression in neurons on the development of the neurological syndrome and cerebellar pathology of sPrP. We show that sPrP overexpression activates the stress-associated kinases ERK1-2 in reactive astroglia, p38 and the phosphorylation of p55, which leads to the death of cerebellar neurons in mutant mice. We found that the expression of sPrP in cell lines expressing very low levels of PrP\(^{\text{C}}\) strongly induces the activation of apoptotic pathways, thereby leading to caspase-3 activation and cell death, which can be prevented by coexpressing Bcl-2. Finally, we corroborate in vivo that neuronal-directed Bcl-2 overexpression in sPrP mice (sPrP Bcl-2) markedly reduces caspase-3 activation, glial activation, and neuronal cell death in cerebellum by improving locomotor deficits and life expectancy.
Prion diseases are fatal neurological disorders that occur in humans and animals. BSE is the disease of cattle, scrapie of sheep and Creutzfeldt-Jakob of humans. The main characteristic of the disease is the accumulation of an abnormal prion protein (PrPSc). This infectious protein is the converted isoform from a host encoded prion protein (PrP). The disease is proofed by post mortem detection of PrPSc. Cattle older than three years are routinely examined on BSE by immunoblot and ELISA analysis. However, sensitivity and specificity are the limiting factors of these techniques referring to sufficiently accumulated PrPSc in brain. A sensitive proof to detect very low amounts of PrPSc in tissues, body fluids, blood and in the environment does still not exist, and there is great demand on developing new techniques. We established a very sensitive and specific quantitative immuno-PCR assay detecting physiological prions. This method combines the enzyme-linked immunosorbent assay (ELISA) and an amplification by PCR. The specific signals are intensified by amplification of bound target DNA molecules using the PCR technique. This assay attained a 1000-fold increased sensitivity compared with results of the ELISA technique. The use of the high-sensitive quantitative immuno-PCR method with a significant increase of the efficiency in the prion protein analytic may be achievable as an early stage diagnosis of prion diseases and of product contamination.

Prion diseases are fatal neurological disorders leading to motor dysfunction, dementia and death. They are accompanied by neuropathological changes, such as spongiosis, astroglisis, and neuronal death. How prions proceed to damage neurons and whether dysfunctions in distinct brain areas are required for clinical disease is still unknown. Axonal transport of spinal cord projecting motor neurons in mouse cortex and the Red Nucleus (RN) was analyzed by injecting the tracer Fast blue (FB) into the cervical spinal cord. Upon intracerebral challenge with mouse RML prions, we found a significant reduction of FB-labelled neurons in the RN of wild-type and heterozygous mice as well as in mice expressing either wild-type PrP (Tg20) or truncated PrP (PrP30/93) shortly before the onset of clinical prion disease despite different incubation times. To show selective impairments of axonal transport, prions were applied into the right sciatic nerve to target the contralateral side of RN. A reduction of FB-labelled neurons in the RN similar to FB. In infected wild-type mice we found a significant reduction of labelled motor neurons in the cortex. Quantitative analyses of brain sections for altered immunoreactivity of axonal markers are currently performed. Our data suggest that prion-induced impairments of the axonal transport in motor neurons located in the cortex and RN are associated with prion pathogenesis.

Prions are the causative agents of Transmissible Spongiform Encephalopathies (TSEs). In vivo experiments suggest an involvement of the peripheral nervous system in prion spread to the brain after peripheral exposure to prions, but detailed knowledge about intraneural prion propagation remains elusive. Here we show uptake of fluorescently labeled Scrapie Associated Fibrils (SAFs) by primary cultures derived from Superior Cervical Ganglia (SCGs). Furthermore we employ a compartmented culture system which allows the investigation of prion propagation by physically separating the liquid environment of cell bodies and neurite endings of SCGs. We exposed neurites to scrape prions for four weeks and detected prion infectivity in cell bodies. We also inoculated cell bodies with prions and detected prion infectivity in the neurites. Hence, applying this system we show anterograde as well as retrograde intraneural transport of prion infectivity in peripheral neurons without the involvement of accessory cells. Since prion diseases are ultimately fatal, understanding the basic principles of prion transport could lead to strategies blocking prion spread after peripheral uptake and therefore help in the abatement of prion disease.
P03.81

Characterisation of Milk Cells by Immunohistochemistry and Western Blot
Finlayson, J; Eaton, S; Finlayson, J; Steele, PJ; Dagleish, MP; Jeffrey, M; González, L; Chianini, F
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Immune cells are believed to be involved in the pathogenesis of natural scrapie in sheep. Abnormal PrP (PrPsc) could be present in circulating immune cells of animals infected with transmissible spongiform encephalopathy (TSE) and they could be shed during inflammatory diseases such as mastitis. Most cases of mastitis occur in the first month of lactation, and as a consequence the number of immune cells present in milk increases. PrPsc has been shown previously to accumulate in lymphoid follicles close to the milk ducts and PrPsc has been found in milk. Described here is a method to collect cells from milk to be used for Western Blot (WB) or to be labelled by immunohistochemistry (IHC). One hundred millilitres (ml) of sheep milk was collected for up to 3 days after lambing from animals of various genotypes from a natural scrapie infected flock kept at Moredun. On each day the 100mls of milk was divided into five ml aliquots and mixed with 40 ml cold PBS and centrifuged at 800g for 10 minutes at 4°C. The cream and supernatant were carefully removed and the pellets cells resuspended with cold PBS. The cells were washed several times using centrigulation and cold PBS and divided equally into three aliquots. Two of the aliquots of cells were resuspended in 5% gelatine and allowed to solidify into plugs. The third aliquot of cells was resuspended in PBS then frozen at -20°C for WB. The gelatine plugs of cells were fixed in either Zinc Salts fixative or 10% buffered formalin for 24 hours before being processed overnight then embedded in wax. Sections were cut and stained with Haematoxylin and Eosin showing that the cells present were morphologically well preserved. A panel of monoclonal antibodies (CD3, CD79, CD68) was used to characterise the cell subsets by IHC. PrPc was demonstrated in the prepared milk cells by WB using P4 antibody.

P03.82

Early Immune Events Following Experimental Scrapie Infection in Sheep
Eaton, S; Anderson, MJ; Rocchi, MS; Hamilton, S; Finlayson, J; Steele, PJ; González, L; Dagleish, MP; Jeffrey, M; Reid, HW; Chianini, F
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In order to gain a greater understanding of the pathogenesis of natural scrapie in sheep an experimental model was developed to investigate immune system cells in the minutes proceeding scrapie infection. Four susceptible (ARG/ARG) one year old Romney sheep were inoculated at four different peripheral lymph node drainage sites. The lymph nodes were surgically removed at specific time points; submandibular lymph node (LN) at 300 minutes post infection (m.p.i.), prescapular LN at 180 m.p.i., preferminal LN at 90 m.p.i., and the popliteal LN at 30 m.p.i.. Inoculation was delivered via the subcutaneous route with clarified scrapie brain homogenate injected into the left hand side LNs and clarified scrapie-free brain homogenate into the right hand side. Flow cytometric triple labelling was performed on the LN cells to simultaneously detect lymphocytes, prion protein and the major histocompatibility complex II (MHC II) on the cell surface. In addition, immunohistochemistry was used to detect the abnormal form of prion protein (PrPsc) in the resected lymph node samples. Preliminary results show PrPsc was not detectable in the LN samples at any of the time points studied. Flow cytometry results have suggested that the level of PrP and MHC II co-expression on the cell surface differs depending upon the cell phenotype. CD14+ and CD201+ cells expressed PrP and MHC II simultaneously whereas co-expression was rarely detected on the CD4+ and gamma delta T cells cell surface.

P03.83

CJD Associated with the E200K Mutation with Valine at Codon 129 on the Mutated Allele and Methionine on the Wild Type Allele: Report of Two Cases
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The E200K mutation, the most frequent among pathogenic prion protein gene (PRNP) mutations, can be coupled with either methionine (M) or valine (V) at codon 129. Due to their rarity, the CJD phenotype associated with the E200K-129M haplotype has not been fully characterized as yet. We studied two unrelated CJD patients carrying the E200K mutation coupled with valine at codon 129 on the mutated allele and methionine on the wild type allele. Symptoms at onset included disequilibrium which rapidly evolved to severe ataxia, while dementia appeared only late in the course. CSF examination showed pathological levels of 14-3-3 and tau proteins, while a MRI-DWI revealed an hyperintensity in the caudate and putamen nuclei. Neuropathological examination in one of the two cases revealed many features of the V2 sCJD phenotype, namely the widespread subcortical distribution of lesions, the laminar distribution of spongiform degeneration in the deep cortical layers, and the predominant involvement of the granular layer in the cerebellum. In contrast, the most characteristic histopathologic feature of the MV2 sCJD phenotype, which is the presence of kuru-like amyloid plaques in the cerebellum was completely lacking. In line with this finding, PHF immunohistochemistry revealed a synaptic pattern of PHF deposition without any associated plaque-like deposits. Finally, Western blot analysis of brain homogenates showed PrPSc type 2 with a predominant diglycosylated form (e.g. pattern 2B) in all regions analyzed. The present results will be discussed in relation to current knowledge of the molecular basis of phenotypic variability of human prion diseases. Supported by EC (FOOD-CT-2004-506579) and the Gino Galletti Foundation.

P03.84

Degradation of Abnormal PrP in Isolated Gut Loops of Sheep Challenged with Scrapie or BSE
Hamilton, S; Dagleish, MP; González, L; Sisó, S; Hope, J; Reid, HW; Pang, Y; Eaton, S; Finlayson, J; Steele, PJ; Chianini, F; Jeffrey, M
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It is commonly assumed that the natural transmission of BSE in cattle and scrapie in sheep are both via the oral route. In previous studies we have suggested that substantial amounts of abnormal PrP may be digested in the intestine. The aims of this study were to investigate the temporal degradation and absorption of abnormal PrP after inoculating scrapie and BSE sheep brain homogenates directly into the intestine. Ileal gut loops (2 or 4 per animal) were isolated surgically and inoculated with either 10% bovine BSE or 10% sheep scrapie brain homogenate (in 0.32M sucrose solution), or with normal cattle or sheep brain homogenate, respectively. Gut loops were either flushed to eliminate gut contents or left with alimentary fluids in place prior to inoculation. Intestine and loop contents were collected after time intervals of 15 minutes to 24 hours and subjected to immunohistochemistry and Western blot analysis to determine the amount of PrPd or PrPres remaining. In agreement with previous studies of sheep scrapie, immunohistochemistry detected absorption of BSE PrPd to submucosal lymphatics. However, the abundance of BSE PrPd and its short survival, suggested most was digested. Less BSE PrPd was digested than scrapie PrPd. When incubated with gut contents, Western blot analyses suggested that the amount of BSE PrPres appeared to be reduced significantly after 15 minutes, while scrapie PrPres levels showed little reduction after 30 minutes. However, a gradual loss of PrPres over longer time periods was found in flushed guts for both cases. These data suggest that although some abnormal PrP in brain homogenates may undergo transmural transportation, most is digested by alimentary fluids in situ. Overall, there appeared to be a greater rate of digestion of BSE PrPres compared to scrapie PrPres. These data give a preliminary understanding of what happens to abnormal PrP protein after oral challenge.
In collaboration with the VLA (Weybridge) a unique bank of tissue and blood samples have been collected from animals suspected of incubating BSE or scrapie. Tissues collected include spleen and brain (areas predicted to contain high levels of PrPSc) and were prepared as 10% (or 5%) homogenates; where possible tissues have been evaluated for PrPSc content.

Genotypes were determined by sequence analysis of amplified PRNP DNA. Whole blood was collected into citrate (CPD) buffer and separated into plasma, buffy coat and red cell components using protocols and reagents that would most closely mimic those used by the National Blood Service (NBS).

To date, samples have been collected from 3 confirmed clinical cases of scrapie and a control animal. Bovine blood and tissues have been collected from 2 clinical and 6 suspect BSE cases. Diagnosis was confirmed by the VLA. This collection is part of an increasing bank of reagents held by NIBSC (www.nibsc.ac.uk/prp), to be used in the development of TSE diagnostic assays. Animal samples are distributed subject to the Independent Archive Advisory Group (IAAG) approval. This work was funded by DEFRA grant 500426

Pathology and Pathogenesis

Preparation of Standard Materials from Cases of BSE and Scrapie

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A Transgenic Mouse Model for Analyzing the Relevance of Copper Binding Outside the Octarepeat Region in Prion Disease

A Transgenic Mouse Model for Analyzing the Relevance of Copper Binding Outside the Octarepeat Region in Prion Disease

Background: Prion protein (PrP) is known to bind copper in the highly conserved octarepeat region. Recent research suggests another strong copper binding site outside the octarepeats at histidine 95 in mice. This latter region is of particular interest because of its essential role in amyloidogenesis. We established a transgenic construct carrying the coding sequence for a mutant PrP protein in which histidine at amino acid 95 is replaced by glycine (H95G-PrP) under the regulatory elements of the half-genomic PrP locus. The transgenic construct was used for pronuclear injection into fertilized oocytes and gave rise to five independent transgenic lines. These lines were analyzed in various organs using Western Blot, histology and immunohistochemistry. Infection experiments with scrapie strain RML are in progress.

Results: Western Blot analysis of brain homogenates revealed no differences in band pattern between wildtype- and H95G-mice. Furthermore, no differences were observed after PNGaseF digestion, indicating that the cellular processing of H95G-PrP remains unaffected. Histological and immunohistochemical examinations were also indistinguishable. H95G-mice with PrP expression levels comparable to those of wildtypes were infected with scrapie strain RML and monitored for clinical signs of scrapie and survival. First data suggest a shorter incubation time compared to wildtype mice.

Conclusion: Taken together, those lines are ideal models to study the influence of copper binding outside the octarepeat region of the prion protein in healthy and scrapie infected animals.

In vitro- and in-vivo Downregulation of the LRP/LR level by LRP-specific siRNAs using a Lentivirus-based Delivery System

In vitro- and in-vivo Downregulation of the LRP/LR level by LRP-specific siRNAs using a Lentivirus-based Delivery System

Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include scrapie in sheep, BSE in cattle, CWD in cervides and CJD in humans. Prions, the causative agents of TSEs, are known to interact with the cellular prion protein (PrPc) by inducing conformational changes. The 37kDa/67kDa laminin receptor (LRP/LR) acts as the cell surface receptor for both, PrPc and the infectious prion protein (2,3). Additionally, hepan sulfate proteoglycans (HSPGs) were identified as co-factors/ co-receptors for PrPc (4). Furthermore, it has been shown that LRP/ LR is essential for PrPSc propagation in neuronal cells (5). The accumulation of PrPSc in scrapie-infected neuronal cells (N2aSc+) has been prevented by transfection with siRNAs against the LRP/LR mRNA. Vector-based application of siRNAs circumvents the transient effect of downregulation of gene expression and allows persistent suppression and therefore analysis of loss-of-function phenotypes that develop over longer periods of time. Transduction of recombinant HIV-based lentiviral vectors expressing siRNAs directed against defined regions of the LRP mRNA resulted in reduction of both, PrPres and LRP levels in scrapie-infected neuronal cells (N2aSc+) has been prevented by transfection with small interfering (si) RNAs specific for the LRP mRNA (5). These results demonstrate the necessity of the laminin receptor for the PrPSc propagation in cultured cells. Vector-based application of siRNAs circumvents the transient effect of downregulation of gene expression and allows persistent suppression and therefore analysis of loss-of-function phenotypes that develop over longer periods of time. Transduction of recombinant HIV-based lentiviral vectors expressing siRNAs directed against defined regions of the LRP mRNA resulted in reduction of both, PrPres and LRP levels in scrapie-infected neuronal cells. To further enlighten the role of LRP/LR in prion diseases, injection of recombinant LRP-specific RNA interference (RNAi) lentiviral particles into mice using an intracerebral (i.c.) route was performed. Western blot analysis of the cortical brain area of mice intracerebrally injected with lentiviral vectors expressing siRNAs directed against the LRP mRNA showed a downregulation of the 67kDa LR. Subsequent prion inoculation in these mice will prove whether the knockdown of LRP/LR by RNAi might prolong the onset of or even prevent prion disease.


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Transmissible spongiform encephalopathies (TSEs) are neurodegenerative diseases, which include scrapie in sheep, BSE in cattle, CWD in cervides and CJD in humans. Prions, the causative agents of TSEs, are known to interact with the cellular prion protein (PrPc) by inducing conformational changes. The 37kDa/67kDa laminin receptor (LRP/LR) acts as the cell surface receptor for both, PrPc and the infectious prion protein (2,3). Additionally, hepan sulfate proteoglycans (HSPGs) were identified as co-factors/ co-receptors for PrPc (4). Furthermore, it has been shown that LRP/ LR is essential for PrPSc propagation in neuronal cells (5). The accumulation of PrPSc in scrapie-infected neuronal cells (N2aSc+) has been prevented by transfection with siRNAs against the LRP/LR mRNA. Vector-based application of siRNAs circumvents the transient effect of downregulation of gene expression and allows persistent suppression and therefore analysis of loss-of-function phenotypes that develop over longer periods of time. Transduction of recombinant HIV-based lentiviral vectors expressing siRNAs directed against defined regions of the LRP mRNA resulted in reduction of both, PrPres and LRP levels in scrapie-infected neuronal cells. To further enlighten the role of LRP/LR in prion diseases, injection of recombinant LRP-specific RNA interference (RNAi) lentiviral particles into mice using an intracerebral (i.c.) route was performed. Western blot analysis of the cortical brain area of mice intracerebrally injected with lentiviral vectors expressing siRNAs directed against the LRP mRNA showed a downregulation of the 67kDa LR. Subsequent prion inoculation in these mice will prove whether the knockdown of LRP/LR by RNAi might prolong the onset of or even prevent prion disease.

Pathology and Pathogenesis

P03.89
Antibodies Directed Against the Prion protein Receptor LRP/LR Provide Alternative Tools in Prion Diseases
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The 37kDa/67kDa lamin receptor (LRP/LR) acts as the cell surface receptor for the cellular prion protein (PrPc) (1) and the infectious prion protein (PrPsc) (2). We proved that the polyclonal antibody W3 was able to abolish PrPsc propagation in scrapie infected neuroblastoma cells (3), demonstrating that the disruption of the LRP-PrP interaction is a relevant strategy in therapies against TSEs (for review (4)). We injected W3 intraperitoneally into scrapie infected mice. Spleen analysis revealed that the PrPsc content was reduced by approximately 66% demonstrating a strong reduction of the peripheral PrPsc propagation. In addition, we observed a prolongation of survival time by approximately 130% compared to the control group suggesting that disruption of the LRP/LR-PrP interaction by antibodies is a promising therapeutic strategy (5).

For antibody delivery into mice we developed single chain antibodies directed against LRP/LR employing a phage display technique. Two scFvs termed N3 and S18 have been selected and characterized (6). A therapeutic effect of the scFvs on scrapie infected mice was investigated by passive immunotransfer resulting in a reduction of the peripheral PrPsc propagation in brains of infected mice by approx. 40% without a significant prolongation of the incubation and survival times.

As an alternative delivery system we developed recombinant AAV encoding for the scFvs (7) and treated scrapie infected mice intracerebrally by a stereotactic device. Although spleen analysis revealed a reduction of the PrPsc level by approx. 50%, the survival times were not significantly prolonged (7). Since scFv S18 did not significantly prolong incubation and survival times in animals we developed an improved version of the single chain antibody termed iS18, that revealed a ten fold better affinity to the LRP/LR and was able to reduce the PrPsc level in Scrapie infected neuroblastoma cells. iS18 will be delivered to mice by passive immunotransfer and alternative delivery systems such as scFv secreting muscle cells, liposomes packaged with scFvs and lentiviral vectors.


P03.90
Role of Cystatin C in Prion Propagation and Prion Disease Pathogenesis
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Cystatin C is a cysteine protease inhibitor found in all mammalian body fluids and tissues. It is a secreted protein, but it also reaches endosomal-lysosomal compartments and inhibits cathepsin activities intracellularly. Previous studies showed that Cystatin C is overexpressed in the brain of scrapie-infected mice (Brown et al., Neuropathol Appl Neurobiol 30:555-67, 2004) and Cystatin C levels are increased in the cerebrospinal fluid of patients with Creutzfeldt-Jakob disease (Sanchez et al., Proteomics 4:2229-33, 2004). Since it is known that Cystatin C interacts with amyloid proteins such as Aβ of Alzheimer’s disease (Levy et al., J Neuropathol Exp Neurology 60:94-104, 2001), we have carried out an immunohistochemical study on brain tissue of patients with variant Creutzfeldt-Jakob disease (vCJD) and Gerstmann-Sträussler-Scheinker disease (GSS) linked to different PRNP mutations (P102L, A117V, F198S). The analysis showed that Cystatin C is consistently present in PrP amyloid deposits of vCJD and GSS patients, suggesting that this protein might interact with PrPsc and play a role in disease pathogenesis. To investigate this issue, transgenic mice overexpressing human Cystatin C (Tg hu-CC) and non transgenic littermates were infected intraperitoneally with 10% vCJD brain homogenate. The incubation period and survival time were significantly shorter in Tg hu-CC mice (mean ± s.e.m.: 425 ± 11 and 453 ± 13 days) than in non transgenic littermates (516 ± 22 and 550 ± 23 days). Neuropathological examination showed that the Tg hu-CC mice had larger amount of amyloid deposits than non transgenic mice; conversely, the brain regional distribution and extent of spongiform changes were similar in both groups. Immunohistochemistry revealed that Cystatin C co-localized with PrP in amyloid plaques of Tg hu-CC mice. These data indicate that high levels of Cystatin C facilitate prion disease propagation; accordingly, this protein may serve as a target for therapeutic intervention.

P03.91
Toxicity of PrPC Isoforms Dissected
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It is becoming increasingly clear that pathways affecting biogenesis, maturation and degradation of the cellular prion protein (PrPc) can lead to the accumulation of alternate potentially pathologic forms. Two such forms that have been described are transmembrane CmPrP and cytoplasmic PrP (CyPrP). It has been suggested that an inefficient N-Terminal signal sequence (SS) which results in leaky ribosome scanning can lead to the formation of CyPrP and may also contribute to the formation of CmPrP. Alterations of topogenic elements such as the SS, the stop transfer effector (STE) domain and the transmembrane (TM) domain can modulate the formation of these potentially toxic forms.

Here we present a study performed in cultured cells focusing on the relationship of PrPc biogenesis, maturation and degradation to the toxicity associated with CmPrP and CyPrP expression. In general CmPrP shows a similar maturation profile compared to the normal PrPc isoform (SecPrP) as probed by glycan maturation, SS cleavage, and degradation half life. Where as CyPrP is a preferred substrate for proteasomal degradation, remains unglycosylated with an intact SS. While upon proteasomal inhibition of wild-type PrP expressing cells CyPrP rapidly accumulates and cells show signs of cell death. Our results suggest that a smaller fraction of CmPrP which also accumulates upon proteasomal inhibition, in this case with an uncleaved SS, is the cause for cell death. This is most dramatically revealed in a mutant that abolishes the formation of CmPrP but does not affect CyPrP formation which shows no susceptibility to cell death by proteasomal inhibition. Our data indicate that the dependence of cells to undergo cell death is related to the capacity of PrP to form CmPrP, CmPrP does not correlate with CyPrP accumulation. In conclusion our data suggest that leaky ribosome scanning can promote the formation of both CmPrP and CyPrP. However the toxicity observed in these conditions is likely facilitated by CmPrP and not CyPrP in this model system. Furthermore our study provides potential insight for the unaccounted discrepancies observed in different model systems investigating the role of CyPrP.

P03.92
Direct Comparison of vCJD Diagnostic Assays Using Identical Sample Sets
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With the fourth reported case of vCJD transmission through blood transfusion there is an increased urgency to identify and implement a test for use in blood donor screening. Selection of an assay with sufficient sensitivity to detect blood-borne TSE infectivity is accordingly, this protein may serve as a target for therapeutic intervention.

Selection of an assay with sufficient sensitivity to detect blood-borne TSE infectivity is therefore needed. One approach is to take materials prepared from vCJD cases that have been characterised for PrPSc content and spike these materials into plasma at various concentrations. Several strategies for determining assay sensitivity are therefore needed. One approach is to take materials prepared from vCJD cases that have been characterised for PrPSc content and spike these materials into plasma at various concentrations. Several studies using such an approach have been undertaken by NIBSC and have highlighted the strengths and potential weaknesses of such an approach. Identical panel sets were presented. Studies using such an approach have been undertaken by NIBSC and have highlighted the strengths and potential weaknesses of such an approach.

For antibody delivery into mice we developed single chain antibodies directed against LRP/LR employing a phage display technique. Two scFvs termed N3 and S18 have been selected and characterized (6). A therapeutic effect of the scFvs on scrapie infected mice was investigated by passive immunotransfer resulting in a reduction of the peripheral PrPsc propagation in brains of infected mice by approx. 40% without a significant prolongation of the incubation and survival times.

As an alternative delivery system we developed recombinant AAV encoding for the scFvs (7) and treated scrapie infected mice intracerebrally by a stereotactic device. Although spleen analysis revealed a reduction of the PrPsc level by approx. 50%, the survival times were not significantly prolonged (7). Since scFv S18 did not significantly prolong incubation and survival times in animals we developed an improved version of the single chain antibody, termed iS18, that revealed a ten fold better affinity to the LRP/LR and was able to reduce the PrPsc level in Scrapie infected neuroblastoma cells. iS18 will be delivered to mice by passive immunotransfer and alternative delivery systems such as scFv secreting muscle cells, liposomes packaged with scFvs and lentiviral vectors.

**P03.93**

**Stress Inducible Protein 1 is Released by Astrocytes in Exosome Vesicles Acting as a Celluar Prion Protein-Dependent Neurotrophic Factor**

**Martins, VB; Arantes, CP; Lopes, MW; Haji, GN; Lima, FRS; Porto-Carreiro, I; Linden, R; Prado, MA**

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The physiological functions of cellular prion protein (PrP^Sc) are under intense investigation. We have described that PrP^Sc birds to Stress Inducible Protein 1 (STI1) inducing neuronal differentiation via Erk phosphorylation. STI1 can be secreted by astrocytes, even though it lacks a secretory signal sequence. We examined whether exosomes contribute to ST1 release. Exosomes were purified from conditioned medium (CM) from wild-type (Prnp^+/+) and PrPC ablated (Prnp^0/0) astrocytes. The exosomal fraction purification was evaluated by western blot using positive markers such as transferrin receptor, HSPA5 and HSPA90 by and measuring exosome associated acetylcholinesterase activity. Equal amounts of ST1 were detected in exosomal fractions from Prnp^+/+ and Prnp^0/0 astrocytes. Additionally, ST1 secretion was not affected when astrocytes were treated with Brefeldin A or Monensin indicating that this mechanism is independent on classical secretory pathways. When expressed and secreted by astrocytes as a fusion protein (GFP-STI1), ST1 binds to the neuronal surface and is internalized. Moreover, exosomes prepared from CM derived from astrocytes activate Erk signaling pathway in Prnp^+/+ hippocampal neurons but not in Prnp^0/0 ones and an anti-STI1 antibody blocks this activation. Therefore, STI1 is a neurotrophic factor secreted by astrocytes in exosome vesicles not its interaction with PrP^Sc at the neuronal surface triggers signaling pathways associated with neuronal differentiation.

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**P03.94**

**Long Surviving MV2 sCreutzfeldt-Jakob Disease: Clinical, Diagnostic Tests and Autopsy Findings**

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1Instituto de Investigaciones Neurológicas, FLENI, Referal Centre for Creutzfeldt-Jakob Disease, Argentina; 2Instituto de Investigaciones Neurológicas, FLENI, Argentina

Phenotypic heterogeneity in sporadic CJD (sCJD) has been related to biochemical properties of the protease resistant PrP and to the prion protein gene (PRNP) methionine/valine polymorphism at codon 129. MV2 subtype, a low percentage in most series, is characterized by ataxia, dementia and non-typical EEG together with a prolonged course and Kuru-plaques. In a recent report on 26 MV2 sCJD patients, MRI was the most sensitive diagnostic test (90%). CSF 14.3.3 WB positive in 76%, while typical EEG sensitivity was low (8%). We report a 59-year-old male who presented slow and progressive cognitive decline, confusion, memory loss, speech and visuospatial impairment for six months. EEG was normal.14.3.3 CSF WB proved positive. He developed ataxia 7 months after onset. A 2nd MRI performed 11 mo after onset showed increased signal along brain cortex, caudate and putamen. Brain biopsy disclosed spongiform changes and plaque-like immunostaining. PrP^Res WB was type 2 with a normal glycoprofile and ratio codon 129 MV. He developed myoclonia 22 months since onset and akinetic mutism 40 months since onset and thereafter remained in a vegetative state until he died 46 mo after onset. Brain autopsy was performed including duramater, cranial nerves (trigeminal, optic), olfactory bulb; ulnar nerve, muscle as well as sternal were also observed. Severe spongiform changes, with focal status spongiosus, neuronal loss and astroglial hyperplasia were observed throughout the cortex, basal ganglia, thalamic nuclei, hippocampus including Ammon horn and cerebellum with extensive Purkinje cell loss and synaptic, granular and Kuru-like plaques. PrP^Immunostaining was also disclosed in olfactory bulb, but not in the rest of the nerves or muscle examined nor in sternal although there were a few immunostained cells in the vascular lumen of the latter. Duration range of our definite CJD cases is 2-25 mo, so this long surviving MV2 sCJD provides an opportunity to study PrP^Res distribution and accumulation.

FLENI SECYT BID 802 OC AR PID 98 0227 1728 OC AR PID 2003 351

**P03.95**

**Monitoring of Scrapie Infection in a New EGFP-PrP Transgenic Mouse Model**

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**Objectives:** The aim of this study is to further enlighten these pathogenic mechanisms. **Methods:** We generated enhanced green fluorescent protein (EGFP-PrP) transgenic mice by pronuclear injection, carrying the coding sequence for an EGFP-PrP-P protein fusion including a L42-tag under the regulatory elements of the half-genomic PrP locus. The resulting mouse lines were analyzed regarding PrP expression, F35-phenotype rescue and scrapie-infection experiments using confocal microscopy, Western Blot and immunohistochemistry. In addition, the EGFP-PrP fusion protein was tested in an in vitro conversion assay. **Results:** EGFP-PrP expression was observed in all mouse lines with some variation in the expression pattern, most likely due to integration effects of the transgene. EGFP-PrP failed in a functional test regarding disease phenotype rescue in F35-transgenic mice and also was not convertible to the proteinase K (PK)-resistant form (PrP^Sc) in vitro. However, Western Blot analysis of EGFP-PrP mice on a wildtype-PrP background which were infected intracerebrally with RML scabies clearly detected PK-resistant EGFP-PrP using specific L42 antibody. This result was confirmed by immunohistochemistry showing a staining pattern comparable to that of wildtype-PrP. Confocal microscopy showed EGFP-positive aggregates only in the scrapie infected mice, but not in the Mock-control, most likely corresponding to EGFP-PrP^Sc. **Conclusion:** In summary, these lines are ideal models to monitor early changes in the localization of the EGFP-PrP fusion protein during scrapie infection. Experiments to identify the first target cells after oral infection are in progress.

**P03.96**

**Cuprizone as an Experimental Control for Prion-Specific Gene Expression Profiling**

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Identification of genes expressed in response to prion infection may help elucidate biomarkers for disease progression, agent replication and mechanisms of neuro-pathology. In this study, brain gene expression from C57/Bl6 mice infected with RML and agent and age-matched controls were profiled using Affymetrix gene arrays. Expression of transcripts was confirmed by qPCR. Profiles were analyzed by robust multi-array analysis. Of the 99,000 genes whose expression was measured, 139 transcripts were up-regulated at end stage while 71 were down-regulated. Functional gene ontology was used to identify processes that changed in response to prion disease. The majority of the cellular processes identified related to the characteristic neuro-inflammation known to occur during prion disease progression. Cuprizone, a copper chelator, mimics the neuro-inflammation observed in prion disease, causing both spongiform change and astrocytosis. Cuprizone treatment induced spongiosis as well as astrocyte proliferation as indicated by GFAP transcriptional activation and immunohistochemistry. We hypothesize that by utilizing a 0.4% cuprizone diet as a control treatment for comparative expression profiling, prion specific responses may be delineated identifying processes specific to cellular pathology and cellular conversion.
A Novel Model Advances the Understanding of TSE Pathology

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All attempts at models to explain TSE fall short because there is insufficient understanding of the pathology of these diseases. Our discoveries of, and work with, prionins, and implicating them in a re-interpretation of data published by others allowed us to design a new model which is simple to test and which provides new insights into the pathology of TSEs. Guided by this model we were able to identify novel, readily accessible, molecular targets which can be used for early diagnosis and against which therapeutic agents can be directed without concern for toxic effects in the subjects that are treated.

Role of Glycosylphosphatidylinositol Anchors in the Neurotoxicity of PrPSc

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There is increasing interest in the mechanisms by which PrPSc leads to neurodegeneration. The majority of PrPC molecules are linked to membranes via a glycosylphosphatidylinositol (GPI) anchor. The self-aggregation of PrPSc results in the clustering of GPI anchors in lipid raft micro-domains that are enriched in signalling molecules. We demonstrate that the addition of GPI anchors isolated from PrPC or PrPSc mimics some of the effects of PrPSc on neurones, namely activation of phospholipase A2 (PLA2), a key enzyme in the process by which PrPSc kills some neurones, and induction of caspase-3 (Bate & Williams, 2004). The GPIs isolated from PrP in hamster brains are unusual in that they contain high amounts of galactose, mannose and sialic acid (Stahl et al., 1992). Although it is not known whether PLA2 activation is a unique property of GPIs attached to PrP, GPI anchors isolated from Thy-1, decay-accelerating factor (DAF) or CD14 did not activate PLA2 indicating that this activity is not shared by all GPIs.

High concentrations of GPI anchors were required to activate PLA2, perhaps reproducing the locally high concentration of GPI anchors that occurs following the aggregation of PrPSc molecules, or the cross-linkage of PrPc by specific mabs (Solforosi et al., 2004). The activity of GPIs was dependent on a phosphatidylinositol moiety, on ester-linked acyl chains and on a glycan component. There were no obvious physical differences between GPIs isolated from PrPc or PrPSc, nor any differences in their activity. Pre-treatment with glucosamine-phosphatidylinositol (PI) reduced activation of PLA2 by PrPSc and protected neurones against the toxicity of PrPSc. These results are consistent with the hypothesis that glucosamine-PI competes with the GPI anchors of PrPSc for cellular receptors involved in signal transduction. While neuronal death in prion diseases is undoubtedly a complex process, these observations provide insight into the signalling processes that result in PrPSc-induced neuronal loss.

MicroRNA Expression Profiling in Prion Disease

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Global analysis of gene expression in the brains of prion infected humans and animals have led to the identification of numerous genes which exhibit transcriptional alterations associated with disease progression. These genes reflect the pathways and biological processes that characterize the key events in prion pathogenesis. However, a central question is how these genes are controlled. Recent work has revealed that a class of small non-coding RNA species, known as microRNAs (miRNAs) has critical functions across various biological processes, for example miRNAs have been shown to modulate programmed cell death during development.

To address whether miRNAs play a role in prion-induced neurodegeneration, we developed a microarray for miRNA expression profiling and performed an analysis of over 400 mammalian miRNAs from mouse brains infected with scrapie. We identified miRNA molecules that show significant, disease-specific, changes in their levels of expression. These miRNA molecules, in turn, act to control other genes that change in the disease brain. Interestingly, some of these genes are involved in safeguarding brain cells from stress and maintaining their viability and function. We have introduced these miRNAs into brain cells grown in the laboratory and we will present results outlining the potential regulatory function of these molecules. We have previously reported comprehensive analyses of gene expression, and the biological processes which are affected, in brains of scrapie infected mice. MiRNAs can act as key regulators of these changes and their inclusion into regulatory pathways and networks will provide a more complete picture of the events leading to neurodegeneration.
**Pathology and Pathogenesis**

**P03.101**

Polypropenol could Improve Disbalance in Membrane Protein Glycosylation in Prion Infection

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Introduction: Dolichyl Phosphate Cycle (DPC) is one of the possible targets in prion infection. The aim of this study IS to investigate the contributing mechanism of DPC substitute – polypropenol (PP) in this process.

Methods: Dolichol (DoL) and Dolichyl Phosphate (DoLP) contents in brain homogenates, blood and urine were assayed by HPLC in mouse brain infected with scrapie. The intensity of the glycoprotein synthesis in subcellular fractions was estimated based on the number of DoL-P[14C]Mannose Starting Glycosylation Complexes (SGC). In an effort to produce an affect on the DPC regulation the animals were intranasally administered [1-3HPolypropenol (PP)], a substance, which is believed to be a predecessor of endogenic DoLP.

Results: In the early period of disease the neurons therewith cumulate non-glycosylated proteins, morphologically reflected by the cellular hypertrophy. The pathologic process was shown to result in the fall of DoLP in neurons by 20-25%. DoLP increased in serum and later in urine. Intranasal PP application decreased DoLP content in serum and brain. Application was beneficial for revealing 17.8% of PP activity in brain, 8.3% in the form of DoLP. It is well established that the number of SGC in the mitochondria is 10-12 times as high as that in the Golgi apparatus. After PP application this gap attains 1.7-2.5 in favour of the Golgi without DoLP drop in brain.

Discussion: The fact is that in prion infection the resistance of membranes in cerebral neurons is ensured by a sufficient level of the glycoprotein synthesis running through DPC in mitochondria. However in prion infection the mitochondria have to ensure a higher energy consumption of the cells. The subcellular redistribution of the glycosilation sites under extreme conditions for brain is believed to be as a marker of resistance to prion infection According to the obtained information the PP provide a produg which makes up for a disbalance in membrane protein glycosylation in prion infection.

**P03.102**

Investigation of Preclinical Deposition of PrPSc in Peripheral Tissues of Primates

Challenged with vCJD, BSE and sCJD Prions

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Non-human primates have been shown to be a reliable model for the experimental transmission of human prion diseases showing a comparable clinical course and a typical pathology within the brain tissue of terminally ill animals. However, to define the risk of accidental transmission of prion diseases via non-neuronal tissues the preclinical deposition of PrPSc has to be elucidated. The involvement of extraneural tissues such as lymphoreticular system and muscle had been demonstrated in humans and experimentally infected animals with clinical disease. To investigate whether extraneural tissues play a role in the preclinical phase of human prion diseases we infected rhesus monkeys with sCJD, BSE and vCJD via the intraperitoneal route. Animals were taken at defined time points before and after development of first clinical signs. Central nervous tissue showed deposition of PrPSc in a disease specific pattern. The amount of PrPSc corresponded directly to the duration of infection. We investigated the deposition of PrPSc in different muscle samples (skeletal muscle, heart, tongue, tongue root) using highly sensitive detection methods. Interestingly, the amount of PrPSc was highest within tongue tissue of vCJD infected monkeys and could be detected several months before the development of clinical symptoms.

**P03.103**

Intranasal and Aerosolic Prion Transmission

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Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of humans and animals. Prions can colonize hosts by a variety of extracerebral routes, including parenteral injection, transdermal administration after skin scarification, and oral administration. The underlying infectious agent, the prion, was shown to accumulate not only in the central nervous system (CNS) but also in secondary lymphoid organs of affected hosts. Until recently prions were not considered to be transmissible by aerial routes. Here we have investigated the transmissibility potential of prions administered intranasally or by aerosols. Various transgenic mouse models expressing the cellular prion protein (PrPC) in specific compartments or cells of the brain (e.g. exclusively in the CNS) were investigated to identify the cellular and molecular mechanism(s) of prion invasion via the intranasal or aerosolic route. Results of this study identify prion aerosols or prions administered intranasally as a startlingly efficacious pathway of prion transmission, and call for appropriate revisions of prion-related biosafety guidelines. We currently investigate the mechanism of this transmission route.

**P03.104**

Replication of Human Prions in a “Humanized” Mouse Model System

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Prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases affecting both humans and a large variety of animals. Ethical constraints prevent most functional analyses of neuroimmunological aspects of TSEs in humans. By combining transgenesis and xenotransplantation, we have created an accurate animal model that circumvents such constraints [1]. The bone marrow of immune compromised c57BL/6±RAG-2/- mice is reconstituted with CD34+ hematopoietic stem cells derived from the umbilical cord blood of healthy human newborns. By this we establish a “humanized” immune system in the mouse, which can be tested for its capability to replicate human prions in vivo. c57BL/6±RAG-2/- mice reconstituted with human CD34+ cells, homozygote for methionine at codon 129 of the human Prion gene (129MM), were peripherally inoculated with brain homogenate derived from a sCJD patient (129MM). In contrast to unreconstituted control mice, we detected PrPSc in the spleens of reconstituted c57BL/6±RAG-2/- mice already at 60, 90 and 120 days post inoculation. Hereby, we established an in vivo assay to test how different polymorphisms of the engraved human prion protein, expressed on human immune cells, permit the replication of various, potentially human pathogenic, prion strains (e.g. chronic wasting disease; scrapie). In addition, this may yield an extremely sensitive, ethically acceptable and reasonably rapid test for human prion infectivity.

