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# Pathogenesis and Pathology

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# Pathogenesis and Pathology

## PO-058: Knockout of sialoadhesin enhances microglial accumulation during prion pathogenesis

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During the peripheral pathogenesis of prion infection the replication of $PrP^{Sc}$ upon follicular dendritic cells (FDC) in lymphoid tissues is important for efficient neuroinvasion.[1,2] The mechanism of how the agent is transported to and into lymphoid follicles is currently unknown but has been shown to be dependent upon integrin α X (CD11c) expressing mononuclear phagocytes.[3,4] One particular group of these cells reside within the splenic marginal zone or lymph node subcapsular sinus and function to trap blood borne antigen.[5] These cells are classically identified by expression of high levels of sialoadhesin (Siglec1 or CD169).[6] Sialoadhesin binds to sialic acid moieties on N- and O-glycans and functions as a cell-cell or cell-pathogen recognition molecule and possible internalization receptor similar to other Siglec family members.[7] Sialoadhesin expression is restricted to members of the mononuclear phagocyte lineage. Sialoadhesin is constitutively expressed upon the above mentioned splenic and lymph node resident cell populations but is also stimulated in response to inflammatory stimuli[8] upon tissue macrophages or microglia.[9] The prion protein is variably glycosylated and heavily sialylated,[10] with evidence showing that the sialylation status of PrP is altered during infection.[11] To determine if disease-associated PrP is collected by sialoadhesin for transference into the lymphoid follicle we have infected Sialoadhesin-deficient mice with the mouse-adapted scrapie strain ME7. Following intraperitoneal infection no differences were observed upon the early accumulation of $PrP^{Sc}$ within lymphoid follicles. Similarly sialoadhesin-deficient mice showed no deficiency in trapping and retention of preformed immune-complexes upon FDC. Investigation of disease incubation period and pathological outcome following intraperitoneal infection are currently in progress. Data will be presented at the meeting on the effects of sialoadhesin-deficiency on disease susceptibility after peripheral exposure. Following intracerebral infection however, sialoadhesin-deficient mice showed an increase in the accumulation of activated-microglia in the brain compared with wild type mice. This resulted in elevated levels of vacuolation occurring specifically within the hippocampal CA1 and septum brain regions. No statistically significant differences were observed in disease incubation period following intracerebral infection of wild type and sialoadhesin-deficient mice. These results reveal that knockout of sialoadhesin results in increased activated microglial accumulation in brain areas targeted by prion infection, with large multi-cellular foci in sialoadhesin-deficient mice. These results suggest that knockout of sialoadhesin may interfere with microglial cell-cell recognition, phagocytosis of apoptotic neurones, suppression of anti-inflammatory signaling and microglial neuroprotective functions.[12]

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## PO-059: Remarkable reduction of MAP2 in the brains of scrapie-infected rodents and human prion disease possibly correlated with the increase of calpain

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Microtubule-associated protein 2 (MAP2) belongs to the family of heat stable MAPs, which takes part in neuronal morphogenesis, maintenance of cellular architecture and internal organization, cell division and cellular processes. To obtain insight into the possible alternation and the role of MAP2 in transmissible spongiform encephalopathies (TSEs), the MAP2 levels in the brain tissues of agent 263K-infected hamsters and human prion diseases were evaluated. Western blots and IHC revealed that at the terminal stages of the diseases, MAP2 levels in the brain tissues of the scrapie infected hamsters, a patient with genetic Creutzfeldt-Jakob disease (G114V gCJD) and a patient with fatal familial

insomnia (FFI) were almost undetectable. The decline of MAP2 was closely related with prolonged incubation time. Exposure of SK-N-SH neuroblastoma cell line to cytotoxic PrP106–126 peptide significantly downregulated the cellular MAP2 level and remarkably disrupted the microtubule structure, but did not alter the level of tubulin. Moreover, the levels of calpain, which mediated the degradation of a broad of cytoskeletal proteins, were significantly increased in both the PrP106–126 treated SK-N-SH cells and brain tissues of 263K prion-infected hamsters. Our data indicate that the decline of MAP2 is a common phenomenon in TSEs, which seems to occur at an early stage of incubation period. Markedly increased calpain level might contribute to the reduction of MAP2.

**Figure 1:** http://www.eventure-online.com/parthen-uploads/6/12PRI/img1_183283.jpg
**Caption 1:** The decline of MAP2 occurs at early stage in TSEs

**Figure 2:** http://www.eventure-online.com/parthen-uploads/6/12PRI/img2_183283.jpg
**Caption 2:** Increased calpain contributes to reduction of MAP2

## PO-060: Transmission of chronic wasting disease from mother to offspring

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To investigate the role mother to offspring transmission plays in chronic wasting disease (CWD) we have developed a cervid model employing the Reeve's muntjac deer (*Muntiacus reevesi*). Eight muntjac doe were orally inoculated with CWD and tested $PrP^{CWD}$ lymphoid positive by 4 mo post infection. Twelve fawns were born to these eight CWD-infected doe, 3 were born viable, 6 were born non-viable, and 3 were harvested as fetuses (1 each from first, second or third trimester of pregnancy) from CWD-infected doe euthanized at end-stage disease. The viable fawns have been monitored for CWD infection by immunohistochemistry (IHC) performed on serial tonsil and rectal lymphoid tissue biopsies. One fawn that was IHC $PrP^{CWD}$ positive at 40 d of age is now, at 28 mo of age, showing early clinical signs associated with CWD infection. Moreover, CWD prions have been detected by sPMCA in placenta, brain, spleen and mesenteric lymphoid tissue harvested from 5 full-term non-viable fawns, and in fetal placenta and brain tissue harvested in utero from the second and third trimester fetuses. Additional tissues and pregnancy related fluids from doe and offspring are being analyzed for CWD prions. In summary, using the muntjac deer model we have demonstrated CWD clinical disease in an offspring born to a CWD-infected doe, and in utero transmission of CWD from mother to offspring. These studies provide basis to further investigate the mechanisms of maternal transfer of prions.

## PO-061: Highly neurotoxic monomeric α-helical prion protein

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Prion diseases are infectious and belong to the group of protein misfolding neurodegenerative diseases. In these diseases, neuronal dysfunction and death are caused by the neuronal toxicity of a particular misfolded form of their cognate protein. The ability to specifically target the toxic protein conformer or the neuronal death pathway would provide powerful therapeutic approaches to these diseases. The neurotoxic form(s) of the prion protein (PrP) have yet to be defined but there is evidence suggesting that at least some of them differ from infectious PrP ($PrP^{Sc}$). Herein, without making an assumption about size or conformation, we searched for toxic forms of recombinant PrP after dilution refolding, size fractionation and systematic biological testing of all fractions. We found that the PrP species most neurotoxic in vitro and in vivo (toxic PrP, TPrP) is a monomeric, highly α-helical form of PrP. TPrP caused autophagy, apoptosis and a molecular signature remarkably similar to that observed in the brains of prion-infected animals. Interestingly, highly α-helical intermediates have been described for other amyloidogenic proteins but their biological significance remains to be established. We provide the first experimental evidence that a monomeric α-helical form of an amyloidogenic protein represents a cytotoxic species. While toxic PrP has yet to be purified from prion-infected brains, TPrP might be the equivalent of one highly neurotoxic PrP species generated during prion replication. Because TPrP is a misfolded, highly neurotoxic form of PrP reproducing several features of prion-induced neuronal death, it constitutes a useful model to study PrP-induced neurodegenerative mechanisms.

## PO-064: 4-Hydroxy-tamoxifen treatment induces $PrP^{Sc}$ clearance in an autophagy-independent manner

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Prion diseases are fatal neurodegenerative disorders involving the abnormal folding of a native cellular protein, named PrPC, to a malconformed, aggregation-prone state, enriched in β sheet secondary structure, denoted $PrP^{Sc}$. Prion diseases are distinct from other neurodegenerative diseases insofar as they are infectious. Recently, autophagy has garnered considerable attention as a cellular process with the potential to counteract neurodegenerative diseases of protein aggregation such as Alzheimer disease, Huntington's disease, and Parkinson disease. Upregulation of autophagy by chemical compounds has also been shown to

reduce $PrP^{Sc}$ in infected neuronal cells and prolong survival times in mice models. Consistent with previous reports we demonstrate that autophagic flux is increased in chronically infected cell models. However, in contrast to recent findings we show that autophagy is not causative of a reduction in scrapie burden. We report that in infected neuronal cells different compounds known to stimulate autophagy are ineffective in increasing the autophagic flux and in reducing $PrP^{Sc}$. We further demonstrate that the anti-prion effect of tamoxifen and its metabolite 4-hydroxytamoxifen is not dependent on autophagy but rather depends on the ability of these drugs to alter the trafficking of both PrP and cholesterol. Because tamoxifen represents a well-characterized, widely available pharmaceutical our data indicate that it may have applications in the therapy of prion diseases.

## PO-062: Persistent retroviral infection influences neuropathological signature and phenotype of prion disease

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The transport of infectious prions from cell to cell to and within the central nervous system is still poorly understood. The involvement of retroviruses in this process has been postulated, since retroviral super-infection of prion infected cells enhance the spread of prion infectivity and $PrP^{Sc}$ to the culture supernatant in vitro.

To study whether retroviral infection influences the prion disease course in vivo, we developed a murine model with persistent Moloney murine leukemia retrovirus (MoMuLV) infection with and without an additional prion-infection. We investigated the pathophysiology of prion disease in mice inoculated with prions only or infected with both pathogens, monitoring temporal kinetics of $PrP^{Sc}$ spread and prion infectivity, brain lesion profile, as well as clinical presentation.

