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Prion workshop

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Abstracts

On the trail of prions: PrP^{Sc} routing to muscles and differentiation of TSE strains

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PrP^{Sc}, the biochemical marker for infectious agents causing transmissible spongiform encephalopathies (TSEs), or prion diseases, has been detected in muscle tissue of scrapie infected hamsters and sheep prior to the onset of clinical symptoms. Studies in rodent models for scrapie and BSE provide further insights into the routing of TSE agents through the body and will contribute to a better assessment of the risks for public health possibly emanating from “Prions in skeletal muscle”. Recently, the occurrence of BSE in small ruminants under natural conditions has been confirmed for the first time after strain typing of a suspect case of caprine TSE in France. If caprine and ovine BSE is present in the field and if muscles of goats and sheep infected with BSE provided reservoirs for infectivity, a stringent epidemiological surveillance and reliable strain differentiation of TSEs in small ruminants would be of great importance for consumer protection. However, the discrimination between scrapie and BSE in sheep and goats is not straightforward and still constitutes a substantial diagnostic challenge. We addressed this problem by structural characterization of pathological prion protein using Fourier transform-infrared (FT-IR) spectroscopy, which appears as a promising tool for molecular strain typing without antibodies and without restriction to specific TSEs or mammalian species.

The role of PrP’s N-glycans in the protein’s cellular location and biochemistry

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Host encoded PrP is central to the transmissible spongiform encephalopathies (TSE). Indeed the disease associated form of the protein, PrP^{Sc}, has been proposed to be the TSE agent. Highly conserved N-glycosylation of PrP occurs at two sites (N^{180} and N^{196} in mice). However glycosylation appears to be variable such that un-, mono- and di-glycosylated forms of the protein are observed *in vivo*, the importance of these different forms is currently unknown. To address the role of PrP glycosylation in TSEs and the normal function of the protein, mice with amino acid substitutions that prevent N-glycan addition have been produced by gene targeting. These mice have an altered pattern of glycosylation of PrP but have no overt alteration of phenotype. Here we present data on the *in vivo* cellular location and biochemistry of mono- and un-glycosylated PrP, in our transgenics. The cellular localisation of PrP is altered compared to that of the wild type protein, suggesting a functional role for the glycans. However, lack of N-glycans does not appear to alter the biochemical properties PrP, such its solubility or proteinase K resistance. Thus lack of glycosylation *in vivo* does not lead to the spontaneous formation of the disease associated form PrP^{Sc}.

Uptake and processing of prion proteins during the early stages of TSE pathogenesis

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Transmissible Spongiform Encephalopathies (TSEs) are prolonged neurodegenerative diseases of the central nervous system (CNS). Despite the clinical and commercial importance of TSEs, much of what happens in the periphery following exposure to infectious prion material (PrP^{sc}) remains poorly understood. After crossing the intestinal barrier, PrP^{sc} infectivity is initially found in association with follicular dendritic cells in the germinal centres of lymphoid organs, prior to detection in the CNS. Recently, an important role for migrating intestinal dendritic cells (DC) in the transportation of PrP^{sc} has been demonstrated. However, it is still unclear how DCs acquire PrP^{sc} from the intestinal lumen.

In this study, we have used a combination of *in vitro* and *in vivo* models, to investigate how early interactions between PrP^{sc} and host immune cells contribute to prion pathogenesis in the rodent model. We have shown that caecal, but not ileal, M cells take up PrP^{sc} in significant amounts following oral infection. Furthermore, we have investigated the role that DCs, and different subsets thereof, play in the uptake and transport of prions. We present our methodology and preliminary results detailing the importance of initial interactions between PrP^{sc} and host cells in the processing of prions from the intestine to regional lymph nodes.