**P03.105**

**PrP Aggresomes are Poly(A)+-Ribonucleoprotein Complexes that Induce a RNA-Dependent Protein Kinase (PKR)-Mediated Deficient Cell Stress Response**

*Rogaeva, E.; Gogotsi, Y.; Grenier, G.; Brown, A.; treated cell pellet to fresh neuronal cells with a construct encoding PrP without the N- and C-terminal signal peptides.*

**Background:** The cause of neurodegeneration in prion diseases is not well understood, and there is much evidence that argues against the direct neurotoxicity of PrPSc. Some attention has recently turned toward exploring mistransferring and accumulation of PrP in the cytoplasm. Recent studies indicate that prion-infected cells produce juxtanuclear cytoplasmic inclusions termed PrP aggresomes, and that such aggregates may be present in the brain of infected mice. The mechanism of toxicity of PrP aggresomes has not been fully investigated.

**Objective:** We have investigated if PrP aggresomes induce a cellular stress by determining if cells producing such aggregates exhibit a spontaneous stress response.

**Methods:** PrP aggresomes were produced by transiently transfecting neuronal cells with a construct encoding PrP without the N- and C-terminal signal peptides. Results: We report that cytoplasmic PrP aggregates initiate a cell stress response by activating the autophosphorylation of the RNA-dependent protein kinase (PKR) at threonine 446. Activated PKR phosphorylates the translation initiation factor eIF2a on serine 51, resulting in protein synthesis shut-off. However, other components of the stress response, including the assembly of poly(A)+ RNA-containing stress granules (SGs) and the synthesis of heat shock protein 70, is repressed. A phosphomimetic mutant of eIF2a normally sufficient to induce the assembly of SGs, was unable to induce the formation of SGs in cells producing PrP aggresomes. These observations suggested that poly(A)+ RNA are trapped and unable to be recruited into SGs. We verified this hypothesis by in situ hybridization experiments with fluorescent oligo-dT probes and confocal analysis. We observed the clustering of poly(A)+ RNA within PrP aggresomes. Affinity chromatography on oligo(dT)-cellulose demonstrated that PrP aggresomes are purified with poly(A)+ RNA, and are therefore poly(A)-ribonucleoprotein complexes in vivo.

**Discussion:** We have demonstrated that PrP aggresomes are poly(A)+-ribonucleoprotein complexes inducing a spontaneous stress response characterized by the activation of PKR, the phosphorylation of eIF2a, and repression of protein translation. However, aggregation of poly(A)+ prevent a full stress response. These mechanisms may not be obligatory lethal per se, but would likely result in premature cell death in the context of an acute environmental stress that would be otherwise dealt with an adequate stress response.

**P03.106**

**Detection of Chronic Wasting Disease Prions in Saliva, Blood, and Excreta of Deer**


**Background:** The potential presence of prions in body fluids is perhaps most relevant to chronic wasting disease (CWD) of cervids, owing to its facile transmission, geographic expansion, and the relatively large amount of aberrant prion protein in peripheral lymphoid tissues. Nevertheless the exact mode by which the CWD prions are shed and transmitted has remained unknown.

**Objective:** To determine whether infectious CWD prions are present in saliva, blood or urine and feces of CWD-positive deer.

**Methods:** Two bioassay studies comprising three cohorts for a total of n = 6 naïve deer/cohort were exposed either orally to 50 ml saliva, or 50 ml urine and 50 gram feces, or via intravenous transfusion of 250 ml whole blood from CWD-positive deer. Study controls included positive control cohorts totalling (n = 8) deer exposed to brain from a CWD-positive deer and a negative control cohort consisting of (n = 6) deer receiving inocula from CWD-negative deer. The recipient animals were maintained under rigorous indoor isolation conditions to exclude potential adventitious prion exposure and monitored for CWD infection for a minimum of 18 months post infection by serial tonsil biopsy and terminal necropsy.

**Results:** Infectious prions capable of transmitting CWD were detected in saliva (by the oral route) and in blood (by transfusion). PrP<sup>Res</sup> was first detected in tonsils between 3 and 12 months post inoculation. To our surprise, no deer fed urine and feces from CWD-positive donors developed CWD infection, despite multiple exposures.

**Conclusion:** Infectious prions in saliva may explain the efficient transmission of CWD in nature. Infectious prions in the blood of CWD-positive deer establishes a basis for developing an antemortem detection of the disease by blood-based assay methods and emphasizes the widespread distribution of infectivity in CWD-positive deer.

**P03.107**

**Modulation of PK-resistant PrP in Cells and Infectious Brain Homogenate by Redox-Iron: Implications for Prion Replication and Disease Pathogenesis**

*Simhoni, D.; Maradumane, M.; Basu, S.; Luo, X.*

**Case Western Reserve University, Pathology, USA**

**Background:** The principal agent responsible for all prion disorders is a β-sheet rich protein of the cellular prion protein (PrPC) termed PrP<sup>scrapie</sup> (PrP Sc). Once initiated, conversion of PrP C to a PrP Sc-like form (AggrPrP) that co-aggregates with ferritin and there is much evidence that argues against the direct neurotoxicity of PrPSc.

**Objective:** To evaluate the role of redox iron in prion disease pathogenesis.

**Methods:** Cell culture models and prion disease affected human and mouse brain tissue were used for this study.

**Results** We provide evidence that prion protein (PrP<sup>C</sup>) binds iron, a characteristic likely to modulate its susceptibility to oxidative damage and contribute to altered levels of total and redox-active iron in diseased brains. Using PrP<sup>C</sup> expressing neuroblastoma cells (PrP<sup>–</sup> cells), we demonstrate that exposure to ferrous chloride (FeCl<sub>2</sub>) induces the conversion of PrP<sup>C</sup> to a PrP<sup>Sc</sup>-like form (PrP<sup>Res</sup>) that co-aggregates with ferritin and resembles brain derived PrP<sup>Sc</sup> in characteristics such as solubility in non-ionic detergents, reactivity with 3F4 unless denatured, resistance to proteinase K (PK), and limited propagation in cell cultures. Thus, manipulation of iron levels in cells induces the generation of additional PrP<sup>Sc</sup> simulating in vivo propagation in the absence of infectious brain homogenate. On the other hand, depletion of iron from prion disease affected human and mouse brains and mouse neuroblastoma (SnSn)2a cells reduces the amount of PK-resistant PrP<sup>Sc</sup> by 4-10-fold, though PK-resistance of ferritin is not altered significantly by this treatment. Discussion Our results suggest that the iron binding characteristic of PrP<sup>Sc</sup> renders it susceptible to redox-iron mediated damage, resulting in the generation of a PrP<sup>Sc</sup>-like molecule that is itself redox-active and induces a similar change in additional PrP<sup>Sc</sup>. Combined with the fact that depletion of iron from infectious brain homogenates and cells reduces PK-resistant PrP<sup>Sc</sup>, these results suggest that redox-iron is involved in the generation, propagation, and stability of PK-resistant PrP<sup>Sc</sup>. It is plausible that the PK-resistant characteristic of PrP<sup>Sc</sup> is derived through its association with ferritin, iron being an important mediator of this complex.

**P03.108**

**Association of PrP<sup>C</sup> with Cytopathological Changes of BSE Affected Cattle**

*Emesé, C.; Goodman, CM; McGovern, G; Simmons, MM; Jeffrey, M; Ersdal, C.*

**The Norwegian School of Veterinary Science, Department of Basic Sciences and Aquatic Medicine, Norway; Veterinary Laboratories Agency, Lasswade Veterinary Laboratory, UK; Veterinary Laboratories Agency, Veterinary Laboratories Agency - Weybridge, UK**

**Aim:** In this study we have sought to determine the nature of sub-cellular changes in the brain (motor cortex of the vagus (DMNV) and the solitary tract nucleus (NTS)) following oral route) and in blood (by transfusion). PrP<sup>Res</sup> was first detected in tonsils between 3 and 12 months post inoculation. To our surprise, no deer fed urine and feces from CWD-positive donors developed CWD infection, despite multiple exposures.

**Conclusions:** Infectious prions in saliva may explain the efficient transmission of CWD in nature. Infectious prions in the blood of CWD-positive deer establishes a basis for developing an antemortem detection of the disease by blood-based assay methods and emphasizes the widespread distribution of infectivity in CWD-positive deer.

**Methods:** Nine naturally BSE infected cattle and three controls were perfusion fixed by mixing aldehydes. The DMNV and the NTS were identified and processed by standard methods to plastic. 65-mm thin sections were immunogold labeled with the R523.7 PrP antibody.

**Results:** Vacuolation was conspicuous and of two types: those that appeared as dissolution of the matrix of dendrites and those that appeared as expanding single walled structures that impinged and involved other processes. Areas of osmiophilic microvacuoles were found in several animals. In most of the infected animals there were restricted areas of small, irregular profiles that had an increased electron density of the extracellular space. Membrane invaginations were found in dendrites. They often appeared as single structures, but could be found in pairs and have a branching structure. The membrane invaginations could be associated with surrounding areas of increased electron density in the extracellular space, membrane irregularities and other nearby invaginations. In axons and axon terminals, spiral membrane inclusions and synaptic vesicles arranged in clumps were seen. In several animals there were large and branching multivesicular bodies. PrP<sup>Res</sup> was associated to membrane invaginations, irregular process profiles, spiral inclusions and lysosomes, but no association was found to vacuoles or to clumped synaptic vesicles.

**Discussion:** Vacuolation may have more than one cause or morphogenesis. Vacuoles may be caused by the break down of dendritic cytoplasmic content, particularly microtubules, followed by outer membrane ballooning and loss of integrity of the plasmamembrane. Other vacuoles may form due to changes in the membranes of numerous small processes which have an appearance of increased osmiophilia. Cell surface PrP<sup>Res</sup> was associated with an abnormal endocytosis related to cell membrane invaginations which are common in some sheep TSE infections and rare in rodents. Conversely amyloid fibril formations are common in rodents and rare in sheep and cattle TSEs.
**P03.109**

**Different Expression Levels of CK2 Subunits in the Brains of Animal Models Infected with Scrapie Agent 263K or 139A and Human TSE**

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1State Key Lab for Infectious Disease Prevention and Control, National Institute for Viral Disease Control and Prevention, China; 2China CDC, State Key Lab for Infectious Dis Prevent & Control, China

Background: Protein kinase, casein kinase 2 (CK2) is a highly conserved serine/threonine protein kinase which is ubiquitous in eukaryotic organisms. In some neurodegenerative diseases, e.g. Alzheimer disease (AD), the amount and activity of CK2 were reported to be decreased.

Objective: To address the possible alteration of CK2 in transmissible spongiform encephalopathies (TSEs).

Methods: The levels of CK2 in the brain tissues of the hamsters inoculated intracerebrally with scrapie agents 263K or 139A, as well as the C57BL mice infected with scrapie agent 139A were evaluated by Western blots, following quantitative analysis of immunoblot images.

Results: Compared with normal animals, the levels of CK2α in the brains of infected hamsters and mice were significantly decreased, regardless of scrapie agents. In addition, the expressions of CK2α/β in the brains of hamsters infected with agents 263K or 139A, and CK2α/β in the mice infected with agent 139A, were remarkably increased. Comparative analyses on the presences of CK2α or CK2β in the brains of animals infected with scrapie illustrated an obviously species-related, but not strain-related manner. Furthermore, decreases of CK2α and increases of CK2α/β were observed in cerebella homogenates from one familial Creutzfeldt-Jakob disease (CJD) case and one fatal familial insomnia (FFI) case.

Conclusion: These results firstly suggested that alterations of CK2 subunits in brains were illness-correlative phenomenon in TSEs and proposed the potential linkage of CK2 changes with the pathogenesis of prion diseases.

**P03.110**

**Doppel Protein Induced Cytotoxic Effect on Neuron Cells in vitro and PrP Protein was Able to Antagonize Doppel-associated Cytotoxicity**

Dong, X-P; Li, P; Han, J; Lei, Y-J; Shan, B; Dong, C-F; Xiao, X-L; Jiang, H-Y; Gao, C

China CDC, State Key Laboratory for Infectious Disease Prevent, China

Background: Doppel (Dpl) is recently identified as a prion (PrP)-like protein due to the structural and biochemical similarities, however, the natural functions of Dpl and PrP remain unclear yet.

Objective: To assess the potential activity of proteins Dpl and PrP on neural cells in vitro.

Methods: A 531-bp human PRND gene sequence encoding Dpl protein was amplified from human peripheral blood leucocytes. The full-length and various truncated Dpl and PrP proteins were expressed and purified from Escherichia coli. Various proteins were supplied on the cultured cell line SH-SY5Y. The cell growth situations were evaluated by MTT and Trypan blue assays and apoptosis phenomenon were observed by staining of Hoechest33342, cytomtery after staining of Annexin V/PI and TUNER assay.

Results: Supplement of the full-length Dpl protein onto the cultured human neuroblastoma cell line SH-SYSY induced remarkable cytotoxicity and the region responsible for its cytotoxicity was mapped at the middle segment of Dpl (amino acid 81-122). Interestingly, employment of full-length wide type human PrP protein onto the cultured cells antagonized Dpl-induced cytotoxicity. Analysis on fragments of PrP mutants showed that its N-terminal fragment (amino acid 23-90) was responsible for the protective activity. Treatment of a truncated PrP (the full-length PrP from aa 23 to 231 in which the peptide from aa 32 to 231 is deleted), which shared similar secondary structure as Dpl, induced cytotoxicity on the cells like Dpl. Furthermore, copper ion could enhance the antagonizing effect of PrP on Dpl-induced cytotoxicity. Apoptosis assays revealed that cytotoxicity induced in Dpl occurred via an apoptotic mechanism.

Conclusion: These results suggested that function of Dpl is antagonistic to PrP rather than synergistic and are consistent with early observations in wide transgenic mice.

**P03.111**

**Expression of PRPC Isoforms in the Parts of Cattle Brain**

Stadnyk, Vitaliy V; Mayor, Chrystyna Y; Vilko, Vasyl V

Institute of Animal Biology, Scientific Center for Prion Infections Study, Ukraine

Background: Prion diseases appear as a result of normal physiological protein PrPc conversation into the abnormal, disease-causing form PrPsc. The physiological form of prion protein is produced naturally in different cells of all mammals, and is harmless. The normal and abnormal prion proteins differ only in their three-dimensional structures. Once the normal protein has undergone such conversation, it can then transform other normal protein molecules into the pathological form. The PrPc expression level is a decisive factor in prion diseases progression, so far as physiological prion protein is a matrix for PrPsc appearance.

Aim: The aim of present work was to investigate the level of PrPc isoforms expression in several parts of cattle brain.

Methods: We used Dot blot and disc-electrophoresis in PAAG gradient with Western-blot analysis to study general level of PrPc expression in the parts of cattle brain: in frontal pole of cerebrum, olfactory tract, olfactory cortex, mammillary body, cerebellum, epiphysis cerebri and medulla oblongata.

Results: The glycosylform signature of PrPc is in general characterized by the presence of three bands with decreasing intensity, representing the bi-, mono- and unglycosylated isoforms of this protein. We detected two additional isoforms in olfactory tract, olfactory cortex, mammillary body and cerebellum. In frontal pole matter of cerebrum and epiphysis cerebri we detected three additional isoforms. Taking into consideration their electrophoretic mobility these isoforms represent partly glycosylated forms of prion protein.

Conclusion: It was demonstrated that the highest level of this protein posseses in medulla oblongata, olfactory tract and olfactory cortex. It was shown that correlation between PrPc glycosylmers is rather divers in different parts of cattle brain.

**P03.112**

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Withdrawn
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**P03.113**

**Spongiform Change Causes High Intensity Lesion in Diffusion-weighted MRI**

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High intensity lesions in diffusion-weighted MRI (DWI) are important diagnostic marker of Creutzfeldt-Jakob disease (CJD). The pathogenesis that causes the CJD-related high intensity lesion has been discussed, but the conclusions are controversial. Time lag between DWI examination and postmortem pathological examination is the major reason. To describe the pathogenesis, we compared the DWI findings and pathological findings of special types of CJD, genetic CJD with V180I and sporadic CJD with MM2-thalamic. In CJD with V180I DWI demonstrates prominent high intensity lesions in the wide range of cerebral cortex incongruous with slight clinical symptoms. On the other hand, in MM2-thalamic DWI demonstrates no high intensity lesions in the brain. Pathological findings of CJD with V180I are severe spongiform changes in whole layers of cerebral cortex, mild neuronal loss and astrogliaosis, and weak synaptic deposition of PrP and those of MM2-T are severe neuronal loss and astrogliosis but no spongiform changes in the thalamus and inferior olive. Immune-reactivity of PrP are absent in the thalamus. In the cerebral cortex, spongiform changes are absent or limited to isolated foci, and immunoreactivity of PrP is absent or weak. Spongiform changes, neuronal loss, astrogliaosis, and PrPSc deposition are major candidates. High intensity lesions in DWI are best fit to spongiform changes based on our observation. Another characteristic of DWI findings of CJD is that the high intensity lesions with sequential DWI do not always progress with the advance of the disease, and the signal intensity sometimes decreases with the disease progression in some lesions. vacuoles of spongiform changes with a diameter of 5 to 20 µm would best provide T2 prolongation and restricted diffusion, i.e. best depicted as high intensity lesions by DWI. Vacuoles conglutinate each other with the progression of disease and vacuoles become larger in diameter. The larger in diameter, the weaker the signal intensity. Spongiform-change hypothesis can easily explain this kind of signal changes with the disease progression. We conclude again that spongiform change is the cause of CJD-related high intensity lesion in DWI.

**P03.114**

**Prion Disease Database: An On-line Systems Biology Resource for Hypothesis Building and Testing**

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Institute for Systems Biology, USA; 2McLaughlin Research Institute, USA; 3Allen Brain Institute, USA; 4University of California, Department of Pathology, USA

Prions, as infectious agents, cause diseases such as bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease (CJD) in humans. The pathogenesis of prion diseases has been extensively studied in the past decades, and major breakthroughs have been achieved in understanding the disease mechanism. However, the pathogenesis of prion diseases remains largely unknown and controversial. The prion disease database (PDDB) is a novel database that integrates data from multiple sources, including prion-host combinations, prion strains, prion infection models, and clinical data. The PDDB provides a platform for hypothesis building and testing in the field of prion diseases. The PDDB is accessible at http://prion-database.org. The PDDB provides a comprehensive overview of prion diseases, including information on prion-host combinations, prion strains, prion infection models, and clinical data. The PDDB offers a wealth of information for researchers and clinicians in the field of prion diseases. The PDDB is a valuable resource for the study of prion diseases and provides a platform for hypothesis building and testing.
**P03.117**

**Could Alterations in Neuroglial Connections Explain Rapid Progression of Late Clinical Phase?**

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Neuropathogenesis of Prion diseases involves, on the one hand, the Prion protein itself and, on the other hand, neurons, astrocytes and microglial cells, by molecular mechanisms that remain poorly understood. To investigate these mechanisms, we used an integrative biology approach based upon information from public databases and specific data about Prion diseases to develop a theoretical model of Prion neuropathogenesis. Our first model describes successive molecular alterations leading to vicious circles that corrupt physiological stability.

To evaluate the neuropathological mechanisms predicted by this theoretical model, we used two complementary experimental approaches, based on the study of gene expression modifications and on immunohistochemical localization. As predicted by the model, these parameters appear to disorganize membrane-associated cytoskeleton components at an early stage of infection in the neurons, and at a later stage in activated astrocytes.

The implementation of experimental observations in an integrative biological algorithm, led to a new predictive model that describes a major alteration of intercellular connections notably concerning glial cells. Histological evaluations confirm a dramatic increase of neuroglial connections that form an interconnected network near PrPres accumulation brain locations. By using Electronocephalographic (EEG) investigation in infected mice, we demonstrate functional alterations of cerebral activities correlated with the increase of neuroglial connections.

Thus, our results based on convergence between a theoretical approach and experimental observations show for the first time functional alterations of neuronal connections which could lead, by toxic bystander effect, to the unexplained rapid aggravation of the clinical late phase. Neuroglial connections constitute an original target that could be applied to other neurologic disorders.

**P03.118**

**Differential Effect of Several Different Polyanionic Molecules on PrP Metabolism**

Sissors, L; Laramendy-Golzalo, C; Maujeul, M; Bonnet, C; Riffet, C; Papy-Garcia, D; Barateau, D; Deslys, JP

1CEA/DSV/IMETI/SEPIA, France; 2Laboratoire CRRET, CRNS FRE24-12, Université Paris XII-Val de Marne, France; 3OT3 sarl, France

Several anti-prion drugs have a proven efficiency in vitro but have exhibited disappointing results in vivo. Among these compounds, sulfated polysaccharides have been especially well studied because they are structurally related to glycosaminoglycans (GAGs) synthesized by cells and appear to compete with them in prion replication (Caughey et al., 1993). Their main drawback (related to size and degree of sulfation) is an anticoagulant side effect.

We tested the two most efficient previously described molecules (pentosan polysulfate (PPS) and CR36, Laramendy-Golzalo et al. 2007) versus natural molecules of respectively similar molecular weights, i.e. enoxaparin (MW 4.500) and heparin (MW 15.000). We analysed the influence of these compounds on PrP internalization and on PrPres decrease. The heaviest compounds (CR36 and heparin) were the most effective in curing cells. Moreover, we observed a correlation between PrPres decrease and the disappearance of PrPc from the cell surface. A similar subcellular relocalization of PrPc is seen in non infected cells. Three of these four molecules (CR36, heparin and PPS) induced an endo-lysosome of PrPC after treatment and a localization PrPc in rab6 positive compartments corresponding to Golgi network. However, only two of the molecules (PPS and heparin) induced a relocalization of a portion of PrPc with rab11 positive patterns corresponding to recycling endosomes. This could explain why PPS and CR36 act in a different way in vivo (Laramendy et al. 2007).

Synergistic effects differed for different combinations of these molecules. The elucidation of the mechanisms underlying the effect of anti-prion drugs is essential for the exploration of new therapeutic approaches.


**P03.119**

**Biochemical Properties of Wild Type Shadoo Protein from Mice**

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1Alberta Centre for Prions and Protein Folding Diseases, University of Alberta, Canada

Shadoo is a notional PrP-like protein encoded by the SPRN gene. Using a variety of antibodies we demonstrate that Shadoo is expressed as a glycosylated, GPI-anchored protein in the mature CNS. Shadoo undergoes endoproteolytic processing both in cultured cells and in the adult mouse brain to generate a C-terminal fragment somewhat analogous to the PrP “C1” cleavage product. The domain architecture of Shadoo is a notional PrP-like protein encoded by the SPRN gene. Using a variety of recombinant plasmids and was purified to homogeneity. While soluble resembling the domain architecture of PrP23-144: the latter molecule is synthesized in wild-type Shadoo, which comprises a signal peptide, a charged region, a region with somewhat analogous to the PrP “C1” cleavage product. The domain architecture of Shadoo is a notional PrP-like protein encoded by the SPRN gene. Using a variety of recombinant plasmids and was purified to homogeneity. While soluble resembling the domain architecture of PrP23-144: the latter molecule is synthesized in wild-type Shadoo, which comprises a signal peptide, a charged region, a region with somewhat analogous to the PrP “C1” cleavage product. The domain architecture of

Thus, our results based on convergence between a theoretical approach and experimental observations show for the first time functional alterations of neuronal connections which could lead, by toxic bystander effect, to the unexplained rapid aggravation of the clinical late phase. Neuroglial connections constitute an original target that could be applied to other neurologic disorders.

**P03.120**

**Prion Protein Anti-Tyr-Tyr-Arg Antibodies Improve Survival in a Mouse Model of BSE**

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Currently there are no therapeutic procedures for the fatal neurodegenerative prion disorders. Antibodies against the normal cellular prion protein (PrPc) can reduce conversion to the abnormal isomorf (PrPsc) in both in vitro and in vivo models of the disease. Previously we demonstrated that antibodies directed to a prion protein tyrosine-tyrosine-arginine (YYR) repeat motif selectively recognise PrPsc (Paramithiotis et al., 2003). We investigated the ability of anti-YYR antibodies to specifically neutralise infectivity in a murine model of BSE. At limiting infectivity titres pre-treatment of 301v disease. Previously we demonstrated that antibodies directed to a prion protein tyrosine-tyrosine-arginine (YYR) repeat motif selectively recognise PrPsc (Paramithiotis et al., 2003). We investigated the ability of anti-YYR antibodies to specifically neutralise infectivity in a murine model of BSE. At limiting infectivity titres pre-treatment of 301v brain homogenates with purified IgM and IgG anti-YYR antibodies increases the survival rate of VM mice following intracranial inoculation. Histopathological investigation demonstrated a reduction in the presence of vacuoles in the brains of these mice compared with those treated with control antibodies. Infectivity titres are decreased approximately 10 fold and the effects of neutralisation are not dependent on antibody class. Thus, differentially exposed prion protein YYR epitopes provide a putative target for selective binding of therapeutic antibodies during disease whilst potentially leaving the PrPc molecule still functional. These results are the first step in demonstrating the use of anti-YYR specific reagents in an immunotherapeutic model for prion diseases.

Paramithiotis et al., Nat Med 2003 9:893-899
Pathology and Pathogenesis

**P03.121**

**Sporadic Fatal Insomnia with Unusual Biochemical and Neuropathological Findings**

Giaccone, G; Manzieri, M; Priano, L; Limido, L; Brioschi, A; Albani, G; Pradotto, L; Fociani, P; Oss, L; Martara, P; Tagliavini, F; Mauro, A

1 Fondazione IRCCS Istituto Neurologico Carlo Besta, Italy; 2 IRCCS Istituto Auxologico Italiano, Italy; 3 Università di Milano, Ospedale Luigi Sacco, Italy; 4 Università di Torino, Dipartimento di Neuroscienze, Italy

Sporadic fatal insomnia (SFI) is a rare subtype of human prion disease, whose clinical and neuropathological phenotype is very similar to familial fatal insomnia (FFI). SFI patients reported until now were all homozygous for methionine at codon 129 of PRNP with deposition of type 2 PrPres (Parchi classification) in the brain. Here we describe a 56-year-old woman who died after a 10-month illness characterized by progressive drowsiness, cognitive deterioration, autonomic impairment and myoclonus. Polysomnography demonstrated a pattern similar to that described in FFI cases with loss of circadian pattern of sleep-wake cycle. A remarkable finding was that 20 years before the onset of the symptoms, the patient had undergone surgery for a colloid cyst of the third ventricle, and two ventricular shunts were placed, one correctly in the left ventricle, while the second ended in the right thalamus. The PRNP gene showed no mutation and methionine homozygosity at codon 129. The neuropathologic examination revealed neuronal loss, gliosis, and spongiosis that were mild in the cerebral cortex, while relevant in the caudate nucleus, putamen, thalamus, hypothalamus and inferior olives. In the thalami, the mediodorsal nuclei were more severely affected than the ventral ones. PrPres immunoreactivity was consistent in the striatum, thalamus and hypothalamus, patchy and of low intensity in the cerebral cortex and absent in the cerebellum. Western blot analysis confirmed this topographic distribution of PrPres. The bands corresponding to di- glycosylated, mono-glycosylated and non-glycosylated PrPres were equally represented. The non-glycosylated PrPres band had an electrophoretic mobility identical to that of type 1 by Parchi classification, in the multiple cortical and subcortical regions examined. These findings demonstrate the existence of further rare molecular subtypes of human prion diseases, whose characteristic may provide clues for the elucidation of the relation between biochemical characteristics of PrPres and clinico-pathological features of these disorders.

**P03.122**

**Attempts to Develop Cellular Models of Human Prion Infection**

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1 CEAD/IS/IMETI/SEPRA, France; 2 Alliance Biosecure Foundation, France

Cellular responses to prion infection are largely unknown, especially for human cells, which is partially due to the lack of cellular models infected by human Prions. Our goal was to develop cellular models infectable by human strains, using approaches aimed at 1) the development of new infection protocols and methods, 2) the adaptation of Prion strains to cells; and 3) modifications of cells to replicate Prions.

First, we verified on about forty cell lines from different species and tissues, using different infected human brain homogenates, that no PrPres accumulation could be obtained with classical protocols of infection. We then tested several other protocols on MM129 human cells varying methods of homogenate preparation, infectious load and culture conditions. Notably, we tried to infect human neuroblastomas with arrested growth, cultivated alone or with glial lines (human glialbiomas and astrocytomas) to mimic the in vivo situation in the brain.

In the second approach, to mimic the adaptation of strains to the host, we inoculated human cell cultures with different human TSE strains and on passage examined one part of the cell lystate for PrPres and used the remainder to establish the next passage.

As the two previous approaches relied on the assumption that the human cells tested were susceptible to infection (which may not be correct), we have also tested the possibility to modify the susceptibility of the cells to infection. We developed techniques to change the pattern of gene expression and to generate random genetic mutations. The modified cells were then exposed to different strains and PrPres accumulation was evaluated after several passages.

The experiments are ongoing and the preliminary data will be presented.

**P03.123**

**14-3-3 Protein Cerebrospinal Fluid Levels in Sporadic Creutzfeldt-Jakob Disease Differ Across Molecular Subtypes**

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Background: The 14-3-3 protein is a physiological cellular protein expressed in various tissues, and its release to cerebrospinal fluid (CSF) reflects excessive neuronal damage as in Creutzfeldt-Jakob disease (CJD), but also in other neurological diseases. Many studies suggested that the 14-3-3 protein in CSF in the proper clinical context is a reliable diagnostic tool for sporadic CJD. However, the sensitivity varies across molecular CJD subtypes.

Objective: We determined the level of the 14-3-3 protein in CSF from 70 sporadic CJD patients with distinct molecular subtypes by an enzyme-linked immunosorbent assay (ELISA) to prove whether different 14-3-3 levels might be helpful in the identification of different molecular subtypes.

Results: The 14-3-3 levels varied markedly across various molecular subtypes. The most elevated levels of 14-3-3 protein were observed in the frequently occurring and classical subtypes, whereas the levels were significantly lower in the subtypes with long disease duration and atypical clinical presentation. PRNP codon 129 genotype, PrPSc isoform, disease stage and clinical subtype influenced the 14-3-3 level and the test sensitivity. All heterozygous patients had 14-3-3 levels less than 3500 pg/ml irrespective of the PrPSc type and none of MV2 patients reached the level of 2300 pg/ml. At a cut-off of 2500 pg/ml, MV2 patients could be differentiated from MM1 patients. A tendency to lower 14-3-3 levels was observed in patients older than 60 years.

Conclusions: The 14-3-3 protein levels differ across molecular subtypes and in combination with other biomarkers might be used for their identification.

**P03.124**

**Use of Heparin Derivatives for Therapeutic Intervention of Transmissible Spongiform Encephalopathies**

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Several studies have indicated a role of glycosaminoglicans (GAGs) in the pathogenesis of transmissible spongiform encephalopathy (TSE). On the other hand, GAGs derivatives have been proposed for therapeutic approaches to TSE. We investigated the anti-prion activity of two heparin derivatives (ST 2808 and ST1830) in vitro and in vivo models. The two drugs were tested in comparison with low molecular weight heparin, enoxaparin in prion-infected murine neuroblastoma cells, (N2A) and in Syrian hamsters experimental scrapie. We found that ST2808 and ST1630 as well as enoxaparin, in a micromolar range, strongly reduced the accumulation of pathological prion protein (PrPSc) in N2A. Syrian hamsters were inoculated intraperioneally with 50 µl of 263K-infected brain homogenate at 10-2 and at 10-4 dilution and chronically treated with the drugs. The treatment started 1 hour or 24 hours after the inoculation and continued three times a week until the onset of symptoms in the control inoculated group. The treatment with ST2808 and ST1830 (2.5 mg/Kg, i.p) significantly prolonged the survival of the animals inoculated with homogenate at the higher dilution (10-2, median survival: control = 259 days, ST1830 = 475 days p< 0.001, ST2808 = 545 days, p<0.001) and in some cases the treatments completely abolished the cerebral accumulation PrPSc and the development of the disease. In contrast the treatment with the derivatives produced only a slight and not significant increase of the survival time (median survival: control = 203 days, ST1830 = 236 days, ST2808 = 226 days) when the animals were inoculated with the homogenate at lower dilution (10-2).

Although further conditions need to be tested before to draw a final conclusion, the results indicate that the heparin derivatives tested can be considered for therapeutic or prophylactic approach to TSE.
Terry, L1

Differential in gel analysis using DeCyder software (GE Healthcare) was indicative of an acute phase response (APR) occurring early in the disease course in (2D-DIGE). The biomarker for prion infection can be identified. In this study weekly collections of urine ideal fluid for surveillance provided a sufficiently sensitive and specific alternative to its ease of collection and comparatively less complex protein profile, is perhaps the to the development of diagnostic tests or intervention therapies is needed. Urine, due to the fact that it is present in extremely small amounts in accessible tissues, or in body fluids such as cerebrospinal fluid, blood and urine. Specific detection of these small amounts of the PrPd conformer is further exacerbated by the presence of a large amount of PrPd in rectal mucosa lymph nodes from white tail and mule deer is based on a PrPd-specific ligand for antigen capture.

Initial optimisation studies demonstrated that there was no significant loss of signal when biopsy samples of approximately 120mg were used, compared to the standard kit weight of 300mg. Release of PrPd from the tissue matrix was optimised by the addition of a large ceramic bead to the ribolysation step and by increasing the number of cycles. The addition of digestive enzymes was investigated but was found to have no significant effect on signal strength. Finally, an increase in time during the primary incubation step along with the addition of a gentle motion was used to optimise sensitivity.

In conclusion, we have developed a protocol for use on rectal mucosa lymphoid tissue using the IDEXX hercdhek EIA test kit that, once optimised, provided specificity and sensitivity figures of 99.2% and 93.5%, respectively, compared to IHC results in the same samples; sensitivity values reached 100% for sheep subjected to two sequential biopsies four months apart.


P03.126

Genome Scan for BSE Susceptibility and/or Resistance in European Holstein Cattle

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The use of PrPd as a pre-clinical, or general marker for surveillance is limited, due to the fact that it is present in extremely small amounts in accessible tissues, or in body fluids such as cerebrospinal fluid, blood and urine. Specific detection of these small amounts of the PrPd conformer is further exacerbated by the presence of a large excess of endogenous PrP. Thus the identification of alternative biomarkers applicable to the development of diagnostic tests or intervention therapies is needed. Urine, due to its ease of collection and comparatively less complex protein profile, is perhaps the ideal fluid for surveillance provided a sufficiently sensitive and specific alternative biomarker for prion infection may be identified. In this study weekly collections of urine were performed on ME-7 infected C57BL/6 mice and age matched controls. The extracted proteins were analyzed by 2-D fluorescence difference gel electrophoresis (2D-DIGE). Differential in gel analysis using DeCyder software (GE Healthcare) was used to identify differentially expressed proteins throughout disease progression. Gel spots of interest were isolated and identified by tandem mass spectrometry. The pattern of abundance and identity of some of the differentially abundant proteins are indicative of an acute phase response (APR) occurring early in the disease course in infected animals.

The work referenced was performed in partial fulfilment of the study “BSE in primates” supported by the EU (QLK1-2002-01098).
P03.130
Prion Protein Analysis in Familial Human Prion Diseases

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Background: The abnormal prion protein (PrP\textsuperscript{Sc}) found in the central nervous system (CNS) in sporadic CJD and variant CJD is characterised by a predominant protease-resistant core fragment size of either ~21kDa (type 1) or ~19kDa (type 2). These types may be further sub-classified according to the ratio of the three glycoforms as either A, when the mono- or non-glycosylated forms are the most abundant, or B in cases where the diglycosylated form predominates. According to one reading of the prion hypothesis, differences in disease phenotype are contingent on the presence of one of these PrP types in conjunction with the host genotype at codon 129 of the prion protein gene.

Objective: We have sought to examine tissues from cases of human prion disease associated with mutations in the PRNP gene to determine if these too may be easily classified according to the system employed for sporadic and variant CJD.

Methods: We have performed Western blot analysis of the PrP\textsuperscript{Sc} types in the CNS of a cohort of cases of human prion disease associated with mutations in PRNP, including cases of familial CJD, Gerstmann-Strassler-Scheinker disease and fatal familial insomnia. We have also had the opportunity to examine peripheral tissue involvement in a case of Gerstmann-Strassler-Scheinker disease.

Results: The results confirm that familial prion diseases exhibit a very broad range of possible PrP\textsuperscript{Sc} types: Some resemble those found in sporadic CJD, while others differ in fragment size, glycosylation ratio and protease sensitivity to those found in sporadic and variant CJD.

Conclusions: This implies that some mutations associated with familial human prion diseases affect prion protein metabolism in a manner that differs from the course of events that occur in sporadic and variant CJD.
**P03.135**

**Tubulovesicular Structures are a Consistent (and Unexplained) Finding in the Brains of Humans with Prion Diseases**

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Tubulovesicular structures appear to be disease-specific markers for all TSEs, including CJD (Liberski et al., 1990, 2003), GSS (Liberski & Budka, 1994), vCJD, FFI (Liberski et al., 2005), naturally and experimentally induced scrapie (Liberski et al., 1990), and BSE (Liberski et al., 1992). Most recently, virus-like structures closely similar to TVS were observed in cells cultured infected with scrapie and CJD (Manuelidis et al., 2007). Here we report ultrastructural findings in 21 brain biopsy or autopsy specimens from patients with TSEs, and in 18 specimens from patients with other neurodegenerative conditions, including a small coded series examined before the diagnosis was revealed.

In the first (coded) series, TVS were found in all five specimens of CJD and in none of four AD brains. In the second series, TVS were found in 12 of 13 specimens, including the single cases of FFI and vCJD: the only negative specimen was from the case of familial CJD. TVS were also found in all three archival cases of GSS and one autopsy FFI case of the third series, but were not found in any of the 14 non-TSE neurological control specimens.

Morphologically, TVS appeared as clusters of round or tubular 25-35 nm particles with light cores surrounded by darker ‘capsules’. When present, these aggregates were rare – approximately 1-2 TVS-containing neuronal processes per specimen (mostly dendrites, rarely, synaptic terminals). The finding of TVS in 23 of 21 cases of human TSE but in none of 18 non-TSE neurological control specimens extends our previous studies and confirms their specificity in both naturally occurring and experimentally induced TSEs.

The nature of TVS remains enigmatic and their pathogenetic role remains to be determined, but three observations are worth of comment: first, the appearance of TVS precedes that of other pathological phenomena, and their number increases through the incubation period in both rodent scrapie and CJD models; second, the 27-35 nm size of TVS corresponds well to 27-35 nm cut-off of ultrafiltration studies for infectivity; and third, TVS are not composed of the abnormal isomor of PrP.
Prion Protein Regulates the β-Secretase Cleavage of the Alzheimer’s Amyloid Precursor Protein

Aim: To investigate the mechanism by which PrP inhibits the action of BACE1.

Results: Neither PrPΔlGPI, which is not membrane attached, nor PrP-CRM, which is anchored by a transmembrane domain and is excluded from cholesterol-rich lipid rafts, reduced cleavage of APP, suggesting that to inhibit the BACE1 cleavage of APP PrP has to be localised to lipid rafts. Coimmunoprecipitation experiments demonstrated that PrP physically interacts with BACE1. However, PrP did not alter the activity of BACE1 towards a fluorogenic peptide substrate nor perturb the dimerisation of BACE1. Using constructs of PrP lacking either the octapeptide repeats or the 4 repeats, we investigated whether PrP alters the proteolytic processing of APP.

Conclusions: These data reveal a novel function for PrP in regulating the processing of APP through inhibition of BACE1. The increase in APP processing in cells expressing disease-associated forms of PrP and in scrape-infected brains raises the possibility that the increase in Aβ may contribute to the neurodegeneration observed in prion diseases.