Unexpectedly, the additional infection of MoMuLV challenged mice with prions did not change incubation time to clinical prion disease. Therefore, retroviral particles do not seem to be efficient vectors for prion transport and probably also not for prion transmission. Interestingly, however, clinical presentation of prion disease was altered in mice that were infected with both pathogens. This was paralleled by remarkably enhanced astrogliosis and pathognomonic astrocyte morphology in the brain of these mice. Therefore, we conclude that persistent viral infection might act as a disease modifier in prion disease.

## PO-063: Scrapie pathogenesis following experimental intra-tonsillar infection in a sheep model

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**Introduction.** The key pathogenetic event in sheep scrapie, the human and animal prion disease (PD) "prototype," is represented by the deposition, within lymphoreticular system (LRS) and nervous tissues, of the disease-associated isoform ($PrP^{Sc}$) of the normal host-encoded cellular prion protein ($PrP^{C}$). Ileal Peyer's patches (PPs) are known to be, among host's lymphoreticular system (LRS) tissues, the earliest $PrP^{Sc}$ accumulation site. The scrapie agent, corresponding largely, if not entirely, to $PrP^{Sc}$, gains access to the central nervous system (CNS) from ileal PPs by the sympathetic and vagal nerve routes. Although palatine tonsils (PTs) are another scrapie agent's early colonization site, no information exists regarding the possibility that neuroinvasion may independently occur from such LRS tissue district. Therefore, we tested the hypothesis that $PrP^{Sc}$ may colonize CNS directly from PTs, either traveling along the neural network and/or by the lympho-hematogenous route.

**Material and Methods.** To test this we injected 50 µl of a 25% classical scrapie brain homogenate from clinically affected Sarda breed sheep into the PT of 40 d-old Sarda breed lambs carrying resistant (ARR/ARR) and susceptible (ARQ/ARQ) PrP genotypes. First three lambs at seven days post-inoculation (pi), and then other three lambs, each at two-months time intervals starting from the first month pi, were euthanized. Immunistochemical and Western-blotting examinations for $PrP^{Sc}$ were carried on a consistent number of LRS and nervous tissues from these animals.

**Results.** We first detected $PrP^{Sc}$ within the inoculated PT and in the ipsilateral retropharyngeal lymph node in 3 ARQ/$ARQ_{wildtype}$ lambs at three months pi. At this step, no $PrP^{Sc}$ was detected in the sympathetic and parasympathetic ganglia connected to PTs. In the subsequent ARQ/$ARQ_{wildtype}$ euthanized lambs, $PrP^{Sc}$ deposition occurred in several other LRS districts, with the ileal PPs being colonized by $PrP^{Sc}$ at seven months pi. Within the brain, the earliest $PrP^{Sc}$ deposition was detected in the substantia reticularis of the obex region at nine months pi. All ovines carrying resistant genotypes did not display any detectable $PrP^{Sc}$ deposition in both nervous and LRS tissues. The experiment is still ongoing and no clinical signs have been observed so far in any animal, at 10 mo pi.

**Conclusion.** These preliminary results suggest that intra-tonsillar inoculation is an effective route for scrapie infection to become established. Our data do also argue in favor of a preliminary $PrP^{Sc}$ replication first in loco-regional LRS tissues from which an "autonomous" neuroinvasion process is likely to

develop, in a time spam similar to that reported in orally infected lambs. In addition, the effectiveness of our low-dose scrapie homogenate inoculation is a matter of special concern with reference to the dose-response relationship in PDs. Finally, our data suggest that the parasympathetic vagal and sympathetic splanchnic nerves could not represent the unique nervous route by which the scrapie agent gains access to the CNS.

## PO-065: Cell-surface expression of $PrP^c$ and the presence of scrapie prions in the blood of goats

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Scrapie, a transmissible spongiform encephalopathy of goats and sheep, is characterized by conversion of a normal cellular prion protein ($PrP^c$) to a protease-resistant isoform ($PrP^{Sc}$). Cell-surface $PrP^c$ expression in ovine peripheral blood mononuclear cells (PBMCs) and prion infectivity in blood from scrapie-infected sheep have been demonstrated earlier. However, such studies are not reported in goats. Therefore, the aim of this study is to identify which components in normal caprine blood express cell-surface $PrP^c$ and also to determine whether blood from scrapie-infected goats harbors prion infectivity. The relative levels of cell-surface $PrP^c$ expression in PBMCs subsets were measured by flow cytometry after double labeling PBMCs with cell type-specific monoclonal antibodies (mAbs) and anti-$PrP^c$ mAbs. Higher levels of cell-surface $PrP^c$ expression were found in PBMCs while lower levels of $PrP^c$ expression were found in platelets. However, $PrP^c$ expression was not detected on polymorphonuclear cells and erythrocytes. Though all PBMCs subsets expressed cell-surface $PrP^c$, the highest cell-surface $PrP^c$ expression was found in $CD2^+$ T-lymphocytes whereas $CD21^+$ B-lymphocytes (a subset of B-lymphocytes) expressed the lowest levels of $PrP^c$. Transmission of preclinical scrapie was detected in all three recipient goats that received whole blood derived from a goat with clinical classical scrapie. Based on these findings, we conclude that all PBMCs subsets of goats express cell-surface $PrP^c$ and blood-borne infectious prions can be detected in scrapie-infected goats by goat kid bioassay. Therefore, goat blood might be a suitable diagnostic target to identify scrapie infections.

## PO-066: Incunabular immunological events in prion trafficking

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While prions probably interact with the innate immune system immediately following infection, little is known about this initial confrontation. Here we investigated incunabular events in lymphotropic and intranodal prion trafficking by following highly enriched, fluorescent prions from infection sites to draining lymph nodes. We detected biphasic lymphotropic transport of prions from the initial entry site upon peripheral prion inoculation. Prions arrived in draining lymph nodes cell autonomously within two hours of intraperitoneal administration. Monocytes and dendritic cells (DCs) required Complement for optimal prion delivery to lymph nodes hours later in a second wave of prion trafficking. B cells constituted the majority of prion-bearing cells in the mediastinal lymph node by six hours, indicating intranodal prion reception from resident DCs or subcapsulary sinus macrophages or directly from follicular conduits. These data reveal novel, cell autonomous prion lymphotropism, and a prominent role for B cells in intranodal prion movement.

## PO-067: The effects of host age on the transport of complement-bound complexes to the spleen and the pathogenesis of intravenous scrapie infection

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Infections with variant Creutzfeldt-Jakob disease (vCJD) have almost exclusively occurred in young individuals but the reasons for this age distribution are uncertain. Our data suggest that the pathogenesis of many peripherally acquired transmissible spongiform encephalopathy (TSE) agents is less efficient in aged individuals.[1] Four vCJD cases linked to transfusion of vCJD-contaminated blood or blood products have been described. Three cases occurred in elderly patients implying that intravenous exposure is more efficient in aged individuals than other peripheral routes. To test this hypothesis, young (6–8 wk-old) and aged mice (600 d-old) were injected intravenously with a TSE agent. In aged and young mice the intravenous route was more efficient than other peripheral routes of TSE agent exposure. However, in aged mice disease pathogenesis was significantly reduced. Although most aged mice failed to develop clinical disease during their lifespans, many showed histopathological signs of TSE disease in their brains. Thus, the effects of age on intravenous TSE pathogenesis may lead to significant levels of sub-clinical disease in the population. After peripheral exposure many TSE agents accumulate upon follicular dendritic

cells (FDC) in lymphoid tissues before they infect the brain. In aged spleens PrPc expression and TSE agent accumulation upon FDC were reduced. Furthermore, the splenic marginal zone microarchitecture was substantially disturbed adversely affecting the delivery of immune complexes to FDC. This study is the first to suggest that the effects of aging on the microarchitecture that the function of the splenic marginal zone significantly influence the pathogenesis of an important pathogen.[2]

**Figure 1:** http://www.eventure-online.com/parthen-uploads/6/12PRI/img1_188549.jpg


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## PO-068: Transcriptome changes of ovine medulla oblongata during presymptomatic natural scrapie and their association with prion-related lesions


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The pathogenesis of natural scrapie and other naturally prion diseases is still poorly understood. The determination of transcriptome variations in infected vs. control animals might clarify some molecular mechanisms of the prion induced pathology. In addition, it may allow the development of new tools for diagnostics and therapy. We presented here for the first time the natural scrapie associated alterations in the gene expression profiles in caudal medulla oblongata (MO) in ovine affected by the resymptomatic phase of the disease using a custom microarray platform. A total of 86 significant probe sets displayed expression changes greater than 2-fold. From these probes we identified 32 genes with known function, those genes encode proteins that are involved in immune response, cell adhesion, and transcription. Our results confirm earlier published regulated genes found in murine models with induced scrapie. Moreover, we have identified new genes that show diferential expression in scrapie and could be involved in prion neuropathology. Finally, we have investigated the relationship between gene expression profiles and the appearance of the main scrapie related lesions: Prion protein deposition, gliosis and spongiosis. In this context, the potential impacts of these gene expression changes in MO on scrapie development are discussed.

## PO-069: Glial cells and scrapie progress


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Transmissible Spongiform Encephalopathies (TSEs) are a group of neurodegenerative disorders affecting animals and humans and for which no effective treatment is available to date. Vacuolization, neuronal and neurite degeneration, deposit of pathological prion protein (PrPsc) and gliosis, are changes typically found in TSE affected individuals. However, the actual role of this last feature, microgliosis and astrocytosis, has not been precisely determined.

