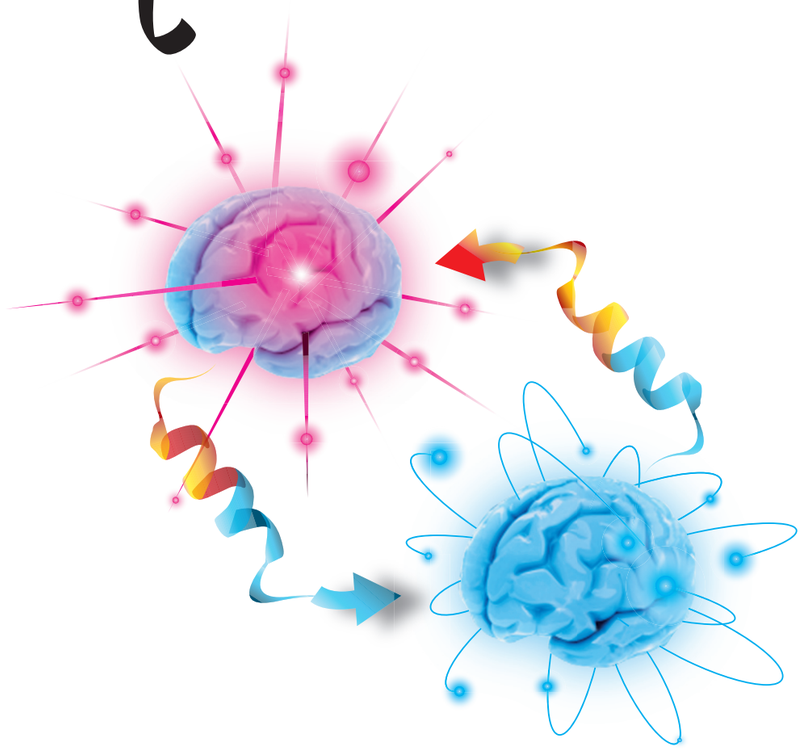
Prion 2009 Book of Abstracts

Greece
Thessaloniki - Chalkidiki
Prion2009



Book of Abstracts

23th - 24th - 25th
September 2009
Porto Carras, Chalkidiki Greece

CERTH
The Centre of Research and Technology Hellas
ARISTOTLE UNIVERSITY OF THESSALONIKI
NeuroPrion

Book of
Abstracts

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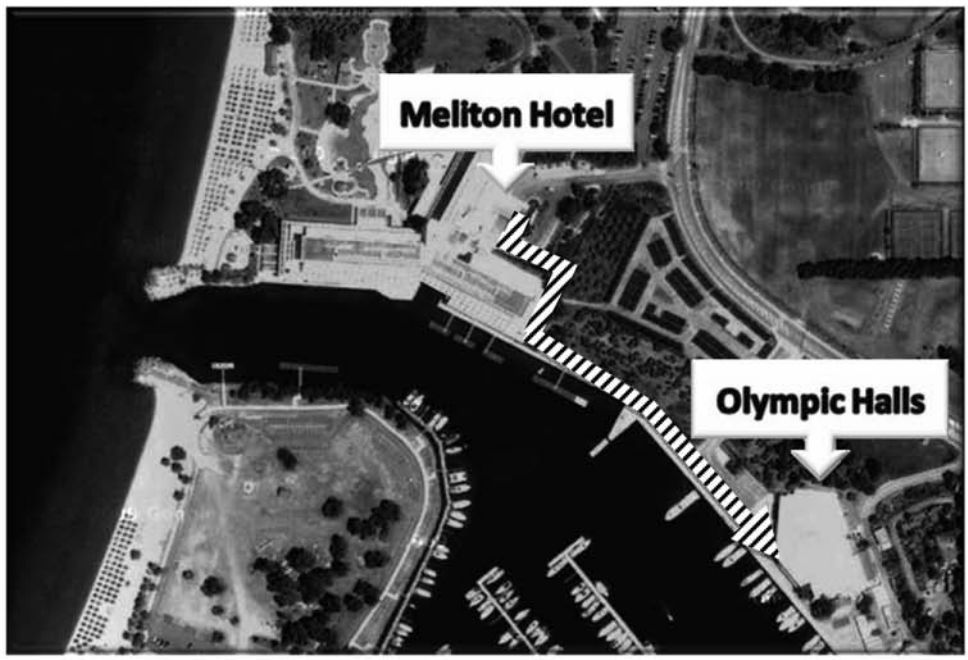
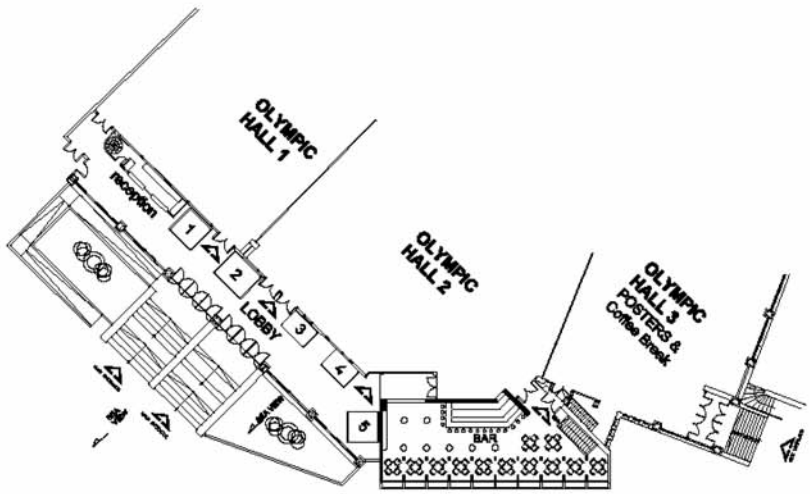
Directions



Olympic Hall Floor Plan



1: Speakers help desk
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Plenary Talks

Heterogeneity of prion populations

Charles Weissmann, Jiali Li,
Shawn Browning, Sukhvir P. Mahal, Anja Oelschlegel

Department of Infectology, Scripps Florida, USA

Prions strains are classically distinguished by their clinical and histopathological properties. It is believed that strain specificity is encoded by distinct conformations of PrP^{Sc}. Some prion strains are very stable when transmitted across species, while others "mutate" to novel forms.

The Cell Panel Assay (CPA) allows discrimination between prion strains by virtue of their capacity to chronically infect some cell lines but not others; it readily distinguishes between 22L, RML, Me7 and 301C strains on a panel of 4 cell lines (Mahal et al., PNAS 104:20908 (2007)).

We found that swainsonine (swa), an inhibitor of complex glycosylation, prevents infection of murine neuroblastoma-derived PK1 cells by certain prion strains but not by others. Prions from 22L-infected brain can infect R33 cells as well as PK1 cells in the presence of swa, however prions from persistently 22L-infected PK1 (PK1[22L]) cells can do neither. Unexpectedly, when PK1[22L] cells were grown in the presence of swa, the resulting prions became fully resistant to the drug. Subsequent prolonged propagation in its absence resulted in a prion population that was again swa-sensitive. Inoculation of mice with swa-resistant or -sensitive PK1[22L] prions resulted in prions indistinguishable from the original brain-derived 22L prions. We determined that even prior to exposure to swa about 0.5% of the prions in PK1[22L] cells were resistant to the drug. This suggests that the 22L prion population consists of more than one "sub-strain", and that, depending on the environment, a different sub-strain is selected as the major constituent. Thus, a "brain-adapted" prion population would have a majority of sub-strain(s) replicating more rapidly in brain than in a cell line, while the opposite would be the case for a "cell-adapted" population; similarly, a swa-resistant sub-strain is selected in the presence of the drug, while in its absence, swa-susceptible sub-strain(s) outgrow their resistant counterparts.

What agent strains tell us

Laura Manuelidis

Yale University Medical School, USA

The essential infectious molecules ultimately causing neurodegeneration in CJD and other TSEs remain unknown. One of the central challenges for any TSE agent hypothesis is to account for the diversity of agent strains. For public health, it is critical to understand the basis for cross-species transmissions to predict and prevent their further spread. As with viruses, TSE agents can adapt to new species and become more virulent, yet maintain fundamentally unique and stable identities, as we will show. To make agent differences manifest, one must keep the host genotype constant. Many TSE agents have revealed their independent identities in normal mice. We transmitted vCJD and primate kuru, a TSE once epidemic in New Guinea, to mice expressing normal and ~8-fold higher levels of murine prion protein (PrP). In both cases, levels of murine PrP did not prevent infection, but instead shortened the incubation time, as would be expected for a viral receptor. Both agents also produced more robust transmissions in wt PrP mice than in humanized PrP mice. Prion advocates had predicted that primate agents would have poor transmission to species with non-homologous PrPs. Clearly host factors other than PrP homology determines transmission susceptibility to specific agents. Sporadic CJD were clearly different from vCJD and kuru in incubation time, clinical signs, brain neuropathology, and lymphoreticular involvement. We also transmitted these and a variety of other TSE agents to simplified monotypic GT1 cell cultures. Abnormal PrP-res band patterns for each agent changed with GT1 cell transmission; thus the PrP-res pattern did not encode the stable strain properties. Moreover, in contrast to sporadic CJD isolates, kuru rapidly and stably infected GT1 cells and hence is not derived from the common sCJD agent. The geographic independence of the kuru agent provides additional reasons to explore causal environmental pathogens (with a nucleic acid) in these infectious diseases.

Therapeutic Approaches for Alzheimer's and Prion Diseases

Thomas Wisniewski

New York University School of Medicine, USA

Alzheimer's and prion diseases belong to a category of conformational neurodegenerative disorders. Treatments capable of arresting or at least effectively modifying the course of disease do not yet exist for either one of these diseases. Alzheimer's disease (AD) is the major cause of dementia in the elderly and has become an ever greater problem with the aging of Western societies. Unlike AD, prion diseases are relatively rare. Each year only about 300 people in the USA and about 100 people in the UK succumb to various forms of prion diseases. Nevertheless these disorders have received great scientific and public interest due to the fact that they can be transmissible among humans and in certain conditions from animals to humans. The emergence of variant Creutzfeld-Jakob disease (vCJD) demonstrated the transmissibility of the bovine spongiform encephalopathy (BSE) to humans. Therefore, the spread of BSE across Europe and the recently identified cases in North America have put a large human population at risk of prion infection. An exciting therapeutic strategy for both AD and prion disease is immunomodulation. My laboratory, as well as other groups, has developed both passive and active immunization approaches for both these disorders. We report the use of synthetic, immunogenic but non-amyloidogenic peptides homologous to amyloid β for induction of a primarily humoral immune response in AD transgenic mouse models. This approach is able to dramatically reduced the amyloid burden, correlating with behavioral benefits, in the absence of any apparent toxicity. For prion disease, we have pioneered the use of mucosal immunization to prevent the entry of the infectious agent via the gut. The GI tract is the natural route of infection in many forms of prion disease. We have shown that in animals with a high IgA and IgG anti-PrP response to our vaccine, complete protection from prion infection via an oral route is possible. In addition we have tested numerous anti-PrP antibodies for therapeutic activity in tissue culture models of prion infection and shown that some of these are effective in vivo to extend the incubation period of prion infection. These approaches, which target abnormal protein conformation that is central to the pathogenesis of AD and prion disease, show great promise as effective, non-toxic therapies.

In memory of Dr Daniel Carleton Gajdusek (1923 – 2008)

Lev G. Goldfarb

Bethesda, Maryland, USA

Dr. Gajdusek was a pioneer and for 40 years a leading authority in transmissible spongiform encephalopathies (TSEs). He was born in Yonkers, NY into a family of recent immigrants from Slovakia and Hungary, went to the University of Rochester, then Harvard Medical School and did his residency in Pediatrics at the Children's Hospital in Boston. He subsequently spent several postdoc years at Caltech working with Linus Pauling and Max Delbróck, and at Harvard with John Enders. While completing his military service at the Walter Reed Army Medical School under Joe Smadel in the early 1950s, Dr. Gajdusek was sent to post-war Germany and later to Iran, Afghanistan, Turkey, Bolivia, Amazonia and Peru to collect data on epidemics of rabies, plague, hemorrhagic fevers and arbovirus infections. On these trips, he became increasingly fascinated with discovering rare diseases in little known remote isolated populations. While studying immunology and virology at the Hall Institute in Melbourne with Macfarlane Burnet, Carleton traveled to investigate kuru, a deadly disease that by the mid-1950s reached epidemic proportions in Fore people of New Guinea. Under his direction kuru was experimentally transmitted and became the prototype of a group of human TSEs. His NIH laboratory also uncovered molecular causes and underlying pathogenic mechanisms of the TSE disorders and established the fact that dura mater transplants and the use of contaminated human growth hormone preparations transmit CJD to human recipients. Several unrelated infectious and neurological disorders were also successfully investigated, including hemorrhagic fever with renal syndrome, tropical spastic paraparesis, ALS/PD on Guam, other motor neuron disorders in Western Pacific, spinocerebellar ataxia and Viliuisk encephalomyelitis in Siberia. The amyloid precursor protein (APP) gene playing a critical role in all forms of Alzheimer's disease was discovered. Dr. Gajdusek received his highest recognition in 1976, when he was awarded the Nobel Prize in physiology or medicine for discoveries of "new mechanisms for the origin and dissemination of infectious diseases". The prize itself he used to set up a trust for the education of the Fore people. He was a member of more than 40 academies around the world and received numerous awards. Carleton had wide interests in classic literature, music, fine arts, history, ethnography and human development. His early paper "The Making of Musics for Man" was a study of human learning. He was a very warm and kind man, sensitive and understanding. Highly demanding of himself, he found ways to forgive failures of others, and never lacked for ideas to share. His staff and students were given complete freedom and encouraged to progress in their chosen projects and prove their talents. Over the years, he brought 48 orphaned teenagers from New Guinea and Micronesia to his home in the U.S. and spent a fortune on their education. Some subsequently returned to their homeland to offer services at the highest levels of Government and society, others have become productive U.S. citizens. The world inherited Dr. Gajdusek's published books, more than 1000 research papers, and printed multi-volume personal journals of a passionate and productive scientist, a thinker and generous human being.

Oral Presentations

O.1.1

Oxidation of Met residues on PrP: An early event in prion formation and disease manifestation

Tamar Canello[1], Silvia Lisa[2], Jackob Moskovitz[3], Maria Gasset[2], Ruth Gabizon[1]

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Background: We have recently shown that PrPSc from all species exhibits a covalent signature in the form of sulfoxidation of M213. This modification could not be found on brain PrPC as well as on its recombinant models. Moreover, computational methods indicated that oxidation of Met residues in helix 3 of PrP, as is the case for Met213, destabilize the structure of helix 2, which present as beta sheet in PrPSc.

Objectives: To investigate the role of PrP methionine oxidation in prion formation and in prion disease manifestation.

Methods: We used several antibodies which specifically recognize either oxidized or non oxidized Met residues on PrP, and tested their activity on wt and mutant recombinant PrP polypeptides as well as on cell culture systems expressing PrPC, PrPSc and mutant PrPs. The oxidation status of mutants and scrapie isoforms of PrP were also tested in cells subjected to oxidative conditions.

Results: We show here that oxidation of Met 213 is not only present in "old PrPSc" accumulated in the brains of prion infected subjects, but also in newly formed PrPSc present in cell lines infected with prions, suggesting that oxidation of Met residues in PrP may be an early event in prion formation. In addition, we also show that substitution of glutamate for lysine at position 200, as is the case in the most common form of genetic prion disease, results in increased sensitivity of helix 3 M residues for oxidation. This could be seen by the spontaneous oxidation of recombinant E200K PrP, and most interestingly, by the fact that in CHO cells cultured in the presence of copper ions, mutant PrP, as opposed to wt PrP, presents in an oxidized form.

Discussion: Oxidation of Met residues on helix 3 of PrP may be the key event in the conversion of PrPC to PrPSc. Most interestingly, mutations causing radical charge changes around helix 3, as is the case for the E for K substitution at codon 200, may predispose the prion protein to such oxidations, thereby inducing the appearance of genetic prion diseases.

Invited by the scientific committee

O.1.2

Studies on the interaction of membrane-bound PrPC with PrPSc

Agnieszka Salwierz[1], Eva Birkmann[1,2], Detlev Riesner[1]

Heinrich-Heine Universitaet Duesseldorf, Germany; [2]Forschungszentrum Juelich

Background: The conversion of the cellular prion protein (PrPC) into the disease-associated PrPSc is the primary event in the course of prion diseases. There are different clues demonstrating that the conversion process might take place at the membrane surface. They all are strengthened by the fact that in vivo PrPC is attached to special plasma membrane domains, called rafts. This attachment is mediated by the glycosyl phosphatidyl inositol (GPI-) anchor, which is linked posttranslationally to the C-terminus of prion protein. It was also shown, that PrPC molecules form intermolecular beta-sheets structures when inserted in to the membrane (1).

Objectives: We studied the interaction of the membrane-anchored PrPC with PrPSc in order to identify the site of the final interaction of PrPC and PrPSc.

Methods: Posttranslationally modified PrPC was purified from transgenic CHO-cells (2, 3) and the interaction processes between membrane-bound PrPC and invading PrPSc particles were studied by surface plasmon resonance (SPR) technique applied in the Biacore instrument. Different types of aggregates were used including non-infectious amorphous aggregates, infectious particles selectively purified by NaPTA precipitation method (4) and prion rods.

Results: At first a saturation of the rafts-like lipid bilayer with CHO-PrPC was established. Onto this PrPC-covered membrane a third component in form of different types of aggregates was exogenously added and the resulting interaction was observed. We could demonstrate a specific binding of prion particles to the PrPC-covered membrane and conclude that the first step of PrP conversion occurs when PrPSc meets PrPC on the membrane.

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(2) Blochberger, T.C. et al. (1997) Prot. Eng. 10, 1465-1473

(3) Elfrink, K. Riesner, D. (2004) ed. by S. Lehmann and J. Grassi (Birkhaeuser Verlag Basel), 4-15

(4) Safar, J. et al. (1998) Nat. Med. 10, 1157-1165

Selected by the scientific committee from the submitted abstracts

O.1.3

Structural intolerance of PrP α-fold for Helix-3 methionine oxidation: the link of theory and experiment

María Gasset

IQFR-CSIC, Spain

Background: Many lines of evidence support PrPSc as the only essential component of the prion. PrPSc differs structurally from its physiological form, PrPC, in the C-terminal domain. This difference consists in a drastic conformational change and the non quantitative irreversible sulfoxidation of conserved methionine side chains. In the absence of a pre-existing template that would assist the change, the involvement of a covalent modification, transient or irreversible and affecting either all or a minor subset of molecules, as a covalent trigger was postulated and never discarded.

Objectives: To test whether methionine sulfoxidation could play any regulatory role in the multi-step structural transition, we have analyzed the impact of this covalent modification on the conformation, stability and aggregation propensity of PrP α-fold.

Methods: Computational studies (molecular dynamics simulation, essential dynamics, correlated motions and signal propagation analysis) has been used to address the structural impact of sulfoxidation of the conserved Helix-3 methionines on the HuPrP(121-229) α-fold, both with sulfur-to-sulfoxide replacement and with oxidation-mimicking mutations. Conformational, stability and aggregation propensity of PrP chains containing the oxidation-like mutations was studied using a combination of biophysical and biochemical tools.

Results: Computational studies show that the substitution of any of the conserved Helix-3 methionines (M206 and M213, singly or in combination) by their sulfoxides or by serines impacts on the α-fold stability allowing a structural flexibility that can facilitate the pathway of pathogenic conversion. Mutation of helix-3 methionines in HaPrP(23-232) causes an α-fold structural intolerance that is accompanied by an enhancement in the aggregation tendency. The formed aggregates display amyloid staining properties, shapes compatible with annular protofibrils arrangements and proteinase K resistance properties reminiscent of PrPSc.

Discussion: The sulfoxidation of Helix-3 methionines emerges as the molecular switch for the α-fold destabilization required for the productive pathogenic conversion.

Selected by the scientific committee from the submitted abstracts

O.1.4

Partial unfolding of the prion protein: early steps on the Path to Misfolding

Will Guest[1], Li Li[1], Olivier Julien[2], Subhrangsu Chatterjee[2], Brian Sykes[2], Wenquan Zou[3], Steven Plotkin[1], Neil Cashman[1]

[1]University of British Columbia, Canada, [2]University of Alberta, Canada, [3]Case Western Reserve University, USA

Background: It is hypothesized that certain regions of PrPC must transiently lose structure to enable it to undergo misfolding to PrPSc. Molecules that selectively bind these regions when unfolded may interrupt the conversion process and are therapeutic candidates for prion disease.

Objectives: We determined regions of PrPC with a small energy barrier to unfolding that are likely to lose structure early in prion conversion. Antibodies capable of recognising these regions in the unfolded state were prepared as inhibitors of conversion.

Methods: The free energy of unfolding for all subsequences of the prion protein in the folded domain were calculated from all-atom molecular dynamics simulations, continuum electrostatic models of solvation with a locally varying dielectric constant, and analytic descriptions of configurational entropy effects. These calculations were performed for all species and mutants of prion protein with available structure data and were used to predict regions of instability in the protein, as well as secondary unfolding events enabled by loss of structure in unstable domains. Urea denaturation NMR studies of bovine prion protein experimentally identified unstable regions as well. Protease sensitivity experiments determined if these regions were exposed in the misfolded conformation.

Results: The unfolding free energy calculations identified five parts of PrPC with reduced stability: the first B strand, part of A helix 1, the loops between B strand 2 and A helix 2, the loop between A helix 2 and A helix 3, and the C-terminus. Urea denaturation NMR spectroscopy confirmed the instability of the first B strand. Protease sensitivity data indicate that parts of three of these regions are exposed in PrPSc. Studies of unfolding-specific antibodies to inhibit misfolding and selectively label PrPSc are ongoing.

Discussion: A detailed understanding of the unfolding of PrPC elucidates early events in the prion conversion pathway. This work suggests a new immunotherapeutic strategy for prion disease.

Selected by the scientific committee from the submitted abstracts

O.1.5

SAXS analysis of PrPSc and oligomeric recombinant mouse PrP(89-230)

Federico Benetti[1], Heinz Amenitsch[2,3], Matthijn Vos[4], Adriana Ramos[5], Peter Peters[6], Giuseppe Legname[1,3], Jesús Requena[5]

[1]Scuola Internazionale Superiore di Studi Avanzati, Italy; [2]Austrian Academy of Sciences, Austria; [3]ELETTRA Laboratory, Sincrotrone Trieste S.C.p.A, Italy; [4]The FEI Company, Netherlands; [5]University of Santiago de Compostela, Spain; [6]Netherlands Cancer Institute, Netherlands

Background: Despite continued incremental advances, the structure of PrPSc continues to be largely unknown, which prevents an understanding of prion transmission, strains, and transmission barriers at the molecular level.

Objectives: We used small angle X-ray scattering (SAXS) to probe the structure of PrPSc and recombinant PrP oligomers generated in the presence of transition metal ions.

Methods: PrP27-30 fibers were isolated from scrapie-infected Syrian hamsters; recombinant mouse PrP (recMoPrP) oligomers were formed by incubation of recMoPrP(89-230) at a concentration of 2 mg/mL and in the presence of copper, zinc and manganese. The various protein solutions were analyzed using a capillary sample holder kept at 25°C; PrP27-30 fibers were also imaged by cryo-electron micrsocopy after plunge-freezing, on a Titan electron microscope and by negative-stain transmission electron microscopy.

Results and Discussion: PrP27-30 appears, under the cryo-EM quasi-native conditions, as twisted 10-14 nm wide fibers composed of two ~5 nm wide individual fibrils. SAXS scattering patterns of good quality were obtained. Data were best fitted to a three-term model consisting of interacting polydisperse cylinders, with a radius of 5 nm and a cylinder to cylinder distance of 10.6 ± 2 nm. The distance distribution function calculated from the scattering patterns for oligomeric recMoPrP showed a maximum at 10 nm, which was interpreted as the cross sectional diameter of a longer cylindrical structure. SAXS analysis reveals that PrP27-30 in aqueous suspension consists of cylindrical structures with a radius of 5 nm, in excellent agreement with cryo-EM images. These fibers are composed of two twisted or intertwined parallel protofibrils, each ~5 nm wide. Small aggregates of recMoPrP generated in the presence of transition metal ions, representing the first intermediates in the process of recPrP fibril formation, also display a tendency to pair in 10 nm-wide structures, conceivably a way to bury hydrophobic regions away from the aqueous solvent. Of note, the diameter of individual recPrP molecules is 5 nm, which strongly suggests that each of the two individual protofibrils in PrP27-30 is a single stack of PrPSc subunits.

Selected by the scientific committee from the submitted abstracts

O.1.6

The PrP-like Shadoo protein converts to amyloid at neutral pH

Nathalie Daude[1], Vivian Ng[2], Joel Watts[3], Sacha Genovesi[1], Serene Wohlgemuth[1], Jean-Paul Glaves[1], Howard Young[1], Gerold Schmitt-Ulms[2], Joanne Mclaurin[2], Paul E. Fraser[2], David Westaway[1]

[1]University of Alberta, Canada; [2]University of Toronto, Canada; [3]University of San Francisco, USA

Background: In prion infections cellular prion protein (PrPC) is refolded into an infectivity-associated form (PrPSc). The related Shadoo protein (Sho) has sequence homology to PrPC and is also expressed in the adult central nervous system as a GPI-anchored glycoprotein. Wild type mouse Sho consists of an Arg-rich basic region encompassing up to six tetra-repeats, a hydrophobic central domain of tandem Ala/Gly AAAG amino acid repeats with similarity to the containing AGAAAGA palindrome of PrPC, and a short C-terminal domain containing one N-linked carbohydrate.

Objectives: Guided by the propensity of alanine-rich proteins and mutant PrPs with shortened C-terminal domains to form amyloid, we investigated the conformational properties of Sho.

Methods: Amyloid-like characteristics were revealed by Thioflavin binding, formation of fibrillar structures by electron microscopy and accumulation of SDS-resistant complexes in semi-denaturing gel electrophoresis. Fibrils formed in vitro exhibited Congo Red birefringence.

Results: Full-length recombinant wt murine Sho(25-122) converted from random-coil to a β-sheet enriched conformation in a variety neutral pH buffers, and without recourse to prior chemical denaturation. A similar propensity was apparent for four allelic forms of ovine Sho In mammalian cells the GPI-linked Sho protein is associated with cholesterol-rich rafts, with a fraction of the full-length molecules and an N-terminal fragment released from the cell surface. Wild type Sho protein is more sensitive to proteinase K than PrPC and exhibits a shorter half-life in N2a neuroblastoma cells.

Discussion: Although wt Sho is predicted to form a helical hydrophobic domain, recombinant Sho is unstructured and converts to an amyloid like form. We infer that the latent propensity of wt Sho to form amyloid is offset in healthy neurons by rapid turnover and presence of a GPI-anchor.

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O.1.7

Conformational dynamic of the PrP minimal domain involved in the oligomerization process

Human Rezaei[1], Stephanie Prigent[1], Nesrine Chakroun[2], Celine Chapuis[1], Cecile Dreiss[2], Franca Fraternali[2]

[1]INRA, France; [2]King's College, UK

Prion diseases are deadly neurodegenerative diseases affecting human and mammalian species. According to the 'protein-only' hypothesis, the key event in the pathogenesis is the conversion of the α-helix-rich monomer (PrPc) into a β-sheet-rich pathogenic conformer in a polymeric state (PrPsc).

We have used a range of biophysical techniques in combination with Molecular Dynamics simulations (MD) to elucidate the molecular mechanisms of PrPc unfolding and PrPc/PrPsc conversion. Under well established conditions, three β-sheet-rich soluble oligomers were generated from the partial unfolding of the monomer, which form in parallel rather than in one sequential process. Single mutations were also performed, which affected dramatically and selectively the PrP protein oligomerization pathway. We have now identified the minimal region of PrP that leads to the same oligomerization profile as the full-length protein, namely, H2H3. We also demonstrate a stereo-selectivity of H2H3 during the polymerization process.

The existence of at least three distinct oligomerization pathways and the effect of single mutations reveal the conformational diversity of PrP and a possible relationship with prion strain phenomena. The identification of domains involved in the conversion process may lead to a better understanding of the effect of mutations or gene polymorphism on the evolution of prion pathology.

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O.1.8

Nuclear trafficking of STI1, a prion binding protein, induced by SUMOylation

Fabiana Caetano[1,4], Iaci Soares[1], Grace Pereira[1], Marilene Lopes[2], Nicolle Queiroz[2], Stephen Ferguson[3], Vania Prado[1,3], Vilma Martins[2]; Marco Prado[1,3]

[1]Universidade Federal de Minas Gerais, Brazil; [2]Ludwig Institute for Cancer Research, Sao Paulo, Brazil; [3]University of Western Ontario, Canada

Background: The Stress Inducible Protein 1 (STI1) is a specific ligand for the Prion protein (PrP^C) and this interaction promotes neuroprotection and neuritogenesis through different signalling pathways. STI1 is secreted by astrocytes suggesting that this protein acts as a soluble neurotrophic-like factor through its interaction with PrP^C at the neuron surface.

Objective: In order to understand possible physiological functions of STI1 and eventually PrP^C, we used a yeast two hybrid assay to identify STI1 ligands.

Results and Discussion: We performed two screens using a human or a murine brain cDNA libraries respectively and the N-terminus region of STI1, comprising the interaction site for PrP^C, as bait. Amongst the various clones isolated that interacted with STI1, we identified several involved with the post-translational pathway for protein SUMOylation, including several E3 ligases (distinct PIAS and also PC2) and the E2 ligase UBC9. We confirm the interaction between STI1 and the all the above proteins in mammalian cells through co-immunoprecipitation assays. We found in cells expressing CFP-SUMO and STI1-HA that a large fraction of immunoprecipitated STI1 is SUMOylated and by using an in vitro assay with recombinant Ubc9 we also found that STI1 is SUMOylated preferentially by SUMO2/3. These SUMOylation assays revealed that PIAS1 enhanced the incorporation of SUMO onto STI1, indicating that PIAS1 acts as an E3 ligase for STI1 SUMOylation. In agreement with these data, STI1 was able to co-immunoprecipitate PIAS1 specifically in HEK-293 co-transfected cells. To test for a possible role of SUMOylation we studied STI1 localization after overexpression of SUMO and PIAS1. Interestingly, co-expression of STI1 and SUMO3 or SUMO1 and the SUMO E3 ligases PIAS1 and PIASy increased the localization of STI1 in the nucleus detected both by confocal microscopy and sub-cellular fractionation.Future experiments will address the role of SUMOylation in STI1 physiology, its interaction with PrP^C and signalling functions.

Support: PrioNet-Canada, FAPESP, CAPES, CNPq and Howard Hughes Medical institute

Selected by the scientific committee from the submitted abstracts

O.2.1

Clinical diagnosis of CJD: are there predictors of treatment response?

Inga Zerr

National TSE Reference Center, Göttingen, Germany

The progress in understanding disease pathology and phenomenology in prion disorders and recent advances in diagnostic techniques might allow researchers to think about therapeutic trials in CJD patients. Some attempts have been made in the past. Drugs tested involved a variety of compounds, which belong to antimicrobial, anti-inflammatory or analgesic substance classes. Most papers on this subject describe single case reports. Controlled trials are virtually not available and double-blinded studies have been performed for single compounds only. Despite it has been demonstrated that clinical trials are feasible, the clinicians might face several specific problems when evaluating the efficacy of the drug in CJD, such as rareness of the disease, lack of appropriate preclinical test and heterogeneous clinical presentation in humans. These problems have to be carefully addressed in future trials by appropriate design. Currently, the clinical diagnosis of CJD is supported by cerebrospinal fluid tests and brain MRI imaging. Both techniques might be helpful to identify patients at early stages and potentially define subgroups in treatment response. An overview on advances in clinical diagnosis in humans, recent clinical trials and observational data on doxycyclin in CJD will be given.

Invited by the scientific committee

O.2.2

vCJD infection in an asymptomatic UK haemophilic patient

Alexander Peden[1], Graham Fairfoul[1], Suzanne Lowrie[1], Linda McCardle[1], Mark Head[1], Seth Love[2], Hester Ward[1], Simon Cousens[3], David Keeling[4], Carolyn Millar[5], FGH Hill[6], James Ironside[1]

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We describe a study of 17 UK patients with haemophilia considered to be at increased risk of vCJD through exposure to UK plasma products.

10 autopsy cases and 7 biopsy cases were analysed for disease-associated, protease-resistant prion protein (PrPres). The tissues available from each case were variable, ranging from a single biopsy sample to a wide range of autopsy tissues. A single specimen from the spleen of one autopsy case gave a strong positive result on repeated testing for PrPres by Western blot analysis. This tissue came from a 73 year-old male with no history of neurological disease, who was heterozygous (methionine/valine) at codon 129 in the prion protein gene. He had received over 9,000 units of Factor VIII concentrate prepared from plasma pools known to include donations from a vCJD-infected donor, and some 400,000 units not known to include donations from vCJD-infected donors. He had also received 14 units of red blood cells and had undergone several surgical and invasive endoscopic procedures. Estimates of the relative risks of exposure though diet, surgery, endoscopy, blood transfusion and receipt of UK plasma products suggest that by far the most likely route of infection was receipt of UK plasma products.

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O.2.3

Detection of prion particles in body fluids of humans and animals

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A major structural difference between the cellular isoform of the prion protein PrPC and the pathogenic isoform PrPSc is the much higher state of molecular aggregation of PrPSc. PK-resistance as normally used for PrPSc-characterization is not reliably valid for all species, strains and sources of prions. The method of suface-FIDA (fluorescence-intensity distribution analysis) was developed avoiding PK-digestion and detecting specifically disease related PrP-aggregates (Birkmann et al 2007, J. Vet. Microbiol 123, 294-304). After partial purification PrP-aggregates are bound to a chip covered with the capture-antibody SAF32, labeled with two types of antibodies against two different epitopes and carrying two different fluorescent dyes. A dual-color laser beam is scanning the chip surface, and the fluorescence signals are evaluated in respect to local coincidence, intensities and particle size. The method was applied to brain homogenate of sCJD-victims (post mortem), to CSF-samples of BSE-afflicted cattle (ante mortem) and blood plasma of scrapie sheep (ante mortem). Particles of PrP-aggregates could be detected in all samples, exhibiting a diameter range of 300 nm (optical resolution) to 1µm. PrP-aggregates were detected with 100% sensitivity in the sCJD-brain homogenate samples; studies on CSF are ongoing. PrP-aggregates were detected in CSF of BSE-cattle, but the number of samples was too little to evaluate the sensitivity. Only one antibody was available to detect scrapie-PrP-aggregates from blood plasma; the sensitivity of 60% will be improved in the ongoing experiments with a second antibody. PrP-aggregates can be used as seeds for fibril formation with recombinant PrP as template (Stφhr et al., 2008, PNAS 105, 2409-14). This system will be used as amplification of the particle detection method.

Invited by the scientific committee

O.2.4

Detection of prions in blood leucocytes

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Background: Infected human blood has been implicated in the iatrogenic transmission of vCJD in four reported cases. Experimental transmission studies have demonstrated that blood from scrapie and BSE infected sheep also contains infectivity. Rodent models of prion disease implicated both cellular and plasma fractions. However, direct detection of PrPsc from blood in the absence of in vitro amplification or bioassay has proved difficult. Methods for the direct detection of PrPsc in blood would be advantageous for the study of the pathogenesis of TSEs and as a basis for a blood test.

Objectives: To develop a method for the direct detection of PrPsc in blood cells from scrapie and BSE infected sheep; to study the temporal distribution of PrPsc in blood and to determine the identity of the cells bearing prions in blood.

Methods: Peripheral blood mononuclear cells (PBMC) were isolated from sheep naturally infected with scrapie or experimentally infected with BSE at the clinical stage of disease and from scrapie infected sheep from 3 months of age through to clinical end-point. PBMCs were tested for PrPsc content by a direct immunoassay based on the IDEXX CWD HerdChek kit. Different subsets of PBMCs were isolated by subset specific cell surface markers and magnetic bead separation and analysed for PrPsc content.

Results: PrPSc was detected in 54% of sheep with clinical scrapie and 71% of sheep with clinical BSE. A longitudinal study of the temporal distribution of blood PBMC associated PrPsc showed that the detection rate increases during the course of disease and is more likely to be observed during the second half of the incubation period. Additionally detection is more likely in scrapie infected sheep if they carry the PRNP genotype of VRQ/VRQ. Cell separation studies showed that the PrPsc is associated with a specific cell subset implicating a subset of B lymphocytes.

Discussion. This is the first report of the direct detection of PrPsc in cells isolated from sheep blood in the absence of in vitro amplification or bioassay. Since PrPsc can be detected from as early as 3 months of age in sheep naturally infected with scrapie, correlating with initial replication in the gut-associated lymphoid tissue, the assay could be the basis of a preclinical test. The identification of the cell subset carrying PrPsc progresses our understanding of the pathogenesis of the disease. However, it remains unclear whether this cell subset is responsible for the dissemination of prions or in clearance of circulating PrPsc. Funded by defra, UK and IDEXX.

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O.2.5

Decontamination of prion infected metal surfaces employing the photo-Fenton reagent

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Background: Transmission of iCJD due to ineffective decontamination constitutes a public-health risk for surgical patients and hospital laboratory personnel. Current protocols are, in many cases, hazardous to equipment, operators and the environment thus, pointing out the need for new methods of routine reprocessing of surgical instruments.

Objectives: The aim of this study is the evaluation of a user-, instrument- and environmentally friendly, yet drastic photocatalytic process, mediated by the photo-Fenton reagent, regarding its ability to decontaminate prion infected metal surfaces.

Methods: Stainless steel and titanium wires, incubated with hamster brain homogenates infected with the 263K prion strain, were treated with the photo-Fenton reagent (Fe^{3+}/H_2O_2/UV-A). Immunoblotting has been performed to evaluate the potential of the photocatalytic oxidation to degrade PrP. A Scanning Electron Microscope (SEM) has been used to assess the removal of adsorbed organic contaminants. Fluorescence Miscoscopy has been employed to monitor the removal of protein contamination from the wires' surface. Groups of golden Syrian hamsters have been implanted i.c. with both types of wires, to evaluate the efficacy of this treatment *in vivo*.

Results: Immunoblotting of PrP eluted from the surface of both types of wires, after photocatalytic oxidation, showed rapid degradation of prions bound on metal surfaces. SEM images of wires treated at different time points, suggest that elimination of all organic contaminants requires longer treatment times. Fluorescence Microscopy indicated that removal of protein contamination requires similar treatment times. The ongoing bioassay will assess the efficiency of the photocatalytic process *in vivo*.

Discussion: Photo-Fenton could be a powerful tool for disinfection. The use of a low-cost catalytic system, combined with the simplicity of the necessary equipment, can offer alternative, economically reasonable, user- and environmentally friendly solutions to the decontamination of non-disposable surgical instruments.

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O.2.6

Human urine and PrP

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Background: The presence and the characteristics of prion protein (PrP) in human urine under normal conditions are controversial. Similarly, there are no definite data on the presence of infectivity in urine in the course of naturally occurring human prion diseases.

Objectives: 1) To definitely determine the presence and characteristics of PrPC in normal urine. 2) To evaluate the prion infectivity in human urine in sporadic Creutzfeldt-Jakob disease (sCJD), we have carried out a set of bioassays in humanized transgenic mouse with urine samples collected from sCJD subjects.

Methods: 1) Advanced mass spectrometry and experimental treatments have been used to demonstrate the presence, primary structure and posttranslational modifications of purified urinary PrPC (uPrP). 2) Bioassays were performed by intracerebral inoculation of 100 times concentrated and dialyzed urine, collected from three sCJD-MM1 cases to humanized transgenic mice and from appropriate controls.

Results: We found that human urine contains significant amount of PrP (approximately 10 ng/ml) that is truncated with the major N-terminus at residue 112 as the PrPC fragment identified as C1, and it carries an anchor, which is soluble because likely lacks the phosholipid component. None of the humanized transgenic mice inoculated with sCJD concentrated urine had evidence of prion disease during a period of over 700 days (their normal life expectancy) leading to the conclusion that prion infectivity in sCJD urine, if present, must be less than 6 infectious units/100ml.

Discussion: The issues raised in the discussion will include: 1) The origin of the truncated uPrP; 2) How the present data compare with the experimental studies published to date that indicate presence of infectivity; 3) The practical implications of our findings.

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O.2.7

Rational design of anti-prion compounds targeting the PrP* characteristic sites

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Background: Conformational conversion of the cellular form of prion protein (PrPC) to a scrapie form (PrPSc) plays a central role in the pathogenesis of prion diseases. Recently we created lots of synthetic compounds which can specifically bind with PrPC and interfere with the PrPSc production. However we found that their chemical structures are quite diversed.

Objectives: Objectives are to understand their precise inhibitory mechanism and to further optimise their chemical structures rationally.

Methods: The chemical shift alterations upon PrP* formation have been observed using the liquid state NMR to identify the specific sites involved in the pathogenic conversion reaction. We have also developed a unique software, in which quantum and classical simulations are included, and applied it for the rational design of anti-prion compounds targeting these characteristic sites.

Results: The regions extended from C-terminal of the helix B to central part of helix C have exhibited the unusual chemical shifts alterations, suggesting the PrP* formation. According to the simulated results based on these observations, we have designed and synthesized a series of compounds, which showed the strong anti-prion activity, and identified the common scaffolds among them.

Discussion: Intriguingly, the amplitude of chemical shift perturbations upon binding with these anti-prion compounds have dramatically increased upon decrease of IC50 of compounds, indicating the local stability of the prion-ligand interaction sites is crucial for their inhibitory action. Application of these findings may well lead to the way for the development of therapeutics for prion diseases.

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O.2.8

A PrPSc specific peptide derived from an antibody against PrPC/Sc

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Background: The prion protein, PrP, exists in several stable conformations, with the presence of one conformation, PrPSc, associated with transmissible neurodegenerative diseases. PrPSc is the most obvious target for diagnostics and therapy of prion disesases and while several PrPSc-specific ligands have been reported, so far, for immunotherapy targeting PrPC rather than PrPSc seemed promising.

Objectives: We intended to define the smallest functional antibody fragment of an antiprion active monoclonal antibody.

Methods: A single chain variable fragment (scFv) was cloned from hybridoma (W226) cells secreting a universal anti-PrP mAB recognizing helix 1. In addition, we derived a peptide from scFvW226 consisting of the CDR3 of the heavy chain (CDR3H), which was weakly reactive to recombinant PrP in ELISA. The antibody fragments were analyzed regarding their binding to PrP and antiprion activity.

Results: scFvW226 exhibited strong binding to both PrPC and PrPSc and was antiprion active with an EC50 in the low nanomolar range as confirmed by bioassay (scFvW226; Móller-Schiffmann et al., 2009, Mol. Immunol. 46: 532). Surprisingly, CDR3H selectively precipitated PrPSc from brain homogenates of scrapie infected mice but not from normal mice. However, CDR3H had lost antiprion activity. A retro inverso D-peptide (riCDR3H), mimicking side chain topology of CDR3H while exhibiting increased protease resistance, exhibited also PrPSc-specific binding and reinstated antiprion activitiy. The 16 amino acid PrPSc-specific peptide was cloned behind an Ig-kappa secretion signal as an EGFP fusion protein to enable live in vivo PrPSc tracking; first results will be reported.

Discussion: To our knowledge riCDR3H is the first PrPSc-specific ligand with antiprion activity demonstrating that PrPSc remains a valid therapeutic target. We believe that this unique, non-PrP derived PrPSc-specific peptide will also be able to replace current PrPSc-specific mABs in prion diagnostic tests.

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O.2.9

N-Terminal amino acid profiling (N-TAAP) shows unexpected and distinct differences between PrPres from atypical and classical scrapie

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Background: Differences in prion protein misfolding between TSE strains can be demonstrated by biochemical analysis of differential sensitivity to proteinase K (PK). LC-MS based methods (N-TAAP) have enabled detection of the exact PK cleavage sites of PrPSc and their proportions and revealed subtle differences not identified by Western blotting [1]. We describe substantial sensitivity and resolution enhancements of N-TAAP facilitated by chip-nano LC-MS which better enable differential diagnosis.

Objectives: To evaluate differences in PrPSc misfolding between atypical and classical scrapie from sheep by N-TAAP and compare BSE and CH1641. To investigate the impact of fine-tuning of PK digestion conditions on strain differentiation.

Methods: Brain homogenates were prepared from terminal TSE affected animals (atypical and classical scrapie [ovinised mouse model Tg338] and experimental TSEs in sheep), PK digested, precipitated and trypsin digested under denaturing conditions. Peptide analysis by full scan MS identified several previously undetected PrPSc cleavage sites. A total of 13 PK cleavage sites were investigated by N-TAAP using chip-Nano-LC-MS.

Results: PrPres from atypical and classical scrapie samples showed not only the anticipated differences in abundance of core PrPSc tryptic peptides but also unexpected differences in PK sensitivity in the octarepeat region. N-TAAP revealed that short PK incubation times best enabled differentiation between BSE and CH1641.

Discussion: The N-TAAP procedure enabled high resolution and sensitive determination of major and minor PK cleavage points providing a basis for diagnosis and discrimination of all strains tested. The diversity of PK site profiles emphasises the variety of misfolded conformations that PrP can adopt. This high resolution profiling of the PK cleavage sites provides a powerful tool for detailed study of TSE type related differences in PrPSc structure.

[1] Gielbert et al., J. Mass Spectrom. 44 (2009) 384

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O.3.1

Novel therapeutic approaches in Alzheimer's disease

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My believe is that neurodegenerative diseases such as Alzheimer's (AD), Parkinson's and Prion disease will be found to share important fundamental mechanisms and that their treatment certainly involves common scientific approaches. For AD, focusing on Aí peptides and tau, the principal components of amyloid plaques and neurofibrillary tangles, respectively, has helped to identify early diagnostic markers. This will eventually enable us to treat patients before irreversible neurological damage has occurred. Successful treatments for a multifactorial disease such as AD will require multiple drugs affecting different pathways and should be devised to target the more tractable "therapeutic window" at an early stage during disease evolution. Such a combinatorial treatment could be based on neurotransmitter modulators addressing APP processing and tau phosporylation, protease and aggregation inhibitors as well as stimulators of protein degradation, passive or active vaccination against Aí and tau, cholesterol lowering and metal chelating strategies, modulation of the regulation of protein folding, metabolism and degradation, treatment with anti-inflammatory agents, scavengers of free radicals, polyunsaturated omega-3 fatty acids such as docosahexaenoic acid (DHA), and treatment with stabilizers of mitochondria such as dimebon. With the establishment of mouse models for AD pathology it became possible to demonstrate attenuation of Aí deposition for these strategies but also for exercise, rich environment, stress reduction, micronutrients such as DHA, caffeine, curcuma, and anti-oxidants. This supports epidemiological findings that a metabolic tune-up through an improved supply of micronutrients combined with a physical and mental tune-up is likely to have great benefits, particularly for those at risk for AD.

Invited by the scientific committee

O.3.2

Good protein, bad protein: A new Aβ variant can be both

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Background: Mutations in the amyloid β (Aβ) precursor protein gene (APP) cause familial Alzheimer's disease with virtually complete penetrance. We found an APP mutation (A673V, corresponding to position 2 of Aβ) that causes disease only in the homozygous state, whereas heterozygous carriers are not affected, consistent with a recessive Mendelian trait of inheritance.

Objective: To investigate the mechanisms by which the A673V APP mutation causes disease only in the homozygous state.

Methods: We analyzed APP processing in fibroblasts from an A673V homozygous patient and controls, and in CHO and COS7 cells transfected with wild-type or mutated APP cDNA. Moreover, we assessed the physicochemical and biological properties of A2V-mutated and wild-type Aβ1-40 and Aβ1-42 peptides.

Results: The study showed that the A673V mutation has two pathogenic effects: it (*i*) shifts APP processing toward the amyloidogenic pathway resulting in enhanced Aβ production, and (*ii*) increases the aggregation and fibrillogenic properties of Aβ. However, co-incubation of mutated and wild-type peptides confers instability on Aβ aggregates and inhibits amyloid formation and neurotoxicity. The finding that the A2V Aβ variant has a dominant-negative effect on amyloidogenesis is consistent with the observation that the heterozygous carriers do not develop disease and offers ground for the development of a therapeutic strategy. As a first step for designing an inhibitor of Aβ aggregation based on the mutated Aβ sequence, we synthesized a peptide homologous to residues 1-6 of Aβ with the A2V substitution and tested its ability to bind to wild-type Aβ and inhibit amyloidogenesis. The study showed that the mutated hexapeptide retains the anti-amyloidogenic properties of the parent full-length Aβ.

Conclusion: The present data have important implications for genetic screening and the potential treatment of Alzheimer's disease based on A2V-modified Aβ peptides or peptidomimetic compounds.

Invited by the scientific committee

O.3.3

Role of RNA processing in the pathogenesis of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurologic disorder in which premature loss of upper and lower motor neurons leads to fatal paralysis with a typical disease course of one to five years. Most efforts to understand ALS pathogenesis over the last 15 years have focused on disease caused by mutations in the ubiquitously expressed superoxide dismutase that represent ~2% of all instances of ALS. However, during the past 14 months, a paradigm shift in understanding ALS has been initiated by the discovery that mutations in two strikingly similar DNA/RNA binding proteins – TAR DNA-binding protein (TDP-43) and Fused in sarcoma (FUS/TLS) – are also primary causes of ALS. While both proteins have been linked to RNA transcription and alternative RNA splicing regulation, their functions in health and disease are not well understood.

We have used a comprehensive genomics approach with cross-linking and immunoprecipitation (CLIP-seq) to identify target RNAs for both TDP-43 and FUS/TLS. High-throughput sequencing of RNAs (RNA-seq) accumulated in nervous system tissues containing, or depleted of, TDP-43 or FUS/TLS is underway to validate putative targets and to determine how loss of either protein affects overall RNA metabolism. Lastly, to decipher the underlying mechanism through which dominant point mutations in either protein cause age-dependent selective loss of motor neurons, mice expressing these point mutations have been generated. We are applying similar genomic methods to samples from our transgenic animals to determine how RNA metabolism is affected in these new ALS models.

Invited by the scientific committee

O.3.4

Autophagy and its role in neurodegenerative diseases

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Autophagy is a fundamental cellular bulk degradation process for e.g. organelles or cytoplasmic proteins which has many implications for physiology and patho-physiology of cells and whole organisms. It is conserved among eukaryotes and has been characterized from yeast to man. Basal levels of autophagy are important for maintaining normal cellular homeostasis. Autophagy contributes to various physiological processes such as intracellular cleansing, differentiation, development, longevity, elimination of invading pathogens and antigen transport to innate and adaptive immune systems or counteracting ER stress and diseases characterized by accumulation of protein aggregates. Several studies suggest a crucial role of autophagy in neurodegenerative diseases, including Alzheimer's disease, Parkinson's disease, tauopathies and polyglutamine expansion diseases like Huntingon's disease. For example, the clearance of mutant huntingtin fragments or mutant forms of α-synuclein can be mediated by autophagy. It is reasonable to assume that autophagy could be a therapeutic target for treatment of these neurodegenerative diseases because of its protective role. The systematic analysis of autophagy in prion infection scenarios is still rather incomplete. From what is found so far there is good experimental evidence from in vitro and partly also in vivo studies that induction of autophagy can have beneficial effects on prion infection. The cellular load of PrP^{Sc} and prion infectivity is reduced, most probably by increase in lysosomal degradation, shifting the equilibrium between prion propagation and clearance towards the latter. The biological function of autophagy per se in prion infection and disease is still searching answers, although preliminary data indicate that the cellular level of autophagy can be a modifier of susceptibility to prion infection. Taken together, there seems to be also a fascinating interplay between prion infections and autophagy.

Invited by the scientific committee

O.4.1

TSE strains and their transmission within and between animals

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There are still major fundamental issues surrounding the Transmissible Spongiform Encephalopathies (TSEs). How many strains of animal and human TSEs are there? How does a TSE strain mutate? Can the "new" TSEs transmit to humans? How many carriers are there of variant Creutzfeldt-Jakob disease and can human to human transmissions result in a self sustaining epidemic? Moreover, there is still no blood test for TSEs, the nature of infectivity remains controversial and basic mechanisms of disease are not yet understood. We aim to address these important questions using our unique small and large animal model systems.

Our murine models in which we have introduced targeted mutations into the *Prnp* gene have been used to address the role of *Prnp* controlling susceptibility to disease. We have established mutations in *Prnp* and the glycosylation state of host PrP can have a profound influence on host susceptibility. We have altered the species of PrP expressed in mice and used these models to examine cross species transmissions and the range of TSE strains in humans. We have developed models in which we can temporally and tissue specifically alter the expression of PrP to determine the role of PrP in Schwann cells, neurones and gut epithelium in the transport of infectivity to the CNS and we have developed and used large animal models to assess the potential for human to human spread of TSE agents by blood transfusion. These model systems have given important insights into the basic mechanisms of these diseases.

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O.4.2

TSE genetic resistance and allelic interference in sheep

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In conventional mice, *Sinc a / Sinc b* allelic dimorphism (codon 108 of the *PRNP* gene) strongly influences TSE incubation period. Curiously, in heterozygotes *Sinc a/b* mice incubation can be longer than in *Sinc a* or *Sinc b* homozygous animals. This phenomenon is highly dependent of the considered TSE strain.

Two mechanisms could explain this phenomenon. Firstly, *Sinc a* and *Sinc b* PrP^C conversion process could be completely independent; the incubation period being the result of the agent replication on each PrP variant. Secondly, one of the PrP variant could interfere with the PrP^C to PrP^{Sc} conversion of the other.

In this study, using quantitative mass spectrometry, we first established that in heterozygote sheep both PrP^C variants are expressed at similar level in tissues. We then showed that distinct TSE agents can differentially convert PrP^C variants in such heterozygous individuals.

Finally, a natural TSE agent that, *in-vivo,* target the VRQ allele was inoculated to ARQ/VRQ and AHQ/VRQ sheep (orally or intra-cerebrally).

In this model, neither ARQ nor AHQ PrP^{Sc} variants were detected. Incubation periods and PrP^{Sc} accumulation dynamics were dramatically different between both animals groups. However in terminally affected animals, brain infectious titres were similar.

This experiment demonstrates the existence of a PrP mediated interference phenomenon on the *in-vivo* PrP^C to PrP^{Sc} conversion process.

Invited by the scientific committee

O.4.3

Spread of BSE prions in cynomolgus monkeys (Macaca fascicularis) after oral transmission

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Background: BSE-infected cynomolgus monkeys represent a relevant animal model to study the pathogenesis of variant Creutzfeldt-Jacob disease (vCJD).

Objectives: To study the spread of BSE prions during the asymptomatic phase of infection in a simian animal model.

Methods: Orally BSE-dosed macaques (n=10) were sacrificed at defined time points during the incubation period and 7 orally BSE-dosed macaques were sacrificed after the onset of clinical signs. Neuronal and non-neuronal tissues were tested for the presence of proteinase-K-resistant prion protein (PrPres) by western immunoblot and by paraffin-embedded tissue (PET) blot technique.

Results: In clinically diseased macaques (5 years p.i. + 6 mo.), PrPres deposits were widely spread in neuronal tissues (including the peripheral sympathetic and parasympathetic nervous system) and in lymphoid tissues including tonsils. In asymptomatic disease carriers, PrPres deposits could be detected in intestinal lymph nodes as early as 1 year p.i., but CNS tissues were negative until 3 – 4 years p.i. Lumbal/sacral segments of the spinal cord and medulla oblongata were PrPres positive as early as 4.1 years p.i., whereas sympathetic trunk and all thoracic/cervical segments of the spinal cord were still negative for PrPres. However, tonsil samples were negative in all asymptomatic cases.

Discussion: There is evidence for an early spread of BSE to the CNS via autonomic fibres of the splanchnic and vagus nerves indicating that trans-synaptical spread may be a time-limiting factor for neuroinvasion. Tonsils were predominantly negative during the main part of the incubation period indicating that epidemiological vCJD screening results based on the detection of PrPres in tonsil biopsies may mostly tend to underestimate the prevalence of vCJD among humans.

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O.4.4

PrPSc distribution pattern in cattle experimentally challenged with H-type and L-type atypical BSE

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Background: After the detection of two novel BSE forms designated H-type and L-type BSE, the question of the pathogenesis and the agent distribution in cattle affected with these forms was fully open. From initial studies, it was already known that the PrPSc distribution in L-type BSE affected cattle differed from that known for classical BSE (C-type) where the obex region always displays the highest PrPSc concentrations. In contrast in L-type BSE cases, the thalamus and frontal cortex regions showed the highest levels of the pathological prion protein, while the obex region was only weakly involved. No information was available on the distribution pattern in H-type BSE.

Objectives: To analyse the PrPSc and infectivity distribution in cattle experimentally challenged with H-type and L-type BSE.

Methods: We analysed CNS and peripheral tissue samples collected from cattle that were intracranially challenged with H-type (five animals) and L-type (six animals) using a commercial BSE rapid test (IDEXX HerdChek), immunohistochemistry (IHC) and a highly sensitive Western blot protocol including a phosphotungstic acid precipitation of PrPSc (PTA-WB). Samples collected during the preclinical and the clinical stages of the disease were examined. For the detection of BSE infectivity, selected samples were also inoculated into highly sensitive Tgbov XV mice overexpressing bovine prion protein (PrPC).

Results: Analysis of a collection of fifty samples from the peripheral nervous, lymphoreticular, digestive, reproductive, respiratory and musculo-skeletal systems by PTA-WB, IDEXX-HerdChek BSE EIA and IHC revealed a general restriction of the PrPSc accumulation to the central nervous system.

Discussion: Our results on the PrPSc distribution in peripheral tissues of cattle affected with H-type and L-type BSE are generally in accordance with what has been known for C-type BSE. Bioassays are ongoing in highly sensitive transgenic mice in order to reveal infectivity.

Selected by the scientific committee from the submitted abstracts

O.4.5

Recent results on the transmission, detection, and pathogenesis of chronic wasting disease

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Background: Chronic wasting disease (CWD) of cervids is distinguished by its high level of transmissibility. We have previously shown that body fluids and excretions contain infectious CWD prions. The precise means by which these prions may access, traffick and cause disease in cervids (or other species) remains to be elucidated. Here we present current results of studies employing cervid and cervidized mouse bioassays and serial protein misfolding cyclic amplification (sPMCA) to address these questions.

Objectives: We sought to determine: (1) which components of blood and saliva carry prion infectivity; (2) whether long term, very low level CWD infection undetectable by conventional assays may exist in cervids; (3) whether CWD can be transmitted via aerosol or minor oral lesions; and (4) potential alternate pathways of CWD prion entry and dissemination exist in vivo.

Methods: The studies described utilized cervid and cervid-PrP-expressing transgenic mouse bioassays, serial protein misfolding cyclic amplification (sPMCA), and high resolution immunostaining.

Results: We present data to demonstrate: (1) localization of infectious CWD prions chiefly to the circulating CD21-expressing B/DC cell fractions of blood; (2) very low level subclinical CWD infection in cervids detectable by sPMCA and bioassay; (3) CWD transmission by aerosol and minor oral epithelial lesions; and (4) evidence of villous autonomic neural uptake and dissemination of PrP^{CWD}.

Discussion: These findings contribute insights into CWD prion transmission, trafficking, and dissemination. The results also help direct efforts toward ante-mortem detection of CWD in cervids and raise interesting questions regarding duration of sub-clinical prion infection in cervids or other species.

Selected by the scientific committee from the submitted abstracts

O.4.6

All separated components, prepared from BSE-infected sheep blood, are infectious upon transfusion

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Background: The possibility that vCJD may be transmitted by blood transfusion is serious public health issue, of which 4 probable (3 clinical) cases have been attributed. Recently a case of asymptomatic vCJD infection was identified in a haemophiliac; following treatment with clotting factors from UK plasma pools. Sheep orally infected with BSE provide a suitable model, to assess vCJD infection in humans & risk reduction methods, as the distribution of PrPSc & infectivity in lymphoid tissues resembles that of vCJD patients.

Objectives: To determine qualitative and quantitative data on the changes in infectivity in blood and its clinically relevant components with time, to assess the effect of leucodepletion of such products and the potential for secondary transmission by blood transfusion.

Methods: We orally infected sheep with bovine BSE brain homogenate and collected two full-sized donations of whole blood, before the onset of clinical signs. The following components were transfused into naive recipients: whole blood, buffy coat and leucoreduced and non leucoreduced plasma, platelets and red cells. A sub sample of all components was inoculated into TgShpXI mice for determination of infectivity titers. A unit of whole blood from selected primary recipients was transfused into secondary recipients. We are creating a blood archive throughout this study.

Results: 33% of the infected donors have been confirmed as having BSE. We have 4 transmissions of BSE-infectivity following the transfusion of whole blood, buffy coat and plasma. Short incubation times were recorded in these recipients (468, 513, 567 and 594 days) & were similar to those seen in their respective donors (534, 628, 614 and 614 days). The donor of buffy coat also donated both leucodepleted and non leucodepleted blood components to other recipients.

Discussion: Our study will provide invaluable data on the safety of blood products, in relation to TSE infection, used in human medicine (DoH 007/0162)

Selected by the scientific committee from the submitted abstracts

O.4.7

Pathogenesis of scrapie in goats: modulation by host PRNP genotype and effect of coexistent conditions

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Background: Following a severe outbreak of clinical scrapie, a large dairy goat herd was culled, and 200 animals were examined to investigate diagnostic, epidemiological, genetic and pathogenetic aspects of the infection.

Objectives:

Methods: Brain, lymphoreticular system (LRS) tissues and other organs were examined for disease-associated PrP (PrPd) by immunohistochemistry. The whole open reading frame of the PRNP gene was sequenced, and antibodies to lentiviral (CAEV) infection were determined.

Results: Seventy-two goats (36%) showed PrPd accumulation: four only in brain, 34 only in LRS, and 34 in both. Susceptibility to scrapie was clearly associated with polymorphisms at codon 142: isoleucine homozygotes (II) showed a higher rate of infection (40/80, 50%) than methionine carriers (IM+MM; 32/120, 27%). Moreover, the vast majority of goats showing definite signs of scrapie (at and prior to the cull) were II. Surprisingly, however, it was M carriers that more often accumulated PrPd in the brain (24/32); only 14/40 II did so, the other 26 being positive in LRS tissues alone. Chronic enteritis was observed in 98 of the 200 goats examined, with no association with either scrapie infection or presence of PrPd in the gut. CAEV infection was found in a similar proportion (around 40%) of scrapie positive and negative goats. Lymphoproliferative mastitis was observed in 13/31 CAEV positive scrapie infected goats, and never in CAEV negative goats; PrPd in the mammary gland was detected in five of those 13 goats.

Discussion: Scrapie in II goats appeared to follow a "conventional" pattern, with early LRS involvement followed by progressive PrPd accumulation in the brain and clinical disease. Unlikewise, infection in M carriers followed a more "erratic" pattern, with indiscriminate, mostly low level affection of the brain and/or the LRS, and rare disease. There was no evidence that chronic enteritis could play a role in scrapie, while accumulation of PrPd in the mammary gland was clearly associated with mastitis due to CAEV infection.

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O.4.8

Induction of macrophage migration by neurotoxic prion protein fragment

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Background: Prion diseases are characterized by accumulation of protease resistant isoforms of prion protein (PrP), and infiltration and activation of mononuclear phagocytes at the brain lesions. Interactions between prion proteins and immune cells during disease progression are still not very well understood.

Objectives: In the present study, multiwell chamber chemotaxis assay was carried out to assess the migratory response of macrophage cell line Ana-1 to a synthetic peptide homologous to residues 106-126 of the human prion protein. Specific protein kinase inhibitors were used to elucidate the signaling events underlying PrP106-126-induced macrophages migration, and a comparaison with the signaling pattern of macrophage migration induced by substance P (SP) and N-formyl-methionyl-leucyl-phenylalanine (fMLP), respectively was carried out.

Results: The results showed that PrP106–126 had a potent chemotactic effect on murine macrophage cell line Ana-1; that multiple signaling pathways might be involved in the PrP106-126-induced macrophage migrations; and that PrP106-126-induced chemotactic activity was similar to that induced by SP.

Discussion: Several lines of evidence have established that follicular DC networks in lymphoreticular tissue play a prominent role in prion transmission to neuronal tissue (Aucouturier and Carnaud, 2002; Glatzel et al., 2003; Prinz et al., 2003b). Given macrophages migratory capabilities and their close anatomic and functional relation with lymphoid tissue, they may play a crucial role in the propagation of the PrPSc.

Signaling pattern underlying PrP106-126-induced migration on Ana-1 cells mimicked that of substance P, and was different from that of fMLP. Similar results were reported for DCs (Kaneider et al., 2005). These findings provide new insights into the mechanisms underlying the interaction between the PrP and the marcophages.

Selected by the scientific committee from the submitted abstracts

O.5.1

Molecular neuropathology of TSEs

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Transmissible spongiform encephalopathies (TSEs) or prion diseases are fatal neurodegenerative conditions in humans and animals. Here I try to summarize the molecular background of phenotypic variability, the relation of prion protein (PrP) to other proteins associated with neurodegenerative diseases, and the pathogenesis of neuronal vulnerability. PrP exists in different forms that may be present in both diseased and non-diseased brain; however, abundant disease-associated PrP together with tissue pathology characterizes prion diseases and associates with transmissibility. Prion diseases have different etiologies with distinct pathogenesis and phenotype. Mutations of the prion protein gene are associated with genetic forms. The codon 129 polymorphism in combination with the Western blot pattern of PrP after proteinase K digestion serves as a basis for molecular subtyping of sporadic Creutzfeldt-Jakob disease. Tissue damage may result from several parallel, interacting or subsequent pathways that involve cellular systems associated with synapses, protein processing, oxidative stress, autophagy, and apoptosis.

Invited by the scientific committee

O.5.2

Patterns of abnormal PrP drainage and amyloid formation in scrapie infected mice expressing GPI-anchorless PrP

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Background: The TgGPI-/- transgenic mouse has a prion protein which lacks a GPI anchor. When TgGPI-/- mice are infected with scrapie, large numbers of amyloid plaques are formed, mainly around blood vessels. We describe here the nature and distribution of lesions and abnormal prion protein accumulation in brains of scrapie infected TgGPI-/- mice.

Objectives: We wished to determine the effect of scrapie on cells lacking cell membrane anchored PrP when compared with classical scrapie and to investigate potential morphological changes which might correlate with clinical deficits in TgGPI-/- mice.

Methods: Scrapie infected TgGPI-/- mice at clinical stages of disease were perfused with mixed aldehydes, and examined by light and electron microscopy and by immunogold electron microscopy for disease associated forms of PrPd.

Results: Numerous angiocentric vascular amyloid plaques were evident throughout the brain. Immunogold labelling showed non-fibrillar, disease associated PrP (PrPd) between cells of the perivascular neuropil, apparently representing diffusion of monomeric or oligomeric forms of anchorless PrPd within the extracellular space. Non-fibrilar PrPd was also seen within basement membranes of endothelial cells, smooth muscle cells and pericytes. Some vessels were found in which non-fibrillar PrPd was detected only in the adventitia and others in which only smooth muscle basement membranes were involved.

Discussion: The lesions of scrapie infected TgGPI-/- mice are substantially different from those of other classical TSEs insofar as they lack much of the membrane pathology found in the latter. The pattern of PrPd accumulation is similar to that found in CAA of Alzheimer's disease suggesting that disease in TgGPI-/- mice is also caused by failure to eliminate excess proteins through the interstitial and extracellular drainage pathways.

Selected by the scientific committee from the submitted abstracts

O.5.3

Sequence-dependent pathobiology of prion proteins: lessons from rabbits, mice and hamsters

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Background: In Prion diseases the normal cellular prion protein (PrPC) converts into a pathological isoform (PrPSc) with distinct biochemical and structural properties. Most mammalian species have shown their susceptibility to prion disorders; however, rabbits have proven resistant to the disease. We reasoned that this intriguing phenomenon could help us to better understand the rules governing PrP misfolding and pathogenesis.

Objectives: Does the primary sequence of prion proteins command its neurotoxic potential? Do Rabbit (Rb), Hamster (Ha) and Mouse (Mo) prion proteins behave similarly when expressed in a new prion-free model organism? Do they display differences regarding sub-cellular distribution and/or biochemical properties?

Methods: We recently created a fly model of sporadic prion disease by expressing wild-type HaPrP in the Drosophila brain (PLoS Genetics, 2009). Here we compared the biochemical, conformational and neuropathological properties of HaPrP with those of flies expressing MoPrP and RaPrP. To do so, we used genetic (targeted expression), behavioral (locomotion), biochemical (insolubility, conformational antibodies, FPLC chromatography) and histopathological (IHC, TEM) approaches.

Results: The three proteins display dramatically distinct behaviors in flies when expressed at similar levels. While HaPrP induces severe spongiform pathology and locomotor dysfunction, RbPrP does not and MoPrP seems to present an intermediate phenotype. In addition, HaPrP and MoPrP accumulate in secretory pathways, while RaPrP displays a diffuse and uniform distribution. This is consistent with the recovery of large HaPrP and MoPrP aggregates by size-exclusion chromatography. Finally, conformational antibodies recognize pathological isoforms only in flies expressing the hamster and mouse proteins.

Discussion: Our data suggest that the PrP primary sequences strongly influence protein misfolding and neurotoxicity, and unravel the potential of the fly model to study species-specific residues of mammalian PrPs.

Selected by the scientific committee from the submitted abstracts

O.5.4

Endogenous PrP conversion is required for prion-induced neuritic alterations and neuronal death

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Background: Mechanisms involved in prion induced neuronal death are still enigmatic. In vivo, both neurons and astrocytes support prion propagation leading to abnormal production of a misfolded protein called scrapie prion protein (PrPSc). Although crucial for prion replication and accumulation of aggregated PrPSc, the role of the normal form of prion protein, the cellular PrP (PrPC), is still controversial in prion-induced neurotoxicity.

Objectives: In this study we investigated the role of neuronal PrPC and the contribution of astrocytes in prion-induced neuronal death.

Methods: We have previously shown that prions could be efficiently propagated in primarily cultured neurons and astrocytes (Cronier et al. 2004). Here, we have set up a model in which neurons devoid of PrPC or expressing PrPC from different species are cocultured with prion-infected astrocytes that continuously deliver physiological concentrations of PrPSc.