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Pathology and Pathogenesis

P03.137

Transmission of BSE to Cynomolgus Macaque, a Non-human Primate; Development of Clinical Symptoms and Tissue Distribution of PrPSc

Background: The normal cellular function of the prion protein (PrP), the causative agent for scrapie and kuru, depends on the tertiary structure of the protein. The normal function of PrP is known to be regulated by the metabolism of the host cell, including the processing of APP through inhibition of BACE1. The increase in APP processing in cells expressing disease-associated forms of PrP and in scrape-infected brains raises the possibility that the increase in Aβ may contribute to the neurodegeneration observed in prion diseases.

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P03.139

Cellular Prion Protein Regulates the β-Secretase Cleavage of the Alzheimer’s Amyloid Precursor Protein

Aim: As both APP and PrP are cleaved by zinc metallocarboxypeptidases of the ADAM family, we investigated whether PrP alters the proteolytic processing of APP.

Results: Here we show that expression of PrP in SH-SYSY cells dramatically down-regulated the cleavage of APP by BACE1 and reduced the secretion of Aβ peptides into the conditioned medium by >92%. Conversely, siRNA reduction of endogenous PrP in 293 cells led to an increase in secreted Aβ. Furthermore, levels of Aβ were significantly increased in the brains of PrP null mice as compared with wild type mice. Two mutants of PrP, PrPΔG and PrPΔInA16, that are associated with familial human prion diseases, did not inhibit the BACE1 cleavage of APP. To investigate whether the Val/Met129 polymorphism in human PrP could alter the production of Aβ, brains from APPΔ30 mice with the human PrP Val/Met129 polymorphism were analysed. In the MM mice there was a significant increase in Aβ in the brains as compared with the wild type mice. In the brains of two strains (79A and 87V) of scrapie-infected mice there was complete correlation between those that could restore BACE1 cleavage of APP and those that bound PrP.

Conclusions: These data suggest a possible mechanism by which PrP regulates the β-secretase cleavage of APP through the N-terminus of PrP interacting via GAGs with one or more of the heparin binding sites on BACE1 within a subset of cholesterol-rich lipid rafts, thereby restricting access of BACE1 to APP.

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P03.140

Prion Protein Regulates the β-Secretase Cleavage of the Alzheimer’s Amyloid Precursor Protein through Interaction with Glycosaminoglycans

Aim: To investigate the mechanism by which PrP inhibits the action of BACE1.

Results: Neither PrPΔlGPI, which is not membrane attached, nor PrP-CRM, which is anchored by a transmembrane domain and is excluded from cholesterol-rich lipid rafts, reduced cleavage of APP, suggesting that to inhibit the BACE1 cleavage of APP PrP has to be localised to lipid rafts. Coimmunoprecipitation experiments demonstrated that PrP physically interacts with BACE1. However, PrP did not alter the activity of BACE1 towards a fluorogenic peptide substrate nor perturb the dimerisation of BACE1. Using constructs of PrP lacking either the octapeptide repeats or the 4 repeats, we investigated whether PrP alters the proteolytic processing of APP.

Conclusions: These data reveal a novel function for PrP in regulating the processing of APP through inhibition of BACE1. The increase in APP processing in cells expressing disease-associated forms of PrP and in scrape-infected brains raises the possibility that the increase in Aβ may contribute to the neurodegeneration observed in prion diseases.

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P03.138

Clustering of PrPres in Central Brain Regions of BSE-infected Macaques (M. Fuscicattus)

Aim: To investigate the mechanism by which PrP inhibits the action of BACE1.

Results: Neither PrPΔlGPI, which is not membrane attached, nor PrP-CRM, which is anchored by a transmembrane domain and is excluded from cholesterol-rich lipid rafts, reduced cleavage of APP, suggesting that to inhibit the BACE1 cleavage of APP PrP has to be localised to lipid rafts. Coimmunoprecipitation experiments demonstrated that PrP physically interacts with BACE1. However, PrP did not alter the activity of BACE1 towards a fluorogenic peptide substrate nor perturb the dimerisation of BACE1. Using constructs of PrP lacking either the octapeptide repeats or the 4 repeats, we investigated whether PrP alters the proteolytic processing of APP.

Conclusions: These data suggest a possible mechanism by which PrP regulates the β-secretase cleavage of APP through the N-terminus of PrP interacting via GAGs with one or more of the heparin binding sites on BACE1 within a subset of cholesterol-rich lipid rafts, thereby restricting access of BACE1 to APP.

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P03.148

Pathogenesis of Clinical Symptoms and Tissue Distribution of PrPSc

Two of three cynomolgus monkeys developed abnormal neurological behavioral signs at 30-97 (78±110) days after inoculation. Of these monkeys, one monkey lost weight and showed movement difficulty, and another monkey walked with an ataxia-like gait. However, the movements of the third monkey were normal, but it showed tremor and hyperkinesia. At 102±118 days after inoculation, one monkey lost weight and showed movement difficulty, and another monkey showed tremor. The third monkey showed neither weight loss nor movement difficulty. All three monkeys eventually died. The post-mortem findings revealed that PrPSc was accumulated in the CNS and in lymphatic organs such as lymph nodes, spleen, adrenal glands and thymus although PrPSc was barely detected in the submandibular lymph node of #7 monkey. Such confined distribution of PrPSc was compatible to that reported on the Cynomolgus macaques infected with BSE by oral inoculation. The lesion profile resembles that of vCJD. Recently, oral infection of M. fascicularis with macaque-adapted BSE material was reported. In cooperation with five European partners a quantitative study for the transmission of the BSE agent to M. fascicularis was initiated to assess the risk of vCJD infection in humans through contaminated food products (EU study QLK1-CT-2002-01089). Titration was performed orally and intra-naturally to determine the minimal infectious dose for cynomolgus monkeys.

Here we report the outcome of the intracerebral infection with 50 mg BSE brain homogenate in six non-human primates. All animals showed clinical symptoms of TSE within an average of 1100 days. Using immunohistological and biochemical methods prior prion protein (PrP) deposits were confirmed in the brains of all animals. Using Western blot analysis the glycosylation pattern was compared to the inoculum and to the pattern of different CJD subtypes. Simian PrPres was detected with the monoclonal anti prion antibody 11C6, which revealed a higher sensitivity in comparison to 12F10 and 3F4. We found that the PrP glycopattern in BSE-infected cynomolgus macaques resembled human CJD type 2. We further analysed the distribution of PrPDep by microdissection of seven different brain regions of all infected macaques. High concentrations of PrPDep were detected in central brain regions, as gyrus cinguli, nucleus caudatus, cerebellum and basis pontis. In contrast, in the peripheral regions gyrus frontalis, gyrus parietalis and gyrus occipitales PrPDep was hardly detectable.

Thus, the incubation period related to the life expectancy, the PrPres glycosylation pattern as well as the distribution in certain brain regions resemble those in vCJD patients. The relative abundance of PrPres in macaques will be compared to that of orally infected animals.
**P03.141**

Aspects of the Cerebellar Neuropathology in Nor98

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Nor98 is a prion disease of old sheep and goats. This atypical form of scrapie was first described in Norway in 1998. Several features of Nor98 were shown to be different from classical scrapie including the distribution of disease associated prion protein (PrP\(^{\alpha}\)) accumulation in the brain. The cerebellum is generally the most affected brain area in Nor98. The study here presented aimed at adding information on the neuropathology in the cerebellum of Nor98 naturally affected sheep of various genotypes in Sweden and Norway. A panel of histochemical and immunohistochemical (IHC) stainings such as IHC for PrP\(^{\alpha}\), synaptophysin, glial fibrillary acidic protein, amyloid, and cell markers for phagocytic cells were conducted. The type of histological lesions and tissue reactions were evaluated. The types of PrP\(^{\alpha}\) deposition were characterized. The cerebellar cortex was regularly affected, even though there was a variation in the severity of the lesions from case to case. Neuronal vacuolation was more marked in the molecular layer, but affected also the granular cell layer. There was a loss of granule cells. Punctate deposition of PrP\(^{\alpha}\) was characteristic. It was morphologically and in distribution identical with that of synaptophysin, suggesting that PrP\(^{\alpha}\) accumulates in the synaptic structures. PrP\(^{\alpha}\) was also observed in the granule cell layer and in the white matter. The pathology features of Nor98 in the cerebellum of the affected sheep showed similarities with those of sporadic Creutzfeldt-Jakob disease in humans.

**P03.143**

Effectiveness of Capillary Electrophoresis Fluoroimmunoassay of Blood PrPSc for Evaluation of Scrapie Pathogenesis in Sheep

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Background: Development of a blood test for transmissible spongiform encephalopathies (TSEs) remains a high priority in order to minimize the possibility of iatrogenic transmission through blood transfusion, to further understanding of pathogenesis, and to facilitate pre-clinical diagnosis and management of disease in both humans and livestock. An immuno capillary electrophoresis (ICE) technique (capillary electrophoresis fluoroimmunoassay) has shown appreciable promise for detection of abnormal prion protein, and presents the possibility of diagnostic application and of advancing our limited understanding of the relationship between PrP genotype, infectivity, and the presence of PrP\(^{\alpha}\) in blood.

Aim: To apply the ICE technique to investigate in detail the temporal occurrence of blood PrP\(^{\alpha}\) with disease progression and thus to establish the potential of the test for pre-symptomatic scrapie diagnosis.

Methods: ICE was used to analyse PrP\(^{\alpha}\) in the white cell (buffy coat) fraction of blood, in a routine laboratory environment. The test sheep population was of various breeds (Swaledale, Welsh Mountain and Dorset) and PrP genotypes (ARQ/ARQ, ARQ/VRQ and VRQ/VRQ) which had been exposed to natural infection from birth. Control sheep were direct progeny of scrapie-free New Zealand stock.

Results: Analysis of extracted blood from test sheep (n=87, 347 samples) at various stages of incubation, and from control sheep (n=194, 489 samples), indicated that overall, test values for these populations were not significantly different, and that a similar proportion of control (7%) and test (10%) sheep were classified as positive. Stages of incubation, and from control sheep (n=194, 489 samples), indicated that over 2-3 month intervals from birth until clinical disease, test specificity and sensitivity and detection of PrP\(^{\alpha}\) in cases where standard western immunoblot detection do not rely on proteolysis are required.

Methods: By immunisation with an altered conformation of the human prion protein, PrP\(^{\alpha}\), is a reliable marker of prion disease and its detection has become a standard diagnostic method. A major limitation of this strategy is that amino acid sequence identity between the normal cellular form of the prion protein, PrP\(^{\beta}\), and PrP\(^{\alpha}\) results in cross-reactivity in immunoassays. The low abundance of PrP\(^{\alpha}\) against a background of ubiquitously expressed PrP\(^{\beta}\) typically requires depletion of PrP\(^{\beta}\), usually by proteolytic digestion of the diagnostic specimen, limiting the sensitivity of diagnostic assays. It is also now apparent that protease-sensitive pathological isoforms of PrP\(^{\alpha}\) may have a significant role in prion diseases and therefore new diagnostic tests that do not rely on proteolysis are required.

Results: Use of this novel antibody allows accurate diagnosis of vCJD with 100% sensitivity and specificity and detection of PrP\(^{\alpha}\) in cases where standard western blot detection fail to detect protease resistant PrP\(^{\alpha}\).

Discussion: We have isolated and characterised an antibody that is selective for disease-associated PrP\(^{\alpha}\) and we describe a method for the diagnosis of CJD which does not require PrP\(^{\beta}\) depletion by proteolysis or other methods. This antibody may facilitate the sensitive detection of PrP\(^{\alpha}\) in peripheral tissues or fluids where the ratio of PrP\(^{\alpha}\) to PrP\(^{\beta}\) is high and proteolysis does not provide sufficient discrimination.
In transmissible spongiform encephalopathies, neurons are supposed to be the main target for different strains of the infectious agent. However, it is possible that some strains may replicate in different cell types before spreading into neurons. To investigate this hypothesis, we have used a non neuronal cell line. These cells responded differently to infection with a number of scrapie strains suggesting that strains may have different cellular tropism for different cell types of the brain. Surprisingly, persistently infected cells were then able to kill wild type or PrP knock-out primary neurons in co-culture showing how some strains may spread between different cells with PrP independent mechanisms. Moreover, we are also using different co-culturing systems between persistently infected non neuronal cells and primary neurons to investigate the mechanisms by which different strains can cause neurodegeneration.

The data presented here may provide new information about TSE infectious process showing how different TSE strains can have alternative mechanisms to infect cells and cause neurodegeneration. These results may also be important for future therapeutic and diagnostic approaches for this kind of infectious diseases.
**Pathology and Pathogenesis**

**P03.149**

PRP Expression in Schwann Cells is not Required for Transmissible Spongiform Encephalopathy Neuroinvasion

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Transmissible spongiform encephalopathies (TSEs) such as scrapie are neurodegenerative diseases characterised by long incubation periods and spongiform vascular pathology in the central nervous system (CNS). The expression of prion protein (PrP) is a requirement for the establishment and development of TSE disease. The process of agent replication in the periphery and invading the CNS is termed neuroinvasin. The exact mechanism of transport of the infectious agent is still unknown, and the roles of various cell types involved in this process are still to be determined. The TSE neuroinvasive process is postulated to be dependent upon a continuous chain of PrP expressing tissue between the infection site and the CNS, with the most likely candidate being the peripheral nervous system (PNS). However, measured transport rates of infection do not match rates of fast or slow axonal transport suggesting some other mechanism of transport. Myelinating Schwann cells of the PNS express PrP when associated with PrP expressing nerve axons and are capable of serially propagating the agent in vitro.

Transgenic mice possessing a Schwann cell specific knockout of the PrP gene were produced using the Cre / loxP system to test the hypothesis that Schwann cells are involved in TSE neuroinvasion. Following Cre-mediated recombination in these mice a 90 % reduction in total PrP expression and an altered PrP glycoform pattern were observed in peripheral nervous tissue. These mice were infected with mouse-adapted scrapie agents ME7 and 139A via intracerebral, intraperitoneal and oral routes of infection. No differences in incubation period or central nervous system pathology were observed when compared with peripherally infected control genotype mice. These results suggest that PrP expression in Schwann cells is not required for TSE neuroinvasin.

**P03.150**

There is Differential Gene Expression in Cattle White Blood Cells During Preclinical BSE Infection

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Differential gene expression within peripheral tissues of BSE infected cattle could provide the means of identification for early BSE infection. The oral route of infection by the infected agent (PrPres) passes from the gastrointestinal system to the CNS via the lymphoreticular system. Little is known of the effect on gene expression of the host organs during this early infection period: although there is some evidence of decreased levels of erythroid differentiation-related factor (EDRF) in the spleen and erythroid cells in the blood of TSE infected mice. Therefore there is a possibility of using differential gene expression in peripheral tissues as an early diagnostic test for BSE infection. In this project we are investigating the differential gene expression between normal and BSE infected cattle using RNA isolated from white blood cells. Blood samples were collected from a BSE challenge set up at Greifswald, Germany using Simmental cattle. Samples were collected from 7 months post infection and subsequently every 2 months during the BSE incubation. Initially samples were fractionated into PBMs (peripheral blood monocytes) and at later time points individual white blood cells were separated into B cells, T cells and macrophages. RNA isolated from the blood samples was used to probe the Affymetrix Bovine Genome Array. Analysis of the microarray experiments produced gene lists of significantly differentially expressed genes. There are many genes which have varying expression patterns throughout the time course however there are some genes which are consistently down regulated across most cell types for example TRYP8(pancreatic anionic trypsinogen), QPCR studies have confirmed the TRYP8 expression patterns and further QPCR experiments are being performed to follow up related proteins to investigate the significance of this finding. Other genes found to be significantly up regulated include members of the chemokine family, both ligands and receptors and immune response pathways. Again these have been confirmed by QPCR and are being further investigated using related proteins by QPCR. It is hoped that these findings are due to the specific disease pathology of BSE during preclinical infection and if this is the case then the differential gene expression could be used as an early signal for the incubation of BSE in cattle.

**P03.151**

A Multicentre Virtual Bank of Animal Tissues and Fluids

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One of the major activities of this Network of Excellence has been the establishment of a virtual bank of animal TSE samples. This objective has been achieved, and is being supported by more than twenty institutes representing sixteen countries of NeuroPrion members, and some associate members. The principal goal of this work is to facilitate contact between research groups by publicising the generic content of stores of potentially available TSE material, enabled by centralised data collection software. The database is located within eDoc on the NeuroPrion website. Researchers can access pdf files that describe the type of bovine, ovine or caprine samples held by each institute, collection protocols, blood stabilisers used and storage media. Email addresses provide links to the primary points of contact at each site, although the publication of such information does not guarantee access to samples, and will be subject to the release conditions operated by each institute.

**P03.152**

Polymorphism at Codon 129 of PRNP in a Population of Patients Initially Suspected of Creuzfeldt-Jakob Disease

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Human prion diseases are rare neurodegenerative fatal diseases. The most frequent is Creuzfeldt-Jakob disease (CJD) that exists in a sporadic, inherited or acquired form (iatrogenic or variant). A biallelic polymorphism exists at codon 129 of the prion protein gene (PRNP), located in 20ter-p12, coding either a valine or a methionine. In patients with sporadic CJD, homozogyous represent about 80% with a predominance of 129 Met/Met, while in controls, they represent 50% in accordance with the Hardy Weinberg equilibrium. Moreover, all patients with clinical variant CJD up to day are 129 Met/Met.

The aim of this study was to determine the repartition of 129 polymorphism in a French population initially suspected of CJD (retrospective study: 1997-2008). The genotype was determined either by PCR-RFLP or DGGE in 1015 patients for which CJD diagnosis was finally discarded at posteriori (mean age : 68 y) and in 391 patients with definite sporadic CJD (mean age : 63.8 y).

This is the first study conducted in this type of population. The methionine allelic frequency was estimated at 0.63 in non-CJD patients; heterozygous patients represent 50%, Met/Met patients 40%, Val/Val 10%. This distribution is quite similar to that previously observed on a population of blood donors in France [1] or in other European studies[2].

In conclusion, PRNP 129 genotype differs in two population of similar age, patients initially suspected of CJD, and definite sporadic CJD. This study confirms the specificity of overrepresentation of homozogyous in CJD. Moreover, it confirms that PRNP genotype repartition do not vary with the age class of patients.

Analysis of Gene Expression Profiles in CNS of Naturally Scrapie Infected Sheep Using a Sheep cDNA Microarray

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The underlying mechanisms of scrapie pathogenesis and many neurodegenerative diseases are still poorly understood. The identification of genes with differential expression in CNS of infected animals might provide clues to clarify the molecular mechanisms that lead to neuronal loss, being useful for future therapies and to identify molecular biomarkers that might be the basis for new diagnostic tests. We present here an initial study on the transcriptional differences in cerebellum obtained from naturally infected Scapie sheep using cDNA microarray hybridizations. We have used the sheep cDNA microarray generated at CIDC-Lelystad (communication of Bosser et al., Prion2006). Total RNA of cerebellum was isolated from 5 control sheep and 9 infected sheep. According to IHC characteristics on cerebellum (CER), spleen (SP) and mesentery lymph node (MN) of infected sheep their RNAs were grouped into 4 pools (1: +CER, +SP, +MN; 2: -CER, -SP, -MN; 3: +CER, +SP, -MN; 4: -CER, +SP, +MN). The remaining (5) pool was formed with the 5 controls. The five RNA pools were hybridized against a universal reference RNA, after cDNA synthesis and fluorescent labeling. We compared “in silico” gene expression of the 4 positive groups against the control group. One common clone was identified to be up-regulated within the 4 diseased groups and two clones identified as down-regulated. In the two groups with PrPSc deposit on cerebellum (1 and 3) we found 2 up-expressed clones and 4 down-expressed clones in common. The other two groups (2 and 4) shared 6 clones with a significant expression increase and 3 clones with decreased expression. The differentiated clones were compared with the GeneBank database and some of them showed similarity with known human and bovine genes. Small sequences of genes GMPS, RPL32 and ATP6AP2 aligned with down-expressed clones. By contrast, the genes GNB2L1, HSAP1, RPS3 and FN1 have similarity with up-expressed sequences. The expression of 11 common clones has been analyzed by Real Time PCR in order to confirm these previous results. Moreover, the expression of these genes has been analyzed in 5 controls and 9 scrapie animals; GMPS and two un-known sequences showed significant differences between both groups.

Immunotherapy- Active and Passive Immunisations in Prion Disease

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Background: The observation that vCJD is transmissible by blood transfusion has highlighted the need to find a treatment for prion diseases. Although no immune response is observed in prion disease due to tolerance of nascent PrPSc, active immunisation in some models has provided partial protection. We have already produced monoclonal antibodies (mAb) ICSM18 (IgG1 to epitope 142-153) and ICSM33 (IgG2b to epitope 93-105) to recombinant (r) human and mouse PrP and their corresponding amplitudes were determined. At post infection day (PID) 57-75 splenic PrPSc decreased in r PrP immunised FVB/N and SJL mice. Conclusion: (1) Passive immunisation remains a realistic therapy for prion disease, particularly after optimising the dose and timing of mAb therapy, and investigating possible side-effects. Studies are underway to determine the potential use of Ab fragments and translocation across the blood brain barrier. (2) Although r PrP immunised SJL mice survived longer than untreated mice, PrPSc levels and infectivity eventually increased leading to disease. These promising results suggest that active immunisation has potential as a therapeutic modality.

A Highly Sensitive and Specific ELISA for the Determination of Prion Infection in Human Samples without the Use of Proteases

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Background: A highly sensitive assay for the detection of PrPSc is vital for the early diagnosis of Prion disease, as well as for the screening of blood and organs. Currently the best diagnostic tool for prion disease is fonsil biopsy and Western Blotting. Proteinase K (PK) digestion is required to distinguish between PrPSc and PrP, however, protease digestion can degrade up to 70% of the target antigen. Rapid detection of PrPSc in blood without protease digestion requires the development of a more sensitive assay.

Aims: Our aim was to increase the sensitivity and throughput of immunoonsays for the detection of PrPSc in blood. In particular, identify conditions for the discrimination of PrPSc from PrP without protease digestion. The assays have been optimised for the detection of vCJD brain homogenate spiked into whole blood from unaffected donors. In addition the ELISA assay has been coupled to the immunoprecipitation (IP) of vCJD tissue from large volumes of blood.

Methods: vCJD brain homogenate diluted into whole blood was analysed by enzyme-linked immunosorbent assay (ELISA). PrPSc was selectively captured without PK digestion, and detected with a fluorogenic substrate. PrPSc was recovered by IP of vCJD brain homogenate spiked into up to 8ml of whole blood and analysed by ELISA.

Results: The optimised ELISA, which utilises unique antibodies, allows detection of PrPSc from vCJD brain homogenate serially diluted into whole blood down to picogram levels. Combining the ELISA with quantitative recovery of vCJD homogenate by IP, indicates detection of PrPSc at extremely low concentrations is possible from large volumes of whole blood.

Discussion: We have developed an assay which allows the rapid, sensitive and selective detection of PrPSc at biologically relevant levels. Coupled with selective capture of PrPSc from clinically practical volumes of blood allows detection at a level equivalent to a 10^6 fold dilution of brain.

Flash Visual Evoked Potential Recordings Reveal No Evidence of Visual Impairment in Bovine Spongiform Encephalopathy

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Background: Disease-associated prion protein has been detected in the visual pathways of sheep with natural scrapie and human patients suffering from sporadic or variant Creutzfeldt-Jakob disease. In addition, bovine spongiform encephalopathy (BSE)-sensitive transgenic mice challenged with retina and optic nerve from a terminally diseased cow with BSE have succumbed to disease. Over-reactivity to visual stimuli is also a common clinical feature of BSE. This suggests that the visual pathways are affected by spongiform encephalopathies, which could be assessed clinically by measuring flash visual evoked potentials (FVEP).

Objective: To determine if BSE causes abnormalities in the visual pathways that could be detected by measuring FVEP.

Material and Methods: As part of a pilot study, FVEP were recorded from 16 adult Friesian steers from an experimental BSE study where steers were orally dosed with various single doses of BSE brainstem homogenate: Six steers displayed neurological signs consistent with BSE and with confirmation of the disease by immunohistochemistry and Western immunoblot, six were culled due to intercurrent diseases without confirmation of the disease, and four were control cattle, either unchallenged environmentally or orally challenged with BSE-free brain (n=1). Animals were not sedated during the recording. The stimuli were flashes of white light at 1.5 Hz, and one hundred evoked responses were averaged twice for each eye. The latencies of the positive and negative peaks (P1-3 and N1-2) and the corresponding amplitudes were determined.

Results: The generated waveforms were highly variable between individual cattle. The differences in the peak latencies and amplitudes between steers affected by BSE and all steers without pathological confirmation or control cattle were statistically not significant (P>0.05, Mann-Whitney U test).

Conclusion: A dysfunction of the visual pathways in cattle with BSE was not evident when based on single measurements of FVEP. The high variability of waveforms makes interpretation very difficult but may be overcome if repeated measurements are taken during the course of the disease.
P03.157
Monoclonal Antibodies Define Conformational Heterogeneity of the Normal Prion Protein
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The physiological function of the cellular prion protein PrPC is still unclear. Seemingly opposing functions, namely toxic or protective ones have been proposed. These effects could be explained by PrPC adopting several stable conformations which was shown in vitro translation studies; here, PrP mRNA is translated into secretory GPI-anchored secreted PrP and the two transmembrane isoforms NTMPrP and CTMPrP. Due to the absence of specific ligands to these isoforms definite proof of conformational heterogeneity of PrPC in vivo has not been demonstrated.

The aim of our study was therefore to provide such ligands and use them to characterize the cell biology of these conformers. Immunoprecipitation of in vitro translated PrP yielded two hybrodina cell lines secreting:

a.) mAb1B910 exclusively recognizing a conformational epitope of an unglycosylated PrP subpopulation overlapping with the NTMPrP from in vitro translation studies. Immunofluoresence staining of cells by overexpressing NTMPrP-favoring PrP constructs showed distinct staining patterns defining a distinct cellular compartment for NTMPrP. Pull-down experiments with recombinant antibody fragments of these antibodies enabled us to identify a specific ligand for NTMPrP.

b.) mAb19C3 recognizing CTMPrP. Using 19C3 we were able to selectively immunoprecipitate CTMPrP from scrapie-infected, but not normal mouse brains suggesting close association of CTMPrP with prion infection and suggesting neurotoxic potential of this conformer.

We thus provide evidence that “normal” PrPC consists in at least three different conformers in vivo. PrPCs conformational heterogeneity is used to differentially carry out biological functions.

P03.158
Transport and Clearance of Hamster-adapted Transmissible Mink Encephalopathy following Intraperitoneal Inoculation
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Background: Following peripheral routes of infection, the abnormal isoform of the prion protein (PrPsc) is initially detected in secondary lymphoreticular system (LRS) tissues. Prion replication within secondary LRS tissues is thought to aid in the efficiency of neurotropism. Intraperitoneal (i.p.) inoculation of the dowry (DY) strain of hamster-adapted transmissible mink encephalopathy (TME) resulted in a failure to detect DY PrPsc in spleen, submandibular lymph node or mesenteric lymph node by Western blot analysis, immunohistochemistry or animal bioassay at 60 days post-infection.

Objectives: The aim of this study is to determine the distribution of PrPsc in secondary LRS tissues during the early stages of infection.

Methods: Following i.p. inoculation of the hyper (HY) and DY TME agents, peritoneal cells, medial iliac lymph node, mesenteric lymph node, and spleen were collected from 1 hour to 20 weeks post-infection. We have analyzed these tissues for the presence of PrPsc by Western blot analysis. We also employed a macrophage cell culture model system to study the uptake and degradation of PrPsc following TME infection of these cells.

Results: Following i.p. inoculation, HY PrPsc is present in the spleen, medial iliac lymph node, and mesenteric lymph node through 32 hours post-infection, DY PrPsc is detectable in the spleen and medial iliac lymph node through 4 hours and 32 hours post-infection, respectively. In all tissues, both HY and DY PrPsc fall below the levels detectable by Western blot analysis by 64 hours post-infection. In the cell co-culture model system, PrPsc levels of the cells peak at 24 hours post-infection, while levels of PrPsc in the media decrease through 72 hours post-infection.

Conclusion: HY and DY PrPsc are detected in secondary LRS tissue at early time points post-infection, indicating that both agents are transported to these tissues. We have also studied degradation of PrPsc using an in vitro macrophage model. We have shown that there is no significant difference between HY and DY PrPsc uptake and degradation in this model system. These combined findings suggest that the inability of HY TME to cause disease following i.p. inoculation may be a result of retention and/or replications within secondary LRS tissues.

P03.159
RNAi-Mediated Knockdown of Genes Essential to Prion Protein Pathogenesis Enhances Neuroblastoma Resistance to PrP<sup>Sc</sup> Cytotoxicity
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The resistance of PrP knockout mice (prnp<sup>−/−</sup>) to prion infection has demonstrated that the product of the prnp gene, PrP<sup>SC</sup>, is essential to prion pathogenesis. Nonetheless, the molecular mechanisms resulting in prion-induced neurodegeneration remain poorly understood.

We hypothesize that reduced expression of genes essential to prion pathogenesis will render neuronal cells resistant to PrP<sup>Sc</sup> peptide mediated toxicity. Initial results targeting prnp mRNA demonstrate that the degree of prnp mRNA knockdown correlates to neuronal cell resistance to PrP<sup>Sc</sup>-induced exposure. Furthermore, the region of mRNA targeted and the structure of the inhibitor plays a role in determining the efficiency of mRNA knockdown. The knowledge gained from this pilot study was applied to the high throughput screening of a library of mRNA inhibitors.

In this study, we have used complementary RNA inhibitors to reduce the levels of expression of specific mRNA. Multiple sites along the mRNA were targeted to ensure that at least one reduced mRNA expression sufficiently. Pure neuronal cell populations containing the targeted inhibitors were selected via antibiotic resistance and then were exposed to 80µM PrP<sup>Sc</sup>-15 peptide. Resistance to PrP<sup>Sc</sup>-15 induced cell death was determined by the ability of resistant cells to re-grow following challenge. PrP<sup>Sc</sup>-15 resistant inhibitor expressing clones permitted identification of genes non-essential for host viability, but required for prion-induced cytotoxicity.

P03.160
Treatment of Scrapie-Infected Mice with the γ-Secretase Inhibitor LY411,575 and Quinacrine Prevents Spread of PrP<sub>Sc</sub> and Neuropathology
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PrP<sub>Sc</sub> is linked to dendrite loss in prion disease by activation of Notch-1 signaling pathways. Notch-1 activation is a multistep process culminating in release of its Intracellular Domain NICD by γ-secretase cleavage. Here we tested the hypothesis that dendritic degeneration is primarily the result of PrP<sub>Sc</sub>-driven activation of Notch-1 signaling pathways. CD1 mice were intra-thalamically inoculated with RML prions. Mice were treated with γ-secretase inhibitor (G) alone or in combination with quinacrine (Q). The drugs were given ad libitum in chocolate drink continuously for 60 days beginning 50 days postinoculation. Results: (1) PrP<sub>Sc</sub>: G alone did not reduce neocortical (Nc), hippocampal (H), or thalamic (Th) PrP<sub>Sc</sub>. In Nc, G alone produced ~50% decrease in PrP<sub>Sc</sub> and G+Q a significant 95% decrease. In Th, G alone increased PrP<sub>Sc</sub> by ~30%, which taken together with the ~50% decrease in Nc, argue that G represses axonal transport of PrP<sub>Sc</sub>. (2) NICD: In Nc, G alone did not reduce NICD signaling pathways. CD1 mice were intra-thalamically inoculated with RML prions. Mice were treated with γ-secretase inhibitor (G) alone or in combination with quinacrine (Q). The drugs were given ad libitum in chocolate drink continuously for 60 days beginning 50 days postinoculation. Results: (1) PrP<sub>Sc</sub>: G alone did not reduce neocortical (Nc), hippocampal (H), or thalamic (Th) PrP<sub>Sc</sub>. In Nc, G alone produced ~50% decrease in PrP<sub>Sc</sub> and G+Q a significant 95% decrease. In Th, G alone increased PrP<sub>Sc</sub> by ~30%, which taken together with the ~50% decrease in Nc, argue that G represses axonal transport of PrP<sub>Sc</sub>. (2) NICD: In Nc, G alone reduced NICD levels ~90% relative to untreated RML-infected mice, consistent with its known effects on γ-secretase activity. In Th, no significant effects were found, although G alone reduced NICD to levels seen in infected controls. (3) Dendrite loss: Golgi silver stained dendrites were quantified. G did not prevent dendrite loss in any region, even though it reduced NICD by 90%. This argues that the Notch-1 repressor pathway is not the only cause of dendritic degeneration. G prevented 80% of the dendrite loss, suggesting that Q affects not-Notch-1 pathways which mediate dendrite degeneration. Dual G+Q treatment increased the number of dendrites 10-20% above that in untreated controls in all regions studied, suggesting that it not only prevented dendrite loss but also stimulated new growth. These results argue that G+Q act synergistically to prevent spread of scrapie in the brain. (Supported by: NIH Grants AG021601, AG02132, AG10770, and AG023501 and by the Stephen C. and Patricia A. Schott Foundation).
**Pathology and Pathogenesis**

**P03.161**

**Species-specificity of a Panel of Prion Protein Antibodies for the Immunohistochemical Study of Animal and Human Prion Diseases**

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Monoclonal antibodies to the prion protein (PrP) have been of critical importance in the neuropathological characterization of PrP-related disease in men and animals. To determine the influence of species-specific amino-acid substitutions recognized by monoclonal antibodies, and to investigate the immunohistochemical reactivity of the latter, analyses were carried out on brain sections of cattle with bovine spongiform encephalopathy, sheep with scrapie, mice infected with scrapie, and human beings with Creutzfeldt-Jakob disease (CJD) or Gerstmann-Sträussler-Scheinker disease (GSS). Immunoreactivity varied between the antibodies, probably as the result of differences in the amino-acid sequence of the prion protein in the various species. Some monoclonal antibodies against mouse recombinant PrP gave strong signals with bovine, ovine and human PrP(Sc), in addition to murine PrP(Sc), even though the amino-acid sequences determined by the antibody epitope are not fully identical with the amino-acid sequences proper to the species. On the other hand, in certain regions of the PrP sequence, when the species-specificity of the antibodies is defined by one amino-acid substitution, the antibodies revealed no reactivity with other animal species. In the region corresponding to positions 134-159 of murine PrP, immunohistochemical reactivity or species-specificity recognized by the antibodies may be determined by one amino acid corresponding to position 144 of murine PrP.

**P03.162**

**Neurodegeneration in a Transgenic Mouse Model of Inherited Prion Disease**

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Impairment of the ubiquitin-proteasome system (UPS) is proposed to play a pathogenic role in prion diseases. In this study we monitored the functional status of the UPS in TgPG14(+) mice, which express a nine-octapeptide insertion associated with an inherited prion disease. These mice produce a misfolded form of the mutant protein in their brains. As this form accumulates the mice develop a fatal neurological illness characterized by massive apoptosis of cerebellar granule neurons. To test whether neurodegeneration in TgPG14(+) mice was associated with reduced function of the UPS, we generated mice co-expressing PG14(+) and a green fluorescent protein (GFP) reporter substrate of the UPS (UbG76V-GFP) (Lindsten et al., Nat Biotechnol 21, 897-902, 2003).

TgPG14(+) mice were crossed with two independent lines of UbG76V-GFP mice, which differ in basal levels of reporter expression, to produce TgPG14(+)UbG76V-GFP and TgPG14(+)UbG76V-GFP offspring. As an additional control, we generated TgWT(+)UbG76V-GFP mice, overexpressing transgenically-encoded wild-type PrP. Mice of the different genotypes were culled at various stages of the TgPG14(+) illness (i.e. 90, 150, and >300 days of age), and analyzed by Western blot and immunohistochemistry with anti-GFP antibodies. No increased levels of the GFP reporter were found in the brains of TgPG14(+)UbG76V-GFP mice compared to TgPG14(+)UbG76V-GFP controls. Some GFP-immunopositivity was detected in Purkinje neurons of the cerebellum of double transgenic mice expressing higher basal level of the reporter. However, this was detected also in TgPG14(+)UbG76V-GFP and TgWT(+)UbG76V-GFP mice.

Our results indicate that the neurodegeneration caused by accumulation of mutant PrP is not associated with dysfunction of the ubiquitin-proteasome system, arguing against the hypothesis that perturbation of the proteasomal pathway plays a pathogenic role in inherited prion diseases.

**P03.163**

**Imaging Probes for Prion Detection**

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Objective: Prion diseases are characterized neuropathologically by deposits of prion protein amyloid fibrils (PrPSc) in brain. These cerebral amyloid deposits are colocalized with a microglia mediated chronic inflammatory response. In this study, we examined the possibility of 6-iodo-2-(4-dimethylamino-phenyl)imidazo[1,2-α]pyridine (IMPY): a β-amyloid probe and the peripheral benzodiazepine probe PK11195, for imaging of progressive prion deposits and activated microglia in a scrapie-infected mice model.

Materials and methods: Scrapie-infected mice model was obtained by intracerebral infection with C506-M5 scrapie strain homogenate in C57BL/6J mice. The binding of [125I]IMPY and [3H]PK11195 to prion deposits and activated microglia were evaluated by in vitro autoradiography on brain frozen sections of 20 µm at different time points postinoculation (dpi). Plaque binding was confirmed by histoblots with prion protein-specific monoclonal antibody 2D6.

Results: Detection of prion deposits, colocalized with activated microglia as early as 60 days dpi, in the right thalamus. The staining spread then asymmetrically to the hippocampus and cortex at 90 days dpi. At 120 and 150 days dpi, an intense and widespread binding of [125I]IMPY and [3H]PK11195 were observed on infected mice brain sections. Radiolabelled regions of [125I]IMPY are consistent and correlated with the signals obtained by histoblots staining. Similar labeling performed with [125I]IMPY and [3H]PK11195 on normal brain sections showed no specific binding.

Conclusion: These observations indicate that a colocalisation of PrPSc deposition and activated microglia suggests a possible role for microglia in the propagation of prion pathology.

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**P03.164**

**Cloning and Transcription Profiling of Shadow of Prion Protein in Sheep**

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Objective: Prion diseases are characterized neuropathologically by deposits of prion protein. Until now, SPRN has not been described in sheep, a highly relevant species in prion matters. Aim: The aim of this research was to clone and characterize the SPRN gene in sheep. Methods: A BAC mini-contig containing SPRN was built by chromosome walking and mapped by FISH. The SPRN sequence in sheep was then obtained by sequencing with primers based on the bovine SPRN sequence and the 3' end was determined by sequencing cDNA from cerebellum mRNA. In addition, the transcription profile of SPRN was determined by RT-PCR in 21 tissues. Results: Comparative mapping revealed the presence of the genes ECHS1, PAOX, MTG1, SPRN, LOC619207, CYP2E1 and at least a part of SYCE1 in the BAC mini-contig. The two most exterior BAC clones of this contig have been mapped by FISH. The SPRN sequence in sheep was then obtained by sequencing with a BAC clone that has been localized by FISH. The transcription profile of SPRN in sheep was determined by RT-PCR, showing high levels of SPRN mRNA in cerebellum and cerebral cortex, and low levels in testis, lymph node, jejunum, ileum, colon and rectum. Conclusions: Annotation of a mini-contig containing SPRN suggests conserved linkage between Oari22q24 and Hsap10q26. The ovine SPRN sequence, described for the first time, shows a high level of sequence identity with the bovine and with a lesser extent with the human SPRN sequence. In addition, transcription profiling in sheep reveals expression of SPRN mainly in brain tissue, as in cow, man, rat and mouse.
**P03.165**

**A Proteomics Approach to Establishing New Surrogate Markers for the Diagnosis of TSE Disease**

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The prion protein as a single marker protein is the basis of all commercially available diagnostic tests for the detection of TSEs in animals and humans. Whilst this has been successful in post-mortem tests, it is difficult to detect in blood thus restricting use as an ante-mortem diagnostic. There are also doubts, with the emergence of atypical strains of scrapie and BSE, that it may lack the sensitivity required for a definitive diagnostic marker of TSE disease.

Considering the above and our interest in understanding the processes involved in the neurodegenerative changes taking place within the CNS of affected animals, we have used a proteomics approach using surface enhanced desorption/ionisation SELDI mass spectrometry to establish new surrogate markers of TSE disease. Our approach to finding new surrogate markers uses and SELDI-MS-TOF technology in brain samples from a well-characterised murine scrapie model to establish a panel of markers for scrapie diagnosis. The murine model used in this experiment (intracerebrally injected with ME7 scrapie isolate) displays severe pathology in the hippocampus where it was thought that the highest number of disease-specific markers would be found. Many potential biomarker profiles were detected by this method, some in the early stages of disease before clinical signs were obvious. The proteins could be used collectively as a panel of markers without formal identification however, by identifying individual proteins we can also establish potential single markers and their role in TSE pathogenesis. We have purified and identified individual proteins using mass spectrometry including SELDI, and western blotting techniques. The identified proteins were then localised in brain sections using immunocytochemical techniques.