The objective of this work is to deal with this role by assessing the involvement of the glial cells in natural Scrapie affected animals at different stages of the disease. To analyze the possible correlation between the glial reaction and the progress of the disease is proposed here. In order to achieve this aim, immunohistochemical techniques are performed to be applied on Scrapie samples from different sources and corresponding to different genotypes. With this specific aim, a descriptive study about the distribution and/or the morphology of glial cells in transversal cerebellum sections differs according to the clinical stage is developed here.

All advances achieved in the frame of this approach result especially relevant for the study about prion pathologies since some other components than PrPsc could be essential to the neurodegenerative progression associated with TSEs and therefore, other alternative strategies for their treatment could be considered.


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## PO-070: Evaluation and quantification of prion infectivity of saliva by sPMCA, sheep and bank vole bioassay


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Scrapie is a prion disease of small ruminants that is transmitted horizontally or by contact with environmental reservoirs of infectivity. In Chronic Wasting Disease (CWD) saliva carries significant levels of infectivity contributing to disease spread.[1,2] We recently demonstrated that salivary glands of scrapie affected sheep accumulate $PrP^{Sc}$.[3] The aim of this study was to evaluate and possibly quantify the prion infectivity in saliva from scrapie affected sheep by using sPMCA, sheep and bank voles bioassay.

For sheep bioassay, saliva was collected from 2 ARQ/ARQ sheep with clinical scrapie and $PrP^{Sc}$ positive salivary glands and from 2 ARQ/ARQ healthy controls. Seven ARQ/ARQ sheep were challenged per os with 100 ml crude saliva (5 from scrapie positive sheep and 2 from negative controls); 2 ARQ/ARQ sheep were orally challenged with scrapie brain homogenate as positive control. Finally, 7 saliva samples (three from natural scrapie cases, two from experimental scrapie and two negative controls) were lyophilized, concentrated 10×, dialysed and used for sPMCA[4] and bank voles challenge.[5] Groups of 8–30 bank voles were inoculated i.c. with saliva inocula and with serial dilutions (from $10^{-1}$ to $10^{-6}$) of a reference positive brain homogenate. Infectivity titers were estimated as $ID_{50}$ per gram by end-point titration.[6] Prion positivity in voles was confirmed in brain tissue by Western Blotting using mAb SAF84.

Sheep inoculated per os with crude saliva are still asymptomatic at 55 min.p.i. and no $PrP^{Sc}$ was detected in tonsil biopsies taken at 34 m.p.i. Positive controls developed clinical scrapie and were sacrificed at 25 m.p.i.

In vole bioassay, no clinical cases indicative of prion disease were observed up to 586 d.p.i. All voles were negative by Western Blotting for $PrP^{Sc}$. The titer was $10^{5.7}$ $ID_{50}$ U/g.

sPMCA of the inocula used for vole bioassay resulted in positive signals starting from the 6th round. After 8 PMCA rounds, 2 out of 3 saliva from experimental scrapie cases and 1 out of 2 saliva from natural scrapie were positive. Negative controls remained negative after 10 PMCA rounds.

Saliva of ARQ/ARQ scrapie affected sheep seems to carry some prion infectivity as detected by sPMCA. However, the lack of scrapie transmission in vole bioassay indicates that scrapie infectivity in saliva should be less than 0.5 $ID_{50}$ U/ml (i.e., 100 ml saliva contains at least 4 log less infectivity than 1 g of brain). The negative transmission observed so far after oral challenge in sheep might suggest that in ARQ/ARQ sheep, infected with the scrapie strain here used, the role of saliva in scrapie spread could be less important compared with CWD in cervids. Further data are needed to support this conclusion.

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## PO-071: Scrapie disease increases plasma level of pregnancy-associated glycoprotein-1 (PAG-1) during the first half of gestation in sheep


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Progressive neurological clinical signs are typical of transmissible spongiform encephalopathies (TSEs) but disruption of endocrine homeostasis has also been observed. Endocrine alterations described in natural and experimental models of TSE are usually explained to be a consequence of cerebral rather than peripheral organ damage. In scrapie-infected sheep, the placenta, which is a transient endocrine organ, presents both $PrP^{Sc}$ and infectivity even in the preclinical stage of the disease. Pregnancy-associated glycoproteins (PAGs) constitute a multigene family and are good indicators of both the pregnancy and the feto-maternal well-being. These PAGs are classified into two main groups, the PAG-1 subgroup are expressed primarily in binucleated trophoblastic cells. This study was designed to establish whether this prion disease could affect the functionality of the placenta and plasma hormone levels during the first half of gestation in sheep. The study population included eight healthy and five scrapie-infected ewes. Peripheral blood samples were collected at gestation days 15, 22, 29, 40, 50 and 75 after mating. Factors affecting plasma level of PAG-1 concentration were established by generalized linear model GLM repeated measures analysis of variance. Increased plasma levels of PAG-1 from day 40 onwards ($p < 0.01$) was registered in scrapie-infected sheep. The higher plasmatic levels on day 75 of gestation suggest that the placental function at this time of $PrP^{Sc}$ accumulation is altered.

## PO-072: Brainstem and spinal cord involvement in a novel neurological syndrome affecting primates exposed to prion contaminated blood products


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**Background.** We investigated the risk of vCJD from blood products components (BPC) in a reliable model of non-human primate (*Macaca fascicularis*) in view of the 4 human cases of vCJD secondary to transfusion of non-leucodepleted blood products.

**Materials and Methods.** Primates were injected by intracerebral or intravenous routes with BPC derived from vCJD infected donors from different sources (human, simian) and types (whole blood, plasma, buffy coat). Histology was performed at different clinical stages on 9 animals that received BPC, 7 inoculated with brain extracts and 4 normal controls, using classical methods.

**Results.** Eight animals that were exposed to BPC developed a unique neurological syndrome. They all showed bilateral necrotic lesions in the lower cervical spinal cord involving all, or part, of the anterior horn. Lesions appeared as a loss of ground substance filled with macrophages and a few floating neurons and surrounded by astrocytes; no lymphocytes were noted. Seven of these animals also showed the same bilateral necrotic foci localized in the inferior part of the trigeminal nucleus and the adjacent inferior cerebellar peduncle. There was no PrPres identifiable by immunohistochemical techniques in sections of cerebral hemispheres of any of these animals. Nevertheless, PrP was visualized in the substantia gelatinosa of their cervical and upper thoracic spinal cords, in higher amounts than in control animals. Other lesions included Wallerian degeneration of the posterior columns of the spinal cord and parts of the optic tracts. Cortical and cerebellar hemispheres were very mildly involved but with no spongiosis. Histology of these lesions excluded a vascular, inflammatory or auto-immune origin for this syndrome and other approaches made other etiologies (infectious, toxic, metabolic and vitamin deficiency) unlikely.

Conversely, these lesions were not present in any of the 7 animals inoculated with brain extracts which all exhibited classical vCJD clinical signs. One of the primates transfused with blood also developed a classical vCJD while all the controls remained normal.

**Conclusions.** We report here unique neuropathological lesions which have never previously been reported in our knowledge in simian pathology. They differ markedly from those observed in classical human myelopathies but might be compared with clinical FLAIL syndrome and to progressive necrotic myelopathy. Spinal cord lesions in prion diseases are anecdotal: if the probable prion etiology of the lesions described here is confirmed, the diagnosis and classification of degenerative myelopathies should be revisited.

## PO-073: Multiple routes of prion transepithelial transport in the nasal cavity following inhalation

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**Introduction.** Inhalation of either prion-infected brain homogenate or aerosolized prions has been shown to cause disease, and in the case of inhalation of infected brain homogenate, the nasal route of infection has been shown to be 10–100 times more efficient than the oral route. The cell types involved in the in vivo transport of prions across the nasal cavity epithelium have not been determined. M cells in the follicular associated epithelium have been shown to mediate transcellular transport of prions in vitro and in the gut of experimentally infected mice. We tested the hypothesis that M-cell mediated transport was responsible for prion entry across nasal cavity epithelium following inhalation.

**Materials and Methods.** Hamsters were inoculated extranasally with 50 or 100ul of infected (n = 31) or mock-infected (n = 13) brain homogenate. Control animals were inoculated with buffer (n = 4) or were untreated (n = 5). Following survival periods ranging from 15 to 180 min, animals were perfused, skulls were decalcified and nasal cavities were embedded in paraffin. Tissue sections were cut and processed immunohistochemically for glial fibrillary acidic protein to identify brain homogenate, or for the disease-associated form of the prion protein. Tissue sections not further than 112 um apart through the entire extent of the nasal cavity were analyzed using light microscopy; photomicrographs were obtained wherever inoculum was observed on the surface of, within, or deep to the nasal mucosa for each animal.

**Results.** Infected or uninfected brain homogenate was identified within the nasal cavities of animals at all time points and was seen crossing the nasal cavity epithelium within minutes of inoculation; the transepithelial transport of brain homogenate continued for up to 3 h after inoculation. Infected or uninfected brain homogenate was seen adhering to, or located within, M cells at all time points. However, larger volumes of infected or uninfected brain homogenate were identified crossing between cells of the olfactory and respiratory epithelia in multiple locations. In addition, infected or uninfected brain homogenate was identified within the lumen of lymphatic vessels in the lamina propria beneath the nasal mucosa at all time points.