Results: In these conditions, we showed that scrapie-infected astrocytes exacerbate prion-induced cell death solely in neurons expressing transconformable PrPC. Although infectious, conditioned medium of prion-infected astrocytes did not display any acute neuronal toxicity. However PrPSc accumulation in neurons led to neuritic damage and cell death associated with an increased sensitivity to glutamate and reactive oxygen species.

Discussion: Our results indicate that interaction between neuronal PrPC and PrPSc is not sufficient to induce neuronal death but that PrPC transconformation is required for prion-associated neurotoxicity. In addition, it is unlikely that a dysfunction of prion-infected astrocytes is involved in the initiation of neuronal death process induced by prions. Altogether, our findings support the view that prion infection and subsequent PrPC transconformation trigger impairment of neuronal homeostasis by sensitizing neurons to environmental stress that are regulated by neighbouring cells including astrocytes.

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O.5.5

Prion replication sensitizes neurons to pro-inflammatory cytokines

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Background: Activation of glia in prion diseases contributes to TSE pathogenesis through the release of chemokines and pro-inflammatory cytokines acting as neurotoxic mediators. While it is now established that expression of the cellular prion protein (PrPC) at the surface of neuronal cells is mandatory for the scrapie isoform (PrPSC) to exert its toxicity, whether PrPSC replication in neurons potentiates their sensitivity to inflammatory cytokines is unclear.

Objectives: Our goal was 1) to assess the impact of prion infection in neuronal cells on TNFα-mediated toxicity and 2) to relate these effects to the normal function of PrPC.

Methods: Our study is based on RT-PCR, western blot and immunofluorescence experiments. We take advantage of the neuroectodermal 1C11 cell line endowed with the capacity to differentiate into bioaminergic neuronal cells. 1C11 cells combine functional expression of endogenous PrPC and the ability to chronically replicate several prion strains. Experiments were also carried out on primary cerebellar granule neurons obtained from FVB WT or PrP-null mice and FVB infected mice with 22L prions.

Results: In 1C11 precursors and their neuronal progenies, infection with the Fukuoka or 22L strains renders cells more vulnerable to exogenous TNFα. This higher cell sensitivity to TNFα relates to an increased expression of the death receptor TNFR1 at the surface of infected cells, itself resulting from both an upregulation of TNFR1 transcripts and an inhibition of metalloproteinase-dependent TNFR1 shedding. The raise in TNFR1 was also monitored in brains of 22L infected mice. Finally, we provide evidence that PrPC depletion promotes a deregulation in TNFR1 that compares that observed within an infectious context.

Discussion: We substantiate an increased vulnerability of infected neuronal cells to inflammatory cytokines, that reflects a loss of PrPC function on TNF-R1 regulation. We propose that the altered cell response to TNFα-mediated toxicity contributes to exacerbate prion-induced pathogenesis.

Selected by the scientific committee from the submitted abstracts

O.5.6

The investigation of dysregulated microRNAs in prion – induced neurodegeneration

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Background: The molecular events triggered by replicating prions that lead to the damage and ultimate death of neurons are poorly understood. Our previous studies have identified numerous host factors affected during prion disease. One factor is the overexpression of cAMP response element binding (CREB) protein in neurons of scrapie infected mice. CREB is a transcription factor that has been shown to play an important role in neuronal survival. In addition, previous data showed that CREB is able to activate transcription of at least one microRNA (miRNA). Deregulation of miRNAs has been implicated in various diseases (cancer and Alzheimer's), thus, miRNAs induced by CREB may be essential in understanding the pathobiology of prion disease and other neurodegenerative disorders.

Objectives: Identify dysregulated miRNAs specific to prion infection and investigate the function of these miRNAs on prion related neurodegeneration.

Methods: The identification of dysregulated miRNAs was performed by isolating the CA1 region of the hippocampus using laser capture microdissection. RNA was extracted and analyzed by real-time PCR, identifying a number of deregulated miRNAs. Bioinformatic analysis was used to identify potential gene targets of candidate miRNAs that were confirmed by dual-luciferase reporter assays. Subsequent primary tissue culture experiments followed by Western blot validations will alleviate the neurodegenerative functions of the candidate miRNAs.

Results: We observed numerous miRNAs to be temporally dysregulated throughout prion disease. In vitro studies confirmed that some of these miRNAs were regulated by the transcription factor CREB. Potential mRNA targets for the dysregulated miRNAs were identified based on the bioinformatic approach supplemented with functional studies.

Discussion: Investigating the specific function of these miRNAs may lead to a better understanding of neurodegeneration in prion disease. Transcripts involved in neuronal survival that are targeted by these miRNAs will be presented.

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O.5.7

Endogenous proteolytic cleavage of disease-associated prion protein to produce C2 fragments is strongly cell- and tissue-dependent

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Abnormally folded prion protein (PrPSc) accumulating in the nervous and lymphoid tissues of prion-infected individuals can be naturally cleaved to generate a N-terminally truncated fragment called C2. Cell cultures permissive to infection provide useful models in which biochemical events associated with prion replication can be studied. Rov and MovS cell lines, primary cultured cerebellar granule neurons (CGN) and astrocytes (CAS) established from ovine PrP transgenic mice tg338, in which natural sheep scrapie agent can be propagated, and CAD neuronal cell line susceptible to mouse-adapted scrapie strains. We examined the N-terminal processing of PrPSc in such cell models and in mouse tissues. Using a combination of cell and biochemical approaches we showed that: i) the efficiency of trimming varies considerably depending on the infected cell or tissue. In primary neurons and in brain tissue, PrPSc accumulated essentially as full length (FL) species, whereas nearly exhaustive trimming occurred in Rov and MovS cells, and in spleen tissue. An intermediate situation, i.e. accumulation in amounts of both C2 and FL PrPSc was observed in primary astrocytes and CAD cells. ii) while C2 is generally considered to be the counterpart of PrPSc PK-resistant core, N-termini of in vivo and in vitro cleaved fragments can actually differ, as evidenced by a different reactivity toward antibody Pc248 that recognizes the octarepeat domain with very high affinity. iii) impairment of lysosomal activity suffices to block C2 formation in Rov and CAD cells, arguing for primary involvement of hydrolases of the acidic cell compartment; however, fairly efficient propagation of prions can occur in subpassaged cultures under conditions of C2 inhibition. Our findings bring new information on the natural, cell-dependent processing of PrPSc molecules, which has substantial implications in terms of prion cell biology, PrPSc molecular analysis and subtyping of TSE agents.

O.6.1

Synergy of polybasic structure and copper co-ordination in the PrP23-89-mediated stress protection response

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Background: Beta cleavage of the prion protein (PrP), producing N and C-terminal fragments designated N2 and C2 respectively, is associated with protection against reactive oxygen species (ROS). Evidence of exacerbated ROS damage and increased beta cleavage of PrP are found in prion diseased brains and, therefore, this cleavage is likely to be part of a cellular protection response.

Objectives: To investigate the cellular interactions through which a synthetic peptide equivalent to murine N2 (PrP23-89) initiates an intracellular reduction in stress-induced ROS.

Methods: We have utilised a combination of cell biology and biophysical techniques.

Results: The N2 fragment contains two notable regions; a polybasic glycosaminoglycan (GAG)-binding motif found in its most N-terminal amino acids (23-28) and a copper-binding octameric repeat sequence from amino acids 51-89. Our previous results have demonstrated that N2 is a biologically active PrP cleavage fragment. Through exoplasmic association, specifically mediated by the N-terminus, N2 can ameliorate intracellular stress-induced ROS. This reaction is dependent upon copper saturation of the octameric repeat region, cellular lipid raft integrity and cell surface heparan sulphate-containing proteoglycans. Additionally, mutational analysis of the polybasic-proline motif within the GAG-binding N-terminus shows the structure imposed upon the peptide by the steric constraints of the prolines is required for the ROS reduction reaction to proceed. Further, this change alters the association of the copper-saturated octapeptide repeats with binding partners at the cell membrane as well as the tendency of N2 to oligomerise.

Discussion: We conclude that the octapeptide repeat, through variable copper binding, may function as a bio-sensor for activation of the protective N2 region, with the structure and charge specificity of the polybasic domain ensuring the correct transducing receptor engagement. Here we will present our latest research into the mechanism of this reaction.

Selected by the scientific committee from the submitted abstracts

O.6.2

Family reunion - encounter of molecular cousins uncovers founder and ancient function of prion gene family

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Background: In the more than 20 years since its discovery, both the phylogenetic origin and cellular function of the prion protein (PrPC) have remained enigmatic.

Objectives: Insights into the function of PrPC may be obtained through a characterization of its molecular neighborhood.

Methods: A quantitative investigation of the PrPC interactome was conducted in a cell culture model permissive to prion replication. To facilitate recognition of relevant interactors, the study was extended to Doppel and Shadoo, two mammalian PrPC paralogs and followed by in-depth bioinformatic analyses.

Results: Interestingly, this work not only established a similar physiological environment for the three prion protein family members, but also suggested direct interactions amongst them. Furthermore, multiple interactions between PrPC and the neural cell adhesion molecule (NCAM), the laminin receptor precursor (LRP), Na/K ATPases and protein disulfide isomerases (PDI) were confirmed, thereby reconciling previously separate findings. Surprisingly, this work further revealed the spatial proximity of a subset of metal ion transporters of the ZIP family to mammalian prion proteins. A subsequent bioinformatic analysis revealed the presence of a prion-like protein sequence within the N-terminal, extracellular domain of a phylogenetic branch of ZIPs. Additional structural threading and ortholog sequence alignment analyses consolidated the conclusion that the prion protein gene family is phylogenetically derived from a ZIP-like ancestor molecule.

Discussion: A simple hypothesis is presented which accounts for the majority of interactions observed and suggests that PrPC organizes its molecular environment on account of its ability to bind to adhesion molecules harboring immunoglobulin-like domains, which in turn recognize oligomannose-bearing membrane proteins. Our data further explain structural and functional features found within mammalian prion proteins as elements of an ancient involvement in the transport of divalent cations.

Selected by the scientific committee from the submitted abstracts

O.6.3

Proteasome inhibitors induce accumulation of a detergent-insoluble PrP26K and formation of PrPSc aggresomes in prion–infected CAD cells.

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Background: Dysfunction of the ERAD/proteasome system in aging is believed to contribute to the initiation or aggravation of neurodegenerative disorders associated with protein misfolding. There is also previous evidence to suggest that failure of ERAD and/or impairment of proteasomal functions might be implicated in prion disease.

Objectives: We investigated effects of proteasome inhibitors on PrP expression in both infected and unfected CAD neuronal cells.

Methods: Biochemical approaches, immunoblotting and confocal immunocytochemistry were used.

Results: We observed the induction of an unglycosylated 26 kDa PrP species insoluble in detergents and weakly resistant to proteinase K digestion that we named PrP26K. PrP26K was also induced upon proteasome inhibition in PC12, in N2A cells and in primary cultured, postmitotic neurons. In CAD cells infected by either 139A or 22L strains of prion, PrP26K induction occurred to a similar extent than in uninfected cells. However PrP26K was apparently not, or at least not efficiently, converted into highly proteinase K resistant PrPSc. Thus the abnormal PrP species produced following proteasome impairment was not particularly prone to prion conversion. In addition, proteasome inhibitors impaired the traffic of regular PrPC leading to accumulation in the Golgi apparatus of both infected and non-infected cells. Proteasome inhibition also increased the intracellular aggregation of PrPSc, which deposited into large perinuclear aggregates exhibiting characteristic features of aggresomes.

Discussion: Sequestration of PrPSc into a structure depending on microtubule transport of cytosolic aggregates raises the question of how PrPSc, which is known to be accumulated in, and trafficked by, vesicles of the endo-lysosomal pathway might also reach the cytosol of the cells. Our data strongly suggest an involvement of proteasomal activity for both control of correct PrPc expression and reduction of intracellular PrPSc aggregation.

Selected by the scientific committee from the submitted abstracts

O.6.4

Neuroimmunoendocrine regulation of the prion protein in neutrophils

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Background: The prion protein (PrPc) is a highly conserved glycosylphosphatidylinositol (GPI)-anchored cell surface protein, expressed mainly in the central nervous system. Abnormal conformers of PrPc (PrPsc) play a central role in prion diseases. Neuroinvasion following peripheral infection with PrPsc requires amplification of the latter in lymphoid organs, dependent on the expression of PrPc by immune cells. Pro-inflammatory cytokines modulate the expression of PrPc, and systemic inflammation accelerates experimental prion diseases. PrPc also modulates peripheral inflammation induced by ligands of either toll-like-receptors or Fas. However, the mechanisms that link the expression of the protein with inflammation are still unknown.

Objectives: To determine the role of inflammatory mediators in the regulation of the expression of PrPc in immune cells.

Methods: Flow cytometry, cell sorter, protein immunoblot, confocal microscopy and several models of induced inflammation and stress, as well as funtional assays.

Results: We show that systemic inflammation induced by lipopolysaccharide (LPS) leads to massive up-regulation of PrPc in mouse neutrophils, an effect that does not depend on pro-inflammatory cytokines, but rather on the anti-inflammatory mediators transforming growth factor (TGF)-β and glucocorticoids (GC). Up-regulation of PrPc was also found in restrained animals, a condition of behavioral stress that increases the serum levels of both TGF-β and GC, and, strikingly, in dexamethasone only treated mice. In vitro experiments with purified neutrophils provided evidence for a direct regulation of PrPc by GC and TGF-β. Functional assays reveal a negative modulation of neutrophil functions by PrPc, such as migration and adhesion.

Discussion: The data demonstrate an interplay of the nervous, endocrine and immune systems upon the regulation of PrPc, which may have significant impact over the physiological functions of the prion protein, and may be involved with progression of neurodegeneration.

Selected by the scientific committee from the submitted abstracts

O.7.1

Understanding genetic susceptibility to human prion diseases

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Prions are lethal pathogens of mammals which appear devoid of nucleic acid and composed of aggregated conformational isomers of a host-encoded glycoprotein, the prion protein (PrP). Human prion disease may be inherited, acquired from dietary or iatrogenic exposure to transmissible prions, or arise as a result of an apparently sporadic process. Recognition that human PrP gene (PRNP) coding mutations could lead to the spontaneous formation of prions provided strong evidence for the protein-only hypothesis although whether this phenomenon can be reproduced in transgenic mouse models remains controversial. The common polymorphism at residue 129 of human PrP has a profound effect on susceptibility to, and phenotype of, human prion diseases and influences prion strain propagation via conformational selection. Other coding and non-coding PRNP polymorphisms are also involved and will be discussed. However, it is clear that multiple additional loci affect both prion disease incubation period and susceptibility in mouse and human, and these prion modifier genes are now being identified and their roles explored. Those relatively small number of UK individuals affected with clinical variant CJD from amongst the millions potentially exposed to BSE prions have not been demonstrated to have unusual dietary or occupation exposure to BSE and are likely to be enriched in genes affecting susceptibility or producing shorter incubation periods. We have also been studying individuals in Papua New Guinea who had multiple dietary exposures to kuru prions but who remain well over half a century following the cessation of exposure at mortuary feasts, and who may be expected to be enriched for resistance genes. In parallel, we have localised genes affecting prion incubation periods in inbred mouse strains and performed comparative genomic studies. Such prion modifier loci may provide insights into population risks of prion infection, disease and subclinical carrier states and identify novel disease pathways and mechanisms. A proportion of such genes and pathways identified in this prototypic protein misfolding disorder, and indeed a neurodegenerative disease to which laboratory rodents are naturally susceptible, are likely to be of wider significance in understanding diseases of protein misfolding. These include the principal causes of degenerative dementia; accumulation of misfolded host proteins is also a feature of normal brain ageing.

Invited by the scientific committee

O.7.2

Properties of the Shadoo protein in health and Prion disease

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While PrP knockout mice are ascribed a variety of subtle deficits, one area of consensus concerns the ability of PrP^C to protect against neuronal insults. Besides action against chemical toxins this property encompasses blocking cerebellar degeneration arising from the expression of internally deleted forms of PrP ("DE and DF PrP") and wt Doppel in the CNS. Indeed, this paradigm of antagonistic action of PrP^C/DPrP action has implicated the existence of a hypothetical PrP^C-like protein denoted pi (p). The *SPRN* gene was sighted by bioinformatic techniques and is now known to encode a neuronal glycoprotein, shadoo (Sho). Sho is a plausible candidate for p as it has a central PrP-like hydrophobic domain (HD) prefaced by an N-terminal repeat region and followed by a short C-terminal domain and a GPI anchor. Sho expression in the adult mouse CNS has some overlaps with PrP^C, but is low in cerebellar granular neurons (CGNs). In CGNs from $Prnp^{0/0}$ k/o mice, wt Sho transgenes mimic the action of wt PrP transgenes in counteracting the toxic effect of expressing Doppel or , PrP. Prompted by these PrP^C-like properties we have extended our studies of Sho to define (i) the extent of covalent and conformational variation of this protein, (ii) attributes of the protein during the course of prion infections, and (iii) the phenotypic correlates of *SPRN* gene mutations.

Invited by the scientific committee

O.7.3

A novel protective prion protein variant co-localises with kuru exposure

Simon Mead, Jerome Whitfield, Mark Poulter, Paresh Shah, James Uphill, Tracy Campbell, Huda Al-Dujaily, Holger Hummerich, Jon Beck, Charles A Mein, Claudio Verzilli, John Whittaker, Michael Alpers, John Collinge

Medical Research Council, UK

Background: Kuru was a devastating epidemic prion disease affecting a highly geographically restricted area of the Papua New Guinea highlands which, at its peak, predominantly affected adult women and children of both sexes. Its incidence has steadily declined since the cessation of its route of transmission, endocannibalism.

Objectives: Identification of acquired human prion disease resistance factors

Methods: Genetic and selected clinical and genealogical assessments of over 3000 individuals from Eastern Highland populations, including over 632 participants of cannibalistic mortuary feasts, 152 of whom subsequently died of kuru.

Results: Kuru-exposed survivors of the epidemic in PNG are predominantly heterozygotes at the known resistance factor at PRNP codon 129. We now report a novel PRNP mutation at codon 127 found exclusively in the kuru region, and present in half of the otherwise susceptible PRNP codon 129 methionine homozygous women from the region of highest exposure. Whilst common in the area of highest kuru incidence, this allele is absent from kuru patients and unexposed population groups. Genealogical analysis reveals a significantly lower incidence of kuru in 127V pedigrees when compared with geographically matched control families.

Discussion: The 127V polymorphism is a new acquired prion disease resistance factor - selected during the kuru epidemic, rather than a trigger of the kuru epidemic. Variants at codons 127 and 129 of PRNP demonstrate the population genetic response to a prion disease epidemic and document the strongest episode of recent human balancing selection. By drawing parallels with variant Creutzfeldt-Jakob disease, this study highlights the contemporary relevance of kuru research now 50 years after its first description.

Selected by the scientific committee from the submitted abstracts

O.7.4

Inherited prion disease with 4-octapeptide repeat insertion – disease requires the interaction of multiple genetic risk factors

Jon Beck[1], Diego Kaski[1], Catherine Pennington[2], Mark Poulter[1], James Uphill[1], Matthew Bishop[2], Jackie Linehan[1], Catherine O'Malley[1], Sebastian Brandner[1], Richard Knight[3], John Collinge[1], Simon Mead[1]

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Background: Genetic factors are implicated in the aetiology of sporadic late onset neurodegenerative diseases. Whether these genetic variants are predominantly common or rare, and how multiple genetic factors interact with each other to cause disease is poorly understood. Inherited prion diseases (IPD) are highly heterogenous and may be clinically mistaken for sporadic Creutzfeldt-Jakob disease (sCJD) because of a negative family history.

Objectives: Here we report our investigation of UK patients with four extra octapeptide repeats (4-OPRI) which suggests that manifestation of the clinical disease requires a combination of a rare mutation with multiple common risk alleles.

Methods: Clinical, genetic and pathologic analysis from 10 patients and 9 families.

Results: The predominant clinical syndrome is a progressive cortical dementia with pyramidal signs, myoclonus and cerebellar abnormalities which closely resemble sCJD. Autopsy shows perpendicular deposits of PrP in the molecular layer of the cerebellum. Identity testing, PRNP microsatellite haplotyping and genealogical work confirm no cryptic close family relationships and multiple progenitor disease haplotypes. All patients were homozygous for methionine at polymorphic codon 129. In addition, at a SNP upstream of PRNP thought to confer susceptibility to sCJD (rs1029273), all patients were homozygous for the risk allele (combined P=5.9 x 10-5).

Discussion: These analyses suggest that manifestation of the clinical disease requires a combination of a rare mutation together with multiple common risk alleles. These families demonstrate a classical example of epistasis. The findings may provide a precedent for the understanding sporadic neurodegenerative disease caused by rare but high-risk mutations.

Selected by the scientific committee from the submitted abstracts

O.8.1

Variant CJD and plasma products

Robert G. Will

National CJD Surveillance Unit, Edinburgh, UK

Evidence from the Transfusion Medicine Epidemiology Review (TMER) project indicates that variant CJD is transmissible through transfusion of labile blood components. The question as to whether plasma products sourced from vCJD contaminated plasma pools has been addressed by a number of risk assessments, with conflicting conclusions. Recently a case of possible vCJD infection in an individual with haemophilia has been described and analysis has suggested that infection may have been related to prior treatment with vCJD implicated Factor VIII. The details of this case will be described together with an analysis of plasma product exposures in UK clinical cases of vCJD.

O.8.2

Blood safety: from screening tests to prion removal

Marc Turner

Scottish National Blood Transfusion Service and Department of Haematology, Royal Infirmary, Edinburgh, UK

Although the number of clinical cases of variant CJD continues to fall, concern remains within UK and Western European Blood Services in relation to the risk of transmission of variant CJD due to the estimated prevalence of sub-clinical infection in the general population and the clinical cases of transmission of variant CJD prions by blood components and plasma products. The UK Advisory Committee on the Safety of Blood, Tissues and Organs (SaBTO) has considered a number of further precautionary measures including reducing exposure to blood transfusion, importation of blood components, implementation of prion assays and prion reduction for red cell concentrates. The latter two technologies are currently under independent evaluation and it is expected that contingent on the outcome of these an initial decision on whether or not to recommend implementation of these technologies will be made by SaBTO in Autumn 2009.

O.8.3

Quantification of PrPSc in different blood fractions, brain and peripheral tissues at distinct stages of prion disease

Claudio Soto, Marcelo Barria, Baian Chen, Dennisse Gonzalez-Romero, Abhisek Mukherjee, Rodrigo Morales

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PrP^{Sc}, the main component of the infectious agent in prion diseases, is also the only validated surrogate marker for the disease and its sensitive detection is critical for minimizing the spread of the disease. We have developed the Protein Misfolding Cyclic Amplification (PMCA) technology to sustain the autocatalytic replication of infectious prions in vitro and enable their ultra-sensitive detection in diverse tissues and biological fluids. Using PMCA, we can replicate prions indefinitively in vitro, leading to generation of infectious material. PMCA is useful to study the nature of the infectious agent, its mechanism of replication, and the phenomena of prion strains, species barrier and pspontaneous emergence of new prions. The technology enables detection with high sensitivity and reproducibility of up to the equivalent of a single particle of PrP^{Sc}. This astonishingly high sensitivity led to detection of PrP^{Sc} in blood and urine in both symptomatic and presymptomatic phases of the disease. Recently, we have been using PMCA to semi-quantify the amount of PrP^{Sc} in various samples at distinct stages of the disease in experimental animals. In this presentation we will describe the multiple applications of PMCA and provide a critical discussion of conceptual and technical aspects of this technology.

O.8.4

Structural analyses and detection of prion proteins: from rapid diagnosis to therapeutic developments

Byron Caughey

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A key challenge in coping with prion diseases is the development of prion assays that are practical for routine testing for medical or agricultural purposes. We have developed relatively rapid and highly sensitive assays based on hamster PrP^{res}-seeded conversion of recombinant hamster PrP^{sen} into protease-resistant amyloid fibrils. PrP^{res}-seeded fibrils can differ from spontaneously seeded fibrils in terms of protease-resistance, infrared spectrum, and H/D exchange patterns, providing evidence of the distinct templating activity of PrP^{res}. We have adapted the quaking *i*nduced conversion (QuIC) assay to the detection of human variant CJD and sheep scrapie. Prion-positive and negative brain homogenate samples from humans and sheep were discriminated within 1-2 days with a sensitivity of 10-100 femtograms PrP^{res}. The assay also distinguished CSF samples from scrapie-infected and uninfected sheep, providing evidence of prions in the CSF of scrapie-infected sheep.

Another daunting challenge is the treatment of prion diseases. To better understand the activity of one of the most potent anti-TSE compounds, we performed NMR and CD spectroscopic studies of the flexible amino proximal region of PrP in the presence and absence of pentosan polysulfate (PPS). In the absence of PPS, PrP residues 23-106 had a highly flexible, largely random coil structure. In contrast, in the presence of PPS, this peptide yielded NMR-derived distance restraints that allowed the calculation of a unique serpentine loop-turn structure for the four (PHGGGWGQ) repeats. A stacked alignment of Trp sidechains in this structure suggested the PPS-induced exposure of a repetitive hydrophobic surface that may mediate PrP oligomerization and the binding of a variety of ligands of physiological and/or pharmacological significance.

O.8.5

Ethical issues in the communication of Prion disease risk

Conrad G. Brunk

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This presentation discusses some of the central ethical issues in the communication of risks of prion diseases to persons who have tested positive for pathological prion protein. These ethical issues arise from a number of decisions that must be made by researchers, physicians, and public health officials. Among these are:

Interpreting test results given the many uncertainties created by lower than desired sensitivity and/or specificity of available tests for prion protein.

Interpreting the uncertainties in the understanding of prion infectivity and resulting disease.

Estimating the risk to any individual of becoming ill (or dying) with prion disease or transmitting the disease to others (e.g., via blood transfusion).

Communicating complex scientific information, fraught with the above uncertainties, to test subjects who have varying degrees of scientific literacy and understanding.

Deciding what information to communicate to test subjects when it is not clear to the health professional what course of action would be indicated by that information, or when communication of the information may lead to actions the professional considers unwarranted or unwise.

Deciding what advice is appropriate to positive screening test subjects who are potential blood donors or surgery patients.

These decisions are guided by a number of important assumptions and considerations, among which are the following:

The capability of lay people to understand and appreciate issues of uncertainty, confidence, and probabilities essential to interpreting screening results.

How lay people differ from experts in their understanding of risk and risk acceptability.

The ability of people to make responsible decisions about their own health and the health of others affected by their actions (e.g., blood donation).

The appropriate role of the health professional in influencing the decisions of persons regarding their health (the issue of paternalism vs. respect for autonomy).

This presentation critically examines the nature of these decisions and the assumptions that typically underlie them, in order to propose ways for handling the communication of health risk in the most ethically responsible manner. It is argued that the first, and perhaps the most important, responsibility of scientists and health professionals is to be as transparent as possible among themselves about the nature of the scientific uncertainties and to characterize them in the most accessible ways. This responsibility is typically left unfulfilled, greatly complicating the communication task. It is then proposed that, if this task is done well, it is more likely that risks can be communicated in ways that non-experts can understand, at least at the level necessary for responsible personal decision making. It is argued that non-scientists have a greater capacity to understand well-articulated scientific issues, including uncertainties and probabilities, than is typically recognized. Experts tend to interpret the judgements made by non-experts about the *acceptability* of risk as misjudgements about the *magnitude* of risk. Accordingly, health professionals also tend to view patients' judgements about risk acceptability that differ from their own as reflecting irrational or incomplete understanding of the risk magnitude itself. This often is the basis of the argument that screening test subjects ought not to be given information that leads them to make "irrational" judgements.

The presentation includes recommendations for the kind of ethical advice appropriate for screening subjects testing positive for prion proteins who are potential blood donors or patients in surgery, and who could be sources for the transmission of infectious prion disease.

O.9.1

No evidence of contagiousness of atypical scrapie: results from a 12-country European study

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Background: During the last decade, active surveillance of transmissible spongiform encephalopathies in small ruminants has been intensive in Europe. In many countries this has led to the detection of cases of atypical scrapie. A question has been raised: is the atypical form contagious?

Objectives: The aim of this study was to investigate if the occurrence of secondary cases in flocks affected by atypical scrapie could help in clarifying the potential contagious nature of the disease by using surveillance data collected in Europe.

Methods: Questionnaires were used to collect, at country-level, data on control measures, on the results of active surveillance and of the within-outbreaks testing from 12 European countries. Generalised linear regression mixed models and meta-analysis were used to model prevalence data taking into account the random effect of country.

Results: The mean prevalence of atypical scrapie was six cases per ten thousand in abattoir surveillance and eight cases per ten thousand in fallen stock. By using meta-analysis, on 11 out of the 12 countries, we found that the probability of detecting secondary cases of atypical scrapie in positive flocks was similar to the probability observed in animals slaughtered for human consumption (odds ratio, OR=1.07, CI95%: 0.70-1.63) or among fallen stock (OR=0.78, CI95%: 0.51-1.2). In contrast, when comparing the two scrapie types, the probability of detecting secondary cases in classical scrapie positive flocks was significantly higher than the probability of detecting secondary cases in AS positive flocks (OR=32.4, CI95%: 20.7-50.7).

Discussion: These results suggest that atypical scrapie is not contagious or has a very low transmissibility under natural conditions compared with classical scrapie. Furthermore this study stressed the importance of standardised data collection to make good use of the analyses undertaken by European countries in their efforts to control atypical and classical scrapie.

Invited by the scientific committee

O.9.2

Survival and limited spread of TSE infectivity after burial for one year

Robert Somerville, Karen Fernie, Allister Smith

University of Edinburgh, UK

Background: Scrapie and Chronic Wasting Disease appear to spread via environmental routes, although there is little evidence that BSE or CJD do. Nevertheless there are concerns about reservoirs of BSE infection remaining in the environment after carcass burial or waste disposal.

Objectives: We are determining the survival and migration of TSE infectivity when buried for up to five years in two soil types; and either buried within bovine skulls, or as a point source bolus of TSE infected brain.

Methods: Two demonstration experiments have been set up. In one experiment boluses of TSE infected mouse brain (301V strain) have been buried in lysimeters containing either a sandy soil or a clay soil. Migration from the boluses is being assessed from soil cores taken from the lysimeters over time and assayed for TSE infectivity. In the other experiment ten bovine heads have been spiked with TSE infected mouse brain (301V strain) and buried in the two soil types. Two heads are exhumed annually and assessed for residual infectivity within and around them.

Results: After one year very small amounts of infectivity have been detected 25cm from the point source bolus in both soils. No infectivity was detected up to 9 months after burial. In the other experiment brain-like tissue was still apparent within the crania after one year. Large amounts of TSE infectivity were detected from samples of the intracranial contents, and also detected in very small amounts in soil samples in the soil immediately surrounding the heads.

Discussion: These data show that TSE infectivity can survive burial for up to a year but migrates very slowly in these soils. Results from future years will likely further illustrate the long term survival and migration properties of these infective agents. Risk assessments of TSE infectivity in the environment should take into account the likely long survival rate of foci of infectivity when large amounts of infected material have been buried.

Selected by the scientific committee from the submitted abstracts

O.9.3

Updated risk assessment of variant Creutzfeldt-Jakob disease (vCJD) risks for recipients of plasma-derived blood clotting products in the U.S.

Hong Yang, Richard Forshee, Mark Walderhaug, Steven Anderson

US Food and Drug Administration, USA

Background: A recent announcement by UK health authorities of a case of vCJD infection in a >70 year old person with hemophilia has prompted the US Food & Drug Administration (FDA) to re-evaluate vCJD risks in the U.S. via plasma-derived Factor VIII (pdFVIII) and to update its 2006 risk assessment. As of May 2009, confirmed vCJD deaths have occurred in persons who are homozygous methionine (MM) at codon 129 of the PRP gene. Several reports in the last few years have indicated signs of vCJD infection in persons of methionine-valine (MV) and homozygous valine (VV) genotypes. FDA updated risk assessment by assuming all genotypes are susceptible to vCJD and modeling the incubation periods for all three genotypes.

Objectives: To evaluate the vCJD risk for pdFVIII recipients with severe hemophilia and vonWillebrand diseases.

Methods: The model assumed equal susceptibility of three genotypes, a median incubation period of 12 years for the MM and 32 years for MV and VV genotypes, and vCJD infectivity was present in the blood of infected donors during the last 50% to 90% of incubation period. Model used statistical distributions for inputs including susceptibility to the disease, donation rates, frequency and duration of travel to the UK, France and other countries in Europe since 1980, the effectiveness of donor deferral policies and infectivity clearance during manufacturing processes.

Results: For severe hemophilia patients at the highest risk (prophylaxis, with inhibitor, with immune tolerance) the model estimated annual mean exposure to be ~7 x 10-8 iv ID50 or ~1 in 270,000 with the lower prevalence (4 per million) assumption, and ~1 x 10-4 iv ID50 or ~1 in 12,000 with the higher prevalence (1 per 4,225) assumption. Donor deferral policies reduce the risk by >92%.

Discussion: Due to limited data and knowledge of vCJD, the model estimates are uncertain. However, it suggests the risk is small, and donor deferral and manufacturing processes greatly reduce the risk.

Selected by the scientific committee from the submitted abstracts

O.9.4

Is Nor98 scrapie contagious? Results from Norwegian flock studies

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Background: Nor98 scrapie, also called atypical scrapie, has been reported in sheep from many European countries, the Falkland Islands, USA and Canada. Although the transmissibility of the Nor98 agent has been proven in ovine transgenic mice and sheep, there is scarce knowledge about transmission under natural conditions.

Objectives: The aim was to investigate whether the scrapie agent spreads between sheep in flocks where Nor98 scrapie has been detected.

Methods: The sheep originated from seven flocks with an index case of classical scrapie or from 58 flocks with an index case of Nor98 scrapie. The flock mates were examined either at destruction immediately after detection of the index case or when found dead or sent to slaughter up to six years after the diagnosis. When available, four brain regions (obex, cerebellum, midbrain and cerebral cortex) and the retropharyngeal or mesenteric lymph nodes were analysed for the presence of PrPSc by using IHC with F89.160.1.5 mAb or ELISA test (Platelia/TeSeE Sheep and Goat, Bio-Rad)

Results: In the classical scrapie flocks up to 35% of the sheep were positive for classical scrapie. In contrast, only one single case was found in 57 out of the 58 Nor98 flocks. In one flock, two positive sheep were found, the second case was Nor98 scrapie, but the index case could not be PrP genotyped or WB analysed. Nevertheless, the IHC analysis suggested that it was a Nor98 case.

Discussion: This study shows that Nor98 is clearly less contagious than classical scrapie under natural conditions. Although a secondary case of scrapie is probable in one single flock, considering the total of flock mates examined this does not exclude that scrapie Nor98 might be a spontaneous disease occurring without an external contamination source.

Selected by the scientific committee from the submitted abstracts

O.10.1

Transmission of uncommon forms of bovine prions to transgenic mice expressing human PrP: questions and progress

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The active, large-scale testing of livestock nervous tissues for the presence of protease-resistant prion protein (PrP^{res}) has led to the recognition of 2 uncommon PrP^{res} molecular signatures, termed H-type and L-type BSE. Their experimental transmission to various transgenic and inbred mouse lines unambiguously demonstrated the infectious nature of such cases and the existence of distinct prion strains in cattle. Like the classical BSE agent, H- and L-type (or BASE) prions can propagate in heterologous species. In addition L-type prions acquire molecular and neuropathologic phenotypic traits undistinguishable from BSE or BSE-related agents upon transmission to transgenic mice expressing ovine PrP (VRQ allele) or wild-type mice. An understanding of the transmission properties of these newly recognized prions when confronted with human PrP sequence was therefore needed. Toward this end, we inoculated mice expressing human PrP Met129 with several field isolates. Unlike classical BSE agent, L-type prions appeared to propagate in these mice with no obvious transmission barrier. In contrast, we repeatedly failed to infect them with H-type prions. Ongoing investigations aim to extend the knowledge on these uncommon strains: are these agents able to colonize lymphoid tissue, a potential key factor for successful transmission by peripheral route; is there any relationship between these assumedly sporadic forms of TSE in cattle and some sporadic forms of human CJD are among the issues that need to be addressed for a careful assessment of the risk for cattle-to-human transmission of H- and L-type prions.

Invited by the scientific committee

O.10.2

Generation of diverse synthetic prion strains capable of infecting mice that express full-length prion protein

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Background: Recombinant (rec) prion protein (PrP) composed of residues 89–230 was refolded into an amyloid conformation and found to contain prion infectivity upon inoculation into Tg9949 mice, which overexpress the same N-terminally truncated PrP construct (Legname, et.al., Science, 2004). The conformational stability of prions correlates directly to the disease incubation period (Legname, et.al., PNAS, 2006).

Objectives: We hypothesized that new synthetic prion strains with short, medium, and long incubation periods could be generated from PrP amyloids with low, medium, and high conformational stability, respectively.

Methods: We generated several PrP amyloid preparations at different temperatures, in the presence of varying concentrations of denaturant, and using two different recPrP constructs: full-length PrP(23–230) and N-terminally truncated PrP(89–230). Amyloids were screened for conformational stability by a conformation-dependent immunoassay, and select PrP amyloids exhibiting a broad range of conformational stabilities were inoculated into Tg4053 mice, which moderately overexpress full-length PrP.

Results: Tg4053 mice inoculated with all but one PrP amyloid preparation developed prion disease after incubation periods of >500 d. The brains of infected mice had typical prion neuropathology and harbored protease-resistant PrPSc. Negative control mice inoculated with monomeric recPrP, albumin, or PBS showed no sign of disease. Upon serial transmission, the newly created synthetic prion strains had incubation periods and conformational stabilities that correlated with the conformational stability of the amyloid used to create them.

Discussion: We found that the properties of synthetic prion strains may be controlled by the conditions used for their formation. Our findings provide direct evidence that mammalian prion strains arise from conformational differences in the PrP polypeptide chain.

Selected by the scientific committee from the submitted abstracts

O.10.3

Separation of prion strains from a mixture by discriminatory bioassay. The example of a natural case of mixed infection of classical scrapie and Nor98

Umberto Agrimi[1], Michele Di Bari[1], Claudia D'Agostino[1], Elena Esposito[1], Maria Mazza[2], Simone Barocci[3], Geraldina Riccardi[1], Shimon Simson[1], Paolo Frassanito[1], Barbara Iulini[2], Pier Luigi Acutis[2], Cristina Casalone[2], Hubert Laude[4], Sylvie Benestad[5], Gabriele Vaccari[1], Romolo Nonno[1]

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Background: The possible coexistence of different strains in natural prion isolates is the subject of enduring discussions and might have implications for prion strains surveillance. Nonetheless, this possibility has not yet been proved. In 2007, a case of ovine scrapie with features compatible with a mixed infection of classical scrapie and Nor98 was reported in Italy.

Objectives: To characterize by bioassay the prion agent(s) involved in this unusual scrapie case.

Methods: Previous studies showed that voles are susceptible to the prevalent Italian scrapie agent and resistant to Nor98, while tg338 mice show the contrary. Taking advantage of this differential susceptibility, we inoculated voles and tg338 with the brain stem (BS) and the cerebral cortex (CC) of the unusual case. Discriminatory WB showed a predominance of classical scrapie PrPres in BS, while CC mainly contained Nor98 PrPres. A classical scrapie isolate (ClS) from the same flock of the unusual case, as well as Nor98 isolates from Italy and Norway were used as controls.

Survival times and attack rates were calculated in voles and tg338. Brains were analysed by discriminative WB for PrPSc type, by IHC and PET-blot for PrPSc deposition and by E&E for lesion profile.

Results: Both CC and BS induced disease in tg338, with survival times similar to Nor98 controls. As expected, Tg338 were resistant to ClS control. In contrast, voles developed disease following inoculation of ClS control and BS, but not of CC and Nor98 controls.

In voles, the neuropathological and molecular phenotypes upon infection with the unusual case were the same of those observed with ClS, while in Tg338 were identical to Nor98 controls.

Discussion: Our results demonstrate that two independent and separable prion agents coexist in the isolate investigated. This implies that:
- mixed prion infections can occur
- classical scrapie and Nor98 are distinct entities
- the isolation of prion strains from a mixture is affordable.

Selected by the scientific committee from the submitted abstracts

O.10.4

Significance of murine scrapie strains: ME7 changes phenotype when inoculated in sheep and then back into mice

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Background: The original concept of scrapie strains arose from the identification of different disease phenotypes in mice inoculated with natural sheep scrapie isolates . Distinct phenotypes are recognised only after serial passage into inbred mouse lines. ME7 murine scrapie has been isolated from sheep scrapie of different PRNP genotypes but the stability of this strain on re-isolation and therefore its significance for sheep is untested.

Objectives: To determine i) whether ME7 produces a unique disease phenotype on passage in sheep of different PRNP gene background, and ii) whether or not its characteristic phenotype is recovered when re-isolated in mice.

Methods: Twenty-two Cheviot or Suffolk sheep homozygous for either VRQ or ARQ PRNP alleles were dosed with ME7. Sheep were monitored for neurological signs of scrapie and killed at clinical end point. Representative samples of CNS and LRS tissues were collected and labelled for disease-associated PrP (PrPd) by immunohistochemistry (IHC). In 2007, selected brain inocula from "sheep ME7"-confirmed cases were inoculated back into C57BL mice (n=45).

Results: ME7 attack rates were 75% in VRQ/VRQ Cheviot sheep, and 87% and 83% in ARQ/ARQ Cheviot and Suffolk sheep, respectively. Differences in incubation periods and PrPd IHC phenotypes were detected, mostly in relation to the PRNP genotype of the recipient sheep. When mice were challenged with these different sheep phenotypic sources, a donor associated variation in disease phenotype were found in recipients.

Discussion: In this experiment, the well characterized, cloned –and therefore presumed single- ME7 murine scrapie strain resulted in variable disease phenotype interpreted as instability when inoculated into sheep. When re-isolated back into mice, a consistent ME7 disease phenotype was absent at first passage. These findings have profound implications for the prion strain concept, and suggests that strains result from an interaction between the agent and the host.

Selected by the scientific committee from the submitted abstracts

O.10.5

A novel human prion disease affecting subjects with the three prion protein codon 129 genotypes: could it be the sporadic form of Gerstmann-Sträussler?

Pierluigi Gambetti

Case Western Reserve University, USA

Background: We recently described a novel prion disease, named protease-sensitive prionopathy or PSPr, characterized by the presence of an abnormal prion protein (PrP) that was 60 fold less protease resistant than that of sporadic Creutzfeldt-Jakob disease (sCJD) and on immunoblot generated a distinct ladder-like profile. All affected subjects where homozygous for valine at codon 129 (VV) and had no mutation in the PrP gene.

Methods: We have characterized several new cases in our surveillance and received from Europe.

Results: 1) A disease overall similar to that reported in the 129VV subjects also affects subjects that are methionine/valine heterozygous (MV) and methionine homozygous (MM) at codon 129 and have no PrP gene mutation; 2) The clinical and histopathological features of the new MV and MM PSPr cases are similar but distinguishable from those of the original VV cases; 3) The electrophoretic profiles generated by the abnormal PrP isoforms associated with the MV and MM cases are similar to VV cases but show increasing levels of protease-resistance; 3) abnormal tau is present in all three genotypic forms of PSPr with features apparently similar to those of primary tauopathies placing PSPr at the intersection of tauopathies and prion diseases.

Discussion: Will focus on: 1) the features of the abnormal PrP in the newly discovered 129MV and 129MM PSPr; 2) the effect of the 129 polymorphism on PSPr compared to that on sCJD; 3) the relationship of PSPr with tauopathies; 4) whether PSPr now with the three 129 genotypic forms is the long sought sporadic form of GSS. (Supported by NIH AG-14359, NS052319, CDC UR8/CCU515004).

Selected by the scientific committee from the submitted abstracts

O.10.6

Biological typing of sporadic Creutzfeldt-Jakob disease isolates and comparison with animal prion isolates

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Background: Our incomplete understanding of the nature of TSE agents, along with the current technical limitations in the analysis of PrP^{Sc} structure, prevent the direct typing of prion isolates. The characterization of prion strains still relies upon bioassay in rodents. Bank vole (*Myodes glareolus*), being susceptible to a wide range of prion sources, offers the opportunity to investigate the biological properties of prion isolates from different species in a single model.

Objectives: To study the biological properties of sCJD subtypes and compare them with animal TSEs.

Methods: We analysed the phenotype of transmission of MM1, MV1, MM2, MV2, and VV2 sCJD subtypes to voles, in comparison with BSE, BASE and classical scrapie isolates from different EU countries. Molecular analysis of PrP^{Sc} from the original isolates preceded voles inoculation. Survival time and attack rate were calculated upon primary transmissions and subsequent passages.

The brain of voles were analysed by WB for PrP^{Sc} type, by Gnd-HCl denaturation for PrP^{Sc} conformational stability, by immunohistochemistry and PET-blot for PrP^{Sc} deposition pattern and by E&E for lesion profile.

Results: This study demonstrated that prion diseases induce in voles a variety of molecular and pathological phenotypes. CJD isolates were grouped into 4 categories: i) MM1/MV1 (n=3), ii) MM2 (n=1), iii) MV2 (n=2) and iv) VV2 (n=1). Scrapie isolates were categorised in at least 4 groups, with no overlapping with sCJD isolates. BSE was distinct from scrapie and sCJD phenotypes. Finally, BASE gave a phenotype distinct from BSE and scrapie but indistinguishable from VV2 sCJD.

Discussion: Overall, the biological classification of sCJD subtypes concurs with their clinico-pathological classification. Similarities in the transmission pattern of prion isolates from different host species were very rare, with the notable exception of BASE and VV2 sCJD. Herein, the meaning of such similarities is discussed in the context of current knowledge on strains and of available tools for their typing.

Selected by the scientific committee from the submitted abstracts

O.11.1

Prions in feces of asymptomatic deer

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Background: Chronic wasting disease (CWD) of several species in the deer family and scrapie of sheep are infectious prion diseases that are transmitted naturally within affected host populations. Even though several potential sources of infectivity have been identified in secretions and excretions from symptomatic animals, the biological importance of these sources in sustaining epidemics remains unclear.

Objective/Methods: Feces from mule deer (Odocoileus hemionus) were periodically collected before and after oral inoculation with CWD prions until the deer developed clinical signs of CWD. Fecal samples were irradiated and intracerebrally inoculated into transgenic mice overexpressing cervid PrP.

Results: We report that asymptomatic CWD-infected mule deer excrete CWD prions in their feces long before they develop clinical signs of prion disease. Intracerebral inoculation of irradiated deer feces into transgenic mice overexpressing cervid PrP revealed infectivity in 14 of 15 fecal samples collected from 5 deer at 7–11 months before the onset of neurological disease. Even though prion concentrations in deer feces were much lower than those in brain tissue from the same deer collected at the disease terminus, the estimated total infectious dose excreted in feces by an infected deer over the disease course may approximate the total contained in brain tissue.

Discussion: Fecal prion excretion over long periods of time by infected deer provides a likely natural mechanism that may explain the high incidence and efficient horizontal transmission of CWD within deer herds, as well as prion transmission among susceptible cervid species.

Selected by the scientific committee from the submitted abstracts

O.11.2

Transmission of bovine-passaged TME prion strain to macaque

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Background: The origin of Transmissible Mink Encephalopathy (TME) remains controversial, with historical evidence for either scrapie or BSE as the source of separate outbreaks. The case for BSE is supported by the experimental transmission of BSE from cattle to mink, whereas scrapie failed to transmit from sheep to mink. Transmission of TME from mink to cynomolgus macaque is inefficient, suggesting a low risk of TSE to human health. Because only typical and atypical BSE prion strains have been shown to be easily transmissible from non-primate to primate species, we have investigated transmissibility to monkeys of a cattle-passaged strain of TME.

Objectives: To compare the transmissibility of cattle-passaged TME prions to the transmissibility of other cattle-passaged prions.

Methods: Monkeys (cynomolgus macaques) were intra-cerebrally infected with classical BSE, atypical BSE strains (BASE and BSE H), and a cattle-passaged TME strain. Animals were regularly monitored for clinical signs, and extensive biochemical and immunohistochemical studies were performed on lymphoid and neural tissues of animals that have already died.

Results and discussion: The animal infected with the cattle-passaged TME strain developed neurological clinical signs after a very short incubation period of 20 months, with a clinical picture that is clearly different from that of BSE/vCJD-infected animals, but similar to that of BASE (the animal is still alive at the time of this writing but post-mortem histopathological and immunohistochemical analyses will provide a more complete characterization of the disease). This new transmission reinforces the notion of human vulnerability to prion diseases passaged through cattle, perhaps due to a low species barrier.

Selected by the scientific committee from the submitted abstracts

O.11.3

Infectivity in skeletal muscle of BASE-infected cattle

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Background: BASE is an atypical form of bovine spongiform encephalopathy caused by a prion strain distinct from that of BSE. Upon experimental transmission to cattle, BASE induces a previously unrecognized disease phenotype marked by mental dullness and progressive atrophy of hind limb musculature. Whether affected muscles contain infectivity is unknown. This is a critical issue since the BASE strain is readily transmissible to a variety of hosts including primates, suggesting that humans may be susceptible.

Objectives: To investigate the distribution of infectivity in peripheral tissues of cattle experimentally infected with BASE.

Methods: Groups of Tg mice expressing bovine PrP (Tgbov XV, n= 7-15/group) were inoculated both i.c. and i.p. with 10% homogenates of a variety of tissues including brain, spleen, cervical lymph node, kidney and skeletal muscle (m. longissimus dorsi) from cattle intracerebrally infected with BASE. No PrPres was detectable in the peripheral tissues used for inoculation either by immunohistochemistry or Western blot.

Results: Mice inoculated with BASE-brain homogenates showed clinical signs of disease with incubation and survival times of 175±15 and 207±12 days. Five out of seven mice challenged with skeletal muscle developed a similar neurological disorder, with incubation and survival times of 380±11 and 410±12 days. At present (700 days after inoculation) mice challenged with the other peripheral tissues are still healthy. The neuropathological phenotype and PrPres type of the affected mice inoculated either with brain or muscle were indistinguishable and matched those of Tgbov XV mice infected with natural BASE.

Discussion: Our data indicate that the skeletal muscle of cattle experimentally infected with BASE contains significant amount of infectivity, at variance with BSE-affected cattle, raising the issue of intraspecies transmission and the potential risk for humans. Experiments are in progress to assess the presence of infectivity in skeletal muscles of natural BASE.

Selected by the scientific committee from the submitted abstracts

O.11.4

The use of zebrafish in prion biology: lessons and perspectives

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Neurodegeneration in prion disease is often regarded as a consequence of abnormal PrP function, yet amazingly little is known about the normal, physiological role of PrP. In particular, the absence of clear phenotypes in PrP knockout mice has made it difficult for scientists to address this important question. Our research is aimed at departing from the use of traditional mammalian models in prion biology. Using morpholino knockdown approaches, we have previously produced strong PrP loss-of-function phenotypes in zebrafish embryos. Morphological and molecular analyses of these phenotypes have revealed that PrP can modulate E-cadherin-based cell-cell adhesion, thereby controlling essential morphogenetic cell movements in the early gastrula. Our experiments with fish, mammalian and invertebrate cells also have shown that PrP itself can elicit homophilic cell-cell adhesion and trigger intracellular signaling via Src-related kinases. Notably, we found that these molecular functions of PrP are conserved from fish to mammals. Using a combination of methods in biochemistry and developmental cell biology, we are now investigating the molecular details of PrP function and its physiological relevance. Here we discuss the use of the zebrafish in prion biology, and how it may help improve our understanding of the roles of PrP in health and disease.

Invited by the scientific committee

O.11.5

Small critical RNAs in the scrapie agent

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Unconventional infectious agents cause transmissible spongiform encephalopathy (TSE) diseases including scrapie and bovine spongiform encephalopathy (BSE) in animals and Creutzfeldt-Jakob disease in humans. The protein only hypothesis claims that the TSE agent is composed solely of the protein called prion (PrPsc). This protein is the misfolded form of a host-encoded cellular protein, PrPc exerting presumably a vital role at the synapse. Even though now widely accepted, the prion concept fails to provide in certain circumstances, a satisfying interpretation of the infectious phenomenon. Using the 263K scrapie-hamster model, we conducted a transmission study to search for a putative prion-associated factor indispensable for infectivity. Here we show that innocuous recombinant prion protein (recPrP) was capable, in a reproducible manner, of transmitting scrapie disease when the protein was β–sheet converted in a solution containing PrPsc-derived RNA material. Analysis of the PrP-RNA mixture revealed the association of recPrP with two prominent populations of small RNA molecules having an average length of about ~27 and ~55 nucleotides. First sequencing data has been obtained and will be discussed. We conclude that the nature of the TSE agent seems to be composed of a nucleoprotein molecular complex, in which informative RNA molecules of small sizes are associated with the misfolded prion protein (PrPsc).

Invited by the scientific committee

Posters

P.1.1

What is the role of amyloid in TSE disease? Examining the accumulation of non infectious PrP amyloid in vivo

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Background: Human P102L Gerstmann-Strδussler-Scheinker (GSS) disease is a familial TSE which is associated with a single amino acid mutation at codon 102 in human PrP. One characteristic of the disease is the accumulation of PrP amyloid plaques in the brain, however two distinct phenotypes of disease are associated with this single amino acid mutation. In classical P102L GSS amyloid plaques and diffuse PrP deposition are accompanied by spongiform degeneration of the brain. In atypical forms of P102L GSS, amyloid deposition occurs in the absence of any spongiosis. We have previously demonstrated that inoculation of atypical P102L GSS into transgenic mice expressing the equivalent mutation in murine PrP (101LL) induces the production of PrP amyloid plaques in recipient mice in the absence of clinical signs of disease or the spongiform degeneration associated with TSEs. Therefore it was concluded that PrP amyloid may not be infectious and may instead seed further PrP amyloid accumulation in the brain, resulting in an outcome unrelated to TSE disease.

Objectives: To further understand this relationship between amyloid and infectivity we aim to identify the role of amyloid in TSE disease by inoculating 101LL Tg mice with recombinant wild type or 101LL murine PrP which has been refolded into either the alpha-monomeric, beta-oligomeric or amyloid isoform.

Results: This experiment is currently in progress and therefore no results are available as of yet.

Discussion: This research will determine whether we can induce amyloid formation in transgenic mice in the absence of infected tissue inoculum, and whether such amyloid forms of PrP are infectious. These experiments could prove that PrP misfolding can be separated from TSE infectivity, and that abnormal forms of PrP can exist in the brain without causing a spongiform encephalopathy.

P.1.2

In vitro species barrier in the SDS-seeding-system

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Background: The conversion of the cellular isoform of the prion protein PrPC to the pathogenic isoform PrPSc is the key event in prion diseases. In earlier studies we presented an in vitro conversion system which simulates the structural transition of recombinant PrP to amyloid fibrils in the presence of low concentrations of SDS.

Objectives: Within our in vitro conversion system we want to analyze in vitro the natural species barrier. We use seeded fibril formation as model system for the infectious disease mechanism and simulate the species barrier by choosing seed and template by different species. We characterize species barrier passage by the kinetics and structure of fibrils.

Methods: The amyloidic character was verified by electron microscopy and fibril formation was obtained by Thioflavin T as specific amyloid marker. The species barrier was simulated utilizing the conversion ability of recPrP into fibril from one species by seeding with prepurified PrPSc from another species and visualized by Thioflavin T fluorescence. Optimum conditions for the conversion of recPrP into fibrils had to be identified and PTA-precipitated prions as seeds.

Results: We present a detailed characterization of the in vitro species barrier, using PTA-precipitated prions as seed and recombinant PrP as Substrat. We analyzed multiple combinations including hamster, sheep and bovine. We could mimic different known species barriers in our in vitro system. We can predict species barriers for unknown combination. By analyzing the conformational state of the template we expect to get insight into infectious prion disease.

Discussion: We applied the conversion assay established in our group for different PrP-species to analyze species barrier. This seeded amyloid forming assay does not require any cellular components, and so we can analyze exclusive by the proteinous components. Our in vitro results reflect the natural species barrier.

P.1.3

Role of Helix-3 methionine redox cycle in the PrP α-fold stability

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Background: The conversion of PrPC into its disease-associated form PrPSc involves a major conformational change and the non quantitative sulfoxidation of methionine side chains. Computational studies have predicted that the substitution of the sulphur atom by a sulfoxide groups at any of the conserved Helix-3 methionines (M206 and M213, singly or in combination) impacts on the α-fold stability allowing a structural flexibility that can facilitate the pathway of pathogenic conversion.

Objectives: To test these predictions, we have designed a mutational approach consisting in M-to-S substitutions in the HaPrP(23-232) chain.

Methods: Molecular dynamics was used to compare the effects of sulfoxidation and M-to-S replacement on the flexibility, stability and energetics of the α-fold of HuPrP(121-229). Site-directed mutagenesis, protein chemistry and spectroscopical techniques were used to characterized the a-folds and stability of HaPrP(23-232) wt and their mutants.

Results: Molecular dynamics studies using HuPrP(125-229) α-fold show that both sulfoxidation of M206-M213 side chains and the M206S-M213S substitution increase the flexibility of the α-fold allowing the population of previously prohibited states. At the experimental level, the use M-to-S mutants, DLS and CD spectroscopy measurements show that replacement of helix3 methionines, singly or in combination, impedes the proper folding of HaPrP(23-232) into the conventional α-state. The found state is featured by the lack of tertiary native contacts, a reduced in secondary structure content and a drastic decrease in thermal stability (Tm shifts from 59°C to 37°C)

Discussion: Mimicked by mutation, oxidation of PrP helix-3 methionines behaves as a conformational switch that destabilizing the native fold favours the conversion into alternative states.

P.1.4

Generation of new prion species by in vitro prion replication

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Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative disorders affecting both humans and animals. TSEs can be of genetic, sporadic or infectious origin. The infectious agent associated with TSEs, termed prion, appears to consist of a single protein which is an abnormal conformer (PrPSc) of a natural host protein (PrPC) that propagates by converting host PrPC into a replica of itself. One of the characteristics of prions is their ability to infect some species and not others and this phenomenon is known as the transmission barrier. In general, the transmission barrier is expressed by an incomplete attack rate and long incubation times which become shorter after serial inoculation passages. The absence of natural TSE cases and/or failed experimental transmissions has suggested that certain species could be resistant for prion diseases. Unfortunately, the molecular basis of the transmission barrier phenomenon is currently unknown and we cannot predict the degree of a species barrier simply by comparing the prion proteins from two species. We have conducted a series of experiments using the Protein Misfolding Cyclic Amplification (PMCA) technique that mimics in vitro some of the fundamental steps involved in prion replication in vivo albeit with accelerated kinetics. We have used this method to efficiently replicate a variety of prion strains from, among others, mice, hamsters, bank voles, deer, cattle, sheep and humans. The in vitro generated PrPres possess key prion features, i.e. they are infectious in vivo and maintain their strain specificity. We are using the PMCA technique to generate infectious PrPres from species hitherto considered to be resistant to prion disease. The correlation between in vivo data and our in vitro results suggest that PMCA is a valuable tool for studying the strength of the transmission barriers between diverse species and for evaluating the potential risks of the newly generated prion species to humans and animals.

P.1.5

MD investigation of the effects of oxidation on the native dynamics of PrP

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Background: The conversion of the cellular prion protein (PrPC) into the infectious form (PrPSc) is the key event in prion induced neurodegenerations. This process is believed to involve a multi-step conformational transition from an α-helical (PrPC) form to a β-sheet-rich (PrPSc) state. In addition to the conformational difference, PrPSc exhibits as covalent signature the sulfoxidation of M213. To investigate whether such modification may play a role in the misfolding process we have studied the impact of methionine oxidation on the dynamics and energetics of the HuPrP(125–229) α-fold.

The experimental verification of computational hypotheses has been carried out by partner 47 (Dr. Maria Gasset).

Objectives: The objectives of our work aim to investigate whether such modification may play a role in the misfolding process. To this end, we have studied the impact of methionine oxidation on the dynamics and energetics of the HuPrP(125-229) α-fold.

Methods: Using molecular dynamics simulation, essential dynamics, correlated motions and signal propagation analysis, we have found that substitution of the sulfur atom of M213 by a sulfoxide group impacts on the stability of the native state increasing the flexibility of regions preceding the site of the modification and perturbing the network of stabilizing interactions. Together, these changes favor the population of alternative states which maybe essential in the productive pathway of the pathogenic conversion. These changes are also observed when the sulfoxidation is placed at M206 and at both, M206 and M213.

Results: The results of our work provide a model for the molecular understanding of the perturbative influence of methionine oxidation on the dynamics of the native state of the Prion protein.

Discussion: The sulfoxidation of Helix-3 methionines emerge as the molecular switch for triggering the initial α-fold destabilization required for the productive pathogenic conversion. The details of the possible conversion pathways will be discussed.

P.1.6

Self-association properties of PrP folding intermediates originated by mutations in Helix-3 methionines

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Background: The conversion of PrPC into its disease-associated form PrPSc involves a major conformational change that obligatory requires the destabilization of the native, α-fold. Computational and biophysical studies have shown that the singly or combined replacement of M206 and M213 impair the α-fold stability.

Objectives: To test whether the mutation-induced α-fold destabilization favours the productive conversion into PrPSc-like polymers we have studied the self-association process triggered by ionic strength at neutral pH.

Methods: Self-association was followed by turbidity, solubility and ThT-binding measurements. The formed polymers were characterized attending to their proteinase K resistant properties as well as their shape as judged by EM and AFM.

Results: Mutation of helix-3 methionines in HaPrP(23-232) causes an α-fold structural intolerance that is accompanied by an enhancement in the aggregation tendency. The formed aggregates display amyloid staining properties, shapes compatible with annular protofibrils arrangements and proteinase K resistance properties reminiscent of PrPSc.

Discussion: Sulfoxidation-mimicking mutation of helix-3 methionines in HaPrP(23-232) provide an excellent framework to experimentally decipher the conformational switches impairing protein native state stability and triggering polymerization events.

P.1.7

Species specificity of the 3F4 epitope and methionine at codon 112 of prion protein

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Background: The epitope of a widely used 3F4 antibody had been believed to contain Met-Lys-His-Met (MKHM) (human PrP109-112) in a critical transition region, mainly based on the indirect sequence alignment observation that 3F4 is able to detect human and hamster PrP but not PrP from other species such as cattle, sheep and cervids, in which M at residue 112 is replaced by Val (V), consistent with data from antigen competition ELISA. But, to date no direct evidence to display binding of 3F4 to synthetic PrP109-112 has been reported.

Objective: To investigate molecular basis of the species specificity of the 3F4 epitope.

Methods: Approaches include peptide membrane array, pepscan analysis, and antigen competition experiments with Western blotting and ELISA. PrP from brains of human, hamster, cervids, and synthesized human and elk prion peptides were examined with various anti-PrP antibodies. Recombinant full-length elk and human PrP (ErePrP and HrePrP) were also examined.

Results: The 3F4 antibody binds to PrP106-110 (KTNMK) highly efficiently but not to PrP109-112 (MKHM) at all. It notably reacts with synthetic elk peptide and ErePrP containing KTNMKHV although substituting V for M at residue 112 lowers its immunoreactivity. Remarkably, while PrP in uninfected elk brain is virtually undetectable, 3F4 readily detects two PrP bands in prion-infected elk samples treated with protease, migrating at ~29 and ~17-18 kDa. In contrast, another anti-PrP antibody 8H4 against human PrP175-185 detects not only the two but also two additional bands migrating at ~23-25 kDa and ~21 kDa.

Discussion: We provide the direct evidence that the minimal residues required for the 3F4 epitope are KTNMK rather than MKHM. However, Met at residue 112 apparently affects the binding of 3F4 to the epitope. (Supported by the CJD Foundation, NIHR01NS062787, NIA AG-14359, CDC UR8/CCU515004, Dutch Ministry of Agriculture, Nature and Food Quality, UK project DEFRA SE1700, and NeuroPrion EC FOOD-CT-2004-506579.)

P.1.8

Conformation-dependent covalent modification of PrP

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Background: The differences between the overall conformation of PrPc and PrPsc are significant enough that the normal form is soluble in non-denaturing detergent, while the diseased form remains insoluble. This difference in propensity for interaction suggests that the surface structures of the two forms differ significantly.

Objectives: We have used a variety of covalently-reactive small molecules to probe this difference.

Methods: We react the proteins with hydrophilic compounds that readily decorate surface accessible peptides but cannot diffuse into the protein core. Then we reduce, alkylate, and trypsinize the protein, as usual for proteomic study. Mass spectrometry then provides a readout of the extent of amino acid residue modification.

Results: We found many differences in surface accessibility for different PrP conformations. Some residues remain completely unreacted in one form and become completely reacted in another form.

Discussion: The structural information provided by characteristic differences in reactivity should assist us in developing a conformation-specific assay. We have outlined our discovery in US Patent Application Serial Number 12/251,456, and here present data supporting our hypothesis and claims.

P.1.9

Correlating prion disease susceptibility in animals with the tendency of their prion proteins to adopt β-sheet rich structures in vitro

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Background: Prion diseases successfully propagate in animals when host, α-helical prion proteins (PrPC) are converted to aggregated or oligomeric, toxic forms that are rich in β-sheet structure (PrPSc). This conversion process is thought to be facilitated by PrP folding intermediates that contain high β-sheet content. Human prions and sheep scrapie have successfully been propagated in susceptible animals such as hamsters and mice, yet rabbits show a greater resistance to prions. Our hypothesis draws a correlation between species susceptibility and the tendency of the animal's prion protein to adopt β-sheet, equilibrium folding intermediates.

Objectives: 1) To compare the relative populations of β-sheet, equilibrium folding intermediate(s), or 'β-State PrP', between hamster, mouse and rabbit PrP. 2) To determine the molecular weight(s) of species that form β-State PrP. 3) To determine the toxicity of β-State PrP.

Methods: 1) The relative abundance of PrP in the 'β-State' was calculated by performing equilibrium, urea-denaturation on recombinant PrP 90-231, monitored by circular dichroism at wavelengths 220 and 229 nm between pH 7 and 4. 2) Equilibrium analytical ultracentrifugation (AUC) and size exclusion chromatography (SEC) experiments were performed on β-State PrP. 3) Cell toxicity assay were performed on β-State PrP.

Results: At pH 4.5 and 4.0, both hamster and mouse promote greater fractions of β-State PrP than rabbit. Hamster, mouse and rabbit PrP all populate both an octameric and monomeric species containing high β-sheet content. Cell toxicity assay results are expected soon.

Discussion: Our results indicate that although hamster, mouse and rabbit PrP are all capable of forming very similar β-sheet, equilibrium folding intermediates, hamster and mouse PrP thermodynamically favour β-State PrP in comparison to rabbit PrP due to key amino acid differences. Thus, the stability of β-State PrP depends on the amino acid sequence of PrP, and is correlated with prion disease susceptibility in animals.

P.1.10

Conformational plasticity of the recombinant bovine PrP

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Background: The existence of prion strains and their capacity of overcoming species barriers seem to point out high conformational adaptability of prion protein. Amyloid fibrils formed by the same prion protein show structural polymorphism which appears to underlie prion strain diversity. It is important to determine how structural polymorphism develops during the process of amyloid fibrils formation by the prion protein.

Objective: The goal of the present study is to characterize the fibrilization pathways of the truncated bovine recombinant prion protein (rPrP) at different concentrations of urea, GdnHCl, pH and compare formed fibrils with those of other rPrP.

Results: Bovine rPrP protein (102-240 a.a.) spontaneously assembled in 7 types of fibrils under acid conditions (pH 3,7- 4.5) in presence of 2-4 M of denaturing agents (urea or GdnHCl). After one hour of incubation, numerous flexible worm-like (WL) fibrils(width ~ 12 nm) with no obvious periodic substructure along their axis appeared. After 5 hours of incubation, rigid polimorphic fibrils (RP) start to form on the background of WL fibrils. RP fibrils presented: a) straight and flexible ribbon-like twisting structures (length 150-300 nm, crossover spacing 130-160 nm); b) side-by side associated two filaments (length 120-250nm, width 25-34nm), c) straight, non-periodic fibrils (length 240nm, width 16nm); d) short rod-like(RL) structures (length 25-80nm, width 7.5-24 nm).WL persisted in the solution until the end of incubation. Partial resistance to standard PK-digestion was demonstrated for all types of named fibrils.

Discussion: The assumption is that the observed aggregation mechanism is concurrent self-assembly for recombinant mammalian prion protein into WL and RP fibrils under above mentioned conditions. We propose two routes of forming WL and RP fibrils. One pathway represents non-nucleated growth of WL fibrils. In contrast to WL structures, the second pathway occurs by nucleation - dependent growth, characterized by lag-phase, resulting in the formation of RP fibrils. Structural polymorphism of fibrils, generated by bovine rec-PrP in vitro, may correlate with the unusual expanded multi-species tropism of infectious BSE-prion protein variants and partially may explain the overcoming of interspecies molecular barriers in vivo.

P.1.11

Biophysical characterization of the prion conversion process in vitro

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Background: Multiple methods can be used to convert prion protein from its native, monomeric, highly helical form (called PrPc) to a largely β-sheet, multimeric, soluble aggregate (called PrP β), which is an intermediate in conversion from PrPc to PrPsc.

Methods: In this study, we used 3 methods for converting truncated recombinant Syrian hamster prion (shPrP(90-231)): 1) acid titration, 2) incubation with salt and urea and 3) reduction with TCEP. NMR spectroscopy was used in combination with other biophysical techniques (circular dichroism, proteinase K digestion, electron microscopy, dynamic light scattering and fluorescence) to characterize each conversion process and the resulting aggregate in detail. This comprehensive characterization allowed us to compare pathways for conversion to provide insight into the mechanisms of prion protein misfolding.

Results: While early stages of conversion for all three methods were consistently associated with changes in particular areas of the protein, specifically destabilization of regions of Helix 2 and Helix 3 followed by destabilization of Beta strand 2, the conversion processes differed in certain important details as well. There were also morphological differences in the structure of the aggregates generated by each process. For instance, conversion with urea and salt caused fibril formation, while pH-based conversion did not.

Discussion: This suggests that subtle differences in the intermediates of PrPc to PrP β conversion may influence the PrP β to PrPsc conversion process, potentially resulting in differing final assemblies of PrPsc.