In collaboration with the German Primate Centre, we have also examined simian CSF from TSE-infected animals using differential protein expression profiling, for protein markers of disease.

**P03.166**

**Pathogenesis Studies of SSBP/1 Scapie in New Zealand Sheep of a Range PrP Genotypes Show Susceptibility Differences from UK Sheep**

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SSBP/1 is a source of experimental scrapie which has been used in NPU Cheviot sheep for many decades. It causes clinical disease in sheep with PrP genotypes encoding at least one VQ allele. Following subcutaneous (sc) inoculation, VRQ/VRQ animals succumb to scrapie in around 160 days, whereas heterozygotes have longer incubation periods (~260 days VRQ/ARQ and ~360 days VRQ/ARR and VRQ/AQH). All other NPU Cheviot genotypes including ARQ/ARQ are resistant to SSBP/1 (sc). However, New Zealand (NZ) sheep were different: VRQ/VRQ animals had shortest incubation periods (~150 days) but VRQ heterozygotes (we tested VRQ/ARQ and VRQ/ARR) had the same incubation periods (~260 days). Pathogenesis studies of SSBP/1 in the susceptible genotypes have revealed the timing of spread of infection (using PrP<sup>Sc</sup> as a marker) from peripheral lymphoreticular tissue to the brain is much slower in VRQ/ARR animals (NZ sheep) than VRQ/VRQ, with implications for development of diagnostic tests.

Why do VRQ/ARR animals in the NPU Cheviot flock have 360 day incubation periods whereas NZ VRQ/ARR animals develop disease around 100 days earlier? In addition, ARQ/ARQ NZ sheep were not resistant to SSBP/1 challenge but became scrapie affected >1,000 days following challenge. This is likely not to be related to the SSBP/1 titre - SSBP/1 is being titred for the first time in transgenic mice and shows very high levels of infectivity. There may be additional genetic differences between the two groups of sheep (NPU and NZ), other than the PrP gene three codon genotype. We have carried out single nucleotide polymorphism analyses of the PrP gene non-coding regions and it is clear that the VRQ, ARR and ARQ alleles are not single haplotypes so it is possible that this heterogeneity contributes to the biological effects observed.

**P03.167**

**Effect of Intraventricular Infusion of Anti-PrP mAbs on the Disease Progression in Scrapie-Infected Mice**

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Anti-PrP monoclonal antibodies (mAbs) have been shown to inhibit PrP<sup>Sc</sup> formation in cells persistently infected with prion, and passive immunization could protect the animals from prion infection through peripheral challenge when anti-PrP mAbs were administered in the early stage of infection. These results suggest that the mAbs are candidate for a treatment of prion diseases, however, the effect of mAbs on disease progression at middle-stage of the disease remains unclear. Therefore, to evaluate the therapeutic effect of anti-PrP mAbs, we carried out intraventricular infusion of the mAbs before and just after the onset of the disease. Scrapie Obihiro and Chandler strains were used in this study. Anti-PrP mAbs were infused into lateral ventricle using Alzet mini-osmotic pump. For the analysis of the effect on PrP<sup>Sc</sup> accumulation and neurodegeneration, infusions (for 4-week) were started at 120 days post infection (dpi) and mice were sacrificed at 150 dpi. For evaluation of the effect on the prolongation, infusions (for 2-week) were started at 60, 90, (before onset) and 120 dpi (just after onset). After the 4-week infusion started at 120 dpi, anti-PrP mAbs reduced PrP<sup>Sc</sup> level to 70-80% of mice treated with negative control mAb. This reduction was due to the deceleration of PrP<sup>Sc</sup> accumulation by anti-PrP mAbs. Spongiform changes and astrocytosis in hippocampus and thalamus of mice treated with anti-PrP mAbs appeared milder than those of mice treated with negative control mAb. The anti-PrP mAb prolonged the incubation period of mice infected with Obihiro strain when infusion was started at 60 dpi, while prolongation was not observed when the infusion was later stage. In contrast, infusion of anti-PrP mAb to mice infected with Chandler strain at 60, 90, and 120 dpi, prolonged the incubation periods for 14, 13.5 and 12 days, respectively. No adverse effect was observed in these mice, in addition, antibody-induced neuronal apoptosis was not observed even when the mAbs were stereotaxically inoculated into hippocampus. Although the effect was dependent on the prion strain, the mAb infusion could partly prevent the disease progression even when the infusion was started after clinical onset, suggesting that antibody therapy still be a candidate for the treatment of prion diseases.

**P03.168**

**Biochemical Identification of Bovine Spongiform Encephalopathies in Cattle**

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Important changes have occurred in the post-mortem diagnosis of Bovine Spongiform Encephalopathy (BSE) in recent years. The methods used for confirmatory diagnosis of BSE should follow the OIE guidelines and continually need to be reassessed. We have evaluated a commercially available Western blot method (TeSeE® Wb) as a potential means of confirming BSE. This method was (i) highly sensitive, compared to previously used biochemical confirmatory methods and (ii) more sensitive than 2 routinely used highly sensitive rapid tests (TeSeE® Eisa, HerdCheck® BSE). We show that this high sensitivity is mainly due to the antibody used (ShaA1). Interestingly, TeSeE® Wb was also able to diagnose the two currently recognised deviant BSE phenotypes (H-Type and L-Type or BASE). The initially described molecular features of these atypical forms of BSE were also readily recognised, although sensitivity of the method may be differently affected by the chosen Ab compared to typical BSE. This method is thus of potential interest for future evaluations of BSE confirmatory methods.
P03.169
Improved Neural Stem Cell Model for Prion Propagation
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The abnormal prion protein (PrPSc) plays a central role in the transmission of prion diseases. PrPSc is produced by conversion of the cellular isoform of the prion protein (PrPC) which is essential for the pathogenesis of prion diseases. Accumulation of PrPSc in the central nervous system leads to neurodegeneration with neuronal cell death and gliosis. The cellular requirement for the conversion of PrPC, strain specificity and propagation of PrPSc are still unclear. To address these questions and investigate the molecular basis of prion diseases it is a necessity to find new cell culture model or improve those already existent. We recently described that, after differentiation, neural stem cells (NSCs) from the central nervous system are able to convert the cellular isoform of the prion protein into its pathologic scrapie isoform in cell culture. Thus, using this original model we have sustained our investigations to optimise these culture conditions. We hypothesized that changing the culture conditions by modifying a given factor or a combination of factors could increase PrPSc production in NSCs. The first results showed that the infection can be strongly influenced by supplements added to the culture medium. Those supplements enabled neuronal but also astroglial differentiation of NSCs and increased prion propagation and PrPSc production. These results and additional data will add efficiently to our understanding of prion diseases and more importantly allow us to develop more powerful models.

P03.170
Towards the Development of a Neural Stem Cell Model of Human Prion Disease
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Introduction: To date, most prion research has been undertaken using animal models. However, modelling prion disease using cell culture has proven more difficult. Prion susceptible cell lines do exist, but are limited to the propagation of either mouse-adapted or sheep scrapie prions. There are currently no reported cell lines able to stably propagate human prions. The development of such a cell line remains key to better understanding the cellular and molecular basis of human prion disease, the development of anti-prion therapeutics and to establish diagnostic assays for prion screening. We are currently developing a unique model of human vCJD using differentiated human neural stem cells.

Aim: To develop a cell line able to stably propagate human prions associated with vCJD.

Methods: Human neural stem cells were derived from 10-week foetal ventral mesencephalon and immortalised by overexpression of the v-myc oncogene. They expand indefinitely in culture as stem cells, but upon removal of key growth factors, differentiate predominantly to a stable, mature neuronal phenotype. Anti-PrP antibodies and metabolic labelling were then used to provide evidence of de novo PrPSc production in these cells following exposure to vCJD prions.

Results: The human neural stem cells used differentiate into stable cultures of neuronal cells with a strong phenotypic resemblance to primary neurones in the human brain. Furthermore, they express increased levels of PrPΔ. This makes them a promising candidate for modelling the propagation of human prions. Undifferentiated cells are not able to propagate prions. However, preliminary data will be presented on the susceptibility of differentiated cultures to human prions and their ability to produce de novo PrPSc. On-going work is being undertaken to characterise these cells more fully.

Conclusion: Taken together, the data suggest that differentiated human neural stem cells appear able to stably propagate human prions. Successfully developing such a model should provide a better understanding of the cellular pathophysiology of human prion disease.

P03.171
Infection of Minute Virus of Mice (MVM) Increases the Binding and Internalization of Exogenous PrPΔ into A8 Fibroblasts
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The possible effect of intercurrent viral infections on prion disease pathology is poorly understood. In this study, we show that fibroblasts infected with Minute Virus of Mice (MVM) bind and internalize significantly more exogenous PrPΔ as compared to naive cells. This was further confirmed by the observation of enhanced localization of the internalized prion protein to the lysosomes in MVM-infected cells. In addition, the internalized PrPΔ was distributed differently in the MVM-infected cells, as demonstrated by the shift in migration of PrPΔ through floatation gradients toward the lighter fractions where the Rattus residents ganglioside GM1 and cavelin 1 are present. Interestingly, the MVM-infected fibroblasts demonstrated a considerable increase in both Raft markers, suggesting that viral infection can significantly alter the cell lipid composition. Such an alteration may facilitate the incorporation of PrPΔ molecules into the Rafts. Since the conversion of PrPΔ to PrPSc is believed to occur in such membrane micro domains, changes in cell lipid composition due to viral infection may affect the first steps of prion disease initiation.

P03.172
Direct Detection of Disease-Associated Prions in Brain and Lymphoid Tissue Using Antibodies Recognising The Extreme N-Terminus of PrPΔ
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A simple diagnostic test is described for the detection of TSE in bovine, ovine and human brain and lymphoid tissue that obviates the use of proteinase K as a discriminating reagent. The immunoassay utilises high affinity anti-peptide antibodies that appear blind to the normal isoform of prion protein (PrPC). These reagents have been produced with novel N-terminal chimeric peptides and we hypothesise that the retention and stability of the extreme N-terminus of PrP in the disease-associated aggregate makes it an operationally specific marker for TSE. Accordingly, the assay involves a simple one-step capture of PrPSc followed by detection with a europium-labelled anti-PrPΔ antibody after homogenisation of the tissue directly in 8M-guanidine hydrochloride. This rapid assay clearly differentiates between levels of disease-associated PrP extracted from brain and lymphoid tissues taken from histology-confirmed TSE positive and negative cattle and sheep. In being PK independent, this assay has application to the detection of PrPSc in atypical scrapie where the disease-associated prion is more susceptible to proteolysis.
Pathology and Pathogenesis

P03.173
Cytoskeletal Disruption and the Role of The MAP Kinase Pathways in Two Neuronal Loss Models of Murine Scrapie
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One of the characteristic pathological changes observed in transmissible spongiform encephalopathies (TSEs) is neuronal loss. Studies in experimental mouse scrapie models suggest that neurons die through an apoptotic mechanism, which may be related to early cytoskeletal disruption. In this study two contrasting scrapie mouse models were used: the ME7/CV model in which neuronal loss is targeted to CA1, and the 87V/VM model, where neurodegeneration targets the CA2 of the hippocampus. Using immunocytochemical and Western blot techniques the distribution of cytoskeletal proteins MAP2 (microtubule associated protein 2) and tubulin, and the expression of mitogen activated protein kinases (MAPKs) and their role in the cytoskeletal disruption was investigated. Results have shown MAP2 and tubulin immunoreactivity to be decreased in both scrapie models, whilst an increase in the the phosphorylated form of the MAP kinase ERK1/2 was observed in both models, and phosphorylated P38MAPK was increased in the ME7/CV model. The MAP Kinases in their phosphorylation state can switch on or off the activity of substrate proteins, such as cytoskeletal proteins. The increase in phosphorylated MAP kinases, observed in both scrapie mouse models, may in turn lead to the decrease in MAP2, destabilization of microtubules and subsequent loss of tubulin. These results suggest that neuronal death may be mediated by cytoskeletal damage, but further studies are now underway to identify the details of the relationship.

P03.174
Normal Prion Protein in Human Placenta
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The emergence of variant Creutzfeldt-Jakob disease (vCJD), which is thought to be caused by consumption of products containing neural tissues form cattle infected with bovine spongiform encephalopathies (BSE), along with recent suggestions that BSE transmission to human may also be linked to sporadic CJD, rises a question about the correct causal attribution of disease. The key event during pathogenesis of transmissible spongiform encephalopathies (TSE) is the conversion of normal prion protein (PrPC) to abnormal, disease-associated isoform PrPSc. Therefore the presence of PrPSc in tissues plays important role in TSE pathogenesis. To date, high levels of PrP expression have been reported to be found in neural, lymphatic and muscle tissues. Our aim was to search for PrP in placenta tissue. To achieve that placenta tissues and three brain tissues were used in experiment. Western blotting was used to investigate and compare the expression levels of PrP in both tissues and immunohistochemistry was used to show exact localization of PrP. All placenta samples used in experiment revealed abundant expression of PrP with levels of PrPconcentration similar or even higher to those observed in brain samples. PrP was observed in the syncytiotrophoblast and the cytotrophoblast. This result along with epidemiological data, which supports the possibility of perinatal transmission of the scrapie in sheep, bring up an issue for discussion whether placentas may be involved in spreading CJD in human population, although, yet, neither epidemiological nor pathological evidence seems to confirm that.

P03.175
Gene Expression Profiling during the Progression of BSE in Cattle
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The molecular pathogenesis of TSEs is still not understood. The aims of this study were to elucidate the effect of prion pathogenesis on gene expression and to identify differentially regulated genes during the progression of BSE infection in cattle. We have analyzed brain tissue from time course samples (8, 21, 27, 36, 39 months p.i.) of cattle orally infected with BSE as well as terminally ill animals and healthy controls. The microarray analysis was carried out using Affymetrix Bovine Genome GeneChips, which contained probes for 24,128 sets of transcripts. The data were normalized and analyzed using the GeneSpring software. Clustering analysis showed that there is a correlation between the disease progression and expression patterns. The mRNA of 205 genes was found to be differentially regulated by ANOVA with a p-value cutoff being 0.05. To validate the microarray data, quantitative reverse transcriptase-PCR (qPCR) of six genes was carried out and the qPCR showed similar profiles to those of microarrays analysis. Many of these 205 genes encode proteins involved in immune response, apoptosis, cell adhesion, stress response and transcription. Several genes and their protein products were already described in previous studies as having a link with prion diseases. Amongst them are s100 proteins, 14-4-4 proteins, collagen, integrin, the major histocompatibility complex and ribosomal proteins. The combination of ANOVA with fold change analysis revealed that most changes in gene expression occur between the negative controls and the animals 21 month post inoculation, suggesting that there are many pathogenic processes in the animal brain prior to the clinical onset of BSE. This is consistent with the findings of gene expression studies in TSE-infected mice as well as with findings of early cognitive deficits and neurophysiological dysfunction in the similar murine TSE-models. The diagnostic potential of TSE-specific gene expression profiles is currently under evaluation.

P03.176
Presymptomatic Impairment of Glutamate Excitotoxicity in the Cerebellum of Transgenic Mice Expressing a PrP Insertional Mutation
Senatore, A1; Collecchi, S2; Restelli, E1; Garofoli, A1; Forloni, G1; Gobbi, M1; Chiesa, P1; D’ubbeco Telethon Institute-Mario Negri Institute, Neuroscience, Italy; 2Mario Negri Institute, Biochemistry, Italy; 3Mario Negri Institute, Neuroscience, Italy

The expression of mutant PrP in the brains of transgenic mice increases the risk of developing a fatal neurological disorder characterized by ataxia and pathologically by dramatic cerebellar atrophy due to loss of synaptic endings in the molecular layer and apoptosis of granule neurons. In this study we tested the hypothesis that mutant PrP deposition induces early synaptic dysfunction, which precedes neurodegeneration and clinical symptoms. We carried out biochemical analyses of PrP and tested the functional status of glutamatergic and GABAergic neurotransmission in isolated nerve endings (synaptosomes) from TgPG14 mice at different stages of neurological illness (between 30 and 400 days of age). Biochemical characterization demonstrated relative enrichment of PrP in cerebellar synaptosomes where the mutant protein was highly aggregated. Glutamatergic synaptosomes from TgPG14 cerebellum, but not from cortex, showed impaired depolarization-induced release already in presymptomatic 30 day-old mice. By the time mice had developed clinical disease (>250 days of age) there was complete impairment of depolarization-induced release. Cerebellar synaptosomes from >150 days old TgPG14 mice showed also a significant increase of basal glutamate release. Importantly, there was no impairment of glutamate uptake, excluding non-specific synaptosomal damage as a cause of these findings. No significant differences were found in GABA uptake or exocytosis.

These results indicate that PG14 PrP deposition is associated with an inherited prion disease, accumulate a form of the mutant protein in their brains that is aggregated and weakly protease-resistant. As this form accumulates the mice develop a fatal neurological disorder characterized clinically by ataxia and pathologically by dramatic cerebellar atrophy due to loss of synaptic endings in the molecular layer and apoptosis of granule neurons. In this study we tested the hypothesis that mutant PrP deposition induces early synaptic dysfunction, which precedes neurodegeneration and clinical symptoms. We carried out biochemical analyses of PrP and tested the functional status of glutamatergic and GABAergic neurotransmission in isolated nerve endings (synaptosomes) from TgPG14 mice at different stages of neurological illness (between 30 and 400 days of age). Biochemical characterization demonstrated relative enrichment of PrP in cerebellar synaptosomes where the mutant protein was highly aggregated. Glutamatergic synaptosomes from TgPG14 cerebellum, but not from cortex, showed impaired depolarization-induced release already in presymptomatic 30 day-old mice. By the time mice had developed clinical disease (>250 days of age) there was complete impairment of depolarization-induced release. Cerebellar synaptosomes from >150 days old TgPG14 mice showed also a significant increase of basal glutamate release. Importantly, there was no impairment of glutamate uptake, excluding non-specific synaptosomal damage as a cause of these findings. No significant differences were found in GABA uptake or exocytosis.

These results indicate that PG14 PrP deposition is associated with early functional alterations of presynaptic glutamatergic nerve endings in the cerebellum, which degenerate in the later stages of disease. Our analysis supports the hypothesis that aggregation of PrP affects the mechanisms governing neurotransmitter release as an early event in the pathogenesis. Studies are in progress to clarify the specific mechanism by which PG14 PrP leads to defective depolarization-induced glutamate excitotoxicity.
P03.177
Microglia Depletion Increases Prion Replication in Organotypic Brain Slices
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Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases characterized by accumulation of PrPSc, an abnormal isoform of the cellular protein PrPC. Microglia is associated with PrPSc plaques in TSE brains, but their role in prion turnover is unknown. We have established a prion organotypic slice culture assay (POSCA) to investigate the contribution of microglia to prion replication in CNS tissue. Thirty-five days after contact with prions, cerebellar slices from wild-type mice had amplified disease-associated prion protein >10³-fold, similarly to terminally sick mice. PrPSc accumulated predominantly in the molecular layer, similarly to infected mice. We then performed POSCA on slices from CD11b-HSV-TK transgenic mice from which microglia can be selectively removed. Microglia depletion led to a 15-fold increase in prion and PrPSc concentration and to increased susceptibility to infection. These results support a protective role for microglia against prion replication.

P03.178
Endocytosis of the Cellular Isoform of the Prion Protein and Neuronal Zinc Uptake
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Background: Whilst an exact physiological function remains elusive for the cellular isoform of the prion protein (PrPc), an involvement in metal homeostasis is an extensively studied concept. To date, this work has centered upon copper metabolism, evaluating roles in binding and uptake of the ion. However, closer examination of available literature has revealed a possible role for PrPc in neuronal zinc homeostasis. Zinc has been shown to be able to bind at the octapeptide repeat sequence and induce endocytosis of the protein. Furthermore, an increase in zinc-bound PrPc was determined in affinity purified material from prion-infected mice. As there was a significant decrease in total zinc measured for the whole brain homogenate, it would imply a redistribution of zinc ions occurs within the brain during disease progression.

Aim: To ascertain whether expression of PrPc influenced zinc uptake and metabolism in neuronal SH-SYSY cells.

Results: Using the zinc binding fluorochrome Zinpyr-1, we have been able to demonstrate a significant increase in staining following zinc supplementation in cells which were stably expressing PrPc. This staining was shown to be zinc specific as cells supplemented either with Cu, Fe, Mn or Ca showed no increase in fluorescence. The pattern of increased staining was consistent with an increase in zinc uptake in the cells expressing PrPc. Further studies to address the effect of mutation in PrPc and the role of the recently identified endocytic partner for PrPc, LRPI, on the uptake of the ion are being investigated.

Conclusions: Taken together these data demonstrate a role for PrPc in the cellular uptake of zinc ions potentially demonstrating a novel physiological function for the protein.

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P03.179
Coexistence of Both PrPSc Type 1 and 2 in sCJD: Does it Affect the Phenotype?
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In sporadic Creutzfeldt-Jakob disease (sCJD) five phenotypically distinct subtypes have been identified based on the methionine (M)/valine (V) polymorphic genotype of codon 129 and two PK-resistant scrapie prion protein (PrPSc) types, which migrate in gel to either 21 kDa (PrPSc type 1) or 19 kDa (PrPSc type 2). sCJD is characterized by phenotypic heterogeneity. The co-existence of both PrPSc types has recently been reported and may complicate the diagnosis.

In the present study we analyze the distribution of the two PrPSc types in various brain areas as well as the PrPSc resistance to PK digestion using a rigorous procedure according to Notari et al. [Human PrPSc “Typing” pitfalls associated with the use of type 1 selective antibodies combined with relative inefficient hydrolysis of PrPSc by proteinase K, poster presentation, NeuroPrion 2006, Torino], with the intent of assessing the co-occurrence of fully PK-resistant PrPSc types 1 and 2 in a ratio of 50:50 or 60:40 while ascertaining that intermediate fragments are completely digested. We are studying the effect of the co-existence of the two PrPSc types, which occurs in either the same or different brain regions, on the sCJD disease phenotype by examining clinical history and neuropathological changes.

{Supported by NIH AG-14359 and CDC UR8/CCU515004 awards, and the Charles S. Britton Fund}

P03.180
Characterisation of Leukocyte Surface PrP Epitope Exposure between PBMCs and Leukocytes from Lymph Nodes of Sheep Affected with Natural Scrapie
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Scrapie pathogenesis in sheep is known to involve the early accumulation of PrPSc within the lymphoreticular system (LRS), notably by follicular dendritic cells and macrophages within lymph nodes. Transfusion experiments have provided evidence that blood also contains infectivity for TSE disease. The components of blood that bear infectivity are less well understood but there is accumulating evidence that some infectivity is cell associated. In order to determine whether infectivity in blood may be associated with transport of cells from the lymph node, we have examined the PrP expression on cells from lymph nodes and peripheral blood mononuclear cells (PBMCs) at terminal stages of scrapie disease. By using a panel of anti-PrP antibodies directed to a range of PrP epitopes, we have used flow cytometry to analyse the PrP epitope characteristics of these cells both from sheep with naturally acquired scrapie and healthy controls. Comparison of immunostaining of PBMCs from scrapie terminal sheep with age and genotype matched controls indicated that the mean fluorescent intensity and the percentage of positively stained cells were both decreased for the majority of antibodies tested, suggesting that the expression of PrP is decreased on PBMCs from scrapie terminal sheep. Our results also indicate that there are significant differences in cell surface expression of PrP between leukocytes prepared from peripheral blood and from lymph nodes.
**Pathology and Pathogenesis**

**P03.181**

**Adenovirus Recombinant for MHC I-restricted PrP Epitopes Induce T CD8+ Cytotoxic Cells in Wild-type Mice: Role of CTL in Prion Disease Protection**

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1ENVA, UMR 1161, France; 2INSERM 712/Hopital St-Antoine, France; 3ENVA, Dept of Histopathology, France

**Background:** Good evidence now exists that the anti-PrP antibodies are efficient at preventing prion disease in mice. However, no information exists on the possibility to induce CD8+ CTL in wild-type mice, and on their influence on prion diseases. In a previous work, we demonstrated that co-injection of PrP-derived MHC I-restricted nonapeptides and CpG/IFA to C57Bl/6 wt mice elicited T cells secreting IFNγ in response to the peptide, provided that they were modified to increase their binding affinity for H-2Kb. Two of these peptides induced T cells cytotoxic for peptide-pulsed RAMS cells in vitro and for splenocytes loaded with the modified peptide in vivo. Yet, immunization stimulated a low frequency of peptide-specific CD8+ measured by pentamer staining.

**Aims/Objectives:** To improve the efficiency of CTL generation, recombinant adenovirus (rAd) were used as immunogens in C57Bl/6 wt mice to deliver PrP peptides; the capacity of these rAd to protect against prion diseases will be evaluated in mice challenged with murine scrapie.

**Methods:** Based on these results, we have engineered rAds expressing minigenes encoding these modified peptides. The constructions were optimized to be independent of proteasome cleavage and of TAP transport.

**Results:** All wt mice immunized with one rAd developed T to 4% pentamer-positive CD8+ T cells the level of which remained high during the asymptomatic period without clinical signs of autoimmune reactions nor histopathological tissue destruction. These T cells were cytotoxic against PrP+ expressing cells and secreted specifically IFNγ measured by intracellular staining.

**Conclusion:** This strategy proves particularly efficient in eliciting peptide-specific CD8+ CTL without inducing autoimmune reactions. Their protective capacity is under investigation in 139A-infected mice.

**P03.182**

**Role of T CD8+ in the Control of Prion Diseases Assessed by Prnp−/− T CD8+ Transfer into C57Bl/6 Wild-type Mice**

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**Background:** Cytotoxic T lymphocytes (CTL) directed against PrP-derived peptides may possibly clear PrPSc accumulating cells and thus confer protection to scrapie-infected mice. Up to now, few works have explored the CD8+ CTL repertoire specific for PrP in wild-type mice. Yet, as the PrP is a self-Ag, the major problem is the immune tolerance which must be bypassed at the risk of auto-immune manifestations. In a study presented in another poster, we demonstrated that injection of class I-restricted PrP peptides (227NP) to C57Bl/6 wild-type mice induced T cells to secrete IFNγ and to lyse peptide-pulsed RAMS cells in vitro and peptide-loaded splenocyte in vivo.

**Aims/Objectives:** We try to evaluate whether or not CTL against PrP could be an immune effector mechanism capable of controlling prion diseases.

**Methods:** To bypass natural tolerance, CTL against PrP were obtained by immunizing Prnp−/− Ly5.1 mice either with spleenocytes from C57Bl/6 Ly5.1 wt mice or with the 227NP peptide in CpG/IFA. These cells were able to lyse PrP positive or peptide-pulsed cells in vitro and in vivo. CD8+ T cells were purified and transferred in sub-lethally irradiated C57Bl/6 Ly5.2 wild-type mice. The capacity of anti-PrP CD8+ cells to protect against prion diseases will be evaluated after infection of these recipient mice with 139A murine scrapie.

**Results:** In both conditions of donor immunization, CD8+ Ly5.1 cells reconstituted irradiated recipient C57Bl/6 Ly5.2 and their level remained high during the asymptomatic period. No clinical signs of autoimmune reactions were observed and histopathological studies did not reveal significant cell infiltrations or destruction in secondary lymphoid organs and brain. We are currently monitoring in the blood the number and function of 227NP-specific CD8+ T cells by pentamer staining and IFNγ specific secretion. Their potential efficiency in controlling the progression of scrapie will be checked on spleen PrPSc accumulation and survival of 139A-infected mice.

**Conclusion:** These experiments will analyse the role of PrP-specific CTL in the protection against prion diseases and might validate new immunological strategies.

**P03.183**

**Functional Genomic Analysis of Preclinical Scrapie in Sheep Using EST Microarrays**

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**Functional genomics of peripheral tissues of scrapie infected sheep has been used for the identification of surrogate markers of prionical expression as well as understanding disease pathogenesis.** Our study used a 20,000 EST microarray to assess differential gene expression in spleen follicular dendritic cells of VRQ/VRQ New Zealand Cheviot sheep taken at 75 days post infection with SSBP/1 scrapie. Normalization was carried out on each chip separately using the print-tip loess method from the Limma package of the Bioconductor project. Analysis of these microarray data indicated down-regulation of the most significant differentially expressed genes in the diseased samples for the majority of the genes the analysed. A series of candidate genes were selected from the genes differentially expressed in control (mock-infected) and infected animals. PCR primers were designed, products were cloned and sequenced to verify gene identity and a real time quantitative RT-PCR assay was optimised in order to validate the microarray data. A panel of housekeeping genes were assayed for expression stability and those exhibiting the least variation were selected as normalization controls. In order to assess the utility of each candidate as a surrogate marker of infection the expression level of each gene was quantified in spleen samples taken from SSBP/1-infected and mock-infected VRQ/VRQ sheep at 6 time points, from pre-clinical through to clinical disease (10, 25, 50, 75, 100, 125 d.p.i.). Fully validated candidates have now been used to assess their use as diagnostic markers by screening blood buffy coat samples from the same animals.

**P03.184**

**An Advantageous Method Utilizing the BioMasher and a Sensitive ELISA to Accurately Detect Bovine Spongiform Encephalopathy and Ovine Scrapie from Brain Tissue**

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1Nippon Research Institute of Biomatrix, Japan; 2University College Dublin, Ireland; 3Immuno-Biological Laboratories Inc, Japan; 4National Institute of Animal Health, Japan; 5National Institute of Infectious Diseases, Japan; 6Immuno-Biological Laboratories Co, Japan

**We developed a new screening method to detect bovine spongiform encephalopathy (BSE). Our method is advantageous because it has a simpler and safer protocol than commercial kits. We developed a new device, termed the BioMasher, to homogenize brain tissue by passing it through a porous rigid propylene filter. A purification step is not involved in the sample preparation. Thus, the time needed for sample pretreatment is substantially shortened, and the risk of infection during sample processing is effectively reduced. We created monoclonal antibodies to prion protein, and used them to construct a sensitive sandwich enzyme-linked immunosorbent assay system. The sensitivities of this assay kit for BSE-positive brain and scrapie brain are comparable or more sensitive than commercial kits. Key words: BSE, ELISA, screening kit.**
**Pathology and Pathogenesis**

**P03.185**  
**Biological Activities of the Shadoop Protein**  
Westaway, D1; Watts, J1; Ng, V1; Daude, N1; Yang, J1; Horne, P1; Strome, B1; Young, R1;  
Carlson, GA1; Fraser, PE1; Schmidt-Ulms, G1  
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Although the function of PrP<sup>C</sup> has remained enigmatic, it is neuroprotective in a number of paradigms. In particular, several labs have demonstrated a potent protective activity against degeneration of the cerebellum mediated by CNS-expressed Doppel or internally deleted forms of PrP ("iPrP"). These studies have facilitated mapping of activity determinants in PrP<sup>C</sup> and implicated the action of a cryptic PrP<sup>C</sup>-like protein termed "pi". Shadoo (Sho) is a hypothetical GPI-anchored glycoprotein encoded by the SPRN gene, exhibiting homology and domain organization similar to the N-terminus of PrP. In situ hybridization, histology and blot analysis demonstrate the presence of Sho in the adult CNS of wt mice. Sho expression has overlaps with PrP<sup>C</sup> but is low in cerebellar granular neurons (CGNs) containing PrP<sup>C</sup> and high in PrP<sup>C</sup>-deficient dendritic processes of the hippocampus and cerebellum. In PrP<sup>C</sup>-deficient cerebellar granular neurons (CGNs), transgene-encoded wt Sho resembles wt PrP<sup>C</sup> in countering the toxic effect of expressing either Doppel or iPrP. This PrP<sup>C</sup>-like neuroprotective activity indicates an overlap in the biology of Sho and PrP<sup>C</sup> and begs the question of Sho’s activity in prion disease. Here it is notable that immunohistochemistry defines Sho in some neuroanatomical structures that become clinical target areas in experimental scrapie. Furthermore, wt mice infected with RML prions exhibit a dramatic reduction in endogenous Sho protein versus healthy controls. Our data define Sho as a bona fide neuronal protein and a plausible candidate for pi.

**P03.186**  
**Adenovirus-mediated Vaccination Induces Antibody and T CD8<sup>+</sup> Responses to PrP in Wild-type Mice: Role in Protection of Prion Disease**  
Elizalde, M1; Sacquin, A1; Adam, M1; Crespeau, F1; Rosset, M1  
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Background: Anti-PrP antibodies (Abs) are able to inhibit PrP-to-PrP<sub>Sc</sub> conversion in vitro and prion pathogenesis in vivo. Other effectors with potential to clear PrP<sub>Sc</sub> producing cells are Cytotoxic T Lymphocytes (CTL) directed against PrP-derived peptides, but it is currently unknown whether they can confer protection. Yet, as the PrP is a self-Ag, the major problem is the immune tolerance which must be bypassed at risk of autoimmune manifestations.

Aim(s)/Objective(s): To induce in C57BL/6 wt mice an humoral or CTL responses directed to PrP by using recombinant adenoviruses vectors encoding entire or fragments of PrP<sup>C</sup> gene expressing CD8 T cell or B-cell epitopes and assess their efficiency against challenge.

Methods: Different rAd constructs expressing xenogenic PrP<sub>C</sub> or minigenes encoding peptides with high affinity for MHC1, together with different schemes of immunization were tested in wild-type mice to induce anti-PrP immune responses in wild-type mice: parental injection of the rAdks, ex vivo transduction of dendritic cells by rAdks and elimination of CD4<sub>+</sub>CD25<sup>+</sup> regulatory T cells before immunization.

Results: We will present results from a series of vaccination experiments in C57BL/6 mice with the different rAd constructs. Specificity and intensity of antibody responses in immunized mice were analysed by ELISA and cytotoxicity methods using cells expressing different PrP<sub>C</sub> species. We were able to induce antibodies against the native murine PrP<sub>C</sub> and/or peptide-specific T CD8<sub>+</sub> cells cytotoxic in vitro and in vivo, without deleterious autoimmune reactions. Protection studies are currently ongoing in mice infected with 139A scrapie.

Conclusion: This strategy proves particularly efficient to elicit cross reactive antibodies and CTL against murine PrP<sub>C</sub> whose protective capacity is under investigation in prion infected mice.

**P03.187**  
**Neuropathogenesis and Neuroinflammation in ME7 Murine Prion Disease: Implications for Alzheimer's Disease**  
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The neuropathology of prion disease is characterised by tissue vacuolation, loss of neurons, the presence of PrP<sub>Sc</sub>, reactive astrocytes and activated microglia. The molecular events that lead to loss of neurons and the contribution of inflammatory processes to disease progression are poorly understood. We have sought to discover the neuropathological changes that underpin the earliest behavioural changes in the ME7 model of mouse prion disease and to characterise the associated inflammatory response. The earliest neuropathological changes associated with behavioural abnormalities are in the hippocampus where there is a loss of synapses from CA3 Schaeffer collaterals onto pyramidal cells of CA1. Biochemical analysis shows that components of the pre-synaptic vesicles are the first to show reduced expression. In response to this large-scale synaptic degeneration the microglia adopt an activated morphology and increase in number. However, despite their activated morphology their phenotype is dominated by receptors expressing immunomodulatory tyrosine inhibitory motifs (ITIMs) characteristic of an anti-inflammatory phenotype and the cytokine profile is also anti-inflammatory and dominated by the cytokine TGFβ1. In contrast, a systemic inflammatory challenge (LPS) in animals with prion disease switches the microglia to a proinflammatory response and leads to upregulated expression of receptors with activating motifs (ITAMs). Previous studies have shown that systemic challenge with LPS in animals with prion disease leads to an increase in the number of neurons undergoing apoptosis (Cunningham et al 2005). These data serve to focus attention on the possible role of systemic inflammation in prion disease and other neurodegenerative diseases.

**P03.188**  
**Gain and Loss of PrP<sub>C</sub>-mediated Functions in Infectious Prion Disease**  
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1University of California, Physiology, USA; 2University of California, Institute for Neurodegenerative Disease

Substantial evidence indicates that the cellular prion protein (PrPC) plays a central role in the pathogenesis of transmissible prion diseases. However, it remains unresolved if its role results from gain or loss of PrP<sub>C</sub>-mediated functions. We propose a hypothesis by which both gain and loss of PrP<sub>C</sub> functions can occur in infectious disease. At the basis of our hypothesis is the detection of two topological isoforms of PrPC in wild-type mice and the isolation of their respective functions: Fully secreted SecPrP reveals a neuroprotective function against reactive oxygen species (ROS) whereas transmembrane CtmPrP triggers caspase-3-mediated apoptosis. To identify the roles of both CtmPrP and SecPrP in prion disease pathogenesis, transgenic mice favoring expression of either the CtmPrP or SecPrP isoform were inoculated with PrP<sub>Sc</sub>. Mice favoring CtmPrP expression develop symptoms within 7 weeks and show pathology for both apoptosis and oxidative stress-mediated neurodegeneration. In contrast, despite substantial accumulation of protease-resistant PrP<sub>Sc</sub>, mice favoring expression in the SecPrP form develop symptoms after ~50 weeks with indications of oxidative stress and non-apoptotic cell loss. Our data suggest PrP<sub>Sc</sub> accumulation leads to upregulation of CtmPrP which triggers apoptosis. Additionally, the SecPrP-to-PrP<sub>Sc</sub> conversion leads to a loss of the neuroprotective function of SecPrP resulting in a higher susceptibility of infected cells to ROS. Therefore, pathophysiology of infectious prion disease is likely facilitated by both the apoptotic effects of CtmPrP<sub>Sc</sub> and the loss of the protective effects of SecPrP<sub>Sc</sub>. 

Acknowledgement: This work was supported by the National Institute of Health (AI076286 and AG042422) and the David and Lucille Packard Foundation.
PrP-Mediated Myopathy in an Inducible Transgenic Mouse Model
Liang, J; Huang, S; Li, Xinyi; Zheng, M; Wang, M; Hays, A; Gambetti, P; Booth, S; Kong, Q

1Case Western Reserve University, Pathology, USA; 2Columbia University Medical Center, Pathology, USA; 3National Microbiology Laboratory, Division of Host Genetics and Prion Diseases, Canada

The prion protein (PrP) level in muscles has been reported to be elevated in patients with inclusion-body myositis, polymyositis, dermatomyositis and neurogenic muscle atrophy, but it was not clear whether the elevated PrP accumulation in the muscles is sufficient to cause muscle diseases. We have generated transgenic mice with muscle-specific expression of PrP under extremely tight regulation by doxycycline, and demonstrated that over-expression of wild type PrP in skeletal muscles is sufficient to cause a primary myopathy with no signs of peripheral neuropathy, possibly due to accumulation of a cytotoxic truncated form of PrP and/or PrP aggregation. Here we will report our progress in investigating the molecular mechanism of PrP-mediated myopathy, especially on the regulation of several critical genes in association with the PrP-mediated pathogenic process in skeletal muscles. One of the genes that are down-regulated in affected skeletal muscles is transcription factor MEF2c, which may underline the central nucleus localization observed in many muscle fibers. The potential role of truncated PrP fragments in natural muscle diseases will also be explored.
**Epidemiology, Risk Assessment and Transmission**

**P04.01**

**Chronic Wasting Disease in a Captive White-Tailed Deer Farm**

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1*University of Wisconsin, USA; 2US Department of Agriculture, USA; 3US Department

of Agriculture, USA; 4USDA ARS-ADRL, Washington 1State University, USA; 5Veterinary

Diagnostic Laboratory, Colorado State University, USA

A white-tailed deer farm in Portage, Wisconsin, was depopulated in January 2006, after

chronic wasting disease (CWD) had been initially discovered on the property in

September 2002. Prior to the depopulation, a total of 22 positive animals had been

removed from the property; one in 2002; six in 2003; ten in 2004; four in 2005 and one

in 2006. At the time of depopulation a total of 76 animals remained: 47 females and 29

males. Age was assessed by visual examination of teeth at the time of death and

revealed 26 adult, 8 fawn and 42 yearling animals. The following tissues were

examined by immunohistochemistry for PrPCWD using Ab89/97.6.1: oesx, tonsil, retropharyngeal, submandibular, parotid, prescapular, axillary, inguinal, prefemoral and

popliteal lymph nodes, recto-anal mucosal tissue and eye. Seventy-nine percent of

animals (sixty) were found to be positive in at least one tissue; 49 were oesx positive,

58 retropharyngeal positive, 56 tonsil positive, 48 recto-anal mucosal associated

lymphoid tissue positive and 4 animals were positive for PPRCWD in the retina. Prion

genotype was determined for all animals.