**Conclusion.** Transepithelial transport of prions across nasal cavity mucosa begins within minutes of inhalation and can continue for up to 3 h. While M cells appear to transport prions across the follicular associated epithelium, larger amounts of prions are transported between the cells of the respiratory and olfactory epithelia, where they immediately enter the lymphatic vessels in the lamina propria. Thus, inhaled prions can be spread via lymph draining the nasal cavity and have access to somatic and autonomic nerves in the lamina propria of the nasal cavity. The increased efficiency of the nasal cavity route of infection compared with the oral route may be due to the rapid and prolonged transport of prions between cells of the respiratory and olfactory epithelia.

## PO-075: Overexpression of peripherin enhances progression of prion disease in mice

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Peripherin is a member of the type III intermediate filament protein, forms part of the cytoskeleton in a subset of neurons, most of which have peripheral fiber projections. We identified peripherin as a highly expressed protein in a $PrP^{sc}$-susceptible cell line using by two-dimensional difference gel electrophoresis (2D-DIGE) method.

Peripherin overexpressed by peripherin expression plasmid did not recover the $PrP^{sc}$-permissivity in a $PrP^{sc}$-unsusceptible

subclone. However, overexpressed peripherin enhanced the $PrP^{sc}$ uptake from the culture medium in a $PrP^{sc}$-insusceptible subclone. The enhancement of uptake the $PrP^{sc}$ from the culture medium, which was induced by transiently overexpressed peripherin, was also confirmed in a PrPSc-susceptible subclone. From this result, we hypothesized that overexpressing peripherin in mouse will increase sensitivity to $PrP^{sc}$ infection. To address this, we created peripherin transgenic mouse (peripherin TG mouse) that overexpress mouse peripherin throughout the whole body under the control of the CAG promoter. Then, we inoculated the mouse adapted C-BSE derived $PrP^{sc}$ in the brain. In C57BL/6 normal mice, the median incubation time was 225 d after intracerebral inoculation with 20 ul of 0.02% brain homogenate. In contrast, in peripherin TG mice, the median incubation time was 206 d. Additionally, the shorten duration of the incubation time in peripherin TG mice in comparison with C57BL/6 normal mice was further prominent when mice were inoculated with 20 ul of 0.002% brain homogenate. These results suggested that peripherin TG mice will be a good tool for highly sensitive bioassay of prion infectivity.

## PO-076: Pathogenic prions deviate $PrP^C$ signaling in neuronal cells and cause imbalances in Aβ clearance

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The subversion of the normal function exerted by the cellular prion protein $PrP^C$ in neurons by pathogenic prions is assumed to play a central role in the pathogenesis of Transmissible Spongiform Encephalopathies. However, our understanding of the molecular events underlying prion-induced neurotoxicity is still far from complete. To address this issue, we exploited two models of prion infection, the 1C11 neuronal cell line and neurospheres. 1C11 cells have the capacity to differentiate upon induction into 1C115-HT serotonergic neuronal cells. They endogenously express $PrP^C$ and can replicate several prion strains.[1] These cells have been instrumental in uncovering $PrP^C$-dependent signal transduction events. Some of these are neurospecific and may have implications as to the neurotoxicity of pathogenic prions.[2,3] Our study also takes advantage of neurospheres isolated from ED14 whole brains of wild-type or PrP-knockout mice. PrP-expressing neurospheres have been demonstrated to efficiently replicate prions when induced to differentiate.[4] We document that, in both 1C115-HT neuronal cells and $PrP^C$-expressing neurospheres, prion infection is associated with an overactivation of the signaling targets normally coupled with PrPC, including the Fyn kinase, the MAP kinases ERK1/2 and the CREB and Egr-1 transcription factors. The deviation of this cascade is associated with oxidative stress conditions, leading to the recruitment of the p38 and JNK stress-associated kinases. Downstream from CREB, prion infection correlates with a decrease in the activity of the MMP-9 metalloprotease, and, in turn, in the cleavage of the $PrP^C$ partner β-dystroglycan. MMP-9 mediates catabolism of the amyloid A-β peptide and we observed that the reduction in MMP-9 activity in prion-infected cells leads to an impairment of Aβ clearance. Further, by exploiting 1C11 infected cells accumulating high or moderate levels of prions, we show that all the prion-induced changes are dose-dependent. Finally, we observed a dose-dependent increase in cerebro-spinal fluid Aβ levels following inoculation of mice with these infected clones. Altogether, our study sheds light on the molecular changes imparted by prions in neurons and discloses a new connection between PrPC and Aβ.

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## PO-077: Monitoring the time-course of prionemia in hamsters fed with scrapie by quantitative PMCA

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Several cases of vCJD transmission between humans via blood transfusion have been reported. Prion infectivity or its biochemical surrogate marker, the pathological isoform of the prion protein, $PrP^{TSE}$, could be detected in blood and blood fractions from different animal species in pre- and subclinical or clinical stages of infection. Additionally, the detection of $PrP^{TSE}$ in blood samples from symptomatic vCJD patients was reported recently. Against this background it would be also informative to quantitatively determine the amount of $PrP_{TSE}$ in the blood at different time-points during the incubation period.

In this study, blood samples from hamsters perorally infected with 263K scrapie (an animal model which in the past has provided key informations about the spread of prions through the body in naturally acquired prion diseases) were analyzed for the amount of $PrP^{TSE}$ seeding activity in different stages of scrapie incubation by quantitative protein misfolding cyclic amplification (qPMCA).

Our results showed, that already at 50 d post infection (dpi), corresponding to approximately one third of the incubation period, low amounts of $PrP^{TSE}$ seeding activity could be found in blood in some of the analyzed animals. At 85 dpi, a time-point

well before the onset of clinical disease, an up to one hundred-fold increase in the concentration of $PrP^{TSE}$ seeding activity could be detected, which thereafter raised only moderately until the late clinical stage of disease.

If these findings in Syrian hamsters would mirror blood-associated prion pathogenesis in vCJD, a blood screening test for this disease would appear feasible already in preclinical stages of infection.

## PO-078: Protease-activated receptor—Two deficient mice exhibit prolonged survival after intracerebral inoculation with scrapie

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**Introduction.** Proteinase-activated receptor-2 (PAR2) belongs to the family of G protein-coupled receptors activated by the site specific proteolysis. Action of trypsin or certain other proteinases unmasks tethered ligand sequence within the N-terminal part of the molecule leading to intracellular signaling. In brain, PAR2 is playing both, protective and damaging roles. Recent reports demonstrated its involvement in the survival and death of brain cells in different models of neurodegenerative disorders including Alzheimer disease. The aim of our study was to evaluate the role of PAR2 in pathogenesis of mouse scrapie.

**Material and Methods.** Homozygous PAR2 knockout ($PAR2^{-/-}$) mice and wild-type (WT) mice (n = 12) were inoculated intracerebraly with 30 µL of 1% RML brain homogenate. The mice were monitored for the development of clinical signs and sacrificed at the terminal stage of the disease. The scrapie status of mice was verified by western blot and the levels of brain infectivity compared by scrapie cell assay utilizing CAD5 cells. The spongiform changes, deposits of $PrP^{TSE}$ and astrogliosis were evaluated after staining of paraffin embedded sections of the brains.

**Results.** $PAR2^{-/-}$ mice demonstrated delayed onset of clinical symptoms including significant difference in the onset of weight loss. In addition, $PAR2^{-/-}$ mice exhibited moderate, but highly significant prolongation of survival over WT controls: 166 ± 8 vs. 150 ± 8 d (mean ± SD, $p < 0.001$). The comparison of the distribution of spongiform changes, pattern of $PrP^{TSE}$ deposits and level of astrogliosis did not reveal any obvious differences between the groups. Similarly, the level of brain infectivity measured with scrapie cell assay and the western blot pattern of brain $PrP^{TSE}$ were similar in both studied groups.

**Conclusion.** Our study demonstrated that the deletion of PAR2 delays the onset of clinical symptoms and prolongs survival of mice after intracerebral inoculation with high level of RML prions without gross perturbation of scrapie pathogenesis. This finding identifies PAR2 as a potential pharmacological target in the development of therapeutic strategies for prion diseases.

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## PO-079: Increased serum amyloid A (SAA) levels in blood from sheep with classical scrapie, evident at both protein and mRNA level

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**Introduction.** Classical scrapie is a fatal neurodegenerative prion disease in sheep, and has a complex epidemiology including long incubation period, genetic susceptibility, variable clinical manifestation and different prion strains. The prion is perceived as the animal's own protein, and there is no detectable immune reaction. Here we present the detection of an acute phase protein in serum of sheep with clinical scrapie.

**Materials and Methods.** *Animals*. Nine lambs (homozygous V136R154Q171) were divided into two groups (control and scrapie) and inoculated orally with 1 g of pooled brain material from healthy sheep and confirmed cases of classical scrapie respectively. Blood samples (plain and PAXgene) were drawn every fortnight from birth until euthanasia at 23 (scrapie) and 25 (control) weeks of age.

*Proteomic analysis*. Fractionated (ProteinChip® Q Spin Columns) serum samples were analyzed using SELDI-TOF-MS and ProteinChip® Array technology.

*Protein identification*. Fractionated serum samples were run on a 16% ClearPAGE gel and one band at around 12 kDa was subjected to tryptic digestion (OMX). Peptide sequence was determined by LC-MS/MS (LTQ-Orbitrap XL). Protein identification was achieved by Proteome Discoverer 1.0 software with SEQUEST algorithm searching against even toed ungulates database.