P.1.12

Aggregation and amyloid fibril formation induced by chemical dimerization of recombinant prion protein in physiological-like conditions

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Background: Substantial efforts have been devoted to reconstitute de novo misfolding of PrP-C and prion infectivity and/or toxicity. Yet, misfolding of recombinant PrP-C in the absence of PrP-Res template, cellular factors, denaturing agents, or at neutral pH has not been achieved. A number of studies indicate that dimerization of PrP-C may be a key step in the aggregation process.

Objectives: In an effort to understand the molecular event that may activate misfolding of PrP-C in more relevant physiological conditions, we tested if enforced dimerization of PrP-C may induce a conformational change reminiscent of the conversion of PrP-C to PrP-Res.

Methods: We used a well described inducible dimerization strategy whereby a chimeric PrP-C composed of a modified FK506-binding protein (Fv) fused with PrP-C and termed Fv-PrP is incubated in the presence of a monomeric FK506 or dimerizing AP20187 ligand.

Results: Addition of AP20187 but not FK506 to recombinant Fv-PrP (rFv-PrP) in physiological-like conditions resulted in a rapid conformational change characterized by an increase in beta-sheet structure and simultaneous aggregation of the protein. Aggregates were partially resistant to proteinase K and induced the conversion of soluble rFv-PrP in serial seeding experiments. rFv-PrP aggregates could also induce the conversion of soluble rPrP into a proteinase-K resistant isoform. As judged from thioflavin T binding and electron microscopy, rFv-PrP aggregates converted to amyloid fibres after 24 hours of incubation with AP20187. Aggregates were toxic to cultured cells whilst soluble rFv-PrP and amyloid fibres were harmless.

Discussion: This study strongly supports the proposition that dimerization of PrP-C is a key pathological primary event in the conversion of PrP-C and may initiate the pathogenesis of prion diseases. This proposition will be tested in transgenic mice expressing Fv-PrP. In future studies, it will be important to determine what triggers the dimerization of PrP-C in vivo.

P.1.13

Efficient in vitro amplification of murine BSE (301C) in spleen and blood

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Background: Transmissible spongiform encephalopathy (TSE) is a fatal neurodegenerative disorder, which is a so-called as prion disease due to the causative agent(PrPsc). Recently, it has become to detect very small amounts of PrPsc by combining the protein misfolding cyclic amplification(PMCA)and Western blotting.

Objectives: Utilizing PMCA, the in vitro conversion of PrPC into PrPSc in spleen and blood of which mice are inoculated intraperitoneally with murine BSE strain 301Cand sequentially culled.

Methods: Total of 58 C57BL mice were inoculated intraperiotoneally with 0.02ml of a 1% (w/v) 301C BSE strain. Five mice were sacrificed sequentially (30 – 450 dpi). Half of the brain and spleen were immediately frozen for WB and PMCA or fixed in 10% formalin for IHC . Normal and BSE affected brains were removed and the homogenates(10%, W/V) were prepared in PMCA conversion buffer. Blood was prepared as buffy coat or plasma. Positive samples were first diluted to 1:5 or 1:10 in normal brain homogenate (NBH). One hundred microliter if each incubated at 37°C in a Misonix sonicator 3000 programmed for 48 cycles of 30 seconds pulse (at power level 5) plus 30 minute incubation. The samples (20ul) after each round of amplification were subjected to eletrophoresis and immunoblotting.

Results: The PMCA generated PrPSc from spleen and blood efficiently convert the conformation of PrPC using brain as substracts. Some biochemical features of PMCA-generated PrPSc are alike the original murine PrPBSE. At the early stage of the incubation period (30dpi), we detected PrPSc in the blood was probably originated from the pheripheral replication of prions. Detection time (30, 180, 240 dpi) in blood was similar to one in spleen.

Discussion: The PrPSc generated from peripheral tissues can further propagate in the brain. It suggests the possibility to substitute PMCA with mice bioassay to investigate the propagation of prion.

P.1.14

Monitoring the prion protein stability by NMR

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Background: Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a group of fatal neurological diseases that affect both humans and animals. The generally accepted protein-only hypothesis suggests that the infectious agent causing prion diseases is composed mainly, if not entirely, of endogenous protein. There are two major forms of prion protein: the native non-infectious form (PrPC), and the misfolded infectious form (PrPSc). The structure of PrPC is mainly α-helical with the exception of a small anti-parallel β-sheet, whereas PrPSc corresponds to an assembly of β-sheet forming amyloid fibrils. About 30 structures of the globular portion of PrPC have been characterized for diverse organisms with different TSE strain susceptibilities. The few, minor structural differences between the PrPC proteins suggest that the key to understanding prion formation lies in the conversion between PrPC and PrPSc.

Objectives: To identify the possible regions responsible for the conversion of PrPC into an unfolding or fibril state, we are using nuclear magnetic resonance (NMR) spectroscopic methods to characterize the stability of PrPC.

Methods: We have monitored the chemical environment changes in the C-terminal domain of bovine and rabbit PrPC during urea-induced unfolding using 1D and 2D proton NMR spectroscopy.

Results and Discussion: Our results suggest that the stability of the small β-sheet of bovine PrPC is perturbed early in the denaturation process, whilst helix α3 remains in contact with helix α1 and helix α2 even in the presence of high concentrations of urea. These results support the hypothesis that a destabilization of the β-sheet is an important step in order for PrPC to structurally transform into PrPSc. This could have significant implication in our understanding of the different polymorphisms and mutations that occur naturally in the PRNP gene, where a destabilized β-sheet would promote conformational changes that lead to PrPSc. A comparison between rabbit and bovine PrPC is also presented.

P.1.15

Formation of protease-resistant PrP aggregates in neuroblastoma cells devoid of prion infectivity

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Background: Prions, the infectious agents of TSEs consist of an abnormal form of host-encoded prion protein (PrPc) and replicate in a self-perpetuating process by protein conversion of PrPc. PrPSc, a beta-sheet rich conformer of PrPc is partially resistant to proteases and is believed to represent the infectious prion.

Objectives: To develop a cellular model for the identification of susceptibility factors. To distinguish bona fide prions from spontaneously formed PrP aggregates.

Methods: Infectious titres were determined by mouse bioassay. The kinetic of prion propagation was assessed by Scrapie Cell Assay (SCA).

Results: Unexpectedly, PrPc overexpression gave rise to PK-res PrP aggregates, as detected by SCA. PK-res PrP was also detected after treatment with lactacystin (LC), an inhibitor of the proteasome that leads to PrP aggregation in the cytoplasm. However, homogenates from cells producing spontaneous PK-resistant PrP were not infectious on bioassay, in contrast to prion-propagating cells. Similarly, in the SCA, only incubations with mouse prions resulted in a progressive increase of the number of PrPSc-positive cells, indicative of de-novo prion replication. An increase of PK concentration from 2 to 9 mU/ml fully overcame PK-resistance in LC-treated and PrPc-overexpressing, but not in prion-infected cells, which enabled us to determine the relationship between PrPc expression levels and prion susceptibility. Remarkably, overexpression of PrPc did not render revertants susceptible, whereas reconstitution of Prnp-silenced PK1 cells with PrPc restored their susceptibility, indicating that PrPc is necessary, but not sufficient for prion propagation in neuroblastoma cells.

Discussion: Resistance of spontaneously formed PrP aggregates to PK did not correlate with prion infectivity. PrP aggregates could be distinguished from bona fide prions by their lack of accrual over time by SCA. Cell-based assays with susceptibilities to a wider range of prion strains may be used to distinguish proteinopathies from prion diseases.

P.1.16

Characterization of the molecular heterogeneity of mutant prion proteins

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Background: Human inherited prion diseases including Creutzfeldt-Jakob disease (CJD), Gerstmann-Strδussler-Scheinker (GSS) syndrome or fatal familial insomnia (FFI), are fatal neurodegenerative disorders, linked to point or insertional mutations in the prion protein gene and associated with distinct clinical and neuropathological phenotypes. The molecular mechanism of such heterogeneity is not clear. It is postulated that different pathogenic mutations induce PrP to fold into distinct abnormal structures that are selectively toxic to specific neuronal populations of the brain.

Objectives: Our project is directed toward illuminating the correlation between the folding of mutant PrP molecules and their selective toxicity.

Methods: We generated transgenic mice expressing the mouse PrP homologues of a nine-octapeptide insertion associated with GSS and the D178N/V129 and D178N/M129 mutations linked respectively to CJD and FFI.

Results: We have found that these mutant proteins acquire biochemical properties typical of pathogenic PrP, including insolubility in non denaturing detergents and protease resistance.

We developed a procedure for purifying mutant PrP aggregates from the brains of Tg mice by sequential centrifugations and immunoprecipitation. Here we describe two new assays for studying the conformation of mutant PrP molecules based on surface plasmon resonance and mass spectrometry.

Discussion: These approaches are starting to identify the structural diversity of different mutant PrP aggregates and their soluble precursors. Our approach offers a new opportunity to defining the structural features of mutant PrPs underlying inherited prion disease heterogeneity.

P.1.17

Structural characterization of PrP(106-126): insights into prion and amyloid formation

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Background: The formation of fibrillar aggregates is involved in many diseases including Alzheimer's and Creutzfeldt-Jakob. While these fibrillar aggregates have long been the indicators of these diseases, it has recently been theorized that small, oligomeric forms of proteins involved in amyloid diseases are the true toxic entity. The peptide comprising residues 106-126 of the human prion protein forms amyloid fibrils as well as small spherical oligomers, thus making it a good model for amyloid disease. Furthermore, this region has been proposed to be involved in the conversion of full-length, nascent prion protein (PrPC) to the disease form (PrPSc).

Objectives: The objective of this work is to characterize the molecular structure of amyloid oligomers formed by PrP(106-126).

Methods: Structural characterization is achieved through the use of solid-state nuclear magnetic resonance (NMR), solution NMR and transmission electron microscopy. This is the first report of solution NMR constraints for the determination of amyloid oligomer structure in the absence of detergents or exogenous solutes.

Results: We have recently reported the molecular structure of the amyloid fibrils, as well as the morphology of the stable oligomers formed by this peptide. Here, we present a detailed structure of the basic subunit of the oligomers of PrP(106-126). Solid state NMR experiments indicate that the oligomers contain parallel β-sheets in an anti-parallel stacking similar to that of the fibrils. Solution NMR data reveal details into the dynamics of the N-terminus of PrP(106-126) oligomers.

Discussion: Our results present the first detailed structure for amyloid oligomers of PrP(106-126). The realization of a basic fibrillar subunit for soluble oligomers of this peptide provides a basis for the further study of its conversion pathway, as well as the conversion of small molecular weight oligomers into fibrils.

P.1.18

Solvation of prion proteins studied by three-dimensional molecular theory of liquids

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Background: Accurate theoretical description of biomolecules in physiological conditions requires explicit accounting for solvent degrees of freedom in all-atom molecular dynamic simulations. Even with modern computers such simulations are unfeasible for large biomolecules such as prion proteins. Simplified continuum methods based on the implicit solvation models give good estimation only for electrostatic part of solvation free energy.

Objectives: To develop a theoretical foundation for proper treatment of both electrostatic and non-polar solvation effects at the statistical-mechanical level; to explore the free energy landscape of amyloids in periodic boundaries and beta-sheet prion oligomers; to study all stages of oligomerization based on the coarse-grained models with full account for molecular solvation structure and thermodynamics.

Methods: The integral equation theory of molecular liquids, (aka 3D-RISM-KH). The method is based on the first principles of statistical mechanics and provides complete information about solvation thermodynamics and hydration structure of macromolecules. The approach has been proven to be efficient and capable of predicting self-assembly, conformational stability and transitions of synthetic organic nanoarchitectures as well as microtubular architectures of tubulin.

Results: We implemented a combination of molecular dynamics and 3D-RISM-KH theory and studied the association thermodynamics and hydration effects for prion amyloid fibrils and beta-sheet oligomers under different solvent conditions (composition, level of pH, ionic strength), including structural stability, self-assembly and possible locations of water molecules and solvent ions in the interior cavities.

Discussion: The 3D-RISM-KH theory explicitly accounts for the effects of chemical specificity of molecular and ionic species on solvation structure and dynamics (in particular, on aggregation and fibril formation), including hydrogen bonding and hydrophobic effects, provides information on internal hydration.

P.1.19

Probing prion protein unfolding by molecular dynamics and three-dimensional molecular theory of solvation

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Background: Misfolding and oligomerization of prion proteins (PrP) are associated with transmissible spongiform encephalopathies. Insolubility and propensity to form aggregates complicate experimental study of infectious isoform of prion proteins. Molecular mechanisms of PrP conversion and molecular structure of infectious prions remain unknown.

Objectives: To study pathways of human PrP misfolding at different solvent conditions, to provide insight into thermodynamics of conformation transitions of prion proteins upon unfolding.

Methods: The three-dimensional molecular theory of solvation (also known as 3D-RISM-KH), molecular mechanics and all-atom molecular dynamics (MD) simulations.

Results: In the all-atom explicit solvent MD simulations, the C-terminal parts of helices H2 and H3 show a tendency for unfolding. In long run, beta-strands may appear in these parts of protein. C-terminus of helix H1 repeatedly unfolds and refolds during the early stages of unfolding, but remains relatively stable, being detached from H2-H3 bundle. Beta-sheet S1-S2 extends to the proline containing part of the protein. Helix H1 may dissolve into two small helices and remains very flexible in the course of simulations. The quantitative description of the correlations between gains in solvation free energy, losses in intra-protein energy from the one hand, and the helical content changes upon unfolding, solvent accessible surface area of the hydrophobic residues and hydrophathy index, has been made. A possible unfolding transition state has been identified.

Discussion: We found that the pathways of prion protein unfolding at elevated temperatures are sensitive to the solvation models used in MD simulations. This can partly account for the discrepancy between the results of some molecular dynamics studies and experimental data. In our study, to avoid the bias due to a sampling method, we analyze the thermodynamics of prion protein unfolding based on reliable structural descriptors.

P.1.20

Thermodynamics of amyloidogenesis: molecular theory of solvation perspective

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Background: Solvation effects play a crucial role at different stages of amyloidogenesis. Whereas the initial misfolding of prion proteins was studied based on the all-atom explicit solvent molecular dynamics simulations, the formation of oligomers and their conversion into amyloid fibrils have been analyzed so far using course-grained models with implicit solvent.

Objectives: Accurate statistical-mechanical description of solvation without compromising the microscopic structural and non-polar solvation effects for studying all stages of amyloidogenesis, from misfolding to fibrillization.

Methods: Molecular mechanics, molecular dynamics and the statistical-mechanical three dimensional molecular theory of solvation (3D-RISM-KH).

Results: Enthalpic desolvation barrier has been identified as an important feature of the free energy landscape of amyloid fibrils. Inter-protein interface is characterized by a high degree of shape complementarity as it follows from a significant contribution of dispersion interactions to the association free energy. A second important physical factor favoring stability of fibrils is solvation entropy. Water and ion channels are identified for different fibrillous aggregates, including HET-s prion amyloid fibril, with implication that the internal hydration may play an important role in association thermodynamics of beta-sheet oligomers and fibrils.

Discussions: The study provides the exact balance between different contributions to the association free energy and quantitative characterization of the solvation entropic (hydrophobic) effects for prion fibrils and beta-sheet oligomers. We show that in acidic conditions (as well as for some point mutations) the changes in solvent organization, solvation entropy and dispersion interactions due to conformational reconstruction caused by destabilization of salt bridges, may dominate over changes in electrostatic interactions. We show that the above can explain the differences in pathways of oligomerization at different solvent pH conditions.

P.1.21

Role of plasma co-factors in misfolding and /or propagation of misfolded prion proteins

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Background: Accumulating evidence suggests that accessory factors may play an important role in the misfolding of protease-resistant prion protein (PrPSc). Data collected in our laboratory demonstrates that plasma proteins appear to modulate the misfolding and templating properties of PrPSc and may contribute to the species barrier associated with disease. Furthermore, we have observed apparent de novo synthesis of PrPSc when plasma and normal brain homogenates from the same animal are combined together and amplified in vitro.

Objectives: Characterize the species-specific effect of plasma proteins on amplification and templating properties of PrPSc.

Methods: Protein misfolding cyclic amplification (PMCA) was used to characterize the effects of conspecific (i.e., same species) and xenospecific (i.e., different species) plasma on amplification of 263K hamster scrapie in vitro and formation of de novo synthesis of PrPSc from different species of animals.

Results: Similar to what has been reported in the literature, we have observed an inhibitory effect of xenospecific plasma (bovine, sheep) on amplification of PrPSc in the PMCA assay seeded with 263K scrapie. However, when conspecific plasma (i.e., 10% hamster plasma) is used in assays, the PMCA is greatly enhanced and formation of PrPSc in vitro is accelerated. Moreover, apparent de novo synthesis of PrPSc by PMCA can be readily achieved by mixing normal hamster brain homogenates with conspecific plasma (hamster) but is not as efficient with xenospecific plasma. Similar findings were observed when normal brain homogenates and conspecific plasma were mixed from a variety of different animal species and subjected to PMCA.

Discussion: The species-specific effects of conspecific plasma suggest that the infectious particle may represent a complex aggregate of multiple host specific molecules, of which PrP is essential, but may require accessory host-specific proteins to confer templating properties for propagation of the infectious particle.

P.1.22

Molecular studies of Abeta amyloid aggregation in Podospora anserina

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Background: Aggregation of amyloid proteins is involved in neurodegenerative disorders such as Alzheimer's disease (AD), type 2 diabetes, Parkinson's disease, and prion diseases. Appearance and transmission of several prions in yeast and fungi are known being supported by amyloid aggregates of particular proteins, for ex. Ure2p in S. cerevisiae and HET-s in Podospora anserina. However, this concept of infectious protein may be extended to other amyloid proteins or peptides. Indeed, it has been recently shown that Abeta amyloid deposits from human patients are able to induce AD like phenotype in a mouse model. Our previous works on the HET-s prion of P. anserina allowed to demonstrate infectivity of HET-s protein and to correlate in vitro structural elements with in vivo aggregates propagation.

Objectives: In AD, it becomes obvious that a crucial point in designing therapeutics and molecular markers is to define the features of in vivo Abeta aggregation.

Methods: In order to study in vivo aggregation of Abeta amyloid peptides, we expressed them as Green Fluorescence fusion protein in P. anserina. We observed effects of site direct mutagenesis of Abeta sequence on the fluorescence distribution. Aggregation state of Abeta peptides were analyzed by centrifugation and gel electrophoresis on crude extracts.

Results: We found that Abeta peptides are able to aggregate spontaneously and uniformly when expressed in Podospora. The level of spontaneous aggregation was depending of the protein fused to Abeta. In vivo Abeta aggregation can be suppressed by replacement of some key amino acids with a proline. At the opposite, Familial Alzheimer Disease mutations affecting Abeta sequence enhanced in vivo aggregation of Abeta peptides.

Discussion: Our results indicated that a systematic mutagenesis of Abeta sequence should permit to correlate in vivo aggregation with in vitro structural elements. Our molecular studies should bring significant advance in the understanding of in vivo Abeta aggregation.

P.1.23

Relationship between protease resistance and aggregation state of the scrapie prion protein in sporadic CJD

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Background: Previous studies, mainly focusing on animal prion disease, identified a PK-sensitive form of PrPSc, which was found to be less aggregated than its resistant counterpart.

Objectives: To investigate relationship between PK resistance and aggregation state in PrPSc and determine whether the PK-sensitive form of PrPSc constitutes a significant component of abnormal prion protein aggregates in sporadic CJD.

Methods: PrPSc from the frontal cortex was purified through sample dilution in sarkosyl and sequential ultracentrifugations in sucrose cushion and pellet resuspensions by sonication. PrPSc aggregates of different size were isolated after ultracentrifugation in sucrose gradient and collection of 12 fractions. An aliquot from each fraction was digested with PK 50 µg/ml at pH 7.4. Protease treated and untreated PrPSc from each fraction were measured semiquantitatively by Western blotting.

Results: In all CJD subtypes PrPSc was mainly distributed in fractions 5-12. However, a fully protease-sensitive minor component accounting for less than 10% of total signal was also collected in fractions 1-4. PrPSc in fractions 4-5 to 12 showed a partial protease resistance which seems to progressively increase from fractions 5 to 9. Preliminary analyses showed that PrPSc distribution among fractions did not significantly differ among subtypes, except for a low increase of the protein in fractions from 1 to 6 in some subtypes.

Discussion: Our results seems to confirm the existence of a PK-sensitive PrPSc form in sCJD. PrPSc seems to require a minimum of aggregation size to become PK-resistant and its resistance seems to at least partially parallel its aggregation size. Differences in PrPSc aggregation pattern among sCJD subtypes and their possible influence on the relative protease resistance of the abnormal protein remain to be better investigated.

P.1.24

Structural characterization of β-octamers formed by ShaPrP(90-231)F198S

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Background: Non-fibrillar assemblies of the mammalian prion have been implicated as important species for infection and cell death, however little is known of their molecular structure. A structural model for smaller oligomers of PrP will allow us to better understand their relationship with PrPSc, and to determine their potential role in the pathogenesis of prion diseases.

Objectives: Using a biophysical and structural biology approach, we are working towards a detailed characterization of the molecular structure and dynamic behaviour of β-sheet rich octamers formed during the misfolding of Sha PrP(90-231)F198S.

Methods: Several biophysical methods, including transmission electron microscopy, circular dichroism, dynamic light scattering and analytical ultracentrifugation, have been used to define the secondary structure and morphology of β-octamers of PrP. More detailed structural information is obtained from solid state NMR of uniformly and selectively 13C, 15N enriched samples.

Results: We have identified sample conditions under which folded ShaPrP(90-231)F198S will form an apparently stable β-sheet containing octamer, which exhibits proteinase K resistance similar to that reported for PrPSc and for amyloid fibrils formed by ShaPrP. Details of local structure, dynamic behaviour, and quaternary interactions between PrP monomers within the octamer have been obtained from solid state NMR, and allow us to compare initial models for these non-fibrillar assemblies with the structure of monomeric PrPC, and with the proposed models for the fibrillar forms of PrP.

Discussion: As initial progress towards characterizing putative misfolding intermediates or small, non-fibrillar assemblies of PrP, we have used an array of biophysical methods, focusing on solid state NMR, to characterize stable β-octamers formed by ShaPrP(90-231)F198S under slightly acidic conditions. Current models for this non-fibrillar assembly of misfolded PrP will be presented, based on our current structural and morphological data.

P.2.1

Studies of immune responses to PrP during prion infection: role of regulatory T cells

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Background: Infections are usually diagnosed by detecting the pathogenic agent or a specific immune response. In Creutzfeld-Jakob disease, the strong immune tolerance to self-PrP prevents specific antibody response to PrPSc and precludes the analysis of immunological events developing during the incubation period. Our recent findings suggest that accumulation of PrPres in the spleen during infection with murine scrapie(139A)interfere negatively with the development of T and B cell responses to a major class II-restricted PrP peptide(P9: PrP158-187). CD4+CD25+ Foxp3+ regulatory T (Tregs) cells are one mechanism of peripheral tolerance that may explain specific unresponsiveness.

Objectives: Here, we investigated the impact of Tregs on the development of anti-PrP immune effectors either during the natural course of disease or after vaccination with P9. Such a phenomenon has never been explored in prion diseases.

Methods: Foxp3EGFP mice were used in Trequantification and transfer experiments

Results: We found that CD4+Foxp3+ T cells accumulate in secondary lymphoid organs but not in blood of mice infected ip with 139A. We also found that in vivo depletion of Tregs by anti-CD25 mAb increase the accumulation of splenic PrPres at 10 weeks post 139A infection. In contrast, transfer of CD4+ Foxp3+ in newly infected mice reduced the PrPres load indicating that Tregs are unexpectedly implicated in the control of infection. The suppressive activity of Tregs derived from infected and non-infected mice is currently being tested. Last, in P9 vaccination studies, we showed that Treg depletion leads to a complete recovery of the T cell response to the P9 epitope.

Discussion: Our results suggest that the adaptive immune system and more particularly Foxp3+ regulatory T cells react to prion infection and have a direct impact on the natural course of the disease. Characterization of the role and specificity of Foxp3+ regulatory T cells will be useful to clarify the mechanisms of peripheral tolerance to PrPSc and design adequate immunotherapy.

P.2.2

Vaccination with class-I restricted PrP peptides induces cytotoxic CD8+ T cells and prolongs the clinical phase duration of murine scrapie

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Background: Vaccination of C57BL/6 wt mice with self-PrP may induce protective antibodies against prion disease but also inflammatory or cytotoxic T cells which may result in brain damages

Objectives: We hypothesized that CD8+ T cells might also eliminate cells expressing high levels of PrPc or PrPSc.

Methods: We first designed PrP peptides restricted to MHC class I (H2-Db) able to generate anti-PrP specific CD8+ T cells. To circumvent central tolerance, we chose PrP-derived peptides with medium affinity for the H2-Db molecule that are more likely able to recruit a PrP-specific CD8+ T cell repertoire in periphery. Peptides affinity was increased by modifications that improve their binding to H2-Db and thus their immunogenicity.

Results: Some of these "modified" peptides , when given together with CpG, recruited T cells in vitro and in vivo cytotoxic for target cells pulsed with the modified peptide, or, at a much lower level, with the corresponding "natural" peptide. Thus, it was necessary to improve further their immunogenicity. We used adenoviral vectors (Ad) expressing minigenes that induce overexpression of our peptides associated with H2-Db at the surface of infected cells. This approach dramatically enhanced the frequency of peptide-specific CD8+ T cells. Next, we determined the benefice/risks of such immunization protocols. Peptides associated with CpG, did not influence prion infection, in terms of incubation or clinical stage durations. In contrast, Ad strategy significantly increased clinical stage duration but not incubation; control Ad also increased the duration of symptoms but at a lesser extent. No clinical signs of autoimmunity were observed in Ad-immunized mice. Histological analysis revealed CD3+ T cell brain infiltration in Ad-control immunized compared to unimmunized infected mice but the number of T cells was significantly higher in peptide-expressing Ad-immunized mice.

Discussion: Altogether, the data suggest that prolongation of the neuroinvasion period is associated with T cell activation.

P.2.3

Antisense oligonucleotides decrease the cellular prion expression in vitro

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Background: Until now effective medicines for prophylaxis and treatment of prion infections was not developed. One of perspective strategies of development of these drugs is a search of compounds which will be able to reduce of cellular prion expression level, because this protein is a necessary component in the process of pathogenesis of transmissible spongiform encephalopathies.

Objectives: In agreement with this, the aim of our study was the decreasing of cellular prion expression level under the influence of complementary to prion mRNA antisense phosphorothioate-modified oligodeoxyribonucleotides (psODN), conjugated with polydymethylaminoethylmetacrylate (pDMAEM) nanoparticles.

Methods: L1210 (mouse lymphocytic leukemia) cells were treated with psODNconjugated with pDMAEM nanoparticles and after 4, 10 and 24 h incubation the level of cellular prion was measured.

Results: It was shown that psODNs complementary to the cap area, start codon area and short middle part of the open reading frame (ORF) of the prion protein mRNA are able to reduce the level of cellular prion expression on 95 % in the cell line L1210. The psODNs complementary to the start codon area was the most effective after 4 h incubation of cells with psODN. On the other hand, after 10 h incubation psODN complementary to the small part of ORF had highest efficiency against prion mRNA. After 24 h of incubation all investigated psODN was showed the very similar efficiency (5 % from a control level of prion expression).

Discussion: These results give a ground for the future investigations of asODNs as therapeutic drugs for prion infections prophylaxis and treatment.

P.2.4

Ante mortem diagnosis of sheep scrapie by single particle counting

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Background: Prion diseases can be transmitted via body fluids. Four cases of variant CJD had recently been reported, that were most likely caused by transfusion of contaminated blood. This raised serious concerns about blood product safety, and emphasizes the need of a robust test system to detect prions in living humans or animals.

Objectives: Our present goal is to detect prions in blood of Scrapie-afflicted sheep. We present the application of an ultra-sensitive diagnostic assay based on surface-FIDA that allows us to count single prion particles.

Methods: Prion aggregates are immobilized via a capture antibody to a chip surface, are labelled with fluorescent antibodies, and detected by confocal laser scanning. To optimize the surface-FIDA assay in respect to characteristic parameters of prion aggregates, we employ a Dual-Color Fluorescence Correlation Spectroscope (FCS) equipped with an XY-scanning piezo unit. The fluorescence intensities were recorded dependent on focus position and can be translated into image data. Using antibodies with different fluorescent labels and evaluating the crosscorrelation increases the specificity of detection.

Results: In order to estimate the applicability of our assay to blood plasma we added in a first step recombinant prion protein aggregates to ovine plasma. PrP epitopes are masked by plasma components such as LDL and thus antibody detection is impaired. To address this issue we developed a purification method employing Sarkosyl and a mixture of lipases. This treatment leads to a recovery of signal intensity suggesting that PrP epitopes become accessible for both capture and detection. A serial measurement of blinded plasma samples from clinical sheeps was conducted. Preliminary data showed that 6 out of 10 positive samples could be clearly differentiated from 5 negative control samples.

Discussion: Next we will analyze samples from preclinical stages. Moreover, we aim to apply our cell-extract-free amplification system in order to increase the sensitivity of the assay.

P.2.5

Single partikel counting of Creutzfeldt-Jacob disease particles

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Background: The agents of prion diseases are composed primarily of the pathogenic isoform of the prion protein designated PrPSc, which is generated by a conformational change of the cellular isoform PrPC. In contrast to its cellular isoform, the pathogenic isoform PrPSc forms insoluble aggregates. Hitherto approved prion tests use the Proteinase K (PK) -resistance of PrPSc as a marker for the disease. Because of varying portions of PK-resistant PrPSc we developed a test, which does not rely on PK-resistance. Our diagnostic approach is based on the aggregated state of PrPSc as marker.

Objectives: Processing of samples without Proteinase K digestion - Detection and counting single prion particles - Development of the test system for early diagnostic of Creutzfeldt-Jakob disease (CJD).

Methods: Our diagnostic approach is based on surface-FIDA (fluorescence intensity distribution analysis). To develop the method prion particles from brain homogenate of sCJD-patients are pre-purified by PTA-precipitation and immobilized by capture antibodies to a chip surface. The prion particles are labelled by two different fluorescent labelled antibodies. The surface is scanned by two laser beams systematically which allows us the imaging of even single prion particles. The sensitivity might be increased by seeded fibrillization.

Results: In the past we were able to distinguish Scrapie-infected hamster as well as BSE-infected cattle in the clinical stage of disease from healthy control groups. Preliminary data showed that we are able to detect disease associated PrP-aggregates in the cerebrospinal fluid of BSE-afflicted cattle. In the present study we optimized the assay in respect to image single PrP-aggregates and developed a multi-parameter analysis.

Discussion: At present we could successfully adapt the application to PrPsCJD-particles from human brain tissue, and will direct the future research to use CSF- and blood samples.

P.2.6

Confirmation of substances that show affinity binding to PrPres in scrapie-infected hamster brain

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Background: As transfusion-associated infection of vCJD was found in the UK, immediate development of a presymptomatic test using blood (Blood Test) became one of the most important problems in TSE research.

Objectives: The Acidic SDS Precipitation , ASP, test which was developed by us was composed of precipitation of the PrPres under acidic conditions and detection of the molecule by highly sensitive chemiluminescence immunoblot. In this method, PrPres precipitates seemed to form an aggregate with heterogeneous molecules. To make the test method more certain, we searched for a partner molecule that showed affinities to the PrPsc, res using more abundant source of PrPres.

Methods: 10% homogenate of Sc237 infected and mock infected hamster (sc- mc- respectively) brains were inactivated by SDS-heat and used as the source of the PrPres. As the possible partner molecule, 3 glycoproteins, one sulfated polysaccharide, mc-plasma (mcPl), 2 synthetic nucleotides and one native DNA with unrelated sequence were tested. Sc- or mc-brain homogenates were mixed with these molecules, incubated at 37, and detect the PrPres as the ASP precipitate using chemiluminescence immunoblot.

Results:

1. All substances seemed to show an affinity to the PrPres.

2. By treating the the glycoprotein molecules by PNGase F, both plasmin and plasminogen showed the affinities to the PrPres.

3. By denaturing the nucleic acids, Poly A and salmon sperm DNA showed the strongest affinities to the PrPres, although presence of some protein affect the acquisition of theaffinity by the SSpDNA.

Discussion: The obtained results suggest that the PrPres seemed to associate the nucleic acids with some sequence specificities. Denatured poly A but not poly C seemed to be useful for the carrier molecule of the acidic SDS precipitation method.

P.2.7

Brain magnetic resonance imaging findings in E200K Creutzfeldt-Jakob disease from Argentina

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Background: The E200K mutation is one of the most frequent mutation found in genetic Creutzfeldt-Jakob (CJD) disease. In Argentina, cases of Chilean origin or ancestry with clinical and neuropathological features similar to sporadic CJD, represent 12%(29/242) of all Definite and Probable cases. Brain MRI appearance of E200KCJD has been thoroughly described in Libyan Jews but not in those of Chilean origin. Magnetic resonance imaging (MRI) including fluid-attenuated inversion recovery (FLAIR) and diffusion-weighted imaging (DWI) with apparent diffusion coefficient (ADC) measurements became extremely useful in CJD surveillance

Objectives: To evaluate the diagnostic value of brain MRI in E200K CJD cases from the Argentine CJD surveillance and compare it with reported cases of Lybian origin.

Methods: Brain MRI findings were evaluated retrospectively in eleven cases (six men and five women, age range 48-80yrs, mean 59yrs, 3 definite and 8 probable E200K genetically confirmed cases; codon129 was MM in 8 cases and MV in 3.In 6/11 cases, mean time between onset and MRI was 3.5mo and between MRI and death 2.2mo. T2 and FLAIR were available in all cases and DWI was available in eight.

Results: On T2 and FLAIR, abnormal bilateral bright signal was seen in the basal ganglia in nine cases. In the same sequences, abnormal thalamic signal in pulvinar area was seen in three cases. DWI showed bright signal in basal ganglia and in pulvinar.

Discussion: DWI was the most sensitive sequence to see abnormal bright signal along the brain cortex, with positive findings in the eight cases. FLAIR disclosed abnormal bright signal along the cortex when DWI wasn't available in two cases. Different resolution as well as timing since clinical onset between referred MRI's is a critical point to evaluate sensitivity and specificity. These findings are consistent with previous reports but future prospective brain MRI studies with a significative number of cases and ADC maps are necessary.

P.2.8

MRI evidence of early cerebellar involvement in dura mater graft-associated Creutzfeldt-Jakob disease

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Background: Recently, sensitivity of diffusion-weighted images (DWI) in MRI of dura mater graft-associated Creutzfeldt-Jakob disease (dCJD) was reported to be high in particular regions such as the cerebral cortex, basal ganglia or thalamus. Although cerebellar ataxia is known to be an early sign of dCJD and the possible relationship between grafted sies and the clinical signs was pointed out, the detailed correlation between MRI lesions and clinical signs has not been established.

Objectives: To clarify the relationship between the early signs of dCJD and MRI findings.

Methods: We analyzed MRI findings of 70 dCJD patients who had been registered by the CJD Surveillance Committee Japan, between April 1999 and October 2008.

Results: About 80 % of the total dCJD patients showed gait disturbance or cerebellar ataxia, but involvement of the cerebellum in DWI was identified only in two patients. The clinical courses of the two patients were relatively slow. One patient received temporo-parietal graft in over 20 years ago, and the DWI signal abnormality in the cerebellum became gradually unclear along with the disease progression.

Discussion: It is controversial whether initial signs of dCJD were correlated with the grafted site or not. On the contrary, early ataxia was reported in patients with most of grafted patients. In this study, the hyperintensity in the cerebellum was detected only in the two patinets. There have been only a few number of cases reported to have hyperintensity in the cerebellum of dCJD patients. Pathological involvement of cerebellum in dCJD was reported mainly in the molecular and granule cell layers, which was associated with disease duration. It is probable that cerebellar hyperintensity in dCJD can be independent of signs and pathological findings, and that the hyperintensity is detectable only in the limited cases, such as plaque type.

P.2.9

The prevalence of human endogenous retroviruses in cerebrospinal fluids as a potential biomarker for sporadic Creutzfeldt-Jakob disease

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Background: About 8% of human genome is constituted by retroviral sequences, termed human endogenous retroviruses (HERVs), which have been implicated in both health and disease. Recently, indirect evidence for a possible role of retroviral elements in neurological diseases has been provided by several studies.

Objectives: In the present study, we aimed to evaluate the relationship between HERVs and sporadic Creutzfeldt-Jakob disease (CJD) known as human form of prion diseases.

Methods: Here, we investigated the prevalence of HERV families by RT-PCR in cell-free cerebrospinal fluids (CSFs) samples from normal controls, patients with sporadic CJD and other neurological diseases (OND).

Results: We found that the incidence rate of some HERV families were significantly different in CSF samples from the group of sporadic CJD compared to samples from normal individuals; HERV-W (P=0.001), T (P=0.039), FRD (P<0.001), L (P=0.003) and ERV-9 (P<0.001) and the incidence rate of HERV-W (P=0.021) and HERV-L (P=0.049) were significantly increased in CSF samples from the group of sporadic CJD compared to samples from OND group. Moreover, our results from combination frequencies of two HERV families indicated that the prevalence of many combination groups were significantly different between sporadic CJD and normal CSF samples and between two patients CSF samples. In addition, a large number of HERV sequences were newly identified in CSFs from normal and diseased individuals.

Discussion: Our study about distinct prevalence patterns of HERVs reflects that some HERVs families may be associated with the development of prion diseases and used as a candidate marker in the diagnosis of sporadic CJD.

P.2.10

A confirmatory assay for the presence of PrPtse protein in human plasma

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Background: Variant of Creutzfeldt-Jakob disease (vCJD), the human counterpart of BSE, is a neurodegenerative infectious disorder, characterised by the prominent accumulation of the prion protein pathological isoform (PrPTSE) in the brain and lymphoid tissues. This distribution raised concern about potential inter-individual transmission of PrPTSE by blood transfusion. Since the publication in the UK of 4 apparent cases following red blood cell transfusions and 1 apparent case following factor VIII injection, the presence of vCJD infectivity in the blood cannot be excluded. The estimated femtomolar sensitivity level required to detect PrPTSE in the plasma of a donor in the pre-clinical phase of infection has been a caveat towards the development of blood screening assays.

Objectives: To develop a sensitive and specific test that enables the confirmation of a PrPTSE positive blood sample.

Methods: The assay comprises 3 steps: 1) selective capture of PrP molecules by ligand-coated beads; 2) in vitro amplification of PrPtse by Protein Misfolding Cyclic Amplification (PMCA), in which minute amounts of the captured PrPtse are amplified in a cyclic process using brain of transgenic mice as substrate; 3) detection by Western Blot of the amplified PrPtse after PK digestion. Optimisation of the assay was performed with plasma samples spiked with serial ten-fold dilutions of 10% vCJD infected brain homogenate.

Results: After capture of PrPtse spiked in plasma, and 3 rounds of PMCA, a 5log increase in sensitivity was obtained in comparison with western blot detection only.

Discussion: We have developed a test that combines a concentration step and in vitro amplification of PrPTSE, which reaches the sensitivity required for the detection in blood. The future prospects are: 1) to realise epidemiological studies on at risk population samples; 2) to analyse any repeatedly positive result that would obtained after screening of blood donations for confirmation.

P.2.11

Differential removal of prion amyloid and general tissue proteins from surgical stainless steel surfaces with commonly used enzymatic chemistries.

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Background: Transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases with no cure to this day, and are generally believed to be transmitted simply by the absorption or ingestion of self-aggregating protease-resistant prion protein (PrPSc), which can be detected in the form of amyloid plaques in the brain and other tissues of affected individuals. There is evidence that PrPSc is very resilient to most standard decontamination techniques; therefore there is a potential risk of iatrogenic transmission, even after standard reprocessing through sterile service departments (SSDs). In addition, the emergence of new variant Creutzfeldt-Jakob disease, an acquired TSE with a relatively long asymptomatic incubation period and unknown prevalence or incidence, renders the option of instrument segregation for this class of patients infeasible.

Objectives: To evaluate the efficacy of various enzymatic chemistries currently employed in SSDs for the removal of potentially prion-infected tissue contamination.

Methods: Surgical stainless steel surfaces were spiked with prion-infected brain homogenates, and Episcopic Differential Interference Contrast / Epi-Fluorescence (EDIC/EF) was applied to assess the amount of residual prion amyloid and other proteins after decontamination with various enzymatic chemistries used currently in SSDs.

Results: Cleaner efficacy varied between manufacturers. The best cleaners appeared very effective against general tissue contamination, but less so against prion amyloid deposits.

Discussion: The findings show that most chemistries are only partially effective under the recommended conditions, and that PrPSc is indeed particularly resilient and constitutes most part of the remaining traces of tissue contamination left on these surfaces. This should be taken into account when assessing relative instrument cleanliness and making related risk assessments as a guide to healthcare policy.

P.2.12

Detection of PrPsc in blood from sheep infected with scrapie and bovine spongiform encephalopathy using a rapid ligand-based immunoassay

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Background: Iatrogenic Creutzfeldt-Jakob disease (iCJD) is caused by CJD transmission through medical and surgical procedures. Differentiating iCJD from sporadic CJD (sCJD, the most common form of human prion diseases) is apparently of importance in prevention of human-to-human transmission of CJD. But without clinical history currently it is difficult to distinguish the two because no differences have been identified in their phenotypes and pathological prion protein (PrPSc).

Objective: To dissect the differences in physicochemical and biological features of prions from iCJD and sCJD.

Methods: Approaches used included one- and two- dimensional SDS-PAGE, neurohistology, gel filtration, sucrose step gradient sedimentation, enzymatic fragmentation, serial protein misfolding cyclic amplification (sPMCA), and transmission study.

Results: Of eight iCJD cases examined, five cases exhibit gel profile similar to sCJD type 1 whereas three show sCJD type 2-like gel profile. No differences in oligomeric sate of PrPSc between the two are detected using gel filtration and sedimentation in sucrose gradients. No significant differences in infectivity of PrPSc from sCJDMM1 and iCJDMM1 are detected by transmission study using humanized transgenic mice expressing human PrP-129MM. However, using sPMCA, the ability of PrPSc to convert PrPC is notably higher in iCJD than in sCJD. Moreover, most of iCJD cases show no detectable protease-resistant C-terminal fragment 12/13 (CTF12/13) that are detected in all sCJD (Zou et al., 2003) but not in variant CJD.

Discussion: Our study indicates that the differences in PrPSc between iCJD and sCJD may be reflected mainly in their different etiologies. Whether PrP-CTF12/13 is a molecular hallmark for distinguishing the acquired CJD including iCJD and vCJD from the spontaneous CJD including sporadic and familial forms needs further study.

P.2.13

Two-dimensional analysis of prion protein charge-isoforms in human CSF of sCJD-patients with different codon 129 polymorphism

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Sporadic Creutzfeldt-Jakob-Disease (sCJD) cases are currently classified according to the methionine/valine polymorphism at codon 129 of the PRNP-gene. In the study we plan to perform a two-dimensional mapping of PrPc isoforms in cerebrospinal fluid (CSF) of sCJD-patients by using a panel of PrP-antibodies. Our main focus will be on the identification of characteristic PrPc isoforms or truncated PrPc-forms, which are correlating with a single codon 129 polymorphism (MM, MV, VV).

We compared PrPc-charge isomers of each sCJD codon 129 type with control patients without dementia diseases. Every antibody revealed a characteristic recognition pattern and we found slight codon 129 dependent varieties in the PrPc-banding pattern. Irrespective of the sample two significant glycoforms patterns were determined with predominant signal-intensity of glycosylated PrPc-forms when using antibodies against the octarepeat region of PrPc such as 3B5, or 8G8. In contrast antibodies raised against the core-region of PrPc (amino acid 140-160) revealed a higher affinity to the unglycosylated PrPc-form. In addition, SAF70 was able to detect truncated PrPc-forms such as the C1 and C2-fragment in human CSF. Both are generated by physiological cleavage of PrPc. Until now very few studies about different PrPc-isoforms in human CSF of sCJD-patients are available and also the function of these forms remains unknown. Particularly, their appearance and their physiological function in neurons may be interesting to study. Nowadays, the CSF, where biomarker proteins such as 14-3-3 are differentially regulated during the progression of sCJD, is a highly supportive tool for the diagnostic. Therefore, our strategy of the analysis of the PrPc charge-isomer heterogeneity may provide novel perspectives in the identification of new potential markers for prion diseases.

P.2.14

Analysis of PrPC/PrPTSE expression on monocytes and circulating dendritic cells of healthy and TSE infected cynomolgus macaques (Macaca fascicularis)

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Background: The ability of blood to transmit TSE was recently confirmed by four secondary vCJD infections in UK recipients of blood transfusion. The infectivity in blood appears to concentrate in leukocytes, while its association with red cells and platelets seems to be quite limited. We hypothesize that prion infection may lead to expression of PrP in changed conformation on a sub-population of distinct blood cells to the level which may allow its detection by flow cytometry.

Objectives: The objective of the study was to analyze surface and intracellular expression of prion protein(PrP) on sub-populations of peripheral blood cells in healthy and BSE/vCJD infected macaques with a focus on monocytes(MC) and circulating dendritic cells(DC).

Methods: Quantitative three color flow cytometry with PE-conjugated anti-PrP monoclonal antibodies(MAb) AG4 (PrP 31-51), 3F4 (PrP 109-115), AH6 (PrP 159-175), V5B2 (PrP 214-226) and 4C2 (YYR epitope) has been used. Native and paraformaldehyde fixed saponin permeabilized cells were analyzed. DC were detected as lineage markers negative (CD3, CD8, CD14, CD20) and HLA-DR positive cells. MC were detected as CD14 and HLA-DR positive cells.

Results: We have found significantly higher binding of MAbs AG4 and AH6 to permeabilized monocytes of vCJD infected macaques (n=6) in comparison with healthy controls (n=10). In the same group a small, but significant increase of binding of PrPtse specific MAbs V5B2 and 4C2 to native nonpermeabilized monocytes was found. No significant differences were recorded in BSE group (n=5) or on circulating dendritic cells.

Discussion: Our study has identified monocytes as blood cells with impairment of PrP expression and/or epitope exposition in the course of vCJD infection. Our inability to detect similar differences in BSE infected macaques might be caused either by the existing difference in the time interval from inoculation of animal cohorts or by behavior of BSE and vCJD strains. Experiments aimed on clarification of these issues are being carried out.

P.2.15

sCJD-associated proteome changes in human CSF – panning for biomarker

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Background: There is no accurate noninvasive test available for sCJD in living patients. The definite diagnosis can only be done by post-mortem brain histopatological analysis. 14-3-3 protein is commonly used to support the diagnosis, but as patients with other neurodisorders may also have elevated level of this protein and usefulness of this test strongly depends on exclusions of other neuropathologies.

Objectives: Cerebrospinal fluid (CSF) could be a potential source of biomarker(s), since it has a direct contact with CJD-affected brain tissue. However, due to high dynamic range of protein abundances in CSF and relatively low dynamic range of detection technologies applied in proteomics, the enrichment of target proteins is indispensable.

Methods: 12 major proteins were partitioned out from selected CSF samples from MM1 and VV2 CJD subtype as well as from non-demented neurological controls. Low abundant protein fractions were separated by 2-D DIGE. A protein spot was recognized as differentially regulated, when its denistometric analyses showed at least 2-fold change in abundance.

Results: We found 81 and 76 differentially regulated protein spots for subtype MM1 and VV2, respectively. 45 up-regulated protein spots were common for both subtypes. 36 protein spots from MM1 and 31 protein spots from VV2 were only regulated in the respective subtype. Out of MM1-regulated proteins, 22 were up- and 14 down-regulated protein spots. In contrast, 29 protein spots showed VV2-specific up- and only 2 down-regulation. Subsequently regulated proteins were analyzed by tandem mass spectrometry. The major alterations were found among proteins involved in glycolysis. The others groups of regulated proteins belongs to signal transduction or apoptosis. We also identified proteins such as 14-3-3, NSE or aldolase C, which are already associated with CJD.

Discussion: We anticipate that a detailed elucidation of CSF proteome alterations by CJD might provide a deeper insight into pathological changes and mechanism underlying prion diseases.

P.2.16

Anti-prion camel antibodies with the ability to immunodetect cytosolic prion protein

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Background: Prion diseases such as CJD and BSE, Alzheimer's disease and certain forms of Parkinsonism are examples disorders caused by protein misfolding and in prion disease, an abnormally folded protein (called prions or PrPSc), derived from a normal cell surface protein (called PrPC), accumulates in the brains and other organs of affected animals. Although there is currently no effective treatment, significant advances in suppressing the infection using antibodies to block the conversion of PrPC into PrPSc has been achieved.

Methodology/Principal Findings: We have generated anti-prion antibodies (called PrioV) following immunisation of camels with murine scrapie adsorbed to immunomagnetic beads. The PrioV antibodies have been shown to display different specificities, some recognizing the PrP27-30 proteinase K-resistant fragment, others specific for PrPC and few with dual binding specificity. PrioV anti-prion antibodies, independent of their conformation specificity for PrPs, were shown to target the cytosol of neurons following cross-linking PrPC; in sharp contrast, conventional anti-prion antibodies produced in mouse against similar target antigen were unable to enter the cell membrane of neurons and formed a ring around the cells instead.

Discussion: We sought to investigate whether camel antibodies possess the ability to bind intracytoplasmic PrPC following entry into the cytosol of neuroblastoma cells by keeping the integrity of the cell membrane intact. An effective therapeutic strategy for prion diseases would crucially rely on the development of PrPC/PrPSc-specific binders capable of entering the cell cytosol and neutralize the disease-associated agent, PrPSc, in vivo.

Conclusions/Significance: The PrioV anti-prion antibodies could prove to be a valuable tool for the neutralisation/clearance of PrPSc in intracellular compartments of affected neurons and could potentially have wider applicability for the treatment of so-called protein-misfolding diseases.

P.2.17

Performances of three rapid post mortem tests for active surveillance of TSE in clinically healthy sheep

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Background: Seven rapid tests were approved according to the Commission Regulation (EC) 162/2009 for active surveillance of TSE in sheep and goat.

Objectives: Aim of the study was to evaluate the diagnostic sensitivity (Se) and analytical sensitivity of three approved rapid tests: Bio-Rad TeSeE Sheep/Goat (test A), Idexx HerdChek BSE-Scrapie Antigen Test kit (test B), Prionics check western Small Ruminants (test C).

Methods: A sample size of 138 positive samples, confirmed by confirmatory western blotting (WB), was obtained through the screening by the three rapid tests of 970 asymptomatic sheep of various genotype and breed, aged over 18 months and belonging to 22 Italian Scrapie flocks. To assess the detection limits of each test, dilutions from 1:5 to 1:5000 of positive brainstem homogenate (belonging to 20 classical scrapie infected sheep representative of Italian genotypes and breeds) in negative homogenate were prepared both according to the IRMM protocol (50%w/v) and to the manufacturers protocols. The dilution series were analyzed in two replicates with the rapid tests in comparison with the confirmatory WB.

Results: The data indicate an excellent diagnostic sensitivity for the test A and B (test A, Se = 97.10%, 95%CI:92.74-99.20; test B, Se = 99.28%, 95%CI:96.03-99,98) and a good diagnostic sensitivity for the test C (Se= 92.75%, 95% CI:87.08-96.47).The results of analytical sensitivity confirmed that all tests could detect positive samples belonging to all Italian ovine genotypes and breeds , but they differed in analytical sensitivity, in particular test A and e test B showed the best performances.

Discussion: Our findings show that the tests are valid and robust tools for scrapie diagnosis in clinically-healthy sheep, however, considering their performances to detect small PrPsc concentration, a difference in the efficacy of the active surveillance system, based on the test adopted, can be expected.

P.2.18

Camelid anti-prion antibodies inhibit prion replication in vitro and in vivo

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Background: The development of effective therapy for prion diseases is of major importance for public health. The antibody-based therapy appears to be the method of choice as has been demonstrated by the use of these molecules, directed against specific prion epitopes, in chronically infected Neuroblastoma (N2a) cells.

Objectives: To characterise a panel of camelid anti-prion antibodies effective at inhibiting PrPSc replication in vitro and in vivo.

Methods: PrP inhibition studies using camelid anti-prion antibodies were conducted using prion-permissive neuroblastoma cell lines (ScN2a), and were assessed for PrPSc replication by Sandwich ELISA and Western Blotting. Neurotoxic effect of camelid antibodies was also investigated with N2a cells by TUNNEL staining. For in vivo studies, wild type mice were challenged intraperitoneally (IP) with Rocky Laboratory Mouse brain homogenate, and treated weekly with camelid anti-prion antibodies via IP injection at day 30 post inoculation (p.i).

Results: A significant reduction in PrPSc replication in vitro was observed using camelid anti-prion antibodies. An isotype matched control had no effects on PrPSc replication. Prion replication was not seen to recur following treatment termination after 3 or 6 days, indicating that these antibodies lead to indefinite depletion of PrPSc in prion-permissive cell lines. In vivo results, as per Western Blotting and ELISA, revealed a significant reduction of PrPSc both within the spleen and brain parenchyma. Cognitive decline was prevented in RML-infected mice treated at day 7. Mice treated at day 30 and already showing cognitive changes displayed behavioural improvements.

Discussion: In this study we show that our camelid anti-prion antiobodies are effective in treating chronically infected ScN2a cells. Furthermore, they were shown to be effective in inhibiting PrPSc replication in the spleens of prion-infected mice.

P.2.19

Anti-prion antibodies with specific discrimination patterns for atypical scrapie in sheep

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Background: Currently there is no ante-mortem test available to discriminate between classical and atypical scrapie.The production of antibodies specifically raised against atypical scrapie would potentially lead to immunodetection of this particular strain and could have applicability in detecting the disease in the pre-symptomatic phase.Here we demonstrate that antibodies raised against the native forms of atypical scrapie can differentiate it from classical scrapie.Work is underway to develop and validate such screen that would lead to a diagnostic test screen in blood.

Objectives: To develop anti-prion polyclonal and monoclonal antibodies to immunodetect atypical scrapie and to discriminate between atypical and classical scrapie and then assay validation to achieve a highly sensitive and specific sandwich ELISA as a platform for the detection of prions in blood.

Methods: Prn-P-/- mice were subcutaneously immunised with emulsified native atypical scrapie immunoadsorbed to immunomagnetic beads using anti-PrP monoclonal antibodies raised against rPrP.The antibody responses were characterised using a battery of experimental procedures,including ELISA,Immunopreciptation,FACS,western blotting and Dot blot.

Results: ELISA results using human and murine rPrP have demonstrated that our polyclonal antibodies that were raised against atypical scrapie are able to immunodetect PrPSc in atypical scrapie-infected tissues.The antibody response was of the IgG isotype,in contrast with previous work using native RML that led to an exclusive IgM response.The immunoblot patterns of PrPSc derived from atypical scrapie displayed the distinct 7-12kDa band associated with atypical scrapie;in contrast the classical PrP electrophoretic profile lead to the 3 glycoforms ranging between 15-30 kDa.

Discussion: The use of these polyclonal and subsequently monoclonal antibodies will help to improve the diagnosis of atypical scrapie from classical scrapie and also to discriminate between the normal and pathological PrP.

P.2.20

Towards simultaneous broad-range disinfection of bacteria, viruses, fungi and prions

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Background: Effective disinfectants are of key importance for the safe reprocessing of surgical instruments. However, instrument-compatible and routinely applicable formulations that are simultaneously effective on a broad range of pathogens including bacteria, fungi, non-enveloped viruses, mycobacteria and prions are scant.

Objectives: To test whether newly developed formulations containing SDS, NaOH and n-propanol are active against the pathogens specified above despite the theoretical concern that alcohols may potentially fix proteins and stabilize prions.

Methods: The efficacy of SDS and NaOH in alcohol against bacteria, viruses, fungi and prions was examined in suspension and on carriers using coagulated blood or brain homogenate as organic load. Furthermore, protein fixating effects were assessed by Coomassie-blue staining of processed test carriers.

Results: We found that a formulation of 0.2% SDS and 0.3% NaOH in 20% n-propanol exerted a potent decontaminating activity on PrPTSE, the biochemical marker for prion infectivity, attached to steel carriers by contamination with brain homogenate from 263K scrapie hamsters, or from patients with sporadic or variant Creutzfeldt-Jakob disease. The formulation decreased the infectivity of 263K scrapie agent on steel wires below the threshold of detection (reduction factor: , 5.5 log10 units [logs]). The mixture also inactivated adeno- and poliovirus in suspension tests, and achieved a reduction factor of , 4 logs when applied on polio- or hepatitis A virus embedded in coagulated blood on the surface of carriers. For bacteria (E. faecium and M. avium) reduction factors were , 6 logs. Fungi (spores of Aspergillus niger) were also efficiently inactivated. Protein fixation by the formulation was not stronger than that observed with water alone.

Discussion: Addressing the disinfection of prions and conventional pathogens in an integrated approach opens a new avenue for the development of effective broad-range decontaminants.

P.2.21

Comparative study of prions associated with iatrogenic and sporadic Creutzfeldt-Jakob disease

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Background: Iatrogenic Creutzfeldt-Jakob disease (iCJD) is caused by CJD transmission through medical and surgical procedures. Differentiating iCJD from sporadic CJD (sCJD, the most common form of human prion diseases) is apparently of importance in prevention of human-to-human transmission of CJD. But without clinical history currently it is difficult to distinguish the two because no differences have been identified in their phenotypes and pathological prion protein (PrP-Sc).

Objective: To dissect the differences in physicochemical and biological features of prions from iCJD and sCJD.

Methods: Approaches used included one- and two- dimensional SDS-PAGE, neurohistology, gel filtration, sucrose step gradient sedimentation, enzymatic fragmentation, serial protein misfolding cyclic amplification (sPMCA), and transmission study.

Results: Of eight iCJD cases examined, five cases exhibit gel profile similar to sCJD type 1 whereas three show sCJD type 2-like gel profile. No differences in oligomeric sate of PrPSc between the two are detected using gel filtration and sedimentation in sucrose gradients. No significant differences in infectivity of PrPSc from sCJDMM1 and iCJDMM1 are detected by transmission study using humanized transgenic mice expressing human PrP-129MM. However, using sPMCA, the ability of PrPSc to convert PrPC is notably higher in iCJD than in sCJD. Moreover, most of iCJD cases show no detectable protease-resistant C-terminal fragment 12/13 (CTF12/13) that are detected in all sCJD (Zou et al., 2003) but not in variant CJD.

Discussion: Our study indicates that the differences in PrPSc between iCJD and sCJD may be reflected mainly in their different etiologies. Whether PrP-CTF12/13 is a molecular hallmark for distinguishing the acquired CJD including iCJD and vCJD from the spontaneous CJD including sporadic and familial forms needs further study.

P.2.22

Clinical features and diagnostic investigations of the rare VV2 subtype in sporadic Creutzfeldt-Jakob disease

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Background: Six molecular subtypes of sporadic Creutzfeldt-Jakob disease (sCJD) have been identified on the basis of the prion protein type (1 or 2) and the methionine (M) / valine (V) polymorphism at codon 129. Until now only limited information on clinical syndrome in VV2 subtype has been published.

Objectives: To establish clinical and diagnostic findings of the rare VV2 subtype in sCJD.

Methods: Our study included fifty-five VV2 patients (34 females). The clinical symptoms and the following diagnostic tests were investigated: magnetic resonance imaging studies, electroencephalograms and the cerebrospinal fluid markers 14-3-3 protein, tau-protein, neuron-specific enolase, S100 protein and í-amyloid 1-40 and 1-42.

Results: The onset of the disease at 66 years of age and the disease duration with 6 months are similar to classical sCJD. The most frequent first symptom in the VV2 subtype was ataxia. In addition to a rapid progressive dementia and cerebellar symptoms, almost all VV2 cases showed concentration and attention deficits and aphasia during the disease duration. The 14-3-3 protein investigation was the most sensitive diagnostic test and positive in all cases. Only in 8% the electroencephalogram revealed periodic sharp wave complexes and approximately the half of the patients displayed the typical signal increase of the basal ganglia on magnetic resonance images.

Discussion: To our knowledge this is the first clinical and diagnostic description of the rare VV2 subtype in sCJD in a big study population. For the clinical diagnosis of VV2 subtype an ataxia at the onset of the disease is indicative and can be best supported by the 14-3-3 protein investigation in the cerebrospinal fluid.

P.2.23

Active immunization prolongs the survival time of hamsters intracerebrally infected with 263K-scrapie

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Background: So far, there exist no possibilities to cure fatal prion diseases.

Objectives: To examine whether active immunization against PrP may prevent, delay or stop sporadic or hereditary forms of prion diseases which are thought to originate focally in the brain. We intended to mimic a focal cerebral onset of PrP misfolding by intracerebral (i.c.) implantation of steel wires loaded with a very low amount of scrapie infectivity into hamsters. This approach aimed at avoiding confounding dissemination of prions into the periphery in order to study the effect of PrP immunisation specifically in the brain.

Methods: Recombinantly expressed SHaPrP90-232 was used to immunize two groups of hamsters with or without Montanide as adjuvant, respectively. Control groups were treated with PBS or Montanide alone. After the development of high antibody titers against PrPrec in immunized animals, hamsters were i.c. implanted steel wires carrying about 30 LD50i.c. of 263K-scrapie infectivity.

Results: Hamsters treated with PrPrec in Montanide lived ~35% longer than those in the group treated with Montanide alone (191 +/- 25 [SD] days [d] versus 140 +/- 16 d). Hamsters immunized with PrPrec alone showed a mean survival time of 218 +/- 52 d, which was ~25% longer than that of the respective controls treated only with PBS (174 +/- 34 d). When comparing the control groups that received Montanide or PBS without PrPrec, the adjuvant itself was found to shorten survival times (140 +/- 16 d vs. 174 +/- 34 d, respectively), possibly by complex stimulation of the immune system.

Discussion: We showed that active immunization with PrPrec prolongated the incubation time of hamsters that were infected by i.c. implantation of steel wires carrying a very low dose of 263K-scrapie agent in order to trigger a strictly focal onset of infection in the brain. This suggests to further probe the protective potential of active or passive immunization against PrP with respect to sporadic and notably hereditary CJD.

P.2.24

In vivo detection of prion amyloid plaques using [11C]BF-227 PETv

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Objectives: In vivo detection of pathological prion protein in the brain is potentially useful for accurate diagnosis of transmissible spongiform encephalopathies and for early therapeutic intervention. However, there are no potent antemortem means for sensitive detection of pathological prion protein in the brain. To evaluate the amyloid imaging probe BF-227 for noninvasive detection of prion protein amyloid in the brain, a clinical PET study was performed using [11C]BF-227.

Methods: Five patients with transmissible spongiform encephalopathies, including 3 patients with Gerstmann-Strδussler-Scheinker disease (GSS) and 2 patients with sporadic Creutzfeldt-Jacob disease (CJD) underwent [11C]BF-227 PET. Data from 10 normal controls and 17 patients with Alzheimer's disease (AD) were additionally analyzed for comparison. The regional to pons standardized uptake value ratios were calculated as an index of BF-227 retention.

Results: A significantly higher retention of BF-227 was detected in the cerebellum, thalamus, and lateral temporal cortex of GSS patients compared with that in corresponding tissues of normal controls. GSS patients also showed a higher retention of BF-227 in the cerebellum, thalamus, and medial temporal cortex compared with AD patients. In contrast, two patients with CJD showed no obvious retention of BF-227 in the brain. Selective binding of BF-227 to prion protein plaques was confirmed using brain samples from autopsy-confirmed GSS case.

Discussion: These findings suggest that BF-227 is useful for in vivo detection of prion protein plaques in the human brain. [11C]BF-227 PET provides a potential means of facilitating both early diagnosis and noninvasive disease monitoring of transmissible spongiform encephalopathies.

P.2.25

A direct comparison of commercially available prion decontamination reagents using a highly sensitive, quantitative cell-based assay

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Background: Prions comprise aggregates of misfolded host proteins and cause fatal transmissible neurodegenerative disorders of humans and animals, such as vCJD and BSE. Prions pose significant public health threats from the contamination of blood products and surgical instruments, require laborious and often insensitive animal bioassay to detect, and resist conventional hospital sterilisation methods. Prions bind avidly and specifically to surgical steel and can efficiently transfer infectivity to a suitable host, and much research has been performed to develop and validate effective prion decontamination of metal surfaces. We developed a novel bioassay method which exploits the marked binding affinity of prions to steel surfaces in combination with an adapted scrapie-cell end-point assay to achieve, for mouse RML prions, a sensitivity 100 times higher than standard mouse assay: the Standard Steel Binding Assay (SSBA).

Objectives: To apply this assay system to perform a direct comparative study of commercially available decontamination reagents and validate our findings using i.c. inoculation of steel wires into indicator mice.

Methods: Dilutions of RML-infected homogenates of known titre were adsorbed to surgical steel surfaces and subsequently treated with a variety of commercially available decontamination reagents. The remaining infectious units bound to the wires following treatment were quantified via the SSBA.

Results: The SSBA assay is capable of detecting infectivity in a 1010 fold dilution (or 100pg) of RML infected brain. Using the SSBA we have been able to compare directly the efficacy of marketed prion decontamination reagents on metal bound prions.

Discussion: This novel assay offers a new methodology for the evaluation and validation of prion decontamination methodologies. We have demonstrated that the efficacy of marketed prion decontamination reagents is highly variable and the SSBA provides a standard assay for reagent validation and comparison.

P.2.26

Effective treatment of prion infection by anti-PrP monoclonal antibodies

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Background: Many people have been exposed to blood products from donors incubating vCJD, and a treatment strategy is now a priority. We have shown passive transfer of anti- PrP monoclonal antibody (mAb) in mice delays onset of prion disease indefinitely. By further studying this immunotherapeutic strategy in mice, we wish to exploit its potential for translation to patients.

Objectives: To determine a minimal effective dose of mAb for immunotherapy that leads to peripheral clearance of prion infectivity, without casing immunosuppression as a side effect.

Methods: FVB/N mice were inoculated intraperitoneally (ip) with RML prions ($10^{4.9}$ LD50 units) or normal brain homogenate. They were treated biweekly ip at post-infection day (PID) 30, with 4, 1, or 0.25mg/ week of mAbs ICSM18 (PrP epitope 143-153), ICSM35 (PrP epitope 93-105), isotype controls or buffer (PBS) alone. At PID69 and at clinical end points, spleens and brains were tested for infectivity and PrP^{Sc} accumulation. To ensure integrity of the immune system after anti-PrP mAb therapy, the ability to mount an anti-ovalbumin Ab production was challenged at PID69.

Results: ICSM18 and ICSM35 clear splenic PrP^{Sc} and infectivity at PID69 in a dose-dependent manner, but ICSM18 is x3-4 more effective, as at 1mg/week, it produces an improved survival outcome- 40% of mice surviving 300 days after death of controls. Treatment with 4mg/week of ICSM18 and ICSM35 did not lead to immunosuppression or affect the T helper arm of the immune system- splenic CD4+ and CD4+CD25+ T cell numbers remained similar in anti-PrP mAb treated and untreated mice. After immunisation, anti-ovalbumin Ab production was similar regardless of treatment.

Discussion: ICSM18 at 1mg/week is more effective than ICSM35 in decreasing infectivity, removing peripheral PrP^{Sc} and extending lifespan of RML-infected mice. These findings highlight the potential of mAb prion immunotherapy treatment for prion infection, and support further investigation of this strategy.

P.2.27

Effects of Riluzone in C57/BL/6J mice inoculated with the mouse scrapie strain RML

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Background: Transmissible spongiform encephalopathies are fatal neurodegenerative disorders affecting to humans and animals. Several treatments have been explored. Riluzole is a benzothiazolic derivated and it has been tested as neuroprotective agent.

Objectives: To evaluate the neuroprotective effect of riluzole in C57/BL/6J mice inoculated intracerebrally with the mouse Scrapie strain RML.

Methods: All studies were performed in 25 male C57/BL/6J aged 6-8 weeks in accordance with the European and National regulations of animal experimentation. Mice were randomized into 4 groups: A) 5 inoculated with 10%RML; B) 10 inoculated with 10%RML and treated with riluzole; C) 5 inoculated with 10% of normal brain homogenated (NBH) and treated with riluzole and D) 5 inoculated with 10%NBH. Riluzole(Sanofi-Aventis)was administrated at 30 days post-inoculation(dpi), by oral way every 15 days at a dose 0,4gr/l coupled with 1% hydroxi-β-cyclodextrin. We evaluated the weight gain, food, water intake, length of stride, time in hanging thread and open field activity at 0, 15, 30, 45, 90, 105 dpi. Brains were analyzed by immunoblotting, histology and immunohistochemistry using 2G11,6H4 and GFAP antibodies. Lesion profile was performed according to Fraser & Dickinson 1968. Statistical analysis was performed by ANOVA followed by post hoc tests for the continuous variables and by Fisher´s test and Chi-Square for semi quantitative parameters.

Results: The treatment with riluzole normalized the performance in open field behaviour in RML inoculated mice and significantly reduced the vacuolization score in treated animals. We observed a reduction of PrP pattern of bands in 6/10 treated mice in comparison with positive control group. Histology analysis shows no differences in the PrPd immunorreactivity pattern and reactive gliosis between the treated and positive control group.

Discussion: A partial protective effect of riluzole was observed in RML mice.

P.2.28

Detection of multimeric forms of misfolded proteins in blood could provide a sensitive method for the early diagnosis of various misfolded diseases

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Background: In many progressive neurodegenerative diseases, TSE, AD, PD, HD and ALS, multimeric aggregated forms of their respective hallmark protein have been investigated as biomarkers for the post-mortem diagnosis of the diseases using brain tissues. Prion was identified for TSE, and others are amyloid beta (Aβ) peptide, synuclein, Apolipoprotein E, Tau proteins, Huntintin, and superoxide dismutase. It has been suggested that the detection of these aggregated or multimeric forms of various biomarkers in bodily fluids would provide as robust targets for the early detection. Multimer Detection System (MDS) was initially developed for the detection of infectious prion protein in blood. MDS became powerful technique in differentiating preclinical scrapie infected lambs from normal lambs.

Objectives: Here, MDS was adapted for AD.