Cellular targeting of infection in the peripheral lymphoid system in mice infected with different TSE strains

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Although TSE infection results in dramatic pathological changes in the central nervous system (CNS) replication of infectivity also occurs in spleen and lymph nodes early in the disease. In previous studies using the ME7 mouse-passaged scrapie strain we have shown that mature follicular dendritic cells (FDCs) of the spleen support replication and are critical for neuroinvasion following moderate dose peripheral challenge. However, studies elsewhere using other mouse-passaged TSE isolates have suggested that other cell types may support replication in lymphoid tissues and/or facilitate neuroinvasion.

Using mice with immune system defects we tested the hypothesis that different TSE strains may target different cell types of the immune system. Immunodeficient (µMT and TNFR-1$^{-/-}$) and immunocompetent (C57BL/6) mice were challenged peripherally with four experimental TSE strains (79A, 139A, ME7 and the BSE derived strain 301C). Pathogenesis of disease and replication of infectivity in spleen was impaired in TNFR-1$^{-/-}$ and µMT mice following peripheral challenge with all TSE strains tested.

As TNFR-1$^{-/-}$ mice lack mature FDCs but have normal B and T cell populations this would suggest that B cells are not directly responsible for neuroinvasion in the TSE strains tested and show that functional FDCs are required for normal pathogenesis. This finding is also supported by studies in µMT mice, which lack B cells and as a result functional FDCs. This provides further evidence for the involvement of FDCs, not just in the pathogenesis of the ME7 strain of scrapie but in a range of TSE strains.

Comparative evidence for a link between Peyer's patch development and susceptibility to transmissible spongiform encephalopathies

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The incidence of natural cases of transmissible spongiform encephalopathies (TSEs) is related to age with the majority of cases occurring in young individuals. There is considerable evidence for the involvement of Peyer's patches and associated lymphoid follicles in orally transmitted TSE infection. In young sheep, cattle and humans, ileal Peyer's patches are the major component of the gut-associated lymphoid tissue. Involution of these patches occurs at around puberty in all three species. Our hypothesis is that although the relationships between Peyer's patch development and age and between susceptibility to TSE infection and age differ in sheep, cattle and humans, there should still be a correlation between Peyer's patch development and susceptibility for each species. In this study, specimens of terminal ileum were collected from NPU Cheviot sheep of different ages and mixed genotypes. Image analysis software was used to calculate areas of intestine and Peyer's patch tissue. Lymphoid follicle density was recorded as the average number of lymphoid follicles per cm^2 of ileum. Peyer's patch data for cattle and humans were obtained from previous studies. The age-susceptibility function for sheep was determined using a recently published method that derived the age risk function for vCJD in humans. Estimates of the relative risk of BSE infection and vCJD infection were obtained from previously published results.

A comparative analysis revealed a significant correlation between Peyer's patch development and estimated risk of infection for sheep, cattle and humans. This implies that knowledge of exposure and PrP genotype are not, by themselves, sufficient to identify which individuals are most at risk of TSE infection; age per se must also be taken into account.

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Expression of PrP^C on cellular components of blood in sheep

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Transmissible spongiform encephalopathies (TSEs) or prion diseases are neurodegenerative diseases that occur in a variety of species, including sheep (scrapie), cattle (bovine spongiform encephalopathy - BSE) and humans (e.g. Creutzfeld-Jacob disease - CJD). A characteristic feature of these diseases is the accumulation of PrP^{Sc}, a post-translationally modified form of the host glycoprotein, PrP^C, in the central nervous system (CNS). PrP^C is expressed in many tissues other than the nervous system, including the lymphoid system, although its precise function(s) remains unclear. Experiments in PrP null mice, which are resistant to TSE infection, highlight a key role of PrP in the pathogenesis of TSE disease. We have previously demonstrated that TSEs can be transmitted by blood transfusion in sheep, and plan to identify which components of blood carry infection. As an initial step, we examined the distribution of PrP^C on cellular components of sheep blood to identify potential targets for infection. Peripheral blood mononuclear cells (PBMC), granulocytes, erythrocytes and platelets were separated by density gradient centrifugation. Fractions were then analysed for PrP^C using either western blotting or flow cytometry. Cell surface expression of PrP^C was found only on PBMC; however, platelets also contained significant amounts of