**P04.02**

**Control Measures for Atypical Scrapie in Portugal**

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As a consequence of the intensive surveillance for TSE in small ruminants, until March

2007, all cases of scrapie were found in sheep and goats. In 2007, the number of positive

cases increased dramatically, with 576 cases notified. This unexpected situation led

to the implementation of new control measures against scrapie. Portugal has developed

an alternative strategy to the compulsory culling policy. Briefly, the affected flocks are placed under intensive surveillance for a 2- year period with movement restrictions, Prnp genotyping of all adult sheep, electronic

identification of all all small ruminants and PrPres testing of all small ruminants over 18

months slaughtered for human consumption and over 12 months in the fallen stock.

The experienced control measures during 3 years will be presented with preliminary

results regarding the cohorts testing and Prnp genotyping as well as the faced

difficulties.

**P04.03**

**Bov5 Cells: A Permanent Bovine Cell Line which is Susceptible to Ovine Scrapie**

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Only few stably prion infected cell lines are available to date and these lines are almost

exclusively coming from rodent species. Apart from these lines a large collection of the

commonly used as well as many unknown permanent cell lines have been tested

without finding a susceptible cell line, e.g. from ruminant species. The factors

necessary for a proficient replication as well as for determining strain effects are still

unknown. In the study presented here, we were able to infect a non-transgenic bovine

cell line (Bov5) with natural sheep scrapie prions. Scrapie infected Bov5 cells (Bov5Sc)

were consistently passaged. The accumulation of PrPres could be increased by

selection and PrPres was still detectable after more than 200 passages. PrPres signals

were detected by dot-blot, western-blot and by a “Cell ELISA”. PrPres from Bov5Sc

cells showed a similar PK resistance as PrPres derived from murine RML infected cell

lines (ScN2a, SMBRC040). However, Bov5Sc were only extremely slowly cured by

chemical compounds known to effectively inhibit the prion replication, e.g. Suramin

and MAI. Synthetic polypeptides - fragments of bovine prion protein and antibodies against

them have sufficient activity and specificity for the PrPBSE detection with

immunoblotting. This work reports the scrapie epidemiological situation in Portugal and the control

measures that have been adopted to the portuguese scenario.

The portuguese scrapie situation will be characterized by the sampling and analytical

procedures, the geographical distribution, mean age, clinical signs and Prnp profile of

the detected positive samples. Based on those different features and the fact that BSE

was excluded, Portugal developed an alternative strategy to the compulsory culling

policy. Briefly, the affected flocks are placed under intensive surveillance for a 2- year

period with movement restrictions, Prnp genotyping of all adult sheep, electronic

identification of all all small ruminants and PrPres testing of all small ruminants over 18

months slaughtered for human consumption and over 12 months in the fallen stock.

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results regarding the cohorts testing and Prnp genotyping as well as the faced

difficulties.

**P04.04**

**BSE Epizootic Situation in the Russian Federation**

Dybakov, S; Alekhina, A; Ryabokon, A; Meltin, A; Yegorov, A;

FGI Federal Centre for Animal Health, Russia

To assess epidemic situation on bovine spongiform encephalopathy (BSE) specific
diagnostic laboratory was established in the FGI “All-Russian Research Institute for

Animal Health” in 1997 where diagnostic testings for the disease using

histopathological method were started.

During the first years the most part of samples were submitted from various parts of

Russia within the programme on passive monitoring. About 500 bovine brain samples

were tested annually.

BSE neuropathological signs are very similar to the signs of many diseases including

rabies. Since rabies has been registered in some Russian animal farming areas “solid-

phase” method for the rabies virus detection in formalin-fixed brain tissues was
developed for the differential diagnosis of rabies and BSE in the laboratory.

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results regarding the cohorts testing and Prnp genotyping as well as the faced

difficulties.
**Epidemiology, Risk Assessment and Transmission**

**P04.05**

Heat Resistance of BSE Infectivity by Dehydration of Materials
Fujita, Y; Matsuura, Y; Ishikawa, Y; Somerville, R; Kitamoto, T; Yokoyama, T; Mothi, S
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Transmissible spongiform encephalopathy (TSE) is highly resistant to inactivation by heat. In particular, TSE infected tissue is more difficult to inactivate by autoclaving when it has become dried onto surfaces. To demonstrate the effect of hydration on the inactivation of bovine spongiform encephalopathy (BSE) infected tissue by heating, we measured the surviving infectivity after autoclaving using follicular dendritic cell (FDC) bioassay of the knock-in mice expressing the bovine prion protein gene (Ki-Bov mice).

Three different dehydration levels of the BSE affected cattle brain tissue were prepared: homogenate, macerate and dry. The homogenate sample contained 5-fold higher water content than the macerate. The dry sample was air dried at 65 °C for 2 hours and contained 0.6-fold of water content compared to the macerate by weight. The samples were subjected to gravity-displacement autoclave at 132 °C, 134 °C, 136 °C or 138 °C for 20 min and then were prepared as 10% inocula by re-homogenisation and sonication in PBS. At 75 days post intraperitoneal inoculation, spleens of Ki-Bov mice were examined by western blot analysis for the presence of the protease K resistant prion protein (PrP(K)) and by immunohistochemistry for PrP(K) deposition in splenic FDC. No infectivity was detected in homogenate after autoclaving at 132 °C, though macerate showed infectivity after autoclaving at 132 °C but not 134 °C. Surprisingly, infectivity was detected in the dried samples after autoclaving at 138 °C.

Our findings indicate that inactivation of the BSE agent may be ineffective by heating at 138 °C or more if the infective material is in a poorly hydrated state.

**P04.07**

Learning to Diagnose Scrapie by Means of a Probabilistic Approach
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1Istituto Zooprofilattico Sperimentale di Piemonte, Liguria e Valle d’Aosta, Italy; 2Faculty of Veterinary Medicine, Animal Pathology, Italy

So far from a clinical point of view scrapie is still a puzzling disease. In particular in Italy the passive surveillance does not work so well yet. Nevertheless it shows some quite peculiar clinical features.

Aim of this study is to provide a list of clinical signs (features) that allows the classification of a sheep as scrapie or non-scrapie affected minimising the probability of error (Pe). This is the first step in the construction of bayesian classifiers to estimate the likelihood of being scrapie affected or not in presence or absence of certain features.

282 sheep coming from outbreaks have been visited during a four-years period. On the basis of a standardised clinical examination, the presence or absence of 29 features and the disease were recorded. The work is based on the assumption of accuracy of the features and their non total independence. A bayesian approach was adopted, then we calculated the Pe in classifying a sheep as scrapie or non-scrapie affected given that feature; the same operation was repeated for grouped features.

In order to define a stair of relevance to the disease, single or grouped features were ranked by their Pe using 2 methods: single best method (SB) and sequential forward selection method (SFS).

The results show that the bigger the information (i.e. larger is the subset of feature), the smaller the Pe: a subset of 4 features (applying SFS: nibble, depression, abnormal gait from clinical exam, ataxia; applying SB: nibble, ataxia, depression, prunus) allows the reduction of Pe lower than 10%, while if we consider 12 feature then Pe decreases around 5%. Then SFS and SB methods may create subsets with same size but different features.

The mandatory report of scrapie suspects is a very important step in the struggle for its eradication. For this reason supplying a brief list of features to the practitioners in order to identify a scrapie affected sheep with low probability of error is very useful.

Two aspects of the present study are relevant: first, data come straight from the on field practice and refer to individual clinical signs; second, Bayesian approach allows to attach a Pe to a subset of a fixed number of features.

**P04.08**

Environmental Persistence of TSEs - Extraction of PrPSc from Soil
Smith, A; Fernie, Karen; Somerville, R
Neuropathogenesis Unit, UK

Background: There are concerns about the potential spread of transmissible spongiform encephalopathies (TSEs) by environmental routes following, for example, burial of infected carcasses or the disposal of waste water. The extent to which TSE infectivity survives or is disseminated within soil and soil water is unclear as is the likelihood of ensuing infection.

Aim: As part of this environmental project, soil samples are being collected from lysimeters containing either infected bovine heads or boluses of infectivity. The aim of this experiment is to devise a method for the extraction of PrPSc from soil and examines the interaction between soil and its components and TSE infectivity.

Methods: Samples from two soil types (clay and sandy loam) were spiked with known amounts of TSE infected brain homogenate and subjected to various extraction methods including combinations of freeze/thaw, boiling, sonication and mixing with various solvents and detergents. Any recovery was determined on western blot using PrPSc as a surrogate marker for the presence of TSE infectivity.

Results: These experiments have shown that PrPSc binds strongly to both sandy and clay soil, and to pure sand (quartz). Elution from quartz and the soils was only achieved in the presence of the detergent sarkosyl, and in the case of clay soil, satisfactory elution was only achieved if PrPSc was digested with protease K. This finding suggests that components in clay soil may bind differently to PrP than those of sandy soil, and that the N-terminal domain of PrP is involved in this binding.

Conclusion: These results form the basis of a method for the extraction of PrPSc from soil and will be used to assay samples from a large scale lysimeter experiment. Samples testing positive for the presence of PrPSc will be selected for bioassay in mice. Results to date suggest that TSE infectivity may bind strongly to soil components and could therefore persist in the environment for long periods of time.
**P04.09**

**Modelling the Within Herd Prevalence of Scrapie Goats in Italy**

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Natural scrapie in goats was first reported in France. Other cases were then described in UK, Canada, Cyprus and Greece. Some of them occurred after contact with scrapie-affected sheep but some did not. In Italy, the first case of natural scrapie in goats was diagnosed in 1997. In that year a sudden rise in scrapie incidence involving both sheep and goats was attributed to a potentially contaminated vaccine. Such spikes in the incidence incite to investigate on the reasons why epidemic changes happen.

Aim of the study was to investigate risk factors accounting for the variability of within herd prevalence among the different outbreaks of natural scrapie in goats since it was confirmed in Italy.

A generalized linear latent mixed model was applied to model within herd prevalence (cases per 10,000 tests) using the data coming from the 45 goat scrapie outbreaks (14 mixed flocks of sheep and goats and 31 goat-only herds) that have been reported since the first case of natural scrapie to date. Using a hierarchical logistic regression, this model allows to multiply random effects not just by a single variable but by a linear combination of variables. The statistical unit was established to be the sheep. The levels we included in the model were: herd, region and the use of a particular vaccine; the explanatory variables were age, breed, husbandry and genotype.

As all the scrapie cases had the same genotype (222Q/Q), showing a significant association with scrapie status any further statistical analysis which included the genotype as a variable was precluded. Moreover the results show a difference in the presence of the disease associated with the fixed effects; however no large random effects were evident.

An adequate statistical modelling of the disease in goats has to account for the clustering of the animals in herds and regions; the mixing model applied in this work has contributed to identify factors associated to the spreading and the persistency of scrapie in the Italian goat herds during the last years.

**P04.11**

**Plaque and Non-plaque Types of Dura Mater Graft-associated Creutzfeldt-Jakob Disease: Clinicopathological and Molecular Analysis**

Yamada, M1; Noguchi-Shinohara, M1; Hamaguchi, T1; Kitamoto, T2; Sato, T3; Nakamura, Y4; Mizusawa, H5; CJD Surveillance Committee, Japan6

The plaque and non-plaque types presented with type 1 PrP res on Western blot. (2) respectively, except for a non-plaque type patient with a Glu/Lys at codon 219. Both sensitivities for plaque as well as non-plaque types. The gender, age, or sites of dura and CSF 14-3-3 protein and neuron specific enolase showed high diagnostic sharp-wave complexes (PSWCs) on EEG. MRI, especially diffusion-weighted images, initial manifestation, relatively slow progression, and no or late occurrence of periodic classical CJD features, the plaque type commonly presented with ataxic gait as an initial manifestation, relatively slow progression, and no or late occurrence of periodic classical CJD features, the plaque type commonly presented with ataxic gait as an initial manifestation.

**P04.12**

**Prion Protein in Milk**

Francescini, N1; El Gedaly, A; Matthey, U; Franitza, S; Zahn, R
Alicon AG

There is increasing evidence that prions are present in body fluids and that prion infection by blood transmission is possible. Using the technology developed by alicon to detect (alicon ProMax®) and to remove (alicon PrionMin®) prion protein, we are able to show that PrP® is present in milk from humans, cows, sheep, and goats. The absolute amount of PrP® differs between the species, from μg/l range in sheep milk, to ng/l range in human milk. Off-the-shelf milk contains significant amounts of endogenous prion protein even after ultra-high temperature treatment. In view of a recent study showing evidence of prion replication occurring in the mammary gland of scrapie infected sheep suffering from mastitis, the appearance of PrP® in milk implies the possibility that milk of TSE-infected animals serves as a source for PrP®. The study of the biosafety of milk with regard to TSE has been recently recommended by the European Commission and the European Food Safety Authority.

ProMax® is able to detect minuscule amounts of PrP® in milk spiked with brain homogenate. We are currently adapting ProMax® for detection of endogenous PrP® in milk from scrapie infected sheep. First results may be presented during the conference.

Epidemiology, Risk Assessment and Transmission

P04.13 Variant CJD: A Review of Individuals Designated “at Risk” due to Exposure through Medical Procedures or Blood Transfusion

Noguchi-Shinohara, M; Nakamura, Y; Sato, T; Kitamoto, T 1; Sneddon, J; Reilly, J; Chow, Y; Soldan, K; Knight, RSJ; Will, RG
1National CJD Surveillance Unit, UK; 2Health Protection Scotland, UK; 3Health Protection Agency, UK

Introduction: Variant CJD (vCJD) is caused by human infection with the bovine spongiform encephalopathy (BSE) agent. Concerns about the possibility of a large UK epidemic of vCJD due to the past extensive exposure of the human population to BSE have receded, with recent mathematical models predicting that the primary outbreak of clinical cases may be relatively restricted. However, reports of four incidents of transmission of vCJD infection via blood transfusion raise the possibility of a self-sustaining secondary epidemic.

Methods: The CJD Incidents Panel (CJDIP) was established by the UK Chief Medical Officers in 2000 with the remit of managing incidents involving potential transmission of CJD between patients through invasive medical procedures, including blood transfusion, surgery and organ and tissue transplants. Potential healthcare exposures are reported to the CJDIP and, depending on the estimated level of risk, public health measures are implemented, including quarantining of instruments and notification of possible ‘contacts’ of their status as “at risk of CJD for public health purposes”.

Results: To date, there are five groups of individuals who have been designated “at risk of CJD” through potential exposure to vCJD: 1) those exposed to potentially contaminated healthcare instruments; 2) recipients of blood from donors who later developed vCJD; 3) recipients of certain plasma-products; 4) donors of blood to people who developed vCJD, and 5) recipients of blood from certain ‘at-risk’ donors. This paper describes the process of notification and risk assessment, the “at risk” categories, including the numbers designated “at risk” and their follow up, and considers the policy implications.

Conclusion: The follow up of these groups is essential to determine whether transmission of vCJD has occurred through routes other than through blood transfusion. This will inform public health policy in the UK and elsewhere.

P04.14 Modelling the within Herd Prevalence of Scrapie in Goats

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Natural scrapie in goats was first reported in France. Other cases were then described in UK, Canada, Cyprus and Greece. Some of them occurred after contact with scrapie-affected sheep but some did not. In Italy, the first case of natural scrapie in goats was diagnosed in 1997. In that year a sudden rise in scrapie incidence involving both sheep and goats was attributed to a potentially contaminated vaccine. Such spikes in the incidence incite to investigate on the reasons why epidemic changes happen.

Aim of the study was to investigate risk factors accounting for the variability of within herd prevalence among the different outbreaks of natural scrapie in goats since it was confirmed in Italy.

A generalized linear latent mixed model was applied to model within herd prevalence (cases per 10,000 tests) using the data coming from the 45 goat scrapie outbreaks (14 mixed flocks of sheep and goats and 31 goat-only herds) that have been reported since the first case of natural scrapie to date. Using a hierarchical logistic regression, this model allows to multiply random effects not just by a single variable but by a linear combination of variables. The statistical unit was established to be the sheep. The levels we included in the model were: herd, region and the use of a particular vaccine; the explanatory variables were age, breed, husbandry and genotype.

As all the scrapie cases had the same genotype (222Q/Q), showing a significant association with scrapie status any further statistical analysis which included the genotype as a variable was precluded. Moreover the results show a difference in the presence of the disease associated with the fixed effects; however no large random effects were evident.

An adequate statistical modelling of the disease in goats has to account for the clustering of the animals in herds and regions; the mixed model applied in this work has contributed to identify factors associated to the spreading and the persistency of scrapie in the Italian goat herds during the last years.

P04.15 Ophthalmic Surgery in Prion Diseases

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Background: Ophthalmic surgery may have a role in transmission of prion diseases because eyes carry high prion infectivity. However, little information is available about ophthalmic surgery in patients with prion diseases.

Methods: We investigated the number and clinical features of the patients with ophthalmic surgery around or after the onset of prion diseases in 597 patients with definite or probable prion diseases registered by the Creutzfeldt-Jakob Disease Surveillance Committee in Japan from April 1999 through March 2005. For gathering information about the ophthalmic surgery, we mailed questionnaires to the ophthalmologists who operated on these patients.

Results: Eleven (1.8%) of 597 patients with prion diseases, comprising sporadic Creutzfeldt-Jakob disease (n=10) and Gerstmann-Sträussler-Scheinker disease (n=1), underwent ophthalmic surgery for cataract (n=10) or detached retina (n=1) around or after the onset of prion diseases. The sporadic Creutzfeldt-Jakob disease cases were performed ophthalmic surgery around or after the onset of Creutzfeldt-Jakob disease (n=10) and Gerstmann-Sträussler-Scheinker disease (n=1), respectively.

Conclusions: Some patients with prion diseases may undergo ophthalmic surgery for often coexisting cataracts without diagnosis of prion diseases, and early visual impairment due to prion diseases would prompt ophthalmologists to perform surgery. To prevent transmission through ophthalmic surgery, ophthalmologists should pay attention to the possibility of prion diseases for their patients with visual symptoms, and the surgical instruments should be disposable.

P04.16 A Rapid, Quantitative Assay for the Presence of Steel-bound Prion Infectivity in Cultured Cells

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Background: Although in many cases PrP Sc is an accurate surrogate marker for the presence of prion infectivity in some circumstances it can be dissociated from biological activity. The standard method for direct assay of infectivity has historically been rodent bioassay. However, recent advances have allowed the use of highly sensitive cell-culture assays to detect the presence of prions. Such assays have proved extremely useful for the investigation of many aspects of prion biology but have not been appropriate for use in prion decontamination studies due to the need to study the behaviour of infectivity bound to steel surfaces.

Aims & Objectives: To develop, optimise and validate a cell based bioassay that can sensitively and quantitatively detect RML prions bound to stainless steel wires with sensitivity equivalent to or exceeding conventional whole animal bioassay.

Methods: Dilutions of RML-infected homogenates of known titre were adsorbed to surgical steel surfaces pre-treated in various ways to give optimal transfer of infectivity. The susceptible subclone of murine neuroblastoma cells (N2A-FK1) were grown in contact with the wires and harvested. The cells were cultured and assayed for the presence of propagating PrPSc and from this the number of infectious units bound to the wires quantified.

Results: The new assay has improved sensitivity and is capable of determining infectivity in a 10⁴ fold dilution of RML infected brain. Application of this assay facilitates the rapid study of how prion infectivity behaves when bound to surgical steel. This has confirmed that prions adsorbed to steel surfaces have an altered behaviour to those in solution, including enhanced resistance to thermal inactivation.

Discussion or Conclusion: This novel assay offers a new methodology for the evaluation and validation of prion decontamination methodologies and we have demonstrated that an enzyme detergent method for prion decontamination can reduce infectious titre by over 10⁴ fold.
P04.17
Sequence Variants in the Prion Protein of European Moose, Roe Deer, Fallow Deer and Reindeer in Scandinavia

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The prion protein sequence from cervids in Scandinavia such as European moose, roe deer, fallow deer and the semi-domesticated reindeer have high homology to the prion protein of North American cervids. Allelic variants exist in the European moose at amino acid position 36 as TT or TN and at amino acid position 209 as MM, Ml or II, in the reindeer at amino acid position 176 as DD or NN and position 225 as SS, SY or YY. Certain variants that are connected to a suggested susceptibility to Chronic Wasting Disease (CWD) are present in Scandinavian cervids and a corresponding susceptibility to CWD may also be expected.

P04.18
Is Natural Prion Disease in Sheep, Goat and Cervids Triggered by a Microbial Protein?

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Though there is overwhelming evidence for a conformationally altered form of PrP being the causative agent of prion diseases the reason why scrapie of sheep and goat and chronic wasting disease (CWD) of cervids – in contrast to prion disease in any other species – spread by contact remains elusive. Observations made by research on investigating the transmissibility of scrapie that only sheep and goat with natural scrapie transmit prion disease horizontally suggest that a vector with a limited host range is responsible for “contact transmission”. Since many microbes are well-known to have a limited host range and reside in the alimentary tract – where natural infection with scrapie and CWD initiates – a microorganism would make a promising candidate for the vector. A recent report by Mathiasson et al. /Science 2006, 314, 133-36/ that natural CWD is transmissible with saliva only if it is administered parenterally but not if given by injection provides additional evidence. When delivered parenterally the microbe should be capable of establishing long-term colonization of the alimentary tract and one of its proteins might gain access to PrPc to alter its conformation. In contrast, if the microbe is introduced parenterally it will soon be destroyed by host defenses.

There could be many additional degenerative diseases developing subsequent to infection with microbes showing similar pathomechanisms. E.g. rheumatic fever caused by the M protein of Streptococcus pyogenes displaying an approximately 40 per cent homology to cardiac myosin may develop after extended streptococcal disease of the upper respiratory tract or of the skin but never following short-term septicemia. Moreover, streptococcal M protein has multiple serotypes, displaying slightly diverse amino acid sequences and eliciting rheumatic fever with distinct efficiencies. The microbial protein triggering scrapie and CWD might also command several serotypes that could account for the existence of the strains of prion.

P04.19
First Results from a Study on Prion Diseases in European Mouflon (Ovis Gmelini Musimon) in Germany

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Background: The European mouflon is an ancestor of the domestic sheep and is originated from Southwest Asia. During the 18th century mouflon were introduced to Central Europe for hunting. Currently, Germany has the second largest free ranging population in the world and mouflon game is consumed on a relatively large scale. Since natural occurrences of transmissible spongiform encephalopathy (TSE) in mouflon from England have been reported and prion protein nucleotide sequences can be identical in mouflon and sheep, it is likely to assume that transmission of TSE from sheep to mouflon is possible. Moreover, transmission may be facilitated by the fact, that TSE infected cattle and sheep share the same habitat with mouflon.

Aims: The objectives of our study are (1) to define TSE occurrence in European mouflon in Germany and (2) to evaluate the applicability of rapid tests for mouflon samples.

Methods: Thus, in August 2006 we started a two-year survey based on a previous study on cervids. Since the prevalence of TSE in mouflon is expected to be low, special risk areas have been defined by the abundance of sheep, mouflon, and cases of scrapie. Areas with high risks were favoured for sampling. In order to detect both typical and atypical scrapie cases, samples from the oesophagus, cerebellum, and retropharyngeal lymph nodes are taken. These are analysed by a highly sensitive BSE-scrapie enzyme immuno assay (EIA).

Results and Conclusion: Over 300 mouflon samples were analysed by the EIA so far; all animals were tested negative. The final results from our ongoing study will allow evaluating the potential risk of occurrence of TSE in mouflon in Germany.

P04.20
Results of TSE Surveillance in the Belgian Sheep Population

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The European breeding program favouring scrapie resistant alleles is confronted to the appearance of atypical cases, e.g. in scrapie-resistant genotypes. In this context an overall view of the characteristics of the TSE found in sheep is essential. We therefore present latest data on TSE positive sheep found in Belgium. Since 2001, all dead or slaughtered sheep older than 18 months undergo a rapid test for TSE. All 2009 suspected and fallen stock sheep are tested with histopathology, immunohistochemistry, electron microscopy and since 2002 Western blotting. Since 2002, all sheep from positive farms have their PRNP gene sequenced to evaluate their scrapie resistance status. In the atypical cases, in addition to codons 136, 154 and 171, we also control codon 141. Furthermore we carried out 35 discriminatory diagnostics using the CEA Bio-Rad Western blotting, for which we are recognized by the CRL/IAQ. We also genotyped all atypical scrapie and the latest classical scrapie case for 5 extra PRNP polymorphisms associated with higher PRNP mRNA expression in blood (see poster Willemsack et al.).

From 1992, we observed 24 primary cases for a total of 77 scrapie-affected sheep from 33 farms. We observed 8 atypical cases (7 Nor/8 + 1 PK sensitive). We observed sheep from all susceptibility groups except group 4 (ARR/RG). From 2002 to 2004, the 10 positive sheep belonged to groups 3 and 5. However since the end of 2004 they came from groups 1, 2 and 3 (N=8). This difference could be related with the replacing of classical scrapie with atypical cases. Indeed we observed a unique atypical case before 2004 and 7 atypical cases out of 8 positive sheep from 2004 onwards. All atypical scrapie sheep were homogoytale LL for codon 141, except one Nor98, which was L/7. Seven atypical cases didn’t possess any of the 5 polymorphisms associated with higher PRNP mRNA expression in blood, 1 atypical case was heterozygous for all 5 polymorphisms and the classical case was homozygous for all 5 polymorphisms. The discriminatory tests on the non-atypical cases showed that all samples corresponded to classical scrapie, excluding the possibility of BSE cases in sheep in Belgium till now.

Finally there were no more scrapie cases than statistically expected in herds bearing an atypical strain.

According to our results the proportion of atypical cases is increasing through time. This could be due to the selection for resistant genotype but this hypothesis needs further data to be confirmed.
The removal of Central Nervous System (CNS) tissues from the food chain as part of the Bovine Spongiform Encephalopathy (BSE) specified risk material is one of the highest priority tasks to avoid the transmission of BSE to humans. This study compared the diagnostic applicability of a recently developed real-time PCR assay with the Ridascreen® Risk material ELISA (raw meat), the Ridascreen® Risk material ELISA (heat treated meat products) and the ScheBo Brainostic GFAP ELISA Kit. Artificially contaminated minced meat as well as different types of moderate and strong heat treated cooked sausages (“Leberwurst-Kesselkonserven”, “Dreiwerkel- and Vollkonserven”) were prepared as internal reference material. The detection limit for bovine brain material was examined throughout a storage time of 14 days for freshly minced meat, 12 months for frozen minced meat and 24 months for the heat treated meat products. The real-time PCR method and the ELISA kits proved to be suitable for the detection of 0.1% CNS tissues in fresh and frozen minced meat. Correct results were obtained even at the end of the storage periods for the different storage conditions. Bovine brain containing heat treated meat products could be reliably identified with all three test systems used. Based on the comparative study and a conducted multi-center trial, the real-time PCR assay has been proven to be useful as a routine diagnostic test for the detection of CNS tissues in meat and meat products.

It is commonly considered that only three polymorphic codons, namely 136, 154 and 171 of the PRNP gene are of fundamental importance in moderating resistance/susceptibility to scrapie. As in other breeds, the wild type ARQ sequence of 112 of the PRNP gene are of fundamental importance in moderating resistance/susceptibility to scrapie. Bovine brain containing heat treated meat products could be reliably identified with all three test systems used. Based on the comparative study and a conducted multi-center trial, the real-time PCR assay has been proven to be useful as a routine diagnostic test for the detection of CNS tissues in meat and meat products.
Epidemiology, Risk Assessment and Transmission

P04.25

The EFSA Geographical BSE Risk Assessment Methodology
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Background: The Geographical BSE-Risk assessment (GBR) is an indicator of the BSE risk in a given country/region. The Scientific Steering Committee (SSC) of the European Commission (EC) developed a GBR methodology (SSC GBR) in 1998. As a result of its application, firstly by the SSC and from 2003 by the European Food Safety Authority (EFSA), all EU Member states and a number of non-EU states have been classified within four categories. The experience obtained by EFSA while carrying out the most recent assessments, highlighted the need for a review of the SSC GBR. As a result, an updated model - EFSA GBR - has been developed.

Aim: To present the EFSA GBR focusing on the assumptions and inputs of the model and on the differences with the SSC GBR.

Methodology: The model is based on two assumptions: (1) BSE arose in the United Kingdom (UK) from an unknown initial source; (2) it was exclusively propagated through the recycling of contaminated bovine tissues into animal feed. Thus, for all countries other than UK, imports of Meat and Bone Meal (MBM) or live cattle are the only possible initial sources of BSE to be taken into account for this methodology. The main two components of the EFSA GBR are: (i) External Challenge, which is the likelihood and the amount of the BSE agent entering into a geographical area in a given time period through infected cattle and/or MBM; (ii) Stability, which is the ability of a BSE/cattle system to prevent the introduction and to reduce amplification and spread of the BSE agent within its borders. The interaction between stability and challenge determines how the GBR develops over time. A specifically designed Excel worksheet has been created by EFSA to simulate the interaction.

Discussion: The methodology provides a framework to assess the likelihood of BSE being present in a specific country/region over a given time period. If the presence of BSE is likely, the EFSA GBR identifies the risks linked to the imports over time. It allows assessing periods of increasing and decreasing BSE risk and predicting the trend of the infection. The methodology does not depend on BSE surveillance data, as availability varies and is lacking in association the model, the EU BSE risk status, as it does not categorise a country/region. However, it provides a methodological framework that could be used for the risk assessment prescribed within the OIE procedure.

P04.26

Animal TSEs in Poland - High Prevalence of Atypical BSE in Cattle with no Scrapie in Small Ruminant Population
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B. The prevalence of bovine spongiform encephalopathy (BSE) and scrapie within European Union Member States differs although general trend shows constant decline in the number of cases found despite high testing volume within active surveillance. New forms of both diseases termed as atypical are reported.

A. The aim of the study was to describe the current status of BSE and scrapie in Poland.

M. Testing for BSE in Poland started in 1996 when histopathology was introduced within passive surveillance scheme to test clinical suspects. However, due to a low number of samples tested no cases were found. Active monitoring using rapid test was initiated in January 2001 and it continued on low scale until 2002 when 5 regional labs took over the regular testing of healthy slaughtered and risk animals. R. After testing around 110,000 samples mostly from slaughterhouses first case of BSE was diagnosed and confirmed in May 2002. Since then 49 cases were found within active surveillance while two additional cases were found in clinical suspects under passive monitoring. Due to the fact that majority of samples tested came from healthy slaughtered animals, 76% of total 51 cases confirmed so far were found in this group of animals. Surprisingly, atypical cases of BSE comprised 14% of all cases, which is relatively high when compared with other countries where this form of BSE was also found. On the other hand despite testing sheep and goats for scrapie since May 2002, Poland is still free from this disease. Small ruminant population in Poland is roughly 300,000, but majority of animals are slaughtered as lambs for meat, and testing volume is very low (4563 animals tested until the end of 2006 with 56% comprising fallen stock and the rest of samples coming from healthy slaughtered sheep and goats).

D. One of possible explanations for the phenomenon of high frequency of atypical BSE in Poland is generally higher mean age of BSE cases (7.6 years), which favours the detection of atypical form of the disease. Referring to the negative results for scrapie, the relative resistance of sheep to classical scrapie has to be considered, since 73% of Polish breeds belongs to very resistant and resistant genotypes (type 1 and type 2 according to British standards).

P04.27

Experimental BSE Infection of Non-human Primates: Efficacy of the Oral Route
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Background: In 2001, a study was initiated in primates to assess the risk for humans to contract BSE through contaminated food. For this purpose, BSE brain was titrated in cynomolgus monkeys.

Aim: To present the results of this study and discuss the implications for the risk assessment prescribed within the OIE procedure.

Methods: Five groups of macaque were orally dosed with different doses of BSE brain. The monkeys were monitored for clinical signs of infection and for the presence of BSE by histology and biochemical methods.

Results: In an ongoing study, a considerable number of high-dosed macaques already developed clinical symptoms of BSE by 5g oral and 5mg i.c. The difference in the incubation period between 5g oral and 5mg i.c. is only 1 year (5 years versus 4 years). However, there are rapid progressors among orally dosed monkeys that develop simian vCJD as early as 1 year after challenge. The work referenced was performed in partial fulfilment of the study “BSE in primates” supported by the EU (QLK1-2002-0198).

P04.28

PRNP Polymorphisms Associated with PRNP mRNA Expression in Blood in Belgian Sheep Population
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Out of 169 bi-allelic polymorphisms, found by sequencing the complete PRNP gene of 40 sheep showing differential PRNP mRNA expression in blood, 5 single PRNP polymorphisms (4 in intron 2 and 1 in 3’-UTR) were found to be associated with higher PRNP mRNA expression in blood, using a non-parametric multifactor dimensionality reduction (MDR) method.

In order to estimate the frequency of these polymorphisms in the Belgian sheep population, 350 unrelated sheep, constituting 7 different breeds (Ardenise Voskop, Bleu du Maine, Hampashire Down, Rouge de l’Ouest, Suffolk, Texel and Vlaams Kuddeschaap), were genotyped.

For each of the 5 polymorphisms an assay (PCR-GE, PCR-RFLP or sequencing) was designed and performed on genomic DNA of 350 sheep, isolated from blood by alkaline lysis.

All 5 polymorphisms could be detected in all tested breeds, except in Bleu du Maine (N=61) and Rouge de l’Ouest (N=28), with an averaged prevalence of 18.02%. Sheep homozygous for the polymorphisms were only found in Vlaams Kuddeschaap (highest prevalence of 30%; N=53). Haplotype analysis shows that all 5 polymorphisms are in strong linkage disequilibrium. Genotyping was also performed on 9 scrapie cases in Belgium (8 atypical and 1 classical; see poster Dobly et al.). Two of the polymorphisms will be used for genotyping a larger number of TSE infected/non-infected sheep in order to detect a possible association with TSE susceptibility/resistance based on the hypothesis that lower mRNA expression results in higher TSE resistance. All polymorphisms are currently subjected to a characterisation assay in vitro to study their role in PRNP mRNA expression.
P04.29
Crisis in Slow Motion: The Community Impact of BSE
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The bovine spongiform encephalopathy (BSE) crisis in Canada in 2003 resulted in economic losses exceeding 2.5 billion dollars. Although much concern and effort has been placed on the economic impact of BSE on the cattle and dairy industries, little has been done to address the psychosocial implications of the BSE crisis in particular on the mental and social health of individuals from rural and farm communities. Moreover, to date there has been a dearth of psychosocial Canadian research that has approached the BSE crisis from a population health point of view. This study recognizes the complex interaction and impact of the social determinants of health on the mental health of populations affected by BSE.

This paper presents the research methodologies and results to-date of the PriOnNet funded study, “The Socio-economic Impact of BSE on Rural and Farm Families in Canada”. We identify the economic and psychosocial impact of BSE on rural and farm family health and socioeconomic well-being, including community resiliency, in Canada. We also assess lessons learned from other countries about effective risk management practices for BSE.

The methods used to achieve the above objectives include: a) preliminary literature review and synthesis on the impact of BSE/TSE on the quality of rural life, and the health and socio-economic well being of rural families and communities in Canada and internationally; b) capturing and analyzing existing information on the impact of BSE on rural families and conducting surveys with key informants; and c) cross country focus groups with farm and rural Canadian households to understand the impact of BSE on farm women, children, families and communities from a risk assessment point of view.

In this process, we build upon and expand existing rural and farm research and engage rural and farm communities in a dialogue to identify key issues, gaps, and solutions to the BSE crisis.

The study findings contribute to the development of a gender-sensitive risk management model for BSE that is encompassed within an ecological framework. Furthermore, in collaboration with project partners at the McLaughlin Center for Population Health Risk Assessment, we are contributing to an Integrated Risk Management Framework to address deficiencies in past practices in managing priority disease risks as well as mitigating future risks involved in potential crisis caused by infectious disease impacting farm families and rural communities.

P04.30
Use of Back-Calculation Method to Estimate the Past Incidence Rate of BSE and Predict the Future Course of vCJD
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Due to the long and variable incubation period of prion diseases including BSE and vCJD, current reported clinical cases do not necessarily reflect number of cases incubating the disease. In addition, there is great uncertainty in the future size of both BSE and vCJD because several epidemiological features of these diseases are still largely unknown. Therefore, it is critical to develop mathematical and simulation models to estimate retrospectively the number of animals infected with the etiologic agent and then estimate the extent of human exposure to infected cattle tissue.

The Back-calculation method was originally developed to obtain estimates of past HIV-infection numbers based on the incidence of AIDS cases, and to predict the future course of the disease. The method was successfully adapted for the analysis of BSE incidence data and it relies on the principle that the known number of clinical cases of BSE is due to an unknown number of animals infected in the past and the disease’s incubation period. The method incorporates important parameters affecting the likelihood of infected cases eventually seen as clinical cases including probability of survival, time-dependent case reporting rate and age-dependent susceptibility/exposure of animals. The method can be also used to predict the future size of any vCJD in Humans.

The method has been used to estimate the rate of BSE infection in the UK, France and other European countries. The results showed significant underestimation of the epidemic in both France and the UK. In France, passive surveillance identified only 20% of infected case compared to complete case identification by the active surveillance system adopted in July 2001. Since Canada, USA and other countries still rely on the passive surveillance system and targeted screening for case reporting, it would be practical to apply this method to evaluate the extent of case underreporting, estimate the real incidence rate of BSE infection, provide estimates of the future scale of the epidemic, and assess the risk of vCJD.

P04.31
Risk Management Case Study of the French BSE Outbreak
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The emergence a new infectious disease with serious economic, psychosocial and health consequences (like BSE) creates an enormous pressure on scientists, public health practitioners, and policy makers to evaluate the magnitude of the problem and identify the appropriate measures to minimize the risks. Several countries experienced a full-scale BSE epidemic and already surpassed the peak of the disease. In those countries, successes and failures of risk management practices that shaped their BSE crisis represent a valuable experience, and several lessons could be derived and applied to other countries in which the disease is still emerging.

France was the country where the first case of BSE was diagnosed outside the UK in February 1991 and currently has the second highest prevalence of vCJD. Throughout the epidemic, lack of appropriate risk assessment procedures and delay in adopting the necessary control measures contributed to the sharp increase of BSE cases in the late 90s. For example, early in the epidemic, the decision-making process was not based on scientific evaluation. Instead, scientific community was mobilized ex post in order to legitimize the decision. Another important feature in managing the BSE epidemic was that the French always lagged behind the UK in taking the proper precautionary actions. For example, British banned MBM for animal consumption in July 1988, while only in 1990 France banned MBM only for cattle. This has caused widespread cross-contamination of animal feed for different species. Also France banned the consumption of BSE-infective parts (brain, spinal cord, offal...etc) in July 1996, almost six months after the British government adopted the same measure. This had probably led to the alarming increase of vCJD incidence at the beginning of 2000.

Despite these drawbacks, the French government accomplished great success in 2001 when they brought the epidemic under control after imposing and enforcing a complete ban on the use of MBM in animal feed.

The important lessons that can be learned from the French BSE experience are: relying on the available scientific knowledge in adopting decisions, acting rapidly in application and enforcement of the necessary control measures to prevent the spread the disease and protect human health, including other countries’ experiences in managing BSE risks, and mobilizing the scientific community to embark on active and sound research on BSE basic science and epidemiology.

P04.32
Prion Inactivation using a New Generation of Gaseous Hydrogen Peroxide Low-Temperature Sterilization Process
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Background: Prions are unique infectious agents which have been shown to be transmitted intracerebrally through contaminated surfaces. Specific decontamination methods have been recommended due to the unique resistant nature of prions, but they are harsh and corrosive. There is an urgent need to propose alternative methods compatible with fragile devices. A previous study has shown that cleaning followed by treatment with vaporized hydrogen peroxide (VHP) under atmospheric conditions was an effective prion decontamination method.

Objective: This report investigated the effectiveness of a new generation of gaseous hydrogen peroxide low-temperature sterilization process.

Methods: In vitro and in vivo test methods have been used. For in vitro studies, glass slides were contaminated with infected homogenates including scrapie, BSE, vCJD, sCJD and analysed by a standard Western blot method. For in vivo studies, stainless steel wires were artificially contaminated with scrapie or BSE material, dried, exposed and evaluated in animal models. The process was designed to sterilize devices under vacuum in a dedicated chamber. Surfaces were exposed to the vacuum process at 30°C for 3 pulses or 6 pulses.