Methods: In AD, Aβ peptides are generated by proteolytic processing of the amyloid precursor protein with the beta,gamma secretases. Aggregation of insoluble Aβ peptide makes senile plaque suggesting the formation of multimeric form of the Aβ peptide in CSF and also in blood. Hence, the development of the sensitive detection methods for Aβ aggregates or multimers in CSF or blood could be important for the therapeutic strategy and for the evaluation of the drugs, especially with upcoming releases if disease-modifying drug candidates.

Results: Multimeric Aβ peptides were detected with sensitivity at upper nanogram/ml quantity. MDS, in short, utilizes overlapping epitope antibody to create competition to discriminate multimers from monomers as for scrapie. Hence, MDS could only detect multimeric forms of Aβ peptide.

Discussion: In conclusion, the detection of various multimeric biomarkers with MDS could offer faster development of early diagnostic and prognostic assay systems for screening candidates, testing drug efficacy, and monitering therapies in their respective diseases.

P.2.29

An in-depth investigation of PrPd distribution in the different brain regions of BSE cases in Great Britain

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Background: BSE has been monitored globally using diagnostic techniques based on pathology and the detection of disease-associated prion protein (PrPd). Transmission to mice indicated that the BSE agent was a single strain that differed from characterised strains of scrapie. Uniformity of the pathology among cattle during the epidemic suggested a consistent disease phenotype. Active surveillance starting in 2001, using tests for detection of PrPd, gave rise to reports of variable pathology and different molecular profiles of BSE, indicating the possible strain variation. Such cases have been detected in several European countries, Japan, and North America and originate mostly in cattle over 6 years of age. These cases are of two distinct types, a higher molecular mass (H-type) and a lower molecular mass (L-type) based on the unglycosylated protein band detected by Western blot analysis relative to the equivalent protein band in classical BSE. Transmission studies in mice show that each type is different from the classical BSE agent.

Objectives: Atypical BSE cases have only so far been found in active surveillance where whole brains are not readily available for further histopathological examination. This present investigation of 523 classical BSE (C-type) cases, derived from archived case material from passive surveillance in Great Britain, was targeted at obtaining comprehensive PrPd distribution data from clinical cases.

Methods: Histopathological examination, immunohistochemistry and Western immunoblotting.

Results and Discussion: No separate phenotypic entity was detected among the 523 brains. The observations and statistics have consolidated our understanding of the phenotype of classical BSE (C-type). Experimental transmission of field cases of H- and L-type into calves has recently been performed at our laboratory so that the distribution of the PrPd throughout the brain can be mapped and eventually compared to the results in this present study of C-type BSE.

P.2.30

Detection of the abnormal prion protein in cerebral spinal fluid using capillary immunoelectrophoresis assay

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Background: Detection of the abnormal prion protein in cerebral spinal fluid (CSF) has been elusive because of the limited amount of material available and the low amounts of abnormal prion present in this fluid. Capillary electrophoresis has excellent sensitivity as well as the possibility of automation. A successful method needs to be sensitive, rapid, and be relatively easy to use.

Objectives: 1) To design a test that uses commercially available reagents and instrumentation.

2) To develop a test that was rapid and accurate for detection of the abnormal prion protein.

3) To establish conditions that eliminated false positives in the cerebral spinal fluid.

Methods: Capillary Electrophoresis equipment was obtained from Beckman Coulter. Protein A magnetic beads were obtained from Invitrogen. The monoclonal antibodies, SAF32 and 12F10, were obtained from Cayman Chemical, Inc. The fluorescein-labelled peptides were synthesized by Multiple Peptide Systems. Samples were prepared by treating first with proteinase K, followed by treatment with 1% formic acid at 65C for 15 min. After neutralization, the samples were incubated with antibodies and the protein A beads for 1hour. The beads were washing 4x with a BSA-TAPS buffer. After washing, the beads with incubated with the fluorescein-labelled peptide. An aliquot of the peptide was analyzed on the capillary electrophoresis.

Results: The untreated recombinant prion protein showed inhibition for the fluorescein peptide binding. But when treated with PK and formic acid the inhibition was lost. When scrapie brain samples were used, the test was able to distingush normal and scrapie infected samples. When a relatively small number of CSF samples were used, the test could distinguish between non CJD patients and CJD patients. About 50% of the CJD CSF samples test positive on this test.

Discussion: The test is a rapid, straightforward assay. Presently, the number of samples is being expanded in order to obtain good statistic analysis of the capabilities of the test.

P.2.31

Identification of chemotactic factors for migration of mesenchymal stem cell to brain lesions of mice infected with prions

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Background: Bone marrow-derived mesenchymal stem cells (MSCs) have been reported to migrate to brain lesions in neurodegenerative diseases, and to ameliorate functional deficits. Recently, we have shown that immortalized human MSCs (hMSCs) migrate to neuropathological lesions of prion diseases and their transplantation prolongs the survivals. However, the precise mechanisms for the migration of MSCs remain to be elucidated.

Objectives: We investigated factors that are involved in the migration of hMSCs to brain lesions of prion diseases.

Methods: Migration of hMSCs to the brain extracts was analyzed using a QCM 96-well cell migration assay kit. To analyze involvement of cytokines/chemokines and growth factors in MSCs migration, we used antibodies for 10 receptors on hMSCs and 13 their ligands. hMSCs were intracerebrally transplanted into mock- or prion-infected mice at 120 days post-inoculation (dpi). Cryosections of brains of the mice were prepared at 2 and 7 days after transplantation for immunohistochemistry.

Results: The hMSCs constitutively expressed CCR3, CXCR3 and 4, and antibodies against CCR3, CXCR3 and 4 decreased hMSCs migration to brain extracts from both mock- and prion-infected mice at 120 dpi. In contrast, expressions of IGFR, PDGFR, CCR4, 5, and CX3CR1 in hMSCs were low before stimulation but were up-regulated after the stimulation with brain extracts from prion-infected mice, and antibodies against these receptors decreased hMSCs migration only to brain extracts from prion-infected mice. Among them, blocking of CCR4 and 5, and CX3CR1 resulted in more than 50% decrease in migration. Treatment brain extracts from prion-infected mice with antibodies against the corresponding ligands (CCL17 for CCR4, CCL3~5 for CCR5, and CX3CL1 for CX3CR1) also decreased the migration of hMSCs. In fact, hMSCs migrated to the brain lesions expressed CCR4, 5, and CX3CR1.

Discussion: Our results revealed several cytokines/chemokines and growth factors that are involved in the migration of MSCs to brain lesions of prion diseases.

P.2.32

Degradation of scrapie infected brain homogenate by a novel bacterial keratinase

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Background: Prion protein is central to Transmissible Spongiform Encephalopathy (TSE) pathogenesis. Characteristically prion protein is resistant to conventional methods of sterilization and the most effective means of its degradation are incineration and alkaline hydrolysis. These methods are limited by environmental acceptability, application compatibility, cost and loss of reusable materials. Enzymatic degradation provides a viable alternative for decontaminating animal carcasses, specified risk materials, as well as surgical and dentistry instruments.

Objectives: The objective of this research was to isolate and characterise microbial keratinases and to investigate their ability to degrade keratinaceous materials and possibly scrapie prions.

Methods: Microbial isolates from farmyard waste were grown on feather meal medium and the synthesised keratinase characterised by MALDI-MS and SDS-PAGE. Keratinolytic activity was determined using keratin azure, casein and melanised feather as substrates. Degradation of scrapie prion was evaluated by western blotting analysis.

Results: A Gram-positive bacterium has been isolated whose keratinase demonstrated considerable promise. The molecular weight of the enzyme was , 28KDa, with optimum pH and temperature at 8.0 and 50 °C respectively. This novel keratinase demonstrated significant activity on keratin azure (14U/mL) and casein substrates, and completely degraded melanised feather within 48h. Western blotting analysis shows significant reduction in prion signal and immunoreactivity for scrapie infected mouse (ME7) brain homogenate after incubation with this keratinase. Inclusion of a biosurfactant also further enhanced degradation of scrapie prion.

Discussion: The ability of this novel bacterial keratinase to degrade keratin materials and scrapie prion suggests its potential use as an environmental alternative in prion decontamination and other applications.

P.2.33

Peptidylarginine deiminase-mediated protein citrullination in brains of sporadic Creutzfeldt-Jakob disease

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Background: Protein citrullination is a post-translational modification that is markedly increased in disease conditions. The occurrence of citrullinated proteins is associated with disease development or progression, and it can serve as a useful marker or therapeutic target for human diseases. However, how the increased citrullination impacts the pathophysiological cellular status in diseases including prion diseases remains unknown.

Objectives: Recently we reported pathological characterization of PAD2 and citrullinated proteins that were abnormally accumulated in various brain regions of ME7-scrapie infected mice. For human diseases, the role of citrullination remains to be assessed.

Methods: To assess pathogenesis of citrullinated proteins and PAD2 in sporadic Creutzfeldt-Jakob disease (sCJD), we performed immunohistochemistry and Western blot analysis using anti-modified citrulline and anti-PAD2 antibody. To identify citrullinated proteins from brain tissues of patients with sCJD, we performed two-dimensional electrophoresis and MALDI-TOF mass analysis.

Results: In brains of patients with sCJD, we found that various proteins were citrullinated and that these proteins accumulated excessively in reactive astrocytes. Increased expression and activity of PAD2 were found in the reactive astrocytes that contained increased levels of citrullinated proteins. We identified various citrullinated proteins that were structural and energy metabolism-associated proteins such as glial fibrillary acidic protein, myelin basic protein, vimentin, enolase, and phosphoglycerate kinase.

Discussion: Based on these findings, our investigations suggest that PAD2 and citrullinated proteins could play a role in pathogenesis and have value as a diagnostic marker for patients with sCJD.

P.2.34

The study of surface topography of the prion biosensor by means of the atomic force microscopy

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Background: The diagnosis of prion diseases depends on neuropathological examination of brain tissue after application of autopsy or brain biopsy. Therefore the diagnosis of amyloid´s diseases in the early stages can be difficult. The promising strategy for early diagnosis of the prion diseases could be based on acoustic biosensors based on monoclonal antibodies immobilised at the surface of quartz crystal transducer and by measurement the oscillation frequency changes caused by affinity interactions.

Objectives: The formation of the sensing layer is of crucial important for biosensor sensitivity and selectivity. The properties of the surface can be analysed by AFM and cyclic voltammetry methods.

Methods: We used antibodies PRI 308 that selective bind to 106-126 amino acid residues of recPrP (SPIbio, Montigny, France). The antibodies were immobilized on smooth gold surface modified by poly(amidoamine) dendrimers of fourth generation (G4) and by protein A. The interaction of recPrP (Human PrP (23-230), Alicon, Switzerland) with the sensor surface was performed by means of Atomic Force Microscopy (5500 AFM, Agilent Technology, USA). The electrical properties of the surface have been studied by cyclic voltammetry method using potentiostat CHI 440 (CH Instruments, USA).

Results: The crosslinking of G4 molecules and neutravidin with glutaraldehyde allowed oriented immobilization of the biotin-modified protein A and PRI 308. Time dependent AFM images performed in solution during interaction of recPrP with the PRI308 suggest that the surface roughness are changed during the immobilization process. The integrity of the layer was investigated by cyclic voltammetry at the presence of redox couple Fe(CN)64-/3-.

Discussion: Our measurements indicate that the sensitive layer with the PRI 308 is impermeable for redox probe species. The obtained results demonstrate that AFM time dependent images provide useful information on the distribution of the prion proteins at the surface.

P.2.35

Investigation of the efficacy and biocompatibility of pulsed power electrotechnologies for the decontamination of prion-contaminated medical devices

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Prions cause fatal neurodegenerative conditions and their unique resistance to front-line methods of medical equipment sterilization poses significant control challenges. There are no effective decontamination methods currently available for their removal on high-risk medical devices and surgical instruments. Prions are known to resist conventional sterilization procedures that have been developed over the years to deal with traditional pathogens and consequently there is an urgent need to re-evaluate routine sterilization methods and to develop new ones that meet the challenges in health care. This multidisciplinary study will present new novel information on development of a low temperature pulsed- plasma gas-discharge in liquid systems to sterilize biomaterials contaminated with murine BSE and scrapie destined for medical device manufacture. Other related important issues as efficiency of energy delivery using this pulsed power approach and ecotoxicology/biocompatibility post treatment will also be addressed.

P.2.36

Pathogenic prion protein is degraded by a serine protease in lichens

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Background: Lichens are unusual, symbiotic organisms formed from a fungus and partner algae or cyanobacteria. The geographic distribution of lichens is extraordinarily wide and many species thrive in extreme environments such as arctic tundra, desert rock, pure sand or toxic slag heaps. Few biological systems have been identified that degrade the pathogenic form of the prion protein (PrPTSE) and the remarkable biology of lichens and their need to capture and conserve nutrients in harsh conditions suggests that lichens may be capable of unique metabolic activities.

Objectives: We tested the hypothesis that lichens have the potential to degrade PrPTSE.

Methods: We used extracts from three lichens, as well as freshly-collected live lichens, to assess PrPTSE degradation as measured by immunoblotting or protein misfolding cyclic amplification.

Results: We found that extracts of the three lichen species caused approximately a 100-fold loss of prion protein immunoreactivity in samples containing PrPTSE from hamsters or white-tailed deer. Intact lichens exposed to infected brain homogenate reduced PrPTSE levels approximately 70% following 24h incubation. Some common lichen chemicals were excluded from being the active substance(s) and treatments to interfere with redox reactions or metalloenzymes were not effective at blocking degradation. Two protease inhibitor cocktails, however, were each partially effective at preventing lichen extract-induced PrPTSE degradation. Screening a panel of individual, specific protease inhibitors revealed that a serine protease of lichens is the likely agent that degrades prion protein.

Discussion: While animal bioassay experiments to determine the effect of lichen extracts on infectious titer are ongoing, these data suggest that lichens could promote prion degradation in the environment and may have utility for prion inactivation in medical or biotechnological settings. Additionally, the role of lichens in prion environmental biology should be investigated.

P.2.37

Detection of prion proteins using immuno sensor based on quartz crystal microbalance and signal amplification by gold nanoparticles

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Background: The early clinical diagnosis of the prion diseases can be performed by the sensitive detection of the prion proteins. However, cellular prions PrPC and especially their pathogenic form PrPSc should be detected with sensitivity down to 1 pM. Among various assay, those based on direct detection using biosensors are most useful for practical applications.

Objectives: In this work we present simple method of detection prion proteins in a blood plasma based on quartz crystal microbalance (QCM) method and measurements of frequency changes of the piezoelectric transducer modified by specific antibodies selective to PrPC. The amplification of the signal has been achieved using gold nanoparticles.

Methods: The following antibodies were used: PRI 308 and BAR223 that selective bind to 106-126 and 141-152 amino acid residues of recPrP. We used also AG4 and AH6, antibodies that selective bind to N or C terminal of recPrP, respectively (TSE Resource Center, Newbury Breks, UK). The antibodies were immobilized on a surface quartz crystal covered by gold that was modified by poly(amidoamine) dendrimers of fourth generation (G4) and by protein A. The interaction of recPrP (Human PrP (23-230)) with the sensor surface was performed by means of QCM.

Results: The binding of PrP to a sensor surface resulted in decrease of the oscillation frequency of the crystal that served as a sensor response. The detection limit depended on the antibody used and was 6.4 nM for PRI 308 and BAR223, 21 nM for AG4 and 43 nM AH6. We used gold nanoparticles for detection amplification. For this purpose the PRI308 was immobilized on a surface of quartz crystal and AG4 was conjugated with gold nanoparticles. In this assay first the recPrP was added in flow format to a sensor surface and then the Au-AG4 conjugate was added.

Discussion: This method allowed to substantially amplified PrP detection (d.l. 1.6 nM). The detection of PrP in blood was also performed with detection limit 9 pM.

P.2.38

Inhibition of scrapie prion replication by polyelectrolyte nanogold particles

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Background: Prion diseases are invariably fatal maladies. To date, no effective therapy is available for the treatment of prion-infected humans and animals. Therefore, the identification of a novel pharmaceutical regimen would be beneficial to an increasing number of people suffering from prion-related central nervous system degeneration.

Objectives: Finding novel potent anti-prion drugs based on using nanotechnology for inhibition of prion diseases.

Methods: The layer-by-layer technique was used to build up nanoparticles with charged polyelectrolytes (polyallylamine hydrochloride (PAH) and polystyrenesulfonate (PSS)). The potency of anti-prion of the nanoparticles was measured by immunoblotting and ELISA detecting scrapie prion protein (PrPSc) levels from ScGT1 and ScN2a cell lysates. Cell viability was performed by calcein-AM assay. Coated nanogold cellular uptake was studied by confocal fluorescence microscopy. In vitro effect of nanoparticles on prion fibril formation was detected by amyloid seeding assay.

Results: The efficiency of particles in inhibiting prion replication was found to be dependent upon the charge of the outermost layer and less prominent to the number of layers. The particles efficiently hampered the accumulation of PrPSc in ScN2a cells and showed curing effects on ScGT1 cells with a nanoparticle concentration in the picomolar range. In addition, the nanoparticles significantly delay the fibril formation in in vitro conversion model with templates as recombinant mouse PrP proteins.

Discussion: In this study, we report on the build up of nanoparticles with oppositely charged polyelectrolytes (PAH and PSS) and on their application as novel anti-prion compounds. Different coatings, finishing either with a positive or a negative layer, as well as different numbers of layers were investigated for their ability to interact and reduce the accumulation of PrPSc in ScGT1 and ScN2a cells.

P.2.39

Decontamination of surfaces exposed to the infectious agents of TSEs

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Background: TSE agents are difficult to inactivate after drying on surfaces, posing a risk for contaminating work surfaces and equipment used to manufacture biologics and for surgical instruments. Several decontamination methods used in research laboratories were recommended for manufacturing and clinical use but their effectiveness for removing TSE agent in tissue dried on surfaces remains incompletely validated.

Objectives: We developed 2 assays to estimate ability of various chemical and physical treatments to remove or inactivate TSE agents dried on surfaces.

Methods: We used suspensions or pastes of brain tissue from hamsters infected with 263K scrapie agent; we assayed infectivity in hamsters inoculated by the intracerebral route. One model, adapted from a virucidal assay, detects residual TSE agent dried on glass and the other on stainless steel needles; both yielded reproducible infectivity titrations. We evaluated methods recommended by a WHO Consultation in 1999 (WHO Infection Control Guidelines for Transmissible Spongiform Encephalopathies. WHO/CDS/CSR/APH/2000.3) to decontaminate surfaces exposed to TSE agents followed by routine cleaning and terminal sterilization commonly used in clinical settings.

Results: Some infectivity repeatedly survived vacuum autoclaving for 90 min at 134ÍC. Infectivity rarely survived exposure to solutions of NaOH or NaOCl combined with autoclaving. Several procedures recommended by WHO eliminated large amounts of scrapie agent, to limits of detection, from both glass and steel surfaces. Exposures to NaOH, NaOCl and concentrated formic acid at room temperature were also very effective. Sonication of glass and steel objects for 90 min in a hot alkaline detergent solution commonly used to clean instruments markedly reduced but did not completely eliminate infectivity.

Discussion: Several WHO-recommended methods effectively removed substantial amounts of TSE infectivity dried on both glass and steel surfaces.

P.2.40

Detection of total prion concentrations in CSF from confirmed and possible CJD patients in Korea

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Background: Detection of 14-3-3 protein in the cerebrospinal fluid (CSF) has been used as WHO standard marker for some Creutzfeldt-Jakob Disease (CJD) in human, such as sporadic CJD(sCJD), sporadic familial insomnia (sFI), familial CJD (fCJD), fatal familial insomnia (FFI), Gerstmann-Strδussler-Scheinker Syndrome (GSS), iatrogenic CJD (iCJD), variant CJD (vCJD), and Kuru. However, the elevated levels of 14-3-3 were also found in other neurodegenerative diseases, like AD, ASL, and Japanese Encephalitis.

Objectives: Due to the low specificity of 14-3-3 protein, ~ 50-60 %, for CJD diagnosis, other alternative prion (PrP) assays with blood and CSF are being developed. Since CSF was found to contain infectivity, the utilization of PrP in CSF could provide useful diagnosis tool for the diagnosis.

Methods: PrP in CSF from confirmed and possible CJD patients in comparison with non-CJD patients were characterized in this study. CSF was obtained by direct lumber puncture, centrifuged for 10 min at 1500 g, stored at –20 °C until usage. Various PrP antibodies were screened for the detection of PrP in CSF and composed ELISA for the quantitation of total PrP. The buffer, pH, detergent, and protease conditions for CSF were also optimized.

Result: The sensitivity of the assay was found to be ~ 1-10 pg/ml concentration using recombinant human PrP. The difference between the confirmed and possible CJD patients and non-CJD patients as negative control will be presented. The 14-3-3 protein was also analyzed by Western Blot and compared with PrP concentrations.

Discussion: The developed ELISA for total PrP in CSF could be used to in conjunction with 14-3-3 for the CJD diagnosis. The development of assay for detecting infectious PrP in CSF is in process, which could be used as confirmation assay.

P.2.41

Specific detection of prions in sheep blood fractions by modified PMCA

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Background: There is still high demand for routine-tests capable to detect infectious prions in blood or blood-products. Detection of prions in the blood of experimentally infected hamsters has been described by using the protein misfolding cyclic amplification (PMCA) method (Castilla, 2005) as well as for sheep (Terry, 2008).

Objectives: Here we describe our efforts to use PMCA to detect prions in blood fractions of naturally infected sheep, an excellent model for vCJD in humans since scrapie in sheep involves the lymphoid system in similar settings as vCJD in humans. With this in vitro amplification technique very small amounts of disease associated prion protein (PrPSc) can be amplified using multiple cycles of sonication and incubation using negative brain homogenate as substrate (one round PMCA). By using multiple rounds of PMCA further amplification and ultra sensitivity can be achieved (serial PMCA).

Methods: We collected blood from well defined scrapie 'free' and scrapie infected sheep of various PrP genotypes. Blood samples from both adult sheep and lambs were taken at different time-points from both flocks allowing the investigation of disease incubation time on prion detection in blood. Highly susceptible animals of the VRQ/VRQ genotype normally succumb to natural scrapie in 24 + 3 months of age.We fractionated sheep blood into plasma, platelets and white blood cells (PBMC component) and used this material to detect prions using modified serial PMCA (sPMCA). This modified sPMCA protocol made it possible to perform 8 rounds of 48 cycles of PMCA in only 4 days.

Results: We were able to reproducibly detect prions in VRQ/VRQ sheep PBMC samples at various time points near clinical disease development. We were able to preserve specificity of the reactions/detection using solely well defined negative brain homogenates of healthy sheep as substrate without extra additions.

P.2.42

Acoustic biosensor for detection of prions based on DNA aptamers

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Background: The risk of possibility of transmission of Creutzfeld-Jacob disease (CJD) by transfusion [1] justify urgent need to develop rapid test of prions in the blood. So far mostly post time consuming mortem test of brain tissues were available based on protein kinase (PK) resistance of PrPSc and on immunochemical reagents. The alternative rout of detection can be based on biosensors composed of DNA aptamers or antibodies. The objective of this work is in development of biosensors based on DNA aptamers for rapid detection of PrPC.

Objectives:

Methods: The biosensor was based on multiwalled carbon nanotubes (MWNTs) formed by electropolymerisation at the surface of AT-cut quartz crystal onto which the DNA aptamers against recPrP were immobilised. We used also neutravidin covered surface onto biotinylated aptamers were attached. DNA aptamer against recPrP was created based on the work by Takemura et al. [2]. The interaction of recPrP (Human PrP (23-230), Alicon, Switzerland) with the sensor surface was performed by means of quartz crystal microbalance (QCM).

Results: The binding of PrP to a sensor surface resulted in decrease of the oscillation frequency of the crystal that served as a sensor response [3]. The limit of detection (LOD) was rather low: 50 pM for aptamer based biosensor immobilised on MWCNTs. However, neutravidin based immobilisation resulted in lower sensor sensitivity, which is probably due to less aptamers density at the surface in comparison with MWCNTs and due to specific physical properties of MWCNTs. The non-specific interactions of the sensor with considerably higher concentrations of human serum albumin (HSA) as well as with blood serum from health individuals suggests high selectivity of biosensors.

P.2.43

Prion protein detection via direct immuno-quantitative real-time PCR

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Background: Prions are detected for diagnostic purposes via immunoassays and are quantified through dilution-based bioassays by monitoring the onset and development of clinical symptoms in animal models. These bioassays, however, are slow and costly, and established immunoassays for detecting PrPSc are substantially less sensitive than bioassays. Immuno-PCR has been shown to provide increased sensitivity over conventional immunoassays but has not yet enabled the detection and quantification of trace amounts of prion molecules (PM).

Objectives: While investigating PM persistence in animal mortality compost, we sought to advance the methods presently available for detecting and quantifying PM by developing a direct immuno-quantitative real-time PCR (iQ-RT-PCR) assay.

Methods: A direct conjugate was created that comprised a prion-specific antibody (ICSM35) and a synthetic 99-bp DNA tail that included a ScrFI restriction site. Following conjugate binding, tails were restricted and quantified using real-time PCR. The assay was tested with scrapie prions bound to membranes and to 96-well plates coated with a capturing antibody from a commercially detection kit (TeSeE). To validate iQ-RT-PCR detection, transgenic mice were inoculated intracerebrally with 10^-2, 10^-3 and 10^-4 dilutions of the scrapie-positive sheep brain homogenate.

Results: Detection sensitivity of this iQ-RT-PCR assay was increased 1000-fold over the commercial assay and was linear for brain homogenate dilutions 10^-2 to 10^-4 bound to membranes. Sensitivity of PM detection was at an order of magnitude equal to that of bioassay using mice.

Discussion: This simple and robust assay enabled quantitative detection of scrapie prions in brain homogenates whether captured by primary antibody or by membrane. The PCR-amplifiable, separable tail enabled nearly exponential target amplification. This sensitive and versatile iQ-RT-PCR has potential to enable rapid and reliable detection of agents causing transmissible spongiform encephalopathies.

P.2.44

Removing prions from medical devices: an alternative to unfriendly inactivation procedures

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Background: The presence of infectivity in both the CNS and peripheral organs of patients with prion diseases poses a still-unresolved dilemma of decontamination for instruments that cannot withstand the harsh decontamination procedures recommended for optimal inactivation, and in many cases require that they are not re-used.

Objective: We have explored an alternative strategy based on 'dissociating' the infectivity removal and inactivation stages of decontamination. We evaluated the ability of a preliminary cleaning step to eliminate surface-bound prion infectivity before its inactivation in the effluents.

Methods: As a worst-case scenario, rough surfaces of medical devices were modeled by artificially ageing steel wires through physical and chemical processes. After immersion in infectious brain homogenate and extensive drying, the wires were treated with different commercially available enzymatic cleaners, and then bioassayed for infectivity by intracerebral implantation in recipient hamsters.

Results: None of the tested treatments exhibited any direct inactivating effect on prion infectivity, nor were most of them effective in removing infectivity from irregular surfaces. However, one powder-formulated cleaner was able to remove 3-4 logs of surface-bound prion infectivity, by detergent action according to epifluorescence microscopy and PrPres detection analyses. Infectivity in the cleaning bath could then be separately inactivated using standard prion inactivation treatments.

Discussion: Depending on the selected formulation, cleaning solutions can be used to remove and transfer significant amounts of infectivity from instruments that cannot withstand harsh prion inactivation protocols to the cleaning bath, which can then be treated separately using standard inactivation methods. This double-step strategy reconciles prion inactivation and device safeguarding; it could be systematically applied whatever the classification of the patient without profound modifications of current processing.

P.2.45

MRI diagnostic accuracy in Creutzfeldt-Jakob disease: results of a multicenter study

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Background: The clinical diagnosis of prion diseases may be challenging, especially in its early stages. Typical sporadic Creutzfeldt-Jakob disease (sCJD) can be confused with other forms of rapidly progressive conditions with cognitive impairment. Subtypes of sCJD can progress slowly and can be misdiagnosed as Alzheimer and Lewy Body diseases. Early diagnosis of sCJD is required for effective treatment should it become available. Diffusion-weighted magnetic resonance imaging (MRI) has been shown to be very sensitive for identifying signal hyperintensity in the cortex and basal ganglia. Nevertheless, MRI was not included among the clinical diagnostic criteria set forth by the WHO in 1999.

Objectives: The aim of this multicenter study was to measure the diagnostic accuracy of MRI in a large population of patients with CJD.

Methods: The MRIs of 270 patients referred with the diagnosis of rapidly progressive dementia were collected in a prospective study. Diffusion-weighted MRI was available in 189 patients (70%); FLAIR was available in all patients. The pathological diagnosis was available in 138 (51%). Two neuroradiologists, who were not aware of the clinical and pathological diagnosis, evaluated the MRIs for signal abnormality in 15 anatomic gray matter regions.

Results: Diagnosis of CJD was made in 185 patients. In the cohort of patients with Diffusion-weighted MRI and FLAIR the diagnostic accuracy was 90% (sensitivity=87%; specificity=94%). In the cohort with FLAIR but without Diffusion-weighted MRI the diagnostic accuracy was 81% (sensitivity=81%). Associated signal abnormalities in cortex and striatum was seen in over 60% of patients. Interobserver agreement was high for the two neuroradiologists (kappa=0.93).

Discussion: Diffusion-weighted MRI should be included in the diagnostic criteria for the diagnosis of probable sCJD. Accuracy of MRI can be very high in the diagnosis of CJD; however, it heavily depends on the quality of diffusion-weighted sequence.

P.2.46

Western blot method for detection and quantification of PrPres in edible tissues from sheep naturally infected with scrapie

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Background: Detection of abnormal prion protein remains the accepted method to definitively diagnose any TSE. Histology and western blot are commonly used together to confirm disease and also to further characterise the disease phenotype. Frequently, a rodent bioassay is carried out to correlate abnormal PrP with infectivity.

Objectives: Our objective was to quantify the detection of PrPd by immunohistochemistry (IHC) and PrPres by western blot (WB) and relate these levels to infectivity titre in bank voles, in a range of scrapie infected sheep tissues that could enter the human food chain. Commonly, methods are optimised for one tissue type; our aim was to simultaneously examine brain, nerve, tongue, lymph node, heart, liver, pancreas, kidney and muscles. This poster describes a WB method suitable for quantification of PrPres from a range of different tissue samples.

Methods: We employed a lysis protocol previously used for cardiac muscle followed by NaPTA precipitation. For comparison, the same tissues were subjected to the BioRad TeSeE WB kit, according to the manufacturer's protocol.

Results: The NaPTA method successfully detected PrPres in brain, nerve, lymph node, kidney and some muscle samples. Very similar results were achieved using the BioRad TeSeE with brain, lymph node and kidney although false negatives were observed from nerve samples. The sensitivity of both tests was similar and neither was able to detect PrPres from certain muscle samples which were PrPd positive by IHC. This occurred where there were a low number of widely dispersed, PrPd positive muscle spindles.

Discussion: The NaPTA method used here is suitable for testing simultaneously several different sheep tissue samples. As a method of detection and marker for potential infectivity, WB is effective, but only if the sample is taken from the correct organ, area or micro-anatomical region and assuming the extraction procedure is suitable for the tissue type. WB paired with IHC gives the most accurate picture of PrPd deposition.

P.2.47

Metallic nickel powder concentrates PrPSc efficiently from sheep urine

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Background: We have previously observed that PrPSc binds to different steel powders with different degrees of affinity. These differences were partly dependent on the presence of nickel or not in the steel alloys. We then observed that PrPSc efficiently binds to metallic nickel powder.

Objectives: Here we tested whether nickel powder could be used as a rapid, user-friendly and inexpensive means to concentrate PrPSc from body fluids to increase the detection level of prions.

Methods: Western blottings on elutes from nickel powder that had been incubated with scrapie suspensions in various body fluids.

Results: Serum and milk was found to reduce the binding of PrPSc to nickel. We therefore tested whether the nickel powder could extract PrPSc from urine. First we added lysates from the 22L prion strain to sheep urine and incubated with nickel power at room temperature for 1h. After rinsing, SDS loading buffer was added to the powder and boiled for 10 min. By Western blotting using the Fab D13, PrPSc bands could then be detected with a higher degree of sensitivity then in the original suspension. Using a similar method to concentrate PrPSc with nickel powder, PrPSc could also be concentrated from sheep urine spiked with a scrapie-infected sheep brain homogenates at various concentrations. In these series of experiments, 10 ml of spiked urine was mixed with 0.2 g nickel powder (100 mesh, 99.99% Sigma). PrPSc bands could be detected with the Ab P4 by Western blotting with an increased sensitivity at all sheep brain homogenates tested as compared to the original suspensions.

Discussion: Incubation with nickel powder could therefore be a simple procedure to concentrate prions from large volumes of liquids, like urine, to facilitate their detection.

P.2.48

Inactivation of surface-bound prion with low pressure gas plasma: perspectives for application on medical devices

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Background: Harsh and corrosive methods are recommended for inactivation of prions, but they can profoundly damage many surfaces and therefore remain unusable for medical device processing: alternative methods are then required to conciliate efficiency against prions and device preservation. Gas plasma technology showed interesting results on conventional agents, and refinements have recently been proposed using low-pressure conditions that involve multiple mechanisms of action (UV, physical etching, chemical sputtering, oxydizing reactive species…).

Objectives: We evaluated the ability of different conditions of low-pressure gas plasma protocols to eliminate and/or inactivate surface-bound prions, and tried to identify the effects of the different process components.

Methods: A low pressure-gas plasma reactor prototype was built up within the framework of the European Project "BIODECON". Thermo-resistant (steel wires) and thermo–sensitive (silk suture) supports were contaminated by immersion in prion (263K or 6PB1)-infected brain homogenate, and then exposed to different gas plasma conditions. Treated supports were then bioassayed by intracerebrally implanting the supports in recipient animals (hamsters and mice respectively).

Results: Low-pressure gas plasma treatment totally eliminated infectivity bound to the silk suture supports. The method was not nearly as effective for the wire supports, although recipient animals died after longer incubation periods than those implanted with untreated wires. It is possible that the greater heat conductivity of the wire supports caused them to 'fix' infectivity (as, for example, happens after exposure to formaldehyde, or drying before autoclaving).

Discussion: Gas plasma technology is a new and potentially promising method for prion decontamination of some materials that suffer deterioration following harsh chemical or physical treatments . However, parameters and conditions of use will need to be optimized for heat-conducting surfaces, such as metallic instruments.

P.2.49

Investigating the relationship between abnormal prion protein and the TSE agent using in vitro amplification techniques

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Background: Transmissible spongiform encephalopathies (TSEs) are fatal, neurodegenerative disorders that affect both humans and animals. The prion hypothesis states that a misfolded form (PrPSc) of the host glycoprotein, PrPC, acts as the infectious agent. Based on this hypothesis current post-mortem diagnostic tests rely on the presence of PrPSc, however recently models of TSE disease have been identified where no PrPSc deposition is evident. One such disease model is a murine transgenic model of Gerstmann Straussler Scheinker (GSS) disease with a point mutation at residue 101 in murine PrP (Tg101LL). Inoculation with either human GSS or hamster 263K scrapie, produces a clinical and pathological TSE disease with high titres of infectivity but low or undetectable levels of PrPSc (Barron et al, 2007). Thus this model questions the correlation between PrPSc and the infectious agent.

Objectives: In this project, this correlation will be investigated further through the in vitro amplification techniques; protein misfolding cyclic amplification (PMCA) and quaking-induced conversion (QUIC) assays. These assays mimic the conversion of PrPC to PrPSc in vitro through cycles of sonication or shaking to break up PrPSc, increasing the number of converting units followed by an incubation period to allow aggregate growth.

Methods: PMCA and QUIC will be optimised for amplification from mouse tissues. Tissues from the above model will be used to 'seed' the amplification reactions and the products will be analysed through both in vivo and in vitro techniques to identify if PrPSc and/or infectivity has been amplified.

Results: Brain tissue from Tg101LL infected with 263K or GSS have been characterised for levels of PrPSc deposition through immunocytochemistry and have been scored for PrPSc levels. Discussion: This project will examine the correlation between PrPSc and infectivity within this mouse model and will compare the in vitro amplification techniques.

P.2.50

Towards primary cell cultures for the titration of 263K scrapie agent

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Background: 263K scrapie prions are frequently used as a model agent in TSE research. However, titration of 263K scrapie infectivity is not yet possible in cell cultures and requires bioassays in animals.

Objectives: To establish primary cultures of astrocytes, neurons and neurosperes from hamsters and screen conditions for their efficient infection with 263K scrapie agent.

Methods: The cell cultures were exposed to 263K scrapie brain homogenate from hamsters and subsequently examined for accumulation of PrPTSE by western blotting. Protein markers specific for the different types of cultures were characterised by immunhistochemstry.

Results: Preliminary results showed progressing accumulation of PrPTSE in cultures of neurons and astrocytes but not in neurosphδres after exposure to 263K infectivity.

Discussion: Primary cultures of astrocytes or neurons may provide a new tool for the detection and titration of 263K scrapie agent which requires further investigation.

P.2.51

Optical imaging of apoptosis in organs of prion-infected mice

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Background: Early studies proposed apoptosis as the predominant cell death mechanism in rodent models of prion disease based on characteristic cellular morphology and end-labelling of fragmented DNA. Immunohistochemistry analysis of brain sections has confirmed activation of caspase 3, the major effector caspase, in human CJD patients.

Objectives: To employ optical imaging methodologies to detect apoptotic cell death in a murine model of prion disease.

Methods: Wild type mice in the terminal stages of prion disease, induced by intracerebral inoculation with the M1000 strain of mouse-adapted prions, were administered a red-fluorescent irreversible pan-caspase inhibitor (sulforhodamine B-Val-Ala-Asp(OMe)-fluoromethylketone) via the tail vein. Mice were euthanized following intraperitoneal administration of ketamine/xylazine and the brain and peripheral organs excised, sectioned and/or homogenised.

Results: Fluorescence imaging identified increased activation of central apoptosis effector caspases in the thalamus ($p<0.001$), occipital lobe ($p<0.01$), hippocampus ($p<0.01$) and pons ($p<0.05$) using fluorescence microscopy (two-way ANOVA, $F=4.06$, $p=0.0068$). The greatest apoptotic cell death colocalised with regions of highest M1000-induced pathology, the thalamus and hypothalamus. Immunoshistochemical analysis indicated that pan-caspase activation was localised within and adjacent to regions of PrPSc deposition. Western blotting of whole brain homogenates indicated both caspase 3 ($U=0.000$, $p=0.0143$) and caspase 6 ($U=1.000$, $p=0.0286$) were increased in prion-infected mice (Mann-Whitney, one-tailed). Quantification of red fluorescence in homogenates prepared from peripheral tissues indicated significant effector caspase activation in prion infected mice ($p<0.01$, Wilcoxon paired rank test), with specific activation in lung tissue where PrPSc has been shown to accumulate in this model of prion disease.

Discussion: Optical imaging can detect pathological changes in whole organs and has potential application to in vivo imaging.

P.2.52

Discrimination between prion-infected and normal brain with monoclonal antibodies specific for C-terminally truncated PrP fragment

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Background: We have previously described the monoclonal antibody (mAb) V5B2 and a panel of related mAbs, raised against a peptide from the C-terminal part of the prion protein (PrP). These mAbs are capable of discriminating between CJD-affected and normal brain tissue without proteinase K (PK) treatment.

Objectives: In the present study we aimed to map the epitopes of four of these mAbs. We anticipated that the identification of PrP residues, important for recognition, will yield information about the exposed residues in PrPSc.

Methods: Analysis of synthetic peptide binding as well as screening of a phage-displayed random peptide library was used to map the epitopes. The C-terminally truncated version of PrP was expressed in E.coli and analysed by western blotting (WB). The naturally occurring version of this fragment in TSE-infected brain samples was detected by WB and indirect immunofluorescence (IF).

Results: Both epitope mapping approaches showed the importance of two C-terminal Y residues for mAb recognition, as well as some more subtle differences between the described mAbs. Surprisingly, an aromatic residue had to be located free at the C-terminus and the addition of a single residue after Y226 completely prevented antibody binding. Recombinant PrP analysis confirmed that our mAbs do not bind to the whole-length PrP, but readily label the truncated version. Furthermore, WB and IF analysis of brain tissue revealed that the truncated form of PrP can serve as a marker for prion disease, as it is far more abundant in infected brain compared to uninfected controls.

Discussion: We have shown that the described C-terminally truncated PrP is characteristic for TSE positive brain. Our observation that the fragment is located predominantly at the periphery of the plaques suggests that its occurrence might be a late-stage event, possibly linked to the increased protease expression. The fragment can be selectively labelled with our mAbs and this offers a novel approach for TSE diagnostics without the need for PK treatment.

P.2.53

Enzymatic degradation of pathological prion proteins from human, cattle, sheep and hamster

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Background: Prion diseases as the human Creutzfeldt-Jakob disease (CJD) and variant CJD are characterized by a neuronal accumulation of a pathological protein (PrPSc) which is believed to be the infectious agent. During pathogenesis the host encoded cellular prion protein (PrPC) is converted into PrPSc which is remarkable resistant to standard inactivation procedures. Efficient decontamination requires harsh conditions, but many medical devices will be damaged under these conditions. In the prevention of disease transmissions there is great demand for product friendly decontamination procedures relating to medical instruments as well product and food safety. Preserving methods have been described, mostly combined of pre-denaturation and proteolysis.

Objectives: In this study we pursued these techniques and report on a PrPSc decontamination method consisting of predenaturation by heat in the presence of SDS followed by serial enzymatic treatment with four common, commercially available and purified proteases as chymotrypsin, proteinase K (PK), bromelain and papain.

Results: PrPSc degradation resulted in specific banding patterns because of the different enzymatic cleavage sites. We systematically followed serial proteolyses, and the degradation efficiency was appraised on immunoblots and ELISAs with applications of sensitive precipitation techniques. Unlike other studies we evaluated the application of the PrPSc degradation method with different prion proteins, because isolates and strains derived from different species may considerably vary in stability and resistance. Isolates, derived from human sporadic CJD, cattle BSE and sheep scrapie, as well the hamster adapted scrapie strain 263K were degraded so that no residual signals were detectable when using a panel of high sensitive detection antibodies.

Discussion: Our results emphasize that a combination pre-denaturation followed by serial enzymatic treatment is a promising tool for prion decontamination.

P.2.54

Tissue specific phenotypes of cellular prion protein analysed by one-step glycotyping

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Prion diseases are fatal neurodegenerative disorders affecting humans as Creutzfeldt-Jakob disease (CJD). They are characterized by the conversion of a host encoded cellular prion protein (PrPC) into it's partially proteinase K (PK) resistant pathological isoform (PrPSc).

Many PrPSc preparations have been characterized using the glycoprotein typing technique with regard to the distribution of the relative staining intensities of each of the three resulting PK resistant PrPSc bands which represent di-, mono- and non-glycosylated isoforms. Within a species, PrPC in healthy and diseased animals is also not uniform in its banding pattern thus it is conceivable that some PrPSc conformations may interact more or less efficiently with subspecies of PrPC differentially expressed in various brain regions and tissues.

We have analysed the distribution and glycoprotein patterns of eight different mouse tissues by a novel one-step glycotyping technique in order to find out differential expression and subtypes.

Intensity of immunoreactive signals was quantified by means of a photo imager and resulting data were statistically ensured. For glycotyping different antibodies which recognize the amino- and the carboxyl-terminal sequences of prions were used. These primary antibodies derived from different species, thus the colour change of different substrates could impartially be detected on one immunoblot. The mono-glycosylated PrPC of heart, muscle, lung, kidney and liver was most abundant using the aminoterminally binding antibodies R522 and R528 while in brain the di-glycosylated PrPC of brain reacted relatively strong. With SAF60 which recognizes the carboxyl terminus high signal intensities were detected for non-glycosylated PrPC of brain while the mono-glycosylated isoforms of heart and muscle and the di-glycosylated isoforms of kidney and lung were most abundant.

In conclusion, the regional PrPC glycoform distributions are strongly dependant on the tissues and the used antibodies.

P.2.55

Negative optical densities for atypical scrapie positive samples

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Background: Since the implementation of a surveillance plan for scrapie in small ruminants by the European Union in all member states in 2002 (according to EU regulation 999/2001 and subsequent alterations) until January 2009, a total of 340 atypical scrapie cases were detected in Portuguese small ruminants (339 in sheep and 1 in goat), corresponding to 0,1138 % prevalence rate in fallen stock and 0.0767% in healthy slaughter. All the confirmed Atypical scrapie cases resulted positive in the screening test, except 4,5%.

Objectives: This work aims to report and characterize atypical scrapie samples that were confirmed as positive despite of a first negative OD in the screening test, between 2003 and January 2009. Additionally, the false positive samples will also be presented.

Methods: Cases that have shown negative optical densities (OD) were further investigated as these were relatively higher when compared to the other negative samples.

All the samples were screened by the rapid test TeSeEâ BIORAD and confirmed by histopathology, immunohistochemistry and TeSeE BIORAD Western Blotä. The Prnp genotype (136/141/154/171) was determined by PCR and automated cycle sequencing.

Results: Under this assessment, a total of 0,007% samples have shown a negative OD higher than usual. They were further studied, resulting 17 as positive and 12 as negative. The PrPres distribution and the electrophoretic profile will be presented as well as the Prnp genotype. In the same period, a total of 0,003% false positives samples were found.

Discussion: The negative OD obtained in the screening test of samples that were confirmed as Atypical scrapie cases could be related to a lower and variable distribution of PrPres in brainstem in the cases of this form of the disease. The screening negative OD could turn out positive with a screening test more specifically designed for small ruminants. An extended study should be carried out by several laboratories in order to establish guidelines to evaluate weak Atypical scrapie positive samples.

P.2.56

Trapping prions in pre-amyloid forms by thienyl pyrimidines compounds

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Background: Prion diseases are characterized by the deposition in the brain of an abnormal form, termed PrPSc, of the cellular prion protein PrPC. According to the prion hypothesis, PrPSc can trigger the autocatalytic conversion of the PrPC protein into PrPSc. Numerous studies support the propagation of an ordered beta-sheet rich form of the protein, commonly referred to as amyloid. This conformational-based replication involves several intermediate forms such as dimers and trimers of PrPSc, depicting the preamyloid state, that further assemble into small oligomers. The replication cycle proceeds by self-assembling of these oligomers into proto-fibrils, which in turn grow into amyloid fibrils.

Objectives: These different conformational states likely participating in the replication cycle of prions, can be specific targets for therapeutic interventions. Our objective was to develop a rational drug design approach to specifically identify chemical molecules that could trap small oligomers through stabilization of dimers, or trimers, or small oligomeric entities, thus preventing their recycling in the pathological process.

Methods: Virtual screening using recHuPrP protein and cellular screening on prions-infected cells was done. Immunoblot performed on cellular and brain homogenate extracts. Bioassay experiments and in vivo experiments were realized.

Results: By virtual screening followed by cellular screening, we identified a family of thienyl pyrimidine derivatives that remarkably triggered the oligomerization of PrPSc molecules in prion-infected cells. This oligomerization is highly specific of the pathogenic isoform, PrPSc. Bioassay experiments performed with prions infected-cells and brain homogenates treated with the thienyl pyrimidine compound, showed that this drug diminish the prion infectiosity.

Discussion: For the first time, we showed that trapping prions in oligomeric forms by thienyl pyrimidine compounds is a new promising approach for the development of therapeutic of prion diseases.

P.2.57

Engineered adeno-associated-viruses as new therapeutic strategy for prion diseases

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Background: Prion diseases are fatal neurodegenerative disorders for which no effective treatments are currently available. Vector based on Adeno Associated Viruses (AAV) are emerging as one of the most promising gene delivery vehicles to treat neurological disorders, since AAV do not express any viral protein and are therefore devoid of inflammatory or immunogenic properties, and are able to infect post-mitotic cells including neurons which maintain viral genome for prolonged periods of time.

Objectives: The aim of this study was to investigate the diffusion pathway and the transduction efficiency of different AAV serotypes in adult mouse brain. To this aim, three AAV serotypes (2, 8, 9) were engineered to express β-galactosidase reporter gene (AAV-LacZ). The AAV with the highest intracerebral diffusion and transduction efficiency was further studied.

Methods: Three groups of mice have been stereotaxically inoculated with either AAV2-LacZ or AAV8-LacZ or AAV9-LacZ (5x1011 genome copies/ml, 5-10 ml/mouse). 2, 4 and 6 weeks after inoculation, the mice were sacrificed and X-gal staining was performed on brain sections.

Results: The study showed that AAV9-LacZ has an higher power of transduction than the other two AAV serotypes and that the diffusion efficiency is greatest one months after injection. Furthermore, we observed that AAV9 is able to transduce neuronal cells. To improve the AAV9 diffusion efficiency, we inoculated mice in four different regions (i.e., hypothalamus, thalamus, hippocampus and cerebral cortex, 2µl/site).

Discussion: Inoculated mice did not show any neurological dysfunction. Serial MRI and neuropathological assessment did not reveal brain changes, neuronal damage or neurotoxicity following AAV9 treatment. On this ground, AAV9 has been engineered to express an anti-PrP antibody (ScFvD18) and experiments are in progress to investigate the effects of treatment of prion-infected mice on disease progression.

P.2.58

Effect of long-term fixation in the immunohistochemical detection of PrPSc in atypical scrapie

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Background: In 1998, an encephalopathy of sheep similar but distinguishable from classical scrapie was identified in Norway and was named Nor98 and therefore considered to be an "atypical" form of scrapie. Further similar cases have been reported in a large number of countries. These Nor98 cases have little or no abnormal PrP (PrPSc) in the obex, but most of them show an intense cerebellar PrPSc deposition. It is well known that PrPSc from atypical cases is less protease-resistant than PrPSc from classical scrapie, this being the reason for the initial difficulties in confirming atypical scrapie cases by standard immunohistochemical (IHC) and Western blot (WB) methods. In order to determine the possible existence of different phenotypes in atypical scrapie identified in several countries, several cases were examined by the "SheepEuroStrains TSE strain typing in the natural host" subgroup. As a result, an effect of the fixation time of the tissues was observed.

Objectives: This work aims to study the effect of long-term fixation of atypical scrapie samples on IHC reactivity of PrPSc, in order to establish an adequate IHC procedure.

Methods: Fixed brain samples from 12 atypical scrapie cases (seven from Norway, four from Portugal and one from Sweden), originally detected between 2000 and 2007, were trimmed again, processed and embedded paraffin. Sections from freshly embedded tissues (long-term fixation) and from the original blocks (short-term fixation) were examined by IHC using seven different PrP antibodies (R145, 2G11, F89, F99, 8G8, P4 and L42) in different laboratories. For R145, 2G11, F89/160.1.5, F99/97.6.1 and 8G8 sections were pre-treated with formic acid followed by autoclaving. In the case of P4 and L42, and for some F89, sections were also pre-treated with proteinase K.

P.2.59

Removal of exogenous prion infectivity in leucoreduced human red blood cells by MacoPharma P-CAPT TM filter

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Background: Four vCJD cases have been related to probable contamination through blood transfusion in United Kingdom. In February 2009, it was announced that PrPres was detected in the spleen of an +70 year old haemophiliac, who died from unrelated reasons, after receiving plasma derivatives issued from a vCJD-affected donor. These five patients establish the vCJD transmissibility of blood donated by asymptomatic carriers, whose number remains unknown due to lack of sensitive and specific antemortem tests. Blood transfusion must urgently be safeguarded, and removal of prions from blood is one of the most promising strategies. An affinity resin produced by Pathogen Removal and Diagnostic Technologies Inc. (PRDT), in association with the American red Cross, that demonstrated high levels of TSE infectivity reduction from brain-spiked leukoreduced red blood cells concentrates (LR-RBCC) and endogenous whole blood infectivity, was incorporated into membrane layers that were developed by MacoPharma into a prion capture filter termed P-CaptTM.

Objectives: To test the ability of the P-CaptTM filter to remove 263K brain-derived infectivity spiked into human LR-RBCC unit.

Methods: Two different spikes were used corresponding to the supernatants from scrapie-infected hamster brains sonicated and centrifuged either at 14 000 g or 188 000 g. Human LR-RBCC units were spiked with the two supernatant preparations and applied to the P-CaptTM filters. Pre- and post-filtrated samples were inoculated intracerebrally into hamsters to estimate removal of infectivity.

Results: PRDT affinity resin was previously tested in column format. The evaluation of the filter developed with this resin for human transfusion, which is the first available CE marked product for prion capture, was performed on the most soluble infectious fractions to mimick endogenous infectivity. The results of this bioassay will be available for Prion2009.

P.2.60

Adoptive CD4+ T cell therapy of prion diseases

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Background: Designing efficient and safe immunotherapy against TSE means to overcome immune self tolerance to PrP, to select the most appropriate effectors (humoral, cell-mediated or cytotoxic) according to the disease situation and to avoid autoimmune complications. In order to explore those issues, a model of adoptive cell therapy has been developed, whereby lymphocytes primed in non-tolerant PrP-/- mice are infused into compatible infected hosts.

Objectives: Our present focus is on CD4+ Th cells which have the capacity to differentiate along various pathways -Th1, Th2, Th17 or Treg- and therefore to modulate the mode of response. In order to increase the resolution of the model, a transgenic (Tg) mouse has been produced which expresses the b-chain of a TCR recognizing a PrP epitope in a class-II context. Although the alphas rearrange freely, anti-PrP CD4+ T cells resulting from free a-chain pairing with the Tg b-chain are 100-fold more frequent than in non-Tg PrP-/- mice and respond in consequence.

Methods: We wish to assess the extent of protection afforded by immune CD4+ T cells at lympho- and neuroinvasion. See which Th profile is most effective and less harmful. Evaluate the risk of autoimmunity or disease exacerbation after transfer of Th17 or of Treg cells.

Results: Immune T cells raised in PrP-/- Tg or non-Tg mice are infused into PrP+ mice infected 1 day later with 139A. The endpoint at 90 dpi shows that Tg T cells prevent the formation of PrPSc in the spleen. Ongoing experiments examine the CDR3 diversity of the alphas in protective T cells, the functional properties of engrafted T cells and neurological protection.

Discussion: This model will provide clues regarding the agents mediating protection. The model will help deciding which polarization profile should be given to Th cells at a given stage. It will tell which profile(s) should be definitively avoided to prevent brain complications. It will pave the way for adoptive T cell therapy in clinical assays.

P.2.61

A comparative analysis of urine biomarkers in two cohorts of BSE infected cattle

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Background: The bovine spongiform encephalopathy (BSE) epidemic and the emergence of a new human variant of Creutzfeldt-Jakob Disease (vCJD) have led to profound changes in the production and trade of agricultural products. The rapid tests currently approved for BSE in cattle used for human consumption are all based on the detection of the disease related isoform of the prion protein, PrPd, in brain tissue and consequently are only suitable for post-mortem diagnosis. In instances such as assessing the health of breeding stock for export purposes where post-mortem testing is not an option, there is a demand for an ante-mortem test based on a matrix or body fluid that would permit easy access and repeated sampling. Previously, we have demonstrated that a single differentially abundant protein identified by 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) and mass spectrometry was able to descriminate between urine produced by control and infected cattle with 100% accuracy as early as 8 months post-infection.

Objectives: To determine whether the result observed in the urine of Simmental cross-breed calves residing in Germany (Friedrich-Loeffler-Institute)and perorally infected with BSE brain homogenate generated in the United Kingdom could be extended to a different breed of cattle raised in a different geographical location.

Methods: 2-D DIGE permits three separate protein samples, each labelled with one of three size and charge matched Cy-Dye fluors, to be run on the same 2-D gel. An internal standard is run on all gels within an experiment thereby creating an intrinsic link across all gels.

Results: Urine obtained from BSE infected Friesian calves and age matched controls were obtained from the Veterinary Laboratories Agency, Weybridge/United Kingdom. 2D-DIGE and mass spectrometry were used to examine the modulation of the urine protein profile caused by TSE disease in this different cohort. The results are compared and contrasted with those previously reported.

P.2.62

A randomized, double-blind, controlled study of the efficacy of quinacrine in the treatment of sporadic CJD

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Background: Quinacrine eliminates prions in vitro, but its effect in sCJD is unknown. In 2005, we began a quinacrine treatment study for sCJD.

Objectives: To provide an update on our study results.

Methods: This NIH-sponsored study was randomized, double-blinded, & placebo-controlled (50:50). Primary outcome was survival from randomization. Other outcomes included change in neurological exam, neurocognitive testing, brain MRI, EEG, and MEG. Subjects with suspected sCJD were evaluated on an inpatient research ward, randomized to study drug, monitored for 1–2 days, and then returned to UCSF at months 2, 6, and 12. All subjects who returned to UCSF at 2 months were offered open-label quinacrine. Biweekly or monthly monitoring (blood work & phone caregiver assessment) was conducted.

Results: From February 1, 2005 to December 31, 2008, we were referred 426 suspected CJD cases. Diagnoses were: 43% (N=185) sCJD, 33% (N=141) potential CJD (insufficient records), 4.5% (N=19) genetic prion disease, 19% (N=79) non-prion diagnoses (many of which were treatable) and 0.5% (N=2) iatrogenic CJD. Study enrollment closed on January 14, 2009. 69 subjects were consented and 54 randomized to the study drug. For the 15 subjects not randomized, 4 had sCJD but chose not to be randomized, 3 had sCJD but were ineligible, 1 was ineligible due to vCJD, 6 did not have CJD, and 1 did not meet our sCJD diagnostic criteria and refused brain biopsy. Three randomized subjects later were found to have PRNP mutations (data not included). Survival from randomization was: mean 6.1 (SD 6), median 4.4, range 0.4 -27.1 months. Survival from first symptoms was: mean 18.5 (SD 11.3), median 17.2, range 2 -50 months. Study data collection formally ended on May 1, 2009, with 5 patients still alive.

Discussion: Data is being analyzed. Implementation of a randomized, placebo-controlled treatment study is feasible for sCJD. Longitudinal data collected will be important for future trials. Other diagnoses must be ruled out for patients with suspected CJD.

P.2.63

Quantification of TSE agents spiked in plasma derived products by either bioassay or conformation-dependent immunoassay result in comparable prion red

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Background: Currently, the prion reduction capacity of the manufacturing process of plasma-derived products should be determined by a bioassay considered as the "gold standard" for the detection of TSE agents.

Objectives: In order to demonstrate the suitability of biochemical/serological assays to assess the prion reduction capacity, quantification of spiked prion material (hamster-adapted scrapie strain 263K) by bioassay and conformation-dependent immunoassay (CDI) in different process intermediates were compared.

Methods: Plasma product intermediates (from a Von Willebrand/Factor VIII product) were spiked with prion material either in the form of microsomes or purified (not membrane-associated) PrPSc and the prion spiked starting material and product intermediate were processed according to the manufacturing conditions based on a valid down-scale model. The prion reduction factors were determined as the difference of the prion load in the spiked starting materials and in the respective final samples using the CDI and a bioassay in hamsters (dose dependent incubation period measurement), respectively, for quantification of PrPSc.

Results: An overall prion reduction factor of 6.2 log10 and 5.9 log10 could be demonstrated using the biochemical assay and the bioassay, respectively, for the microsome preparation; preliminary data on the reduction capacity of the purified PrPSc preparation will be presented.

Discussion: The data demonstrate that a large amount of animals used for bioassays (hamster model) can be saved in investigational studies on the prion reduction capacity of the manufacturing process of plasma-derived products when the spiked prion material is quantified by the conformation-dependent immunoassay.

P.2.64

Combined diffusion imaging and MR Spectroscopy in human Prion diseases

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Background: While brain magnetic resonnance imaging (MRI) has become a tool of choice in the diagnosis of several forms of prion diseases, the physiopathological bases underlying the signal changes observed in patients with prion diseases (human transmissible spongiform encephalopathies, TSEs) are still poorly understood.

Objectives: We hypothesized that combining magnetic resonance spectroscopy (MRS) to diffusion and conventional brain imaging could help better understand the mechanisms underlying signal alterations and changes in the apparent diffusion coefficient of water (ADC)regularly observed in the brain of TSEs patients.

Methods: We designed a prospective study of multimodal MRI in patients with suspected TSEs. Fourty five patients with a suspicion of TSE and 11 age-matched normal volunteers were included. The MRI protocol included T1, FLAIR and diffusion sequences. MRS was performed on the cerebellum, pulvinar, right lenticular nucleus and frontal cortex. MR images were assessed visually and ADC values were calculated.

Results: Among the 45 suspected TSE, 31 fulfilled the criteria for probable or definite TSEs (19 sporadic, 3 iatrogenic, 2 variant CJD and 7 genetic TSEs) and 14 were classified as alternative diagnoses. High signals in the cortex and/or basal ganglia were observed in 26/31 TSEs patients on FLAIR and 29/31 patients on DWI. In the basal ganglia, DWI high signals corresponded to a decreased ADC. Metabolic alterations, increased myo-inositol and decreased NAA, were observed in all TSEs patients. ADC values and metabolic changes were not correlated suggesting that neither neuronal suffering (vacuolization) nor astrogliosis contribute to the decrease of ADC.

Discussion: Metabolic changes were detected in all patients with sporadic, inherited or infectious prion diseases in at least one of the studied voxels, even in areas that looked normal on conventional imaging. No metabolic ratio was correlated to ADC values, pointing out that these two imaging modalities explore different pathological processes.

P.2.65

Targeted delivery of small regulatory RNAs to the brain using a peptide derived from rabies virus glycoprotein

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Background: The recent discovery of a group of small regulatory RNA termed micro-RNAs (miRNAs) has altered our understanding of gene regulation. These miRNAs work by binding to target sequences in mRNAs which prevent their translation; a single miRNA can bind to as many as 200 mRNA targets. Therefore, the number of regulatory circuits affected by miRNA is enormous perhaps as extensive as transcription factors. MiRNAs play a role in neurodegeneration and specifically in terms of prion disease they have been identified as having a protective role in human neurodegenerative disease. To explore the specific miRNAs during prion disease a system for their delivery to the brain in vivo would be advantageous. The blood brain barrier is the difficulty in this strategy. A novel method to deliver RNA molecules to the brain was recently reported using a modified rabies virus glycoprotein (RVG).

Objectives: To deliver small RNAs to the brain to determine their function and potential to alter the transcriptome during prion disease and outcome of infection.

Methods: RVG and a control peptide (RV-MAT) were complexed to a miRNA labeled with fluorescein isothiocyanate or FAM. In vitro assays were performed to test delivery to Neuro-2a cells while in vivo assays were performed by injecting the RVG-miRNA complex intravenously to CD1 and Tga20 mice. Uptake of RNA was determined using fluorescent microscopy and flow cytometry and by monitoring the knockdown of target genes.

Results: Both the in vitro and in vivo assays demonstrated the selective uptake of RVG-miRNA, but not RV-MAT-miRNA, complexes into neurons of the brain.

Discussion: The establishment of this RNA delivery system provides a means to modulate transcription during the disease process. We will be able to correlate information regarding the functionality of key miRNAs both in vitro and in vivo. Further, de-regulated RNA species in prion disease, whether mRNA or miRNA, are prime candidates for therapeutic intervention and using RVG delivery to the brain will allow us to test this.

P.2.66

Immunization with recombinant murine prion proteins provides partial protection against TSEs

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Background: Transmissible Spongiform Encephalopathies (TSEs) are fatal neurodegenerative diseases, linked to the conversion of the normal prion protein (PrP^C) to an abnormally folded, disease associated isoform (PrP^{Sc}). Although passive immunization against PrP was proven effective *in vitro* and *in vivo*, poor immunogenicity of PrP in wild type (wt) animals hampers active immunization against TSEs.

Objectives: We assessed the immunogenicity and possible protective role against TSEs of purified murine recombinant PrP (mrPrP) inclusion bodies (AgPrP), solubilized mrPrP, hybrid mrPrP-DnaK proteins and an mrPrP and DnaK mix.

Methods: We immunized mice with the recombinant proteins and evaluated the presence of anti-PrP antibodies by western blotting and ELISA. To evaluate the elicited immune response we isolated splenocytes from immunized mice and assessed their mitogenic response and cytokine expression profile following incubation in the presence of antigens. Furthermore we checked the FDCs, B and T lymphocytes populations in the spleens. We then challenged the mice with RML and monitored splenic PrP^{Sc} accumulation and disease progression.

Results: Mice immunized with the mrPrP and DnaK mix developed antibodies against PrP^C and mrPrP and their splenocytes expressed Th-2 associated cytokines when incubated in the presence of mrPrP. Nonetheless these mice failed to survive longer than control mice. Mice immunized with AgPrP accumulated less splenic PrP^{Sc} and survived significantly longer than control mice, despite antibodies against PrP were not detected. Splenocytes from these mice displayed low cytokine expression and poor proliferation in the presence of the antigen. The mature FDCs population in the spleens was depleted, whereas B and T lymphocytes populations remained unaltered.

Conclusions: Although generation of anti-PrP antibodies in wt mice was achieved, these failed to exert a protective role against TSEs. Protection, possibly linked to the induced FDC depletion, was achieved following immunization with AgPrP.

P.2.67

Inactivation of mouse brain Infectivity and associated BSE / Scrapie prion protein through subcritical wet oxidation

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Background: Subcritical wet oxidation at high pressure and moderate temperature (hydrothermal oxidation) can be applied to the disposal of ruminant by-products from slaughterhouse. After oxidation of sheep tissue samples, the main oxidation by-products identified were acetic acid and ammonia.

Objectives: Investigate the efficacy of hydrothermal oxydation to inactivate Scrapie or BSE in highly infected mouse brains using adapted mouse models.

Methods: Groups of 6-7 mice were used for the titration of (1) PG127S and C506M3 Scrapie strains in Tg338 transgenic mice carrying the sheep VRQ Scrapie susceptibility allele and (2) VM-adapted BSE strain in VM mice (strain 301V). The 301V strain is known as having high capacities of resistance to elevated temperatures. The same mouse lines were IC inoculated with mouse brain homogenates (0.1%) having been treated (10MPa, 250°C, 20min, 150ml vol) or not into a pilot apparatus introduced in a L3 laboratory. In addition, treated and control BSE infected sheep brain homogenates were inoculated in bovine transgenic (Tg110) mice.

Results: After hydrothermal treatment, Scrapie and BSE prion protein were no more detectable through Western blot. IC inoculated control mice were clinical at expected time from 87d + 5 for Tg338 mice, 141d + 5 for VM mice to 304d + 8 for Tg110 mice; PrPsc was WB detected into their brain. All mice inoculated with hydrothermal oxidation treated homogenates are still living (>450-496d).

Discussion: Still ongoing titration experiments suggest reduction in infectivity titres superior to 5-7 log according to the model. Although pilot experiments with larger volumes have to be performed, hydrothermal oxidation should be an interesting procedure for the inactivation of TSE infectivity in waste from slaughterhouse or animal facilities.

P.2.68

Characterization of a resin-nonwoven separation medium for removal of prions from complex suspensions

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Background: Risks associated with transmission of prion diseases through blood-related products still exist despite the mitigation efforts currently in place. These risks can be further minimized through the removal of infectivity via adsorption to ligands. Small interstitial hydraulic radii of typical packed-bed columns can preclude separations from sources containing particles, such as red blood cells. Entrapping resin particles in a nonwoven membrane support circumvents this limitation, allowing particles to pass unimpeded while maintaining high surface contact with the resin.

Objectives: To demonstrate the use of a resin-nonwoven medium for prion capture from suspensions containing particulate.

Methods: Particle-impregnated membranes (PIM) were produced by spreading a layer of polymeric resin between two layers of nonwoven membrane. The membranes were bonded together, stacked and packed into columns. Challenge suspensions (human red blood cell concentrate, cell suspensions, and plasma solutions) were passed through the columns. Prion binding, interstitial porosity and other parameters were determined.

Results: PIM columns displayed good flow distribution while yielding interstitial porosity of 0.485. The columns were able to pass RBC at a linear flow velocity of 0.04 cm/s with hemolysis well below the acceptable levels. Dynamic binding studies showed that the PIM had the same prion and BSA binding capacity as columns packed with the resin alone. It was also shown that the presence of 7.5% human plasma had no effect on the prion binding capacity to the PIM columns.

Discussion: PIM materials exhibit the necessary properties to handle mobile phases containing large particles while retaining the desirable binding characteristics of chromatographic resin and can be utilized for the affinity removal of prions, adding an additional layer of protection to blood and plasma products.

P.2.69

Removal of exogenous (soluble) and endogenous infectivity from red cells by 22 layer variant of Pall leucotrap prion reduction filter

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Background: The transmissible agent of variant Creutzfeldt-Jakob disease (vCJD) is present in the blood of infected individuals and can transmit the disease to recipients of blood components. In the absence of a screening test, prevention of transfusion transmission relies on the combination of low-risk sourcing, leukodepletion, and infectivity-reducing processing steps.

Objectives: To increase the safety of red blood cell transfusion with respect to vCJD, we evaluated the capacity of a novel filter designed by Pall Co. to remove prion infectivity from red cell concentrate (RCC).

Methods: In the endogenous infectivity study, we passed a bag of leukodepleted RCC from 263K scrapie-affected hamsters through Pall prion removal filter. Pre and post filtration samples were tested for infectivity by inoculation into recipient hamsters. In the exogenous infectivity study, we spiked a unit of human RCC with soluble infectivity (dilution 1:10) prepared from brains of 263K scrapie-affected hamsters (Berardi V. et al., Transfusion, 2006). Spiked RCC were leukodepleted and then passed through Pall prion removal filter. Infectious titers were bioassayed by the end-point dilution method.

Results: In the endogenous infectivity study, the prefiltered RCC transmitted the disease to 5/87 hamsters (ongoing study now at 335 dpi), giving an infectious titre of 1.18±0.52 ID/ml (according to Gregori L. et al., Lancet, 2004 and 2006). The postfiltration RCC did not trasmit the disease (infectious titre <0.23±0.23 ID/ml). The removal factor is >0.71 log ID, or >80.5%. In the exogenous infectivity study we estimated a reduction from 5.6 to 4.0 log LD50/g in the post filtration sample, yielding a removal factor of 1.6 log LD50/g.

Discussion: Our results show that the combination of a leukodepletion filter with Pall prion-specific removal filter is effective in reducing endogenous prion infectivity and exogenous soluble infectivity spiked into RCC. The use of this filtration setting can reduce the risk of vCJD transmission through blood.