*ELISA.* SAA was determined using Phase™ Range Multispecies SAA ELISA kit.

*mRNA analysis.* Total RNA was extracted from PAXgene blood tubes (PAXgene® Blood miRNA Kit). Quantity and quality of extracted RNA was estimated using NanoDrop Spectrophotometer and Agilent RNA 6000 Nano Kit. QuantiTect® Reverse Transcription kit from Qiagen was used for cDNA synthesis. Two specific SAA primer pairs were used to relatively quantify SAA mRNA concentration by the use of 48.48 Dynamic Array™ IFC with BioMark™ HD real-time PCR system.

*Data analysis.* Statistical analysis of SAA data was performed using Microsoft Excel 2010, SigmaPlot and JMP®. qPCR data were processed and analyzed in GenEx software.

**Results.** Clinical signs of classical scrapie were evident in the scrapie group from 22 weeks of age. The higher intensity of the SELDI peak (m/z 12680 Da) in the scrapie group as compared with the control group, was statistically significant. The protein

identified by LC-MS/MS was SAA, is likely the protein behind this SELDI peak. Detection of SAA at protein level from the two groups revealed a significant rise from 22 weeks of age, and increasing until euthanasia without showing a decline or plateau phase. The same pattern was evident at mRNA level.

**Conclusion.** Animals suffering clinically from classical scrapie have an increased SAA level in blood, both at protein and mRNA levels. We have demonstrated an ongoing acute phase response during the clinical stages of classical scrapie.

## PO-080: Infectivity in peripheral muscle but not in lymphoreticular system of cattle intracranially challenged with L-type or H-type BSE

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After a higher zoonotic potential of L-type BSE (BASE) has been demonstrated by transmission studies in transgenic mice and in nonhuman primates, it is important to analyze the agent distribution in the periphery of L-type BSE infected cattle. We have therefore performed a mouse bioassay in highly sensitive bovine PrP transgenic mice (Tgbov XV) using peripheral tissues of the muscular, neuronal and lymphoreticular system of cattle that were intracranially challenged with L-type and H-type BSE. It could be confirmed that, like in classical BSE, the lymphoreticular system plays no role in the agent distribution, since all spleen samples turned out to be free of infectivity. Interestingly, the truncus vagalis ventralis of clinically affected cattle was shown to contain low levels of infectivity, albeit the intracranial inoculation route of the cattle. After inoculation of M. semitendinosus samples from the same cattle, attack rates in transgenic mice of up to 41% were observed for L-type BSE and of up to 36% for H-type BSE with incubation times of 378 and 399 d for L-type BSE and 481 and > 600 d days respectively for H-type BSE. However, the PrPSc concentration in these samples remained below the detection limit, as we were unable to visualize PrPSc depositions by western blot or immunohistochemistry in the bovine muscle samples.

We can therefore conclude that the pathogenesis of atypical BSE basically follows the same pattern that is known for classical BSE. However, the detection of infectivity in the muscle sample with incubation times comparable to those observed after challenge with a 10–5 dilution of a L-type BSE positive brain sample, deserves further analysis.

## PO-081: Chronic wasting disease in the cat—Similarities to feline spongiform encephalopathy (FSE)

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**Background and Introduction.** Chronic wasting disease (CWD) is an efficiently transmitted prion disease of cervids with an as yet to be fully defined host range. Moreover, the risk that CWD poses to feline predators and scavangers, through cross-species consumption and subsequent transmission, is unknown. Previous and ongoing studies in our laboratory evaluating the susceptibility of domestic cats (*Felis catus*) to CWD (Mathiason et. al., NeuroPrion 2011, Nalls et. al., NeuroPrion 2012) have documented the susceptibility of domestic cats to CWD following intracerebral (IC) inoculation. However, many of the pathologic features of feline-adapted CWD, including the neural and systemic patterns of $PrP^{CWD}$ accumulation and neuropathology, remain unknown.

The chief objectives of this work were: (1) to design a sensitive, enhanced immunohistochemical (E-IHC) protocol for the detection of CWD prions ($PrP^{CWD}$) in feline tissues; (2) to document the systemic distribution of $PrP^{CWD}$ in CWD-infected cats through E-IHC; (3) to utilize single and multiple-label immunostaining and laser scanning confocal microscopy (LSCM) to provide insights into the subcellular patterns of $PrP^{CWD}$ accumulation and neuropathologic features of CWD-infected cats; and (4) to compare feline CWD to the other known feline TSE

**Materials and Methods.** Periodate-lysine-paraformaldehyde (PLP)-fixed, paraffin-embedded (PLP-PE) from terminal, IC-inoculated (n = 9) and sham-inoculated (n = 2), 1st and 2nd passage, CWD-infected cats were examined by E-IHC for the presence of $PrP^{CWD}$ and its association with markers of cell phenotype and organelles.

**Results.** The most sensitive E-IHC technique for the detection of $PrP^{CWD}$ in feline tissues incorporated a combination of slide pretreatment with proteinase-K (PK) in concert with tyramide signal amplification (TSA). With this protocol, we identified $PrP^{CWD}$ deposits throughout the CNS, which, in the 1st passage cats was primarily restricted to the obex, but increased in distribution and severity upon 2nd passage to include a number of midbrain nuclei, cortical gray matter, the thalamus and hypothalamus, and the hippocampus. Peripheral $PrP^{CWD}$ deposits were detected only in the 2nd passage cats, and included the enteric nervous system, the Peyer's patches, and the retropharyngeal and mesenteric lymph nodes. $PrP^{CWD}$ was not detected in the sham-inoculated cats.

Moreover, using multi-label analysis, intracellular $PrP^{CWD}$ aggregates were seen in association with neurofilament heavy chain (NFH)-positive neurons and GFAP-positive astrocytes. In addition, large aggregates of intracellular $PrP^{CWD}$ were identified within LAMP1-positive lysosomes.

**Conclusions.** Feline $PrP^{CWD}$ is present in CNS neurons, astrocytes and LAMP-1-positive lysosomes. The morphologic overlap between the $PrP^{CWD}$ deposits in feline CWD and BSE-origin feline spongiform encephalopathy (FSE), implicates the importance of the host as a key determinant in the development of prion neuropathology and suggest a signature for detection of potential spontaneous feline prion disease.

## PO-082: Analysis of the UPR transducer IRE1α in neurodegenerative disorders: Further evidence against a major role of ER stress in prion disease pathology

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**Introduction.** Synthesis and correct folding of proteins is one of the main functions of the endoplasmic reticulum (ER). Cellular stress conditions can interfere with this process causing accumulation of unfolded or misfolded proteins in the ER lumen. In an adaptive response to this, several pathways are activated in the ER, which are, taken together, termed the unfolded protein response (UPR). Some years ago, Katayama at al. and Hoozemans et al. suggested that ER stress and activation of the UPR plays a crucial role in neuronal death in Alzheimer disease (AD).[1,2] While upregulation of the UPR in AD has since then been confirmed in a number of studies, any participation of ER stress in prion diseases is still a matter of debate. In a previous study, analyzing the expression of the UPR transducer PERK and its downstream effector eIF2α, we demonstrated that mere prion diseases differ significantly from AD, as well as Creutzfeld-Jakob disease (CJD) with concomitant AD-related pathology, by the lack of prominent ER stress features.[3] In a follow-up study, we now analyzed phosphorylation and activation of the most conserved UPR transducer, inositol-requiring enzyme 1 α (IRE1α), a bifunctional transmembrane kinase/endoribonuclease enzyme located in the ER membrane, comparing the expression profile of phospho-IRE1α in different types of prion diseases to that of AD, as well as different forms of tauopathies and synucleinopathies.

**Materials and Methods.** Immunohistochemical expression analysis of serin 724-phosphorylated IRE1α in cortical, hippocampal, pontine and brain stem sections from human autopsy material of the following diseases: sporadic and familial CJD (8 cases), sporadic CJD with concomitant AD-related pathology (3 cases), AD (5 cases), tauopathies (2 cases), synucleinopathies (4 cases), motoneuron diseases (2 cases), and controls (5 cases).

**Results.** We observed a strong neuronal immunoreactivity for phosphorylated IRE1α in particular in the hippocampal region of advanced cases with AD and sporadic CJD with concomitant AD-related pathology. Similar to our previous study, immunoreactivity for the UPR transducer IRE1α correlated well with the extent of neuronal tau pathology (measured according to the staging by Braak and Braak), with widespread upregulation of phospho- IRE1α in high and very limited upregulation in low Braak and Braak stages. In contrast to these findings, cases with mere sporadic or familial CJD showed virtually no immunoreactivity for phospho-IRE1α in all but one case. Of note, this later case with a strong but restricted neuronal immunoreactivity for phospho-IRE1α in the CA2 and CA1 region of the hippocampus displayed in addition a mild tau pathology (Braak and Braak stage II), again linking the upregulation of ER stress markers to the intracellular accumulation of pathological protein aggregates like tau protein. In line with this, we observed neuronal and glial immunoreactivity for phospho- IRE1α in different tauopathies and synucleinopathies, each time linked to the area of disease-specific pathology known for the intracellular accumulation of pathological tau and α-synuclein protein.

**Conclusions.** Confirming our previous results, our study provides further evidence that ER stress and activation of the UPR do not play a major role in the CNS pathogenesis of prion diseases in general.