Results: Sterilization under vacuum with VHP was effective in a short time, even in the absence of cleaning. Reduction of infectivity was>5.6 logs both on the 263K and 6P81 strains similar to the reduction induced by classical recommended procedures (Fichet et al., Lancet 2004). The mechanism of action of gaseous peroxide suggested protein unfolding, some protein fragmentation and higher sensitivity to proteolytic digestion in contrast to the hydrogen peroxide liquid showing a degree of protein clumping and full resistance to protease degradation.

Conclusions: The use of gaseous peroxide in a standard low temperature sterilization vacuum process presents a useful method for prion inactivation. It is rapid (less than 1 hour), efficient as the WHO recommended procedures, validated on scrapie and BSE models and compatible with fragile devices. With the development of new processes that are effective at removing and inactivating prions, in addition to more conventional microbial pathogens, it would seem likely that such precautions will be practical and widely used in the future.
A Powerful Cell Sorter at the Service of the Prion Research Community

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The foundation Alliance Biosecure aims to sponsor research on the diagnosis, comprehension and management of risks for public health, linked to microbiological agents, in particular prions. Recognized as a Public Utility in December 2006 by the French Government, Alliance Biosecure promotes the discovery of technological solutions aimed at improving the detection, elimination and inactivation of these agents within biological products used in human therapy.

Indeed biological safety regarding prion risk constitutes a major public health issue, as publicized by the report of a fourth human variant Creutzfeldt-Jakob Disease (vCJD) infection attributed to blood transfusion, in the UK.

For this purpose, Alliance Biosecure has launched in 2007 a first call for grant applications and acquired laboratory equipment that will be made available to selected projects. Of special note is the availability of a three-laser Influx® cell sorter, located in a BSL-3 containment facility (NeuroPrion CEa Research Platform, Fontenay-aux-Roses).

This cell sorter was developed by Cytopeia (Seattle, WA, USA), with 12 detectors and a modified design suitable for Prion experiments, including specially adapted decontamination procedures.

Here, we present several different experiments that illustrate the capabilities of this cell sorter. We show its capacity to separate complex cell populations and to isolate cells with particular characteristics, including cell count, protein, IgG index, oligoclonal bands (OCBs), 14-3-3, NSE, T-Tau in patients with probable or definite sporadic CJD and non-prion rapidly progressive dementia (RPD), most of whom were referred to our center with a potential diagnosis of CJD. For probable sCJD diagnosis, we used U5C criteria that are modified from WHO to substitute MRI for 14-3-3. T-Tau and NSE were considered positive if > 1300 pg/ml and > 35 ng/ml, respectively. For this analysis, ambiguous 14-3-3 results were considered as negative.

Results: 14-3-3 protein (n=149) sensitivity was only 48% (47% for definite; 50% for probable sCJD), NSE (n=49) sensitivity was 63% (66% for definite; 59% for probable sCJD); T-Tau (n=28) was the most sensitive at 68% (69% for definite; 67% for probable sCJD). The specificity of these biomarkers among our CJD and RPD controls (n=72) was 66% for 14-3-3 (n=47), 81% for NSE (n=21), and 100% for T-Tau (n=7). The 14-3-3 had the lowest sensitivity and specificity. Mildly elevated CSF protein (<100 mg/dl) is common in sCJD; High WBC; >20, is uncommon in sCJD.

Conclusions/Relevance: In a cohort from a major U.S. CJD referral center, the 14-3-3 sCJD criteria should be revised; by eliminating 14-3-3 and including brain MRI into the criteria. We are currently analyzing the effects of disease duration and codon 129 polymorphism on these CSF biomarker results.

Enhanced Surveillance of Persons Identified as at Increased Risk of CJD Due to Blood Transfusion or Healthcare Procedures

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Introduction: Reports of four iatrogenic transmissions of variant-CJD (vCJD) infection in the UK (all due to transfusion of blood from donors who later developed vCJD), evidence from iatrogenic transmissions of sporadic CJD and experimental work on CJD infectivity in tissues and on healthcare instruments have given rise to concern about the risks of iatrogenic transmission of CJD. This risk warrants a) certain public health precautions, and b) follow-up of individuals with identified risks in order to gain evidence about their risks and ensure appropriate management of these risks. Evidence of transmission via iatrogenic routes is important to inform public health measures and so prevent ongoing transmission of CJD.

Methods: The Health Protection Agency and Health Protection Scotland holds details of persons identified as ‘at-risk’ of CJD due to blood transfusion or from other healthcare procedures. The GPs/clinicians of all persons identified as ‘at-risk’ for public health purposes are provided with: information; risk assessment updates; advice on public health precautions and advice on referral to specialist care. Procedures are being established to obtain enhanced surveillance data on these individuals, including: clinical status updates, date and cause of death, survival tissue and blood specimens, and post-mortem investigations.

Results: Persons ‘at-risk’ of vCJD have experienced a range of exposures. Estimated risks are uncertain and overlapping. Some individuals - recipients of vCJD implicated blood components - are considered to be at a clearly higher risk of infection: active follow-up is currently conducted for these individuals. In time, the enhanced surveillance of persons at increased risk of CJD will provide estimates of transmission risks and of the impact of iatrogenic exposures on mortality.

Conclusions: Knowledge about iatrogenic transmission of CJD is being gained by the follow-up of individuals who have been identified as ‘at-risk’ of CJD in the UK. This enhanced surveillance may need to be sustained for many years.
**Comparison of the Neuropsychological Profile of Patients with Sporadic Creutzfeldt-Jakob Disease and Patients with Alzheimer's**

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Background: To evaluate the neuropsychological profile of sCJD we administered the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-cog) in order to determine if and how the sCJD-Subgroups (Met/Met, Met/Val, Val/Val) have different results in the item analysis of the ADAS-cog. Furthermore, we studied how the scores differ from that of patients with Alzheimer's disease (AD).

Methods: 33 sCJD patients (11 with definite CJD and 22 with probable CJD) underwent neuropsychological testing with the ADAS-cog and Mini Mental State Exam (MMSE). Of these 31 were genotyped at the Codon 129 (11 Val/Val, 18 Met/Met and 2 Met/Met). The patients were matched in regards to sex and total ADAS-cog score with AD patients. The scores of the 11 ADAS-cog items were compared between the sCJD and the AD groups as well as between the sCJD-subgroups Met/Met, Val/Val and the AD group.

Results: The ADAS-cog total score of the sCJD and AD groups was 22.6±6.5, respectively. Regarding the single items the scores of the sCJD patient group and the AD patient group showed statistically significant differences in the items Constructional praxis, Word-finding difficulty in spontaneous speech and Spoken language ability. When comparing the sCJD subtypes with each other no statistically significant difference was found in the items.

Conclusion: In the speech domain and constructional praxis there is indication of greater impairment in sCJD patients in general when compared with AD patients. A disturbance of the speech appears to be an important characteristic of the Met/Val and Val/Val subtypes of sCJD, and should therefore be the focus of special attention in future neuropsychological studies.

**Economic Impacts of Bovine Spongiform Encephalopathy in Canada and the Effect of Beef Producer Compensation Programs**

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Before the first domestic case of bovine spongiform encephalopathy (BSE) was identified in May 2003, Canada was the world's third largest exporter of cattle behind the United States (U.S.) and Australia. After the BSE disclosure, the U.S. and 40 other countries imposed an immediate ban on imported Canadian beef and cattle products. The interdependence of the Canadian beef industry with that of the U.S. was a critical factor in Canada's market vulnerability with 99.7% of Canadian cattle shipped to the U.S. prior to the outbreak. As the re-opening of the US border was prolonged, beef producers adopted a variety of strategies to deal with the loss of income including refinancing existing loans, extending loan payments, borrowing more money, finding employment off the farm, and selling land or other assets. However these measures taken by individual farmers were not sufficient in completely supplementing their loss of income, thus creating a need for government funding and support. Little research has been done to assess the impact of government subsidies as a tool to offset the economic losses incurred by BSE. Analysis of existing literature shows subsidies did very little to restore economic stability of farmers in Canada. This analysis is extended to other countries to see if government subsidies had a similar impact as compared to Canada.

**Bovine Spongiform Encephalopathy Policy in Australia**

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Australia's geographical isolation has given it an advantage in excluding many agricultural pests and diseases, including foot-and-mouth disease, rinderpest, scrapie, and contagious bovine pleuropneumonia, as well as bovine spongiform encephalopathy (BSE). This natural advantage has been strengthened with import restrictions and a strict quarantine system. Australia's 1966 ban on stockfed imports of animal origin from any country except New Zealand significantly reduced the external challenge to the country's beef industry during the height of the BSE epidemic. External challenges were further reduced in 1988 with a ban on live cattle from countries affected by BSE, and lifetime quarantine for such animals already in the country; however measures to control BSE should it enter the country have not been widely implemented. There is no ban on specified risk materials (SRM) or downer cattle in rendering. A 1997 ruminant-to-ruminant feed ban evolved into a 'specified mammalian material'-to-ruminant ban in 1999 and by 2002 a vertebrate-to-ruminant ban was in place. Surveillance is undertaken according to Office International des Epizooties (OIE) guidelines, and in 2005 the rate of testing was approximately 0.01%. Australia is the world's largest exporter of beef by value and was only recently overtaken by Brazil as the largest exporter by volume. The benefits of BSE-freedom were highlighted after 2003 when the first U.S. case of BSE prompted Japan and some other Asian nations to ban U.S. beef and source their beef from Australia instead. However, while Australia exported nearly $5 billion worth of beef in 2004-2005, it also received nearly $15 billion from exports of cotton, milk products, fish, grain, wool, sugar and other horticultural products. In attempting to encourage its trading partners to adopt consistent, 'science-based', import standards for its other exports, Australia has had to appear to relax its BSE vigilance to some extent, while at the same time providing convincing evidence that the country is taking all appropriate steps to control the disease.

**A Case Study of Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease in Germany**

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Despite the emergence of Bovine Spongiform Encephalopathy (BSE) throughout Europe, Germany considered itself an oasis that was free of the disease. However, on November 26, 2000 the first domestic case of BSE appeared in the country and public concerns over food and health safety ensued. Germany traditionally is known for its large agriculture and farming sectors with health and environmentally conscientious citizens. The occurrence of BSE within the country bordered had dramatic economic and social effects. The response of Germany’s public and politicians far surpassed that of most other countries affected by the disease. In Germany the reaction to BSE and perception of risk was even more intense than that of the United Kingdom, which to date is still the region that has been most affected by BSE and variant Creutzfeldt-Jakob disease (vCJD). Response to BSE was rapid with new policies and high percentage testing of cattle implemented within months. To date, Germany has not had a single case of vCJD.

**Conclusion**

A disturbance of the speech appears to be an important characteristic of the Met/Val and Val/Val subtypes of sCJD, and should therefore be the focus of special attention in future neuropsychological studies.
P04.41
Risk Management of Bovine Spongiform Encephalopathy and Variant Creutzfeldt-Jakob Disease in India
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India is a unique country because of its distinct religious outlook toward cows and beef consumption. India has been assessed as “GBR II” level (unlikely, but cannot be excluded) for BSE by the Scientific Steering Committee (SSC) even though there has not been a single case reported in the country. The assessment is based on the fact that the cattle system in India with regards to BSE is “unstable” as there have been some live cattle imports from BSE affected countries like Germany and Denmark, there is only passive surveillance and the testing rate of suspected cattle is far below the Office of International des Epizooties (OIE) guidelines. India has instituted certain risk management policies to manage BSE in the event of its occurrence and to sustain its status as the third largest beef exporter in the world. It is also imperative that the Indian Government ensures implementation and compliance of already instituted policies to maintain its control system. A country with a population of 1.1 billion people is a challenge on its own for risk management. Moreover, India is becoming globally highly mobile and new emerging challenges to human health are becoming more prevalent, it is driving the pressure for further health service developments and resources. India has a dire need for constituting new national policies to address the growing concern about safe blood supply, iatrogenic transmission of variant Creutzfeldt-Jakob disease (vCJD) through transfusion of blood and blood products and contaminated surgical instruments.

P04.42
Can Retinal Pigment Epithelial (RPE) Cells Be Infected with TSE Agents?
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Retinal pigment epithelial (RPE) cells are in close contact with neuronal retinal cells, and are responsible for phagocytosis of “outer fragments” of photoreceptors. Infectivity in the retina has repeatedly been demonstrated in animals with experimental and natural scrapie, and PrP-TE has been found in the plexiform layers in two cases of sporadic CJD (sCJD) and two cases of variant CJD (vCJD). We aimed to investigate whether RPE cells can be infected with various transmissible spongiform encephalopathy (TSE) agents in cell culture. Adult RPE (ARPE-19) cells were exposed to the mouse-adapted GSS strain (Fukuoka-1), mouse-adapted vCJD (mo-vCJD), vCJD, sCJD and BSE for 96 hours and carried through 15 passages. The presence of PrP-TE and PrP-TE was demonstrated using 5G11 antibodies by cell blot or western blot of the cultured cells 96 hours following inoculation, and after 5, 10 and 15 passages. We showed that ARPE-19 cells express PrP-TE through all passages; but did not find PrP-TE in cell lysates in any passage following inoculation with Fukuoka-1. A small amount of PrP-TE was detected in cells exposed to mo-vCJD at 96 hours after inoculation by cell and western blot. Results of tests to elucidate the susceptibility of ARPE-19 cells to BSE, vCJD and sCJD (not yet completed) will be presented and discussed.

P04.43
Policy and Risk Management of Bovine Spongiform Encephalopathy in Portugal
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The policy and risk management case study of bovine spongiform encephalopathy (BSE) in Portugal represents an excellent example of the difficulty encountered when dealing with uncertainty in decision making. Measures to control the spread of the disease were recommended by the European Union (EU) and national expert groups early on in the crisis. However, initial low compliance to these measures led to increasing numbers of detected cases. By 1998, Portugal was the third most affected area after the United Kingdom and Ireland. In Portugal, the BSE outbreak can be divided into three main periods, each marked by a major event at the national level. First, the disclosure of evidence of BSE cases dating back to 1990 by scientists from the reference laboratory (1993); second, the discovery of the link between BSE and variant Creutzfeldt-Jakob disease (vCJD) (1996); and third, the embargo on all beef exports (1998). The embargo represented a turning point that led to needed changes in the management of the crisis and an eventual decline in the epidemic. Changes included an effective meat and bone meal (MBM) ban on all farm animals, the exclusion of specified risk materials (SRMs) from animal and human feed chains, improved rendering according to the EU recommended conditions, the introduction of active components to the surveillance system and further improvements in the passive surveillance system already in place. As with other countries that dealt with BSE, many lessons can be learned from the case in Portugal. These include the importance of implementing protective measures that have been proven effective in other countries, transparency in decision-making to avoid controversy, investments in research programs to understand the nature of the disease, as well as the consideration of scientific advice in government policies.

P04.44
Bovine Spongiform Encephalopathy Risk Factors in Russia and Preventive Risk Management Strategies
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Bovine spongiform encephalopathy (BSE) is recognized as an animal and zoonotic disease with extreme economic and public health consequences. Currently, BSE has been detected in 23 countries distributed across Asia, Europe, North America and the Middle East. To date, Russia has not reported any BSE cases in its own territory. Qualitative risk assessment has indicated that if BSE occurred in Russia, the economic consequences would be devastating for Russian agriculture and its economy. The Russian veterinary authorities acted much earlier compared to some other countries to protect domestic herds from BSE. A number of preventive strategies and control measures to decrease the likelihood of the introduction and establishment of BSE in Russia included: prohibitions on live animals and beef products imports from BSE and scrapie affected countries; prohibitions on use and import of contaminated feed; introduction of the adequate processing of animal waste; an on-going surveillance program for BSE; and increased education/training efforts. However, analysis of the extensive experience from Europe and North America show that the absence of BSE cannot be ensured by such BSE prevention and detection efforts alone. The purpose of our study was to identify the potential BSE risk factors and risk sources in Russia and review risk management strategies taken by veterinary authorities that reduced the probability of BSE occurring in Russian livestock. Evidence-based recommendations focus on actions to strengthen measures shown to prevent the introduction and spread of the disease.
In Canada a partial feed ban was introduced in 1997. Scientific research had determined that the incubation period for bovine spongiform encephalopathy (BSE) by oral consumption was, on average, five years. As a result it was projected that the number of BSE cases would peak approximately five years later in 2002-2003. The number of infected cattle remaining in the herd after this time was predicted to be very small as many would have already culled, slaughtered or died. With little risk communication or other options most farmers continued to use contaminated feed until it was consumed. This situation extended the potential for infectivity and a small number of additional BSE cases were expected after the feed ban. A number of possible transmission routes for contaminating prions are known but on-farm cross-contamination is thought to be the primary source of exposure. Devastating economic losses occurred when Canada detected its first domestic BSE case in May 2003 and each additional positive case born after the feed ban only reinforces the perception of BSE risk in Canada. The small numbers of BSE cases born well after the feed ban (five years) may have been avoided or reduced with simple interventions and improved risk communication to farmers when the initial partial feed ban was introduced. Continued detection of BSE cattle born after the feed ban requires risk communication to the public to explain why cases are still appearing. Effective risk communication will help maintain the public’s confidence in government and the Canadian beef industry.

The majority of disaster research either concentrates on a particular extreme event, or addresses a category of disaster more generally. The former research projects tend to emphasize the effects of a disaster in a specific time and place and impacting on a particular population of people. The latter kind of research is more likely to aspire to generalize, and is likely to emphasize the appropriate policies that should be implemented to avoid negative impacts from that kind of extreme event (e.g. building code revisions for earthquake-prone areas, prohibiting rebuilding in flood plains). Both kinds of research are obviously of great importance, but we would like to suggest another approach to disaster research that is less common, at least in explicit terms. Using the BSE outbreak in Canada (May 2003-) as a focus, we will argue that we can learn a great deal by looking at series of related disasters or crisis in Canada and worldwide in order to explore how institutional responses and individual resilience are influenced by the sequence. Garcia-Acosta (2002:49) has suggested that the mainstream of disaster studies tends to be “ahistorical and even antihistorical”. We believe that this needs to be changed, that experiences and perceptions of past related events may have a major impact on later responses. For the data paper this is based on extensive document analysis (government reports, other publications, media coverage) and primary data collected from ethnographic interviews conducted in 2006 and 2007 in Alberta, Canada.
Case Report of Variant Creutzfeldt-Jakob Disease in a Macaque after Blood Transfusion
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A fourth human case of probable transmission of vCJD through transfusion has now been reported but a number of features affecting transfusion-related infection remain unexplained, including infectious dose, length of incubation period and critical infectious window of blood donors. We report here the first case of experimental transmission of vCJD in primates by blood transfusion. Experimental infection of Cynomolgus macaque has been demonstrated to be a sensitive model for the investigation of human prion diseases, inducing similar distribution of infectivity in peripheral lymphoid tissues and equivalent brain pathology. In our study, transfusion was performed with 40 ml of whole blood drawn from a vCJD-infected macaque at the terminal stage of the disease. Clinical symptoms of vCJD appeared in the recipient animal after five years of incubation. The total amount of infectivity in the transfused blood was approximately 106 fold lower than in the brain (titration still in progress). In several animals infected intravenously with brain homogenate, the presence of PrPRes in serial lymph node biopsies and in other organs at autopsy was examined and results will be presented.
Epidemiology, Risk Assessment and Transmission

P04.53
Reduction of Prion Infection in Blood by Navigant Prototype Separation and Cell Washing Device
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Although prion diseases are rare in humans, the established link between a new variant form of CJD (vCJD) and the consumption of cattle meat contaminated by BSE have raised concern about a possible outbreak of a large epidemic in the human population. Over the past few years, BSE has become a significant health problem affecting many countries, and it seems now apparent that vCJD can be transmitted from human to human by blood transfusion. Exacerbating this state of affairs is the lack of a reliable test to identify individuals incubating the disease during the long and silent period from the onset of infection to the appearance of clinical symptoms. The purpose of this research study was to evaluate the effectiveness of the Navigant Prototype separation and cell washing device to remove infectious prion scrapie prion protein (PrPSc) from red blood cell (RBC) suspensions in prion spiked samples. Analysis of the treated sample supernatants by Western blot revealed that approximately 88% of PrPSc was removed with the initial plasma expression and the equivalent to 6% was detected in a saline wash. The final sample of RBCs revealed no detectable levels of PrPSc by western blots, but by extrapolation of the removal rate, we calculate that the Navigant blood separation and cell washing device removed 98.5% PrPSc. Further analysis of the treated RBCs using the PMCA assay indicated detectable amounts of PrPSc only after 2 consecutive amplification rounds. Semi-quantitative analysis of PMCA enabled us to estimate that the treated RBCs contained less than 1 x 10^5 LD50 of PrPSc. These results were confirmed by in vivo infectivity studies. Preliminary in vivo data displayed significant differences in the incubation periods of the spiked blood inoculated hamsters (100.1 ± 1.7) versus washed RBCs (135.8 ± 6.7 with an incomplete attack rate). Moreover, a substantial difference in the attack rate (40% in RBC, versus 100% in spiked blood) further indicates a substantial removal of infectious prions. Comparison of this data with results of animals inoculated with different dilutions of infectious material, indicate a >99.0% reduction of infectivity. This data suggest that the Navigant separation and cell washing device represents an efficient method to remove prions from blood fractions and its application may lead to increased safety of blood products prepared in this way.

P04.54
Development of a Bank of Standardized Blood Samples for the Evaluation of Prion Diagnostic Tests
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An accurate screening of blood donors would constitute a major improvement for transfusion safety from vCJD infection. Several companies already claim positive results with new ante mortem tests for prion in blood but no standardized samples are available to allow comparative evaluations. In the context of the NeuroPrion BloodDiag project, we have constructed a library of blood fraction samples from sheep for the evaluation of these new tests.

Sheep were experimentally infected with scrapie and, in parallel with healthy controls, have been sampled regularly since 2004. Blood was fractionated by the French Transfusion Center of Lille (France) using a protocol similar to the one used for humans. With complete information about the animals (genotype, source of contamination, days post-contamination and presence of clinical signs), this library comprises now more than 8000 aliquots from over 110 different blood samples. Assessment of infectivity of the different blood fractions (plasma, platelets, red cells and buffy-coat) has been performed by inoculation of transgenic mice overexpressing ovine PrP gene (tg338). Successful transmissions were observed with the buffy coat of several animals. Now, two years post-infection, some positive results are also being observed with plasma samples inoculated by intraperitoneal route (a still ongoing study).

This bank constitutes a powerful tool for the evaluation of blood tests under development for use as pre-clinical diagnostic screens for TSE infection.
# P04.57
**A Potential Blood Test for TSE by Detecting Carbohydrate-dependent Aggregates of PrPres-like Proteins in Scrapie-infected Hamster Plasma**

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**Background:** PrPres has rarely been detected in blood, except in leukocytes, even in diseased animal models which show a large amount of PrPres in affected organs. It seems likely that PrPres detection in blood is difficult because of the low titer of infectivity of the material in blood.

**Aims/Objectives:** To demonstrate the detection of proteinase K-resistant 3F4-reactive protein in the plasma of scrapie-infected hamsters but not in the plasma of mock infected hamsters.

**Methods:** Partial purification using a novel method termed acidic SDS precipitation and a highly sensitive chemiluminescence detection system after conventional western blotting was used.

**Results:** The chemiluminescence method could show the presence of PrPSc at a concentration equivalent to 1.4x10^-9g of brain homogenate or 1.5x10^-12g (6.5x10^-17mole) of h4PrP. Using the above method, the 3F4-reactive proteins in scrapie-infected hamster plasma were often indicated as multiple Mw protein bands between higher Mw positions than position of di-glycosyl PrP molecule. Mixing of scrapie-infected hamster brain homogenate with mock infected hamster plasma resulted in the formation of similar Mw of multiple 3F4-reactive proteins. Predigestion of carbohydrate chains from the proteins in plasma or brain homogenate before mixing resulted in the failure to obtain these multiple 3F4-reactive proteins.

**Discussion:** These observations suggested that PrPres was aggregated with self or other protein molecules in plasma through carbohydrate side chains. This type of PrPres was successfully detected in the plasma of scrapie-infected hamster. Biological properties of these aggregates with PrPres-like protein in scHApI are not known.

**Acknowledgement:** We gratefully acknowledge Dr. Takashi Yokoyama, Research Institute for Prion Diseases, National Institute of animal Health of Japan, for his greatest support to use scrapie-infected hamster materials.

# P04.58
**Environmental Degradation of the Prion Protein and Impacts of Prion Conformation on Environmental Behavior**

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**Background:** Recent evidence suggesting an environmental transmission pathway for chronic wasting disease (CWD) has led to interest in the environmental fate and transport of prions. In spite of this, there is limited information available about the behavior of prions in the environment.

**Aims and Objectives:** In this study, we investigate the environmental degradation of the prion specific isoform of the prion protein, PrPSC, to determine the most environmentally-relevant form of the protein. Soil sorption experiments were performed to investigate the impact of prion conformation on environmental behavior.

**Methods:** Prion-infected brain homogenate (BH) was added to a series of 200-µl tubes and was incubated at either 22ºC or 37ºC. At pre-determined time points (0, 1, 2, 7, and 35 d), tubes were removed from the incubator and analyzed by Western blot using 3F4, 8B4, and POM19 antibodies. For each experiment, a prion-infected BH (either hamster strain HY-TME or elk CWD) and an uninfected BH were used. Experiments were performed in triplicate and were conducted with pH 4, 7, and 10 to evaluate the influence of solution pH on degradation. Tests were performed to investigate sorption of full-length PrPSc and N-terminal truncated PrPSc onto montmorillonite clay and silicon dioxide microparticles. PrPSc was added to tubes containing sorbent suspended in 10 mM NaCl and were equilibrated by rotating at ambient temperature for 2 hours. Unbound PrPSc was separated from bound PrPSc by centrifugation and analyzed by Western blot.

**Results:** Results suggest that agents capable of PrPSc degradation exist in BH and that PrPSc degradation is evident within 24 to 48 hours. The C-terminus of PrPSc was detected at times up to 35 d at 22ºC, but was significantly degraded by 7 d at 37ºC. Data on sorption of full-length PrPSc onto montmorillonite and silicon dioxide microparticles will also be presented.

**Discussion:** These results indicate that N-terminal truncated PrPSc is the most environmentally-relevant form of the prion protein. PrPSc will be degraded in the environment, resulting in potentially significant conformational changes, which may impact sorption behavior. These results highlight the importance of using the most environmentally-relevant form of PrPs.

# P04.59
**Quantitative Assessment of the BSE Risk from Processing Meat and Bone Meal in Feed for Non-Ruminants in the Netherlands**

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**Background:** Meat and bone meal (MBM) derived from infected cattle is the major route of BSE transmission worldwide. Partial purification using a novel method termed acidic SDS precipitation and a highly sensitive chemiluminescence detection system after conventional western blotting was used.

**Methods:** A stochastic simulation model was constructed that calculates (i) the average number of TSE cases since 1969 is 206. The average yearly mortality from 1996 to 2006 is 1.39 per million, with a peak incidence of 2.7 in 2006. 85% of definite TSEs were classified as sporadic Creutzfeldt-Jakob disease (sCJD). We observed a significant linear increase of the mean age at death of 0.6 years per calendar year (p = 0.016). Based on autopsy results, clinical diagnostic surveillance criteria had sensitivity and specificity, respectively, of 82.7% and 80.0% for “probable” CJD, and a positive predictive value of 80.5% for “probable” and of 38.9% for “possible” CJD. Alternative neuropsychological diagnoses included Alzheimer’s disease with or without Lewy body pathology, vascular encephalopathy, metabolic encephalopathies, and viral or limbic encephalitis. No common risk factors have been detected. However, CJD mortality in Vienna double as high as in the rest of the country.

**Conclusion:** The steady increase of mortality rates especially in old age groups reflects most likely improved case ascertainment due to active surveillance causing higher awareness of the medical community. In comparison with other European countries, it is re-assuring to note that the overall death rate of TSEs does not significantly differ from the Austrian autopsy-controlled data, thus confirming the value of clinical surveillance criteria.
**Epidemiology, Risk Assessment and Transmission**

**P04.61** Survival of PrPSc during Simulated Wastewater Treatment Processes

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Concern has been expressed that prions could enter wastewater treatment systems through sewer and/or septic systems (e.g., necropsy laboratories, rural meat processors, private game dressing) or through leachate from landfills that have received TSE-contaminated material. Prions are highly resistant to degradation and many disinfection procedures raising concern that they could survive conventional wastewater treatment. Here, we report the results of experiments examining the partitioning and survival of PrPSc* during simulated wastewater treatment processes including activated and mesophilic anaerobic sludge digestion. We establish that PrPSc can be efficiently extracted from activated and anaerobic digester sludges with 1% sarcosinate. Activated sludge digestion does not result in significant degradation of PrPSc. The protein partitions strongly to the activated sludge solids and is expected to enter biosolids treatment processes. A large fraction of PrPSc survives simulated mesophilic anaerobic sludge digestion. Our results suggest that if prions were to enter municipal water treatment systems, most of the agent would partition to activated sludge solids, survive mesophilic anaerobic digestion, and be present in treated biosolids. Land application of biosolids containing prions could represent a route for their unintentional introduction into the environment. Our results argue for excluding inputs of prions to municipal wastewater treatment facilities that would result in unacceptable risk of prion disease transmission via contaminated biosolids.

**P04.62** BSE Epidemic Trend in Poland based on Age at Detection of Polak, MP; Skubicki, P; Berkvens, D; Saagerman, C

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B. Bovine spongiform Encephalopathy BSE epidemic in Poland started in May 2002 when first case was diagnosed with rapid test and confirmed. Overall between May 2002 and December 2006, 50 cases were recorded. 2 in passive surveillance and 48 in active monitoring (42 were healthy slaughtered cows and 6 were risk animals). Most of the cases were diagnosed with Bio-Rad test (35), while two additional rapid tests were also used.

A.) The aim of the study was to evaluate BSE epidemic trend in Poland based on Belgian and Great Britain experience with age distribution at time of detection as the main indicator.

M. Epidemiological data was used for the calculations especially: the age at detection, the date of detection, a type of rapid test used for preliminary diagnosis, incidence rate ratios for every quarter of a given year.

R. When age of 50 cases of BSE recorded in Poland between 2002 and 2006 was evaluated in relation to the date they were detected, and all three rapid tests were considered, this turned out to be not an appropriate indicator. However when only the same test was taken into account (Bio-Rad test) and age of 35 cases of BSE in relation to the date they were detected was considered, this was an appropriate indicator with growing tendency of age in time. More BSE cases in Poland were detected in the first quarter of each year and those were not linked to the number of animals tested. This trend was unchanged for all streams and all tests used. Incidence rate ratio (IRR) for the first quarter when compared with other quarters was 3.05 (for all streams) and 3.80 (for healthy slaughtered). There were less cases in animals older than 7 to 9 years (IRR=0.84) and older than more than 9 years (IRR=0.27) than in animals aged 4 to 7 years.

D. When evaluating several tests together, the interpretation of the indicator must be performed for each test separately because each test has its own, specific characteristics. The use of Bio-Rad test is appropriate and indicates an increase of the trend of age at detection of BSE in Poland. For this reason the IRR was calculated for each test individually. The third test had been used only in the first quarter of each year. Further research is required to investigate this finding.

Several hypothesis were formulated to explain this phenomenon like careful supervision of animals by their keepers during that period, making it more likely to notice mild signs of weight loss and decrease in milk yield, and cull the animal, as has been proposed by Wilsmeis (1998).

**P04.63** N154Y and N169S Amino Acid Substitutions in the Prion Protein Play a Major Role in Conditioning the Susceptibility of Rodent Species to Prion Diseases

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The taxonomy of rodents includes all the species under study in different subfamilies. The consistency of transmission findings observed in species belonging to different taxonomic branches reinforces the deduction that amino acid residues 154 and 169 are major determinants of transmission barrier in rodent species. Moreover, the similar susceptibility exhibited by bank voles, field voles and beach mice indicates that the range of rodent models highly susceptible to TSE and easy to breed under laboratory conditions could be much wider than it currently is.

Based in the results of transmission studies, the rodent species under investigation can be ascribed to three different groups. One group includes bank voles, field voles (Microtus agrestis) and beach mice (Peromyscus polionotus) and was characterized by: i) high susceptibility to scrapie, ii) low susceptibility to BSE, iii) extremely short incubation times with adapted strains and iv) glycoprofile’s change of 139A. The second group comprises C57Bl/6 mice, wood mice (Apodemus sylvaticus), and gerbils (Meriones unguiculatus) and displayed rather opposite features: i) low or no susceptibility to scrapie, ii) relatively higher susceptibility to BSE, iii) longer incubation times with adapted strains and iv) no change in the glycoprofile of 139A. The third group includes only spiny mice (Acromys cahirinus) which showed a distinctive resistance to all prion sources.

**P04.64** Studies on the Infectivity Distribution of BSE in the Gut of Pre-clinical Cattle for Definition of Specified Risk Material

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To establish public health protection measures it is important to precisely define the tissues which must be considered as specified risk material (SRM). Intestine from duodenum to rectum is currently considered as SRM in the European Union – a definition which is largely based on the infectivity distribution in TSE infected sheep. In contrast only the distal ileum will be removed as SRM in Canada. In clinically affected cattle (in experimental as well as natural conditions) no infectivity has yet been found outside of the Peyer patches of the distal ileum, neither by conventional mouse bioassay nor by IHC/IB. It must be acknowledged, however, that the sensitivity of these techniques is quite limited and no data are available for those tissues from cattle in the early BSE incubating time. We are therefore studying by when PrPSc and infectivity (if at all) can be found in the small intestine (particularly in the gut associated lymphoid tissue) of cattle which were challenged at the age of 4-6 month. For this purpose samples of the jejenum, the ileum and the ileocecal junction from 22 orally dosed BSE (100g brain stem per animal) infected cattle which were culled after 4, 8, 12, 16, 20 and 24 months respectively, are examined by Immunohistochemistry, PTA Western Blot, IDEXX HerdChek ELISA, BioRad TeSeE and by Tgbov XV bioassay. It must be acknowledged, however, that the sensitivity of these techniques is quite limited and no data are available for those tissues from cattle in the early BSE incubating time. We are therefore studying by when PrPSc and infectivity (if at all) can be found in the small intestine (particularly in the gut associated lymphoid tissue) of cattle which were challenged at the age of 4-6 month. For this purpose samples of the jejenum, the ileum and the ileocecal junction from 22 orally dosed BSE (100g brain stem per animal) infected cattle which were culled after 4, 8, 12, 16, 20 and 24 months respectively, are examined by Immunohistochemistry, PTA Western Blot, IDEXX HerdChek ELISA, BioRad TeSeE and by Tgbov XV bioassay. The systematic approach of this study will allow mapping of the exact temporal and spatial emergence/distribution of PrPSc in the small intestine of cattle in particular in the gut associated lymphoid tissues. Such estimates have a critical role in qualitative and quantitative risk assessments and in providing advice on the designation and removal of certain SRM tissues according to age of cattle at slaughter. This study is funded by Health Canada.
Scrapie of sheep represents the prototype and a suitable model of prion diseases. During the course of infection, the pathological prion protein (PrP\textsuperscript{sc}) progressively accumulates in nervous and several other tissues. PrP\textsuperscript{sc} is a useful marker for prion diseases, but its level in a given tissue does not necessarily correlate with the infectivity titer.

Prions infectivity is quantified by end point titration or by “incubation time assay” in rodent models. However, its measurement in tissues from scrapie affected sheep is hampered by the difficult transmission of scrapie to wild type rodent models.

Recently, we utilized the bank vole (Clethrionomys glareolus) for transmission studies of prion diseases from different species, including human, sheep, goat, deer, mouse and hamster.

Herein we describe the use of the bank vole as a bioassay for strain characterization and titration study on the medulla oblongata and tonsil from an ARQ/ARQ scrapie affected sheep.

Western blot analysis showed that the diglycosylated and unglycosylated bands of PrP\textsuperscript{sc} appeared of higher and lower size, respectively, in tonsil than in brain. Both these sources transmitted efficiently to voles and, in spite of the aforementioned PrP\textsuperscript{sc} molecular differences, the disease phenotype upon primary transmission and second passage into voles, was identical between tonsil and brain suggesting the involvement of the same strain of agent.

The infectivity titer in the brain, assessed in voles by end point titration, was 10\textsuperscript{1.4} i.c. ID\textsubscript{50}/g, which was higher than that measured in wild type mice (10\textsuperscript{1.2} i.c. ID\textsubscript{50}/g). By plotting Western blot signal of tonsil against brain we determined that the PrP\textsuperscript{sc} concentration in tonsil was -0.12% that of brain. This value correlated well with the infectivity titer estimated in tonsil by the “incubation time assay”.

This study shows that: 1) the bank vole is a bioassay more sensitive than wild-type mice for infectivity studies on sheep scrapie; 2) the “incubation time assay” is a good alternative to end point titration; 3) the concentration of PrP\textsuperscript{sc} in brain and tonsil correlates with their infectivity titer; 4) differences in the PrP\textsuperscript{sc} molecular phenotype do not necessarily reflect the involvement of different strains.

### A Correlation of Magnetisation Transfer Ratio Histogram Measures with Clinical Disease Severity in Inherited Prion Disease

A Correlation of Magnetisation Transfer Ratio Histogram Measures with Clinical Disease Severity in Inherited Prion Disease

MacFarlane, RG; Scailhi, R; Youssry, IA; Collinge, J; Vroe, SJ

Inherited prion diseases (IPD) are progressive neurodegenerative disorders in which conventional magnetic resonance (MR) neuroimaging is often unremarkable. We investigated global and regional cerebral MR magnetisation transfer ratio (MTRs) in IPD. Twenty-three patients, recruited into the MRC PRION-1 Trial, underwent MTR and conventional MR imaging. For each patient, whole-brain MTR histogram mean (AVMTR), peak height (PH), peak location (PL) and MTR at the 25th, 50th and 75th percentile (MTR25%, MTR50%, MTR75%) were calculated together with mean MTR for bilateral caudate, putaminal and pulvinar regions of interest (ROI). A clinician’s assessment of disease severity (GIC), clinician’s dementia rating (CDR), Alzheimer’s disease assessment scale (ADAS-COG), activities of daily living (ADL), brief psychiatric rating scale (BPRS), mini mental score examination (MMSE) and Rankin scores were evaluated. Significant (p<0.01) bivariate Spearman rank correlations were found between AVMTR and Rankin (p=0.008), CDR (p=0.002) and ADAS-COG (p=0.004; PH and Rankin (p=0.002); MTR25% and Rankin (p=0.001), CDR (p=0.001), ADAS-COG (p=0.008) and GIC (p=0.006); and MTR50% and Rankin (p=0.004). Mean ROI MTRs did not correlate with clinical scores, and there were no pathological appearances on conventional MR imaging. Whole-brain MTR histogram measures may provide valuable indices of IPD disease severity for future therapeutic trials.
Oral Transmission of Prion Disease Is Enhanced by Binding to Soil Particles

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Background: Ten to 15% of human prion diseases are genetic and include familial Jakob-Creutzfeldt Disease (JCJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), and Fatty Familial Insomnia (FFI). Human genetic prion diseases (gCJD) have varied clinical presentations, including behavioral/psychiatric abnormalities, cognitive dysfunction, parkinsonism, ataxia, dysautonomia and insomnia. Symptoms and age of onset can vary even within a mutation and even within a family. Diagnosis is particularly difficult because symptoms can overlap with more common neurodegenerative diseases. In this analysis, we review the presentations of gCJD cases from a large U.S. prion referral center.

Design/Methods: We review available medical records from patients with, or at risk for, gCJD, referred to our center over the past 5 years. For most patients seen at our center, EEG, MEG, neurologic exam, neuropsychological testing, CSF testing, and PRNP mutation analysis (most performed through the U.S. NPDPSC) are performed. EEGs and MRIs were reviewed by readers blinded to clinical diagnosis.

Results: Among more than 800 referrals for potential prion disease over the past five years, approximately 15% had, or were at risk for, PRNP mutations. 9 PRNP mutations were identified in 44 symptomatic patients among 31 kindreds. There were wide ranges for age of onset and disease duration for JCJD, GSS, and FFI. We found differences in the frequencies of first symptoms for the three diseases. EEG and 14-3-3 protein results were poor diagnostic tools for gCJD, while DWI and FLAIR MRI were often abnormal in JCJD. Several cases were identified who initially had a negative family history for prion disease. We summarize features of symptomatic patients in this genetic prion cohort and some present some representative cases.