P.2.70

Ovine and bovine PRNP transgenic mice allows discrimination between Scrapie and BSE in co-infected mice and sheep.

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Background: Differentiation between Scrapie and BSE infection in small ruminant flocks is currently based on discriminating biochemical tests performed on obex samples. We have previously shown through Scrapie-BSE co-infection experiments in sheep that BSE prion protein is not detected in nervous tissues on the contrary of Scrapie PrPsc. This was true with the 3 Scrapie strains tested. In lymphoid tissues, however, BSE PrPres was easily detected, pointing out the capacity of BSE to disseminate in lymphoid tissues.

Objectives: Confirm BSE and Scrapie strains immunochemical identification through the inoculation of mouse lines transgenic for the bovine (Tg110) or ovine(Tg338) PRNP gene and investigate for silent carriage of BSE strain in nervous tissues of co-infected sheep.

Methods: Groups of 6-7 mice were IC inoculated either with control strains, a mixture of ovine BSE and Scrapie (0.5:0.5 vol/vol) or with spleen and obex tissues from BSE/Scrapie co-infected sheep sampled at terminal stage of the disease.

Results: Clinical signs appeared with shorter incubation period in mice transgenic for the PRNP gene corresponding to the origin of the TSE strain inoculated, i.e. bovine Tg110 in about 230 days after inoculation of BSE containing tissues and ovine Tg338 mice in 200-350 days, according to the Scrapie strain. Tg338 mice were highly resistant to BSE and reciprocally bovine Tg110 mice were highly resistant to Scrapie. Re-inoculation in these two mouse lines of tissues from co-infected sheep allowed evidencing BSE into their obex.

Discussion: Inoculation of transgenic Tg110 and Tg338 mice with BSE-Scrapie mixtures demonstrated their capacities to discriminate between these strains. These lines were used to investigate the carrier status of BSE-Scrapie co-infected sheep and allowed to evidence previously immunochemically undetected BSE in the brain of these animals, thus confirming that their diagnostic was impaired by the development of Scrapie.

P.2.71

Anti-idiotypic antibodies against V5B2, the PrPSc-specific antibody, in prion research

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Background: In certain cases, anti-idiotypic (Ab2) antibodies that recognize an antigen-combining site of an antibody can mimic the structure and/or function of certain nominal antigens. As an alternative to conventional active anti-prion vaccine development, the anti-idiotypic approach offers another way to overcome the unresponsiveness of the immune system.

Objectives: The aim of this study was thus to investigate the applicable ways to induce an Ab2 response to V5B2, the PrPSc- specific monoclonal antibody, and to define the preparation of Ab2 monoclonal antibodies with well-defined strategies for immunization, selection and subsequent characterization.

Methods: Balb/C mice were immunized with Fab V5B2. Stable hybridoma cell lines were prepared with the mouse hybridoma technology. Competition studies were performed using the ELISA and Immunohistochemistry-based tests. Interaction of V5B2 and 5D12, the most promissing Ab2 antibody, was investigated by computer modelling and molecular docking.

Results: Our results demonstrate that it is possible to induce a strong Ab2 immune response against V5B2 in syngeneic experimental system. From the competition seen between polyclonal and monoclonal Ab2 antibodies and the ultimate target antigen, PrPSc, we conclude that selected antibodies bind to the antigen-combining site of V5B2 and might even resemble the PrPSc -specific epitope. The involvement of both antigen-combining sites in the interaction between V5B2 and 5D12, was further supported by molecular docking.

Discussion: According to the convincing results of the competition studies and the computer modelling data, 5D12 is a highly plausible candidate for being an internal image antibody, mimicking the PrPSc-specific epitope. The results of the present study not only provide an example of the successful production of Ab2 monoclonal antibodies based on a well planned strategy for selection, but should also provide a new experimental approach that is applicable to the field of prion diseases.

P.2.72

Sensitive detection of protein folding disorders; proximity ligation-based detection of protein oligomers

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Background: The possibility to detect and analyze proteins in their biological environments with increased specificity and sensitivity will provide opportunities to use also very rare molecules as reliable biomarkers of diseases. Improved analyses are also required to study the molecular basis of diseases.

Objectives: The aim is to apply the proximity ligation assay (PLA) for sensitive and specific detection of proteins relevant in neurodegenerative diseases.

Methods: In PLA, affinity probes, such as antibodies, are attached to oligonucleotides to form proximity probes. Once a target molecule is recognized by a set of proximity probes the DNA strands are connected to each other via enzymatic ligation to form a DNA template that can be amplified and quantified as the measure of the target molecule's concentration.

Results: Here, we present the application of PLA for sensitive identification of oligomers and aggregated proteins. For specific detection of protein oligomers we use a single monoclonal antibody conjugated to two different ssDNA arms, respectively. In this format of PLA only oligomers are detected while monomers with only one available epitope will be excluded. This is illustrated here with specific detection of oligomers spiked in different biological matrices as well as detection of endogenous oligomeric conformations of proteins.

Discussion: 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 very small amount of materials to be tested –, or in a heterogeneous format in which the target molecules to be detected are immobilized on a surface using affinity probes, while other materials are washed away. Proximity ligation can, therefore, provide a powerful molecular tool for detection and study of the biology of protein folding disorders.

P.2.73

Modeling of prion adsorption to an affinity matrix

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Background: Characterization of the transport and binding properties of prions in adsorption processes is an important tool to aid in the design and optimization of operations and devices intended to achieve a desired separation, as well as in the validation of TSE clearance studies.

Objectives: This paper evaluates the transport and binding capacity of prion protein from hamster brain homogenate (HaBH) to a hybrid particle-nonwoven membrane medium (a particle-impregnated membrane or PIM) composed from a polymeric chromatographic resin, containing an affinity ligand for prions, which is entrapped between layers of a nonwoven polypropylene membrane.

Methods: Dynamic binding capacities of prions in saline solution were determined for columns packed with PIM materials, affinity resin only, and the nonwoven membrane only. Quantitative detection of prion protein was possible through an ELISA method. The general rate (GR) model of chromatography was used to evaluate the prion protein breakthrough from columns packed with each of the materials and subsequently used to predict prion binding behavior from HaBH spikes to human red blood cell concentrate (RBCC) and human IgG solutions.

Results: The GR model of chromatography was able to fit the breakthrough behavior of prions for the columns packed with affinity resin, PIM, and nonwoven membrane materials. The GR model was able to predict the binding behavior of prions spiked into RBCC loaded to a column packed with PIM materials, as well as accurately predict the breakthrough of prions spiked into a hIgG solution loaded to a column packed with the affinity resin.

Discussion: These results suggest the GR chromatographic model can accurately predict the binding behavior of prion protein to the affinity resin, PIM and, nonwoven materials and could be utilized as a tool in the design, evaluation, and validation of TSE clearance studies.

P.3.1

GSK-3beta mediates MPTP-induced mitochondrial dysfunction

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Background: Aberrant mitochondrial function appears to play a central role in dopaminergic neuronal loss in Parkinson's disease (PD). 1-methyll-4-phenylpyridinium iodide (MPP+), the active metabolite of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is a selective inhibitor of mitochondrial complex I and is widely used in rodent and cell models to elicit neurochemical alterations associated with PD. Recent findings suggest that Glycogen Synthase Kinase-3beta (GSK-3beta), a critical activator of neuronal apoptosis, is involved in the dopaminergic cell death.

Objectives: In this study, the role of GSK-3beta in modulating MPP+-induced mitochondrial dysfunction and neuronal death was examined in vivo, and in two neuronal cell models namely primary cultured and immortalized neurons.

Results: In both cell models, MPTP/MPP+ treatment caused cell death associated with time- and concentration-dependent activation of GSK-3beta, evidenced by the increased level of phosphorylation at tyrosine 216 residue, the active form of the kinase. Using immunocytochemistry and subcellular fractionation techniques, we showed that GSK-3beta partially localized within mitochondria in both neuronal cell models. Moreover, MPP+ treatment induced a significant decrease of the specific phospho-Tyr216-GSK-3beta labeling in mitochondria concomitantly with an increase into the cytosol. MPP+ induced cell death through the depolarization of mitochondrial membrane potential. Inhibition of GSK-3beta activity using well-characterized inhibitors, LiCl and kenpaullone, and a GSK-3beta knock-down using small interfering RNA, prevented MPP+-induced cell death by blocking mitochondrial membrane potential changes and caspase-9 and -3 activation.

Discussion: These results indicate that GSK-3beta is a critical mediator of the neurotoxicity induced by MPTP/MPP+ and exerts its role at least partially by regulation of the mitochondrial function. Inhibition of GSK-3beta activity might provide protection against mitochondrial stress-induced cell death.

P.3.2

Application of novel phospho-Tau antibodies in a sensitive in vivo diagnostic assay for Alzheimer's disease

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Background: Phosphorylation of Tau protein (p-Tau) represents one of the central and earliest pathogenetic events in Alzheimer Disease (AD). The measurement of p-Tau, especially on serine 181 (p-181), released into the CSF is currently used for in vivo diagnosis of AD. Investigation of further sites of phosphorylation may improve specificity of in vivo body fluid diagnosis of AD and other tauopathies.

Objectives: Our aim is to develop novel p-Tau specific monoclonal antibodies (mabs) and to test their diagnostic applicability in body fluids.

Methods: Mice were immunized using phospho peptides of human Tau sequence conjugated to KLH. Hybridomas were performed by harvesting of spleen cells, fusion to mouse myeloma cells and HAT selection of IgG producing cells. Mabs were selected regarding their reactivity to phospho Tau peptides in ELISA. Specificity was tested using immunohistochemistry, Western Blotting and immunogold electron microscopy using diseased brain tissue from various tauopathies.

Results: Mabs 2B11, 5G7, 9D8 and 4C10 specifically recognize p-231 position and mabs 1E7 and 8B11 react with p-181. Mab 3G3 specifically recognizes p231-235 positions of human Tau whereas mab 1F2 binds to phospho residues independently from the amino acid position. Mabs 5G7, 9D8 and 4C10 prove to be the best in immunolabelling various neuronal and glial disease-associated Tau-profiles in tissue sections of various tauopathies. Mabs 5G7, 9D8, 2B11 and 3G3 appear to be the best in Western Blotting. These latest specifically stain Paired Helical Filaments of AD brains and show specific electrophoretic profiles in brain extracts from several tauopathies.

Discussion: We found sensitive and specific tools able to recognize different phosphorylated epitopes of human Tau. Couples of capture and detection antibodies were built for ELISA format and are ready for CSF application to improve the diagnosis of AD and to distinguish other tauopathies.

P.3.3

Good protein, bad protein: A new Aβ variant can be both

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Background: Mutations in the amyloid β (Aβ) precursor protein gene (APP) cause familial Alzheimer's disease with virtually complete penetrance. We found an APP mutation (A673V, corresponding to position 2 of Aβ) that causes disease only in the homozygous state, whereas heterozygous carriers are not affected, consistent with a recessive Mendelian trait of inheritance.

Objective: To investigate the mechanisms by which the A673V APP mutation causes disease only in the homozygous state.

Methods: We analyzed APP processing in fibroblasts from an A673V homozygous patient and controls, and in CHO and COS7 cells transfected with wild-type or mutated APP cDNA. Moreover, we assessed the physicochemical and biological properties of A2V-mutated and wild-type Aβ1-40 and Aβ1-42 peptides.

Results: The study showed that the A673V mutation has two pathogenic effects: it (*i*) shifts APP processing toward the amyloidogenic pathway resulting in enhanced Aβ production, and (*ii*) increases the aggregation and fibrillogenic properties of Aβ. However, co-incubation of mutated and wild-type peptides confers instability on Aβ aggregates and inhibits amyloid formation and neurotoxicity. The finding that the A2V Aβ variant has a dominant-negative effect on amyloidogenesis is consistent with the observation that the heterozygous carriers do not develop disease and offers ground for the development of a therapeutic strategy. As a first step for designing an inhibitor of Aβ aggregation based on the mutated Aβ sequence, we synthesized a peptide homologous to residues 1-6 of Aβ with the A2V substitution and tested its ability to bind to wild-type Aβ and inhibit amyloidogenesis. The study showed that the mutated hexapeptide retains the anti-amyloidogenic properties of the parent full-length Aβ.

Conclusion: The present data have important implications for genetic screening and the potential treatment of Alzheimer's disease based on A2V-modified Aβ peptides or peptidomimetic compounds.

P.3.4

Comparative immuno-detection of α-synuclein, a biomarker of Parkinson disease by ELISA and immuno-quantitative PCR

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Background: Parkinson disease (PD) is a neurodegenerative movement disorder characterized by the progressive loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) and by the presence of intraneuronal proteinaceous cytoplasmic inclusions, termed "Lewy bodies" (LBs). The main component of these inclusions is aggregated α-synuclein, a presynaptic protein of 140 aminoacids expressed at high levels in nervous tissue. It has been suggested that, because of his primordial role in the pathogenesis, biological fluid levels of α-synuclein may be used as a biomarker for PD. To date there is neither serological nor urine sensitive assay that could confirm the diagnosis of PD in symptomatic patients and the diagnosis remains essentially clinical.

Objectives: In order to improve the sensitivity, a new immunological method called "immuno-quantitative PCR or iqPCR" for the detection of the α-synuclein. This technology, previously described by Zorzi et al. in the patent EP1232283, couples an antibody detection step similar to an ELISA with nucleic acid amplification by real-time PCR procedure. In a previous study, we showed that bovine PrPres can be detected with very high sensitivity by iqPCR.

Methods: The sensitivity of the detection is compared by direct ELISA and iqPCR.

Results: The limit of detection by the direct ELISA for recombinant α-synuclein is about 16ng/ml. By iPCRq, this limit of detection is about 2ng/ml.

Discussion: These data provide evidence that the immuno-quantitative-PCR improve the sensitivity of the detection of the α-synuclein, biomarker for the PD. The iPCRq gives about 8 fold improvement of sensitivity over the direct ELISA. This new sensitive immunological detection method seems to be promising for early diagnosis when the biomarker is present at very low levels. Currently we are in process to investigate the α-synuclein detection in biological fluids.

P.3.5

Severe psychological stress in elderly: a proposed model of neurodegeneration

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Many similarities are observed between PTSD and Alzheimer's Disease, such as (a) Hippocampus is the most vulnerable brain structure (b) The first symptom is memory problems, (c) Increased levels of glucocorticoids (GCs) are observed in both conditions and (d) there is the same prevalence of women/men ratio 2:1 in both diseases . The objective of our study is to examine the possible effect of severe psychological stress on cognitive function in the elderly. Brain MRI studies revealed structural abnormalities in patients with PTSD. These abnormalities include nonspecific white matter lesions and decreased hippocampal volume. (Tsolaki et al., 2009). Previous research (Bremner et al., 1995,1997) suggests significant decreases in the hippocampal volume in combat veterans or sexually abused people who developed PTSD as well as focal lesions of white matter in a PET study (Bremner, J.D., et al., 1999) in which Vietnam veterans with PTSD were exposed to combat sounds and pictures; decreased medial prefrontal blood flow and relatively smaller increases in anterior cingulate activity were found in PTSD patients compared to Vietnam combat veterans without PTSD. In a recent study (Tsolaki et al., 2009) our results showed that the majority of patients reported a past stressful event just before the onset of dementia (n= 990, 77.9%), whereas less patients reported insidious onset (n=281, 22.1%). The prominent stressful event was the announcement of a life-threatening disease (n=472, 37.1%), followed by problems within the family (n=157, 12.4%), spouse death (n=100, 7.9%), and death of a sibling or other beloved person (n=77, 6.1%).

Conclusions: There are some similarities between stress-induced damage and AD. First of all, HC is the first target and memory problems are the first observed symptoms. Furthermore, there are increased levels of the GCs in both stress-induced damage and AD, and there is the same prevalence of women/men ratio 2:1 in PTSD as well as in AD. According to the above results, some elderly people have increased tendency to develop dementia after a stressful event than others. This might be due to a genetic predisposition of a certain elderly population, who carries specific genes, an hypothesis which needs further and thorough investigation. Recent study showed that a deletion variant of ADRA2B, the gene encoding the *a*2b-adrenergic receptor, is related to enhanced emotional memory. The evidence collected to date is consistent that initial cognitive symptoms of a Severe Psychological Stress event may: (a) increase vulnerability to stress, (b) make neurons more sensitive to the GCs as we age, and (c) alter the regulation of the GC receptors. It is well known that plasticity of brain is changed in the elderly and perhaps acute stress is another pathogenic mechanism of neurodegeneration.

P.4.1

Enhanced enteric invasion of scrapie agents into villous epithelium by maternal immunoglobulin

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Background: The uptake and kinetics of infectious prion protein (PrPSc) in the intestine are little understood. Epidemiological studies reported that cows might be exposed to PrPSc at the first 6 months of life in most case of BSE.

Objectives: In the present study, the uptake of mouse adapted scrapie agents in the intestine of pre/post weaning mice was examined, in order to reveal the age-dependent oral transmission risk.

Methods: ICR and SCID mice at 15, 20, and 25-day-old were orally administered twice at 3h interval with 10% brain homogenate infected by mouse adapted scrapie (Tsukuba-1) diluted with PBS or PBS containing 5 mg/ml purified mouse IgG, or normal brain homogenate as a control. After fixation of intestine, embedded into Thechnovit resin and analyzed histopathologically. P8 and T2 antibody was used for PrPSc, while lectins (UAE-1 and WGA) were used for cell-identification.

Results: PrPSc was mainly incorporated through the villous columnar epithelial (UAE-1-/WGA+) cells and then detected in the villous lacteal in 15-day-old mice. This uptake significantly reduced in 20 and 25-day-old mice after weaning. In addition, the uptake of PrPSc in suckling SCID mice lacking maternal antibodies was significantly less than in wild-type ICR mice, whereas the uptake of PrPSc was enhanced by diluted mouse IgG. On the other hand, less PrPSc was detected on the epithelium of Peyer's patches.

Discussion: Villous columnar epithelial cells during suckling periods take a more important role for age-dependent uptake of PrPSc than M cells incorporating foreign substances. Immunoglobulin containing maternal milk may be enhanced the enteric invasion of PrPSc.

P.4.2

Scrapie alters immune complex trapping and the maturation cycle of follicular dendritic cells

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Background: Electron microscopy of the lymphoreticular system (LRS) of scrapie-affected sheep has shown disease-specific morphological changes of follicular dendritic cells (FDCs) and tingible body macrophages (TBMs). Such changes are associated with abnormal disease-associated prion protein (PrPd) accumulations.

Objectives: We aimed to determine and describe any scrapie-related morphological alterations in FDCs and their association with PrPd, immunoglobulins and ubiquitin.

Methods: In this study, mesenteric lymph nodes (MLNs) of ME7 scrapie-affected mice and controls were examined by light and electron microscopy using immunogold techniques.

Results: In normal mouse MLNs, FDCs could be arranged into immature, mature and regressing categories. These categories were also present in scrapie-affected mouse MLNs, however, abnormal mature FDC forms were also present. Different disease-associated FDC forms showed abnormally extended dendrites combined with excess accumulations of immune complex or vesicles within the extracellular space. Disease-associated FDC forms accumulated PrPd at the plasmalemma and either or both abnormal cell membrane ubiquitin and excess globulins. Plasma cells surrounded by PrPd-labelled FDC dendrites were numerous within scrapie-affected follicles. Within germinal centres, TBMs accumulated PrPd, ubiquitin and immunoglobulin within endosomes and lysosomes.

Discussion: The data suggests a sequence in maturation and regression of different abnormal FDC forms. Though small differences due to species and tissue were present, we nevertheless suggest that PrPd accumulation is causally linked to the abnormal pathology observed. We further suggest that scrapie-affected FDCs may shed infectivity on regression and that the dogma that prion infections do not affect the immune system should be revisited

P.4.3

Potential of cell substrates used for production of biologics to propagate transmissible spongiform encephalopathy (TSE) agents

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

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

Methods:We inoculated 3 reference TSE agent inocula, brain suspensions containing agents of bovine spongiform encephalopathy (BSE), variant Creutzfeldt-Jakob disease (vCJD) or sporadic CJD, into several cell lines important in the manufacture of biologic products. Serial dilution of BSE reference material was also inoculated in mice and squirrel monkeys.

Results: We studied these cell lines: (1) actual or candidate substrates Vero, CHO, MDCK, HEK-393, and WI-38 or (2) a control cell line from rabbit (a species resistant to common TSEs). We also studied lines of human neuroblastoma-derived cells (SH-SY5Y) engineered to overexpress wild-type PrP and mutant PrP isoforms. Cells exposed to TSE agents were serially propagated for 30 passages and selected passages tested for TSE-associated PrP (PrPTSE) and for infectivity by intracerebral inoculation into transgenic mice and squirrel monkeys (BSE-exposed cells only). No PrPTSE was found in any exposed cells after 30 passages. Susceptible cells exposed to a mouse-adapted scrapie agent as positive controls accumulated PrPTSE. No exposed cell line tested has transmitted TSE to mice or monkeys to date. One monkey inoculated with BSE reference material has developed TSE.

Discussion: We have found no evidence to date that any candidate cell substrate exposed to 3 TSE agents, including BSE agent, accumulated PrPTSE. Bioassays in susceptible animals are in progress.

P.4.4

Possible iatrogenic Creutzfeldt-Jakob Disease in an adult male 50 years after treatment with human chorionic gonadotrophin

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Background: Known causes of iatrogenic Creutzfeldt-Jakob disease (iCJD) include cadaverous corneal transplants, dural mater grafts, human growth hormone (hGH), neurosurgical depth electrodes, and neurosurgical instrument contamination. Four cases of iCJD from human gonadotrophin have been described to date, all of whom have been women.

Objectives: To present a case of possible iCJD from human chorionic gonadotrophin (hCG) and review data from four other cases

Methods: Case report and descriptive analysis

Results: A 62-year-old Caucasian man developed ataxia that resulted in frequent falls and an initial diagnosis of benign positional vertigo. Further workup including brain magnetic resonance imaging (MRI), electroencephalogram (EEG), and a lumbar puncture were unrevealing. A cerebrospinal 14-3-3 protein analysis was indeterminate. At the end of the third month of his illness, he developed short-term amnesia, disorientation, and confabulation. A repeat EEG showed generalized slowing without evidence of periodic sharp wave complexes and a repeat 14-3-3 analysis was positive. A second brain MRI showed hyperintensity in the basal ganglia on diffusion-weighted images. He died following a four-month illness. Severe vacuolization was noted on microscopic examination and Western blot analyses detected type II prion proteins. Genomic analyses detected a silent polymorphism at codon 117 and valine homozygousity at codon 129 of the prion protein gene. Further review of his medical records revealed a history of cryptorchidism and treatment with hCG as a child in the 1940's-1950's.

Discussion: This case report describes a possible case of iCJD from hCG injections and is unique in that the patient was male and the incubation period approached 50 years. His clinical presentation, EEG findings, and codon 129 homozygousity are similar to previously described cases.

P.4.5

The exchange of single aminoacids in ovine and caprine prion variants influences convertibility of the prion protein in vitro

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Background: The central role in the pathogenesis of prion disease is the conversion of the normal cellular host-encoded prion protein PrPC into an abnormal pathogenic isoform PrPSc. This conversion process can be imitated in a cell-free conversion assay by adding purified PrPSc to recombinant PrPC, which is converted into a PK-resistant isoform PrPres. Polymorphisms of the allele encoding ovine and caprine prion protein are known to influence scrapie susceptibility in sheep and goats.

Objectives: Substitutions in the prion amino acid sequence are directly associated with variations in relative susceptibility to scrapie in sheep and goats. These effects were determined with numerous variants of bacterial full length prion protein in a cell-free conversion assay. Moreover, some polymorphisms exhibited dominant-negative effects on prion replication.

Methods: Ovine and caprine prion alleles were expressed in E. coli and purified over a N-terminal His-tag using a Ni-NTA-column according to standard procedures. Cell-free conversion is carried out by incubating recombinant prion protein with purified PrPSc seeds in an appropriate buffer. Detection of newly converted PK-resistant PrPres fragments is carried out by immunoblotting and incubation with mab P4.

Results: A set of recombinant goat and sheep derived prion constructs was generated by the substitution of single aminoacids at specific positions throughout the whole protein and were subsequently analyzed in the cell-free conversion assay. Our study demonstrates that several newly generated prion alleles show a significantly reduced convertibility or are even inconvertible by PrPSc seeds.

Discussion: The results highlight that specific aminoacid residues induce a resistance of the cellular prion protein with regard to conversion and prion replication. In summary, the data indicate that the in vitro analysis of prion conversion is strongly consistent with the situation in vivo.

P.4.6

Different contribution of PrP glycoforms in modulating TSE infection within and between different hosts

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Background: The mechanisms by which TSE agents are able to infect and cause disease in different hosts remain elusive. It has been proposed that the variation in PrP glycotypes may contribute to determine TSE strain characteristics and the species barrier effect.

Objectives: To establish the contribution of glycosylation of PrP in the infectious process.

Methods: We have generated three lines of gene-targeted transgenic mice with amino acid substitutions at the first (G1), second (G2) or both (G3) glycosylation sites of PrP. These mice express glycosylation deficient PrP species. We inoculated these mice with different murine, human and hamster derived TSE-strains.

Results: We established that mono and un-glycosylated PrP supports clinical and pathological disease in mice inoculated with mouse-adapted TSE strains. We also showed that different TSE agents had dramatically different requirements for glycosylation of host PrP. A significant alteration in transmission was also observed when mice were challenged with TSE agents derived from humans or hamsters. However, in this case transmission of disease was exclusively modulated by host mono-glycosylated PrP (G2 PrP). Mice without a sugar at the second PrP site were efficiently infected with sporadic CJD or 263K strains whereas wild type mice were resistant. However mice inoculated with variant CJD strain became ill after prolong incubation times. These results suggest that glycosylation at the second site of host PrP is a key factor affecting species barrier. Finally, we have further demonstrated a role for PrP-glycosylation in determining some of the TSE strain properties.

Discussion: Using unique transgenic mice we showed the important role that different PrP glycoforms play on TSE pathogenesis. PrP glycosylation is a key factor in this process and N-linked sugars have different roles that influence intra- or inter-species transmission of TSE diseases.

P.4.7

Dynamics of PrPSc accumulation in peripheral organs and blood at different stages of prion disease

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Background: The presence of PrPSc in blood and peripheral tissues is considered a risk for TSE transmission. Although the presence and quantity of infectious prions in peripheral tissues and biological fluids has been previously reported, little is known about the dynamics of PrPSc replication and accumulation in the body.

Objectives: Measure the levels of PrPSc in spleens, blood fractions and brain at different time points after infection.

Methods: PrPSc levels were assessed by PMCA. Tissue extracts were obtained from hamsters intraperitoneally infected with 263K prions. Animals were sacrificed at different time points. Tissues and fluids analyzed include brains, spleens and different blood fractions.

Results: PrPSc from the inoculum disappeared from spleens and blood few days after inoculation. Endogenous replication of PrPSc reached high levels in spleens at early stages after infection, which correlated with their presence in white blood cells. Interestingly, PrPSc quantity decreased in spleens in the middle of the incubation periods, precisely prior to the time in which PrPSc began to appear in the brain. The levels of PrPSc increased again in spleens close to the symptomatic phase. The levels of PrPSc in brain followed a linear relation with time whereas in plasma PrPSc was only detectable at the symptomatic phase.

Discussion: Our findings indicate that the presence of PrPSc in blood has two different sources: peripheral replication in spleen at early stages and brain leakage at late stages. Our results contribute to understand the dynamics of prion replication and accumulation, providing important information to elucidate the prion replication mechanism after infection by peripheral routes.

P.4.8

Distribution and frequency of nerve fibres in ovine palatine and pharyngeal tonsils

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Background: Studies about the uptake and dissemination of PrPSc in natural scrapie showed that Peyer's Patches and tonsils are the first organs contaminated. It's generally well accepted that pathogens reach the central nervous system via an ascending propagation along the autonomic nervous system. Nevertheless, if Peyer's patches are well documented, the pattern of innervation in tonsils is poorly described. In addition, lambs of some particular races and genotypes show inconsistent prion sensibility when compared with others or with older animals.

Objectives: The aim of our study has been to determine the potential role of the lymph reticular system innervation in the transport of prion infectivity to the central nervous system.

Methods: Palatine and pharyngeal tonsils were removed from healthy sheep of which the race, age and PrP genotype are known. Cryosections were processed for immunohistochemistry and incubated in a range of primary antibodies directed against neurofilaments and glial cells surrounding nerve fibres.

Results: After quantitative analyses on palatine and pharyngeal tonsils, it appears that the lymphoid compartments are poorly innervated. The majority of the nerves fibres were located in the connective tissue surrounding these organs. Some GFAP positive fibres and in less proportion, heavy neurofilaments were detected in the interfollicular T cells area. Occasionally, GFAP+ fibres and NFH + filaments were present in the mantle zone and in the germinal centre of lymphoid follicles. Neurofilaments L and M were almost absent.

Discussion: Our preliminary results detect the presence of nerves fibres inside the studied tonsils. The lymphoid compartments seem to be poorly innervated. To complete our overview of the innervation of tonsils in sheep, we must extend our researches to other types of nerves fibres. Additional analyzes are essential to point out possible contacts between nerves fibres and FDCs.

P.4.9

Trafficking of CWD prions via the autonomic nervous system

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Background: The pathway by which CWD prions efficiently transit from the periphery to the central nervous system remains incompletely understood. Here we provide evidence that the autonomic nervous system (ANS) serves as a significant pathway for centripetal and centrifugal CWD spread in cervid PrP transgenic mice (Tg[CerPrP]) infected via oral and parenteral routes of exposure.

Objectives: The chief objective of this work was to determine the role of the ANS in the trafficking of CWD prions by longitudinally mapping the PrPres tropism patterns in neural and non-neural tissues of multi-route inoculated Tg[CerPrP] mice.

Methods: Five groups of n=10 Tg[CerPrP] mice were inoculated with CWD prions via either the intracerebral (IC), intraperitoneal (IP), intravenous (IV), or oral (PO) route. Mice were sacrificed at specified time points post inoculation and at the onset of clinical disease. CWD infection was documented and prion trafficking patterns determined by detection of PrPres using amplified IHC methods.

Results: Early and progressive PrPres depositions were detected in the parasympathetic, sympathetic, and enteric nervous systems (ENS), in particular within the myenteric plexus, vagus nerve, dorsal motor nucleus of the vagus, solitary tract, lateral ventricular nuclei of the hypothalamus, periaqueductal gray matter, and spinal cord. Moreover, PrPres was seen in intimate association with fibers and cells of the ENS, namely enteroglial cells, and sympathetically-innervated lymphoid organs, including the spleen, Peyer's patches, and mesenteric lymph nodes.

Discussion: We present evidence for a temporal-based pattern of PrPres accumulation in the parasympathetic and sympathetic elements of the ANS and ENS, implicating these elements as major pathways for CWD prion neuroinvasion and gastrointestinal prion shedding. These patterns of spread, notably accumulations in the ENS, may explain the ease by which CWD prions are taken up from and shed into the environment, thereby its high degree of horizontal transmission.

P.4.10

BSE/Scrapie phenotype are maintained following co-infection of ovine PrP overexpressing transgenic mice (Tgshp IX)

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Background: In TSE infected sheep a differentiation of the strains is routinely performed to discriminate between BSE and scrapie. Until now it is unclear if a co-infection with BSE and scrapie would be detectable with the validated methods used.

Objectives: Determination of BSE and scrapie in co-infected transgenic mice over-expressing ovine PrP.

Methods: Transgenic mice over-expressing ovine PrP (Tgshp IX) were i.c. inoculated with three well characterized scrapie strains and the ovine BSE pool. At first, a simultaneous co-infection was performed, using three approaches: (i) an equal dilution (10-1) of both the scrapie strains and the ovine BSE (ii) 10-1 diluted scrapie strains combined with 10-3 diluted ovine BSE and (iii) a 10-3 dilution of the scrapie strains with a 10-1 dilution of ovine BSE. Secondly, a consecutive co-infection, in which scrapie (10-1) preceded BSE (10-1) by four weeks and vice versa, was also performed. To characterise the propagated strain the mouse brains were examined by histopathological and immunohistochemical (lesion profile score) as well as biochemical (discriminatory immunoblot, the so called FLI-test) methods.

Results: A co-infection of Tgshp IX mice with ovine BSE and classical scrapie isolates/strains produced phenotypes which resembled either one or the other starting agent or a mixture of both. The determining factor for the outcome of the infection seemed to be the infectivity titre of the respective isolates/strains used in the co-infection. The inoculum carrying the higher infectivity load usually dominated the lesser infectious inoculum.

Discussion: It has to be emphasized that a discrimination of the propagated strains in this co-infection model is not possible without knowing the characteristics of the original strains. Future studies should focus on the question of whether both isolates/strains propagate independently in these mice or whether the overwhelming presence of one is inhibitory for the other isolate/strain.

P.4.11

Detection of subclinical CWD infection in conventional test-negative deer long after oral exposure to urine and feces from CWD+ deer

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Background: Chronic wasting disease (CWD) of cervids is distinguished by its high level of transmissibility, wherein bodily fluids and excretions are thought to play an important role. Using cervid bioassay and established CWD detection methods, we have previously identified infectious prions in saliva and blood but not urine or feces of CWD+ donors. More recently, we were able to identify very low concentrations of CWD prions in urine of deer by cervid PrP transgenic (Tg[CerPrP]) mouse bioassay and serial protein misfolding cyclic amplification (sPMCA).

Objectives: In these experiments, we sought to investigate whether deer previously exposed orally to urine and feces from CWD+ deer, while conventional test-negative, may actually be harboring very low level CWD infection, not evident in the 19 month observation period in the previous studies.

Methods: Brain and lymph nodes from conventional test-negative deer were reanalyzed for CWD prions by sPMCA and cervid transgenic mouse bioassay in parallel with appropriate tissue-matched positive and negative controls.

Results: PrPres was detected in tissues of exposed deer by both sPMCA and Tg[CerPrP] mouse bioassay; each assay revealed very low levels of CWD prions previously undetectable by western blot, ELISA, or IHC.

Discussion: The finding of subclinical infection in deer orally exposed to urine and feces (1) suggests that a prolonged subclinical state can exist such that observation periods in excess of two years may be needed to detect CWD infection and (2) illustrates the sensitive and specific application of sPMCA in the diagnosis of low level prion infection.

P.4.12

Activated prion infected immune cells accelerate the transmission of prion disease

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Background: We have previously shown that when scrapie-infected mice were challenged with EAE, an autoimmune CNS inflammatory insult, they succumbed to a fatal neurological disease long before control scrapie-infected mice perished of prion disease. Pathological and biochemical examinations did not present accelerated accumulation of PrPSc but did reveal uncharacteristic aggregates of PrPSc in spinal cord white matter infiltrates.

Objectives: To test whether induction of EAE activates prions-infected immune-cells in the spleen and lymph nodes. These cells may carry PrPSc into the CNS directly, bypassing the "normal" neuroinvasion pathway and redirecting PrPSc to white matter areas.

Methods: In order to examine this hypothesis, we used an adoptive-transfer EAE protocol. Mice infected with prions were induced for EAE (MOG35-55) and sacrificed 9 days post induction. Cells from spleens and lymph nodes were collected, incubated with MOG for 3 days and injected to naove mice. Control group of naove mice were injected with dead cells representing regular prions infection.

Results: As expected, mice injected with viable scrapie infected and MOG-activated immune cells showed immune-cells infiltrates in the CNS and clinical signs of EAE (29 d.p.i). Subsequently, mice in this group succumbed to progressive neurological disease prior to control group injected with dead cells.

Discussion: Our results suggest that activated immune cells infected with prions can cause accelerated fatal neurological disease. These cells may cause disease by an alternative pathway of neuroinvasion, and in addition, change the clinical pattern of disease related to non conventional sites of PrPSc accumulation, such as the white matter in spinal cord. Further experiments are required to determine the exact target of activated and prion infected immune cells.

P.4.13

Detection and localisation of abnormal prion protein in the liver of TSE-affected sheep

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Background: Transmissible spongiform encephalopathies (TSEs) are commonly associated with the accumulation of disease-associated PrP (PrPd) in nervous and lymphoid tissues of infected animals and humans. Following advances in diagnostic sensitivity PrPd and associated infectivity has been shown in other tissues including muscle, salivary gland, mammary gland, placenta and kidney, as well as blood, urine and milk, indicating they may be potential sources for disease spread.

Objectives: To investigate the accumulation of PrPd in the liver of scrapie infected and BSE challenged ewes.

Methods: We have compared two immunoassays specific for PrPd, one ligand-based and one based on Proteinase K resistance for the detection of PrPd in the liver of sheep naturally infected with scrapie and orally challenged with BSE. Assay results were confirmed by Western blot and immunohistochemistry.

Results: We show that PrPd can be detected in the liver of VRQ//VRQ scrapie infected sheep and ARQ/ARQ BSE-challenged sheep. PrPd was detected in the livers of all sheep showing clinical signs and in the preclinical phase. The presence of PK-resistant PrPd was confirmed by Western blot and IHC showed PrPd deposits in the Kupffer cells.

Discussion: This study showed that liver from both clinical and preclinical sheep naturally infected with scrapie and orally challenged with BSE contained PrPd. The PrPd was localised to isolated cells of myeloid morphology likely to be Kupffer cells and exposure of liver to PrPd is likely to occur via the hepatic portal vein directly from the gut, or from blood, either in leucocytes or in plasma. Although the levels of PrPd in liver are relatively low compared with brain and lymphoid tissue these findings indicate that liver is a source of infectivity in the absence of inflammatory lesions. Estimating the potential risk to human health would depend on whether BSE was transmitted to the GB sheep flock and also on whether accumulation in the liver coincides with the age at which liver is consumed.

P.4.14

Age related alterations influence the susceptibility to prion infection in mice

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Background: Sporadic and genetic forms of Creutzfeldt–Jakob disease (CJD) affect mostly elderly people aged 60 to 70 years. Nevertheless, the infectious forms of CJD, Kuru and vCJD, present a much earlier age of onset. Although susceptibility to infection seems to decrease with age, no apparent age-related epidemiological factor was found, that could explain this bias.

Objectives: To study the effect of age on prion pathogenesis.

Methods: We inoculated young (4 weeks) and aged (16 and 24 months) C57BL/6 mice intraperitoneally with RML prions as well as mock-inoculated age-match controls, and followed them for clinical signs of prion disease. Disease-specific pathological characteristics such as brain vacuolation and gliosis were detected using histological and immunohistochemical methods, and accumulation of the pathogenic isoform PrPSc in spleen and brain was detected by western blot analysis. We also studied the expression profile of age-related and disease-related genes in all groups of mice, by quantitative real time RT-PCR.

Results: We show that prion disease incubation time is significantly prolonged when mice are infected at older age, as compared to mice infected at younger age. In addition, mice infected at older age present milder brain pathology such as gliosis and vacuolation as well as reduced PrPSc accumulation both in the brain and in the spleen. Interestingly, we found significant differences in the expression pattern of disease-related genes in the brain, between mice infected at young age and those infected at old age.

Discussion: Our data suggest that alterations associated with aging, such as the reduced response of pro-inflammatory and stress-response genes to prion infection, decrease the susceptibility to prion infection, delay the disease onset, and result in milder pathology in aged mice.

P.4.15

Cryoprecipitate components induce the solubilization of spiked prion infectivity

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Background: Transmissible spongiform encephalopathies (TSE's) can be transmitted via blood, therefore testing industrial plasma fractionation processes for potentially pass on of prion infectivity into plasma derivatives is essential. Since direct testing of infectivity is impractical, spiking of human plasma with rodent brain prions and subsequently testing for the clearance of PrPSc into individual plasma components is used to test the safety of such reagents. This method is based on the assumption that the disappearance of PrPSc correlates with the removal of prion infectivity.

Objectives: To test the correlation between the clearance of PrPSc and prion infectivity following the fractionation of prion spiked cryoprecipitate fractions.

Methods: Human Cryoprecipitate fractions were spiked with microsomes prepared from prion infected hamster brain and fractionated by several centrifugations and filtration protocols. Designated fractions were tested for traces of PrPSc both by immunoblot analysis and by prion infectivity bioassay. In parallel, the interaction of PrPSc with cryoprecipitate components in the presence and absence of heparin was tested.

Results: Following ultra centrifugation of the spiked cryoprecipitate, PrPSc was detected exclusively in the pellet fraction. Nevertheless, the titers of prion infectivity as determined by a bioassay, were similar in both the pellet and the supernatant fractions. Additional data suggest that cryoprecipitate fractions may solubilize trace quantities of PrPSc, an effect abolished by the addition of heparin.

Discussion: Our results are consistent with previous data indicating that the correlation between PrPSc and prion infectivity is not necessarily quantitative and imply that high titer prion infectivity may be transmitted by low quantities of PrPSc bound to cryoprecipitate components harboring affinity to heparin.

P.4.16

In vitro evaluation of the conversion potential of fish prion proteins

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Background: In transmissible spongiform encephalopathies (TSEs) the key event in disease pathogenesis is the accumulation of an abnormal conformational isoform (PrP^{Sc}) of the host-encoded cellular prion protein (PrP^{C}). While the precise mechanism of the PrP^{C} to PrP^{Sc} conversion is not understood, it is clear that host PrP^{C} expression is a prerequisite for effective infectious prion propagation. The identification of apparent PrP orthologues in lower vertebrates, including fish, raises the question of their susceptibility to prion diseases. While fish PrP-like sequences do not share high homology with their mammalian relatives, they do contain several strongly conserved prion protein structural motifs. Although there have been many studies on TSEs in mammalian species, little is known about TSE pathogenesis in fish.

Objective: The aim of this study was the evaluation of the possible conversion of recombinant fish PrP proteins into abnormal, PK-resistant isoforms in a scrapie-infected cellular environment.

Methods: The genes coding for sea bream (*Sparus aurata*) PrP-1 and zebrafish (*Danio rerio*) PrP-1 and 2 were cloned into pcDNA3.1/Zeo(+) transfection vector and used to transfect 22L-scrapie N2a cells. Fish PrP proteins overexpressed in cell lysates were assayed for resistance to PK-treatment by western blot.

Results: Our results suggest that no PK-resistant isoforms are formed following the expression of the the three fish PrP proteins in the scrapie-infected cells and their intracellular incubation with mouse PrP^{Sc}.

Discussion: The two zebrafish prion proteins and the PrP from sea bream that were used in the study, although efficiently expressed, appear not to convert into PK-resistant isoforms in the 22L-ScN2a cells. These results are in agreement with our *in vivo* findings, which suggested that no PK-resistant deposits are formed in the brains of sea bream orally-challenged with natural scrapie.

P.4.17

Effects of passage history, dose, PRNP genotype and age at exposure on incubation period and attack rates in sheep orally challenged with BSE.

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Background: In the early part of the BSE cattle epidemic, some UK sheep may have been exposed to feedstuffs containing BSE infected tissues but ovine BSE has not yet been detected in field case isolates of UK sheep TSEs.

Objectives: To determine whether the BSE phenotype could change following serial passage over time or as a result of several other factors, a number of transmission experiments were undertaken.

Methods: The abundance and features of PrPd accumulation were determined on samples of brain and viscera of 120 sheep. Data from three different experiments were pooled and incubation periods and attack rates were assessed according to breed (Romney, Suffolk, Cheviot, Poll Dorset), genotype (at codons 136, 154, 171), oral dose (0.5-0.0005g), passage history (primary, secondary, tertiary), and age at challenge (3 or 6 months) of challenge.

Results: No significant modulation of the IHC BSE phenotype was noted either in CNS or LRS tissues, but marked variation of incubation periods and/or attack rates was observed for each factor tested. The presence and magnitude of PrPd accumulation within tissues showed no correlation with attack rate or incubation period. The shortest incubation periods were in AHQ/AHQ sheep and no disease has yet been observed in those challenged with less than 0.05g. No detectable effect was observed for sheep passage history or for the age at challenge.

Discussion: The present results do not show a drift of the disease phenotype under controlled experimental oral challenge, and provide some confidence that sheep BSE has not changed over time and become undetectable against the wider ovine TSE background. For each parameter tested, a marked variation in incubation periods was found, but this did not correlate with the magnitude of PrPd accumulation in brain or viscera, suggesting that there is no simple linkage between PrPd accumulation and clinical disease.

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P.4.18

Uptake of variant Creutzfeldt-Jakob disease brain material by human embryonic stem cells in vitro

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Recent developments in human embryonic stem cell (hESC) biology offer future therapies for a wide range of diseases. However, both the efficacy and safety of therapeutic stem cells require careful evaluation. The history of inadvertent prion transmission by growth hormone therapy, dura mater grafting, and now blood transfusion all indicate that it would be prudent to examine the potential for prion infection of stem cells, in addition to more conventional pathogens. We have begun this process by determining the PRNP codon 129 genotype, by restriction fragment length polymorphism analysis, and prion protein expression levels, by Western blot and immunocytochemistry of a series of hESC lines. We then selected lines with different PRNP codon 129 genotypes and challenged these cells in a relatively undifferentiated dividing state with material derived from variant Creutzfeldt-Jakob disease brain, using material form Alzheimer disease brains as a negative control. PrP uptake was monitored in situ by immunocytochemistry and confocal microscopy. The hESC lines were found to be broadly representative of the population as a whole, with PRNP codon 129 methionine and valine homozygous and heterozygous lines all identified. In their relatively undifferentiated pluripotent state, levels of cellular prion protein expression were found to be low. Immunocytochemistry for prion protein showed that uptake of infectious brain material was extensive and rapid, with the majority of cells exhibiting readily detectable intracellular prion protein accumulation not seen in unexposed cells or in cells exposed to non-Creutzfeldt-Jakob disease brain. Uptake did not appear to depend on the cellular prion protein codon 129 genotype.

Understanding the cell biology of this process, the metabolic processes applied to this material and the potential for establishing true prion infection of these cells will be an important component part of assessing the safety of therapeutic stem cells.

P.4.19

Tracking prion infectivity in the blood of deer with Chronic Wasting disease

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Background: The blood and saliva of deer infected with chronic wasting disease (CWD) contain infectious prions (Mathiason, 2006). Elucidation of the blood component(s) carrying prion infectivity would be valuable in understanding CWD prion trafficking within the host and in focusing development of antemortem assays to detect prionemia.

Objectives: The goal of these studies was to identify the blood components principally responsible for prionemia in CWD infection.

Methods: CD21+ B cells, CD14+ monocytes, and CD41/61+ platelets were enriched from the blood of CWD+ white-tailed deer to >97% purity by magnetic immunoselection. Un-fractionated leukocytes and cell-free plasma were also prepared. Cohorts of n=4 CWD-naove white-tailed deer were inoculated intravenously with each of these purified cell subsets or with cell-free plasma or un-fractionated leukocytes from the CWD+ donors. CWD infection status of the recipient deer was monitored by IHC and western blotting in tonsil biopsies collected at 0, 3, 6, 12, and 15 mos pi. At study termination (19 mos pi) a wider array of lymphoid and nervous system tissues were examined for PrPCWD. Parallel bioassay studies were conducted in cervid PrP transgenic mice using multiple routes of inoculation and clinical disease as study endpoint.

Results: IHC and WB analysis of tonsil biopsies and terminal tissues revealed PrPCWD in 4/4 deer inoculated with the cell-associated fraction of blood from CWD+ donors, while 0/4 recipients of cell-free plasma from the same donors became CWD+. Upon further analysis of the cellular fraction, PrPCWD was detected in 4/4 deer receiving CD21+ B cells and 3/4 receiving CD41/61+ platelets, but 0/4 recipients of CD14+ monocytes. All 8 positive control deer became tonsil biopsy PrPCWD+ between 6 and 12 mos pi while all negative control deer remained PrPCWD negative. Bioassay results in Tg[CerPrP] mice closely mirrored those found in deer.

P.4.20

Limited presence of arginine-171 containing PrP in PK resistant fraction of naturally scrapie infected ARR/VRQ sheep

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Background: The national scrapie control plan by selective breeding in The Netherlands has since 1998 been aiming at breeding with rams of the ARR/ARR prion protein (PrP) genotype that is expected to be effective enough to limit scrapie as well as spread of BSE - if it were present - under natural conditions. This has since 2008 resulted into a decreasing prevalence of classical scrapie.

Objectives: To assess the potential risk of scrapie adaptation to ARR PrP under natural conditions, we tried to determine whether the ARR allele contributes to PrPSc accumulation in naturally classical scrapie infected heterozygous VRQ/ARR animals using biochemical PrP detection techniques.

Methods: We analysed brains of such heterozygous infected sheep to see whether ARR-containing prion protein (ARR-PrP) would be present in the proteinase K resistant PrP fraction (PrPres), using Q171 allele dependent antibodies, recombinant variants of ovine PrP, generation of specific proteolytic cleavage fragments, and with identification of the R and Q allel containing fragment by 2D dimensional gel electrophoresis.

Results: Indeed, we have clear evidence that ARR allele containing PrPres is present in varying amounts in all investigated individuals. However, this ARR PrPres fraction represents only marginal amounts relative to the VRQ-PrPres fraction, and is estimated to be around 1% of the total PrPres material. As forecasted from in vitro conversion assays (Bossers et al. J Virol. 2000 74:1407-14), ARR-PrP has a definite but low tendency to become converted.

Discussion: These data support that in the various (usually older, >6 years) heterozygote VRQ/ARR animals there is a near zero contribution of ARR to the PrPres fraction, indicating poor (if any) adaptation of classical scrapie under natural conditions.

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P.4.21

Tissue homogenate from inherited prion disease A117V is transmissible to transgenic mice expressing PRNP 117-Valine

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Background: Prion diseases are by definition transmissible, but some inherited prion diseases (IPDs) including Gerstmann-Straussler-Scheinker (GSS) disease associated with the A117V mutation in the human prion protein gene (PRNP) have not been shown to be transmissible experimentally. This IPD has a diverse phenotypic spectrum, even among members of the same family. The absence of transmission data, coupled with the inability to detect conventional protease-resistant PrPSc in A117V brain tissue has led to the suggestion that pathogenesis may relate to an aberrant topological form of PrP referred to as CtmPrP.

Objectives: To investigate the transmissibility of inherited prion disease A117V in appropriate transgenic models.

Methods: We generated transgenic mice lacking murine PrP expression but homozygous for transgenes expressing human PrP 117V and challenged them with brain homogenates from A117V IPD and iatrogenic and sporadic (classical) CJD patients. Mouse brain samples were analysed by immunoblotting and immunohistochemistry.

Results: Our data show for the first time that brain homogenates from IPD A117V patients are transmissible and can result in clinical disease and propagation of detectable full-length PrPSc. Although these 117V transgenic mice express the identical protein, there remains a substantial transmission barrier which manifests in low clinical attack rates and prolonged incubation periods. Classical CJD prion isolates propagated readily in these mice, with the production of typical PrPSc. Investigation of PrPSc produced in mice after transmission of IPD A117V and classical CJD showed marked differences in relative PK resistance.

Discussion: We conclude that previous difficulties in detecting PrPSc from A117V patient brain relates to the propagation of a distinct human prion strain involving a relatively unstable PrPSc conformation.

P.4.22

The effect of leucodepletion on transmission of BSE by transfusion of sheep blood components

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In humans affected with vCJD, there is an expansion and dissemination of infectious material within the lymphoreticular system. To date, there have been 4 probable cases of vCJD attributable to infectivity arising from transfusion of blood or blood products. Previously, we have shown that BSE-infected sheep blood is transmissible to naive recipients. This experimental system thereby provides a unique model, suitable to assess the risk of vCJD being transmitted in humans following blood transfusion.

Objectives:

- To prepare the different blood components to the same specificity and within parameter boundaries as those achieved by the UK Blood Transfusion Service
- To examine the efficacy of leucoreduction on the transmission of BSE infectivity by transfusion of various sheep blood components
- To establish which blood components are able to transmit infectivity.

We have undertaken a series of blood transfusions of leucoreduced and non-leucoreduced blood components (using methods and specifications similar to those employed by the blood transfusion services) to assess the distribution of BSE infectivity within both orally-dosed donors and transfused recipients. Westerns were carried out on PK and NaPTA precipitated brain homogenates. Detection of PK-resistant PrPSc was conducted using 'in-house' generated monoclonal antibodies raised to recombinant sheep prion protein.

Within this study, we have performed over 250 blood transfusions. In addition to data on infectivity we have also examined the brain and peripheral tissues for the expression of PrPSc. At terminal disease, our analyses have shown that PrPSc deposition occurs throughout the brain and can also be detected in lymphoid organs such as the spleen and peripheral lymph nodes.

Our current data shows that whole blood, buffy coat and plasma (from BSE blood donors) can transmit infection to recipients. As yet no sheep that have received leucoreduced components have succumbed to infection.

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P.4.23

Transmission of atypical BSE in humanized mouse models

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Background: Classical BSE is a world-wide prion disease in cattle, and the classical BSE strain (BSE-C) has led to over 200 cases of clinical human infection (variant CJD). Atypical BSE cases have been discovered in three continents since 2004; they include the L-type (also named BASE), the H-type, and the first reported case of naturally occurring BSE with mutated bovine PRNP (termed BSE-M). The public health risks posed by atypical BSE were largely undefined.

Objectives: To investigate these atypical BSE types in terms of their transmissibility and phenotypes in humanized mice.

Methods: Transgenic mice expressing human PrP were inoculated with several classical (C-type) and atypical (L-, H-, or M-type) BSE isolates, and the transmission rate, incubation time, characteristics and distribution of PrPSc, symptoms, and histopathology were or will be examined and compared.

Results: Sixty percent of BASE-inoculated humanized mice became infected with minimal spongiosis and an average incubation time of 20-22 months, whereas only one of the C-type BSE-inoculated mice developed prion disease after more than 2 years. Protease-resistant PrPSc in BASE-infected humanized Tg mouse brains was biochemically different from bovine BASE or sCJD. PrPSc was also detected in the spleen of 22% of BASE-infected humanized mice, but not in those infected with sCJD. Secondary transmission of BASE in the humanized mice led to a small reduction in incubation time. The atypical BSE-H strain is also transmissible with distinct phenotypes in the humanized mice, but no BSE-M transmission has been observed so far.

Discussion: Our results demonstrate that BASE is more virulent than classical BSE, has a lymphotropic phenotype, and displays a modest transmission barrier in our humanized mice. BSE-H is also transmissible in our humanized Tg mice. The possibility of more than two atypical BSE strains will be discussed.

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P.4.24

Recycling of PrPSc via retrograde transport pathway from endosome to TGN in Neuro2a mouse neuroblastoma cells

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Background: While propagation of prion in neurons is believed to be tightly associated with neuronal degeneration in central nervous system on prion diseases, the molecular mechanisms of prion propagation have not been fully understood yet.

Objectives: To clarify the mechanisms of prion propagation in cells, we analyzed the intracellular localization of PrPSc.

Methods: Subclone of Neuro2a mouse neuroblastoma cells that was persistently infected with 22L strain, was used. To detect PrPSc with an indirect immunofluorescence assay, cells were pretreated with guanidinium thiocyanate and stained with anti-PrP mouse monoclonal antibodies (mAbs). For the analysis of the intracellular localization of PrPSc, double staining was performed with following organelle markers: PDI, Giantin, Tgn38, Rab5, Rab7, Lamp1 and Shiga toxin subunit B (StxB).

Results: PrPSc could be specifically detected by using mAb132 that recognizes the region adjacent to the most amyloidgenic region of PrP. As a result of the double staining, the granular staining of PrPSc was found at perinuclear regions juxtaposed to trans-Golgi network (TGN). The PrPSc was co-localized with StxB that is known to be transported to TGN via retrograde transport pathway. The perinuclear PrPSc disappeared by the introduction of siRNA against a component of machinery associated with retrograde transport from endosome to TGN. When the cells were cultured at 20, C to prevent the retrograde transport, PrPSc was scattered to cytoplasm, however, PrPSc signals returned to perinuclear regions 30 min after transferring the cells to 37, C. Interestingly, the returned PrPSc signals disappeared from perinuclear regions after the additional 30 min incubation at 37, C and appeared again after the subsequent 60 min incubation.

Discussion: These results suggest that PrPSc is recycled between plasma membrane and the perinuclear regions, and propose an idea that retrograde transport from endosome to TGN may be involved in recycling of PrPSc.

P.4.25

Human susceptibility to atypical scrapie

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Background: Isolates of classical sheep scrapie are thought to pose little risk to humans as there have been no documented links between presence of sheep scrapie and the development of human TSE disease. However, the link between BSE and the development of vCJD in humans proves that a risk does exist from ruminant TSE disease, and therefore all new ruminant TSEs may potentially be transmissible to humans. Due to increased sensitivity of TSE diagnostic assay systems, a new TSE of sheep termed 'atypical scrapie' has been identified. This disease has been difficult to identify, and is found mainly in sheep which are previously thought to have a genetic make-up that made them resistant to scrapie. It is unclear whether this is a new TSE of sheep, an old disease which has only been identified through increased surveillance, or if it represents the phenotype of classical scrapie in so called 'resistant' sheep PrP genotypes.

Objectives: The objective of the study is to assess relative transmissibility of atypical scrapie isolates to humans and the associated risk to the population.

Methods: In order to determine whether atypical scrapie poses a risk to human health we have transmitted isolates from three different sheep PrP genotypes to our gene targeted transgenic mice which express human PrP with the M129V polymorphism known to be important in human susceptibility to disease. Mice of all three PrP genotypes have been inoculated intracerebrally with atypical scrapie isolates.

Discussion: In order to prevent the emergence of a new human TSE, we need to be able to assess the risk to humans from new emerging TSEs in livestock. The study of atypical scrapie infection in these transgenic lines could therefore provide important information on the host range and disease characteristics associated with such isolates. Preventative measures could then be put in place before this disease gives rise to another human disease variant and an underlying level of infection in the population.

P.4.26

Aerosol and intranasal transmission of CWD

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Background: Little is known regarding the potential risk posed by aerosolized prions. Chronic wasting disease (CWD) prions are present in saliva and urine of infected animals and it is clearly established that CWD is transmitted horizontally, almost surely by mucosal exposure. However, the potential transmissibility of CWD by aerosol or nasal routes is not known.

Objectives: The present study was therefore designed to determine whether CWD prions are transmissible by these routes of exposure using the cervid PrP transgenic mouse model of CWD infection.

Methods: FVB mice transgenically expressing the normal cervid PrPC protein [Tg(cerPrP) mice] were exposed to CWD prions by either nose-only exposure to an aerosol generated by nebulizing 0.5 ml of a 5% w/v CWD+ brain homogenate or 10µl of a 10% w/v CWD+ brain homogenate by dropwise instillation into the nostrils. Mice were monitored for signs of clinical disease for up to 755 days post inoculation (dpi). Nasal mucosa, vomeronasal organ, lymphoid tissue, and the brain were assessed for PrPCWD by western blotting and immunohistochemistry.

Results: Six of 7 aerosol-exposed Tg(cerPrP) mice developed clinical signs of neurologic dysfunction between 411 and 749 dpi mandating euthanasia. In all symptomatic mice CWD infection was confirmed by histopathologic lesions and detection of PrPCWD within the brain. Two of 9 IN-inoculated Tg(cerPrP) mice also developed TSE between 417 and 755 dpi, again confirmed by PrPCWD detection within the brain. No evidence of PrPCWD was detected in any Tg(cerPrP) mice examined at any of the pre-terminal time points.

Discussion: CWD is transmissible by aerosol as well as intranasal exposure—potentially implicating exposure via the respiratory system in CWD and potentially other prion diseases. Studies examining very early post-inoculation sampling intervals (1 and 4 hours) are in progress in an attempt to determine initial prion targeting and entry portals.

P.4.27

Minor oral lesions facilitate CWD infection

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Background: While the exact mechanisms of chronic wasting disease (CWD) prion transmission, entry, and trafficking remain incompletely elucidated, transmission by exposure of the oral and/or nasal mucous membranes seems certain. As part of foraging, cervids likely experience minor lesions in the oral mucous membranes; these could have impact on susceptibility to prion entry and subsequent infection.

Objectives: To explore this potential co-factor, we used cervid PrP transgenic mice to assess whether or not micro-abrasions to the tongue may enhance susceptibility to oral CWD infection.

Methods: Two sets of FVB mice transgenically expressing the normal cervid PrPC protein [Tg(cerPrP) mice], with or without abrasions on the lingual mucosa, were inoculated orally with 10µl of a 10% w/v brain homogenate from either CWD-positive or negative deer. Abrasions were created by lightly scratching the dorsal lingual epithelium with a 30g needle. Cohorts were sacrificed at 1, 2, 12, 52, 78, and 104 weeks post inoculation (pi) or when signs of neurologic disease were observed. Tongue, lymphoid tissue, and the brain were assessed by western blotting and immunohistochemistry to detect the CWD abnormal prion protein (PrPCWD).

Results: Between 296 and 430 dpi, 8 of the 9 CWD-inoculated mice with lingual lesions developed clinical signs of neurologic dysfunction mandating euthanasia. The brains of all 8 mice were positive by western blot and immunohistochemistry for PrPCWD. Conversely, all mice without oral lesions remain asymptomatic at >450 dpi. No evidence of PrPCWD was detected in any Tg(cerPrP) mice examined at any of the preterminal time points.

Discussion: Micro-abrasions to the lingual surface substantially facilitate CWD transmission, suggesting a co-factor that may be significant in foraging cervids or other species. Earlier post-inoculation sampling intervals (1 and 4 hours) are in progress in an attempt to determine when and where PrPCWD might be detectable after oral mucosal exposure.

P.4.28

PrPsc is associated with a subset of blood leucocytes in scrapie infected sheep

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Background: Prion infectivity is present in the blood of sheep with scrapie and BSE (Houston et al 2000,2008; Hunter et al 2002) as demonstrated by transfusion studies. Using two different approaches, we have recently shown that PrPsc is associated with peripheral blood mononuclear cells (PBMCs) isolated from scrapie infected sheep (Thorne and Terry 2008; Terry et al submitted) however; the cell subset(s) that harbour PrPsc is yet to be identified.

Objectives: To determine the subset of PBMCs associated with PrPsc in scrapie infected sheep.

Methods: Cell subset specific antibodies combined with magnetic beads (MACs) were used to isolate various sub-populations of blood cells. PrPsc associated with each subset was established using a highly sensitive and rapid plate based immuno-assay.

Results: PrPsc is specifically and consistently associated with a subset of PBMCs from sheep naturally infected with scrapie and orally challenged with BSE. This has been repeated in animals showing clinical signs of disease and during the presymptomatic phase of infection. Antibodies directed to the four distinct but overlapping markers expressed on the surface of this identified cell population have been employed for cell enrichment and have all shown to concentrate PrPsc associated cells. Cells that do not express these markers are PrPsc negative.

Discussion: We have identified a population of PBMCs that is associated with PrPsc in the blood of scrapie and BSE infected sheep. It is unclear, how or where these cells acquire PrPsc, and whether they are important in the dissemination of PrPsc throughout the lymphoreticular system. Various markers including integrins and selectins are important in the homing and trafficking of leucocytes from the blood to lymphoid tissue. Current work aims to determine the expression of these markers on the identified cell population.

P.4.29

Effects of scrapie brain homogenates on the small intestine of isolated gut loops: an ultrastructural study

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Background: In naturally occurring TSEs of sheep, deer and cattle amplification of infectivity is often first detected in Peyer's patches (PPs) of the small intestine. Although these data suggest that infection is acquired via the oral route it remains unknown precisely how infectivity reaches this site and how it affects the structure of PPs.

Objectives: To try to understand how infection reaches PPs and the effects of scrapie on the structure of intestinal lymphoid follicles we have examined scrapie challenged intestinal loops at early stages of exposure and late stages of disease.

Methods: Isolated intestinal loops were created in 4 month old lambs and challenged with a scrapie brain homogenate or given sucrose as a control. Tissues were taken at 15 minutes, 2 hr, 24 hr, 3-5-10-30 days post exposure and at terminal disease (20-24 months). Samples of PPs were prepared for examination by light and electron microscopy and selected samples labeled at light microscopy for PrPd and MHCII and by immunogold for PrPd, ubiquitin and immunoglobulins.

Results: By light microscopy, PrPd was not detected in PPs from 15 min-10 days. Weak PrPd was present in follicles at 30 days and abundantly in dome and follicles of PPs at terminal disease. The light zone of PP follicles showed morphological variation. At terminal stages of disease, the size and number of FDC dendrites and the abundance of extracellular dense material were greater than at earlier stages. This material was labeled for PrPd by immunogold methods.

Discussion: The present results do not show significant morphological changes to PPs prior to 30 days of challenge. However morphological changes to PPs are found at late stages of disease and in relation to secondary follicles of other lymphoid tissues. We cannot exclude the possibility that infectivity reaches the PPs via the blood.

P.4.30

Trafficking of prion proteins via retroviral particles/exosomes

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Background: In prion diseases, pathological changes mainly occur in neuronal tissues, but deposits of PrPSc have been detected in non-neuronal compartments such as lymphoreticular system and muscle. Potential trafficking of PrPSc to and from the periphery to the central nervous system either within one cell or between different cell types remain obscure. Recently the transfer of PrP via nanotubes, as well as retroviral particles and exosomes has been shown in vitro.

Objectives: During retroviral budding, retroviral particles are enveloped by cellular membrane thereby possibly carrying PrPC and PrPSc on their surface. To elucidated the role of retroviral particles in prion protein trafficking we investigated in vitro and in vivo models for retroviral superinfection of prion diseases.

Methods: We first determined the release of PrPC and PrPSc via virus particles and exosomes after acute retroviral infection in cell culture. In our experiments we could demonstrate enhanced shedding of PrPC by viral particles. In contrast, the release of PrPC associated with exosomes was not changed in our system.

Results: To elucidate the impact of retroviral superinfection on prion disease pathophysiology, we established an in vivo model for prion/retroviral double infection. Mice were shown to have persistent viremia, but no retrovirus associated disease within the incubation time of prion diseases (>220 days). RML-prion proteins were inoculated either intraperitoneally or intracerebrally and mice were taken at defined time points. The results of our study will be presented focussing on clinical disease evolution and temporospatial analysis of PrPSc.

Discussion: The investigation of prion protein-retroviral double infection will help to determine co-factors for disese progression as well as potential risk factors for prion protein transmission.

P.4.31

Prion infectivity in milk from ARQ/ARQ sheep experimentally infected with Scrapie and MAEDI-VISNA virus

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Background: Scrapie in sheep is characterized by the deposition of misfolded and aggregated prion protein (PrPSc) in the central nervous system (CNS) and within the lymphoreticular system (LRS). PrPSc was shown to accumulate in organs beyond the CNS and the LRS when lymphofollicular or granulomatous inflammation was also present.

Objectives: Our aim was to determine whether ectopic PrPSc accumulation in the inflamed mammary gland of sheep with scrapie results in infectious prion secretion into the milk.

Methods: We fed approximately 1.1 - 2.1 L of milk from sheep with lymphofollicular mastitis and clinical scrapie to each of 8 ARQ/ARQ lambs derived from scrapie-free flocks. The milk donor sheep had been previously inoculated with Maedi-Visna virus (MVV) intratracheally and intravenously and scrapie brain homogenate orally. In addition, 3 ARQ/ARQ lambs were fed approximately 1.4 – 1.7 L of milk from ARQ/ARQ sheep that had been experimentally infected with only scrapie. Additional control ARQ/ARQ lambs were inoculated with scrapie brain homogenate only, or with milk from uninfected sheep.

Results: Two lambs which had received milk from sheep with mastitis and scrapie developed clinical signs of scrapie at 677 and 745 days post-inoculation. One additional clinically healthy lamb from this group, which was sacrificed for a cause unrelated to scrapie, was found to have PrPSc in brain and tonsil. The control lambs and those which received milk from sheep affected only with scrapie are, to date, clinically healthy.

Discussion: This is the first evidence of clinical scrapie in sheep fed milk from scrapie sick sheep. The experiment is ongoing, however these preliminary results indicate that milk and/or colostrum from ARQ/ARQ sheep with clinical scrapie and lymphofollicular mastitis could contribute to scrapie transmission.

P.4.32

Prional origin hypothesis for cardiac amyloidosis in indian population

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Discussion: Cardiovascular diseases (CVDs) will overtake communicable diseases as leading killers in India by 2020. Cardiac amyloidosis is a chronic, progressive and fatal restrictive cardiomyopathy accounting for nearly 10% of non-ischemic cardiomyopathies, which leads to sudden cardiac deaths. Though, cardiac amyloidosis has multiple etiologies, prion associated cardiac amyloid disease is a newer facet of prion pathobiology, as indicated recently by some animal experiments. This extra neural manifestation of prion pathogenesis may have specific consequence over the prevalence of cardiac amyloidosis in Indian population, as Sheep brain Rabies vaccine (SbRv) was extensively used for control of human rabies in later half of the last century. Scrapie, a classical prion disease of sheep was reported in the Kumaon foothills of Himalayas during the early 1960s, where the Central Research Institute is located, which produced 4-5 million doses of SbRv for years and contributed significantly in controlling the human rabies. Human rabies continued to be endemic to India and males under the age of fourteen years are the major victims of dog bite and hence were effectively protected by SbRv. Thus, I put forward the hypothesis that, some of these victims might have acquired prion infection through SbRv resulting into development of cardiac amyloidosis in later stage of life after incubating prions for many years. Therefore, cardiac amyloidosis in those victims can be of prion origin. Hence, epidemic of acute cardiac death in young male Indian population in the last 25-30 years possibly be correlated with childhood history of dog bite followed by immunization with SbRv. Further, longer incubation period of prion infection and higher incidence of cardiac amyloidosis in males signifies causation. The association needs to be exposed more precisely with detailed investigations to establish conclusive link, which will allow earlier diagnosis for better results of therapeutic interventions.

P.4.33

Effect of route of inoculation and cell washes on TSE blood infectivity titers

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Background: In numerous titrations of endogenous infectivity in whole blood (WB) in the scrapie hamster model we have titered the blood by intracerebral inoculation (IC) immediately following intense sonication to disperse the infectivity. Endogenous WB infectivity measured this way is distributed roughly equally between the cellular and plasma compartments. Incidental observations in our laboratory suggested that intact WB might have a lower effective titer due to compartmentalization of the infectivity into cells and that this cell association might be tenuous.