## PO-083: Analysis of $PrP^{Sc}$ accumulation and glial cell activation in brains of prion-infected mice at the early stage of infection

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Prion diseases are fatal neurodegenerative disorders that are characterized by the vacuolation of neurons and neuropils, reactive astrogliosis, microglial activation and accumulation of an abnormal isoform of prion protein ($PrP^{Sc}$) in the central nervous system. These glial cells are known to be activated before clinical onset; however, how glial cells respond to prion propagation at the early stage of prion infection is largely unknown. Our laboratory has reported that anti-PrP monoclonal antibody 132, recognizing aa119–127 of mouse PrP, is useful for $PrP^{Sc}$-specific detection in prion-infected cells in combination with guanidium salt pre-treatment of fixed cells. In this study, this method was modified for $PrP^{Sc}$-specific detection in frozen tissue sections to analyze glial cell activation in brain regions where the accumulation of $PrP^{Sc}$ becomes detectable in the early stage of infection. First, brain regions were investigated where the accumulation of $PrP^{Sc}$ can be detected in the early stage of infection. $PrP^{Sc}$ became detectable in the medulla and thalamus of Chandler strain-infected mice as early as 30 dpi and 45 dpi, respectively. Double-staining of $PrP^{Sc}$ and glial or pre-synaptic marker molecules revealed that at the early stage of infection, most PrPSc was present in regions close to synaptophysin-positive areas, a marker molecule of pre-synaptic regions. In the thalamus of Chandler-infected mice, astrocyte activation assessed by GFAP became detectable as early as 45 dpi, which was earlier than the appearance of microglial activation assessed by Iba-1. The precedence of astrocyte activation over microglial activation was also observed in the medulla. The same tendency was observed in the exact areas of the thalamus in which PrPSc is present in the early stage

of infection. These results suggest that astrocytes recognize prion propagation directly or subtle changes in neurons by prion propagation and play an important role in neuropathobiology in the early stage of prion infection. Further studies on the activation state of glial cells in the early stage of infection will contribute to elucidate the pathobiological mechanisms of prion diseases.

**Figure 1:** http://www.eventure-online.com/parthen-uploads/6/12PRI/img1_188994.jpg

## PO-084: Signaling pathways involved in dendrite spine formation and neuronal death identified from kinomic screens of scrapie-infected mice

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**Introduction.** Prion diseases are characterized by $PrP^{Sc}$ accumulation and spongiform degeneration. $PrP^{Sc}$ accumulation results in early progressive synaptic degeneration, before gliosis, vacuolation, or neuronal death. Many genes involved in synapse formation are affected by scrapie, as evaluated by genomic analyses of microRNA and mRNA. Consistently, neuronal knockout of $PrP^{C}$ during early synaptic dysfunction prevents disease progression. However, inhibition of dendrite degeneration, by a γ-secretase inhibitor and quinacrine (to inhibit $PrP^{Sc}$ propogation), does not. A better understanding of the signaling mediating synaptic degeneration may allow development of novel therapies to inhibit disease progression.

**Materials and Methods.** We developed a targeted proteomics (kinomics) approach to identify dysregulated signaling pathways. Mice intraperitoneally inoculated with 10% mock- or scrapie-infected (RML) mouse brain homogenate were euthanized at 70, 90, 110, 130 d post-infection (dpi) or at terminal stages (155–190 dpi). Brains were dissected into hindbrain, midbrain and forebrain. Primary screens were performed using multiplex western blots with 1.2 mg of hindbrain homogenate of 3 mock- or scrapie-infected mice per time.

**Results.** The expression levels of the 106 detected protein kinases were normalized to mock-infected mice, log (base 2) transformed, and analyzed by hierarchical clustering, which blindly groups together kinases based on changes in relative levels. Through literature and database searches, we identified four clusters of interest. Cluster 1 consisted of DLK, JNK2 and MST1, expressed to similar levels in scrapie- or mock-infected mice at 70, 90, 110 dpi, or at terminal stages, but to lower levels in scrapie-infected mice at 130 dpi. All participate in a pathway promoting neuronal death. The kinases in cluster 2, HER3, PKD1, c-Abl1 and TrkA, were expressed to similar levels in scrapie- or mock-infected mice at 70, 90 or 110 dpi, but to lower levels in 2 of the 3 scrapie-infected mice at 130 dpi and in 1 at terminal stages. They participate with HER2 in a pathway promoting neuronal survival. Cluster 3 includes PKG1, Lyn, CaMK4β p90RSK1 and p38γ expressed to higher levels in 2 of the 3 scrapie- than in mock-infected mice at 70 dpi, but to similar levels at 90, 110 or 130 dpi, and to lower levels at terminal stages. Cluster 4 includes EphA4, TrkB and p39, expressed to lower levels in 2 of the 3 scrapie- than in mock-infected mice at 70 dpi, to similar levels at 90 and 110 dpi, then lower again in 2 of the 3 scrapie-infected mice at 130 dpi and in 1 at terminal stages. Eight of the 11 kinases in these clusters participate in a pathway promoting dendrite spine formation by γ-secretase-mediated cleavage of EphA4 following NMDA-activated calcium influx.

**Conclusion.** The unbiased screen identified three signaling pathways of interest. One is involved in dendrite spine formation (EphA4/CaMK4β/PKG1) and the two others in neuronal death/survival (DLK/MST1; TrkA/c-Abl1/PDK1). We are testing the proteins in these pathways not included in the primary screens, their activation states, and their correlations with $PrP^{Sc}$ levels. In situ analyses will test relationships between the cells in which signaling is dysregulated and $PrP^{Sc}$ accumulation, vacuolation and gliosis.

## PO-085: Phenotypic characterization of cells participating in transport of prion protein aggregates across the intestinal mucosa of sheep

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The oral route is considered to be the main entry site of several transmissible spongiform encephalopathies or prion diseases of animals and man. Following natural and experimental oral exposure to scrapie, sheep first accumulate disease associated prion protein ($PrP^{d}$) in Peyer's patch (PP) lymphoid follicles. Dendritic cells are considered the first line of defense at mucosal sites, determining the fate of material crossing the gut epithelium. In this study, recombinant ovine prion protein (rPrP) was inoculated into gut loops of young lambs and the transportation across the intestinal wall studied. In particular, the immunohistochemical phenotypes of cells bearing the inoculated prion protein were investigated. The rPrP was shown to be transported across the villi of the gut, into the lacteals and submucosal lymphatics, mimicking the transport route of $PrP^{d}$ from scrapie brain inoculum observed in a previous intestinal loop experiment. The cells bearing the inoculated rPrP were mainly mononuclear cells, and multicolor immunofluorescence procedures showed that the rPrP bearing cells were professional antigen presenting cells expressing Major histocompatibility complex II (MHCII). The further investigation of cell markers used to phenotype antigen presenting cells in sheep showed that rPrP bearing cells labeled with CD205, CD11b and the macrophage marker CD68, but not with the dendritic cell markers CD11c and CD209. The CD68 molecule is a marker of lysosomes useful to identify monocytes and macrophages. Macrophages ingest $PrP^{d}$ in early and late stages of disease. Both in murine scrapie and in sheep scrapie $PrP^{d}$ accumulates in lysosomes.We found rPrP to be transported

in the same manner as $PrP^{d}$ from scrapie infected brain inoculum. Furthermore, the cells associated with uptake of rPrP were MHCII positive antigen presenting cells co-expressing CD68, CD205 and CD11b.

## PO-086: Protective function of physiological shedding of the prion protein in prion disease

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The cellular prion protein ($PrP^{c}$) plays a fundamental role in prion disease, where it is converted to a pathogenic form ($PrP^{Sc}$) and $PrP^{Sc}$ is an essential component of prion-infectivity. $PrP^{c}$ is a glycosylphosphatidylinositol (GPI)-anchored protein with two variably occupied N-glycosylation sites, expressed mainly in neurons. These postranslational modifications are of outstanding importance in $PrP^{c}$ function and in conversion to $PrP^{Sc}$.[1] Cells expressing anchorless $PrP^{c}$ are resistant to prion infection and transgenic animals expressing anchorless $PrP^{c}$ show a delayed onset of disease and atypical clinical and neuropathological features.We and others recently identified the A-disintegrin-and-metalloproteinase 10 (ADAM10) as the main sheddase of $PrP^{c}$.[2] Mice lacking this protease in neural precursor cells died perinatally and showed a posttranslational accumulation of $PrP^{c}$.[3] Furthermore, shedding of $PrP^{c}$ was absent in primary neurons derived from these animals and could be rescued by genetic re-introduction of ADAM10. However, the influence of physiological shedding on the course of prion disease remains enigmatic. We are currently investigating this issue in vivo using a mice lacking ADAM10 in neurons of the forebrain. Upon infection with prions, these mice show a significant reduction in incubation times when compared with controls. Data from neuropathological and biochemical analysis as well as bioassays favor a model in which ADAM10-mediated shedding plays a protective role by lowering the substrate for conversion at the plasma membrane rather than supporting the spread of infection by production of anchorless prions.

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## PO-087: Dendritic spine density in prion-infected cerebellar organotypic cultures decreases 4–5 weeks after infection, analogous to spine loss in vivo

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**Introduction.** Early treatment is the goal in prion disease therapy, therefore understanding early pathogenesis of the disease is essential. How $PrP^{Sc}$ leads to pathology is unclear, but the earliest abnormalities include the development of dendritic varicosities and the loss of dendritic spines in neurons, occurring 4–5 weeks after initial detection of $PrP^{Sc}$ in mice. Functionally, dendritic spines are associated with synaptic learning, and this too is decreased early in prion disease. Spine loss also co-localizes to areas of prion protein pathology. This study uses the prion organotypic slice culture assay as an ex vivo model of prion disease pathogenesis to determine if dendritic spines are altered in a manner analogous to that seen in vivo.