Conclusions/Relevance: Diagnosis of genetic prion disease involves careful attention to family history and consideration of the possibility of a genetic etiology. A reportedly negative family history does not necessarily indicate the absence of a PRNP mutation. Genetic testing should be done for any patient with presumed cCJD and should be considered for anyone with an unexplained neurological disorder, particularly with prominent symptoms of ataxia, parkinsonism, behavioral changes or cognitive dysfunction.

Epidemiology, Risk Assessment and Transmission

PrP in Salivary Glands of Scrapie-affected Sheep

Nonno, R; Vascellari, M; Mutinelli, F; Bigiato, M; Di Bari, MA; Melchiotto, E; Marcon, S; D’Agostino, C; Vacci, G; Conte, M; De Grossi, L; Rosone, F; Pifferi, AR; Agrimi, U

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A long-unanswered question in prion biology is how certain transmissible spongiform encephalopathies (TSEs), such as sheep scrapie and cervid chronic wasting disease, spread from animal to animal. Anecdotal evidence and controlled field experiments have suggested the presence of an environmental TSE reservoir. We, and others, have speculated that soil may harbor TSE agent in the environment and allow its transfer to naïve hosts. TSE infectivity can persist in soil for years, and we previously demonstrated that the disease-associated form of the prion protein binds to soil particles and that, unexpectedly, binding to Mte significantly enhances disease penetrance and reduces incubation period relative to unbound agent. Cox proportional hazards modelling revealed that across the doses of TSE agent tested, Mte increased the effective infectious titer by a factor of 680 relative to unbound agent. Oral exposure to Mte- and soil-bound prions and found that prions bound to Mte are orally bioavailable and that, unexpectedly, binding to Mte significantly enhances disease penetrance and reduces incubation period relative to unbound agent. Cox proportional hazards modelling revealed that across the doses of TSE agent tested, Mte increased the effective infectious titer by a factor of 680 relative to unbound agent. Oral exposure to Mte-associated prions led to TSE development in experimental animals even at doses too low to produce clinical symptoms in the absence of the mineral. We tested the oral infectivity of prions bound to three soils with different texture, mineralogy and organic carbon content, and found soil-bound prions to be orally infectious. Two of the three soils increased oral transmission of disease, and the infectivity of agent bound to the third soil was equivalent to that of unbound agent. Enhanced infectivity of soil-bound prions may explain the environmental transmission of some TSEs despite the presumably low levels shed into the environment.

Validation of a 1.5 Hour TSE Assay: An Ultra-short Microtiter Based EIA

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IDEXX Laboratories, USA

The IDEXX HerdChek® Bovine Spongiform Encephalopathy (BSE) and IDEXX HerdChek® BSE-Scrapie Antigen Test Kits are transmissible spongiform encephalopathy (TSE) antigen-capture enzyme immunoassays (EIA) for the detection of the abnormal conformer of PrP in postmortem tissues. These assays have an approved “Short” protocol in which the EIA portion of the test is completed in ~2.2 hours. For laboratories that require a fast turn-around time, IDEXX has validated an even shorter assay protocol in which the EIA part of the test is completed in 1.5 hours. This new “Ultra-Short” assay protocol uses increased temperature (32-37°C) and a slow plate shaker during the sample incubation step to accelerate PrP capture from viscous brain homogenate samples. The enhanced binding kinetics from the elevated temperature and plate shaker allow for a significant reduction in sample and conjugate incubation times compared to the original “Standard” (4-hour) and “Short” (2.2-hour) protocols. In a study of >2,000 negative bovine brain samples and >2,000 small ruminant samples, the Ultra-Short assay protocol performed with 100% specificity and aligned closely with the original Standard protocol. The population mean OD to cutoff ratio was >15 standard deviations from the test cutoff for both populations. For evaluation of diagnostic sensitivity, twenty BSE positive bovine brain and twenty scrapie positive ovine samples were tested in quadruplicate on the Ultra-Short and Standard assay protocols, resulting in a diagnostic sensitivity of 100% on both methods. In addition, the OD relative to cutoff values for the positive samples strongly correlated between the two assay protocols with R2 values of >0.97 for bovine samples and >0.99 for ovine samples. For evaluation of analytical sensitivity, twenty BSE positive bovine brain samples and twenty scrapie positive ovine samples were serially diluted out to >1:1,000 and tested on the Ultra-Short and Standard assay protocols. In all cases, the limit of detection for these samples was the same on both assay protocols. The IDEXX HerdChek® Ultra-Short (1.5-hour) assay protocol delivers the same high level of sensitivity and specificity as seen with the Standard and Short assay protocols.

HerdChek is a trademark or a registered trademark of IDEXX Laboratories, Inc. in the United States and/or other countries.
Epidemiology, Risk Assessment and Transmission

P04.73
Whole-body Biodistribution and Tissue Uptake Kinetics of PrPSc in the Initial Phase of the Infection
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Although prion diseases have been a public health concern for decades, the lack of knowledge about the pharmacokinetics and biodistribution of prions complicates the risk assessment. In our prior studies, we found that the level of PrPSc in blood was undetectable several weeks after inoculation, then it became detectable during the early pre-symptomatic phase, disappeared from blood right before the symptomatic phase and raised to its highest at the clinical stage of the disease. These data suggest that there are several stages of the movement of PrPSc in the body during the progression of the disease. The aim of the current study was to analyze the biodistribution and tissue uptake kinetics of PrPSc in the initial phase of the infection in mice.

After an intravenous injection of [131I]PrPSc (together with [125I]albumin as a vascular space marker), the levels of [131I]PrPSc in serum decreased biphasically with time, whereas albumin levels did not significantly change during the course of the experiment. Elimination half-lives of [131I]PrPSc and [125I]albumin were 3.44 ± 0.42 and 17.6 ± 8.6 hr, respectively. These results suggest that the level of [131I]PrPSc in serum 24 hr after the injection is less than 1 % of the injected dose (ID). The rate for albumin was consistent with previous reports. The volumes of distribution for [131I]PrPSc (3.34 ± 0.16 ml) suggest that PrPSc was well distributed in the extracellular space in the body, whereas the majority of albumin was in the serum space. [131I]PrPSc showed higher systemic clearance rates than that of [125I]albumin. The uptake of [131I]PrPSc was also investigated in various tissues. The quantity of PrPSc taken up by brain was around 0.2 %ID, indicating that the protein can penetrate across the blood-brain barrier with a medium efficiency compared to other proteins. The higher levels of [131I]PrPSc were found in liver, spleen, kidney, lung, heart, and skeletal muscle when compared to the levels in the brain. Interestingly, TCA-precipitable [131I]PrPSc was clearly detected in urine. These results provide a fundamental pharmacokinetic characterization of PrPSc in animals that may be relevant to estimate tissue risks, mechanisms of prion neuroinvasion and to develop novel therapeutic strategies.

P04.74
Scrapie Strains and Prion Protein Genetics in Italian Ovine Population: Epidemiological Surveillance
Scipio-Ranzacchielia, E; Nonno, R; Vaccari, G; Esposito, E; Chiappini, B; Conte, M; Morelli, L; Fazzi, P; Parisi, C; Bona, C; Agrimi, U; Scavia, G
Istituto Superiore di Sanità, Italy; Istituto Zooprofilattico Sperimentale del Piemonte, Italy

Susceptibility of sheep to scrapie is influenced by polymorphisms of prion protein (PrP) gene as well as by the scrapie strains. The aim of this study is to describe the results of sheep transmissible spongiform encephalopathies (TSEs) surveillance in Italy, with regard to prion protein genotyping and molecular strain typing, between July 2004 and December 2006. Classical scrapie cases were identified in 109 flocks (128 index and 353 secondary cases) while Nor98 in 23 flocks (24 index and 2 secondary cases). In only one flock both strains were diagnosed (1 index Nor98 and 1 secondary classical strain). The ARQ/ARQ genotype was by far the most dominant (411 cases) in classical scrapie infection, followed by ARQ/ARQ (34), ARQ/VRQ (5), AQH/AQH (1) and ARQ/ARQ (1). On the whole, a proportion of 98.6% of affected animals carried the wild type PrP allele (ARQ) and 7.1% the AQH. Polymorphisms other than those at codons 136, 154 and 171, were recorded at codon 141 (L/F) in 4.9% of the sheep carrying the ARQ allele.

As far as Nor98 is concerned, only 29.6% of positive cases carried the ARQ/ARQ genotype. Moreover the ARQ/ARQ, AQH/AQH, ARQ/ARR, ARR/AQH and ARR/ARH genotypes were present in 33.3%, 18.5%, 11.1%, 3.7% and 3.7% respectively of diseased animals. The amino acid substitution L/F at codon 141 was present in 72.7% of sheep carrying the ARQ allele. Worthy of note is that more than 80% of the Nor98 positive sheep carried either the ARQ allele or the AQH allele.

Our data suggest that significant differences between classical and atypical scrapie exist concerning the host genetic targeting. In classical scrapie the most affected genotype was the ARQ/ARQ, while in Nor98 cases, a high proportion of sheep carried either the ARQ allele or the AQH allele.

P04.75
Genetic Susceptibility of Sarda Breed Sheep to Experimental Inoculation of Scrapie, BSE and BASE
Vaccaro, G; D’Agostino, C; Nonno, R; Rosone, F; Conte, M; Di Bari, MA; Chiappini, B; Esposito, E; De Grossi, L; Giordani, F; Casalone, C; Marcon, S; Morelli, L; Agrimi, U
Istituto Superiore di Sanità, Italy; Istituto Zooprofilattico Sperimentale delle Regioni Lazio e Toscana, Italy; IAEA - Istituto Zooprofilattico Sperimentale del Piemonte, Italy

With the aim to investigate the genetic susceptibility of Sarda breed sheep to scrapie and BSE, animals carrying major and rare PrP alleles were inoculated intracerebrally (i.c.) and maintained under observation until the development of clinical signs. Sheep succumbed to scrapie with mean incubation times of: 462±25 (ARQ/ARQ), 703±36 (ARQ/ARQK176/ARQ), 790 (AHQ/AHQ), 1083 (ARQ/ARH) and 1252±87 (AHQ/ARH) days post infection (d.p.i.), showing a clear gradient of susceptibility.

The BSE challenge induced the disease in ARQ/ARQ and in ARQ/ARH sheep with 868±95 d.p.i. survival time. BASE caused disease only in ARQ/ARQ sheep with 868±95 d.p.i. survival time. The susceptibility of Sarda sheep is currently under investigation also with respect to BSE. The 12-bp indel polymorphism in the PRNP gene affects the susceptibility to BSE in German cattle and frequencies of insertion/deletion (indel) polymorphisms within the bovine PRNP gene (Neurogenetics: 5:19-25, 2004). In this study, we investigated the frequencies of indel polymorphisms within two variable sites in the bovine PRNP gene in 206 dairy cattle in Vietnam and in 102 dairy cattle in Thailand. Two variable sites, a 23-bp indel polymorphism (123 bp/100 bp) in the promoter region, and a 12-bp indel polymorphism (103 bp/91 bp) in intron 1, were investigated by PCR. As for the 23-bp indel in Vietnamese dairy cattle, the frequency distributions of the 100-bp allele and 100-bp/100-bp genotype, which were shown to be associated with BSE susceptibility in BSE-affected German cattle, were significantly high (84.7% and 70.7%, respectively). On the other hand, the frequency of the 103-bp allele polymorphism was higher than that of the 91-bp allele polymorphism in the 12-bp indel site in Vietnamese cattle (52.3% and 47.7%, respectively). Besides, As for the 12-bp indel in Thailand dairy cattle, the frequency distributions of the 103-bp allele and 103-bp/103-bp genotype were significantly high (84.8% and 72.5%, respectively), in healthy cattle and 33% and 67%, respectively, in BSE-affected cattle. A high frequency distribution of the 91-bp allele polymorphism was reported to be lower than that of the 91-bp allele polymorphism in German cattle (49% and 51%, respectively, in healthy cattle and 33% and 67%, respectively, in BSE-affected cattle). A high frequency distribution of the 91-bp allele polymorphism was reported to be associated with BSE susceptibility. It has remained unclear how the haplotype consisting of an extremely high frequency of the 100-bp allele on the 23-bp indel polymorphism and a low frequency of the 91-bp allele on the 12-bp indel polymorphism in the PRNP gene affects the susceptibility to BSE in Vietnamese dairy cattle.

P04.76
Frequencies of PRNP Gene Polymorphisms in Dairy Cattle in Vietnam and Thailand for Potential Association with BSE
Tamura, Y; Muramatsu, Y; Ogawa, T; Suzuki, K; Kanameda, M; Horuchi, M; Nakano Gakuen University, Veterinary Public Health, Japan; Japan International Cooperation Agency, Japan; Hokkaido University, Laboratory of Prion Diseases, Japan

In 2004, Sander et al. found significant associations between bovine spongiform encephalopathy (BSE) susceptibility in German cattle and frequencies of insertion/deletion (indel) polymorphisms within the bovine PRNP gene (Neurogenetics: 5:19-25, 2004). In this study, we investigated the frequencies of indel polymorphisms within two variable sites in the bovine PRNP gene in 206 dairy cattle in Vietnam and in 102 dairy cattle in Thailand. Two variable sites, a 23-bp indel polymorphism (123 bp/100 bp) in the promoter region, and a 12-bp indel polymorphism (103 bp/91 bp) in intron 1, were investigated by PCR. As for the 23-bp indel in Vietnamese dairy cattle, the frequency distributions of the 100-bp allele and 100-bp/100-bp genotype, which were shown to be associated with BSE susceptibility in BSE-affected German cattle, were significantly high (84.7% and 70.7%, respectively). On the other hand, the frequency of the 103-bp allele polymorphism was higher than that of the 91-bp allele polymorphism in the 12-bp indel site in Vietnamese cattle (52.3% and 47.7%, respectively). Besides, As for the 12-bp indel in Thailand dairy cattle, the frequency distributions of the 103-bp allele and 103-bp/103-bp genotype were significantly high (84.8% and 72.5%, respectively), in healthy cattle and 33% and 67%, respectively, in BSE-affected cattle. A high frequency distribution of the 91-bp allele polymorphism was reported to be lower than that of the 91-bp allele polymorphism in German cattle (49% and 51%, respectively, in healthy cattle and 33% and 67%, respectively, in BSE-affected cattle). A high frequency distribution of the 91-bp allele polymorphism was reported to be associated with BSE susceptibility. It has remained unclear how the haplotype consisting of an extremely high frequency of the 100-bp allele on the 23-bp indel polymorphism and a low frequency of the 91-bp allele on the 12-bp indel polymorphism in the PRNP gene affects the susceptibility to BSE in Vietnamese dairy cattle.
In Sicily the first two cases of clinical BSE had been observed and confirmed on two limousine animals in 1994; the two animals had been imported from Great Britain 6 months before clinical signs appearance and so they are not considered as autochthon cases. Sicily has also the only confirmed case of human BSE in Italy. Active surveillance program by rapid diagnosis test had been started since January 2001 for bovine population and since April 2002 for goat and sheep. Rapid diagnosis had been performed by Prionics western blot up to June 2003 and by chemiluminescent ELISA Enter since then. This activity resulted in 6 confirmed positive cows on a total of more than 163,000 BSE tests: all positive animals did not show any clinical signs and had been regularly slaughtered. TSE surveillance had shown 25 scrapie outbreaks in sheep and almost 12 outbreaks in goats on a total of more than 65,000 animals tested. Only one ovine outbreak had been detected by clinical signs. For consumer health protection a plan to control also animal feed had been active since the end of 2000 to control feed-ban. On a total of 1299 analysis, 1035 regarded feed for ruminant population and 264 for non-ruminant. All but two resulted negative. The analysis are currently performed by official microscopic method.

The results show the efficiency of control program to guarantee food safety preventing illegal circulation and contamination of meat meals in animal feed and BSE risk on meat consumers.

P04.77
Epidemiology and Surveillance Activity to Prevent TSE Risk in Sicilian Island
Vitale, M; Schiavo, MR; Catanzaro, A; Vitale, F; Caracappa, S
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In Sicily the first two cases of clinical BSE had been observed and confirmed on two limousine animals in 1994; the two animals had been imported from Great Britain 6 months before clinical signs appearance and so they are not considered as autochthon cases. Sicily has also the only confirmed case of human BSE in Italy. Active surveillance program by rapid diagnosis test had been started since January 2001 for bovine population and since April 2002 for goat and sheep. Rapid diagnosis had been performed by Prionics western blot up to June 2003 and by chemiluminescent ELISA Enter since then. This activity resulted in 6 confirmed positive cows on a total of more than 163,000 BSE tests: all positive animals did not show any clinical signs and had been regularly slaughtered. TSE surveillance had shown 25 scrapie outbreaks in sheep and almost 12 outbreaks in goats on a total of more than 65,000 animals tested. Only one ovine outbreak had been detected by clinical signs. For consumer health protection a plan to control also animal feed had been active since the end of 2000 to control feed-ban. On a total of 1299 analysis, 1035 regarded feed for ruminant population and 264 for non-ruminant. All but two resulted negative. The analysis are currently performed by official microscopic method.

The results show the efficiency of control program to guarantee food safety preventing illegal circulation and contamination of meat meals in animal feed and BSE risk on meat consumers.

P04.78
Role of Human Enterocytes and the 37kDa/67kDa Laminin Receptor LRP/LR on the Development of Zoonotic Prion Diseases
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TSEs show a species-specific barrier depending on the kind of the incoming prion strain as well as the kind of target organism to be infected. The species barrier results either from altered disease incubation/survival times or even insusceptibility to specific prion strains.

Recently, we found, that BSE-derived prions bound to and became internalized by human enterocytes, the major cell population of the intestinal epithelium via the 37 kDa/67 kDa laminin receptor LRP/LR (1), acting as a receptor for the cellular prion protein PrP(C) and infectious PrPSc (2). In contrast, mouse adapted scrapie prions failed to bind to human enterocytes (1), which can be explained by a possible failure of this prion strain to bind to human LRP/LR on the cell surface. We investigate the species-specific entry barrier in the enterocyte cell system addressing the question whether different animal prion strains such as scrapie in sheep and chronic wasting disease (CWD) in cervids might have the potential to cause a zoonotic disease. The role of LRP/LR in the binding and internalization processes is investigated by the use of anti-LRP specific antibodies such as W3 (4, 5) and scFv S18 (6), as well as polysulfated glycans targeting LRP/LR (3). Moreover, we show cross-species binding and internalization studies on human and animal enterocytes such as FBU (bovine), DIGNM-R (cervids) and IPEC-J2 (procine) with human and animal prions. With this in vitro cell system we mimic one of the first steps of prions entering the organism. The intestine might represent the crucial barrier for prions deciding upon the development of a zoonotic prion disease, which might be caused by sheep scrapie and CWD.


P04.79
Clinical Surveillance of BSE in Italy: Survey to Investigate the Application of the Quality Checks
Loli Piccolomini, L1; Duranti, A; Bu G1; Binkin, N1
1Regional Veterinary Services, Italy; 2Istituto Zooprofilattico Sperimentale Umbria e Marche, Italy; 3Istituto Zooprofilattico Sperimentale di Piemonte, Italy; 4Istituto Zooprofilattico Sperimentale of Sanità, Italy

Clinical surveillance is an important component of BSE surveillance system in use and, if effective, it can be a good indicator of the presence of the infection if laboratory based surveillance is suspended. Since the year 2000 the Italian veterinary officers were requested of actively looking for clinically suspected animals by visiting each farm. However, the efficacy of the system has been shown to be very low, with few reported clinical suspects. Therefore we conducted a survey to better understand content and quality of the surveillance conducted on farms, the attitudes of public veterinarians involved in this operation and the association of these variables with the training received by the veterinary staff. In 8 regions of Italy a questionnaire was distributed by mail to a sample of local health unit veterinarians. Univariate and multivariate analysis was performed on the 494 completed questionnaires. 75.8% of the interviewed veterinarians (IC 95%: 71.7-79.5) visit each farm twice per year as indicated in the national plan, but the quality of the checks seems to be low. In fact only 24.2% (IC 95%: 20.5-28.3) use more detailed and sensitive screening strategies. Moreover some results indicate a lack of motivation of veterinarians: BSE was only 24.2% (IC 95%: 20.5-28.3) use more detailed and sensitive screening strategies. Moreover some results indicate a lack of motivation of veterinarians: BSE was only 24.2% (IC 95%: 20.5-28.3) use more detailed and sensitive screening strategies.

P04.80
Clinical Observations of BSE Infection in Red Deer
Steele, E; Martin, S; Jeffrey, M; Gonzalez, L1; Sidd, S; Finlayson, J; Hamilton, S; Eaton, Samatha L; Reid, Hugh W; Todd, R; Pang, Y; Chianini, F; Dagleish, M1; Moredur Research Institute, UK; Veterinary Laboratory Agency, Lasswade, UK

Clinical observations of BSE infection in Red Deer

Observation of clinical signs is often the first step in the diagnosis of TSE diseases in experimental, farmed and wild animals. Clinical presentation of chronic wasting disease (CWD) infected deer varies widely as disease progresses and many clinical signs observed can be non-specific to TSE infection, however by terminal stage the majority of cases involve behavioural changes and loss of body condition.

We present here the first description of clinical disease in deer experimentally infected with BSE. These data are part of the results of an ongoing project to investigate the susceptibility of UK red deer (Cervus elaphus elaphus) to BSE infection either by alimentary or intra-cerebral infection.

Eighteen European red deer calves (mean 64 days old) were challenged intra-gastrically with 25g of BSE-infected bovine brain. Six challenged and 2 control deer were culled at 6 and 12 month post infection. These animals showed no clinical signs and no disease-specific PrP (PrPd) on immunohistochemistry (IHC) examination of a wide range of tissues collected at post-mortem. Six BSE-dosed and 4 negative control deer are still alive at time of writing (1384 dpi).

Subsequently, 6 red deer of the same cohort (mean 341 days old) were challenged with 0.05g of BSE positive bovine brain material and 2 with sterile saline by the intra-cerebral route. Currently (1106 dpi), five of the six challenged animals have developed clinical signs and terminal disease confirmed by IHC and western blot detection of PrPd.

Clinical signs similar to CWD cases have been observed including behavioral change, wide stance, lowered head, and excessive salivation. All animals had significant weight loss attributed to inability or unwillingness to feed, with inhalation pneumonia occurring in the case of one animal which is commonly observed in CWD cases. The first animal to show clinical signs was markedly different to the four subsequent cases. This animal had to be culled following several behavioral episodes causing physical injury.

Our results prove for the first time that UK red deer are susceptible to intra-cerebral BSE infection and shows that the clinical presentation of disease shares many similarities to that recorded for CWD.
Epidemiology, Risk Assessment and Transmission

P04.81
High Pressure/Temperature Inactivation of TSE Agents in Specified Risk Materials
De Pasquale, A1; Cardone, F; Berardi, V; Valanzano, A; Graziano, S; Abdel-Haq, H; Heinz, V; Buchow, R; Mathys, A; Kees, M; Knorr, D; Meyer, R; Pocchiari, M; Brown, P

Specified Risk Materials (SRM) include those parts of ruminant animals, such as brain, spinal cord and cranial nerves that represent a potential risk for transmission of transmissible spongiform encephalopathy (TSE) agents. European Union legislation excludes SRM from the human and animal food chain, but in view of a possible future use of these products, an efficient process of decontamination is required. In previous studies we proved that ultra-high pressure/temperature (HPT) treatments can reduce TSE infectivity in meat food up to a million fold. In the present study we investigate the reliability and the efficiency of these treatments for TSE infectivity removal from rendered SRM.

Rendered and desiccated SRM was rehydrated and spiked with high or low amounts of infectivity from mouse-adapted bovine spongiform encephalopathy (mBSE, strain 6PB1) or hamster-adapted scrapie (strain 263K). The samples were sealed in plastic bags and were pressurized for 5 minutes at 690 and 1000 MPa at increasing temperatures from 121 to 145°C. The efficiency of decontamination was evaluated by the reduction of the pathological prion protein (PrPres) by western blot and by infectivity bioassay.

HPT treatments at 690 or 1000 MPa removed 1 log of PrPres from SRM samples spiked with high mBSE or scrapie infectivity. The removal of PrPres was higher at higher temperatures regardless of the pressure and was comparable for both strains. The reliability of these results was demonstrated by the study of replicate samples for which we observed a constant removal of 0.5 logs in eight replicate preparations. The effect of the different HPT treatments on high mBSE infectivity was also tested by the mouse bioassay. All inoculated mice developed disease, with incubation periods that become longer with increasing temperature. Surprisingly, the samples processed at 690 MPa produced longer incubation periods compared to the 1000 MPa samples. Finally, SRM spiked with a low dose of mBSE infectivity was exposed to 690MPa at 134°C, in order to investigate the possibility of reducing infectivity below the level of bioassay detectability. Infectivity was not totally eliminated from the two samples analysed.

These results indicate that HPT treatment can produce a reproducible but limited reduction of TSE infectivity spiked into rendered SRM. The limited reduction compared to HPT treatment of processed meat products may be due to the prior desiccation of the SRM before HPT treatment.

P04.83
Assessing the Feasibility of Accessing Dental Case Records for Detailed Past Dental Histories in CJD Cases and Controls
Smith, A1; Everington, D; Ward, H; Will, R; Mathewson, A; Baggs, J

Iatrogenic transmission of vCJD cannot be ruled out as a risk to public health. Dental interventions account for a significant number of invasive procedures performed in primary care. Therefore, it is important to determine the level of risk dental treatment pose in relation to the transmission of CJD. To date published epidemiological studies have relied on reported dental case histories of CJD cases and controls. Little published work has been performed examining the past dental history of CJD cases and controls by direct review of dental records.

The aim of this study was to assess the feasibility of accessing dental records of CJD cases and controls. For this pilot study, we proposed to randomly select 32 CJD (8 vCJD & 8 sCJD) cases (4 vCJD and 4 sCJD) from England, Scotland, Wales and Northern Ireland) and 24 controls (8 from England, Scotland and Wales), who had consented to have their records accessed and who were registered at the National CJD Surveillance Unit (NCJDSU). Addresses of the dentist treating the cases and controls were obtained from records held at the NCJDSU. For the first part of the study, the dental records were contacted by the research group by letter and then by a follow up phone call. They were invited to submit the patient’s records for photocopying and return to the practice. The case records were reviewed by a dental practitioner. The data collected include age at each dental visit, presence or absence of oral disease, types of treatment performed and additional risk factor information, e.g., use of human dura mater graft material.

The first part of the study found that 50% of controls and 22% of cases dental records were available for viewing by contacting the dental practitioner. The major reasons for failing to retrieve patients dental records is due to incorrect details supplied during the initial data collection particularly as the data is supplied by a relative of the case/control. A second stage of the project to assess the feasibility of accessing records via central records held by relevant dental practice boards is on-going.

P04.82
Screening for vCJD in UK NSHSBT Tissue Donors using a Highly Sensitive Western Blot Protocol
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A characteristic feature of the pathogenesis of vCJD is an accumulation of the disease associated isoform of the prion protein in the lymphoreticular tissues and brain of affected individuals. Since accumulation of prions in lymphoreticular tissue is likely to occur relatively early, before neurological symptoms become apparent, it is possible that tissue donation may be a route of vCJD transmission. A pilot study to screen UK NHS Blood & Transplant deceased tissue donors for vCJD has been underway since April 2006 using tonsil tissue retrieved at the time of tissue donation. The study will be extended to include spleen tissue in the near future.

The test protocol consists of a prion concentration step developed from the Prionics®-Check Western kit followed by Western blot analysis. The prion concentration procedure is rapid, robust and highly efficient at recovering prions from vCJD brain homogenate spiked into tonsil and optic nerve tissue homogenate. The protocol is at least as sensitive as sodium phosphotungstate precipitation against brain and spleen tissue homogenates from vCJD affected individuals and could be readily automated if a high throughput screen is required.

P04.84
Scrapie Surveillance in Iceland – Detection of Nor98 and Natural Scrapie in Healthy Sheep after Implementing Rapid Testing
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In Iceland scrapie in sheep has been a problem for a long time, but the first report of the disease was in 1878. One hundred years later, in 1978, an eradication program against the disease was started. After enhancements of the program in 1986 and 1993, it now includes culling of all scrapie flocks, a period without sheep for at least two years, restocking with sheep from scrapie-free areas after thorough cleaning and disinfection of all premises and equipment on the farm. Quarantine zones, marked by fences or rivers, which were established in the 1930’s to fight “Karakul diseases”, have also prevented spreading of the disease. Six of these zones (out of 36), located in three remote parts of the country, have always remained scrapie free. Incidence of scrapie in the last ten years has been one to eight cases per year (mean: 3.6 cases/year). Most of the cases have been in endemic areas and in some instances scrapie recurs on farms where disinfection and restocking took place years earlier. Most scrapie cases in Iceland are detected through passive surveillance (clinical suspects/fallen stock), although we have had active surveillance of scrapie since 1978. Several thousand abattoir samples from healthy adult sheep were tested every year by using histopathology, but there were, however, no cases detected in this group until 2004, when new rapid testing methods were implemented. Initially the ELISA-method (TeSeI, Bio-Rad) was used in addition to histopathological staining, but is now used for screening all samples from healthy slaughtered sheep. In 2004 a total of eight scrapie cases were detected, of which three originated from the 3000 samples from healthy slaughtered sheep tested by ELISA. One of these positive cases was of the atypical type of scrapie, Nor98 (confirmed by NV, Norway). When that flock of 350 sheep was culled and tested by ELISA, one additional Nor98 case was detected. Among 7000 samples from healthy sheep tested since 2004, we have detected one additional scrapie case.
Epidemiology, Risk Assessment and Transmission

**P04.85**

Transmission Dynamics in a Suffolk Sheep Flock Naturally Infected with Scrapie as Shown by Sequential Biopsies and Necropsies

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Tonsil biopsies and or necropsies were performed on 6 birth cohorts of a scrapie infected Suffolk sheep flock between 1998-2004. The onset of accumulation of abnormal Prion protein in lymphoid tissue, the attack rate and age to clinical scrapie or necropsy were determined for different groups of sheep. Differences in attack rates and/or mean age to necropsy were found for different birth cohorts, for lambs born to scrapie infected sheep when compared to scrapie resistant sheep; for male and female sheep; and for prima gravida progeny compared to lambs born from later pregnancies. When husbandry conditions are taken into account, the data suggest that under natural conditions the highest risk of acquiring infection occurs in the perinatal period, probably by placental contamination of the environment. However, the data also support the probable horizontal scrapie infection occurring at low frequency in older sheep. Naturally infected flocks will experience transmissions to susceptible genotypes by high and low risk events. In this flock accumulation of abnormal PrP in lymphoid tissues occurred at approximately 50% of the age to death. When compared with previous data, this does not suggest that LRS infection is necessary for neuroinvasion.

**P04.86**

Surveillance for Frequencies of Polymorphisms on Bovine Prion Protein Gene of Commercial Sires in Japan

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The recent discovery of significant associations between bovine spongiform encephalopathy (BSE) susceptibility in cattle and the frequency distributions of insertion/deletion (indel) polymorphisms within the bovine prion protein (PRNP) gene prompted an evaluation of 283 commercial sires in Hokkaido, where dairy farming has prospered the most in Japan. Two variable sites, a 23-bp indel polymorphism (123 bp/100 bp) in promoter region and a 12-bp indel polymorphism (103 bp/91 bp) in intron 1, were investigated by PCR. As for the 23-bp indel in the commercial sires, the frequency distributions of 100 bp allele and 100 bp/100 bp genotype which were mentioned the association with BSE susceptibility in the BSE-affected German cattle were 54.8% and 29.7%. On the other hand, the frequency distribution of 91 bp allele and 91bp/91bp genotype in 12-bp intron 1 site which were mentioned the association with BSE susceptibility in the UK cattle were 49.6% and 23.0% in the commercial sires. The frequency distributions of 100 bp allele and 100 bp/100 bp genotype in 23-bp indel site in Japanese sires were different from those of the United States (U.S.) sires. No significant allelic and genotypic differences were detected for the 12-bp indel on intron 1 region between Japanese sires and the U.S. sires. Our results suggest that Japanese sires have low susceptibility against BSE.

**P04.87**

Epidemiology of Atypical Scrapie In Italy

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Since 1995 to 2006, 310 scrapie outbreaks were reported in Italy; 267 sheep flocks, 29 goat herds, 14 mixed flocks. Due to the implementation of the compulsory system of active surveillance in January 2002, many new cases have been recorded leading to a strong increase of scrapie incidence. The shift in September 2004 from using Prionics to BioRisk allowed the identification of atypical scrapie cases, however only on May 2005 a first atypical case in a sheep was detected. Aim of our work was to describe the epidemiology of atypical scrapie in Italy during the period 2005-2006. Outbreaks were characterized on the basis of disease distribution by time, space and animal or flock features. Age of affected animals were compared by type strain (classical vs. atypical) and by species using the Mann Whitney test. 28 cases in sheep and 6 in goats have been confirmed up to December 2006. Only a couple of secondary sheep cases in the same flocks were identified. All the cases were classified as Nor98-like. Compared to classical scrapie that has a more even geographical distribution, the atypical cases seem to be scattered above all in the central-southern regions of the country. Whereas in sheep the atypical cases are clearly older than classical ones, in goat the same age classes are affected by the two strains. The genotype distribution doesn’t show significant differences compared with that reported in the rest of Europe. So far only few hints are available regarding the transmissibility of the disease, its origin or its aetiology. Nevertheless the collection of these data seem to be crucial in order to increase the understanding of the disease and to implement an efficient eradication plan.

**P04.88**

A Rapid, Sensitive Dual Stain Procedure to Discriminate between PrPSc and Other Proteins in Tissue Contaminating Surgical Stainless Steel Surface

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We have previously developed a rapid, sensitive staining procedure for in situ detection of prion amyloid material on surgical stainless steel surfaces using fluorescent thiazole reagents. To increase the resolution of the amyloid material in tissues and on contaminated surfaces, and discriminate between PrPSc and other proteins, we now report the development of a Thioflavine T / SYPRO Ruby dual staining procedure. This is used in combination with our episcopic differential interference contrast/epifluorescence (EDIC/EP) microscope for rapid scanning of instrument surfaces. Double-blind studies using various dilutions of ME7 infected brain in normal brain homogenate produced a dose-related signal on contaminated surgical stainless steel surfaces, as assessed by image analysis. The sensitivity of our method proved over 2-fold better compared to the classic Western blot procedure with the same prepared samples. It is believed that the sensitivity improvement is actually greater since testing instrument surfaces by Western blot relies on swelling the contaminated surface, and we have shown previously that PrPSc binds to surfaces more strongly than other proteins. Moreover, if the instrument is serrated or pitted then it is more difficult to elute biological deposits from the surface for analysis. The detection limit for the new procedure was demonstrated to be <100fg PrPSc; approx. 2 attomoles. Therefore, this new sensitive microscopy procedure is rapid and can be applied directly to instrument surfaces to check for amyloid contamination.
Epidemiology, Risk Assessment and Transmission

**P04.89**

Prion Alleles Influence Disease Progression in Orally Challenged White Tail Deer

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We have shown a statistically significant bias in chronic wasting disease (CWD) prevalence in free-ranging Wisconsin white-tailed deer associated with the presence of specific Prion Protein (PrP) alleles. To determine the extent of influence these PrP polymorphisms exert on susceptibility to and progression of CWD, we initiated oral transmission of CWD agent into white-tailed deer. Deer selected for this study had PrP alleles that were variable at amino acids (AA) 95 and 96 with the most common allele having glutamine at position 95 and glycine at position 96, which we refer to as wild-type (wt). Two other alleles were present in the deer used, a glutamine to histidine change at position 95 (Q95H) and a glycine to serine at position 96 (G96S). All twelve deer used in this study were rescued fawns from outside known CWD-positive regions of Wisconsin, and tested negative for CWD by tonsil biopsy. Two deer were lost to non-disease related death. Of the remaining inoculated deer, all wt/wt deer became end-stage positive for CWD with an average incubation period of 693 days post inoculation (PI); all 95/wt or 95/96 |G96S|/96/96 deer became end-stage positive with an average incubation period of 956 days PI and neither the 95/95 or 95/96/96 deer have demonstrated any clinical symptoms of CWD as of 5/16/2007, 1101 days PI.

**P04.90**

A Rapid Light Microscopy Technique for Sensitive Detection of Prion Infection in Cell Models

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Transmissible spongiform encephalopathies (TSEs) are fatal transmissible neurodegenerative diseases observed in various mammalian species, including humans. The accumulation of an isoform of the host-encoded prion protein (PrP) that has become resistant to degradation by proteases is a common feature of TSEs. Although different species possess their own PrP molecule, these are highly conserved and it has become clear that the protease-resistant PrP (PrPRes) is capable of crossing the species barrier, potentially affecting humans. Diagnostic tests rely on immunohistochemistry, ELISA, Western blotting) using tissues obtained post-mortem. In the mean time, because of the long incubation period, animal models of infectivity are a constraint and pose ethical problems. Therefore, there is a need for a rapid and reliable method allowing the early detection, identification and characterisation of various strains of resistant PrP in animal tissues. In this project, we are assessing an established N2a neuroblastoma prion infectivity model and a Neural Stem Cell (NSC) model developed by our collaborators, which may allow to rapidly assess in vitro the infectivity of various strains of PrPsc. Episcopic Differential Interference Contrast / Epi-Fluorescence (EDIC/EF) microscopy, previously developed in our laboratory, can be used in combination with sensitive fluorescent thiazole dyes to detect proteins particularly rich in beta-sheets. We are using the N2a and NSC models in combination with our detection methods as an alternative to longer and more costly immunohistochemical protocols. This technique may prove faster, more reliable and more cost effective than current immunohistochemical methods for rapid confirmation of PrPsc infectivity and assessment of decontamination protocols.

**P04.91**

A Cellular Automata Approach to Modelling the Transmission of Scrapie by Sheep Movements Between Flocks

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Control measures against scrapie are usually applied to whole flocks, so the most important process in the transmission of scrapie is movement of sheep between flocks. Whole life movements of ewes are of especial interest, because of the long incubation time of scrapie. Cellular automata provide an opportunity to model the transmission of scrapie by sheep movements, including interactions with environmental factors such as farm altitude and the effects of variations in sheep density. They also promote the combination of dynamic spatial modelling with geospatial technologies. This model involved three main areas of technological development:

1. Development of methods to identify birth and death holdings of ewes sampled for scrapie. Animal movement databases allowed the tracing of c. 17000 samples taken from fallen ewes to their birth holdings. Consistent with expert knowledge, over 80% of ewes died where they were born, so scrapie transmission depends on the minority of ewes that are mobile. Similar tracing methods have also been applied to much larger numbers of scrapie samples from abattoir surveys.

2. Generation of simple cellular automata movement models for sheep in the UK from the data on ewes that moved. These included observed contrasts between movements to upland and lowland holdings. Such contrasts can influence the potential distribution of scrapie.

3. Application of recently developed cartogram methods to deal with uneven sheep distributions by transforming between regular equal area projection maps and a space in which sheep are evenly distributed. The cartogram was divided into a grid consisting of 80000 cells, each of which represented a single holding. This allowed inclusion of environmental factors (e.g. hills and coastlines), while keeping model mechanics very simple.

This work has demonstrated the potential for simple simulations to recreate the broad characteristics of scrapie epidemics.