Objectives:

1. Assess effect of dispersion on the titer of TSE infectivity inoculated IC vs IV.

2. Assess effect of cell washes on cell-associated infectivity.

Methods: A pool of endogenously infected WB was collected from hamsters clinically ill with the 263K strain of scrapie. WB was inoculated IC and IV either intact or dispersed by sonication. Buffy coat cells (BC) were titered before and after the washing with a large excess of buffered saline. RBC were titered before and after separation from the other cellular components by centrifugation. Cellular compositions were determined with a hamster-calibrated cell counter. Surviving animals were observed for 18 months and all brains tested by Western blot.

Results: The IC infectivity titer of dispersed WB was twice that of intact WB and the titer dropped ~ 50-fold when the blood was inoculated IV. Washed BC cells lost most of the infectivity even though the cell composition did not change. Separated RBC lost ~1 log10 of infectivity while the RBC recovery was almost 90%.

Discussion: These results confirmed preliminary indications of reduced titer of intact vs. lysed WB and IV versus IC transmissions. Cell-associated infectivity is not tightly bound to the cells and is removed with washes. This observation has practical applications and also warns that cell-associated infectivity released into plasma would render leukoreduction less effective in reducing TSE risk.

P.4.34

BSE in hamsters: An animal model lacking PrPSc accumulation in lymphoid tissues

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Background: Strain- and host-specific pathogenesis are known to occur in prion diseases. In bovine spongiform encephalopathy (BSE)-affected cows, the accumulation of the abnormal isoform of the prion protein (PrPSc) is almost limited to the central nerve system (CNS). However, when the BSE prion was passaged to mice, PrPSc could be detected from CNS, and lymphoid tissues. Consequently, there are no good animal models for mimicking BSE cattle pathogenesis.

Objectives: In this study, we attempted to establish a new experimental animal model for studying BSE pathogenesis.

Methods: BSE prions are not transmitted to hamsters. However, the mouse-passaged prions showed altered species susceptibility and gained transmissibility to hamsters. Then, these altered prions were passaged to hamster-PrP-overexpressing transgenic mice and then intracerebrally inoculated into hamsters. The affected hamsters showed neurological clinical signs after an incubation period of 349.5 ± 6.6 days. The brains of these hamsters were used as the inocula (BSE/Ha) in a pathogenesis study.

Results: The BSE/Ha prions were intraperitoneally inoculated into hamsters, and the incubation period of the prion was 492.6 ± 82.3 days. PrPSc was detected from all the affected-hamster brains, but rare PrPSc accumulation in spleen was confirmed by western blotting. In all the examined tissues, PrPSc was detected only from the brain in one hamster, which may represent the BSE cattle pathogenesis.

Discussion: The resistant animal-adapted prion may mimic the CNS-limited prion pathogenesis. We are presently conducting further time-course studies using orally challenged hamsters to evaluate the usefulness of this rodent model.

P.4.35

Improvement of neural stem cell model for prion propagation

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Background: The pathogenic isoform prion protein (PrPSc) plays a central role in the transmission of prion diseases. PrPSc is produced by conversion of the physiological 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. PrPC is cleaved during its normal processing between residues 111/112 (PrPalpha cleavage), generating an amino-terminal fragment referred to as N1 and its carboxy-terminal named C1, whereas PrPSc, is cleaved at an alternate site around position 90, yielding N2/C2 (PrPbeta cleavage).

Objectives: 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 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.

Methods: Thus, using cells obtained from wild type or transgenic animals, we have pursue our investigations to optimize and characterize this model based on the hypothesis that changing culture conditions by modifying a given factor or a combination of factors could modulate PrPSc production in NSCs.

Results: The first results showed that those supplements act on differentiation of NSC and can facilitate prion propagation and increase PrPSc production when they are added to the culture medium. We are currently investigating the cellular mechanism underlying this phenomenon, including modification of PrP cleavage and intracellular signaling pathway.

Discussion: These experiments will add efficiently to our understanding of prion diseases and more importantly allow us to develop more powerful models.

P.4.36

Prolonged incubation time in sheep with QK171 genotype

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Background: Sheep scrapie susceptibility or resistance is a function of genotype with polymorphisms at codon 171 playing a major role. Glutamine (Q) at 171 contributes to scrapie susceptibility while arginine (R) is associated with resistance. In some breeds, lysine (K) occurs at 171, but its affect on scrapie resistance has not been studied.

Objectives: Charge and structural similarities between K and R would suggest that they may contribute to prion disease susceptibility in a similar way, but studies have not been done to confirm this. The purpose of this study was to determine and compare susceptibility of sheep with QQ171 and QK171 genotypes.

Methods: We inoculated Barbado sheep ARQ/ARQ and ARK/ARQ with scrapie (NADC 13-7) by intracerebral inoculation to assess the susceptibility of these sheep to scrapie. After inoculation, sheep were observed daily for clinical signs and were euthanized and necropsied after clinical signs were unequivocal. Tissues were collected at necropsy for immunohistochemistry and western blot.

Results: QQ171 sheep had clinical signs an average of 12 months after inoculation whereas QK animals had an incubation time of 30 months to average onset of clinical signs. The distribution of PrPSc was similar in QQ171 and QK171 sheep.

Discussion: Sheep with a single K allele at 171 are susceptible to scrapie, but with a prolonged incubation time. Studies are underway to determine the relative susceptibility or resistance of sheep with KK171.

P.4.37

PET Blot versus IHC and WB labelling in a selection of tissues from sheep naturally infected with scrapie

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Background: Certain TSEs can cross the species barrier with relative ease resulting in infection in susceptible recipients. This phenomenon was highlighted by the BSE epidemic in the 1990's which resulted in changes in legislation for abattoirs and the exclusion of "high risk" materials, such as the central nervous system (CNS), from entering any food chain. However, other tissues deemed to be "low risk", such as lymph nodes and kidneys, have since been proven to be both positive for the disease associated form of prion protein (PrPd) and infectivity. The PET blot technique is an intermediary test between western blotting (WB) and immunohistochemistry (IHC) that detects proteinase K resistant prion protein (PrPres) within tissues.

Objectives: To compare IHC and WB with PET blot labelling in brain, lymph nodes and kidneys from sheep naturally infected with scrapie.

Methods: Four Suffolk sheep (ARQ/ARQ) naturally and clinically affected, were euthanised identical to slaughter house methods (electrocution followed by exsanguination). Selected tissues were sampled (brain, kidney and prescapular lymph node) and PET blots and IHC labelling were employed using monoclonal antibodies SAF84 and R145 respectively.

Results: Detection of PrPres and PrPd in CNS tissue demonstrated a high degree of homogeneity between the PET blot and IHC labelling. In contrast, the labelling of PrPres in prescapular lymph nodes by PET blot does not directly correlate with accumulation of PrPd detected using IHC methods. Furthermore, interpretation of the kidney labelling has proven problematic when using the PET blots due to non-specific background labelling.

Discussion: Our investigations have shown a distinct correlation between IHC and PET blots in CNS tissue. However, this is not apparent in LRS tissues with fewer positive follicles detected using PET blots. This result may be due to the fact PrPd visualised by IHC comprises of both PK-resistant and some non-resistant isoforms.

P.4.38

Quantitative assessment of PrPd in placentomes from a pregnant sheep

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Background: Accumulation of the abnormal form of prion protein (PrPd) within the placenta of scrapie infected sheep is dependant on polymorphisms in the PrP genotype. In Suffolk sheep, only ewes with genotypes that are susceptible to scrapie (ARQ/ARQ) and carrying susceptible lambs are affected. Transmission of scrapie is thought to occur during the lambing period via infected placental tissues. However, there is little information on the amount of potential PrPd contamination of the environment during this period.

Objectives: To assess and quantify the amount of PrPd in every individual placentome collected from a pregnant sheep by immunohistochemistry and digital imaging.

Methods: The placenta from an ARQ/ARQ RAMALT biopsy positive pregnant sheep was perfused with 1M PBS containing 0.05M EDTA, pH 7.4 by cannulation of the middle uterine arteries and umbilical vessels. The ewe contained two foetuses (F1 and F2, 39 and 42 placentomes respectively) and all placentomes were fixed in buffered formalin and processed by routine methods prior to embedding in paraffin wax. Tissue sections were subjected to antigen retrieval by autoclaving in citrate buffer, R145 used as the primary antibody and the ABC method (Vector Laboratories) was used to visualise bound primary antibody with a red substrate to aid digital analysis. PrPd was measured using an acetate grid placed under the microscope slide and in each grid square where labelling was present an image was captured using analySIS® software. The percentage of area labelled compared to the whole placentome was then calculated using Image J public domain software.

Results: Every placentome contained positive labelling for PrPd. However, the amount in each was highly variable.

Discussion: These findings show the placenta is a source of contamination of the environment with PrPd during the lambing season and may be the source of infection leading to lateral transmission. This technique should be considered for use where the amount of PrPd within tissues requires relative quantitation.

P.4.39

Transgenic mice brains can efficiently be used to measure species-barriers in vitro by PMCA

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Background: Results from bovine and porcine transgenic mice bioassays demonstrate an increased virulence of sheep-BSE compared to the original cattle-BSE isolate. The protein misfolding cyclic amplification reaction (PMCA) has been used in diverse studies oriented to a better understanding of the underlying molecular mechanism of TSE agent replication, the existence of TSE strain variation, and to explain intra-and inter-species transmission of prion diseases.

Objectives: The work presented here was focussed to assess the usability of transgenic mice brains as substrates in PMCA reactions. We compared the In vivo and In vitro (PMCA) replication of prions of different ovine and bovine TSE isolates using as substrate PrPC preparations obtained from transgenic mice over-expressing bovine and porcine PrP.

Methods: Bovine (Tg110) and porcine (Tg001) transgenic mice brain homogenates were prepared following standards protocols and under PMCA conditions mixed with minimal amounts of PrPres from a panel of ovine and bovine prions.

Results: In general, using Tg110 mice PrPC as substrate, conversion ratios were higher for the BSE in Sheep isolates than for the cattle BSE isolates. In contrast, as expected the conversion ratios of different sheep Scrapie isolates to these selected substrates were generally low. Using Tg001 mice PrPC as substrate, most sheep-BSE isolates showed evident conversion, while with the tested classical cattle BSE isolates no significant conversions were observed as was observed again for most of the tested sheep Scrapie isolates.

Discussion: We demonstrated that brains from transgenic mice over-expressing bovine or porcine PrP can be efficiently used as PrPC substrates for PMCA studies. In general, the conversion ratios obtained with PMCA as measurement for the species-barrier, were similar to those obtained with previous bioassays studies in these transgenic mice.

P.4.40

Role of polyunsaturated fatty acids on cellular cholesterol, prion formation and neurotoxicity

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Background: The role of cholesterol in neurodegenerative diseases has been widely reported. Cholesterol synthesis inhibitors reduced PrPSc formation & protected neurones against prion mediated toxicity. This was attributed to cholesterol depletion affecting formation of lipid rafts. While these drugs prolonged survival of scrapie-infected mice, their limited effects were likely due to poor CNS penetration/short half lives. Effects of polyunsaturated fatty acids (PUFA), long chain fatty acids that reduced cellular cholesterol, were examined.

Objectives: Determine the effects of different PUFA on cellular cholesterol, PrPC trafficking & PrPSc formation. Determine the effects of PUFA on neuronal responses to the prion peptide PrP82-146.

Methods: Prion-infected cells were exposed to PUFA for 7 days. Cell extracts were pK digested & the amount of PrPSc measured by ELISA. In non-infected cells, effects of PUFA on total PrPC content, surface PrPC & the half-life of PrPC were measured. Cortical neurones pre-treated with PUFA were incubated with PrP82-146; activation of cPLA2, synapse damage & cell death were then measured

Results: 1 μM docosahexaenoic acid (DHA) reduced the amount of cholesterol in cell membranes. In contrast to the effect of cholesterol synthesis inhibitors, DHA increased PLA2 activity & PrPSc formation in prion-infected cell lines. DHA also increased the amount of cell surface PrPC & increased the half-life of PrPC 4-fold. Pre-treatment of cortical neurones with DHA increased uptake of PrP82-146 into caveolae, increased activation of cPLA2 & caspase-3 activity, & reduced neuronal survival. We prupose that PrP82-146 triggers the formation of a platform containing caveolin-1, in which it activates cPLA2 resulting in cell death

Discussion: Our studies demonstrate that the membrane cholesterol is not the sole factor regulating lipid raft function, PrPSc formation & PrP mediated neurotoxicity.

P.4.41

Assessment of prion infectivity distribution in primate blood among cell fractions.

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Background: Probable interhuman transmission of vCJD through blood and derived products has been reported in several cases in Great Britain, highlighting the necessity to secure transfusion against prion risk. Experimental models suggest an equal distribution of infectivity among plasma and white cells, but the cellular populations supporting infectivity remain to be clearly identified.

Objectives: Experimental BSE/vCJD infection of cynomolgus macaque constitutes a model of choice to better assess the distribution of prion infectivity among the different blood cell populations.

Methods: Monkeys were experimentally infected with BSE by the oral route, or with primate-adapted BSE by the intravenous route (secondary and tertiary passages). In the context of the PrionBloodPrimate project funded by Alliance BioSecure Foundation, blood was sampled and fractionated according to an adapted protocol to concentrate white cells. Different cell populations were then sorted with the Foundation's secured cell sorter (Influx). Infectivity of whole blood, plasma and the different cell fractions will be assessed by inoculation of transgenic mice overexpressing human PrPMet129 (tg650).

Results: Primates were selected for their presence of peripheral infectivity according to the presence of PrPres in biopsied inguinal lymph nodes. Corresponding granulocytes, monocytes, lymphocytes and dendritic cells fractions were separated and enriched with purity around 90%. tg650 mice that demonstrate a high susceptibility to vCJD and allow endpoint titration of infectivity within relatively short delays, have been inoculated with these samples and the first results will be available for the congress Prion 2009.

Discussion: With detailed information about donor primates (incubation period, presence of peripheral replication or clinical signs), the efficient actors are now gathered to evaluate the distribution of infectivity among the different components of these unique samples, to better assess the risk of transmission of prion by blood transfusion.

P.4.42

Transmission of TSEs to a highly susceptible transgenic mouse model overexpressing ovine PrP (A136 H154 Q171)

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Background: Bioassay of TSE diseases in animal hosts is regarded as the gold standard method for detecting TSE infectivity. Preparation of transgenic (Tg) models expressing foreign PrP can lead to abrogation of transmission barriers and offers the prospect of faster, more economically viable bioassays, which may be more sensitive to TSE detection than wild-type mice and the natural host.

Objectives: The aim of the study was to generate and characterise transgenic mice expressing ovine PrPAHQ and to assess the susceptibility of the PrPAHQ allele as an alternative bioassay model for improved detection and differentiation of ruminant TSEs.

Methods: Transgenic mice were prepared by pronuclear microinjection of an ovine PrP transgene encoding amino acid residues A136 H154 Q171. Founder mice were crossed with PrP null mice to transfer transgene expression to a mouse PrP-ablated background. Transgenic lines were characterised by Southern hybridisation and Western blotting expression analysis. Transmissions of TSE agents (atypical scrapie, classical scrapie and BSE) were conducted and mice were analysed using biochemical, and neuropathological, methods.

Results: The Tg(OvPrPAHQ) model demonstrated remarkable susceptibility to atypical scrapie isolates from sheep, with similar incubation periods of ~160 days on primary passage, irrespective of donor sheep genotype. The Tg mice exhibited early detection of atypical scrapie PrPres at <110 days, an apparent lack of a transmission barrier on secondary passage, and were also susceptible to classical scrapie and BSE.

Discussion: Tg(OvPrPAHQ) mice represent a novel bioassay system with the prospect of high susceptibility, broad specificity and differentiation of TSE agents in the same transgenic model. The development and assessment of improved transgenic PrP models in the transmissibility and pathogenesis of TSE agents, as well as in the evaluation of transmission barriers, is crucial to estimating the potential risks of prion diseases to human and animal health.

P.4.43

PrPsc deposition in placenta from natural infected ewes

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Background: Scrapie in sheep is an endemic disease in many countries. It is widely accepted that classical scrapie spreads mainly by horizontal transmission, through direct or indirect contact with placenta from infected animals. PrPsc deposition in placenta depends mainly on the PRNP genotype of the ewe and the fetus; it occurs in the placenta of scrapie infected sheep carrying a fetus of a susceptible genotype, but it doesn't seem to occur in fetus of resistant genotype (ARR haplotype).

Objectives: The aim of the present study is to assess the PrPsc deposition in placenta from natural scrapie infected sheep carrying fetus of ARQ or ARR haplotype.

Methods: A total of 24 ARQ/ARQ ewes presenting PrPsc deposits in lymphoid tissue biopsies were included in the present study. Infected ewes were mated with ARQ/ARQ or ARR/ARR dams. In addition, 6 pregnant infected ewes from different scrapie infected flocks detected through the Spanish surveillance program were also included. A total of 46 placentas from 37 gestations were collected and were analyzed for PrPsc detection by immunohistochemistry. Briefly, immersion in 98% formic acid, proteinase K treatment and hydrated autoclaving were performed as pretreatments before L42 primary antibody incubation (R-Biopharm, Germany; 1:500 ; 30 min, RT). Envision TM (DAKO) as visualization system and diaminobencidine as substrate chromogen were used.

Results: PrPsc deposits were detected in 16 of 23 placentas from fetus carrying an ARQ/ARQ genotype. In placenta from fetus presenting other haplotypes PrPsc was detected in 12 cases.

Discussion: Immunohistochemical results suggest that when there is a wide spread of the PrPsc in terminal stages of the disease, the prion protein can accumulate in placenta from fetus carrying an ARR haplotype.

P.4.44

Distribution of infectivity in the oral tissues of VM mice and the potential risk of vCJD transmission through dentistry

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Background: Ongoing concerns regarding the prevalence of variant Creutzfeldt-Jakob Disease (vCJD) in the UK population have heightened awareness of the iatrogenic transmission risks. Whilst the individual risk associated with dentistry may be very low, the extensive number of procedures carried out annually in the UK (~75 million) amplifies this significantly.

Objectives: The main aim of this study was to evaluate the risks of prion transmission by dentistry and assess the levels of infectivity in various murine oral tissues following exposure via two different routes.

Methods: VM mice were inoculated with BSE-301V infectious mouse brain homogenate either by direct injection into the small intestine or transient inoculation of the gingival margin and then monitored for TSE clinical symptoms. Oral tissues together with brain and spleen were removed at regular intervals and re-inoculated intracranially (i.c.) into indicator mice to observe for infectivity.

Results: The results from the primary duodenal challenge route were an efficient route of infection with a 100% attack-rate and a mean incubation to clinical disease of 157 ± 17 days (c.f. 120 days for the equivalent titre inoculated i.c.). Infectivity was observed in all oral and control tissues at a range of titres during both pre-clinical and clinical stages of the disease. Results from the gingival margin inoculation route showed that BSE infectivity can spread to a significant proportion of murine oral tissues following transient inoculation from a contaminated instrument. The results demonstrate that a wide range of murine oral tissues contain variable amounts of infectivity.

Discussion: The results from this study show the spread of prion infectivity to the mouse oral cavity from two independent inoculation routes. This study supports the need for risk assessments around the potential for vCJD transmission via dentistry and studies evaluating the effectiveness of decontamination and re-use of dental instruments.

P.4.45

Up-regulation of endothelial nitric oxide synthase expression and mitochondrial dysfunction in the hippocampus of scrapie-infected mice

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Background: The elevation of nitric oxide (NO) within the central nervous system (CNS) in known to be associated with the pathogenesis of neurodegenerative diseases such as HIV-associated dementia (HAD), brain ischemia, Parkinson's disease, and Alzheimer's disease. NO is enzymatically formed by the enzyme nitric oxide synthase (NOS), which are basically divided into two forms; the constitutive forms in endothelial cells (eNOS) and neurons (nNOS) and the inducible form (iNOS) in various cell types including astroglia and microglia of the CNS.

Objectives: To investigate the involvement of eNOS in the pathology of prion disease, we used scrapie-infected mouse as an experimental model for prion disease.

Methods: We found that eNOS immunoreactivity was intensely detected in reactive astroglial cells in the hippocampus of the scrapie-infected mice. Using subcellular fractionation and Western blot analysis, up-regulated eNOS expression was detected in cytosolic and mitochondrial fractions from scrapie-infected brains compared to controls.

Results: In hippocampal regions of scrapie-infected brain, we found abnormal accumulation of eNOS in the mitochondria along with its structural dysfunction. In correlation with this finding, the mitochondrial superoxide dismutase (Mn-SOD or SOD2) was down-regulated both in whole brain and the hippocampal region of scrapie-infected brains compared to controls.

Discussion: These results suggest that up-regulation of eNOS may play a role in mitochondrial dysfunction of prion disease.

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P.4.46

Estimation of Chronic Wasting disease (CWD) infectivity in cell culture

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Background: The rapid spread and high prevalence of chronic wasting disease in North American cervids have raised concerns about a potential threat to human health. Experimental transmission of CWD to various species has been reported in vivo.

Objectives: The establishment of a PrPSc permissive cell culture model for the determination of CWD-infectivity derived from different species, including Elaphus nelsoni, Odocoileus virginanus and Macaca fascicularis-passaged CWD.

Methods: To achieve high levels of ectopically expressed PrP in prion susceptible cell lines, an effective retroviral transduction system (pVPack vector, Stratagene)is used. The PrP knockout cell line HpL3-4 is retrovirally transduced with murine, macaque and human wt-PrP, respectively. Stable ectopic expression of transgenic PrP is validated by qRT-PCR. Subsequent propagation of infectious inocula from CWD-infected E. nelsoni, O. virginanus and M. fascicularis in respect to human- or macacanized cell lines are monitored by Western blot and a Membran Adsorption Assay.

Results: The application of CWD-infected tissues to macacanized cells can be used to simulate the species barrier between cervids and primates. Furthermore quantitative analysis of infectious doses can be performed in a high-throughput 96-well format to assist the results of elaborate in vivo bioassays.

Discussion: To date, the possibility of CWD transmission to humans by the uptake of contaminated food has not been investigated. Transmission studies of CWD to non-human primates are in progress. The described cell culture model provides a different way to elucidate whether transmission of CWD to non-human primates could possibly occur and thus may lead to a more detailed assessment of the current risk of chronic wasting disease.

P.4.47

Determining the role of follicular dendritic cells in scrapie pathogenesis

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Background: Following peripheral infection, TSE agents often accumulate in lymhoid tissues before spreading to the CNS. In mice, follicular dendritic cells (FDCs) expressing the host prion protein (PrP) are essential for TSE agent accumulation in lymphoid tissues. However, it is unknown whether FDCs themselves replicate TSE agents, or simply accumulate them following production by other cells in the lymphoid tissue. This is because there is no way in the models used so-far to dissociate the PrP genotype in FDCs from that in the nervous system and other non-bone marrow derived cell populations. Determining which cell type actively replicates the scrapie agent may provide a potential target for intervention prior to neuroinvasion.

Objectives: To create a transgenic mouse model to switch-off PrP specifically on FDCs and definitively determine their role in TSE pathogenesis.

Methods: To address this issue a transgenic mouse model was created in which PrP expression is switched-off exclusively on FDCs. Expression of cre-recombinase (Cre) under the action of an FDC specific promoter can be used to delete the expression of a target gene in the FDCs. An FDC-Cre mouse line was characterised and crossed with a floxed-PrP mouse line to produce mice in which PrP expression is switched-off specifically in FDCs. These mice have been challenged with scrapie agent to assess the role of the FDCs in peripheral TSE pathogenesis.

Results: Characterisation of the FDC-cre line was achieved by crossing with a ROSA26 reporter strain. Expression of Cre in these mice results in cell-specific expression of b-galactosidase. These mice showed efficient activation of Cre on FDCs in spleen and lymphoid tissues. These mice were crossed with the floxed-PrP line and initial results show a significant reduction of PrP levels on the FDC surface in peripheral lymphoid tissues.

Discussion: Our data suggest this transgenic mouse line provides a useful model to definitively assess the role of FDCs in TSE pathogenesis.

P.4.48

Kinomic studies of in vitro prion pathogenesis

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Background: Protein kinases participate in the pathogenesis of neurodegenerative diseases, but the signaling pathways critical in prion diseases are unknown. Our hypothesis is that prion pathogenesis is mediated by chronic dysregulation of signaling pathways.

Objectives: To develop assays to identify dysregulated signaling pathways and apply them to an in vitro model of prion pathogenesis.

Methods: To reach our objectives, we optimized multiplex Western blot assays for 156 protein kinases using only 1.2 mg of sample. 117 antibodies detected their cognate proteins in mouse brain and 19, against kinases pathologically expressed in neurons, in mouse fibroblasts. Sensitivity and linearity were tested spiking brain with fibroblast lysates, and ability to detect known dysregulated pathways, using HSV. We are applying the assays to an in vitro model of prion pathogenesis, N2a neuroblastoma cells expressing EGFP-fused and truncated PrP. CyPrPEGFP lacks ER-targeting and GPI-membrane-anchoring signals, leading to cytoplasmic PrP and RNA aggregation and inhibition of protein synthesis. Further truncations result in aggregation of RNA but not PrP (CyPrPEGFP124stop), or PrP but not RNA (CyPrPEGFP157-231).

Results: Expression levels of 82 protein kinases in N2a cells expressing the PrP mutants were analyzed by multiplex Western blot and hierarchical clustering. Literature and database searches identified those clusters composed of protein kinases in one pathway. Three dysregulated pathways were identified; we focused on the one involved in protein synthesis. GRK2, PRK2, PKCα, and p70S6K were all expressed to similarly lower levels in N2a cells expressing any cytoplasmic EGFP-PrP than in those expressing EGFP. We are testing all kinases in the GPCR-p70S6K pathway, and their targets involved in protein synthesis, ribosome S6, eIF4B, and eEF2κ.

Discussion: We have developed kinomic assays to identify chronically dysregulated signaling pathways, and are identifying pathways inhibited by cytoplasmic PrP in cultured cells.

P.4.49

Plasmacytoid dendritic cells efficiently sequester prions at early stages of prion disease

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Background: The cellular expression of the prion protein (PrPc) is a pre-requisite for the replication of prions. PrPc expression is reportedly undetectable in plasmacytoid dendritic cells (pDCs), as compared to conventional dendritic cells (DCs), thus rendering pDCs a poor candidate for a role in prion pathogenesis. PDCs are natural type 1 IFN-producing cells, located in the T cell rich periarteriolar lymphoid sheath of lymphoid organs. Like conventional DCs, pDCs are highly specialized to capture, process and present antigens. The number of pDCs is highly variable among mouse strains, with the 129Sv strain showing several fold higher numbers than most other strains. Notably, 129Sv is a commonly used mouse strain due to the fast accumulation of prions in lymphoid organs.

Objectives: To determine the cell tropism of prion accumulation in splenic cells of scrapie-infected mice.

Methods: We monitored the accumulation of prions in 129Sv mice at early stages of prion disease in a cell-specific manner by sequential isolation of splenic cell types, like B and T cells, DCs, pDCs and macrophages, followed by the determination of infectious titres using the Scrapie Cell Assay.

Results: Prion titres of pDCs rapidly increased from 40 Tissue Culture Infectious Units (TCIU)/Mio cells 3 days after intraperitoneal inoculation with a 1% RML brain homogenate to 2250 TCIU/Mio cells after 30 days and exceeded infectious titres of macrophages, B and T lymphocytes and pan DCs by seven, eight, ten and five fold, respectively. Experiments are in progress to investigate the effects of in-vivo depletion of pDCs on splenic prion titres and the onset of disease.

Discussion: We here present evidence for a PrPc-independent mode of prion dissemination that may have a role in prion colonisation of the lymphoreticular system. In this pilot study we determined infectious titres of splenic cells to characterise the cell tropism of prion accumulation and experiments are in progress to assess the contribution of stromal cells.

P.4.50

Successful oral transmission of classical scrapie to ARR/ARQ sheep

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Background: Scrapie susceptibility in sheep is strongly influenced by allelic variation in the gene which encodes the prion protein. As part of the National Scrapie Plan (NSP) for Great Britain ram genotyping and selective breeding has been used to increase the number of sheep in the national flock that are genetically resistant to classical scrapie. According to the NSP, ARR/ARQ sheep are considered 'genetically resistant' to scrapie, although four field cases have been detected since 2002.

Objective: To investigate the susceptibility and pathogenesis of classical scrapie in ARR/ARQ sheep.

Methods: TSE-free lambs were dosed orally with 5g of pooled brain from scrapie clinical suspects. Timed-culls were performed at 12 and 24 months post-inoculation (mpi) then six monthly thereafter. All sheep underwent a detailed clinical examination before culling. At post-mortem 41 tissues were sampled from all major body systems. Detection of disease-associated prion protein (PrP^d) in central nervous system tissues was done by immunohistochemistry (IHC), Western blot and Bio-rad elisa. All other tissues were examined by IHC only.

Results: PrP^d was first detected in the LRS at 24 mpi, in the central nervous system (CNS) at 36 mpi, and in the peripheral nervous system (PNS) at 66 mpi. Throughout the time course PrP^d accumulation in LRS tissues was more restricted and less severe than in CNS and PNS tissues.

The first confirmed clinical case occurred at 72 mpi.

Discussion: We have shown that ARR/ARQ sheep can be infected with classical scrapie via the oral route.The pathogenesis of scrapie in ARR/ARQ sheep appears to be different to that in sheep of susceptible genotypes. While VRQ/VRQ clinical suspects have extensive LRS involvement only one LRS tissue was positive in the ARR/ARQ clinical suspect. This could suggest that infectivity had travelled to the CNS via a non-gastrointestinal route. ARR/ARQ sheep may act as 'silent carriers' of disease. However, PrP^d accumulation in the gut-associated lymphoid tissues was mild and restricted so non-faecal routes may be more important in lateral transmission from this genotype. The tissue distribution of PrP^d accumulation in this study suggest that currently available 'live tests' for preclinical diagnosis – third eyelid and/or rectoanal mucosa-associated lymphoid tissue (RAMALT) biopsy – may be unrewarding in sheep of this genotype.

P.5.1

Detection of cellular prion protein (PrPc) in plasma from healthy cynomolgus monkeys (Macaca fascicularis) and changes observed after BSE infection

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Background: Orally BSE-dosed cynomolgus monkeys represent a valuable model to examine the kinetic of blood infectivity and to assess the risk of blood-borne transmission of variant Creutzfeldt-Jacob disease (vCJD).

Methods: Blood samples were collected monthly from BSE-infected (n = 18) and non-infected female cynomolgus monkeys (n = 8) over a period of up to 9 years. PrPc concentrations were retrospectively analyzed in plasma samples by a dot blot assay and by a sandwich ELISA using a highly sensitive dissociation-enhanced lanthanide fluoro-immunoassay (DELFIA) for detection. Different blood preparation protocols were evaluated to obtain plasma.

Objective: To detect changes in the levels of soluble plasma-derived PrPc.

Results: Different blood preparation protocols had a significant effect on the measured plasma PrPc concentrations. In non-infected macaques, concentrations of soluble, plasma-derived PrPc were at least 10-fold lower compared to plasma concentrations in healthy humans. Levels of plasma PrPc increased 6 – 12 months after experimental BSE infection, remained high during the asymptomatic phase, and dropped towards the clinical phase. Soluble, plasma-derived PrPc molecules were PK-sensitive in BSE-infected macaques.

Discussion: There is a species-specific difference in the PrPc concentrations between human and macaque. At least a part of the plasma-derived PrPc fraction originates from blood cells. Andfinally, BSE infection caused an increase in plasma PrPc levels during the asymptomatic phase of infection. Blood transfusion studies have been initiated to examine whether these PK-sensitive PrP molecules carry infectivity.

P.5.2

Impaired autophagy in prion protein-deficient Purkinje cells

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Background: The ectopic expression of the prion protein homologue Doppel (Dpl) in brain neurons causes progressive cerebellar Purkinje cell death in prion protein-deficient Ngsk mice (NP0/0). The neurotoxicity caused by Dpl involves Bax-dependent apoptotic pathways as well as other yet to be characterized cell death mechanisms in the NP0/0 Purkinje cells.

Objectives: In order to get new insights into the cell death mechanisms triggered by Dpl in the Ngsk Purkinje cells, we investigated an eventual activation of autophagic pathways in these neurons.

Methods: For this purpose, the mRNA and protein expression of several markers of autophagy was analyzed using Wester blott and immunohistochemistry in parallel with ultrastructural analysis of te Ngsk mice cerebellar tissue.

Results: These neurons display increased amounts of several autophagy-related molecules such as the scrapie-responsive gene one (Scrg1), LC3B-II and p62 without showing any changes in mRNA expression, and in addition autolysosomes accumulate in all neuronal compartments including axon terminals.

Discussion: This suggests that Dpl toxicity provokes impairment in the autophagic flux, which may trigger apoptosis in these neurons, similar to the way neurodegeneration is thought to occur in Alzheimer and prion diseases. Purkinje cells feature early axonal autophagy in both NP0/0 and GluRdelta2Lc mutants, but no signs of autophagic flux impairment are evident in GluRdelta2Lc Lurcher suggesting that different pathogenic stimuli (i.e., Dpl versus GluRdelta2Lc) trigger different cell death modalities involving autophagy and apoptosis in the same type of neuron. The interplay between these multiple pathways of programmed cell death needs to be further investigated in animal models of neurodegenerative diseases in order to develop new therapeutic approaches.

P.5.3

Differences in the expression levels of selected genes in the brain tissue of cattle naturally infected with classical and atypical BSE.

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Background: Recently cases of BSE in older cattle named BSE type L and type H were distinguished on the basis of atypical glycoprofiles of PrPres. The nature of those strains is still not fully understood but it is suspected that the atypical BSE cases are sporadic. Hitherto most BSE cases were studied in respect to the features of PrPSc. Here we propose gene expression profiling as a method to characterize and distinguish BSE strains.

Objectives: The aim of the study was to compare the activities of some factors which are known to play a role in TSE's pathogenesis in order to distinguish the differences/similarities between all BSE types.

Methods: 10 % homogenate of brain stem tissue collected from obex region of medulla oblongata from 20 naturally infected BSE cows (8 assigned as classical BSE, other 8 and 4 infected with atypical BSE L type and H type respectively) was used in the study. As negative control animals we've used 8 animals in the age between 2.5 and 13 years. The genes were relatively quantified using SYBR Green real time RT-PCR. Raw data of Ct values was transformed into normalized relative quantities using Qbase Plus®.

Results and Discussion: In most of the tested genes significant differences in the expression levels between the brain stem of healthy cattle and animals infected with different BSE types were observed. In c-type BSE in comparison to healthy and atypical BSE the overexpression of the gene of bcl-2, caspase 3, 14-3-3 and tylosine kinase Fyn was significant. Simultaneously in atypical BSEs type-L and type-H the levels of prion protein, Bax and LPR gene was elevated in comparison to c-BSE. Additionally L-BSE was characterized by the overexpression of STI1 and SOD genes compared to the other of BSE types. The downregulation of the gene encoding NCAM1 was observed in all BSE types in comparison to healthy cows. Different gene expression profiles of bovine brains infected with classical and atypical BSE indicates possible different pathogenesis or source of the disease.

P.5.4

Altered expression of type 1 inositol 1,4,5 trisphosphate receptor in the Ngsk Prnp deficient mice

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Background: Some lines of PrP knock-out mice, which express the prion-like protein Doppel (Dpl), show a significant cerebellar Purkinje cell loss. Little is known about the mechanism of Dpl-induced neurodegeneration. A possible scenario would involve opposite effects induced by Dpl and PrP.

Objectives: To understand the molecular and intracellular pathways that are affected in Purkinje cells of Ngsk, one of the Prnp0/0 mouse lines expressing Dpl, we investigated the regulation of calcium-release channel protein, type 1 inositol 1,4,5-trisphosphate receptor (IP3R1) gene.

Methods: We performed Western blot, Immunohistochemistry, Real time RT-PCR and Electrophoretic mobility shift(EMSA)assay.

Results: We found that the expression level of IP3R1 gene is reduced in the cerebella of Ngsk mice as early as 3 months of age compared with age-matched control animals. Furthermore the nuclear factor of activated-T cells (NFAT), which is a transcription factor of IP3R1 gene, was present as the hyperphosphorylated (inactive) state and had reduced DNA binding activity in Ngsk mice. Interestingly, expression of PrP restored the DNA binding activity of NFATc4 that had been reduced by Dpl. Furthermore, the expressions of BDNF and GluR2, which are regulated by NFATc4 was also restored by PrP expression.

Discussion: Based on the fact that the expression of IP3R1 is regulated by synaptic activity, our present data strongly suggests that Dpl affects neuronal activity by interfering with the normal function of PrP related to the regulation of intracellular Ca2+. A decrease in IP3R1 gene expression leads to functional deficits and death of Purkinje cells in Ngsk mice.

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P.5.5

Impact of prion infection on the proteome of 1C11 cells in relation with serotonergic neuronal differentiation

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Prion-propagating cell lines represent useful tools to assess the impact of PrPSc accumulation on cellular functions. Cellular changes depicted in prion infected cells include increased sensitivity to oxidative stress and reduction of cell viability. We have recently reported on the 1C11 neuroectodermal progenitor as a novel prion-permissive cell line. The 1C11 clone has the ability to differentiate within 4 days into 1C115-HT neuronal cells exhibiting a complete serotonergic phenotype including serotonin (5-HT) synthesis, storage, catabolism and uptake. 1C11Fk cells infected with Fukuoka prions retain the ability to engage into a serotonergic differentiation program. However, prion infection promotes drastic alterations of the overall 5-HT-associated functions.

We are developing a systemic approach to compare the proteome of 1C11Fk infected cells to that of uninfected cells, in the precursor and the differentiated states. 2D-electrophoresis was carried out on whole cell extracts prior to image analysis. The global changes of protein expression were assessed through statistical analysis of 2D gels.

A panel of 1200 spots were detected in whole cell extracts. Statistical analyses of 2D gels reveal that serotonergic differentiation of 1C11 cells is accompanied by significant changes of expression for 77 proteins. Comparative analyses between 1C11Fk precursor cells and its differentiated 1C11Fk5-HT progenies suggest that the implementation of a serotonergic differentiation program is globally preserved in prion infected cells. This observation fits in with our previous findings that in 1C11Fk5-HT cells, PrPSc impairs serotonergic functions primarily by promoting detrimental post-translational modifications. Comparing the proteome of 1C11Fk infected cells to their non infected counterparts may help understand how prion accumulation impacts on the normal function of PrPC, notably at the level of its cell signaling effectors.

P.5.6

Prion infection interferes with cellular prion protein signalling function

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Background: Corruption of the normal function of the cellular prion protein (PrPC) by the scrapie isoform (PrPSc) emerges as a critical causal event in Transmissible Spongiform Encephalopathies (TSE) pathogenesis. Taking advantage of the 1C11 neuroectodermal cell line, endowed with the capacity to differentiate into either 1C115-HT serotonergic of 1C11NE noradrenergic neuronal cells, we previously documented specific effects of infection on neuronal functions. While PrPSc accumulation does not induce any noticeable phenotypic change in the 1C11 progenitor cells nor prevent the entry of cells into neuronal differentiation programs, pathogenic prions deviate the overall neurotransmitter-metabolism in both 1C115-HT and 1C11NE cells.

Objectives and Methods: The aim of the present study was to go further into the mechanisms sustaining prion-associated neuronal damage. We assessed the outcome of infection with Fukuoka prions on PrPC signaling function in the 1C11 cell line by Western Blots and RT-PCR experiments. Additional studies were carried out with neuronal stem cells (NSC) obtained from 129 WT or PrP-null mice and infected with the 22L strain.

Results and Discussion: Our data reveal that, in 1C11 precursor cells and their 1C115-HT progenies, infection with the Fukuoka strain is associated with the activation of several targets of PrPC signaling. The impact is all the more pronounced since the cells accumulate PrPSc. Similar results have been obtained in 22L-infected NSC, while infection has no effect on NSC derived from PrP-/- mice. As a whole, our data indicate that the impact of PrPSc is dependent on PrPC expression and add further support to the notion that TSE-associated neurodegeneration results from distortion of PrPC signaling.

P.5.7

Altered expression of CRMPs in the brain of bovine spongiform encephalopathy-infected mice during disease progression

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Background: The symptoms of transmissible spongiform encephalopathies (TSEs) always develop after a long period post infection and coincide with exponential accumulation in the central nervous system (CNS), of the disease associated form of the prion protein (PrPd). Although PrPd has been reported to be potentially directly toxic in in vitro studies, it is still unclear how the infectious agent alters neural functions or neuronal loss in vivo. However, the description of PrPd accumulation in synaptosomes, the progressive loss of synapses and dendritic spines, the abnormal expression of synaptic proteins and the correlation of synaptic changes with early behavioural signs of neurological dysfunction suggest that neural lesions in TSEs may start by synaptic and dendritic alterations.

Objectives: Thus identifying intrinsic neuronal factors that could mediate such alterations should help to highlight some of the mechanisms involved in prion neurotoxicity. Here the expression of 4 collapsin response mediator proteins (CRMPs), a family of signal transduction proteins involved in brain development and altered in Alzheimer's disease, was studied in the brain of C57Bl/6 mice infected with the BSE strain of prion agent.

Methods: Using RT-PCR and Western-blot methods, CRMP-1, -2, -4 and –5 were analysed quantitatively in C57Bl6 mouse brains at mid-course (90 days post inoculation (d.p.i.)) and at the terminal stage of the disease (180 d.p.i.) induced by BSE strain of agent injected by intra cerebral route.

Results: At the terminal stage of the disease, gene expression of each CRMP had decreased, while at the mid-stage of the disease only CRMP-4 (mRNA and protein) expression had increased, concomitant to the start of PrPd accumulation in the brainstem.

Discussion: Over-expressed CRMP-4 may support the prion-initiated neurite disorganisation or promote the selective remove of damaged neuritis. Altogether our findings picked out originally CRMPs, and especially CRMP-4, as potential contributors to prion pathogenesis.

P.5.8

Cytoskeletal changes and synaptic loss observed in the CA1 neurons of the ME7 infected hippocampus

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Background: One of the characteristic pathological changes observed in transmissible spongiform encephalopathies (TSEs) is neuronal loss. In the ME7/CV scrapie mouse model , 50% of hippocampal pyramidal cells die from day 160 of a 240 day incubation period (Jeffrey et. al. 2000). Prior to this at approximately 100 days post injection (dpi) dendritic spine loss is observed in the CA1 neurons indicating early cytoskeletal damage in these neurons (Brown et. al. 2001).

Objectives: In this study the ME7/CV scrapie mouse model was used to investigate the role of the neuronal cytoskeleton in the dendritic spine loss observed in the ME7 infected CA1 neurons and to relate any changes observed with synaptic dysfunction.

Methods: A time course study was performed using the ME7/CV scrapie mouse model. The distribution of dendritic cytoskeletal protein MAP2 (microtubule associated protein 2) was compared with the expression of Drebrin, a dendritic spine cytoskeletal protein. Synaptic dysfunction was analysed using the pre- and post-synaptic markers, synaptophysin and PSD-95 .

Results: Loss of MAP2 and drebrin was observed at 160 dpi a time point when neurons are known to be lost. A decrease in synaptophysin and PSD-95 expression were not observed until the terminal stage of disease.

Discussion: Cytoskeletal disruption in the post-synaptic dendritic spine plays a major role in the neuronal dysfunction observed in ME7 infected CA1 neurons, although the post synaptic density is not involved. Pre-synaptic changes and disruption to the innervation of CA1 neurons is not apparent until the end stages of disease suggesting that the neuronal loss observed in the CA1 sector was not the result of deafferentation. The trigger for this cytoskeletal disruption and the subsequent neuronal loss may be the early deposition of PrPSc in the extracellular space but the precise mechanisms involved are still to be elucidated.

P.5.9

The ironic role of iron in prion disorders

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Background: Imbalance of iron homeostasis has been reported in prion infected cell and mouse models. The mechanism(s) underlying this abnormality, however, remain unclear.

Objectives: To evaluate the role of PrP function and dysfunction on iron metabolism in normal and prion disease affected cell and animal models and in diseased human brain tissue.

Methods: Measurement of iron and expression levels of major iron management proteins in cells expressing PrPC and mutant PrP forms, Wt and transgenic mice lacking PrPC expression (PrPKO), and in prion disease affected human and animal brain tissue.

Results: Expression of PrPC has a positive effect on cellular iron levels and mutant PrP forms alter cellular iron differentially, suggesting a dominant role for PrP in iron uptake and transport. Selective deletion of PrPC in PrPKO mice results in a phenotype of systemic iron deficiency reflected in hematological parameters and iron levels of major organs including the liver, spleen, and brain, confirming the role of PrPC in iron uptake. Furthermore, introduction of radiolabeled iron (59FeCl3) to PrPKO mice by gastric gavage reveals impaired transport of 59Fe from the duodenum to the blood stream and decreased uptake by parenchymal cells of various organs, including hematopoietic precursor cells. The iron deficient phenotype of PrPKO mice is rescued by expressing PrP on the PrPKO background, confirming the role of PrP in iron uptake and transport. More importantly, aggregation of PrPC to the PrPSc form induces a state of iron dys-homeostasis in prion disease affected human and animal brains, suggesting a role for iron induced oxidative stress in the pathogenesis of prion disorders.

Discussion: The interaction of PrPC with iron and its role in cellular iron uptake has significant physiological and pathological implications since loss of function combined with sequestration of iron in PrPSc-ferritin complexes induces brain iron dys-homeostasis, a condition likely to induce neurotoxicity.

P.5.10

Removal of PrP expression from neurones prior to scrapie infection prevents synaptic loss and neuronal death but not disease.

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Background: The expression of PrP has been shown to be vital for the development and progression of TSE disease. In an attempt to identify which cell types are involved in disease we have produced a model in which PrP expression can be selectively removed from neurones of the central nervous system (CNS). This model has been challenged with the mouse-adapted scrapie strain ME7.

Objectives: 1. Selectively remove PrP expression from CNS neurones.

2. Characterise effect upon PrP expression.

3. Investigate effect upon TSE disease.

Methods: The PrP coding sequence was selectively knocked out from neurones using cre/lox technology. Expression of a tamoxifen-inducible Cre recombinase fusion protein was limited to CNS neuronal populations using the rat neuron-specific enolase (Eno2) promoter in transgenic mice. Inducible neuronal specific PrP knockout mice were created by cross-breeding with floxed PrP mice described previously [1].

Results: Removal of neuronal PrP expression resulted in an average 50% reduction in brain total PrP. Subsequent i.c. ME7 challenges revealed extended incubation periods, with mean increases of +85 days (+57%) in male and +235 days (+162%) in female neuronal PrP knockout mice. Pathological analysis revealed the neurones in the CA1 region of the hippocampus at the level of the anterior thalamus, which were severely depleted in the wild type mice, were protected in the conditional knock out mice. The pattern of deposition of PrP in the female knockout mice also showed a marked alteration from that of the wildtype and male knockout mice.

Discussion: The removal of PrP expression from neurones therefore impacts on neurodegeneration in specific brain regions but does not prevent TSE disease.

Reference:

1. Tuzi, N.L., et al., Cre-loxP mediated control of PrP to study transmissible spongiform encephalopathy diseases. Genesis, 2004. 40(1): p. 1-6.

P.5.11

Oxidation of Met residues on PrP: an early event in prion formation and disease manifestation

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Background: We have recently shown that PrPSc from all species exhibits a covalent signature in the form of sulfoxidation of M213. This modification could not be found on brain PrPC as well as on its recombinant models. Moreover, computational methods indicated that oxidation of Met residues in helix 3 of PrP, as is the case for Met213, destabilize the structure of helix 2, which present as beta sheet in PrPSc.

Objectives: To investigate the role of PrP methionine oxidation in prion formation and in prion disease manifestation.

Methods: We used several antibodies which specifically recognize either oxidized or non oxidized Met residues on PrP, and tested their activity on wt and mutant recombinant PrP polypeptides as well as on cell culture systems expressing PrPC, PrPSc and mutant PrPs. The oxidation status of mutants and scrapie isoforms of PrP were also tested in cells subjected to oxidative conditions.

Results: We show here that oxidation of Met 213 is not only present in "old PrPSc" accumulated in the brains of prion infected subjects, but also in newly formed PrPSc present in cell lines infected with prions, suggesting that oxidation of Met residues in PrP may be an early event in prion formation. In addition, we also show that substitution of glutamate for lysine at position 200, as is the case in the most common form of genetic prion disease, results in increased sensitivity of helix 3 M residues for oxidation. This could be seen by the spontaneous oxidation of recombinant E200K PrP, and most interestingly, by the fact that in CHO cells cultured in the presence of copper ions, mutant PrP, as opposed to wt PrP, presents in an oxidized form.

Discussion: Oxidation of Met residues on helix 3 of PrP may be the key event in the conversion of PrPC to PrPSc. Most interestingly, mutations causing radical charge changes around helix 3, as is the case for the E for K substitution at codon 200, may predispose the prion protein to such oxidations, thereby inducing the appearance of genetic prion diseases.

P.5.12

Electrophysiological studies in TSE-affected cattle

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Background: BSE causes over-reactivity to external stimuli and an increased parasympathetic tone, which are attributed to neuropathological changes (vacuolation, PrPd accumulation) in the brain.

Objectives: To investigate whether nervous system dysfunction in the sensory and autonomic pathways can be measured, we assessed electrocardiograms (ECG), auditory (AEP) and flash visual evoked potentials (VEP) to compare cattle affected by TSE (BSE, scrapie) with TSE-free controls.

Methods: Studied animals were not sedated and comprised 42 (ECGs), 12 (AEP) and 11 (VEP) naturally or experimentally infected cattle with neuropathologically confirmed disease and 59, 38 and 39 TSE-free controls respectively, some of which were assessed repeatedly. Heart rate variability (ECG) parameters were compared by student's t test or Mann-Whitney U test; peak latencies and amplitudes (AEP, VEP) were compared against the reference range established from the control data.

Results: None of the heart rate variability parameters were significantly different. Four TSE-affected cattle presented with either abnormal parameters in AEP (peak absence or longer latencies; two cattle) or VEP (higher amplitudes, longer latencies; two cattle), sometimes only affecting one side.

Discussion: Autonomic nervous system dysfunction was not evident in TSE-affected cattle despite neuropathological changes in parasympathetic nuclei. Similar to CJD in humans, AEP or VEP parameters are not consistently altered in bovine TSEs. Higher VEP peak amplitudes as reported in patients with CJD may be due to increased hyperexcitability. Unilateral AEP or VEP abnormalities were in contrast to the general bilateral symmetrical neuropathological changes in bovine TSEs. These findings suggest that clinical signs are not caused by PrPd accumulation or vacuolation.

P.5.13

Increased cellular proliferation and lisosomal activity in the brains of a transgenic murine model of BSE

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Background: A brain gene expression study was performed on a murine BSE model based on the intracerebral inoculation of BSE in transgenic mice over expressing the bovine prion protein (BoTg110). The results of this study revealed that certain genes had a differential expression when compared to the uninoculated animals and also throughout the disease evolution. The present poster shows the results of the study, now at the protein level, of two of the mechanisms involved in the model: cell proliferation and lisosomal activity.

Methods: Seven mice intracerebrally inoculated with bovine normal brain homogenate were included as controls along with 7 mice inoculated with BSE (BSE2 inoculum). The latter were sacrificed at terminal stage of the disease. The brains where formalin fixed and paraffin embedded for their neuropathological examination. The brain distribution of spongiform change and resistant prion protein (PrPres) deposition were studied along with the immunolabelling of the proliferation marker Ki67 and the lisosomal marker Cathepsin C.

Results: Brain lesion and PrPres deposition profiles showed a parallel distribution mainly affecting the brain stem (medulla oblongata through thalamus) and, less intensely, the rest of the brain. Moreover, an increase of the immunolabelling of both markers studied was observed in the inoculated group when compared to controls which also paralleled the lesion and PrPres distribution.

Discussion: The results of the present study confirm that the differential DNA expression observed in the transcriptional study does translate in differential protein expression. These changes have been localized precisely within the brain anatomy and correlate well with the deposition of PrPres and spongiform change. In both cases the signalling is observed in cells morphologically identifiable as glial cells thus reflecting the glial proliferation and its phagocitic activity, both properties are acquired by this cell type as a response to PrPres deposition.

P.5.14

Silent prion-like forms in human prion diseases

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Background: We recently identified proteinase K (PK)-resistant prion protein (PrPres) fragments termed silent prions in the normal human brain and in uninfected cultured neuronal cells. In sharp contrast with PrPres detected in classic human prion diseases, the newly-identified PrPres exhibited extremely low immunoreactivity with 3F4 but preferential immunoreactivity with 1E4 antibody (Ab) that detects a PrP sequence next to the 3F4 epitope. The pathophysiology of silent prions remains unknown. Moreover, whether there are any changes in silent prions in various human prion diseases remain to be determined.

Objective: To study the pathophysiology of silent prions.

Methods: Taking advantage of the unique features of silent prions that allow preferential detection by the 1E4, but not 3F4 Ab, we analyzed brain tissues obtained at either autopsy or biopsy from atypical prion diseases and classic sCJD by Western blotting and neurohistology.

Results: Silent prion-like forms are readily detected in three pathological conditions: 1) protease-sensitive prionopathy with homozygous Val/Val at codon 129 of PrP exhibiting the ladder-like PrPres migrating between 6 and 26 kDa on the gel; 2) a relative 'early-stage' of sporadic fatal insomnia showing PrPres migrating between 19 and 29 kDa in biopsy brain tissues of three cases; and 3) a subset of sCJD(Met/Met) with co-occurrence of PrPSc type 1 and type 2 exhibiting a 19-kDa PrPres. All the PrPres fragments observed in these conditions share the same epitope immunoreactivity behavior as silent prions present in the normal brain: preferential detection by 1E4, but not by 3F4.

Discussion: Because of the similar epitope immunoreactivity behavior, all silent prion-like PrPres detected in affected brains may originate from silent prions. The mechanism underlying the increase in silent prions remains to be determined. (Supported by the CJD Foundation, NIHR01NS062787, NIA AG-14359, and CDC UR8/CCU515004.)

P.5.15

Protease-sensitive prions in the familial prion disease linked to 144-bp insertion of the prion protein gene

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Background: We recently described a novel prion disease (termed protease-sensitive prionopathy (PSPr) characterized by the presence of protease-sensitive abnormal prion protein (sPrPSc) rather than the protease-resistant isoform (rPrPSc), the molecular hallmark of prion diseases. The correlation between the two conformers and neuropathological changes remains poorly understood.

Objective: To explore the role of sPrPSc and rPrPSc in the familial prion disease linked to 144-bp insertion of PrP gene.

Methods: Approaches used include one and two-dimensional SDS-PAGE and Western blotting, native PrPC and PrPSc capture, neurohistology, PRNP gene analysis in brain tissues obtained at autopsy from subjects with a 144-bp insertion encoding six additional octapeptide repeats.

Results: Seven cases with the 144-base pair (bp) insertion were examined. While six cases exhibit rPrPSc on Western blots, in one case rPrPSc is undetectable even in commonly used PrPSc-enriched preparations. However, a large amount of PrP was captured from this case by the gene 5 protein (g5p) and PTA, regents that specifically capture abnormal, but not normal, PrP. The captured PrPSc is sensitive to protease-digestion, i.e. sPrPSc. Using a combination of a detergent-solubility assay and PrP capture with either the 6H4 antibody, which captures only native PrPC, or g5p, which specifically captures abnormal PrP, we demonstrate that rPrPSc and sPrPSc are composed of both wild-type and mutant alleles. Moreover, independent of the presence of rPrPSc, cases with the insertion mutation show the characteristic "stripe" PrP immunostaining pattern in the molecular layer of the cerebellum.

Discussion: Our study favors the hypothesis that spongiosis is attributable to the deposition of rPrPSc and the stripe pattern is composed of sPrPSc with insertion mutation. (Supported by the CJD Foundation, NIH NS062787, NIA AG-14359, NIH NS052319, and CDC UR8/CCU515004.)

P.5.16

Co-existence of scrapie prion protein type 1 and 2 in sporadic Creutzfeldt-Jakob disease: its effect on the phenotype and prion type characteristics

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Background: The frequent co-occurrence of different types of the scrapie prion protein (PrPSc) type 1 and 2 in patients affected by sporadic Creutzfeldt-Jakob disease (sCJD) adds to the complex spectrum of phenotypes and makes the diagnosis of CJD more challenging.

Objectives: We investigate the prevalence of PrPSc types 1 and 2 co-occurrence, its effects on the disease phenotype and PrPSc molecular characteristics analyzing 34 sCJD cases, all methionine (M) 129 homozygous at the PrP gene (sCJDMM).

Methods: The use of adequate PK concentrations and of several antibodies maximized the detection of both PrPSc types while minimizing the estimation of "false" sCJDMM1-2 cases. The molecular findings were correlated with the clinical and histopathological phenotype.

Results: Our data show that sCJDMM1 and sCJDMM2 cases exist and account for ~ 56% and 5%, respectively; 39% are sCJDMM1-2 cases. There is strong evidence that PrPSc types 1 and 2 co-occurring in the same anatomical region have peculiar conformational and immunoreactivity characteristics: i.e., PrPSc type 1 gains conformational features of PrPSc type 2 while keeping its molecular size (20 kDa); also, a 19 kDa fragment matching the "core" of PrPSc type 2 can be detected exclusively by the antibody 1E4 and is conformationally more stable than PrPSc type 2 immunoreacting with 3F4. The brain distribution of types 1 and 2 appear to be non random but dictated by distinct degrees of type-specific "permissiveness". The prevalence of PrPSc type 2 and sCJDMM2 phenotypes correlate with the disease duration. Clinically, sCJDMM1-2 has an average disease duration intermediate between the other two sCJDMM subtypes. The histopathology is also intermediate but the cerebellum resembles that of sCJDMM1.

Discussion: sCJDMM1-2 is not simply a mixture of two PrPSc types but it has unique features. It seems justified to keep sCJDMM1-2 as a separate subtype of sCJDMM at this time.

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P.5.17

E200K genetic CJD associated with prominent intraneuronal accumulation of disease-associated PrP

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Background: Immunostaining for PrP reveals well described disease-associated deposits mainly located extracellularly in the neuropil and only rarely detected intracellularly.

Objectives: To describe E200K genetic CJD cases with peculiar intraneuronal deposition of disease-associated PrP.

Methods: Immunohistochemistry for PrP using monoclonal antibodies against different epitopes of the PrP. Anatomical mapping of neuropathological changes in several regions. Paraffin-embedded tissue (PET) blotting to demonstrate proteinase-K resistant PrP.

Results: Five cases from two different families were examined. Age at death ranged between 54-74 years, and the duration of illness between 3 and 14 months. Clinical symptoms were not distinct from described E200K genetic CJD cases. Spongiform change was mild to moderate and affected mainly deeper layers of neocortical araes as well as the basal ganglia. Immunohistochemistry for PrP revealed variably dense synaptic type of immunoreactivity in all examined regions. Prominent and unusual PrP immunoreactive globules were seen only in the neurons of the deeper neocortical layers as well as in the subiculum, basal ganglia, and brainstem nuclei. Regions with prominent synaptic PrP deposits did not show intraneuronal globular PrP deposits. PET-blot confirmed that these intraneuronal PrP deposits were Proteinase K resistant. Intraneuronal PrP immunoreactive globules were not detectable using antibodies against the N-terminal fragment of the PrP and were lacking in a cohort of 40 cases with E200K genetic CJD.

Discussion: Our observations support the notion that several truncated forms of disease-associated PrP may be present in the same brain suggesting different processing of PrP in distinct neuronal populations. In contrast to other neurodegenerative disorders, inclusion-body-like intracellular accumulation of PrP is unusual in prion diseases and suggests complex disease pathogenesis.

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P.5.18

From protein misfolding to neurodegeneration

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Background: The TSEs, one of a group of diseases which result in misfolding of a protein in the brain resulting in neuronal degeneration and death, are set apart from other protein misfolding diseases due to the infectious nature of these diseases. To fully understand the TSEs therefore we need to understand both the infectious and neurodegenerative nature of these diseases.

Objectives: We are addressing this using our gene targeted transgenic mice in which the species, glycoforms or expression patterns of PrP the murine PrP gene has been altered. We have examined the role of host PrP in transmission between species and the infectious process within the host from periphery to the CNS. We have examined whether peripheral nerves are the major route of transport and whether glycosylation status of PrP determines delivery of the agent to the CNS. We are also examining the neurodegenerative process within the CNS and the links between PrP and Alzheimers disease.

Methods: We have used a number of different TSE strains, different routes of inoculation and a range of transgenic mice and examined the incubation time of disease and a range of pathological markers of disease.

Results: We have established that PrP can dramatically influence the course of disease. Alterations in host PrP influence cross species transmissions, glycosylation of PrP in peripheral organs is important for dictating both the timing of neuroinvasion and the final targeting in the brain, while removal of PrP from Schwann cells dramatically reduced levels of PrP with a loss of the glycosylated isoforms in the PNS but did not prevent neural invasion. We have further examine the link between APP processing and PrP

Discussion: While host PrP clearly influences pathogenesis and transmission characteristics of disease, its role in this process is clearly unresolved. Removal of PrP from specific cells and tissues yielded unexpected results which question our basic understanding of these diseases.

P.5.19

Characterization of soluble oligomers of prion protein by fluorescence correlation spectroscopy

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Background: The conversion studies in vitro of recombinant PrP (recPrP) which were induced by diluting from a high concentration of sodium dodecyl sulphate (SDS) or by pH shift have been reported. It is, however, difficult to address the conformational alterations of recPrP from the denaturing condition and little is known about the molecular events leading to the oligomerization.

Objectives: To clarify the oligomerization process and conformational transition of recPrP from a natural condition.

Methods: Aqueous solution of enhanced green fluorescent protein (EGFP)-tagged PrP (EGFP-PrP) was prepared and the oligomer formation of EGFP-PrP induced by SDS was monitored by fluorescence correlation spectroscopy (FCS). Moreover, conformational changes of PrP were detected by fluorescence cross-correlation spectroscopy (FCCS) using a panel of anti-PrP monoclonal antibodies (mAbs).

Results: The FCS analysis showed that soluble oligomers were formed between 0.005% and 0.01% SDS and that the basic unit of the soluble oligomers was estimated as a trimer of EGFP-PrP. Conformational changes in both the N- and C-terminal region of PrP and involvement of the N-terminal region in the soluble oligomer formation were indicated by the combination method of FCS/FCCS and mAbs.

Discussion: The oligomerization process and conformational transition of EGFP-PrP was monitored from the natural condition (80 mM NaPi, pH 7.3) in which PrP is expected to possess a native conformation. Our results propose an essential model for the initiation of the spontaneous oligomer formation of PrP in which a trimeric PrP is formed as a basic unit of the oliogmerization and a conformational alteration takes place at the C-terminal region of the PrP oligomers. Our studies also provide the invaluable tool for analyzing the oligomerization and conformational transition of amyloidgenic proteins.

P.5.20

PrPSc degradation pathway in macrophages

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Background: Prions initially infect digestive tissue and transported to the central nervous system. Macrophage is one of the candidates of up taking and transport prions. However, it has been reported that macrophage could degradade PrPSc, and may be involved in the clearance of prions.

Objectives: The aim of this study was to identify PrPSc degradation pathways in macrophages. In particular, we focused on lysosomal and proteasomal pathways.

Methods: In this study, we added PrPSc of Chandler and Obihiro strain onto mouse macrophage J774 and Raw cell line. We studied degradation, cellular localization of PrPSc, and inhibitory effects of lysosomal and proteasomal inhibitors against PrPSc degradation.

Results: PrPSc was increased once after PrPSc addition and then reduced in a time-dependent manner. PrPSc were colocalized with Ubiquitin and lysosomal marker (Lamp-1) by fluorescence confocal laser microscopy. Treatment with lysosomal (Leupeptin) and proteasomal inhibitors (MG132 and ALLN) inhibited PrPSc degradation.

Discussion: Macrophage may degradade PrPSc with both lysosomal and proteasomal pathways.

P.5.21

Parallels between different forms of sheep scrapie and types of Creutzfeldt-Jakob disease (CJD)

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Background: Scrapie in sheep and goats is often regarded as the archetype of prion diseases. In 1998, a new form of scrapie – atypical/Nor98 scrapie – was described that differed from classical scrapie in terms of epidemiology, Western blot profile, the distribution of pathological prion protein (PrPSc) in the body and its stability against proteinase K. In a similar way, distinct disease types exist in sporadic Creutzfeldt-Jakob disease (CJD). They differ with regard to their clinical outcome, Western blot profile and PrPSc deposition pattern in the central nervous system (CNS).

Objectives: The comparison of PrPSc deposits in sheep scrapie and human sporadic CJD.

Methods: Tissues of the CNS of sheep with classical scrapie, sheep with atypical/Nor98 scrapie and 20 patients with sporadic CJD were examined using the sensitive Paraffin Embedded Tissue (PET) blot method. The results were compared with those obtained by immunohistochemistry. With the objective of gaining information on the protein conformation, the PrPSc of classical and atypical/Nor98 sheep scrapie and sporadic CJD was tested for its stability against denaturation with guanidine hydrochloride (GdnHCl) using a Membrane Adsorption Assay.

Results: The PrPSc of atypical/Nor98 scrapie cases and of CJD prion type 1 patients exhibits a mainly reticular/synaptic deposition pattern in the brain and is relatively sensitive to denaturation with GdnHCl. In contrast classical scrapie cases and CJD prion type 2 patients have a more complex PrPSc deposition pattern in common that consists of larger PrPSc aggregates and the PrPSc itself is comparatively stable against denaturation.

Discussion: The similarity between CJD types and scrapie types indicates that at least two comparable forms of the misfolded prion protein exist beyond species barriers and can elicit prion diseases. It seems therefore reasonable to classify classical and atypical/Nor98 scrapie – in analogy to the existing CJD types – as different scrapie types.

P.5.22

Study on the Calretinin-immunopositive cells in the cerebellar cortex of Scrapie-affected sheep

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Background: Calretinin (CR) is a member of calcium-binding protein family that confers protection against the pathogenic mechanisms of neuron involution/disease. A small number of well defined subtypes of calretinin-immunopositive (CRi+) neurons and fibers exist throughout the brain, being the cerebellum the CNS region with the most intense CR-immunopositivity.

Objectives: In this region we have studied the relationship between neuronal CR content and neuronal involution in the cerebella of normal sheep of different ages and of Scrapie-affected animals.

Results: CR-immunoreactivity was seen in subsets of widely varying numbers of cells belonging to all the neuronal subtypes and afferent fibers. Generally, immunopositive cells were seen in very high densities in lobules X and IX, and in low density in lobule VIIb. Unipolar brush cells (UBCs) were 100% CRi+. The Purkinje cells were the most variable neurons in CR-immunoreactivity. Few granule cell perikarya were immunostained but a large number of their axons were CR-immunopositive. Subsets of stellate and basket cells were CRi+. Strongly CRi+ mossy and climbing fibers were observed in all lobules. The aged animals showed reduction in certain neuronal subtypes (mainly UBCs) and hypertrophy / hyperimmunoreactivity in many CR-immunopositive cells of different CR-immunopositive neuronal subsets, also depending on the location. Purkinje cells were the most prone to show increased CR-immunoreactivity. Scrapie sheep showed signs of both neuronal involution and hypertrophic/hyperimmunoreactive responses in the subsets, also depending on the archi- or neocerebellar location.

Discussion: The results suggest that CR expression and/or CR cell content are related to the selective functions of the subtypes of the cerebellar cortical neurons and fibers and local factors rather than to general neuroprotection. Some types of CRi+ neurons are affected by age and Scrapie but other types seem to increase their CR content for neuroprotection.

P.5.23

Autophagy and prion infection: mutual interactions

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Background: In search of cellular mechanisms that play a role in prion diseases and have the potential to interfere with accumulation of intracellular pathological prion protein (PrPSc), we investigated the autophagic pathway. Recently, we showed that drug-induced induction of autophagy accelerates clearance of PrPSc in persistently prion-infected cells.

Objectives: To cover further aspects such as the role of drug-induced autophagy in acute prion infection, autophagy as a general susceptibility factor in prion infection, prion infection as modulator of autophagy levels and in vivo studies towards therapeutic applications.

Methods: Treatments with autophagy inducers, inhibitors and siRNA were performed in vitro and analyzed via Western blotting, confocal microscopy and FACS analysis. Further effects were investigated in vivo.

Results: We show here that manipulation of autophagy does not only have an effect on persistent prion infections, but it also on acute infections. Autophagic induction can strongly interfere with de novo prion infections and restrict the accumulation of intracellular aggregated prion protein. We observed that the ability to restrict acute prion infection is a common feature of diverse autophagy inducers. Moreover, a substance already in use for cancer therapy, is able to induce autophagy and enhance clearance of PrPSc in cell culture models and prolongs survival in animal studies. In a further study we correlated basal levels of autophagy in cells susceptible or non-susceptible for prion infection and monitored changes in basal levels of autophagic activity in cells acutely infected with prions.

Discussion: Our data are of high relevance for elucidating the exact function of autophagy in prion propagation and infection and indicate a general role of autophagy in prion disease scenarios, pointing to a novel potential therapeutic target.

** These authors contributed equally*

P.5.24

Mutant prion protein expression causes impairment of cerebellar synaptic functions in a transgenic mouse model

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Background: Tg(PG14) mice, expressing a prion protein (PrP) insertional mutation associated with an inherited prion disease, accumulate in their brains a misfolded and aggregated form of the mutant protein. As this form accumulates, the mice develop a neurological disorder characterized clinically by ataxia, and neuropathologically by synaptic-type PrP deposition, associated with loss of synapses and granule neurons in the cerebellum.

Objectives: We explored the hypothesis that mutant PrP induces synaptic dysfunction as a primary event in the pathogenesis.

Methods: We carried out functional studies in isolated nerve endings (synaptosomes) from mice at different stages of their neurological illness and in primary cultures of cerebellar granule neurons.

Results: Analysis showed a marked alteration of glutamatergic synaptic functions. Depolarization-evoked release of glutamate was already significantly reduced in cerebellar synaptosomes from presymptomatic mice, and was completely impaired at the time mice had advanced clinical disease. Defective depolarization-induced release was also observed in primary cultures of cerebellar granule neurons from newborn Tg(PG14) mice. To explore the possibility that reduction of glutamate release was due to defective calcium influx upon depolarization, we measured intracellular calcium levels in cerebellar synaptosomes and in cultured neurons, and performed whole-cell patch clamp recordings to measure calcium currents.