**Materials and Methods.** Eleven day old tga20 mouse pups were sacrificed and cerebellar slice cultures were prepared as described previously. Three mice were used for each time point, with 3 representative images from each mouse. Slices were treated with 1ug/mL brain homogenate from uninfected mice or mice infected with RML strain prions. Long-term culture viability was confirmed with propidium iodide (PI). Infection was confirmed by western blotting for Proteinase K-resistant PrP using SAF83. For spine analysis, slices were fixed and stained with PI, DAPI, and anti-calbindin, which selectively labels Purkinje cells. Confocal images were taken using a Zeiss LSM 700, deconvolved using ImageQuant X, and spines were identified and analyzed using Imaris 7.1.1 software.

**Results.** The earliest PK-resistant material was detected at day 14 and remained detectable, increasing in amount, for the duration of the study, 69 d. Slice architecture was preserved, although the slice thickness (350 mm at start) decreased over time. During the first 7–10 d of culture, infected and uninfected slices were more opaque in appearance, but by day 10–14, all slices became more translucent, an appearance they maintained throughout the rest of culturing. At day 14, infected and uninfected slices had similar spine densities of 2.45 ± 0.32 and 2.55 ± 0.44 spines/micron respectively. At day 21, uninfected slices stabilized at 2.0 ± 0.3 spines / micron and remained at this level for the remainder of the study, out to 69 d. In contrast, infected slices demonstrated an increased spine density of 2.85 ± 0.49 at day 21, before returning to the levels seen in the uninfected slices the following week. At day 49, 4–5 weeks after initial detection of PK-resistant PrP, the infected slices started to show a drop in spine density, reaching a significantly different density of 1.38 ± 0.17 spines/micron by 63 d and then 1.06 ± 0.05 spines/micron by day 69.

**Conclusion.** Prion-infected tga20 mouse cerebellar slice cultures recapitulate the early signs of prion disease pathogenesis, with loss of spine density beginning 4–5 weeks after detection of PK-resistant PrP. Interestingly, an increase of spines is

temporarily seen in infected cultures coincident in timing to the first detection of PK-resistant PrP. Further studies are underway to characterize inflammatory and synaptic pathways that may be active during these stages.

## PO-088: Decoding pre-clinical genomic programs in prion disease reveals in vivo evidence for the early induction of nmda receptor-mediated signaling

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In prion diseases neurons ultimately undergo necrosis and apoptosis, yet the up-stream pathways which trigger the damage and dysfunction of nerve cells are as yet unidentified. Assessing the global transcriptome patterns provides a way to unravel the molecular pathobiology of prion diseases by identifying perturbed networks during the illness. We performed a thorough high-throughput microarray screen for genes specifically altered in the hippocampal CA1 region of infected mice and determined a clear temporal genetic response to the challenge of replicating prions.

The earliest transcriptional changes within neurons were of particular interest and we identified a subset of ~400 genes dysregulated in CA1 neurons during pre-clinical disease.Many of these genes were novel to prion disease, and their biological functions in healthy and/or diseased neurons not well annotated. Comparison with published studies did however reveal striking similarities to transcriptional profiles generated from neurons in response to N-methyl-D-aspartate receptor (NMDAR) stimulation.In particular the strongest correlation in early stages of disease was to a genetic profile triggered by nuclear calcium signaling;[1] we identified 97/185 of these genes as being altered over 2-fold during prion disease. Of the remainder, 22 were below the detection threshold and 21 were not represented on our array. These included genes with putative functions in death/survival such as GADD45β GADD45γ and NR4A1, genes involved in nuclear calcium signaling such as BTG2, BCL6 and CAMKIV and novel genes such as HOMER1, a scaffolding protein that interacts with post-synaptic density proteins, and TRIB1 also proposed to be an adaptor or scaffold protein. In a second independent experiment we were able to validate 28/32 chosen genes as being deregulated in CA1 hippocampal neurons early in disease by qRT-PCR confirming these findings.

Mounting evidence suggests that the subcellular location of NMDARs determines the neuronal genomic response to $Ca^{2+}$ entry. Synaptic NMDARs are believed primarily to be transducers of signals promoting a neuroprotective genetic cascade, whereas $Ca^{2+}$ influx through extra-synaptic localized NMDARs appears to directly oppose these effects, and to promote cell death. The transcriptional changes we have identified within neurons appear to reflect the early induction of a genomic survival program induced by synaptic dysfunction leading to the enhancement of calcium signaling in the nucleus.This genetic program appears to be lost later in disease, prior to the onset of clinical symptoms. Treatments to enhance these signaling pathways may represent novel therapies to combat neurodegenerative conditions.

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## PO-089: Experimental atypical BSE—Determination of impact of challenge route and age on clinical presentation and neuropathology

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**Background.** Recently, two novel forms of bovine spongiform encephalopathy (BSE), termed atypical BSE, have been reported in a number of countries. Compared with classical BSE, these new forms show differences in the immunobiochemical characteristics of the prion protein and seem to occur in older animals. In addition, distribution of histopathologic changes and $PrP^{Sc}$ accumulation shows differences between the classical and atypical forms. However, knowledge of clinical presentation is incomplete or absent.

**Objective.** To determine the potential impact of challenge route and age at inoculation on the intraspecies transmission of atypical BSE.

**Materials and Methods.** Hereford/Angus cross animals were divided into 3 different groups. Group 1 consists of calves challenged intracerebrally (ic) at ~5 mo with 1 ml of a 10% brain homogenate from Canadian C-, L-, or H- type BSE (n = 7). Group 2 consists of calves perorally(po) challenged at ~5 mo with 100 g ofbrain homogenate from experimental cases of German L- or H-type BSE (n = 7). Group 3 consists of animals perorally challenged at ~15 mo with 100 g of brain homogenate from experimental cases of Canadian C- or German L- or H-type BSE (n = 10). Each group contains a single similarly sham inoculated control animal.Animals were monitored for clinical disease using a standardized examination protocol.Incubation period, description and progression of clinical signs were recorded and videotaped for objective evaluation.Once definite and progressive clinical disease manifests, animals will be euthanized.Selected brain regions will be tested using IDEXX-HerdCheck EIA, histopathology and immunohistochemistry with mAbs F99 and 6H4.

**Results and Discussion.** All ic H- and L- type animals displayed clinical signs at ~11 mo post inoculation with a slow and constant disease progression. Common clinical signs included hesitation at doors and gates; spontaneous muscle fasciculation; and sensitivity to touch. Teeth grinding and excessive salivation

occurred infrequently. L-type animals were very anxious and showed high levels of sensitivity to hand movement. One H-type animal showed periods of somnolence. Both H-type animals go down during handling with difficulty to rise and some sensitivity to movement in head and neck areas, but less than the L-type animals. In ic C-type BSE, clinical abnormalities started at approximately 18–20 mpi. Clinical disease progressed slowly and intermittently during the initial stages; and was similar to previously reported. Animals challenged po are at 28 and 19 mpi respectively and remain clinically normal at this time. Incubation periods following ic challenge are shorter for H- and L-type BSE as compared with C-type. Once clinical signs begin, progression is slow but relentless in atypical BSE, and more insidious in C-type BSE. A summary of clinical signs of the 3 different BSE types will be presented, and video clips of clinical disease will be displayed, alongside with the neuropathologic comparison and $PrP^{Sc}$ detection by immunohistochemistry and rapid test.

## PO-090: Delay in prion disease incubation time in transgenic mice with a microRNA-146a gene deletion

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Increasing evidence supports the involvement of microRNAs (miRNAs) in inflammatory and immune processes in prion neuropathogenesis. MiRNAs are small, non-coding RNA molecules which have recently emerged as key regulators of numerous cellular processes. However, their importance in the development of disease processes has yet to be fully realized. Lately we established that significant overexpression of miR-146a in prion-infected mouse brain tissues is concurrent with the onset of prion deposition and appearance of activated microglia. *MiR-146a* expression is enriched in immune tissues, and can be induced in various immune cells upon their maturation and/or activation. The recent creation of transgenic mice with a targeted deletion in the *miR-146a* gene have established miR-146a as an important negative regulator of inflammation myeloid cell proliferation, and cancer.[1,2] A significant delay in the incubation time was seen in transgenic mice inoculated by the intraperitoneal route, but not when inoculated by the intracerebral route. Dendritic cells and macrophages as well as brain microglia express mature miR-146a at relatively high levels. Deletion of miR-146a causes these cells to be hyper-responsive to immune activation. Microarray analysis also predicts a regulatory role for miR-146a in cellular morphological changes in microglia, as well as phagocytic mediators of the oxidative burst.[3] We hypothesize that miR-146a ablation may lead to alteration in the ability of phagocytes to capture and transport prions after inoculation. Consequently, the neuroinvasion process and incubation time of prion disease were delayed. These results confirm the importance of miRNAs in the development of disease, and corroborate our hypothesis that miRNA expression is a potential target for manipulation as new means for therapeutic

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## PO-091: A decreased expression of pre-synaptic markers in neurons in differentiated neurospheres infected with BSE-derived prion strain

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**Introduction.** We have reported that differentiated neurosphere cultures containing neurons, astrocytes, oligodendrocytes, NG2-positive cells and nestin-positive cells are susceptible to many prion strains and that $PrP^{Sc}$ was preferentially found in GFAP-positive cells and lesser incidence, in neurons. To further characterize the neurosphere cultures infected with prions, we analyzed the intracellular localization of $PrP^{Sc}$ in GFAP-positive cells and expression of pre-synaptic markers in differentiated neurospheres.