intracellular PrP^C. The level of PrP^C expressed on the cell surface of PBMC was influenced by PrP genotype, with the highest levels found in scrapie-susceptible sheep (PrP genotype VRQ/VRQ) and the lowest levels in scrapie-resistant (ARR/ARR) sheep. In susceptible sheep, PrP^C was expressed at varying levels on all major subsets of PBMC, with the highest levels on the $CD21^+$ subset of B cells, which recirculate through the lymphatic system. PrP expression was also upregulated on $CD21^+$ B cells in some scrapie-infected sheep compared with uninfected sheep of the same genotype. Since we did not have access to monoclonal antibodies that can distinguish PrP^{Sc} from PrP^C, it was not possible to determine whether this was due to accumulation of abnormal PrP^{Sc}, or upregulation of the expression of normal PrP^C. These findings provide valuable baseline data for studies on the distribution of infectivity and PrP^{Sc} in the blood of TSE-infected sheep, and may also contribute to understanding the function of PrP^C and the basis for genetic susceptibility to scrapie.

The implications of new findings for future predictions of the UK vCJD epidemic

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The UK epidemic of vCJD has so far claimed 147 lives. Until 2 years ago, the number of new deaths had been declining steadily from its peak of 28 cases in 2000, down to 8 in 2004. This has led to model projections of small numbers of future cases (e.g. Ghani et al. 2003). However, recent results challenge some of the key assumptions about vCJD used in mathematical and statistical models for predicting the size of epidemic. A recently published study found the prevalence of vCJD infection to be 235 per million, a much higher figure than had been expected (Hilton et al. 2004). In particular, it has been assumed that everyone infected will eventually develop clinical vCJD and die, and that only those people who are methionine homozygous at codon 129 of the prion protein gene are susceptible. We shall talk about how we extended previous models for the vCJD epidemic to relax these assumptions, and the implication this had for predictions of the epidemic size (Clarke and Ghani 2005). Furthermore, following evidence of individuals contracting vCJD through blood transfusion (Llewelyn et al. 2004; Peden et al. 2004), we shall also talk about modelling a potential secondary vCJD epidemic via blood transfusion and introduce mathematical models to predict its size.

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The prophylactic potential of oral pentosan polysulphate in animal TSE models

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vCJD differs from other human TSEs in that infectivity is not confined to the CNS but is disseminated throughout the lymphoid system. Blood transfusion, blood products, re-used surgical instruments and cell and tissue transplantation present potential vCJD transmission risks. At present there is no practical treatment that can be offered to people who may have been exposed to vCJD. We, and others, have shown that high dose injectable polyanions, such as pentosan polysulphate (PPS), significantly increase survival time and reduce disease incidence when given to rodents within weeks of exposure to normally lethal TSE doses. However, PPS is an anti-coagulant and has a variety of other systemic effects which makes long term parenteral use undesirable.
Oral PPS administration is licensed in the US for the treatment of interstitial cystitis and is well tolerated long term. However oral delivery was thought to result in little, if any, systemic uptake of PPS. We report that a single high dose oral PPS bolus can reduce disease incidence and increase survival time in mice following normally 100% lethal oral scrapie exposure. Multiple lower dose oral administration increases survival time even after high dose parenteral scrapie exposure. Survival time also increases when more clinically relevant PPS doses are multiply administered orally to mice orally exposed to scrapie. In addition, PPS given orally increases survival time after high dose intravenous BSE. Long term administration of oral PPS may therefore have some benefit in cases where low dose TSE contamination is thought to have recently occurred, and without the toxicity associated with other drugs.