Discussion: Analysis indicated that depolarization-induced calcium transients were significantly reduced in synaptosomes and cells from Tg(PG14) cerebella, due to defective function of voltage-gated calcium channels. Electrophysiological recordings are in progress to characterize the molecular mechanisms underlying the calcium defect and the consequences of calcium channel impairment on cerebellar synaptic transmission and plasticity.

P.5.25

Generation and characterization of new mouse models of genetic prion disease

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Background: Fatal familial insomnia (FFI) and a subtype of familial Creutzfeldt-Jakob disease (CJD178) are clinically and neuropathologically distinct diseases linked to the D178N mutation in the gene encoding the prion protein (PrP). Disease phenotype is determined by the M/V polymorphism at codon 129 of the mutant allele: D178N/M129 segregates with FFI, while D178N/V129 is associated with CJD. We have generated transgenic (Tg) mice that express the mouse PrP homologue of the CJD178 mutation (D177N/V128) containing the epitope tag for monoclonal antibody 3F4. These mice synthesize a misfolded form of mutant PrP, which is aggregated and protease resistant, and develop pathological features of CJD178, including motor dysfunction, memory impairment, and EEG abnormalities (Neuron 60:1-12, 2008).

Objectives: To generate new Tg lines with a pure C57BL/J background expressing D177N/V128 and D177N/M128 PrP without the 3F4 tag.

Methods: cDNAs encoding mutant PrPs were cloned into the half-genomic PrP transgenic vector, containing the mouse PrP promoter, and microinjected in the pronuclei of fertilized eggs of C57BL/6J x CBA/J F1 mice.

Results: We have identified 9 mice carrying the D177N/V128 and 3 carrying D177N/M128 transgenes, which were backcrossed to PrP knockout mice with a pure C57BL/6 background.

Discussion: We are characterizing the biochemical, neuropathological and clinical features of the new lines. These mice will allow us to study the mechanism of phenotypic variations due to the M/V PrP polymorphism.

P.5.26

Candidate gene analysis in distinct regions of the central nervous system during the development of SSBP/1 sheep scrapie

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Background: Rodent scrapie models have been exploited in order to define a common molecular signature in the central nervous system for the progression of TSE disease.

Objectives: The aim of this work was to assess if gene expression profiles in murine models are of generic relevance or are TSE strain and/or species specific.

Methods: qRT-PCR assays were developed for six candidate genes and used to quantify transcripts in defined brain regions at times after SSBP/1 infection; a scrapie strain that causes defined clinical disease in sheep of Prnp genotypes encoding at least one VRQ allele. All six candidates showed consistent changes in expression levels in relation to progression of disease in murine scrapie models and were chosen on the basis that they represent disparate physiological pathways.

Results: Peripheral infection with SSBP/1 in sheep shows a consistent progression of pathology in the central nervous system as assessed by PrPSc deposition and neuropil vacuolation. The first affected region was the medulla (obex), then the thalamus and finally cerebellum and frontal cortex. However, unlike in mice there were no consistent changes to any of the candidate genes in relation to the progression of pathology in sheep.

Discussion: The dissimilarity between mouse and sheep scrapie is likely to be due to differences in the pathological characteristics of the diseases and highlights the difficulties in the development of a TSE-specific profile of gene expression.

P.5.27

Amino acid conditions near the glycosylphosphatidylinositol (GPI) anchor attachment site of prion protein for the conversion and the GPI anchoring

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Background: Prion protein (PrP) is a glycosylphosphatidylinositol (GPI) -anchored protein, and the C-terminal GPI anchor signal sequence (GPI-SS) is cleaved before PrP presents on a cell surface via a GPI anchor. However, mutations near the GPI anchor attachment site (the ω site) in the GPI-SS of PrP gene have been recognized to date in human genetic prion diseases such as M232R and M232T. Moreover, the ω site of PrP has been identified only in hamster, though it is known that amino acid restrictions are very severe at the ω site (amino acids: G, A, S, N, D and C) and the ω + 2 site (G, A and S) in other GPI-anchored proteins.

Objectives: To investigate the effect of single amino acid substitutions near the ω site of PrP gene on the conversion and the GPI anchoring and to discover the ω site of murine PrP.

Methods: We systematically created mutant murine PrP genes with all possible single amino acid substitutions at every amino acid residue from codon 228 to 232 near the ω site, transfected them into scrapie-infected mouse neuroblastoma cells and compared the conversion efficiencies of each mutant PrP with that of wild type PrP by Western blot analysis using 3F4 antibody. Further, we added PI-PLC in the medium and detected mutant PrPs released from the cell membranes.

Results: Single amino acid substitutions near the ω site in the GPI-SS of PrP altered the conversion efficiencies and the GPI anchoring efficiencies. Especially, amino acid restrictions for the conversion and the GPI anchoring were severe at codon 230 (amino acids: A, D, G, N, P, T and V) and codon 232 (A, C, D, E, G, H, K, N, Q, R, T and V) in murine PrP. Only the conversion competent PrPs with mutations in the GPI-SS were released from the cell membranes by PI-PLC.

Discussion: These findings indicate that codon 230 is the ω site in murine PrP and the amino acid restrictions of the ω and ω + 2 sites for the conversion and the GPI anchoring are less severe in PrP than in other GPI-anchored proteins.

P.5.28

Differential regulation of interferon-gamma responsive genes during prion infection

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Background: Interferon-gamma (IFN-g) is a cytokine produced exclusively by T-cells and natural killer cells to regulate immune responses. Although neither IFN-g mRNA or protein have been detected in the brains of infected animals, gene expression analysis by us and others has identified IFN-g stimulated genes up-regulated in brain during infection.

Objectives: As IFN-g has not been detected in brains of TSE-infected mice, we sought to confirm IFN-g stimulated gene and protein expression. We also tested the hypothesis that IFN-g is directly necessary for progression and presentation of TSE infection.

Methods: Brain gene expression from C57Bl/6 mice infected with RML agent were profiled using gene arrays. Profiles from pre-clinical and clinically infected animals were analyzed and expression of IFN-g stimulated genes was quantified by qPCR. To test the role of IFN-g in TSE infection, IFN-g knock-out (KO) mice and age-matched wild type (WT) mice were infected with RML. Disease progression, clinical symptoms, lesion profiles, PrPTSE deposition, gliosis, and gene expression were assessed.

Results: Twelve IFN-g induced transcripts were identified as up-regulated during infection by microarray, with three confirmed by qPCR. There was no difference in survival of KO and WT mice after RML infection, but there were differences in clinical presentation. Gliosis, PrPTSE deposition and lesion profiles were similar between KO and WT mice. Expression analysis of infected KO and WT mice reveal many genes with altered expression in response to prion infection when IFN-g is eliminated.

Discussion: The up-regulation of IFN-g induced gene expression in the absence of detectable IFN-g in the brain during prion infection was confirmed. In vivo studies using IFN-g knock-out mice indicated that IFN-g is not necessary for TSE infection but its absence does affect clinical presentation of disease. We are currently investigating the JAK/STAT signaling cascade to determine if IFN-g stimulated genes are being induced by an IFN-g independent mechanism.

P.6.1

Defining and understanding intracellular trafficking of PrPSc and the subcellular compartments where PrPSc-mediated neurotoxicity may occur

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Background: Prion diseases are fatal neurodegenerative disorders associated with a conformational change of cellular PrP (PrPC) to abnormal disease-associated conformers (PrPSc). To date, relatively little is known about the cell biology of this process; the cause of prion mediated neurodegeneration is not known, and a major gap exists in understanding how conversion of PrPC to PrPSc causes neuronal dysfunction and death. We are currently using PrP knockout cells, derived from the prion-susceptible N2aPK-1 cell line, enabling the expression of heterologous-tagged PrP and the study of trafficking of newly formed PrPSc without interference from host wild-type PrP.

Objectives: To track the conversion and cellular trafficking of PrPSc, and how it may result in prion-mediated neurotoxicity.

Methods: N2aPK-1 PrP-KO cell lines were transduced with a construct containing mouse PrP modified to contain the mAb 3F4 epitope and a panel of six different moPrP.FLAG constructs. We then generated cells that stably express tagged PrP at close to physiological levels. After exposing these cells to RML prions, prion-susceptible cells were identified using the scrapie cell assay. Susceptible cell clones were stained with the 3F4 antibody and intracellular organelle markers to identify the subcellular localisation of PrPSc.

Results: Immunofluorescence and immunoblot studies indicated that cells expressing 3F4-tagged PrP or the FLAG sequence at position 22 or 30 of PrP contain formic acid and PK-resistant PrP, indicating misfolded PrP propagation. In addition, our preliminary confocal microscopy studies have shown that tagged misfolded PrP co-localises with the plasma membrane, transferrin receptors, early endosomes and lysosomes.

Conclusions: Taken together, our data suggests that prion susceptibility with 3F4 and N-terminal FLAG-expressing PrP has been reconstituted in our cells. Furthermore, using our system will yield insights into PrP conversion, intracellular trafficking and putative pathways of prion-mediated neurotoxicity.

P.6.2

Biarsenical labelling by IDEAL protocol provides new insights into effects of anti-prion drugs on PrP cell biology

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Background: FlAsH is a biarsenical derivative of fluorescein which can specifically label tetracysteine (TC)-tagged cell surface proteins by IDEAL-labeling. This tagging is very useful for investigation of cell biology of PrP because FlAsH-labeled TC-tagged PrP (FlAsH-PrP) behaves similarly as wild-type PrP, and FlAsH-PrP can be analyzed by microscopy or SDS-PAGE followed by laser scanning (fluorescent gel analysis), enabling correlation of morphological and biochemical data. Time-lapse observation of FlAsH-PrP allows pulse-chase analyses.

Objectives: To evaluate direct effects of compounds on PrP, short-term incubations are more favorable than long incubations, because cytotoxicity and indirect effects might emerge to complicate the results. FlAsH-pulse-chase is suitable for short-term experiments because a few hours' incubation is sufficient for detection of changes in levels or localization of FlAsH-PrP. We evaluated acute effects on PrPsen and PrPres of representative anti-prion reagents and compounds which exhibit cytotoxicity during long incubation.

Methods: After labeling with FlAsH or an Alexa Fluor-FlAsH derivative by IDEAL labeling, the cells were incubated with various compounds and then harvested after different periods of time for fluorescent gel analysis, or alternatively were monitored by time-lapse confocal microscopy.

Results: For some compounds including sulfated glycans, fluorescent-gel or microscopic FlAsH-pulse-chase analyses confirmed reported effects observed with conventional methods of analyses along with some novel findings, whereas the results were different for other compounds such as E-64, suggesting involvement of indirect effects in the anti-prion activity observed after long-term incubation.

Discussion: Identification of direct effects of various compounds on PrP by pulse-chase analysis advances our understanding of cell biology of PrPsen and PrPres and underlying mechanisms of anti-prion activity. This will aid the development of efficacious anti-prion drugs.

P.6.3

Cellular prion protein protects T lymphocytes from oxidative stress during thymus development

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Background: PrPC (cellular prion protein) is a highly conserved GPI-anchored glycoprotein whose physiologic functions remain enigmatic particularly in immune system. Although PrPc deficient mice display only minor abnormalities, some mice over-expressing PrPC show alterations of thymus development.

Objectives: We investigated the function of the PrPC redox homeostasis in the cells of the thymus to assess PrPC involvement in T lymphocytes response to oxidative stress.

Methods: Thymocyte subpopulations from C57BL/6 wild type (WT) or PrPc deficient (Prp°) mice were defined flow cytometry for the expressions of CD4, CD8, CD44 and CD25 markers. Reduced glutathione (GSH), a scavenger of reactive oxygen species induced by stress, was measured by the fluorescence after Monochlorobimane incubation. Oxidative stress was induced in vitro by exposure of the thymocytes to 0.2mM of H2O2, and in vivo by a restricted diet consisting in a free access to food and water only for 2 hours per day for 7 days.

Results: Each thymocyte subpopulation is characterized by a precise level of GSH evolving throughout differentiation stages: most immature DN1-DN2 thymocytes contain the highest level of GSH which decreases as the cells differentiate. This evolution is identical in both WT and Prp° mice. Monitoring the level of GSH in thymocyte cultures after H2O2 exposure shows that thymocytes from Prp° mice display a higher susceptibility to oxidative stress than thymocytes from WT mice: WT C with high levels of GSH drop from 83% to 68%, whereas in Prp° mice the decrease is more dramatic from 92% to 20%. Restricted diet, known to provoke oxidative stress, induces a decrease of all thymocyte population that is earlier, more pronounced and for a longer period in Prp° as compared to WT mice.

Discussion: In the absence of PrPc, thymocytes are less able to maintain their pool of GSH, supporting a role in the response to oxydative stress. Taken together, our results clearly ascribe to PrPC a protective function in T lymphocytes against oxidative stress.

P.6.4

Mapping prion protein – tubulin interaction sites

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Background: Tubulin is a building block of microtubules – dynamic cytoskeletal structures that can switch between phases of assembly and disassembly. This property is crucial to microtubule functions in the cell. Previously, we have demonstrated that prion protein (PrP) binds directly to tubulin and inhibits microtubule formation by inducement of tubulin oligomerization.

Objectives: This report is focused on mapping the regions of PrP and tubulin involved in the interaction and identifying PrP domains responsible for tubulin oligomerization.

Methods: In these studies we employed covalent cross-linking, mass spectrometry analyses, cosedimentation experiments, light scattering measurements, electron and fluorescence microscopy.

Results: Using a panel of deletion mutants as well as proteolytic fragments of PrP we identified two microtubule-binding motifs at both ends of the N-terminal flexible part of the molecule. We found that residues 23-32 constitute a major binding site, whereas residues 101-110 represent a weak binding site. We demonstrate that PrP deletion mutants lacking residues 23-32 exhibit very low capability to induce tubulin oligomerization. Moreover, a synthetic peptide corresponding to this sequence mimics the effects of the full-length PrP on tubulin oligomerization and microtubule assembly. Studied at the cellular level, peptide composed of the PrP motive 23-30 and signal sequence (residues 1-22) disrupted the microtubular cytoskeleton. Furthermore, we found that the effect of PrP on tubulin oligomerization may be regulated by microtubule associated proteins (MAPs). Employing proteolytic fragments of α- and β-tubulin we mapped the docking sites for PrP within the C-terminal domains constituting the outer surface of microtubule.

Discussion: We hypothesize that induced by PrP oligomerization of tubulin may explain molecular mechanism of toxicity of cytoplasmic prion protein which concentration increases significantly in TSEs.

P.6.5

Alternative reading of PrP mRNA can give rise to CytPrP

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Background: The sub-cellular sorting and handling of PrPC is surprisingly flexible. The main route for the protein is through the secretory pathway ending at the plasma membrane, from where PrPC is shed or endocytosed. Surprisingly, PrPC has also been observed in significant amounts in the cytoplasm (CytPrP) of several cell types. The mechanistic underpinnings of CytPrP are poorly understood and the focus of this report.

Objectives: We have explored whether alternative reading of PrP mRNA can generate CytPrP.

Methods: We have expressed sheep PrP and fusion proteins of sheep PrP and green fluorescent protein (GFPPrP) in N2a cells. We have manipulated the sequence context surrounding the translation initiation codon Met1 (start codon with surrounding Kozak sequence) and generated amino acid substitutions in the ER-targeting signal sequence. The sub-cellular partitioning of PrP was studied by live confocal microscopy and Western Blots by use of a panel of PrP antibodies.

Results: Manipulation of the Kozak sequence switched the cellular localization of PrP and GFPPrP from peri-cellular membranous to disperse cytoplasmic. When expressing GFPPrP, two cytoplasmic N-terminal fragments of GFPPrP starting in frame at Met17 were found. The appearance of these fragments in WB proved to be a sensitive assay for CytPrP in this cell culture system. Our data suggesting that leaky ribosomal scanning (LRS) occurs also in wild-type sheep PrP with Met17 serving as an alternative start site for translation.

Discussion: Our data show that in cell culture systems sheep PrP allows Met17 to serve as an internal start site for translation. This type of expression can be up-regulated by altering the Kozak sequence, indicating that leaky ribosomal scanning occurs. PrP generated from Met17 is poorly translocated through the ER-membrane, probably due to the shortened ER-targeting signal peptide, resulting in cytoplasmic localization of the protein. To what extent this mechanism operates in vivo is currently under investigation.

P.6.6

Characterization of PrPC in C. elegans by life span

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Background: A fundamental understanding of the characteristics of PrPC is still of major dispute and necessity to comprehend the disease underlying processes of PrPSc. A frequently used and established model system for the analysis of proteins is the nematode Caenorhabditis elegans, the first multicellular organism to have its genome completely sequenced. Recently C. elegans was established as a model organism for transgenic expression of the cytosolic murine prion protein mPrP(23-231), which lacks the N-terminal signal sequence and the C-terminal glycosylphosphatidylinisotol (GPI) anchor site.

Objectives: It was suggested that prion protein folding mechanisms are similar in mammals and C. elegans since the mPrP(23-231) elicits toxic effects in the worms as well. To now further investigate the physiologic characteristics of PrPC we used for the first time C. elegans to express human full length PrPC.

Methods: C. elegans GE24-strain was transformed with three DNAs: 1) prnp in pBY871 under sel-12 promoter for expression in all cells, 2) pha1 rescue plasmid for selection at 25°C and 3) gfp as additional control. Prion expression was verified by Western Blot analysis applying 3F4 and 6H4 monoclonal antibodies as well by GFP Fluorescence and pha-1 linked thermo selection. We then further characterized transgenic worms with respect to their lifespan.

Results: There was no significant difference between wild-type, controls transformed with expression vector lacking the PRNP sequence, and two independently generated PrPC expressing worm strains, regarding life span. While prion expression has been verified for those two strains which were supposed to.

Discussion: We conclude that the model system we created might be a useful tool for the analysis of cellular localization, biochemical properties and all recently discussed physiological characteristics of PrPC, it is further a compatible system to study the infectiology of disease associated misfolded Prion.

P.6.7

Cell culture model to study the neuronal transport mechanism of normal and pathogenic prion proteins

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Background: Transport of disease-specific prion, PrpSc , along nerve fibers and between synaptically linked neurons is central to prion neuroinvasion and virulence. The spread of PrpSc to first- and higher-order neurons is essential for induction of neurodegenerative disease. Despite the integral role of neuronal transport in prion pathogenesis, the mechanism is not understood. Our previous work has used animal models to study the spread of PrpSc by a variety of infection routes. In an effort to increase the resolution of these studies we are establishing an in vitro system to characterize and dissect the mechanisms involved in the neuronal transport of PrpSc.

Objectives: The goal of the proposed studies is to identify the cellular transport pathways (motor proteins, adapters and associated cargos) used by PrpSc to move along axons.

Methods: Recent advances in protein fluorescence labeling and live cell imaging will be used to establish an innovative approach to investigate the movement of PrpSc in axons in vitro and identify cellular pathways involved in its transport. Utilizing fluorescently tagged prion protein and time-lapse microscopy, experiments will be conducted to interfere with known pathways of axonal transport, via lentiviral encoded siRNA and dominant negative proteins, to identify those which are responsible for transporting PrpSc.

Results: We have identified a population of PrpSc associated with synaptic proteins present in cargo vesicles that use kinesin motors for fast transport in axons. An in vitro system has been established with dorsal root ganglion cells (DRGs) in microfluidic chambers.

Discussion: Our system should provide an ideal model for identifying the molecular motors involved in the transport of PrpSc. Characterization of this neuronal transport will greatly increase our understanding of the spread of PrpSc within axons and between neurons. As we will also study the transport of the normal prion protein isoform, it is anticipated that light will also be shed on its mechanism of transport.

P.6.8

Crossvalidation of three different methods to detect CtmPrP

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Background: The physiological function of the cellular prion protein (PrPc) remains enigmatic as controversial funcions of PrP (toxic and protective) have been proposed. These results can be explained by the heterogeneity of PrPc conformations, i.e. secretory-GPI-anchored SecPrP and the two transmembrane isoforms CtmPrP and NtmPrP. So far, CtmPrP could only be detected an rather complicated and error prone assay involving mild proteolysis with proteinase K in the presence of detergent ("cold protease assay").

Objectives: Here, we present simple and reliable methods to detect CtmPrP to examine its physiological function in vitro and in vivo.

Methods: Immunization of PrP -/- mice with recombinant purified mouse PrP, and screening against in vitro translated PrP by immunoprecipitation generated a hybridoma cell line secreting mAB 19C3 specifically recognizing CtmPrP.

Results: The epitope of 19C3 was identified to comprise a linear stretch of a PrP polypeptide. Molecular modelling paralleled our discovery in that this stretch is uniquely exposed in the transmembrane conformation of PrP but hidden in the secretory conformation. Immunization of New Zealand White rabbits and PrP -/- mice with this peptide yielded more specific ABs against CtmPrP thus confirming our findings. The specificity of the polyclonal ABs or mABs against CtmPrP was validated by combining the "cold protease assay" with an immunoprecipitation of the Ctm specific ABs. Furthermore, we identified a chemical that specifically precipitated PrP from brain homogenates of tg(SHaPrP K110I, H111I) mice, but not from brain homogenate of tg(SHaPrP Δ104-113) mice. KHII mice favor CtmPrP expression in contrast to ΔSTE mice that favor SecPrP expression. Cold protease assay of the precipitated PrP confirmed the assumption that the chemical specifically precipitated only CtmPrP.

Discussion: In conclusion, we cross-validated three methods for CtmPrP detection which will greatly accelerate research on this appoptosis-associated PrP conformer.

P.6.9

PrP106-126-induced neuronal cell death inhibits under the hypoxic condition

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Background: Low oxygen concentration, hypoxia is a common environmental stress. Prion diseases are neurodegenerative pathologies characterized by apoptotic neuronal death

Objectives: This study investigates the effect of low oxygen concentrations on the neurotoxicity induced by prion peptide

Methods: After PrP106-126 peptide treatment in low oxygen environment, we tested the cell viability using crystal violet assay and MTT assay.

Results: The results showed that PrP106-126 peptide induced apoptosis in of SH-SY5Y, a cell line derived from a human neuroblastoma and the apoptosis inhibited by hypoxic conditions. We investigated the intracellular signaling responsible for the Prion peptide-induced cell death and the inhibition by hypoxia. As a result, hypoxia inhibited caspase-3 activation, p-38 phosphorylation and NF-kB activation induced by Prion peptide. And hypoxia increased the anti-apoptotic Bcl-2, IAP-2 proteins and activation of Akt phosphorylation.

Discussion: These results demonstrated that hypoxia inhibit the PrP106-126 peptide-induced neuroblastoma cell death with mechanism by Akt activation, p-38 and NF-kB inhibition, and suggested that hypoxic conditions or HIF-1.

P.6.10

Inferring prion partners from Gene-Networks

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Background: Prion diseases are caused by the accumulation of an altered cellular prion protein conformation (PrPSc) that further acts as a catalyst for the recruitment and modification of the normal form (PrPC) in an autocatalytic process.

Objectives: Elucidating the physiological function of these two protein conformations (PrPC and PrPSc) through the identification of their "interacting partners" might unveil the mechanisms involved in prion induced neurodegeneration.

Methods: Here, we use a method (LNI – Linear Network Inference), which aims to reverse engineer the regulatory mechanisms observed in gene expression under steady state conditions. A gene network model is first trained on a large compendium of gene expression measurements. An independent dataset, describing the biological system under the effect of an external agent (i.e. prions), is then used on the top of the previous network. Genes showing an incoherent behavior relative to the initial model are chosen as the best candidates for direct targets of the external agent.

Results: We collected previously published gene expression profiles of Mus musculus brain tissue under many different conditions (1382 experiments), in response to a prion inoculation in wild type and knocking out the Prnp gene. In terms of a mathematical model, inoculated prions can be thought as a hidden variable in our model, while a gene knockout can be interpreted as a node deletion in the original gene-network. In applying LNI to these expression profiles, we identified known PrPC partners, (such as APP), and also predict prion targets and buffering genes. A remarkable result is the ability to identify the PRNP and BACE2 genes among the predicted targets of Prions. Moreover, LNI makes specific new predictions (like the relationship between PrPC and α-synuclein), which we plan to pursue experimentally.

Discussion: This may unveil a key role of previously unsuspected genes interacting with the normal and pathogenic prion forms.

P.6.11

Prion protein regulates heparan sulfate expression

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Background: Despite several decades of extensive research, the normal function of prion protein remains unknown. A close interaction of PrP and heparan sulfate proteoglycans (HSPGs) has been reported. HSPGs (in total termed the 'heparanome') are involved in many biological processes that are regulated through various heparan-binding proteins, including growth factors. The HSPGs comprise a protein core with covalently attached glycosaminoglycans (GAGs). Metabolism of GAGs was shown to be disrupted in prion disease, with GAGs secreted in the urine of prion-infected animals. Significant changes in transcription patterns for HSPG synthesis in prion infected cells have also been reported, suggesting a link between sulfation and PrPSc accumulation. Data presented here are consistent with regulation of heparan sulfate signals by PrP.

Objectives: To further examine the interaction between PrP and HS or HSPGs by investigating protein and HS expression levels in various biological systems.

Methods: Several techniques were used to probe the relationship between HS and PrP.

1) Cell lines grown in vitro were analysed by immunoblot for differences in HS and PrP levels as a function of monolayer confluence.

2) Human cancer cells with high levels of PrP were treated with an anti-PrP antibody, then analysed for HS content.

3) HSPG levels were compared in wildtype and transgenic PrP-knockout mice.

Results: As cell cultures became more confluent, higher PrP expression was observed. Concomitantly, HS expression decreased. Secondly, human cancer cells treated with PrP antibody displayed increased HS expression. In PrP knockout mice HSPG levels were higher compared to wildtype animals.

Discussion: Using three independent techniques we observed an inverse relationship between HS levels and PrP expression. Thus it appears that a function of PrP is the regulation of HS and/or HSPG expression. This may account for many of the complex and diverse interactions of PrP with other proteins.

P.6.12

Development of protein candidates as CJD diagnostic biomarkers

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Background: Creutzfeldt-Jakob disease (CJD) is one of the transmissible spongiform encephalopathies (TSEs) caused neurodegerative disease by conformational conversion of normal forms of prion protein (PrPc) to infectious PrPSc isoform. 14-3-3 proteins in cerebrospinal fluid were used as laboratory diagnostic criteria for CJD. However, the 14-3-3 assay has the problem with high rate of false positive results in sCJD.

Objectives: Proteome of ME7 scrapie infected mouse brain was profiled, and analyzed their interactions and functions to facilitate the differential diagnosis of CJD as new biomarkers with high sensitivity and specificity.

Methods: C57BL/6 mice were intracerebrally inoculated with 10% brain homogenate of scrapie infected mouse and monitored. All mice showed a neurodegerative symptom after 171 dpi, and we performed western blotting to confirm PrPSc expression. For screening specifically expressed proteins among infected samples in comparison with normal controls, we applied 1-dimensional gel electrophoresis and liquid chromatography mass spectrophotometer scan mode for the protein identifications, and EIC or SRM mode for the protein quantifications. In addition, we performed following bioinformatics tools, the SEQUEST, the David, the Cytoscape and GO mapping

Results: We chose 111 proteins with high peptide scores and ratio values (p-value <1.30E-06). These proteins were involved in various biological processes and molecular functions. Three biological processes based on their protein-protein interactions were the transport, the localization, and the establishment of localization. Three descriptions related to neurological process were the transmission of nerve impulse, synaptic transmission, cell-cell signaling. Furthermore, Eno2, Gfap, HspA5, Tcp1, Ncam1, Ywhaz, and Syn1 were identified in this study.

Discussion: This proteomic approach could be applied for the biomarker discovery from plasma and CSF in developing presymptomatic diagnosis of CJD.

P.6.13

Molecular interaction of human prion protein and cytoskeleton associated proteins

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Background: Prion proteins form amyloid fibers in the process of aggregation while converting from normal cellular (PrPC) to its diseased form (PrPSC), a structure of striking similarity with cytoskeletal assembly. Similar or overlapping proteins may support both processes makes it essential to investigate the possible association of cytoskeleton proteins with PrPC.

Objectives: In the present study we aim to identify possible interaction of cytoskeleton proteins with human PrPC.

Methods: We present a novel combinatorial cloning method for the cloning of PrPC in One-STrEP-tag mammalian vector for the expression and purification of PrPC interacting proteins. C-terminus One-STrEP-tag PrPC was transiently expressed into the Prion protein-deficient murine hippocampus HpL3-4 neuronal cell line (1). STrEP-tactin affinity matrix purified proteins were separated on 1DE, In-gel digested and identified by Q-TOF MS/MS analysis and subsequently verified by western blotting.

Results: The result shows both known and novel proteins interacting with the PrPC.

Discussion: Identified interacting partners of PrPC range from structural constituent of cytoskeleton, some of which are involved in cell growth to proteins that are important for cell haemostasis maintenance. These findings could indicate possible interacting roles of cytoskeleton associated proteins with PrPC which may have implication for the onset of Prion diseases.

P.6.14

The prion protein ligand, Stress Inducible Protein 1, interacts with UBC9 and is SUMOylated

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Background: The co-chaperone Stress Inducible Protein 1 (STI1) triggers activation of PKA and ERK1/2 signaling through interaction with prion protein (PrPC), resulting in neuroprotection and neuritogenesis respectively. It was shown that STI1 is secreted by astrocytes suggesting that this protein acts as a soluble neurotrophic-like factor through its interaction with PrPC at the neuron surface.

Objectives: To understand better the regulatory mechanism of this process and to gain further insight into the STI1 and also PrPC biological functions, we carried out yeast two-hybrid screens to identify interactors of STI1.

Results: We found that STI1 binds in the yeast assay with many of the proteins involved in the SUMOylation pathway. SUMOylation is a post-translational modification involved in protein and genome stability, DNA repair, transcription regulation, trafficking and nuclear translocation. We found that STI1 interacts with the unique SUMO E2 ligase Ubc9 and also binds to several SUMO E3 ligases. Herein, we focused on one of these interactions and whether STI1 is indeed SUMOylated. We confirm the interaction between STI1 and Ubc9 in mammalian cells and by using an in vitro assay with recombinant Ubc9 we also found that STI1 can be SUMOylated preferentially by SUMO2/3. Confocal microscopy experiments were performed to investigate if SUMO1 or SUMO3 could modify STI1 intracellular distribution. Interestingly, co-expression of STI1 and SUMO3 or SUMO1 and the SUMO E3 ligases PIAS1 and PIASy increased the localization of STI1 in the nucleus detected both by confocal microscopy and sub-cellular fractionation.

Discussion: In conclusion, the results suggest that STI1 physically interacts with members of the SUMOylation pathway, is a substrate for SUMOylation and changes its intracellular localization when the SUMOylation pathway is upregulated. Future experiments will address the role of SUMOylation in STI1 secretion and if this process could be involved in signaling mediated by STI1-PrPC interaction.

P.6.15

Role of molecular chaperones and cargo proteins in the secretory pathway in metabolism of PrPC and PrPSc

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Background: Prion diseases are fatal and transmissible neurodegenerative disorders characterized by accumulation of the abnormally folded isoform of cellular prion protein (PrPC), termed PrPSc.

Objectives: In this study the influence of cellular quality control mechanisms on metabolism of PrPC, PrP aggregation and PrPSc levels was examined.

Methods: To identify the role of cargo protein VIP36 in the trafficking of PrPC and PrPSc, the cell surface localization of PrPC in N2a cells and PrPSc levels in prion infected N2a-22L and L929-22L cells were analyzed during overexpression of VIP36. Furthermore, we analyzed how induction of ER stress in L929 cells affects folding, expression and localization of PrPC and the involvement of selected cellular chaperones in the PrP quality control. To counteract ER stress and/or to enhance the cellular response to ER stress, the contribution of lectins like ERGIC-53 and EDEM3 to the metabolism of PrPC was examined via overexpression of these molecules under ER stress conditions.

Results and Discussion: Expression of VIP36 caused a decrease of PrPSc in L929-22L cells, whereas PrPC or PrPSc in N2a and N2a-22L cells were not affected, indicative for a cell-type specific function of VIP36. Induction of ER stress by tunicamycin resulted in decreased PrP levels as well as in an interaction between PrP and BiP/GRP78, indicating an involvement of BiP/GRP78 in PrP quality control. Conversely, proteasome inhibition did not induce ER stress, but resulted in detergent insoluble PrP aggregates and increase of the PrP levels. We found a decrease of PrPC and a very pronounced reduction of PrP aggregates pointing to an important role of ERGIC-53 and EDEM3 in quality control of PrPC and PrP aggregates. Overall, we provide new evidence for the contribution of cellular proteins in PrP quality control.

P.6.16

Studying cellular prion protein infection by reverse genetics: development of a siRNA screening assay in a 96 well format

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Background: Prion diseases or transmissible spongiform encephalopathies are fatal neurodegenerative disorders of humans and animals caused by the accumulation of PrPSc, a conformational isomer of the host encoded cellular prion protein PrPC. Despite extensive research, the precise mechanisms of prion internalization and PrPSc formation still remain unsolved. Furthermore, uncertainty exists if prion strains use the same routes for infection and depend on the same cellular pathways for efficient replication. Identification of the exact routes of PrPSc internalization and of subcellular compartments important for PrPSc formation with respect to potential strain differences is fundamental for elucidating potential drug targets for disease intervention.

Objectives: We aimed to develop a fast and sensitive reverse genetics assay utilizing murine fibroblast L929 cells that are permissive to at least three different prion strains.

Methods: To increase sensitivity L929 cells highly susceptible to 22L, RML and ME7 mouse prions were established by two rounds of sequential cloning. Furthermore, we have developed a 96 well assay combining siRNA mediated protein knockdown and subsequent prion infection of cell cultures.

Results and Discussion: This screening system allows for large numbers of samples to be analyzed at the same time under comparable conditions and enables identification of cellular proteins involved in prion propagation in acute or persistently infected cell cultures.

P.6.17

The prion ligand Stress Inducible Protein1 interacts with different enzymes from the SUMOylation pathway

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The Stress Inducible Protein 1 (STI1) is a specific ligand for the Prion protein (PrPC) and this interaction promotes neuroprotection and neuritogenesis through different signalling pathways. In order to understand possible physiological functions of STI1 and eventually PrPC, we used a yeast two hybrid assay to identify STI1 ligands. We performed two screens using human or murine brain cDNA libraries and the N-terminus region of STI1 as bait. Amongst the various clones isolated that interacted with STI1, we identified several involved with the post-translational pathway for protein SUMOylation, including several E3 ligases (distinct PIAS and also PC2) and the E2 ligase UBC9. Here we focused on the question of whether STI1 is SUMOylated in cells and if there is an E3 ligase that preferentially enhances STI1 SUMOylation. We found in cells expressing CFP-SUMO1 and STI1-HA that a fraction of immunoprecipitated STI1 is SUMOylated. Similar results were obtained in cell extracts probed with an STI1 antibody. These SUMOylation assays revealed that PIAS1 enhanced the incorporation of SUMO onto STI1, indicating that PIAS1 acts as an E3 ligase for STI1 SUMOylation. In agreement with these data, STI1 was able to co-immunoprecipitate PIAS1 specifically in HEK-293 co-transfected cells. In contrast, immunoblotting assays showed that overexpression of Pc2 decreased STI1 SUMOylation, suggesting that Pc2 is not a SUMO E3 ligase for STI1. To investigate whether these interactions promoted any changes in cellular distribution of STI1, we performed confocal microscopy experiments in PrP3F4 CF-10 cells. We observed enhanced localization of STI1 in the nucleus when it was overexpressed with PIAS1 and SUMO1 or SUMO3 suggesting that these proteins induce nuclear translocation of STI1. Triple labelling of cells with STI1, Pc2 and SUMO1 showed that Pc2 does not alter STI1 distribution. Future experiments will address the role of SUMOylation in STI1 physiology, its interaction with PrPC and signalling functions.

P.6.18

Involvement of cellular prion protein (PrPc) in erythroid differentiation in vitro

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Background: Several reports suggested possible connection between PrPc/ prion pathogenesis and erythropoesis, but role of PrPc in the process remains still elusive. Regulation of PrPc expression was shown also in murine erythroleukemia cells (MEL) which present in vitro model of erythroid differentiation.

Objectives: Aim of the study was to elucidate the role of PrPc in MEL cells by its silencing with RNA interference.

Methods: Antibodies AH6, AG4 and 6H4 were used for PrPc detection by western blot or flow cytometry. Apoptosis was detected by 7-AAD stain. For quantitation of mRNA (PrPc, AHSP- α- hemoglobine stabilizing protein, HBA- hemoglobine-α) we employed qRT-PCR. Total hemoglobine was measured by TMB spectrofotometric assay.

Results: We observed upregulation of PrP mRNA 72- 120 hours after start of MEL cells differentiation. Surprisingly, level of the protein was highest after 24 hours. Cell lines with ~90 % downregulated PrPc expression showed similar level of total hemoglobine content and normal expression pattern of AHSP and HBA during erythroid differentiation. Previous reports showed that neither physiological nor enhanced expression of PrPc altered percentage of apoptotic MEL cells during differentiation and our data suggest that even downregulation of PrPc probably does not lead to sensitization of cells to apoptosis. If under normal conditions all lines showed equal differentiation, then they could react differentially after exposure to external stress. We induced stress conditions by 3 treatments- elevated temperature (40°C), oxidative stress by 500 μM H2O2 and 125 nM Staurosporin. But even such conditions did not alter the level of apoptosis/ necrosis in lines with inhibited expression of PrPc.

Discussion: We have introduced new cell culture model for study of PrPc function in erythroid differentiation. Our initial observations suggest that downregulation of PrPc does not have direct impact on studied process or minimal expression of PrPc in created lines is sufficient to sustain its normal function.

P.6.19

Prion protein (PrP) and glypican-1- a functional relationship: a hypothesis (Part I)

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Background: Function: Prion protein (PrP) encodes, amplifies and transports specific structural information-containing heparan sequences on glypican-1

Introduction: Glypican-1 is a heparan sulfate proteoglycan which undergoes endocytic recycling with PrPc.

The "Heparanome": The heparan sulfate molecule has a large number of potential structures and has been variously termed "heparanome", "glycome" and the "heparan code". In addition specific heparan sequences bind to a wide spectrum of proteins which include most growth factors, cytokines and chemokines that are the key modulators of development and homeostasis in tissues. Present theories on regulated enzyme expression, encoding fine heparan structure cannot generate the complexity required.

Amplification of Heparan Sequence by PrP dimmer: A specific heparan sequence on glypican-1 binds and folds the first PrPc in a manner which triggers dimerisation with a second PrPc and endocytosis. Central to the hypothesis is that both proteins in the dimer have identical folds and that the N-terminus PrPc which is relatively unstructured, is highly sterically organized on the C-terminus to be identical to that of the PrPc which has already undergone specific folding, thus duplicating the synthetic cleft that is imprinted on the first PrPc, providing a synthetic template.

Encoding of Heparan Sequence by PrP: It is proposed that strength of the binding of RNA to PrP monomer of specific sequences of RNA as demonstrated by RNA aptemer studies is biologically significant and will subtly alter the folding of the PrP, resulting in a specific heparan sequence synthetic template.

Conclusion: The function of PrPc is amplification and encoding of heparan sulfate, and that synthesis of specific heparan sequences result in mis-folded PrPsc.

P.6.20

Prion protein (PrP) and glypican-1- a functional relationship: a hypothesis (Part II)

David Cullis-Hill

Sylvan Scientific, Australia

Background: Specific Sequences of heparan sulfate encode a spectrum of normal folding and mis-folding of prion protein (PrP): hypothesis

Introduction: It is hypothesized here that a family of specific heparan sulfate sequences is the infectious component in prion mis-folding disease.

Discussion: These specific sequences bind to a heparan binding site on the prion molecule, which gives a specific fold to the prion protein. In the case of infectious PrPsc the folding results in a change in solubility due to disclosure of hydrophobic amino acids, and prolonged half-life of the PrP, which progresses to further associations with other identical misfolded dimers and the formation of fibrils. As this sugar sequence may be duplicated by the prion/glypican complex, the heparan molecule takes up an infectious character. This accumulation leads to defects in the heparan recycling, resulting in heparan processing abnormalities. Inter-species barriers to dimerisation occur due to critical changes in amino acid sequence which disallows stable mirror dimerisation. A number of strains of prion mis-folding have been identified. It is suggested that a family of different heparan sequences exist which are capable of mis-folding PrPsc which will have different tissue distribution and incubation time. It is proposed here that the different patterns of PrP mis-folding in nervous tissue are due to the highly spatially restricted expression of specific enzymes involved in the distinct heparan modifications.

Conclusion: It is proposed here that prion diseases result from the synthesis of an error in heparan sequence modifications (controlled by the PrP template) which result in folding the PrP in such a manner that its half-life is prolonged. It is suggested that glypican is "protein X".

P.6.21

Prion protein (PrP) and glypican-1- a functional relationship: a hypothesis (Part III)

David Cullis-Hill

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Background: Prion Protein-encoded heparans have a function in health and disease.

Introduction: It is proposed that the prion group of proteins (PgP), which includes prion, doppel, shadoo and testicular prion, is co-expressed with various heparan sulfate proteoglycans (HSPG), which include glypican 1-5 and perlecan, to form a complex which is the template for the synthesis of specific heparan sulfate sequences.

Development: Prion protein (PrP) and glypican are expressed during embryogenesis and organogenesis in the brain, liver and kidneys and immune system, in a highly regulated manner. Specific heparan sulfate sequences have been associated with organogenesis, neural and vascular development.

Cancer: PgP are up-regulated in many malignant cancer cells. Importantly malignant tumors also upgrade the expression of one or more HSPGs. Malignancy depends partly on the capacity of a tumor to influence the metabolism of the surrounding cells by specific heparan sulfate structures such as fibroblasts, stromal cells and immune cells, in a manner, which supports their malignant characteristics.

Alzheimer's disease: The prominent heparan-containing proteoglycan in Alzheimer's amyloid is perlecan which is excreted in a baso-lateral fashion rather than an apical. In Alzheimer's disease, a specific heparan sequence is encoded or evolves due to errors in encoding, and the prion/perlecan system amplifies this sequence as it binds to the Alzheimer's amyloid related proteins, thereby causing them to form insoluble amyloid species.

Conclusion: The cell-free system (PMCA) amplifies a unique family of heparan sequences which mis-fold PrPsc. Modifications of this technology platform hold promise as a method of amplifying specific sequence of heparan sulfates extracted from various tissues at various stages of development, and pathology.

P.7.1

Lack of association between PRNP 1368 polymorphism and Alzheimer's disease (AD), vascular dementia (VaD) or sporadic Creutzfeldt-Jakob disease (CJD)

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Background: Polymorphisms of the prion protein gene (PRNP) at codons 129 and 219 play an important role in the susceptibility to Creutzfeldt-Jakob disease (CJD), and might be associated with other neurodegenerative disorders. Several recent reports indicate that polymorphisms outside the coding region of PRNP modulate the expression of prion protein and are associated with sporadic CJD, although other studies failed to show an association. These reports involved the polymorphism PRNP 1368 which is located upstream from PRNP exon 1.

Objectives: In a case-controlled protocol, we assessed the possible association between the PRNP 1368 polymorphism and Alzheimer's disease (AD), vascular dementia (VaD) or sporadic CJD.

Methods: To investigate whether the PRNP 1368 polymorphism is associated with the occurrence of AD, VaD or sporadic CJD in the Korean population, we compared the genotype, allele, or haplotype frequencies of the PRNP 1368 polymorphism in 152 AD, 192 VaD and 171 sporadic patients with frequencies in 268 healthy Koreans.

Results and Discussion: Significant differences in genotype and allele frequencies of PRNP 1368 polymorphism were not observed between normal controls and AD & sporadic CJD. There were no significant differences in the genotype and allele frequencies of the PRNP 1368 polymorphism between Korean VaD patients and normal controls. However, in the haplotype analysis, haplotype Ht5 was significantly over-represented in Korean VaD patients. This was the first genetic association study of a polymorphism outside the coding region of PRNP in relation to AD and VaD.

P.7.2

Genetic variability of the prion protein family genes in predator species

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Background: The natural spread of TSEs in sheep and goats is most likely through oral exposure and scarification. In contrast, predator species have an increased risk of exposure through consumption of potentially contaminated tissue from prey. In the open rangeland of Norway, sheep are being predated by bears, wolves and wolverines. Amongst the prey will be sheep with classical or atypical scrapie. The situation in Norway is not unique; in the USA free-ranging deer with CWD are predated by mountain lions. Several carnivorous species have developed TSEs in captivity, eg. mountain lion, domestic cats. Others have succumbed to disease after challenge, eg. ferret, raccoon. However, no dog or bear has ever been reported with a TSE. Susceptibility to TSEs is under the genetic control of the PRNP gene but it is possible that a second prion-family gene, SPRN, encoding shadoo protein also modifies disease.

Objectives: To gain insight into the genetic TSE susceptibility of predator species by analysis of prion family genes.

Methods: Genomic DNA was prepared from blood from free-ranging and captive animals in the USA, Scandinavia and the UK. PCR products were sequenced directly or after cloning in T-vector.

Results: PRNP and SPRN gene open reading frame sequences were determined from several predator species, including brown bear, mountain lion, wolf, wolverine, cat and dog. Gene sequences of all species were compared and polymorphisms recorded from several hundred samples. We have confirmed the unusual insertions in the feline PrP octapeptide region. PRNP and SPRN gene sequences will be presented as groups according to amino acids in specific codon positions.

Discussion: Based on the new genetic data and our knowledge of PRNP genetics in ruminants, man and rodents we discuss the predictive value regarding the susceptibility or resistance of predator species to TSEs.

P.7.3

Transcriptome analysis of human variant Creutzfeldt-Jakob disease autopsy brain specimens

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The pathological mechanisms of variant Creutzfeldt-Jakob disease (vCJD) in the human brain remain poorly understood. Data on differential gene expression in vCJD brains relative to control brains may provide important insights into the molecular mechanisms involved. Three age and gender matched comparison groups, each containing one vCJD case, one other neurological disease (OND) case and one non-neurological disease (NND) case, were used for gene expression analysis. To isolate age- or gender- related gene expression changes, samples were selected to cover a range of ages and both genders. For all brains, replicate RNA samples were prepared from matched areas of the frontal cortex using standard techniques for the preparation of total RNA followed by an extra phenol extraction to ensure removal of any remaining infectious proteinaceous material. All RNA preparations were characterised for their quality and yield. Gene expression data was generated using Superarray GEArray® Focused DNA Microarrays and analysed using the GEArray Expression Analysis Suite and Significance Analysis of Microarray software. A comparison between matched vCJD and NND control cases identified 26 up-regulated and 16 down-regulated genes, showing >1.5-fold change with a false discovery rate of 9%. The gene expression changes observed in this study are consistent with much of the recent literature on the neuropathogenesis of TSEs and highlight changes in genes associated with the folding, processing and degradation of proteins and in particular PrP and the biochemical and membrane events associated with the formation and turnover of lipid rafts.

P.7.4

Cpne8, a copine family member from Mmu15, is a candidate gene for prion disease incubation time in mouse

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Background: Prion disease incubation time in mice is determined by many factors including host genetic background. The prion gene itself plays a major role in incubation time however other genes are also known to be important. Quantitative trait linkage (QTL) mapping studies have identified multiple loci across the genome, however, these regions are often large, making the identification of individual candidate genes extremely challenging. With the exception of Hectd2 (Mmu19), no quantitative trait genes or nucleotides have been demonstrated.

Objectives: We therefore aimed to carry out fine mapping of known QTL and identify the underlying gene.

Methods: Approximately 1000 heterogenous stock mice were inoculated intracerebrally with Chandler/RML prions and genotyped with 20 microsatellite markers from the region of a previously identified BSE-associated QTL on Mmu15. Candidate genes were evaluated by sequencing and quantitative RT-PCR.

Results: Multipoint linkage analysis reduced the locus size from approximately 25 to 1.2cM. We characterised 29 genes from this 3.5Mb region and identified Cpne8, a member of the copine family, as the most promising candidate gene. We also show that Cpne8 mRNA is upregulated at the terminal stage of disease supporting a role for Cpne8 in prion disease.

Discussion: This is the first example of a prion disease incubation time QTL to be resolved that is independent of prion strain. Applying these mapping techniques to other loci will reveal additional modifier genes and identify key pathways in prion disease pathogenesis.

P.7.5

Is the serine for asparagine substitution at codon 171 of prion protein linked to Creutzfeldt-Jakob Disease?

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Background: The asparagine (N) to serine (S) substitution at codon 171 (N171S) of the prion protein (PrP) has been reported as a polymorphism with a prevalence of 5-8% in the African population. It has been also identified in a family with a prion-related psychiatric disorder. However, the association of the substitution with prion disease is far from being conclusive, because no pathological PrP (PrPSc) or prion-related neuropathological changes have been reported in subjects with N171S to date.

Objectives: To report a Creutzfeldt-Jakob disease (CJD) case with N171S substitution.

Methods: Approaches used include one and two dimensional SDS-PAGE and Western blotting, neurohistology, and prion protein gene (PRNP) analysis.

Results: The patient was a 78-year old African-American male with the N171S substitution exhibited the clinical triad of dementia, myoclonus, and ataxia as well as a heterozygous N171S substitution, 129 Val/Val (V/V) polymorphism, and the silent 117alanine/alanine polymorphism. Fine spongiform degeneration was observed using H&E staining with intense synaptic PrP immunostaining as demonstrated by immunohistochemistry with the 3F4 antibody. Western blot analysis showed the presence of proteinase K (PK) resistant PrP (PrP27-30) in the brain tissue. Compared to the PK-resistant PrP associated with sporadic CJD (sCJD), the monoglycosylated fragment in the subject is split into two bands, migrating at ~26 and 25 kDa, respectively. Furthermore, diglycosylated PrP was virtually undetectable and the migration of the non-glycosylated fragment is slower than that of sCJD VV1 control.

Discussion: Our study suggests that the protein with N171S substitution may possess both a unique structure and changed glycans, which favors the hypothesis that this case represents a novel familial form of CJD rather than sporadic CJD. (Supported by the CJD Foundation, NIH NS062787, NIA AG-14359, NIH NS052319, and CDC UR8/CCU515004.)

P.7.6

Resistance to classical scrapie in experimentally challenged goats carrying the mutation K222 of the prion protein gene (PRNP).

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Background: Several European studies indicated that PRNP polymorphisms can modulate susceptibility to classical and atypical scrapie in goats. In Italy and France, case-control studies showed a protective role against classical scrapie given by the PRNP mutation K222.

Objectives: Aim of this work was to investigate the genetic resistance in K222 goats after experimental transmission of classical scrapie.

Methods: Five goats carrying the genotype Q/Q at codon 222 (wild type) and five goats carrying the genotype 222Q/K were intracerebrally inoculated, at 5 months of age, with a brain homogenate from a classical scrapie positive goat with a wild type PRNP genotype. From the animals which succumbed several organs and tissues were collected. Scrapie diagnosis has been carried out by rapid test, Western blot and immunohistochemistry. Differences in survival times between the two groups have been compared by Kaplan-Meyer survival estimates.

Results: At the time of writing all the goats carrying the genotype 222Q/Q died of scrapie, with a mean survival period of 18.6 months (±1.4) while four out of the five 222Q/K goats are alive and without apparent clinical signs (survival period: 31 months). One 222Q/K goat was found dead at 24 months post inoculation but it resulted scrapie negative in the central nervous system and in all other peripheral organs and tissues. Statistical analysis showed that 222Q/K goats present a probability of surviving significantly higher than 222Q/Q animals ($x2 = 9.34$, $p=0.002$).

Discussion: The experimental scrapie transmission confirms that the mutation K222 gives protection against classical scrapie in goats. The continuous monitoring of the challenged goats will allow to understand whether this resistance is a prolongation of the incubation period or a resistance to clinical disease or a resistance to infection, giving information about the possible use of this polymorphism as target for scrapie control strategies based on genetic selection in goats.

P.7.7

Characterization of ovine SPRN gene promoter

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Background: SPRN is a new gene coding for shadoo (Sho), a protein with remarkable similarity to prion protein (PrP), encoded by PRNP. Both genes are expressed in brain. SPRN has recently been associated with CJD susceptibility. It has also been suggested that there is co-regulation of the PRNP and SPRN genes in cerebral and cerebellar cortex of sheep.

Objectives: We have studied the sheep SPRN promoter region to identify which factors contribute to the modulation of gene expression and whether there are similarities to PRNP regulation.

Methods: The SPRN promoter was sequenced between -996 to +39 relative to the exon I start site at 1207 (Genbank DQ870545). The promoter activity was analysed by reporter gene transfection assays in N2a cells, using a series of promoter sequence deletions cloned in front of the luciferase gene. The DNase I footprint technique was utilised to study molecular interactions within the promoter region. PCR fragments of the promoter were produced with 6-FAM labelled primers and then incubated with nuclear extracts from N2a cells. DNase I digested products were analysed with an automated DNA analyzer.

Results: Our reporter gene assays proved for the first time that this region has promoter activity and showed regions that appear to suppress and enhance the expression. We detect seven promoter polymorphisms in sheep, two and five in cattle and deer SPRN, respectively. Our data from DNA protection assays suggested the binding of transcriptional factors within the suppressor / enhancer regions of the promoter.

Discussion: Highly conserved sequences were identified on ruminant, rodent and human SPRN gene promoters, which are likely to be involved in the SPRN gene expression regulation. The homology to PRNP promoters is however low.

P.7.8

Molecular characterization of the putative regulatory regions of the prion protein gene (PRNP) in goats

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Background: Transgenic animal models of transmissible spongiform encephalopathies (TSEs) have demonstrated an inverse relationship between disease pathogenesis/susceptibility and the level of host PRNP gene expression. In cattle and sheep, polymorphisms within the PRNP promoter and intron 1 have been associated with TSE incidence. No basic information currently exists regarding the frequency of genetic polymorphisms within the homologous Putative Regulatory Regions (PRRs) of the goat PRNP gene.

Objectives: The aim of this work was to carry out the first polymorphism and bioinformatics analysis of the PRRs of the goat PRNP gene.

Methods: Genomic DNA from n=32 goats belonging to nine European breeds was submitted to direct PCR sequencing. From selected heterozygous samples PCR products were cloned and sequenced from several clones. Bioinformatics was used on the new primary sequence data to predict the goat PRNP PRRs and to identify transcription factor binding sites.

Results: Sequence analysis of 3 kb of the goat PRNP gene and haplotype cloning revealed 28 novel single nucleotide polymorphisms (SNPs) and three insertion/deletion (indel) polymorphisms in the putative promoter and intron I. Only one SNP was detected within the predicted exon 1. PRNP promoter prediction analysis yielded a single goat PRNP promoter that was homologous to regions of known promoter activity in sheep and cow. No genetic variation was observed within the four conserved PRNP promoter motifs previously described in mammalian species.

Discussion: This study provides novel knowledge on the genetic variability of the goat PRNP. The predicted goat PRNP promoter was, as expected, homologous to the PRNP promoter regions of both cattle and sheep. None of the identified changes were identical to bovine and ovine variants demonstrated to be involved in gene expression. Functional investigations will now be initiated to assess the significance of the polymorphisms and of the predicted motifs and their possible relationship with TSEs susceptibility.

P.7.9

PRNP gene sequencing in Belgian goats: codon frequency

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Background: Contrary to sheep, no strong association with TSE susceptibility has been discovered yet in goat PRNP gene, even if some codons (e.g. 146 and 154) can be associated with resistance/susceptibility to (classical) scrapie in goat. As TSE susceptibility can be influenced by PRNP coding mutations, we sequenced the PRNP gene of Belgian goats and carried out a frequency analysis of the codons suspected to be of importance. In Belgium, there are about 50000 goats in 13000 herds. The main races are Saanen (60%) and Alpine (30%); there are also some Naine, Anglo-Nubian and Poitou goat (Poitevine).

Objectives: Our study aimed at calculating the genetic diversity of the PRNP gene in Belgian goat.

Methods: Genomic DNA was isolated from blood or brain with a commercial kit (Qiagen or MO BIO Labs). Goat PRNP ORF was amplified with primers designed from the published goat PRNP sequence. PCR fragments were amplified using forward and reverse primers (Biometra T1 Thermocycler). Both strands of the PCR fragments were sequenced with forward and reverse primers by using a CEQ8800 sequencer (Beckman Coulter). Sequences were analyzed using the Variant Reporter software (Beckman Coulter). We analysed the codons 102, 110, 127, 142, 143, 146, 151, 154, 163, 168, 211, 222 and 240. We examined 94 goats. Most of them were Saanen.

Results: The goat PRNP gene is very similar to the sheep PRNP (three exons). A third of the whole gene sequence consists of repeats. At least five codons showed variations and seemed important (127, 142, 154, 211 and 240). The poster details our last results on the codon frequency, which are still under analysis.

Discussion: Our study focused on Saanen goats. It would be interesting to compare them to other races. Nevertheless the goat codon frequency can be used for future (comparative) studies concerning, e.g., genetic tools to control (ruminant) TSE (including atypical TSE). We will discuss the implications of our last results on the codon frequency for a possible future selection programme.

P.7.10

Investigating the influence of ovine PrP genotype on lesion profile: beyond the 3 codon genotype

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Backgound: Variation in the coding sequence of ovine PRNP plays a key role in susceptibility to prion disease. Codons 136, 154 and 171 are considered mostly responsible for determining susceptibility to scrapie and BSE in sheep, outside of which the open reading frame (ORF) of ovine PRNP is highly polymorphic. The BSE agent produces a unique, reproducible lesion profile on primary isolation in RIII mice, an approach employed by the World Organisation for Animal Health and the Community Reference Laboratory for prion strain discriminatory purposes. However, we recently reported 10 cases of classical scrapie, all derived from ARQ/ARQ ovine sources, that gave consistent and similar lesion profiles to BSE on primary isolation in RIII mice (termed 1-4-7-scrapie) (Beck et al., 2009). Isolates were subsequently confirmed as scrapie by cluster analysis, additional histopathology and immunohistochemistry. A further 21 cases analysed, consisting of 5 ARQ/ARQ isolates and 16 associated with the V136 allele, gave non-BSE like profiles. Our knowledge that ovine PRNP, and in particular the ARQ allele, is polymorphic lead us to hypothesise that additional polymorphisms may exist in 1-4-7-scrapie cases.

Objectives: To investigate the presence of potential polymorphisms in the PRNP ORF that may influence lesion profile, in the 31 field scrapie isolates that have undergone characterisation through the mouse bioassay.

Methods: DNA from blood samples of all 31 cases has undergone ovine PRNP open reading frame genotyping. Sequence data, generated for each sample is analysed using the Staden Software package.

Results and Discussion: We compare the full ORF of 1-4-7-scrapie samples with ARQ/ARQ samples that do not give the 1-4-7 profile, and with VRQ/VRQ samples, and investigate possible links between PrP genotype and lesion profiles. Ultimately, further genotypic information outside of the traditional 3 codon genotype has the potential to impact on a) further controlling TSE disease in sheep and b) enhancing our understanding of the influence of ovine PrP genotype on strain typing through the mouse bioassay.

P.7.11

N176K and L141F dimorphisms of the PRNP gene regulate PrPSc deposition in placentas of ARQ/ARQ sheep affected by natural and experimental scrapie

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Background: In sheep, resistance or susceptibility to scrapie is strongly modulated by the polymorphisms at codons 136 (A or V), 154 (R or H) and 171 (Q, H or R) of the PRNP gene. In addition, the same polymorphisms characterize some of the pathological aspects of the disease, including PrPSc deposition in the placenta. Indeed, it has been demonstrated that the placentas from foetuses with the Q171R dimorphism are resistant to PrPSc deposition.

Objectives: Recently, a protective effect of other dimorphisms of the PRNP gene in ARQ/ARQ sheep was recently showed. Therefore it could be worthy of interest to know whether other polymorphisms of the PRNP gene of the foetus, beyond that at codon 171, modulate the replication of PrPSc in the placenta of scrapie-affected sheep.

Methods: In this study the placentas of 25 symptomatic or asymptomatic naturally scrapie-affected ewes and 11 symptomatic experimentally scrapie-affected ewes were screened by performing western blotting and immunohistochemistry analysis for the detection of PrPSc. Moreover, the entire PRNP coding region of all the placentas was determined.

Results: In single pregnancy, PrPSc was demonstrated in the placentas of the ARQ/ARQwildtype foetuses but not of the ARQ/ARQmutated foetuses with the amino acid substitution L141F, N176K and, as previously reported, Q171R. However, F141L placenta displayed PrPSc western-blotting signal in a dizygotic twin pregnancy in which the other placenta carried the ARQ/ARQwildtype genotype. In this case, PrPSc accumulation level in ARQ/ARQwildtype placenta was much higher than in that with the F141L mutation.

Discussion: These findings demonstrate for the first time that further dimorphisms of the PRNP gene, other than Q171R, prevent PrPSc placental deposition. This may result in new strategies for the ovine breeding program, which may make more feasible the genetic selection plan for scrapie control.

P.7.12

Genetic analysis of the ribosomal protein SA family in sheep

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Background: Transmissible spongiform encephalopathies (TSEs) are incurable neurodegenerative diseases caused by the accumulation of PrPSc, the alternatively folded isoform of the cellular protein PrPC. It has been demonstrated that the ribosomal protein SA (RPSA) acts as a receptor for both PrPC and PrPSc, and is involved in the propagation of prion diseases. Recently, several new therapeutic approaches based on the blocking of RPSA were developed. RPSA is a member of a complex gene family and is described in human and several animals but the gene family in sheep, an important species in prion research, is not characterized yet.

Objectives: The aim of this study was to identify the members of the ovine RPSA gene family and characterize them.

Methods: The INRA sheep BAC library was screened with primers designed on the ovine RPSA mRNA sequence and mini-contigs were built. For each mini-contig, the RPSA gene family member was sequenced by direct sequencing. The mini-contigs were annotated by comparative mapping. The transcription profile of each RPSA member was determined by RT-PCR in 7 tissues.

Results and Discussion: Twelve ovine RPSA gene family members were found. One gene is the active RPSA gene. The 11 other RPSA gene family members were (partly) processed. In 9 (pseudo)genes all the introns were spliced out but 2 (pseudo)genes still contained a part of intron 4, the intron that holds a small nucleolar RNA (snoRNA). In 9 of the 11 (pseudo)genes, premature stopcodons were introduced due to frameshift mutations or SNP's. Comparative mapping showed that 5 (pseudo)genes have an ortholog in the cattle genome and that 6 (pseudo)genes appeared after the divergence of cattle and sheep. The active RPSA gene is transcribed in the cerebellum, cerebrum, blood, lymph nodes, spleen, muscle and duodenum. The transcription profile of the other RPSA gene family members is diverse and further investigation will show if these members are active genes or pseudogenes.

P.7.13

Spanish goat prion protein gene variability and implications for susceptibility to classical scrapie disease

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Background: Classical scrapie disease is a neurological disorder of the Central Nervous System (CNS) characterized by the accumulation of an abnormal partial resistant protein PrPsc in the CNS and some peripheral tissues. This disease affects domestic small ruminants but statistically the caprine specie seems to be most resistant. Scrapie in sheep is well known, in special the genetic factors that control the susceptibility or resistance to the disease. In contrast, the genetic control in goats is still unknown nowadays.

Objectives: The aim of this study was to determine the PRNP polymorphisms in Spanish and worldwide goat breeds with the objective of getting closer to a hypothetic genetic surveillance program in goats.

Methods: We report here the complete Open Reading Frame (ORF) sequencing study of the PRNP gene in native (Retinta, Pirenaica and Moncaina) and worldwide (Saanen, Alpine and crossbreed) Spanish goats. In this study, 9 scrapie-affected goats and 1057 healthy animals (555 from scrapie-affected herds and 502 from breed survey) were analysed.

Results: In total, fourteen polymorphic sites were identified, including the known amino acid substitutions at codons: G37V, G127S, M137I, I142M, R151H, R154H, R211Q, Q222K, and P240S, and new polymorphisms at codons T18R, M112T, L141F, Q215R and G232W. In addition, the known 42, 138 and 179 silent mutations were detected but new ones are also reported in codons 122 and 201. Genotypic and allelic frequencies showed significant differences between scrapie affected and healthy animals in certain breeds.

Discussion: The results obtained give preliminary bases of polymorphic distribution in Spanish and worldwide goat breeds. Even thought further studies are needed, especially regarding the variability present in scrapie-affected animals, these results give an initial starting point for the application of future eradication programs.

P.7.14

Assessment of genetic susceptibility of sheep to scrapie: comparison between PMCA and in vitro studies

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Background: The susceptibility of sheep to scrapie is mainly influenced by the prion protein polymorphisms A136V, R154H, and Q171R/H. In a previous study, we challenged sheep carrying different genotypes (ARQ/ARQ, ARQ/ARR, ARR/ARR, ARQ/AHQ, AHQ/ARH, ARQ/ARQK176 and ARQ/AT137RQ) with classical scrapie. The survival times observed indicated different levels of susceptibility to scrapie, with the following ranking order: ARQ/ARQ > ARQ/AHQ > AHQ/ARH. The presence of the ARR allele (ARQ/ARR, ARR/ARR) conferred protection from disease. In the same experiment evidences of the protective effect of the AT137RQ and ARQK176 alleles were first reported (Vaccari, G. et al. 2007).

Objectives:

1. To analyze in vitro, by the Protein Misfolding Cyclic Amplification (PMCA), the conversion efficiency of the above-mentioned sheep genotypes triggered by sheep scrapie;

2. To compare the results of PMCA with those from in vivo studies.

Methods: Brain homogenates (10% w/v) from healthy sheep carrying the same genotypes tested in vivo were prepared as sources of PrPC (substrates). Brain homogenate (10% w/v) from a ARQ/ARQ sheep with natural scrapie was used as infectious seed. Dilutions from 10-1 to 10-3 of the seed into the various substrates were submitted to PMCA. The conversion efficiency was assessed by calculating an amplification factor for each genotype.

Results: The ARQ/ARQ substrate showed the highest level of conversion efficiency. All the others genotypes showed lower amplification factors. In particular, the ARQ/AHQ substrate converted more efficiently than the AHQ/ARH. All genotypes which showed to be protective in vivo (ARQ/ARR, ARR/ARR, ARQ/ARQK176 and ARQ/AT137RQ) showed similar and weak amplification by PMCA.

Discussion: The conversion efficiency of PMCA mimicked the ranking order of genotype-related susceptibility observed in vivo. Our results suggest that PMCA represents a high-throughput in vitro alternative to transmission studies for testing the susceptibility of the numerous sheep PrP genotypes to a variety of TSE sources.

P.7.15

Gene expression variations in Spanish natural sheep scrapie disease determined by sheep oligo DNA microarrays

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Background: The pathogenesis of natural scrapie and other naturally prion diseases is still poorly understood. The determination of transcriptome variations in infected versus control animals might clarify some molecular mechanisms of the pathology. In addition, it may allow the development of new tools for diagnostics and therapy.

Objectives: The aim of this work was to identify ovine natural scrapie associated alterations in gene expression profiles in two different tissues: Medulla oblongata (MO), the most affected regions of CNS, and mesentery lymph node (MN), tissue where the prion protein is accumulated without histopathological lesions.

Methods: Eleven Spanish Rasa Aragonesa sheep naturally infected with scrapie at clinical and preclinical phases, besides the group of 6 sheep without scrapie that were collected from different sites of Aragon were included in this study. The transcriptome was analyzed using the CVI custom 4x44K microarray containing 60-mers oligos which were designed from sequences obtained from 13k previously sequenced clones from a custom normalized cDNA library of sheep Peyers Patch, tonsil and brain. The array was supplemented with all publicly available transcripts from NCBI/EBI databases.

Results: Over 500 significant clone expression alterations greater than 2-fold were identified in clinical phase, from which the majority was found in medulla oblongata. In contrast, only 150 clones showed significant alteration in the preclinical animals, the majority was found in mesentery lymph node. Sequences identified in this study encode proteins that are involved in many pathways including immunity and apoptosis.

Discussion: This study confirms the importance of lymphatic tissue in preclinical stage where a different set of genes was regulated compared to clinical disease. In addition we were able to confirm many earlier published regulated genes found in artificial scrapie infected animals. Confirmation the expression changes by Real Time PCR is in progress and preliminary data will be discussed.

P.7.16

Modulated conversion of PrPc into PrPsc in sheep by PMCA based on PRNP genotype

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Background: As is well known, scrapie is a neurodegenerative and transmissible disease in sheep. Experimental and natural infectivity studies have established that susceptibility and incubation periods are conditioned by the prnp gene. Different polymorphisms depends on allele frequency and especially for codons 136, 154 and 171, which are commonly associated with susceptibility-resistance. As well as 141 codon for Nor98. Several studies have shown that resistance to scrapie, or even the transmission of BSE by inoculation can increase depending on other alleles present at other positions. More than twenty of these mutations cause changes in the PrP amino acid sequence haven´t been tested all of then. In order to do it, several difficulties as time for long incubation period in needed. The use of Protein Misfolding Cyclic Amplification (PMCA) can be a useful tool to carrying on these experiments.

Objectives: ¨In vitro¨ study of susceptibility-resistance linked to genotype variability in samples of nervous tissue of sheep.

Methods: PrP amplification by PMCA has been performed on ARQ/ARQ, ARQ/ARH and ARR/ARQ negative substrates with several positive inoculums as PrPSc seeds.

PrPres have been detected by western blot after PK treatment of samples.

Results: Amounts of PrPRes obtained from substrates with similar concentrations of PrPc after PMCA are different when are observed in western blot. Comparison of samples show as ARQ/ARQ amplification rate is 50% higher than ARQ/ARH and this one 50% higher than ARR/ARQ.

Discussion: Conversion efficiency and amplification rate depend on the genotype present in the material used for the PMCA. Substrates with susceptible allele ARQ showed higher amplification rate than and more resistant genotypes ARR. Results observed in the PMCA show as genotypes ARR/ARQ, ARQ/ARH and ARQ/ARQ have a similar behaviour to that observed for in vivo studies.

P.7.17

Prion gene octapeptide insert mutation mimicking Alzheimer's disease

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Background: Genetic prion diseases (gPrDs) can present as rapidly progressive dementias, similarly to sporadic Jakob-Creutzfeldt disease (sCJD), or with longer duration over years, often mimicking Alzheimer's disease (AD) or parkinsonian disorders.