**P04.92**

Assessment of Prion Decontamination Procedures on Surgical Stainless Steel Surfaces

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Recently we have developed a rapid, sensitive staining procedure for in situ detection of prion amyloid material on surgical stainless steel surfaces using fluorescent thiazole dyes. Initial data show that this procedure is several fold more sensitive than conventional Western blot detection of equivalent infected tissue that has not been first dried onto instrument surfaces. In addition, recognised inefficiency in elution of the amyloid off the steel surfaces will further reduce the sensitivity of the Western blot assay compared to the in situ staining procedure. The stain employs a thiazole incubation in addition to a SYPRO Ruby step to detect both amyloid and general tissue protein simultaneously in situ on surgical stainless steel surfaces. This procedure is currently being used to assess various cleaning chemistries from several companies for their ability to remove or inactivate PrPsc. However, in reality, a range of several different detergents or enzymatic cleaning reagents may be used within automated washer-disinfector cycles. As such, we have utilized our dual stain to assess the effects of different pre-treatments and cleaning reagents on prion removal throughout the course of validated washer-disinfector cycles. This work is being performed in collaboration with the CEA, Paris who have an established animal model of infectivity to confirm the efficacy of the disinfection procedures. This collaboration will allow the new rapid, dual stain method to be developed for in situ detection of PrPsc and general protein contamination on surgical steel surfaces and its suitability to assess prion removal (hence disinfection) during SSD cleaning procedures.
Sequence Variability of Noncoding Regions of the Prion Protein Gene in Italian BSE-affecte and Control Cattle
Paletto, S; Maneschi, MG; Grego, E; Maurella, C; Zuccon, F; Riina, MV; Corvona, M; Colussi, S; Caramelli, M; Goldmann, W; Acuts, PL; CEA - Istituto Zooprofittico Sperimentale del Piemonte, Liguria e Valle d’Aosta, Italy; "Istituto di Patologia Animale, Epidemiologia ed Ecologia - Facoltà di Medicina Veterinaria, Italy; "Roslin Institute, Neuroneopathogenus Unit, UK

Prion protein gene (PRNP) polymorphisms are known to modulate susceptibility to transmissible spongiform encephalopathy (TSE) in species such as humans and sheep changing the amino acid composition of the resulting prion protein. Nevertheless, no major association has been established between polymorphisms in the PRNP open reading frame (ORF) and bovine spongiform encephalopathy (BSE) infection in cattle. Previous reports for Swiss and German cattle suggested an association with BSE susceptibility of insertion/deletion (indel) sites outside the ORF: a 23-bp indel in the upper region of exon 1 and a 12-bp indel in the putative promoter region of intron 1. These polymorphisms are thought to influence PrP expression level and the incubation period of certain TSEs in cattle, which has been identified in captive and free-ranging cervids more than 20 years ago (Nonno R. et al. 2006). The second passage of MV2 and V2 scJD is still in course. V210I gCJD was transmitted both at the first and the second passage while P210L gSS was not. On the other hand, E200K gCJD was negative at the first passage and positive at the secondary.

In conclusion, serial transmission of scJD and gCJD cases to C3H mice did not result in a strain adaptation since at the second passage neither the incubation period shortened nor the number of PrPPrPres positive animals increased. Furthermore, positive transmission (MV1 scJD) at the primary passage did not warrant a successful transmission in the second one. Finally, the absence of PrPPrPres positive animals at the first passage does not necessarily imply a negative outcome at the second one (E200K gCJD).
In the course of the mad cow crisis several measures were established in certain member states, and finally, Europe wide. These measures aimed at a reduction of the human exposure risk with the agent of Transmissible Spongiform Encephalopathies (TSE). The highest priority has the removal and harmless disposal of Specified Risk Materials (SRM) to this day. The legal definition of SRM according to the European directive 99/43/EC defines tissues of concern, in particular the central nervous system (CNS: brain and spinal cord) from cattle, sheep and goats over 12 months of age.

Methods for the detection of CNS that are currently available are based on a more or less uniform immunochromatological principle. They only detect tissues of the CNS without further information on species and age of the animal and therefore differ from the given legal definition of SRM. This applies to molecular biological techniques and species-specific immunooassays since these methods do not identify the age of the CNS as well. Moreover, false negative results or at least less precise quantification of the CNS are to be expected due to heat processing effects on the used markers during production of meat products or meat and bone meal (MBM).

In the course of a research project, which was funded by the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) a procedure using gas chromatography mass spectrometry (GC/MS) was developed and optimized. On the basis of CNS-typical fatty acids and their patterns, the detection of the CNS including their age and species was possible additionally to the quantification of the CNS. The complex lipids, containing these fatty acids where shown to be even thermally stable up to extreme conditions not reached during food and feedstuff processing. Currently, this method is being evaluated in a ring test organised by the Institute of Food Hygiene, University of Leipzig. This project is funded by the BMELV.

The present contribution summarises first results on the detection of CNS in meat products and MBM from the ongoing ring test.

Monitoring the Potential Transmission of Chronic Wasting Disease to Humans

P04.97 Validation of a Reference Method for the Detection of CNS in Meat Products by Means of GC/MS

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The present contribution summarises first results on the detection of CNS in meat products and MBM from the ongoing ring test.


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The infectious nature of the pathological prion protein (PrPSc) has been recognized by investigation of kuru (caused by ritualized cannibalism). Since then several animal studies confirmed the infectious potential. Thus, also transmission by iatrogenic procedures was suspected. The most frequent cause of iatrogenic transmission worldwide has found to be following the administration of cadaveric growth hormones and following Dura mater transplants. Transmission by neurosurgery is assumed, but only once described in the literature for three occasions (Will 1985). In Germany, iatrogenic CJD is a rare phenomenon compared to other countries, and no case caused by growth hormones is known. Thus, we analyzed all confirmed and probable CJD patients and controls for history of brain surgery. The frequency in both groups was similar, and therefore it is no epidemiological risk factor for CJD. In the years 1993 to 2006, eight patients with Dura mater grafts were reported. Further analysis of the CJD patients found time-and-space cluster for three patients treated within 15 months in the same hospital by neurosurgical intervention for traumatic head injury (two patients; February 1983, May 1984), and resection of an astrocytoma (September 1983). This cluster raises the question whether a transmission by neurosurgical instruments might be a potential cause of the infection in these cases, or the patients were infected by Dura mater grafts. A comparison of clinical, neurochemical, and morphological findings will be presented.

Monitoring the Potential Transmission of Chronic Wasting Disease to Humans

P04.98 Validation of a Reference Method for the Detection of Chronic Wasting Disease to Humans

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Chronic wasting disease (CWD) has been occurring in Colorado and Wyoming for decades and in Wisconsin for several years. Studies have shown the existence of a substantial species barrier for CWD transmission to humans. However, the transmission of bovine spongiform encephalopathy to humans indicates that this barrier may not be completely protective. In the United States, the Wyoming Department of Health (WDH), the Colorado Department of Public Health & Environment (COPHE), and the Wisconsin Department of Health and Family Services established a follow-up study of hunters to monitor potential CWD transmission to humans. Identifiers from selected hunters are cross-checked with mortality data to determine their mortality status and causes of death. The WDH hunter study includes 966,339 records of licenses purchased during 1996-2005 whereas the CDPHE study contains about 3.9 million licenses purchased during 1995-2005. These licenses represent about 292,873 and 919,684 individual hunters in Wyoming and Colorado, respectively. The median age of hunters was 42 years in Wyoming and 48 years in Colorado. About 19% of the hunters in Wyoming and 11% in Colorado purchased licenses to hunt in known CWD-endemic areas. Most of the other hunters had statewide licenses. A total of 194,206 records were cross-checked with mortality data in Wyoming and 470,046 records in Colorado; to date, 2 (including 1 identified by passive surveillance) and 4 hunters, respectively, died of CJD. In Wisconsin, 142 persons were identified as having consumed venison from CWD-positive deer harvested during 2003-2005. Hunter mortality studies are valuable to monitor possible CWD transmission to humans. CJD deaths unrelated to CWD are expected to be identified through cross-matching with mortality databases. Further epidemiologic and laboratory investigations are required to establish a causal association, if any, of the CJD deaths with CWD.

Tissue Plasminogen Activator’s Level in Cerebrospinal Fluid as a Potential Biomarker for Creutzfeldt-Jakob Disease

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Human tissue plasminogen activator (tPA) is a protease of the St.1 tissyp family which converts plasminogen to active plasmin. Plasmin is important in cell migration, inflammation and tumor invasion and has been shown to be involved in neurodegeneration and neuronal plasticity. Furthermore, it is found to be secreted in response to mental stress. tPA-mediated activation has been shown to be stimulated in the presence of partially denatured proteins including PrP and has also been shown that apoPrP (PrP not bound to Cu) stimulates this system, especially the terminal fragment. Another finding regarding the relationship between PrP and plasminogen is that the later binds to the disease associated isoform of prion protein, as well as to recombinant PrP (rPrP). Previously we have shown that tPA activity and expression levels were higher in Transmissible Spongiform Encephalopathies (TSE) infected animals compared to normal ones. Here we report our data of tPA activity in Cerebrospinal Fluid (CSF) samples from several Creutzfeldt-Jakob Disease (CJD) patients. Preliminary results show that tPA activity levels are elevated in CSF from CJD patients as compared to control ones. Normally, tPA levels do not exceed the range of 1ng/ml limit and are elevated in CSF from patients affected with a variety of other diseases, including Multiple Sclerosis, Post-Haemorrhagic Ventricular Dilation (PHVD) and Bacterial Meningitis. CSF is a potential valuable source for detection of human TSEs.
Epidemiology, Risk Assessment and Transmission

P04.101
Development of a Standardised Approach to Assess the Effectiveness of Current and New Decontamination Technologies against TSE Agents
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Background: The development of inactivation methods for Transmissible spongiform encephalopathies (TSEs) is an urgent requirement in relation to the potential for iatrogenic transmission of variant Creutzfeldt-Jakob Disease (vCJD). The evaluation of the effectiveness of such methodologies requires a highly sensitive and specific assay or a combination of assays. With current cellular and biochemical based assays still in development, the bioassay remains the accepted approach to assess effectiveness; however, careful matching between the TSE strain and host species is required to help ensure that the risks are appropriately evaluated with regard to vCJD transmission.

Aims: The project aims to develop a robust system to assess proposed TSE inactivation technologies focusing on a model using the TSE strain, BSE-30IV, designed to mimic the key features of possible vCJD transmission via contaminated surgical instruments. The dynamic range of the model was determined using a titration series of infectivity which in the first instance was 'tested' using a conventional autoclave based process.

Methods: BSE-30IV infected mouse brain homogenate, previously titrated to 10^12 ID50 per gram, was dried onto the surface of surgical steel suture wires using a standardised process. Wires were implanted i.c. into VM mice and monitored for clinical symptoms for up to 550 days.

Results: For the wire-based titration series clinical symptoms were observed in animals from groups across a 6-log dilution range, however, at dilutions below 10^1 transmission rates fell below 60%, suggesting that the useful range is around 4-logs. Data will be presented comparing the surface bound titration results with the equivalent in-solution titration series. The ongoing results from the decontamination studies will also be presented in relation to the titration data generated.

Conclusions: Methods have been established to ensure a consistent exposure of wires to the decontamination process with no further manipulations of the carriers post processing. Using this protocol a titration series has been established for BSE-30IV on surgical steel that potentially covers a 4-log range. The use of these protocols to evaluate novel prion decontamination methods will be discussed.

P04.102
Has vCJD been Transmitted by Human Blood Plasma Products? 20 Years and Counting
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The diagnosis of vCJD in a patient whose plasma had previously been used in the preparation of blood plasma products by the NHS led to the decision in 1998 that the preparation of plasma derivatives from UK-donor plasma should cease as a precautionary measure. Since then, plasma products have either been manufactured by the NHS, using plasma purchased from the USA and Europe, or purchased directly from commercial companies.

It is now known that donations from 11 individuals, later diagnosed with vCJD, had been included in the preparation of a total of 175 batches of different plasma products that were released for use between June 1987 and September 1998. No cases of vCJD have been associated with these products, although 20 years have elapsed since the first implicated batches were released for use. This contrasts with 3 instances of probable transmission of vCJD by red cells in which symptoms of vCJD developed in recipients 6.5 years, 7.8 years and 8.3 years after transfusion.

There are a number of possible explanations for the apparent absence of transmission by plasma products.

(1) Prion infectivity was not present in the donated plasma.
(2) Prion infectivity was present in the donated plasma but not in the manufactured products, due to dilution or removal of infectivity by the manufacturing process.
(3) Prion infectivity was present in manufactured product(s) but has not resulted in clinical symptoms of vCJD because of either a prolonged incubation period or a lack of susceptibility in recipients.

The methods used for the manufacture of blood plasma products by the Scottish National Blood Transfusion Service have been examined to determine the extent to which removal of prions might have occurred. These experiments indicate a possible overall prior reduction of 2.7 logs for intermediate-purity factor VIII concentrate (ZB), 3.0 logs for intermediate-purity factor IX concentrate (DEFIX), 5.8 logs for thrombin, 26.2 logs for fibrinogen, 26.5 logs for immunoglobulin, 7.4 logs for high-purity factor IX concentrate and >11.5 logs for albumin.

P04.103
Femtograms-Detection of PrPSc in Biological Samples using Chemically Synthesized RNA-Aptamer
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For the safety of biological products, it is one of our major concerns to reduce the TSE-risk of cattle-blood derived materials such as serum and plasma. For the detection of possibly contaminated abnormal isoform of prion protein (PrPSc) in the biological samples, it is indispensable to develop a highly sensitive PFP detection procedure. Here, we have developed an aptamer-beads PFP-concentration procedure by using RNA-aptamer 60-3 which binds to recombinant mouse PFP with high affinity (Kd = 5.6 nM) (1).

The RNA-aptamer 60-3 was chemically synthesized employing a novel RNA synthetic method with a 2-O-(2-cyanoethoxymethyl) protecting group (2), with 2’OMe substitution being indispensable to mimic the H-bonding groups of the native PrPSc. The RNA-aptamer 60-3 was then bound to streptavidin-coated magnetic beads (60-3 aptamer-beads) and was then bound to streptavidin-coated magnetic beads (60-3 aptamer-beads) and used for pull-down assays. The pulled-down PFPSc was analyzed by Western blotting.

The 60-3 aptamer-beads demonstrated the enrichment of PrPSc from the 20-milliliter times diluted scrapie-infected mouse brain (50mg of 50mg brain equivalent ml^-1). Comparing to phosphotungstic acid (PTA) concentration method, the 60-3 aptamer-beads revealed more than 100 times efficiency in concentrating PrPSc spiked in bovine serum. Moreover, the 60-3 aptamer-beads showed binding ability to PrPSc in highly diluted BSE-infected bovine brain.

The present aptamer-beads pull-down procedure enables us to perform a femtograms-detection of PFP. The procedure was also proven to be applicable to BSE-PrPSc. The present aptamer-beads system could serve as a resource for prion-removal column and serum prion assays, and potentially achieve the safety of blood derived biological products.

References

P04.104
Survival of Prion Proteins in Environmental Matrices
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Several publications have suggested the environment as a possible route of transmission, especially for sheep scrapie and cervid Chronic Wasting Disease (CWD). The role of the environment as a reservoir for these disorders is difficult to prove and faces a considerable lack of information. In this work, different methodologies have been developed to evaluate the survival and inactivation of TSE agents in environmental matrices.

Different slaughterhouse and urban sewage samples were spiked with diverse strains of either scrapie or BSE agents and kept under controlled conditions for extended periods of time. Aliquots of every experiment were sequentially collected and concentrated according to a methodology specifically selected for each type of matrix. Sensitivity of the methods developed was estimated among 2-10 µg of infected tissue. PrPres was finally detected by western blot. Films were then transformed into digital pictures, signal intensities were quantified and regression models were computed.

According to the results obtained, scrapie agent showed higher stability than BSE in all the environments studied. However, no significant differences were observed among mouse-passaged scrapie strains and sheep scrapie. The regression models provided t90 and t99 values (times of incubation necessary for 90% and 99% reduction of PrPres levels). In urban sewage, i.e., t99 was estimated as around 50 and 22 days for scrapie and BSE respectively. In general, the effect of the matrix was clearly observed in all the experiments, showing up to a 6-8 fold higher reduction of PrPres levels in comparison to PBS controls.

As some of the inocula were titrated in terms of infectious doses, we approximated the t99 as the incubation time necessary for a 99% reduction of PrPres levels. In comparison to PBS controls, clearly observed in all the experiments, showing up to a 6-8 fold higher reduction of PrPres levels in comparison to PBS controls.
Epidemiology, Risk Assessment and Transmission

P04.105
Transgenic Mice Expressing 5 Octarepeats Bovine PrP Show a Reduced Susceptibility to BSE but the Generated 5OR-BSE Prions Retains its Transmissibility
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Background: Some degree of polymorphism has been described in the number of octapeptide repeats (OR) found in bovine-PrP. The most common allele harbours six OR, but in a lower proportion, five and seven OR variants can be found. By side, PrP from other species have five OR in their amino acid sequences. The number of OR has been related to incubation times after experimental inoculations and the susceptibility to BSE infection of the five OR.

Objectives: The main aims of this work were to assay the susceptibility of transgenic mice expressing bovine PrP with five octarepeat (5OR-BoPrP-Tg mice) to BSE prions and to study the biochemical and biological properties of the new generated 5OR-BSE prions.

Methods: Susceptibility of 5OR-BoPrP-Tg mouse lines in comparison to 6OR-BoPrP-Tg mouse lines was assessed by intracerebral inoculation of the same cattle BSE inoculum. Biochemical and histopathological properties of the new generated 5OR-BSE prions were studied and then re-inoculated in other transgenic mouse lines expressing PrP from different species for its biological characterization.

Results: The attack rate and incubation period found after inoculation of BSE in 5OR-BoPrP-Tg mice indicate that the infection is less efficient than in 6OR-BoPrP-Tg mice expressing similar PrP levels. No biochemical differences were found in the 5OR-BSE PrP molecule from BSE-infected 5OR-BoPrP-Tg mice when compared to 6OR-BoPrP PrP. The new generated 5OR-BSE prions were transferred in all mouse models used. Transmission efficiency of 5OR-BSE prions was similar to those of 6OR-BSE prions in all mouse models tested.

Discussion: The reduced susceptibility for BSE infection found in 5OR-BoPrP-Tg mice reflects a lower efficiency of prion replication, which is probably due to a reduced ability for transformation of 5OR-PrP into 6OR-PrP. This would explain the genetic basis for the low incidence of BSE in cattle expressing 5OR-BoPrP. 5OR-BSE prions preserve the same ability to cross transmission barriers than 6OR-BSE prions.

P04.106
Detection of Single Scrapie- and Bse-Prion Particles by Surface-Fida
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The infectious agents of prion diseases are composed primarily of the pathogenic isoform of the prion protein designated PrP(Sc), which is generated by a conformational change of the cellular isoform PrP(C). In contrast to its cellular isoform, the pathogenic isoform PrP(Sc) forms insoluble aggregates. Hitherto approved prion tests use the PK-resistance of PrP(Sc) as a marker for the disease. Because of varying portions of disease related aggregated PrP which is not PK-resistant [2; 3], these prion tests offer only a limited sensitivity.

We developed a new method for prion detection with two major advantages. First it avoids completely PK-digestion, and second it counts single prion particles. The detection system is based on Fluorescence-Correlation-Spectroscopy (FCS). The partially purified prion particles are labelled by two different antibodies with different fluorescence labels, so that FCS is applied in the mode of Dual-Colour Fluorescence-Intensity-Distribution-Analysis (2D-FIDA) [2]. To increase the sensitivity particles were concentrated on a chip surface by capture antibodies (Surface-FIDA) [1].

With Surface-FIDA we are able to distinguish Scrapie-infected hamster as well as BSE-infected cattle in the clinical stage from a control group [1]. Therefore the sensitivity to identify Scrapie-infected hamster as well as BSE infected cattle in brain samples could be increased dramatically as compared to FIDA in solution [2]. Preliminary data showed that applying Surface-FIDA one is able to detect PrP-aggregates in the cerebrospinal fluid of cattle. We present further optimization of Surface-FIDA in respect of the methodology to enhance specificity and sensitivity.


P04.107
Bovine Prion Protein Gene Expression is Modulated by DNA Polymorphisms in the Promoter Region
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The observation that prion protein gene knock out mice (Prnp-/-) can not be infected with prions and that mice with a single copy of Prnp (Prnp+/-) have a much longer incubation time than normal mice resulted in the hypothesis that prion infection depends on endogenous expression of the prion protein and that incubation time correlates with the amount of prion protein expressed. This hypothesis was further confirmed in transgenic mice with an additional Prnp copy resulting in a shorter incubation time.

DNA polymorphisms in the bovine PRNP promoter were found in known transcription factor binding sites. To study the influence of 13 polymorphisms within the core promoter on prion protein gene expression, naturally occurring combinations were cloned into luciferase expression vectors and transfected into Neuro2A cells as well as transgenic mouse lines expressing similar PrPC levels. No biochemical differences were found in the 5OR-BSE prions and then re-inoculated in other transgenic mouse lines expressing PrP from different species for its biological characterization.

Transmission efficiency of 5OR-BSE prions was similar to those of 6OR-BSE prions in all mouse models tested.

Discussion: The reduced susceptibility for BSE infection found in 5OR-BoPrP-Tg mice reflects a lower efficiency of prion replication, which is probably due to a reduced ability for transformation of 5OR-PrP into 6OR-PrP. This would explain the genetic basis for the low incidence of BSE in cattle expressing 5OR-BoPrP. 5OR-BSE prions preserve the same ability to cross transmission barriers than 6OR-BSE prions.

P04.108
Creutzfeldt Jakob Disease Associated with the R208H-129V Haplotype in the Protein Gene
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Human Transmissible Spongiform Encephalopathies (TSEs) or prion diseases occur in sporadic, acquired and genetic forms. All the genetic forms of human TSEs are linked to point or insertion mutations of the PRNP and several of these genetic forms are transmissible. Among the rarer point mutations, R208H was reported in 3 patient (JA. Mastrianni et al. 1986; S. Capellari et al. 2005; C. Bassett-Leonob et al. 2006). Here we described the clinical and pathological features of the CJD phenotype associated with the R208H-129V haplotype in an Italian patient. Case report A 64-year-old woman was referred to the Italian National Register of the Creutzfeldt-Jakob disease and related disorders, with a clinical presentation characterized by mood disorder and ataxia. Two months after the onset, she showed cognitive decay, cerebellar symptoms, pyramidal symptoms involving the left leg, and a progressive action myoclonus of left hand. Four months later the patient was bed ridden in a state of akinetic mutism. The family history was negative for dementia or neurological disorders. The protein 14-3-3 test gave a positive result. The EEG became typical 4 months after the onset. The MRI test gave a positive result. The EEG became typical 4 months after the onset. The MRI examination showed severe spongiform changes in the cerebral cortex, striatum, thalamus and cerebellum. Conclusions: this report is the first on the R208H mutation found in association with val/val polymorphism in Italy and it strengthens the linkage of the R208H mutation to CJD.
**Methods:** We propose a prospective, unlinked anonymous survey of British blood distribution of the general population (17-70 yrs), and include the ages where the study design and objectives in more detail.

**Introduction:** Current estimates of the prevalence of sub-clinical vCJD infection in the UK are largely based on a single, relatively small retrospective study of appendix/tonsil tissue. The National Anonymous Tonsil Archive (NATA) will provide additional information on prevalence in tonsil tissue. A large proportion of tonsils are from individuals born after the estimated peak of exposure to BSE (i.e. after 1993). The development of tests for PrP\(^{\Delta27}\) in plasma now offers a potential alternative approach to obtaining prevalence estimates. Blood donors are broadly representative of the age-distribution of the general population (17-70 yrs), and include the ages where the incidence of vCJD has been highest (born 1961-1985).

**Methods:** We propose a prospective, unlinked anonymous survey of British blood donors, using available prototype assays for testing for PrP\(^{\Delta27}\) in plasma. A sample size of ~50,000 donations should give an exact 95% confidence interval of ~100-350 on an observed prevalence of 200/million (i.e. 10 positives). A sample of ~5,000 non-British plasma samples will be tested as a negative control panel. Potential assays are being assessed through a tender and evaluation by NIBSC. The selection of screening assays, design of testing algorithms, and interpretation of test results is being overseen by the Expert Advisory Group on Laboratory Testing Strategy for Large Scale Abnormal Prion Studies.

**Results:** The study would provide estimates of blood-borne PrP\(^{\Delta27}\) prevalence derived from reactivity to available prototype plasma-PrP\(^{\Delta27}\) tests. These findings would have implications with respect to estimating blood-borne vCJD infectivity, and total prevalence of sub-clinical vCJD infection, in the population.

**Conclusion:** Despite the limitations, the proposed study (given adequately performing tests currently under evaluation) should generate information about detectable blood-borne PrP\(^{\Delta27}\) that will be the first of its kind and may have implications for strategies to prevent person-to-person transmission of vCJD. This paper will present the study design and objectives in more detail.

**P04.110**

**A Search for Atypical Scrapies in The NPU Sheep Brain Archive**

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There is currently a major concern that new forms of scrapie have been isolated in sheep throughout the UK and Europe during the last few years. The case in Norway in 1998 (Nor98), and other "atypical scrapie" forms, appear to affect genotypes of sheep previously regarded as resistant to classical scrapie. With the current breeding programs aimed at increasing the incidence of resistant genotypes, are these genuinely new and emerging forms of TSE in sheep or are they of much older history and simply being detected now due to the vastly increased levels of surveillance currently in use. To establish whether these atypical forms are newly emerging, this project will take advantage of the large archive of natural scrapie samples available to the NPU-Roslin. Our archive contains around 2000 brain samples from sheep of various ages not only from NPU's Cheviot and Suffolk flocks but also from a range of sites from across the UK, and importantly dates back to the early 1960s. One case has already been isolated in the archive - an animal that died in 1989 in a flock in Scotland ('Nor98-like sheep scrapie in the United Kingdom in 1989' Bruce, M. E., et al. Veterinary Record 2007), while another case has recently been identified within our own NPU Cheviot flock (unpublished data). This prompted the archive search and brings into effect genotyping (establishing which samples were the most at risk from atypical forms of scrapie); Western blotting (using the P4 antibody which identifies the Nor98-like 12kD PrPres band); and the Biorad TeSeE detection kit (which is able to detect atypical forms of scrapie). The aim of this study is to provide an indication as to the frequency of occurrence of atypical scrapie in the UK at dates earlier than those currently published. The presence of a Nor98-like form isolated in 1989 already lends weight to the hypothesis that atypical scrapie has been present for longer than previously thought. Results to date will be reported upon.

**P04.111**

**Speculations on PrP Protein Sequence Variation and Transmission Barriers**

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PrP misfolding is a key molecular mechanism in prion diseases. Amino acid polymorphisms become an important factor in this process because the primary sequence may provide a constraint on the number of conformations that PrP will be able to adopt. This conformational barrier through allelic variation may also lead to a barrier in transmission of prion disease within and between species.

Extensive studies into the genetics of the ovine PrP gene have established that the three polymorphic amino acid codons at positions 138, 154 and 171 are central to determining susceptibility to TSE disease. The revelation that atypical scrapie susceptibility is associated with a codon 141 polymorphism now highlights the shortfall of a PrP genetics system based on only three polymorphic codons and 15 PrP genotypes as used in the UK National Scrapie Plan (NSP).

To answer the question what the true extent of polymorphisms in the ovine PrP gene is we have continued to apply DNA sequencing analysis in the open reading frame of all major PrP alleles in sheep and other species. Most additional amino acid polymorphisms have so far been found on the ARQ allele, considered to be the ancestral allele. In addition we report here variants of the ARQ, ARR and VRQ alleles. We will provide a comparison of the PrP variation of new and previously published sequences from over 100 species to argue that detailed sequence analyses may help to predict key amino acid positions with relevance for species barriers and normal protein function.

**P04.112**

**Decontamination of MBM Infected with Pathogenic Prion Proteins Using a Biodiesel Process**

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The recent epidemic of bovine spongiform encephalopathy (BSE) in cattle is linked to a subsequent surge in incidence of variant Creutzfeldt-Jakob Disease, a transmissible spongiform encephalopathy (TSE) affecting humans. This has led to worldwide drop in the market for beef by-products, such as meat-and-bone meal (MBM) a fat-containing product traditionally used as an animal feed. Our study evaluated a method for producing biodiesel from MBM for decontamination of TSE. We predict that, in addition to producing valuable fuel, the method also destroys any TSE infectivity present, while simultaneously producing a potentially value-added solid residue. As a model for a rendered TSE-infected animal carcass we spiked MBM with scrapie infected hamster brain. Laboratory scale conditions produced fatty acid methyl esters (for use as a biodiesel) using alkaline methanolysis, through incubation of MBM with 0.25 M sodium methoxide at 35°C for 2 hours with vigorous shaking. We evaluated all 3 resulting phases (biodiesel, glycerol, and solids) for residual scrapie TSE in vitro and in vivo. Western blot tests indicate the protein associated with TSE infectivity, PrPSc, is completely destroyed under these alkaline methanolysis conditions. Definitive in vivo tests, involving intra cranial inoculation of hamsters, are pending inoculation.
Environmental Exposure Assessment of Scrapie Infectivity Following the Application of mMBM to Agricultural Land

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Current legislation in the European Union (European Commission regulation No. 1774/2002) allows member-states to use Category 3 waste (including mammalian Meat and Bone Meal (mMBM) derived from slaughtered animals fit for human consumption) as a fertiliser to be spread on non-pasture agricultural land. The objective of this study is to develop an environmental exposure assessment model for scrapie infectivity through environmental pathways following the application of mMBM-derived fertiliser to non-pasture agricultural land. The model uses Monte Carlo simulation techniques to account for parameter uncertainty and variability and consists of four specific modules: Farm, Slaughtering, Rendering and mMBM Landspreading module. All modules are linked in order to calculate the probability of scrapie-infected mMBM being spread on non-pasture agricultural land and to give an estimate of the resulting exposure to scrapie infectivity. The model indicated an average scrapie infectivity of 3.46 x 10^-8 ID50/tonne of soil on non-pasture agricultural land following the spreading of mMBM as a fertiliser. The mean ovine exposure level was estimated to be 3.39 x 10^-9 ID50/annum. The model indicated a slightly higher exposure level during winter months due to the greater probability of soil (and hence infectivity) attachment to forage and subsequent animal consumption. A sensitivity analysis indicated that the initial prevalence of scrapie infected sheep and the rendering reduction were the most important input parameters, with correlation coefficients 0.70 and 0.45, respectively. The latter indicates the important role the rendering industry plays in minimising the transfer of scrapie infectivity. Due to the estimated low exposure levels of scrapie-infectivity on agricultural land, utilisation of mMBM as a fertiliser in Ireland is a feasible option without comprising animal health.

Transmission of Ruminant TSEs to Transgenic Mice Expressing Bovine, Kudu and Sheep PrPs

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Background: Transgenic PrP model mice offer the prospect of bioassays of greater susceptibility and sensitivity for TSE agents compared to conventional mouse lines. Overexpressing transgenic models are potentially more sensitive than the natural host in detecting prion infectivity, and also represent more economically viable approaches than bioassay in cattle and sheep.

Aim: The aim of this Defra-funded study was to produce bioassay models of improved efficiency for the evaluation of BSE and scrapie by generating transgenic PrP mice overexpressing bovine, kudu and sheep PrPs, and to assess the susceptibility of characterised lines by challenge with TSEs.

Methods: Transgenes containing full-length bovine PrP and chimeric forms of kudu and sheep PrPs were prepared and used to generate and breed mice on a FVB/PrP strain. Transgene expression levels in brain and other tissues and copy number were determined. Mice challenged with TSE agents were monitored for the development of clinical signs and prion disease was confirmed by Western blot detection of PrPSc and neuropathology.

Results: Transgenic lines expressing a range of PrPs in brain and other tissues were produced and bred to homozygosity on a PrP null background. Homozygous and heterozygous mice were challenged with a range of TSE agents (BSE, classical and atypical scrapie). Homozygous bovinised mice showed susceptibility to BSE and scrapie, with reduced incubation periods in comparison to wild-type controls and heterozygous mice.

Discussion: Transmission studies of TSE agents in the transgenic lines produced are in progress. Preliminary findings indicated susceptibility and reduced incubation periods in several transgenic lines, but further results are awaited. Western blotting and neuropathological analyses of challenged mice will be conducted and should reveal the characteristics associated with different TSE agents and particular transgenic PrPs.

Genetic Anticipation in v210I CJD Italian Patients

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Genetic Creutzfeldt-Jakob disease(CJD) is a rapidly progressive neurodegenerative disorder that typically affects individuals between the ages of 50-70 years. Five to fifteen percent of CJD cases show a familial pattern corresponding to autosomal dominant inheritance. Over 30 different point and insert mutations responsible for genetic CJD have been identified in the human PRNP gene. The V210I point mutation is the most common mutation in Italy accounting for more than 41% of the genetic TSE cases (Ladogana et al 2005). Genetic anticipation has been shown in several neurodegenerative diseases where the gene linked to the disease is affected by trinucleotide repeat instability, with expansion of repeats clearly correlated with an earlier age at onset. Preliminary evidence has shown anticipation in genetic CJD linked to the E200K mutation among Libyan Jews (Rosenmann H. et al 1999). The same analysis, performed in E200K Italian patients of the Calabrian cluster, has shown a significant difference between the age at onset in two generations (Ladogana et al Abstract GEG-16 in “Prion 2006 Strategies, advances and trends towards protection of society” 3-6 October 2006 Torino Italy). We investigated whether genetic anticipation may occur in V210I Italian patients from the Southern Italy. We recorded the age at onset of 46 parent-offspring pairs from 34 pedigrees. When the parent at risk was alive and neurologically healthy we recorded the time at observation as the age at CJD onset. The paired t-test was performed to test the statistical significance of paired differences.

The age at onset for the carrier generation was 69.3±11.74, while the age at onset of CJD offsprings was 57.5±9.7. The differences between the age at onset in the two generations was statistically significant (p< 0.0001). This result suggests that anticipation is also present in the V210I Italian cases. The basis for the anticipation in genetic CJD is unknown. A number of genetic and environmental factors might play a role in determining the anticipation phenomenon.
Creutzfeldt-Jakob Disease in Recipients of Corneal Transplants

Begue, C; Abusis, G; Gentier, M; Rivadulla, M; Bueri, J; Mattiazzi, M; Kaufmann, M; Amante, M; Botti, D; Cosacou, P; Ghiardi, G; Ferrari, J; Gatto, E; Canto, L; Fernandez Santivanez, N; Ganin, D; D’Angelo, R; Mamprelian, J; Marquez Vigo, C; Morán, D; Salvadori, B; Valenzi, S; Toledo, S; Villagra, J; Andrade, J; Villamos, G; Meichtry, O; Somoza, M; Martetto, H; Pocchiari, M; Equestre, M; Piccardo, P; Taratuto, A

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The neuropeplastic and molecular aspects of 19 patients with E200K representing 12% of CJD in Argentina (19/159). Thirteen of these patients had familial history consistent with autosomal dominant disease (AD) of argyrophilic fibrous astrocytoma (ArgFib). All patients showed PrP129-MM and PrP129-MV; in all these patients type I PrPres was observed by immunohistochemistry. They have been described since 1974, when the first case was reported. Because of the large number of corneal transplant procedures, some sporadic CJD patients are likely to have a history of corneal transplantation. Determining a causal link between transplant and CJD illness may be difficult due to lack of neuropathologic testing to confirm the disease and the absence of available donor data. One long incubation period.

Methods: CJD cases with a history of corneal transplantation were reported to the Centers for Disease Control and Prevention, and medical records for these cases were reviewed to assess each patient’s illness and the presence of any additional CJD risk factors. If possible, records from the eye bank that processed the corneas were examined to gather information on the cornea donors and on the recipients of the donors’ corneas. The National Prion Disease Surveillance Center performed genetic and neuropathologic testing on available specimens. Statistical analyses were performed to determine the occurrence of coincidental CJD among corneal transplant recipients.

Results: Four CJD decedents with histories of corneal transplantation were identified, three of whom were from the United States and one from Japan. The time from transplant to onset of CJD symptoms ranged from about 3 years to 18 years. Available eye bank records did not suggest evidence of neurologic illness in the donors. Based on corneal transplantation and CJD death data from 1990-2006, statistical analyses suggest that, on average, a case of coincidental sporadic CJD in a corneal transplant recipient is expected to occur approximately every 1.5 years.

Conclusions: It is likely that these four recipients of transplanted corneas had sporadic CJD. Because of the high number of corneal transplantations performed each year in the United States, occasional sporadic CJD in this population is expected.
The potential application of Neopredisan 135-1 for the decontamination of TSE-contaminated medical devices has been analysed using the 263K Hamster model recently (1). In order to examine the potency of Neopredisan 135-1 for the inactivation of the BSE agent, we performed similar experiments to those described in the 263K-model with brain material from BSE infected cattle. Briefly, a dilution series of a 10% brain homogenate was incubated with Neopredisan 135-1 at different concentrations. After pH neutralisation the samples were either digested with protease K and analysed by immunoblot or inoculated into Tgbov XV mice overexpressing bovine PrPC. Both experiments indicate only a limited effectiveness of Neopredisan 135-1 for the inactivation of the BSE agent, as only minor differences in the results from samples with and without 1% or 2% Neopredisan 135-1 were observed. These results do not support a practical application of Neopredisan 135-1 for decontamination of animal facilities or slaughterhouses.

Reference: (1) C. Riemer, T. Bamme, S. W. F. Mok, and M. Bauer, 2006:3-Methyl-4-Chlorophenol for Prion Decontamination of Medical Devices. Infect Control Hosp Epidemiol. 27(7):778-80.

The steel wire test has been applied successfully for the determination of prion residual infectivity, and is recommended to evaluate the efficacy of different cleaning procedures. However its use is subtle and results are often affected by consistent variability; moreover [Lioscomb et al., 2006, J Hosp Infect. 64(4):339-43] demonstrated that the contamination is more easily removed from wires than from flat metal surfaces. Therefore we decided to set up a novel model of cell culture infection to reproduce more realistic conditions representative of surgical instruments in term of dimension, shape and size.

The rationale of the “scrapie surface assay” (SSA) is to contaminate plastic or metal facilities or slaughterhouses.

The so-called TSE roadmap, published by the European Commission 15th July 2005, suggests relaxations of BSE measures in the short, medium and long-term. According to the TSE roadmap “any relaxation of BSE measures following the scientific assessment should be initiated by an open discussion with all stakeholders and supported by a strong communication strategy”.

This social scientific project addresses the issue by directly involving stakeholders of five European Member States (Belgium, Great Britain, France, Germany and Portugal) as well as the European governmental level (DG SANCO and EFSA) to investigate their perceptions of the TSE roadmap and its implications for the precautionary consumer protection. Knowledge about the risk perception of the public was gathered by interviewing stakeholders representing the public (such as: farmers, consumers) and juxtaposed by the risk perceptions of food industry and involved governments.

The poster will present first preliminary results of the project. The results of the project contribute not only to the improvement and consolidation of the knowledge base, but support also a better understanding of adequate risk communication. Recommendations on good practices of risk communication, taking into account public risk perceptions, will be formulated for policy makers in the EC and within the Member States.

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Such a highly sensitive detection system was established - the priontype® TSE - combining a peroxidase-based colorimetric ELISA with a detection technique called Immuno-PCR. Using Immuno-PCR, sensitivity of the corresponding classical ELISA was increased 10.000-fold resulting in a detection limit of 10 pg/mL recombinant prion protein even when spiked in body fluids as serum, plasma or cerebrospinal fluid. The sensitivity of priontype® TSE can be further improved by an additionally enrichment of PrP using immunoprecipitation (IP). It was shown that IP can improve the detection limit up to 100-fold. Sensitivity and specificity of both formats were proved by analysing spiked plasma samples (vCJD brain and normal brain) as well as plasma samples from patients with various neurodegenerative diseases and from healthy donors. The results clearly show that the established detection system is an excellent tool for a general screening of blood donations.
Environmental Persistence of TSE Infectivity: Field Studies
Ferrie, K.; Smith, A.; Somerville, R
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Background: There is concern about the consequences of contamination of the environment with TSE infectivity. Infectivity may enter the environment by various routes, persist in the ground and spread from the original source to contaminate an extended area and groundwater.

Aims: We are studying this problem by addressing the following questions: 1. Does infectivity with some containment (e.g. in a carcass) survive in the carcass over time; 2. Does infectivity without containment survive, and is it disseminated into the surrounding soil and water? 3. Do the environmental conditions, e.g. soil type and pH, affect the survival and/or transport of infectivity through soil?

Methods: To address these questions, we are performing two field experiments (with appropriate containment) each using two soil types. Air temperature, rainfall, soil temperature and moisture content are being monitored. In one experiment a series of 10 bovine heads have been spiked with the BSE derived TSE strain 301V and buried in the two soils, contained within individual lysimeters, for exhumation and analysis at yearly intervals. Rainwater flowing through and collected as groundwater is also being analysed. In the second experiment a bolus of infected brain is buried at the centre of two 3 meter diameter lysimeters and soil samples taken from them at regular intervals. Water flow-through is also analysed.

Results: To date, the first two bovine heads have been exhumed and the surrounding soil sampled. Both of the exhumed heads were apparently largely decomposed but on examination of the brain cavity were found to contain significant amounts of brain tissue. These have been sampled and are presently being analysed. The soil samples taken from around the heads and five sets of core samples taken from the soil surrounding the buried brain in the two large lysimeters are presently being analysed for PrPSc, the abnormal protein associated with the TSEs and for infectivity. Water samples have also been collected for analyses.

Discussion: We will use the acquired data to build a predictive model of TSE behaviour in the environment which will inform future risk assessments.
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