**Methods.** Neurospeheres were prepared from fetus brains of ICR/Jcl mice at embryonic day 14. Differentiation of neurospheres was initiated by the withdrawal of growth factors and addition of B27 supplement. Brain homogenates of mice infected with the Chandler, Obihiro, 22L, G1, Fukuoka1 and KUS prion strains were used for the infection of neurospheres. The KUS strains were derived from BSE cattle after serial passage in C57BL6/J mice. 10 to 30 d after differentiation, 0.1% brain homogenates were added to the neurosphere cultures and the cultures were kept up to 15 to 40 d post inoculation. Neurosphere cultures were fixed and subjected to multiple immunofluorescent staining with mAb 132 for specific detection of $PrP^{Sc}$, astrocytes (anti-GFAP antibody) or neuronal markers (a mixture of anti-MAP2 and anti-Î²III tubulin antibodes), and various organelle markers or pre-synaptic markers.

**Results and Discussion.** In the GFAP-positive cells, $PrP^{Sc}$ were co-localized well with Flotillin and Lamp1, indicating that $PrP^{Sc}$ in GFAP-positive cells were mainly present in late endosomes and lysosomes.In addition, some $PrP^{Sc}$ granular stains were found to be merged with the Rab5a or Rab11a stains. This suggests that $PrP^{Sc}$ also exists in early endosomes and recycling endosomal compartments. During the extensive analysis, we observed a tendency that $PrP^{Sc}$ was found in soma as well as neuronal dendrites of MAP2 and βIII-positive neurons in BSE-derived prion strain KUS infected neuroshepre cultures when KUS prions

were inoculated at 30 d after differentiation. Thus we analyzed the expression of pre-synaptic markers in the $PrP^{Sc}$-positive and $PrP^{Sc}$-negative neurons in the KUS prion-infected neurpspheres. Among $PrP^{Sc}$-negative neurons, around 80% neurons were positive for synaptophysin; however, among $PrP^{Sc}$-positive neurons, only about 40% neurons were positive but the remaining neurons were negative for synaptophysin. The similar tendency was observed for the expression of α-synuclein and SNAP25.These results suggest that the expression of several pre-synaptic markers may be affected by the infection of KUS prion strain. There is few cell culture models that can reproduce neuronal degeneration by prion infection so far. The decreased expression of pre-synaptic markers in KUS prion-infected neurons in differentiated neurosphere cultures may provide a cell culture model of neurodegeneration caused by prion infection.

## PO-092: Dendritic cell interaction with $PrP^{Sc}$—Activation, inhibition or no response?

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Dendritic cell (DC) interaction with infectious prion protein ($PrP^{Sc}$) appears to be an important part of prion pathology. DCs are specialized antigen presenting cells that play a critical role in the host immune response by regulating the stimulatory and tolerogenic functions of B and T-cells to antigens. Upon activation by a foreign antigen, DCs secrete specific cytokines and alter the expression of surface markers involved in the immune synapse. This allows the DC to elicit appropriate stimulation of other cells involved in the immune response. Previous studies have shown that DCs interact with $PrP^{Sc}$; however, no specific immune response against $PrP^{Sc}$ is observed. DCs infected by $PrP^{Sc}$ might alter the normal function of the DC to trigger an immune response, contributing to the lack of an immune response against $PrP^{Sc}$. We intend to determine the initial response of the DC when it is exposed to $PrP^{Sc}$. We hypothesize that $PrP^{Sc}$ interaction with DCs will alter the normal function of the DC to elicit an immune response. $PrP^{Sc}$ may cause DCs to become activated or inhibited; or alternatively they may not respond.

The toll-like receptors (TLRs) are one type of pathogen recognition receptors used by DCs to recognize pathogens. Upon TLR activation, DCs produce inflammatory cytokines TNFα and IL-12, alternatively, they can produce the regulatory cytokine IL-10 when exposed to an inhibitory signal. In addition, activated DCs also increase the expression of the co-stimulatory markers CD40, CD80 and CD86, whereas an inhibitory signal causes no increase in these markers. We exposed bone-marrow dendritic cells (BMDC) derived from C57/Bl6 mice to various concentrations of mouse-adapted ME7 scrapie, then measured cytokine production by ELISA. We show that ME7 prions are taken up by BMDCs as early as 24 h after exposure to infected brain homogenates, however, there was no detectable production of TNFα, IL-12 or IL-10. This demonstrates that $PrP^{Sc}$ does not activate BMDCs via TLR pathways. Further studies are needed to determine whether ME7 is having an inhibitory effect on DCs. BMDCs will be exposed to ME7, followed by treatment of a known activator of DCs (LPS), and analyzed for the production of cytokines using ELISA. In addition, possible changes in surface marker expression will be examined using immunofluorescence.

We show that DCs are not activated upon exposure to $PrP^{Sc}$ since there is no detectable TNFαor IL-12 production after exposure to ME7. In addition, there appears to be no regulatory function induced in the DCs since no IL-10 is detected upon exposure to ME7. The lack of cytokine response may mean that the TLR pathway is not involved in $PrP^{Sc}$ infection and that $PrP^{Sc}$ may interact with DCs using an alternate pathway.Further work will determine if $PrP^{Sc}$ affects the DC's ability to respond to other antigens.

## PO-093: The significance of pre-clinical miRNA expression levels in an animal model of prion disease

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Prion diseases are caused by the conversion of the normal prion protein ($PrP^{C}$) to the infectious form ($PrP^{Sc}$) and with time, the accumulation of $PrP^{Sc}$ leads to disease. All prion diseases exhibit three hallmark phenotypic changes as a result of $PrP^{Sc}$ buildup: neuronal dysfunction and loss, plaque deposition, and spongiform formation. Nevertheless, it remains unclear how $PrP^{Sc}$ causes the observed neuronal dysfunctions. Therefore, investigating molecular changes that occur early in disease, when $PrP^{Sc}$ is beginning to accumulate, may hold promise in identifying these disease-related pathways. Only a small proportion of neurons are affected by the presence of $PrP^{Sc}$ very early in prion infection, making the analysis of whole brain samples insensitive to detect these changes. Therefore, we chose to specifically select a neuronal-dense region to assess neuronal-specific molecular changes in response to the increasing presence of $PrP^{Sc}$.

MicroRNAs (miRNAs) are a major class of post-transcription gene regulators that function in two main capacities: as rheostats that fine tune a few targets whose levels are critically important for function and as molecular switches by regulating vital transcription factors which govern entire pathways. Not surprising, deregulation of miRNAs has been implicated in numerous diseases such as cancer, viral diseases and neurodegenerative disorders including prion disease. Understanding the miRNA expression profiles in early prion disease may elucidate mechanisms of prion-induced neuronal death.

To this end, we removed the hippocampal CA1 region from scrapie (RML) infected and control mice using laser capture microdissection at six different time points: 40, 70, 90, 110, 130 and ~160 (end-point) days post inoculation. RNA was extracted

and samples were screened for miRNA expression levels using the TaqMan low density array (TLDA) platform and further validated using individual real-time PCR assays. We found numerous miRNAs to exhibit diverse temporal expression patterns during prion disease where many were either upregulated early or upregulated late. miRNA target prediction programs were employed to curate a list of potential targets for candidate miRNAs dysregulated early in prion disease and this list was further refined using mRNA microarray data. The significance of the pre-clinical miRNAs identified in our animal model system for prion disease will be presented.

## PO-094: Dystrophic neurites accumulating autophagic vacuoles show early stages of neuritic destruction

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The ultrastructural pathology of rodent models of human prion diseases are characterized by spongiform change, dystrophic neurites containing abundant autophagic vacuoles, lysosomal dense bodies and the presence of tubulovesicular structures (TVS), the disease-specific particles of unknown significance. Recently, a role of autophagy in Drosophila flies transfected with a construct encoding Ab42, a major amyloidogenic peptide of Alzheimer disease was published and it demonstrated the presence of dystrophic neurites, not unlike those seen in AD filled with autophagic vacuoles and lysosomal electron-dense bodies, and showing areas of the cytopasmic clearance. The latter finding suggests the leakage of lysosomal enzymes form autolysosomes that initiate degeneration.

The latter publication prompted us to re-examine the electronmicrographs from Echigo-1-infected hamsters to look for the cytoplasmic clearance, not unlike of those of Drosophila model. We reevaluated photographed dystrophic neurites for the presence of cytoplasmic clearance as showed in transgenic fruit flies transfected with Ab42. In several neurites, we found electron-lucent areas not bound by any membranes or only partially bound; which means they were not autophagic vacuoles as the latter are membrane-bound. In some neurites, that could be traced over several sections, the electron-lucent areas were evident to change size, i.e., to expand. In addition, we found some membrane-bound autophagolysosomes which protrude filopodia like structures in a process of engulfing a target.

To evaluate if observed changes are common among other models of TSEs we used hamsters infected either with 263K or 22C-H strains of sections. Blocks that were already examined, were serially sectioned into 5 consecutive grids and dystrophic neurites with signs of clearance were searched for. We observe similar changes and we conclude that leakage of the lysosomal enzymes from neurites undergoing autophagy may initiate destruction of the brain in prion diseases.

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