Objectives: We report 2 different PRNP octapeptide repeat insertion (OPRI) cases with clinical overlap with AD.

Methods: Patients were evaluated at our center with neurologic, neuropsychological, MRI, CSF and other laboratory testing. PRNP analysis was done at the NPDPSC or NIH.

Results: Patient 1 was a 41-year-old man with a history of depression referred for progressive short-term memory problems. Family history is significant for a paternal grandfather who died in his 40s from a neurological disorder and a brother in his 30s with early onset AD (EOAD). Exam showed impairment in multiple cognitive domains and gait ataxia. MRI was significant for parietal, posterior cingulate and precuneus cortical atrophy with thinning of the posterior body of the corpus callosum. CSF 14-3-3 was negative. EOAD was considered, but genetic testing revealed a PRNP 6-OPRI mutation. Autopsy confirmed prion disease. His brother had the same mutation. Patient 2 was a 49-year-old man with a 10-year history of tremor and lifelong depression. Family history is significant for a paternal grandfather who died of dementia in his 80s. He had mild cognitive impairment 2 years before presenting to us. He later developed cerebellar ataxia, myoclonus, and cortical blindness. Initial outside diagnosis was AD with posterior cortical atrophy (PCA). Brain MRI was consistent with CJD. CSF 14-3-3, T-tau and NSE were elevated. EEG showed global slowing with irregular triphasic waves. PRNP analysis revealed a 9-OPRI mutation. Autopsy confirmed CJD.

Discussion: gPrDs, particularly OPRI mutations, can present with a prolonged course and misdiagnosed as AD or other neurodegenerative diseases. Prion gene testing should be considered in AD cases with atypical features.

P.7.18

PRNP mutation at codon 188 associated with atypical CJD

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Background: The human prion disease is inherited as an autosomal dominant mutation in the prion gene (PRNP) in 10-15% of cases. To our knowledge, only one T188R and three cases of T188K have been reported.

Objectives: We present a case of a T188R PRNP mutation with multiple asymptomatic family members harboring the same mutation.

Methods: The patient is a 55-year-old gentleman who presented to UCSF for assessment of rapid cognitive decline. Extensive investigations were performed.

Results: The patient's disease began with behavioral changes. Over the next 2 months, he developed severe behavioral, executive, memory and language impairments. He began playing with his feces and hoarding large quantities of hard-core pornography. Within 4 months, he was almost mute and no longer recognized most family members. He was referred with suspected sCJD. His family history included three paternal relatives with dementia. PRNP analysis revealed a missense mutation of Threonine (ACG) to Arginine (AGG) at codon 188 (T188R) with codon 129 MV (V cis). His asymptomatic father (81; 129 MV) and his brother (57; 129 VV), with MCI, harbor the same mutation; other family members remain to be tested. Examination revealed perseveration, impaired language, verbal memory, and executive function. His MMSE was 12/30. He had a right upper quadrantonopia and bilateral apraxia. Brain MRI showed reduced cortical diffusion in the anterior cingulate gyrus and insula bilaterally with corresponding decreased signal on the ADC map. He did not meet WHO or UCSF criteria for sCJD.

Discussion: Our findings suggest that the T188R mutation may not be fully penetrant or that the pathogenic effect depends on additional factors such as polymorphism at codon 129. Codon 129 exerts an influential role in sCJD and in familial prion disease. No autopsy data is yet available on this mutation, as another patient with the T188R mutation died 16 months after symptom onset without autopsy and our patient is alive. Future study should be undertaken to determine the pathogenicity of T188R.

P.7.19

Polymorphisms of the prion protein gene (PRNP) in Hanwoo (Bos taurus coreanae)

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Background: Bovine spongiform encephalopathy (BSE) is one of fatal neurodegenerative diseases that are known as transmissible spongiform encephalopathy (TSE) caused by infectious prion protein (PrPSc). Recent studies on resistance or susceptibility to TSEs have focused on the genetic structure of PrP gene such as the number of octapeptide repeats, difference of specific amino acids causing conformational change, and polymorphisms in non-coding region involved in PrP expression.

Objectives: To identify the novel mutation(s) and/or polymorphism(s) of bovine prion protein gene (PRNP) in Korean cattle (Hanwoo) and to investigate their functional alterations in prion pathogenesis.

Methods: Genomic DNAs were isolated from the meat of a several hundreds of Hanwoo. Coding and non-coding region of PRNP have been amplified by PCR. The sequences have been determined and analyzed for genotypic analysis.

Results: With synonymous changes in nucleotides of bovine PRNP in the coding region of amplified PCR products of PRNP in Hanwoo, non-synonymous alterations were found in Hanwoo. We report those findings and further implication in pathogenesis including prion expression.

Discussion: It is difficult to compare directly healthy group and BSE cases, because there is no BSE case in Hanwoo at present. The functional analysis on the differences of their genotypes and haplotypes of Hanwoo should be followed for their significance and information in monitoring of the diseases beside the industrial and cultural environment in TSE development.

P.7.20

Cell culture modelling of prion neurotoxicity

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Background: Prion diseases are slowly progressing proteinaceous infectious neurodegenerative disorders. It has been demonstrated that in vivo and in vitro neurotoxicity of oligomeric PrP is independent of PrP expression, indicating involvement other neurotoxic mechanisms. Rodent models and prion strains have been developed to minimize disease progression time, but genome-wide screening for genes essential to prion neurotoxicity remains time and cost prohibitive. The ideal model to identify alternative genes involved in prion neurotoxicity is cell culture based. This would permit a means of screening RNA interference (RNAi) libraries to identify genes required for prion-mediated neurotoxicity, but non-essential to cellular survival.

Objectives: This project seeks to develop a cell culture-based model of prion neurotoxicity, and screen a shRNA library to identify host genes involved in prion-mediated neurotoxicity.

Methods: A human neuroblastoma cell line and the PrP106-126 peptide have been used to model prion neurotoxicity. Ribozymes and shRNA molecules targeting human prnp mRNA have been utilized to test efficacy of mRNA and protein knockdown using real-time quantitative PCR and immunoprecipitation. Retroviral particles carrying ribozyme and shRNA constructs were generated. Neuroblastoma cells were transduced and challenged with the PrP106-126 peptide to select for resistant cells.

Results: Human neuroblastoma cells challenged with the PrP106-126 peptide under the proper conditions exhibit extreme neurotoxicity, providing a model for screening a shRNA library. Efficiency of prnp mRNA and protein knockdown with ribozymes was significantly impacted based upon the helicase recruitment signal.

Discussion: Here it is demonstrated for the first time that a cell culture model challenged with the PrP106-126 peptide can provide a means of modelling prion-induced neurotoxicity, permitting the screening of a shRNA library to identify genes essential to prion neurotoxicity but non-essential to survival of the host cell.

P.7.21

Heterozygosity at polymorphic codon 219 in two variant Creutzfeldt-Jakob disease patients

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Background: Genetic variants of the prion protein gene (PRNP) strongly determine susceptibility to prion diseases. All tested patients with definite variant Creutzfeldt-Jakob disease (vCJD) are homozygous for methionine at the common polymorphism at codon 129. Heterozygosity at codon 219 (E219K), a common variant in Asian populations, has not been reported in sporadic CJD patients and is therefore considered protective.

Objectives: To search for heterozygosity at codon 219 in our heterogenous diagnostic referral series totalling over 1800 samples.

Methods: The classification of vCJD was established according to the WHO criteria. Western blotting was used to detect abnormal prion protein deposition and genomic DNA was extracted from whole blood and the PRNP sequenced.

Results: The E219K polymorphism was found in 11 patients, all with Asian ethnicity or ancestry and 2 patients of non-Asian ethinicity with diagnoses of probable and definite vCJD. The first patient was a 34 year old British male with clinical features and investigations consistent with probable vCJD. The second patient was a 31 year old female of Afro-Caribbean origin in whom a tonsillar biopsy demonstrated abnormal prion protein deposition in a typical pattern for vCJD. Both patients were methionine homozygous at codon 129.

Discussion: This polymorphism has not been reported in a white Caucasian individual. The E219K polymorphism is protective against sporadic CJD but appears to be neutral or may even confer susceptibility to variant CJD. Our observation may be interpreted in the context of the conformational selection model of prion replication. This proposes that the extent of a transmission barrier depends on the degree to which permissible pathological conformations of PrP overlap between inoculum and host.

P.7.22

Genome-wide association study of BSE in European Holstein cattle

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Background: Genetic associations of TSEs have been established in a variety of mammals including humans, mice and sheep with the Prion gene having the largest contribution in these species. However, there is a growing pool of evidence that genomic regions other than the PRNP gene domain are also associated with susceptibility and/or resistance to TSE diseases.

Objectives: To examine the bovine genome of a representative set of, case and control, European Holstein cattle with the highest density SNP chip available for genetic associations with classical BSE. This served to test, with a greater resolution, previously identified loci with associations of classical BSE while simultaneously refining their position and identifying novel loci.

Methods: This whole genome association study was performed with 330 BSE case and control European Holstein. The bovine SNP50 Beadchip, which has approximately 54,000K evenly spaced SNPs, was utilized for this association study.

Results: Various chromosomal regions were identified to have an association with classical BSE incidence. Regions of particular interest are on chromosomes 1 at 29.1Mb (p=3.1 x 10(-5)), chromosome 3 at 32.4Mb (p= 5.9 x 10(-5)), and chromosome 14 at 44Mb (p=5.2 x 10(-5)).

Discussion: Chromosomal regions containing positional and functional candidate genes exhibit an association with classical BSE. Further examinations of these regions and candidate genes in additional species are essential to determine biological significance. Our observations provide important genomic ground work for future biological studies.

P.7.23

The first case of genetic Creutzfeldt-Jakob disease in a methionine / valine heterozygous carrier of a rare mutation R208H of the prion protein gene.

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Genetic Creutzfeldt-Jakob disease (gCJD) with the prion protein gene (*PRNP)* mutation R208H is very rare. All three reported CJD patients were methionine homozygous at codon 129 of *PRNP*. A case of gCJD with mutation R208H (without family history) is described in a patient methionine / valine heterozygous at codon 129. Comparison of preceding methionine homozygous cases with presented heterozygous patient revealed considerable similarity in clinical signs and histopathological brain lesions. The age at onset was 58, 60, 65.5 years in homozygous cases and 73 years in the heterozygous patient. Duration of the disease was 7, 7 and 12 months in previously reported cases, while the heterozygous one was treated in psychiatry for relapsing depressive syndrome more than 9 years, with rapid progression of clinical signs in the last 5 months. Differences observed between homo- and heterozygous carriers of the *PRNP* mutation R208H demonstrate a similar influence of M129V polymorphism on the age at onset and the clinical course of the disease, as was confirmed in sporadic CJD and gCJD with *PRNP* mutation E200K.

P.9.1

Surveillance for Prion diseases in the United States

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Background: Prion diseases are a family of rare progressive neurodegenerative disorders that affect humans and animals. The most common form of human prion disease, Creutzfeldt-Jakob disease (CJD), is believed to occur in many countries of the world. Variant CJD (vCJD), a recently emerged human prion disease, has been causally associated with bovine spongiform encephalopathy.

Objectives: To describe the occurrence of CJD and vCJD in the United States.

Methods: Analysis of prion disease deaths on death certificates of US residents, 1979-2006, and vCJD deaths identified through other surveillance mechanisms, 1996-2008. Since CJD is invariably fatal and illness duration is usually less than one year, the CJD incidence is estimated as the death rate.

Results: During 1979 through 2006, 6911 deaths with CJD listed as a cause were reported in the United States, an annual average of approximately 247 deaths (range 172-304 deaths). The average annual age-adjusted incidence for CJD was 0.97 per million persons (95% CI=0.95-0.99). Most (61.8%) of these cases occurred among persons , 65 years of age for an average annual incidence in this age-group of 4.8 per million persons. Most cases were among whites (94.6%); the age-adjusted incidence for whites was >2.5 times higher than that for blacks (1.04 and 0.40, respectively). Three patients who died since 2004 were reported with vCJD; epidemiological evidence indicated that their infection was acquired outside of the United States.

Discussion: National surveillance continues to show an annual CJD incidence rate of about 1 case per million persons and marked differences in CJD rates by age and race in the United States. Ongoing surveillance remains important for monitoring the stability of the CJD incidence rates, and detecting occurrences of vCJD and possibly other novel prion diseases in the United States.

P.9.2

Genotyping and survey of scrapie in Finnish sheep

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Background: In Finland, sheep husbandry is relatively small with app. 55000 ewes over 12 months kept on about 1000 farms. The Finnish sheep population consists mainly of pure-bred Finnish Landrace but includes a small proportion of other breeds such as Pure-bred sheep of Ahvenanmaa, Grey race sheep of Kainuu and Texel. Before extensive monitoring of scrapie started in Europe in 2002, few hundred sheep were examined yearly with histopathology as routine scrapie diagnostics in Finland. Nothing was known about genetic background and susceptibility of Finnish sheep races to scrapie, but on the other hand, no scrapie had ever been detected in Finland. Whether this was due to PrP resistant genotypes in sheep races or due to relatively small proportion of examined animals could only be speculated at that time.

Objectives: Objectives of the study were to determine PrP genotypes of the main breeds of sheep in Finland and to screen Finnish sheep population for scrapie.

Methods: Genotyping was performed by direct sequencing of the PrP open reading frame. Scrapie monitoring was performed using Bio-Rad TeSeE® test for healthy sheep and Prionics® Check WESTERN test for dead-on-farm sheep (2002-2003; TeSeE® test from 2004-). BioRad TeSeE Western blot was used to identify classical/atypical scrapie of the positive samples.

Results and Discussion: In Finland, over 9600 healthy and over 5800 dead-on-farm sheep have been analysed for scrapie together with at least 100 annual PrP genotype determinations since 2002. The original Finnish breeds were shown to be rather susceptible to classical scrapie as A136R154Q171 was the most common genotype found and A136R154R171 allele frequency was low in each breed studied. Despite extensive screening, no classical scrapie was detected. This was rather surprising since the Finnish sheep seems to be genetically susceptible to classical scrapie. However, five atypical scrapie cases were found which shows clearly that atypical scrapie is present in the Finnish sheep population at low level.

P.9.3

Risk factor analysis for sporadic Creutzfeldt-Jakob disease: surgery, blood, medical profession

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Background: Creutzfeldt-Jakob disease is a transmissible neurodegenerative disorder. For sporadic CJD (sCJD), origin of the disease is not known. Moreover, iatrogenic infection with long-time incubation occurred repeatedly. Thus, we analyzed a large population of sCJD patients for potential risk factors.

Objectives: to identify medical risk factors for sporadic CJD

Methods: Within German CJD surveillance system, all suspected CJD patients or their family members were asked to answer a questionnaire about medical risk factors. Additionally, a matched control for each patient was selected. To detect bias by study design, we performed a separate analysis for population and hospital controls as well as direct and proxy interview. Furthermore, time-lag analysis and genotype distribution was investigated.

Results: The study included 1155 sCJD patients and 1029 controls. By comparison of sCJD with population controls, we found lower rates of exposure factors in cases than in controls for blood transfusion (OR 0.6) and surgery on vertebral column (OR 0.52; not significant after Bonferroni adjustment). Surgery in general (OR 0.7), tonsillectomy (OR 0.61), and appendectomy (OR 0.55) became significant for hospital controls only. While surgery on vertebral column (OR 0.39), tonsillectomy (OR 0.45) and appendectomy (OR 0.43) revealed significant results by direct interview, however it became non-significant by proxy interview. Medical profession revealed non-significant differences between cases and controls.

Discussion: Study design is highly influencing the results for case-control studies in sCJD. This might explain conflicting results in previous studies. In our study, the only consistent significant factor independent of chosen design is blood transfusion, whereas no clear medical risk factor associated with higher risk of sCJD was identified.

P.9.4

Greek National Scrapie Surveillance programme from 2002 up to 2008

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During the implementation of the Greek National Scrapie Surveillance Programme from 2002 to 2008, a total number of 141917 samples originated from small ruminants were checked. From the above samples 103220 were healthy slaughtered and 38697 of risk population. 2159 samples were found positive, isolated from 226 infected flocks. The implementation of the active surveillance programme started in 2002 with a prevalence up to 0.31% and after a progressive increasing, in 2008, reached 2.61% with an average incidence at 74 cases per year.

Positives flocks were increased progressively by an average of 33 new flocks per year up to 2008.

Atypical Scrapie has been detected in 6 sheep flocks, from slaughtered sheep.

Genotype analysis has been done in 1336 positive samples. From the analysis:

- ARQ haplotype was the predominant haplotype founded in 1206 cases.
- VRQ haplotype was found at a percentage of 7.63% either as homozygote or as heterogygote, usually in combination to ARQ haplotype.
- ARR haplotype in combination to ARQ haplotype was found in 5 classical Scrapie samples and in combination to AHQ haplotype was found in 4 atypical Scrapie samples.
- AHO haplotype was found at a percentage of 14.07% and ARH haplotype in 19 cases.

ARQ/ARQ genotype was predominant (75.58%), followed by AHO haplotype either as homozygote or as heterogygote (16.25%) and VRQ/ARQ genotypes (6%), while no ARR/ARR genotype was found.

The distribution of the most significant genotypes in 970 random healthy sampled sheep was:

NSP1 (ARR/ARR): 4.74%, NSP2 (ARR/…):.29.27%, NSP3 (ARQ/ARQ): 49.48% NSP3 (AHQ/AHQ, AHQ/ARH, AHQ/ARQ) 9.79%, NSP4 (ARR/VRQ): 1.64% NSP5 (VRQ/…): 5.05%.

106 positive sheep flocks (29.148 animals) were fully genotyped resulting to: NSPI 6.08% (rams only 79), NSP2 32.44%, NSP3 57.67%, NSP4 0.63% and NSP5 2.61%.

P.9.5

Monitoring of bovine spongiform encephalopathy in the Russian Federation using a kit TeSeE (Bio-Rad) and immunohistochemical method

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Background: A problem of prevention of bovine spongiform encephalopathy and other prion diseases of farm and wild animals is of current interest for the Russian Federation (RF). Since 2005 the Ministry of Agriculture of Russia put into practice the programs aimed at the increase of high-productive bovine animal population on the basis of an increase of a portion of pedigree cattle up to 13%. A rapid increase of pedigree cattle population can be achieved owing to the import of high-productive cattle breeds from other countries. Unfortunately, the Russian cattle-breeding enterprises are compelled to import animals from countries with indigenous BSE. For example, the import of pedigree cattle in 2007 has made 76385 animals; from them 95 % are imported from the counties of BSE controlled risk, mainly Germany, Denmark, the Netherlands and Austria, and 5% from a country of BSE negligible risk – Australia.

Objectives: From 2005 the main surveillance object is the cattle from risk groups (non-ambulatory, emergently slaughtered or dead cattle), imported from countries of BSE controlled risk.

Methods: Diagnostic investigations are conducted predominantly by ELISA using kits TeSeE (Bio-Rad). For the purpose of verification, fragments of brainstem of some samples are additionally tested following formalin fixation by an immunohistochemical method using polyclonal antibodies to synthetic peptides-fragments of bovine prion protein.

Results: The number of monitoring investigations among cattle of risk groups increased in 2006-2008; 634 samples were tested in 2006, 1187 samples – in 2007 and 3081 samples – in 2008. Previously, polyclonal antibodies to peptides-fragments of bovine prion protein from regions PrP(106-126), (108-126), (111-126) and PrP(206-230), (216-230), (214-233) have been received and tested with positive results. At present antigenic and immunogenic properties of peptide PrP(154-169) and constructs on its basis are studied.

P.9.6

Quantitative models to estimate the risk of vCJD: a topical review

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Background: Like BSE, vCJD is an infectious disease typified by long incubation period and asymptomatic infections, two factors making epidemiological investigations particularly difficult. Primary infections are associated with the ingestion of infectious materials. Secondary infections are associated with blood transfusions and surgery.

Objectives: Here we present the mathematical models attempting to quantify the risk of primary and secondary vCJD transmission and describe how those models helped identifying various parameters of vCJD disease.

Methods: Medline search was carried out using the following keywords: vCJD risk, vCJD models, vCJD projection, and vCJD estimation. Studies were reviewed and the mathematical approach used to build the model was explained. A comparison was performed between various models.

Results: Existing models for vCJD can roughly be classified into two types according to the principal source of data: 1-Forward models that relate past pattern of BSE epidemic to the vCJD epidemic based on the estimated number of infected cattle slaughtered for human consumption 2. "Backward" models that use the vCJD epidemic in the UK to estimate important epidemiological parameters. Models for primary infection served two purposes: 1. provided estimates of the magnitude of the vCJD risk 2. Provided insight into the structure and possible control of the vCJD risk by estimating the risk of exposure to BSE infected materials and the subsequent risk of vCJD infection as a function of various control parameters.

Discussion: Risk modeling of vCJD helped in estimating, with reasonably narrow confidence bounds, prevalence of infection and predicted the future size of the epidemic. Furthermore, vCJD risk models reduced several uncertainties related to disease transmission like incubation period, age-dependent susceptibility and genetic predisposition.

P.9.7

Gerstmann-Sträussler-Sheinker syndrome with variable phenotype in a recently detected kindred with PRP-P102L mutation.

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Background: Human Spongiform Encephalopathies constitute a group of entities which includes Creutzfeldt-Jakob Disease, Fatal Familial Insomnia, Kuru and Gerstmann-Strδussler-Sheinker (GSS). Analyzed as a group, 10-15% of prion diseases are dominantly inherited, including 100% of GSS cases and approximately 10% of CJD cases. Since the first description of GSS in 1936, other families have been described in ten countries.

Objectives: The objective is to report the first kindred with GSS in Argentina.

Methods: We studied in the Brain Bank a family of italian descendants with members affected by a neurological disorder displaying two clinically different patterns: cognitive decline (FTD-type) with ataxia or only cognitive decline (FTD-type). We performed the neuropathological examination of two members, and molecular studies in one of them.

Results: Neuropathological examination showed deposits of the prion protein (PrPSc) in the way of plaques in different brain and cerebellar areas. The molecular studies performed showed abnormal PrP and highlighted the previously described P102L mutation in the prion protein gene (PRNP). We confirmed the diagnosis of Gerstmann-Strδussler-Sheinker disease.

Discussion: We report the first family with Gerstmann-Strδussler-Sheinker disease in our country, which members are of italian origin, presented the most frequent mutation associated with the disease, and two different clinical patterns. The detection of this kindred was performed through the Brain Bank implying that it can perform a complementary function with the National Surveillance Center on Prion Diseases.

P.9.8

Comparing BSE risk factors and management of the three largest cattle producers: India, Brazil and China

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Background: India, Brazil and China collectively possess 56% of world's total cattle inventory and to date have not recorded any case of bovine spongiform encephalopathy (BSE). With BSE emerging as a zoonotic disease worldwide especially in Europe, India and Brazil surfaced as major beef exporting regions controlling 30% of world's total beef and cattle exports. Each country was prompted to implement many effective BSE management strategies to sustain their BSE free status.

Objectives: To review the risk mitigation and management strategies for BSE introduced in India, Brazil, and China.

Methods: A comprehensive and structured survey was carried out for available literature published in peer review journals on risk management of BSE in India, Brazil, and China. Sources also included government reports, grey literature, internet web pages and newspaper databases to assess non-expert information on BSE risk mitigation strategies. The information was compiled to synthesize a comparative overview of BSE risk management strategies in the respective regions. **Results:** The study provides the background on the agro-beef sector of India, Brazil and China and draws comparisons between the three regions to assess the comprehensiveness of the instituted BSE risk management measures. An attempt has been made to provide an account of the external and internal challenges faced by these regions with regards to BSE.

Discussion: While the three largest cattle producers have implemented many effective BSE management policies some gaps exist in particular, the lack of specified risk materials (SRM) ban in Brazil and China.

P.9.9

A prevalence study in QQ171 sheep investigating the role of individual and flock-level risk factors for scrapie

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Background: Scrapie is transmitted from one individual to another by direct contact or through a contaminated environment. Moreover a genetic susceptibility depending on the 136, 154 and 171 polymorphic codons of the PRNP gene is an individual risk factor for scrapie. Large numbers of breeding females, introductions of sheep, spreading of sheep compost and contacts with placentas were also reported to be associated with the disease.

Objectives: A cross-sectional study was carried out to identify the main risk factors affecting the scrapie prevalence within sheep flocks.

Methods: The study was carried out on 28 scrapie-affected flocks i.e. a sample of outbreaks identified in Sardinia between 1998 and 2006: the selection was based on the availability of data on genotyping and scrapie testing of a large proportion of animals . Out of 9,767 sheep, the results of rapid testing for 1,657 susceptible (QQ171) animals were available for data analysis. A mixed effect logistic regression model, with flock as random effect, was used to explore the role of scrapie history within the flock, the proportion of susceptible animals, the age of the sheep, and the size of the flock. A subset of 643 animals was used to assess the adjusted effect of M137T and N176K PRNP gene mutations.

Results: A higher risk of scrapie was associated with larger flocks (OR 11.8, P=0.04) and with the 1-4 year age class (OR 4.8 P=0.000) whereas a lower risk was associated with the M137T (OR 0.12, P=0.001) and N176K (OR 0.02, P=0.001) mutations. The effects of a longer history of scrapie and a larger proportion of susceptible sheep identified through a fixed effect logistic model disappeared after applying a flock random effect, due to the clustering of sheep within flocks.

Discussion: This study confirms the importance of age, individual mutations other than those identified at 136, 154 and 171 codons, and of flock size. How flock size affects the probability of disease deserves further

P.9.10

A cell sorter specifically adapted to prion at the service of the Prion research community

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Background: The foundation Alliance Biosecure aims to sponsor research on the analysis, 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 case of vCJD prion protein found at post mortem in the spleen of a person with haemophilia and treated with plasma products.

Objectives: For this purpose, Alliance BioSecure Foundation is proud to make available to scientific community a three-laser Influx® cell sorter and a Nikon confocal microscope, both located in a BSL-3 containment facility. Equipped with a unique modified design suitable for Prion experiments, this cell sorter constitute a powerful tool to study & isolate infected rare cellular sub-populations in blood or in cell culture.

Methods: Here, we present two foundation research projects related to prion that illustrate the capabilities of the cell sorter. First, we show a sort of different cell sub-populations from infected monkey blood checked by a confocal miscroscopy view of these sorted cells. In second, we separate, from cell lines, differentially infected cell populations following the expression of a surface marker.

Results: With a securised cell sorter able to sort infected cell populations at a rate of 5.000 cells/s, with a purity greater than 90%, a combination of 12 detectors and a confocal microscope, the fondation's projects have a real asset for the study of Prion deseases.

P.9.11

Comparative expert judgment elicitation using the classical model and EXCALIBUR under conditions of uncertainty for Prion disease risks

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Background: Formal Expert Panels can take extensive amounts of time to meet, review data, create risk assessment models, complete assessments before reaching consensus answers. For example, the first expert panel convened at the beginning of the United Kingdom's BSE outbreak, the Southwood Working Party, required nearly one year to reach consensus conclusions regarding milk, food, animal health and public safety. Solicited expert opinion using a mathematical formalism for uncertainty gaps in research can provide a robust and faster method to obtain answers that can greatly inform policy decision-making.

Objectives: There is much uncertainty surrounding parameters of secondary prion disease transmission through blood and blood derived products. The "Classical Model" of expert elicitation can be applied to provide expert estimates for uncertainty gaps as well as provide response to real-time, emerging threats as they occur.

Methods: Two separate elicitation exercises were undertaken with Canadian experts (n = 14; held March 2008) and international experts (n = 11; held March 2009). TSE experts were calibrated on a series of seed questions for which answers are known and asked to complete seven target item questions for which answers are not known.

Results: Performance weighted results from both the Canadian and international experts reveal similar estimates and uncertainty ranges for the seven target item questions elicited dealing with TSE prevalence, genotype effects, susceptibility and infectivity.

Discussion: The theoretical basis for the rational pooling of expert opinion is established and we show it can be successfully applied for prion disease uncertainty gaps to inform policy decision-making.

P.9.12

Scrapie surveillance in the Netherlands; the effect of selection for scrapie resistance in sheep.

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Background: The susceptibility to scrapie, a fatal neurodegenerative disease of sheep, is closely linked by the PrP genotype of the animal. The PrP genotype of sheep, modulate the susceptibility to scrapie, with the ARR allel conferring high resistance. In 2004 a scrapie control program was initiated in the Netherlands, based on the selection of ARR homozygote rams for breeding. At the same time new EU regulation required intensive active scrapie surveillance (by testing fallen stock and healthy slaughter) as well as certain control measures in affected flocks.

Objectives: Here we analyze the data on scrapie incidence over the period of 2002 to 2008, obtained from both the surveillance and control activities. At the same time genotyping results for a random subset of test samples from the active surveillance (2005 – 2008) reveal the temporal trend in ARR allel frequency in the Dutch sheep population. The combined data enable us to obtain a quantitative insight into the transmission of scrapie at flock level and its genetic control at population level.

Methods: Data on the scrapie incidence are accumulated within the Dutch active surveillance on TSEs in sheep (2002 – 2008). From 2005 a yearly random sample is taken form the active surveillance, for genotyping of the PrP gene. This data provides both information on the temporal trend of scrapie incidences, and on the genotype frequencies at a national level.The second part of data are results on infection status and genotyping in animals that were culled, as part of the mandatory scrapie control efforts. With this information we could obtain the relative risk of infection across different genotypes.

Results: Genotyping showed a clear trend for increasing ARR allel frequencies in the Dutch population, together with a significant decrease in scrapie incidence. The combined data indicate that the decrease in scrapie incidence is based both on a decrease of susceptible animals, and a decrease of transmission of the infection between susceptible animals. Results from the relative risk of infection of PrP genotypes are presented.

Discussion: Differences with previous results, regarding the relative risk of infection for PrP genotypes, are discussed. Furthermore, the importance of selection for genetic scrapie resistance, compared to culling of infected flocks, in the decrease of scrapie incidence is discussed.

P.9.13

No evidence of contagiousness of atypical scrapie: results from a 12-country European study.

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Background: During the last decade, active surveillance of transmissible spongiform encephalopathies in small ruminants has been intensive in Europe. In many countries this has led to the detection of cases of atypical scrapie. A question has been raised: is the atypical form contagious?

Objectives: The aim of this study was to investigate if the occurrence of secondary cases in flocks affected by atypical scrapie could help in clarifying the potential contagious nature of the disease by using surveillance data collected in Europe.

Methods: Questionnaires were used to collect, at country-level, data on control measures, on the results of active surveillance and of the within-outbreaks testing from 12 European countries. Generalised linear regression mixed models and meta-analysis were used to model prevalence data taking into account the random effect of country.

Results: The mean prevalence of atypical scrapie was six cases per ten thousand in abattoir surveillance and eight cases per ten thousand in fallen stock. By using meta-analysis, on 11 out of the 12 countries, we found that the probability of detecting secondary cases of atypical scrapie in positive flocks was similar to the probability observed in animals slaughtered for human consumption (odds ratio, OR=1.07, CI95%: 0.70-1.63) or among fallen stock (OR=0.78, CI95%: 0.51-1.2). In contrast, when comparing the two scrapie types, the probability of detecting secondary cases in classical scrapie positive flocks was significantly higher than the probability of detecting secondary cases in AS positive flocks (OR=32.4, CI95%: 20.7-50.7).

Discussion: These results suggest that atypical scrapie is not contagious or has a very low transmissibility under natural conditions compared with classical scrapie. Furthermore this study stressed the importance of standardised data collection to make good use of the analyses undertaken by European countries in their efforts to control atypical and classical scrapie.

P.9.14

Quantifying the effect of the EU control measures in 2001 on the decline of the BSE epidemic in Europe

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Background: During the 1990s, the control measures for BSE were very different amongst the EU member states, and the moment of implementation and efficacy of various control measures varied a lot. In 2001 the EU decided on implementing expensive extra control measures, to control the BSE epidemic among cattle. These measures were identical for all member states with a BSE problem. The new regulation included active surveillance among all culled and dead cattle over 30 months of age. This has by now produced a large data set on BSE prevalence in the EU, which allows for an evaluation of the effect of these measures.

Objectives: From this data set, we quantify the effect of the control measures in 2001.

Methods: The growth or decline op the epidemic is quantified in the form of the reproduction ratio and the growth rate of the infection. This was done for the EU and separately for a few member states.

Results: We compare the growth or decline of the national and European epidemics in the birth cohorts born before 2001 and after and find that the European BSE epidemic was declining already before the 2001 birth cohorts. We also find that the actual level of decline differed a lot between countries. Since 2001, all EU member states apply the same control measures, which should lead to a similar rate of decline of the epidemic. We find that the growth rate has declined further substantially and calculate the number of cases avoided due to the new harmonized control measures.

Discussion: The different methods show similar results, which concur with preliminary results from other models. However, we find that for this type of question an analysis by reproduction ratio is much more sensitive than an estimate of growth rate. This is because in estimating the growth rate, there is a smoothing effect of the exposure in transmission level and presence of infectious animals . The reproduction ratio shows the effect very clearly, since it focusses on the level of transmission in a specific year only.

P.9.15

Investigating dental treatment as a possible route of transmission of variant CJD

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Background: Human-to-human transmission of variant CJD (vCJD) has occurred from blood transfusions in four known instances in the UK. There is a possibility that vCJD may also be transmitted through other routes such as through dental treatment. The average person undergoes far more dental treatments than either surgery or blood transfusions. Preliminary findings from a mouse model led the UK's Spongiform Encephalopathy Advisory Committee (SEAC), to conclude that the potential risk of transmission of vCJD via a range of dental procedures may be greater than previously anticipated.

Objectives: To identify vCJD cases that had been treated at the same dental practice, and to report the type and dates of treatments undergone. To investigate whether vCJD cases experienced a greater number of dental procedures compared to controls.

Methods: Cases of vCJD (n= 161) and general population controls (n=~500) with appropriate consent were included in the study. Dental case records were accessed by contacting general dental practitioners (GDPs) directly and from data held by NHS Dental Practice Boards. Treatments and examinations carried out since 1980 were recorded.

Results: Currently, the success rate for retrieval of dental records from cases is 53% and for controls is 87%. Further results will be presented regarding dental record retrieval and preliminary risk analyses.

Discussion: Several problems were encountered in retrieving dental records, the main ones being that in the UK dental records do not 'follow' the patient as medical records do when a person changes their dentist. In addition dental records can be legally destroyed after 11 years. Therefore, accessing the dental history of one person typically involved trying to identify and contact more than one GDP. Given the above methodological caveats, a preliminary statement about possible risk of transmission through dental treatments will be given.

P.9.16

Estimation of the size of the Bovine Spongiform Encephalopathy epidemic in Canada

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Background: The first BSE case appeared in Canada in 1993 was imported from the UK as a five month old calf. In ten years, the first domestic BSE case was discovered in Alberta and then followed by the discovery of the second case as a cow exported to the US. Up to May 2009, 15 typical BSE cases and 2 atypical cases have been discovered. Canada has implemented control measures to eradicate the disease from the national herd like the partial (ruminant to ruminant) feed ban in 1997 and the enhanced feed ban in July 2007 (to rule out cross contamination). Other control measures were already in place before the 1993 BSE case, like the ban of live cattle importation from the UK in 1990 and the ban of meat-and-bone meal (MBM) importation from the UK in mid-70s for foot and mouth disease reason.

Objectives: The main target of our current project is to estimate the risk of contracting vCJD in Canada through the associated route of oral consumption of contaminated meat and the thereafter risk of secondary transmission from human to human. To do so, we are to find the likely number of infected cattle that were culled to one of the natural streams and specially the health slaughter stream before showing up clinical symptoms. We assume that infectivity has entered Canada through importation of live cattle and MBM.

Methods: We have built a stochastic simulation model to find the likely number of infected animals that were culled to one of the streams due to the importation of live cattle and MBM from high risk countries. The simulation model is based on unsophisticated yet valid methods.

Results: We have applied this model to the Canadian data. The results showed a moderate level of infection in the Canadian herd. The infectivity is also on its way to eradication.

Discussion: The model validation and verification are still underway which means that the results are preliminary at the time we are writing this abstract.

P.9.17

Humic acid influences pathogenic prion protein attachment to clay particles

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Background: Soil may contribute to the horizontal transmission of sheep scrapie and chronic wasting disease of North American cervids by serving as an environmental reservoir for the infectious agent. We previously demonstrated that the disease-associated form of the prion protein (PrPTSE) binds to soil particles and that the PrPTSE interaction with the clay mineral montmorillonite (Mte) is remarkably avid. In soils, clay mineral particles are typically associated with natural organic matter (primarily the products of microbial decomposition of vegetation). Humic substances constitute an important fraction of natural organic matter.

Objectives: The objective of this study was to investigate the role of natural organic matter on the interaction of pathogenic prion protein with soil particles.

Methods: Complexes of humic acid and montmorillonite (HA-Mte) were prepared by mixing dissolved HA with a suspension of Mte at ratios of 1:10 and 1:100 (w/w) to obtain different HA loading levels. The attachment of PrPTSE to HA-Mte (1:10) and HA-Mte (1:100) was measured in batch sorption experiments at varied protein:sorbent ratios. We analyzed PrPTSE extracted from HA-Mte and the unbound fraction by immunoblot and ELISA respectively.

Results: We found that HA-Mte complexes have a lower affinity for PrPTSE than Mte alone and that the binding capacity for the protein decreased with increasing HA content. Extraction of humic acid from HA-Mte after PrPTSE sorption suggests that the pathogenic prion protein associates primarily with Mte surfaces and that natural organic matter blocks PrPTSE binding sites on Mte. Immunoblot analysis of PrPTSE incubated with dissolved Elliot soil humic acid indicates cleavage of the protein at the N-terminus. More polar fractions of natural organic matter from soil did not cleave PrPTSE.

P.9.18

Neuropathology-confirmed CJD decedents less than 55 years of age, United States, 1994-2006

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Background: Approximately 10% of US Creutzfeldt-Jakob disease (CJD) decedents are reported to be <55 years of age. Detailed investigation of young CJD cases could provide a better understanding of the occurrence of human prion diseases and help identify variant CJD (vCJD) and other prion disease cases with an exogenous source of infection.

Objectives: To describe neuropathology-confirmed CJD decedents aged <55 years in the United States during 1994-2006.

Methods: CJD decedents are identified from the national multiple cause-of-death data of the National Center for Health Statistics and through reports by physicians, public health personnel, and others. Relevant portions of medical records for these cases are requested from state health departments and medical facilities and reviewed. Neuropathology results for cases, as available, are provided by the National Prion Disease Pathology Surveillance Center.

Results: During 1994-2006, 449 CJD decedents aged <55 years were identified in the United States. For 305 cases with known neuropathological testing status, 176 (58%) were biopsy or autopsy-confirmed. Of these 176 confirmed cases, 81% were diagnosed with sporadic CJD, 17% with familial CJD, and 2% with iatrogenic CJD; none had unsuspected vCJD. Approximately 92% of the cases were white, 6% black, and 2% Asian. For cases with available information on clinical features, most (90%) had dementia reported, 77% had ataxia or poor coordination, 65% had myoclonus, 38% had visual signs, and 70% had pyramidal or extrapyramidal signs. The median illness duration was 6 months. For 118 confirmed CJD cases with electroencephalogram (EEG) results available, 42% had a typical reading.

Discussion: Neuropathologic confirmation of younger suspected CJD cases is especially important because these cases are more likely to be vCJD than older cases and may more readily indicate iatrogenic transmission or a novel prion disease emergence. Additionally, typical EEG may be less commonly reported.

P.9.19

Screening of bovine spongiform encephalopathy in Korean cattle breed, Hanwoo, using real-time immno-PCR.

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Background: Bovine spongiform encephalopathy (BSE) is a fatal neurodegenerative disease caused by pathogenically infectious prion protein (PrPSc). Screening of slaughtered cattle is one of the most important methods to prevent the outbreak of BSE, and a number of countries have been subsequently under surveillance for BSE in over-24-month-old cattle. In Korea the case of BSE has not been reported yet, and the appropriate procedure of surveillance for BSE in nationalwide has been required. We describe the first study for screening of BSE in Korean cattle, Hanwoo.

Objectives: To establish sensitive and reliable screening procedure and apply it to surveillance for BSE in Korea.

Methods: Samples for screening have been taken from both spinal cord and skeletal muscle from around 30-month-old, females or castrated males of Hanwoo cattle. Real-time immuno-polymerase chain reaction (IPCR) method has been developed and applied for detection of PrPres in Hanwoo.

Results: IPCR with 100-fold higher sensitivity compared to the conventional protocol of Western blot or ELISA was carried out in our study. In tested 1,085 Hanwoo cattle, no significant PrPres were detected in our experimental condition.

Discussion: For detection of PrPSc, we have established real-time IPCR method and confirmed no BSE case in Hanwoo so far. As this protocol, however, was using PK digestion for detecting PrPSc, PK sensitive PrPSc cannot be confirmed like other official protocols currently used for BSE.

P.9.20

A retrospective immunohistochemical study reveals atypical scrapie has existed in the United Kingdom since at least 1987

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Background: Atypical scrapie is a relatively recent discovery, and it was unknown whether it was a new phenomenon or whether it had existed undetected in the United Kingdom national flock. Before 1998, the routine statutory diagnosis of transmissible spongiform encephalopathy (TSE) in sheep relied on the presence of TSE vacuolation in the brainstem. This method would not have been effective for the detection of atypical scrapie. Currently, immunohistochemistry (IHC) and Western blot are commonly used for the differential diagnosis of classical and atypical scrapie. The IHC pattern of PrPd deposition in atypical scrapie is very different from that in classical scrapie using the same antibody. It is thus possible that, due to a lack of suitable diagnostic techniques and awareness of this form of the disease, historic cases of atypical scrapie remain undiagnosed.

Objectives: To perform IHC on selected formalin-fixed, paraffin-embedded (FFPE) blocks of ovine brain from the Veterinary Laboratories Agency archives that were submitted for various reasons, including suspect neurological disorders, between 1980 and 1989. To develop a method to obtain PrP genotype from DNA extracted from archival FFPE tissues.

Methods: IHC was performed using the VLA routine diagnostic PrP IHC protocol using mouse monoclonal antibody 2G11. Several scrapie positive cases were identified including some that were originally diagnosed as scrapie negative by examination of haematoxylin and eosin sections only. One case from 1987 was deemed atypical scrapie positive. A method to obtain PrP genotype from DNA from archival FFPE was developed and applied to material from this case.

Results: The IHC patterns identified in the brain material of this case from 1987 are consistent with contemporary atypical scrapie. The PrP genotype of this case was AHQ/AHQ, a PrP genotype commonly associated with atypical scrapie.

Discussion: We have identified that atypical scrapie has existed in the UK since at least 1987. To the authors' knowledge, this is the earliest example of atypical scrapie and further demonstrates that atypical scrapie is not a new condition, but rather has been present in the UK national flock for at least 2 decades. Further studies using older FFPE material in the UK and in other countries may reveal more historical cases of atypical scrapie.

P.9.21

Molecular characterization of BSE in Canada

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Background: Three BSE types (classical and two atypical) have been identified on the basis of molecular characteristics of the misfolded protein associated with the disease. To date, each of these three types have been detected in Canadian cattle.

Objectives: This study was conducted to further characterize the 16 Canadian BSE cases based on the biochemical properties of there associated PrPres.

Methods: Immuno-reactivity, molecular weight, glycoform profiles and relative proteinase K sensitivity of the PrPres from each of the 16 confirmed Canadian BSE cases was determined using modified Western blot analysis.

Results: Fourteen of the 16 Canadian BSE cases were C type, 1 was H type and 1 was L type. The Canadian H and L-type BSE cases exhibited size shifts and changes in glycosylation similar to other atypical BSE cases. PK digestion under mild and stringent conditions revealed a reduced protease resistance of the atypical cases compared to the C-type cases. N terminal-specific antibodies bound to PrPres from H type but not from C or L type. The C-terminal-specific antibodies resulted in a shift in the glycoform profile and detected a fourth band in the Canadian H-type BSE.

Discussion: The C, L and H type BSE cases in Canada exhibit molecular characteristics similar to those described for classical and atypical BSE cases from Europe and Japan. This supports the theory that the importation of BSE contaminated feedstuff is the source of C-type BSE in Canada. It also suggests a similar cause or source for atypical BSE in these countries.

P.10.1

First cases of scrapie in Poland

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Background: Scrapie is a prion disease of small ruminants which has been known for over 250 years. An unusual isolate of scrapie was identified for the first time in Norway in sheep in 1998 and it was designated "Nor98". Despite recording cases of BSE in cattle since 2002, Poland was free of scrapie until 2009. In early 2009, the first two suspect cases of scrapie were detected in Poland.

Objectives: The aim of the study was to classify the PrPSc patterns of the first two cases of scrapie diagnosed in Poland on the molecular level using western-blot technique.

Methods: Both samples were derived from dead-on-farm sheep, 109 and 84 months old. The brain stem tissue from both cases provided for analysis was partially autolysed. A small portion of the cerebellum was available from the second sheep and it was used only for Biorad WB. Confirmatory western blot applied was based on a 1 hour digestion of 10% homogenate followed by precipitation of PrPres using 1 propanol. Additionally, the TeSeE Western Blot Assay (Bio-Rad) and VLA Hybrid WB to discriminate BSE from scrapie were used.

Results: The PrP banding profile observed appeared to be different from the typical triple-band profile known for classical scrapie. Four to six major PK-resistant bands were detected which were all situated below the expected positions. Additional analysis with the TeSeE WB Assay revealed a PrP banding profile comparable to that described for atypical scrapie with bands migrating between 7 kDa and 18 kDa.

Discussion: This study confirmed the usefulness of the WB technique in detecting and distinguishing atypical scrapie from classical scrapie in obex samples. Despite of the multi-banding pattern usually observed for atypical scrapie in different WB systems, the presence of abundant band(s) below 15 kDa position can be regarded as the unique feature of this form of scrapie. Discriminatory WB when atypical scrapie is suspected requires using a suitable primary antibody because this selection can influence the final diagnostic result.

P.10.2

C-terminal prion fragments as a novel tool for the molecular discrimination of prion strains

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Background: Recent transmission studies suggested that BSE could have originated from the recycling of an atypical, putatively sporadic, form of BSE, called L-type BSE or BASE. This could have happened after a first cross-species transmission in an intermediate host, possibly in sheep. Given their unusual molecular properties, close to BSE and even more to L-type BSE, recently identified "CH1641-like" scrapie isolates might represent candidates for the origin of BSE.

Objectives: To investigate and compare the molecular features of the prion protein, from ruminants TSEs transmitted in an ovine transgenic mouse model.

Methods: A variety of TSE sources from ruminants was bioassayed by intra-cerebral route in a transgenic mouse model (TgOvPrP4) that overexpress the ovine PrP protein (A136R154Q171 allele). Cerebral and splenic PrP from TgOvPrP4 mice were isolated (i) in the absence of protease digestion and (ii) after digestion with proteases (PrPres), before Western blot analyses with a C-terminal anti-prion antibody (SAF84).

Results: Our main finding is the identification of a novel C-terminal PrP fragment that readily distinguishes natural scrapie from classical and L-type BSEs. This fragment (PrPres#2) resisted to digestion with proteases (PrPres#2), and was also detected in the absence of any protease digestion (C3). PrPres#2 was particularly abundant in "CH1641-like" scrapie isolates, but was absent in both classical and L-type BSEs. Interestingly, PrPres#2 was undetectable in the spleen of TgOvPrP4 mice, contrary to PrPres#1.

Discussion: Our results provide an additional method for the discrimination of prion strains, through the identification of C-terminally cleaved PrP fragments, especially useful to distinguish "CH1641-like" scrapie from bovine classical and L-type BSEs. Similar C-terminal prion fragments were also recently reported in H-type BSE or in sporadic CJD in humans, which suggests the involvement of common proteolytic pathways in these disorders.

P.10.3

Atypical scrapie cases in Italy: neuroanatomical distribution of pathological prion protein with different antibodies

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Background: An increasing number of scrapie cases with atypical features, designed Nor98 have been recognized in many countries. The molecular characteristics of these cases have been well defined but detailed descriptions of PrPsc distribution in the whole brain are rare.

Objectives: The aim of the present study was to investigate PrPsc deposition pattern and distribution by immunohistochemistry with different antibodies on atypical scrapie cases (46 sheep and 5 goats) submitted to our laboratories for TSEs confirmatory tests.

Methods: Immunohistochemistry was performed on the whole brain of 39 animals and different brain regions were considered : telencenphalon, diencephalon, mesencephalon, pons cerebellum and medulla. In other 12 cases only brainstem and cerebellum were studied. PrPsc was detected using a panel of 4 monoclonal antibodies (F99/97.6.1, L42, 2G11, R145) and the immunoreactive PrPsc was visualized using a commercial immunoperoxidase tecnique (ABC; Vector Laboratories). Results of immunohistochemistry labelling were analysed using a semi-quantitative scale for the intensity of staining (-,+,++,+++) and the PrPsc deposition patterns were studied.

Results: The antibody F99/97.6.1 disclosed very well the following patterns: aggregates, granular and globular. L42 evidenced globular pattern better than 2G11 and R145 but it immunolabelled neuronal bodies aspecifically. L42 always showed less granular staining than F99/97.6.1. R145 showed weak signal in the majority of samples. 2G11 showed less positive labelling than the other three antibodies. PrPsc distribution in the brain areas was not homogeneous in the cases considered.

Discussion: The present study showed that the pattern and the distribution of PrPsc were different from classical scrapie. No differences have been observed between sheep and goats. The antibody F99/97.6.1, routinely used in our laboratory for confirmatory test, appeared the best to reveal both PrPsc deposition and immunolabelling intensity.

P.10.4

Efficient infection of murine stromal cell cultures of spleen origin with mouse-adapted human TSE agents, vCJD and Fukuoka-1, but not BSE

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Background: The quest for cultures universally susceptible to various TSE agents continues. We recently reported that murine stromal cell culture of spleen origin with features of mesenchymal stem cells (SP-SC) is susceptible to mouse-adapted vCJD (mo-vCJD) and GSS-isolate, Fukuoka-1 (Fu). The SP-SC was easily infected with Fu but not with mo-vCJD.

Objectives: Establish the rate of infection in SP-SC after inoculation with Fu or mo-vCJD by single cell cloning. Evaluate susceptibility of SP-SC clones to various TSE agents.

Methods: First, cells were exposed for 72 hours to 1% brain homogenate from mice infected with either mo-vCJD or Fu or BSE. Single-cell cloning was performed and cells were propagated and collected for Western blotting (WB). Second, 200 SP-SC clones were generated by single-cell cloning and propagated to obtain sufficient numbers for ex vivo inoculation. Each cell clone was inoculated with mo-vCJD or Fu or BSE adapted to transgenic mice expressing bovine PrP (BoTg-BSE), propagated through five passages and tested for PrPres. The PrP was confirmed in proteinase K (PK)-untreated and PK-treated samples by WB using 6D11 antibody.

Results: The rate of infection was 4% for Fu and 7% for mo-vCJD, in spite of the fact that the original SP-SC culture could always be infected with Fu, but not with mo-vCJD, and the level of PrPres was always higher in Fu-infected that in mo-vCJD-infected SP-SC. One clone that persistently generated PrPres was tested for infectivity by bioassay in mice. None of 60 clones selected after inoculation with BSE produced sustainable levels of PrPres. Multiple clones derived from the original SP-SC culture tested positive for PrPres at passage 5 following inoculation with Fu or mo-vCJD, but tested negative after exposure to BoTg-BSE.

Discussion: We envision that developed cell models will be used in the future to address the questions of cell susceptibility to TSE infection.

P.10.5

Titration and characterisation of a Catalan BSE isolate in a transgenic murine model of BSE

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Background: Experimental murine models are valuable tools to study TSE pathogenesis. Usually these models are challenged with an inoculum obtained from field case isolates with known infectivity titter and phenotypic features. Wild type strains of mice, such as RIII, C57 or VM, have been used. The use of a transgenic model over-expressing the bovine prnp on a murine K.O. background (Botg110, Castilla et al. 2003) allows omitting the transmission barrier existing between cattle and mice and reduces the incubation period.

Objective: To titrate and characterise a cattle BSE isolate.

Methods: Groups of 10 Botg110 transgenic mice were intracerebrally inoculated with six dilutions (10-1 to 10-6) of a brain homogenate. TSE related clinical signs were monitored twice a week from 300 days post inoculation (DPI) onwards. Mice were sacrificed when the end point criteria was reached or at 525dpi. The brains were removed, formalin fixed and paraffin embedded. PrPres deposition was assessed by means of immunohistochemistry.

Lesion profiles were assessed as well as the brain PrPres distribution curve (BPDC). The molecular profiles were assessed by western blotting.

Results: The Log10 DL50 was 4.3; calculated using the Spearman Krabber formula.

Survival curves, spongiform change brain profiles, BPDCs and PrPres molecular profiles of the six groups were compared to that obtained with a reference inoculum (BSE2) and to the neuropathological and molecular characterisation of the original BSE field case.

Conclusion: An inoculum from a cattle BSE field case isolate has been successfully prepared. The further titration and characterisation have validated its usage in experimental inoculations. An available stock of this inoculum is kept in the Animal Tissue Bank of Catalunya (BTAC, http://btac.uab.cat/) and is already being used in several neuropathogenesis experiments.

P.10.6

Preservation of proteinase K-sensitive prion infectivity using pronase

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Background: Disease-related prion protein PrP is classically distinguished from its normal cellular precursor, PrPC, by its detergent insolubility and partial resistance to proteolysis. Molecular diagnosis of prion disease has historically relied upon detection of protease-resistant fragments of PrP using proteinase K (PK); however, it is now apparent that a substantial fraction of disease-related PrP is destroyed by this protease. Recently, using thermolysin and the mouse RML prion strain, we demonstrated heterogeneity in PK-sensitive disease-related PrP isoforms and found that the majority of prion infectivity (80 %) appeared to be associated with a minor fraction of PK-sensitive PrP. (1)

Objectives: To proteolytically degrade PrPC while retaining the full spectrum of PK-sensitive isoforms of disease-related PrP.

Methods: To digest uninfected- and RML-infected mouse brain homogenate over time with varying concentrations of proteases and measure PrP content by western blotting and ELISA and RML infectivity by the Scrapie Cell Assay. (1)

Results: We found that pronase (a mixture of proteinases isolated from Streptomyces griseus) can destroy PrPC in RML brain homogenate without affecting infectious prion titre.

Discussion: Degradation of PrPC with pronase will facilitate detailed characterisation of PrP structures associated with the highest specific prion infectivity that are readily destroyed by exposure to PK.

References:

1. Cronier,S., Gros,N., Tattum,M.H. et al. Detection and characterization of proteinase K-sensitive disease-related prion protein with thermolysin. Biochem J 416, 297-305 (2008)

P.10.7

Serial passage of sCJD in humanised transgenic mice indicates two major transmission strains associated with PrPSc of either type 1 or 2

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Background: Questions remain about the aetiology of sporadic CJD and whether phenotypic variation is solely controlled by factors such as codon 129 genotype and biochemistry of PrPC. Variation in infective strain has not been clearly demonstrated in sCJD.

Objectives: By serial passage of sCJD in transgenic mice expressing human prion protein with MM, MV, and VV codon 129 genotypes we aimed to understand strain transmission characteristics for the three most commonly observed phenotypes of sCJD.

Methods: We performed intracerebral inoculation of humanised transgenic mice with brain homogenates derived from similar mice previously inoculated with frontal cortex from sCJD patients of subgroups MM1, MV2, and VV2. These mice were assessed for clinical TSE signs, for TSE vacuolation, and deposition of PrPSc.

Results: sCJD(MM1) passage via all mice showed transmission profiles similar to primary inoculation.

sCJD(MV2) passage via HuMM and HuVV mice showed a transmission profile similar to primary inoculation. Passage via a HuMV mouse showed transmission properties similar to not only the primary inoculum but also sCJD(MM1).

sCJD(VV2) passage via HuMV and HuVV mice showed transmission profiles similar to the primary inoculation. Passage via a HuMM mouse showed transmission properties similar to not only the sCJD(VV2) primary inoculum but also sCJD(MM1).

Cluster analysis of the lesion profile data showed that three clusters seen after primary inoculation were reduced to two following second passage, identified by the biochemical type of PrPSc (1 or 2) found in the host mice.

Discussion: Serial passage of sCJD subgroups MM1, MV2, and VV2 shows that PrPSc type and mouse codon 129 genotype determine the secondary transmission profile, independently of the originating inoculum strain. There are associations between type 1 PrPSc and C129-Met, and type 2 PrPSc and C129-Val. This should allow us to investigate further the relationship between PrPSc, genotype, infection, and pathology.

P.10.8

Biochemical characterization of animal and human prion strains in bank voles (Myodes glareolus)

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Background: The zoonotic potential of BSE spurred studies on the possible link between animal and human prion strains. Molecular strain typing methods are based on the immunobiochemical properties of PrPSc. These methods allow PrPSc typing, but can be inappropriate for comparing PrPSc types among different species.

Objectives: To analyse and compare the biochemical features of PrPSc associated with human and animal prions after transmission and adaptation to voles.

Methods: PrPSc from voles infected with 45 TSE isolates, including classical scrapie, BSE, BASE and sCJD subtypes, was analysed by semi-quantitative WB i) after PK digestion (PrPres-epitope mapping) and ii) after denaturation with increasing GdnHCl concentrations and PrPC/PrPSc differential centrifugation (conformational stability assay).

Results: By PrPres analysis we observed the following molecular types:

- type A, with three PrPres fragments (19kDa, 14kDa, 11kDa), was found in BASE, VV2 and MM1/MV1 sCJD. PrPres amount in BASE and VV2 sCJD was distinctly much higher than in MM1/MV1 sCJD;

- type B, distinctive of classical scrapie, was characterised by a single PrPres fragment of 18kDa;

- type C, characteristic of MV2 sCJD, showed a doublet of 17-18kDa;

- type D, characteristic of BSE, with a single PrPres fragment of 17kDa;

- type E, found in MM2 sCJD, was characterised by a 17kDa fragment, accompanied by a minor fragment of 14kDa.

Preliminary results by conformational stability assay confirmed the categorization obtained by PrPres analysis.

Discussion: Scrapie, BSE, MM1/MV1 and MM2 sCJD, but not VV2 sCJD and BASE, preserved their PrPres pattern after transmission to voles.

The conformational stability assay did not allow to further discriminate PrPres types.

All animal TSEs investigated showed molecular features distinct from sCJD types, with the notable exception of BASE.

Similarities between VV2 sCJD and BASE encourage further studies on the nature of the sporadic forms and on the possible relationships between animal and human TSEs.

P.10.9

Infectivity and abnormal prion protein in edible tissues: bioassay versus lab detection

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Background: The validation of abnormal prion protein (PrPd) detection techniques is fundamental to allow the use of high-throughput laboratory based tests, avoiding the limitations of bioassays. The data generated are vital to risk assessors and will allow the optimisation of strategies to prevent exposure of humans to TSE material via the food chain, in scrapie infection and by extrapolation in other ovine TSEs.

Objectives: This project aimed to define the infectivity and abnormal prion protein distribution in edible tissues of sheep naturally infected with scrapie.

Methods: Sheep with terminal clinical signs of scrapie were euthanised by electrocution followed by immediate exsanguinations (to reflect commercial slaughter practice). Post-mortem tissue collection included: brain (for use as a standard), prescapular lymph nodes and samples from semitendinosus, and ocular muscles, tongue, sciatic nerve, heart and kidney. Abnormal prion protein distribution was assessed by immunohistochemistry (IHC) and western blot (WB) in samples from four animals, and infectivity was measured by bank vole bioassay in samples from two selected animals.

Results: Overall, there was a good correlation between IHC, WB and preliminary bioassay results. Discrepancies were observed in tissues with very low levels of abnormal PrP; IHC was the more sensitive method and was able to visualise very small foci of PrPd within the muscles spindles for example in semitendinosus muscles. These were negative by WB and interestingly negative by vole bioassay.

Discussion: These results show that even where animals are at terminal disease, variable amounts of PrPd can be found in edible tissues. Such PrPd and putative infectivity may have a focal or multifocal distribution within a tissue. Sampling is critical to avoid potential false negative rapid tests and bioassay results. Tissues with high PrPd accumulations are more of a risk to the consumer whilst negative tests do not imply absence of infectivity.

P.10.10

Biochemical variability of murine scrapie strains re-isolated in sheep

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Background: Murine scrapie strains originally derived from several sheep scrapie isolates, have remarkably stable phenotypic characteristics after serial passage. The Western Blot (WB) glyco-profiles of different murine strains have shown diverse relative amounts of di-, mono-, and a-glycosyl bands, so that this biochemical analysis is regarded as a useful tool for the discrimination between prion strains.

Objectives: To determine the biochemical stability of four cloned murine scrapie strains when passaged into sheep of different breeds and PRNP genotypes.

Methods: Cloned murine strains ME7, 22A, 79A and 87V were inoculated into ARQ/ARQ Suffolk and Cheviot sheep and VRQ/VRQ Cheviot sheep. Oral, intracerebral and subcutaneous routes were used individually or combined. Brain obex from sheep at clinical end point was examined by WB.

Results: Preliminary results indicate that (1) For some strains like 79A, the glyco-profiles obtained from sheep obex samples remain similar to those of the murine strain itself; (2) For some other strains like ME7, 22A and 87V, the WB glyco-profiles obtained from sheep brains not only diverged from the murine ones, but were also variable and apparently independant of the sheep genotype; (3) The route of challenge does not seem to affect the glycosylation pattern.

Discussion: Complete analysis of the experimental results will provide fundamental understanding on the concepts of stability of TSE strains and strain adaptation depending on host factors.

P.10.11

Biological typing of sporadic Creutzfeldt-Jakob disease isolates and comparison with animal prion isolates

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Background: Our incomplete understanding of the nature of TSE agents, along with the current technical limitations in the analysis of PrPSc structure, hamper the direct typing of prion isolates. The characterization of prion strains still relies upon bioassay in rodents. Bank voles (Myodes glareolus), being susceptible to a wide range of prion sources, offer the opportunity to investigate the biological properties of prion isolates from different species in a single model.

Objectives: To study the biological properties of sCJD subtypes and compare them with animal TSEs.

Methods: We analysed the phenotype of transmission of MM1, MV1, MM2, MV2, and VV2 sCJD subtypes to voles, in comparison with BSE, BASE and a variety of classical scrapie isolates from different EU countries. Molecular analysis of PrPSc from the original isolates preceded voles inoculation. Survival times and attack rates were calculated upon primary transmission and subsequent passages.

The brains of voles were analysed by discriminative WB for PrPSc type, by immunohistochemistry and PET-blot for PrPSc deposition pattern and by EE staining for lesion profile.

Results: The results of this study demonstrated that prion diseases can induce in voles a wide variety of molecular and pathological phenotypes. Isolates from sCJD were grouped into 4 categories: i) MM1/MV1 (n=3), ii) MM2 (n=1), iii) MV2 (n=2) and iv) VV2 (n=1). Scrapie isolates were categorised in at least 4 groups, with no overlapping with sCJD isolates. BSE was distinct from scrapie and sCJD phenotypes. Finally, BASE gave a phenotype clearly distinct from BSE and scrapie but indistinguishable from VV2 sCJD.

Discussion: Overall, the biological classification of sCJD subtypes concurs with their clinico-pathological classification. Similarities among prion isolates from different host species were very rare, with the notable exception of BASE and VV2 sCJD. Herein, the meaning of such similarities is discussed in the context of current knowledge on strains and of available tools for their typing.

P.10.12

Separation of prion strains from a mixture by discriminatory bioassay. The example of a natural case of mixed infection of classical scrapie and Nor98

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Background: The possible coexistence of different strains in natural prion isolates is the subject of enduring discussions and might have implications for prion strains surveillance. Nonetheless, this possibility has not yet been proved. In 2007, a case of ovine scrapie with features compatible with a mixed infection of classical scrapie and Nor98 was reported in Italy.

Objectives: To characterize by bioassay the prion agent(s) involved in this unusual scrapie case.

Methods: Previous studies showed that voles are susceptible to the prevalent Italian scrapie agent and resistant to Nor98, while tg338 mice show the contrary. Taking advantage of this differential susceptibility, we inoculated voles and tg338 with the brain stem (BS) and the cerebral cortex (CC) of the unusual case. Discriminatory WB showed a predominance of classical scrapie PrPres in BS, while CC mainly contained Nor98 PrPres. A classical scrapie isolate (ClS) from the same flock of the unusual case, as well as Nor98 isolates from Italy and Norway were used as controls.

Survival times and attack rates were calculated in voles and tg338. Brains were analysed by discriminative WB for PrPSc type, by IHC and PET-blot for PrPSc deposition and by E&E for lesion profile.

Results: Both CC and BS induced disease in tg338, with survival times similar to Nor98 controls. As expected, Tg338 were resistant to ClS control. In contrast, voles developed disease following inoculation of ClS control and BS, but not of CC and Nor98 controls.

In voles, the neuropathological and molecular phenotypes upon infection with the unusual case were the same of those observed with ClS, while in Tg338 were identical to Nor98 controls.

Discussion: Our results demonstrate that two independent and separable prion agents coexist in the isolate investigated. This implies that:
- mixed prion infections can occur
- classical scrapie and Nor98 are distinct entities
- the isolation of prion strains from a mixture is affordable.

P.10.13

Guanidinium chloride unfolding of PrPSc exhibits marked differences between genotypes

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Background: Various methods have been promoted as to their potential to differentiate TSE isolates (strains). Traditionally a panel of inbred mice has been used to differentiate TSE isolates, and while this approach has proven rigorous it is slow. Biochemical methods employed to date are rapid in comparison but for the most part are limited to proteinase K cleavage point differentiation as determined by the molecular weight of the protein on a Western blot. This approach is used extensively with BSE and has been employed by various groups to reliably differentiate H-type, L-type, and classical BSE. In the context of the protein only hypothesis, the differences must arise due to different folding parameters (structure, stability, or both). It is not surprising then that denaturant unfolding of PrPSc monitored by circular dichroism has been used to differentiate various TSE isolates.

Objectives: Here we adapt denaturant unfolding of PrPSc to be monitored by a commercial, ELISA based diagnostic platform.

Methods: Brain homogenate from experimental sheep scrapie is incubated in guanidinium chloride prior to evaluation on a commerical ELISA based diagnostic platform allowing quantitation of the fraction of PrPSc remaining in the folded form at various denaturant concentrations.

Results: Marked differences in denaturant unfolding parameters are observed between sheep of different genotypes inoculated with a single sheep scrapie isolate. Considerable differences between isolates are also observed.

Discussion: These results indicate that denaturant unfolding coupled with PrPSc analysis on a commercial ELISA may prove useful for strain typing of TSE isolates. However the results also highlight the difficulty of adapting approaches developed for rodent models of TSEs to a natural host system, as well as the potential for passage in a new host of different genotype to drive strain differentiation.

P.10.14

PrPSc type determines whether cross-seeding occurs with prion strain mixtures in vivo

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Background: During a prion infection, multiple PrPSc subtypes with distinct proteinase-K-resistant core sizes may co-exist in the central nervous system and are thought to indicate strain specific conformations. These PrPSc mixtures have been shown to occur naturally in sporadic Creutzfeldt-Jakob disease (sCJD) and experimentally when infectious prions are transmitted into a new species.

Objectives: We explored the interactions of distinct PrPSc subtypes in vivo using three mouse-adapted prion strains to determine whether cross-seeding would lead to hybrid strains or whether strains would remain distinct. We used luminescent conjugated polymers (LCPs), which emit conformation-dependent fluorescence spectra, to distinguish the prion strains as well as the PK-resistant PrPSc core sizes, PrPSc aggregate morphology, and lesion profiles in the brain.

Methods: We infected mice intracerebrally with defined mouse-adapted prion strains singly or as a 50:50 mixture consisting of (1) scrapie and BSE, (2) CWD and BSE, or (3) CWD and sheep scrapie. All mice developed terminal prion disease. We characterized the resulting PrPSc aggregates by their LCP emission spectra, morphology, and PK-resistant core size.

Results: We found that the incubation period to terminal disease was significantly delayed by the presence of a second prion strain. Based on the LCP emission profile, plaque morphology, and PK-resistant PrPSc core size, multi-strain infections led to three outcomes depending on the strain mixture: (1) cross-seeding to form hybrid plaques, (2) remaining as distinct conformers, or (3) the predominance of one strain.

Discussion: The outcome of prion strain interactions in vivo depend on the PrPSc subtypes involved and in some cases can lead to an increase in the diversity of prion strains.

P.10.15

Adaptation of chronic wasting disease (CWD) into hamsters: evidence of a novel strain of CWD

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Background: Prion strains are well-characterized for scrapie and BSE. Little is known about the potential for strains in chronic wasting disease (CWD). Different CWD strains could have different patterns of shedding infectious agent as well as differential detectiblility and interspecies transmissibility.

Objectives: We identified prion protein variants in white-tailed deer populations and demonstrated that Prnp genotype affects the susceptibility/disease progression of white-tailed deer to CWD. Cervid prion protein variants raise the likelihood of distinct CWD strains. We hypothesize that the prion protein variability results in different PrPCWD conformers producing different CWD strains upon interspecies transmission.

Methods: Hamsters were intracerebrally inoculated with brain homogenate or phosphotungstate concentrated preparations from CWD positive hunter-harvested (Wisconsin CWD endemic area) and from experimentally infected deer with known Prnp genotypes.

Results: Primary passage of concentrated CWD agent resulted in clinical disease at approximately 1 year post-infection. Subclinical infection was established with the other primary passages based on the detection of PrPCWD in the brains of hamsters and the successful disease transmission upon second passage. Second and third passage resulted in a decrease in incubation period to approximately 280 days. Inocula from deer with wt/wt and wt/G96S Prnp genotypes did not result in any differences in incubation period or clinical symptoms.

Discussion: Inocula from deer with the wt/wt and wt/G96S prion proteins resulted in similar disease upon transmission to hamsters, likely due to the presence of the wt prion protein. Comparison of the incubation period and clinical symptoms of the hamsters infected with inocula from Wisconsin white-tailed deer to transmission studies using different CWD inocula (Western white-tailed deer; elk and mule deer; Raymond et al. 2007) indicates that the CWD agent present in US Midwest is different from the strain(s) present in the endemic region of the western US.

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