Novel immunisation strategy for the generation of therapeutically-relevant immune responses for the treatment of CJD

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Creutzfeldt-Jakob Disease (CJD) is a relatively rare form of human dementia presenting as a familial, sporadic or iatrogenic disease. The disorder is part of a family of related diseases, the transmissible spongiform encephalopathies (TSEs), characterised by a progressive and invariably fatal neurodegeneration associated typically with a spongiform brain pathology at post-mortem. The emergence of a variant form of the disease (vCJD) presenting in younger individuals, possibly due to ingestion of prion-contaminated meat products has emphasised the need for novel therapeutic strategies

Using a novel prime-boost peptide immunisation strategy we have generated high titre antibodies against specific domains of the prion protein. In BSE-301V VM mouse bioassays this strategy has generated a short, but statistically significant extension to lifespan using a very difficult post-exposure challenge model. This extension to lifespan was accompanied by a change to the plaque profile in brain sections suggesting that the immunisation was having a therapeutically relevant effect on the accumulation of PrP^{Sc} in the brain. Characterisation of the immune response generated by the novel strategy and the results of ongoing experiments to refine and improve on the therapeutic effect will be presented.

Use of a new immunoassay reveals PrP genotype-specific differences in the level of PrP^{Sc} deposition in sheep brain

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The diagnosis of prion diseases has traditionally relied upon the identification of the disease-associated form of the prion protein, PrP^{Sc}, based on its resistance to digestion by proteinase K (PK). A recent development is the conformation-dependent immunoassay (CDI), which distinguishes between PrP^{Sc} and PrP^{C} based on their differing solubility in guanidine hydrochloride rather than resistance / sensitivity to PK. Using the CDI-format, we have developed a sandwich immunoassay for measuring PrP^{Sc} in sheep brain. The immunoassay was calibrated using full-length recombinant ovine PrP purified from *E. coli*., and the monoclonal antibodies FH11 (epitope spanning residues 54-57 PrP molecule) and europium-labelled 8H4 were used for antigen capture and detection respectively. The assay was applied to brain homogenates from Poll Dorset sheep (PrP genotypes VRQ/VRQ, VRQ/ARQ and VRQ/ARR) infected with an experimental scrapie source (SSBP/1). The incubation periods of VRQ/VRQ, VRQ/ARQ and VRQ/ARR sheep infected with SSBP/1 were 151 ± 19, 196 ± 15 and 220 ± 26 days post inoculation respectively. Using the immunoassay, clinically affected VRQ/VRQ and VRQ/ARQ sheep could be distinguished from uninfected controls of the same PrP genotypes. In addition, we have shown that PrP genotype appears to have a significant influence on the amount of PrP^{Sc} deposited in the brains of sheep experimentally infected with scrapie, with the highest levels found in VRQ/VRQ sheep and the lowest in VRQ/ARR sheep. Infected and uninfected sheep with the VRQ/ARR genotype could not however be distinguished using our immunoassay, despite the fact that all infected sheep were positive for PK-resistant PrP^{Sc} by Western blot. This may be partly due to the small numbers of animals tested, but suggests that the assay might not be sufficiently sensitive to discriminate infected from uninfected sheep of all genotypes in its current format. We aim to increase the sensitivity of this assay by testing a new panel of monoclonal antibodies (generated against truncated ovine PrP) for antigen capture and detection. In conclusion, we have developed an immunoassay for the detection and quantification of PrP in sheep brain homogenates. The method should also be readily applicable to non-neuronal tissues, such as lymphoid tissues. Our findings have potentially important implications for understanding how PrP genotype controls susceptibility and resistance to TSEs *in vivo*, as well as for the further development of diagnostic tests for scrapie.

Is PrP^{Sc} Associated with TSE Infectivity?

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The Prion Hypothesis predicts that the aetiological agent of the Transmissible Spongiform Encephalopathy diseases is an abnormally folded isoform of a host glycoprotein PrP. This abnormal proteinase K (PK) resistant isoform (PrP^{Sc}) is deposited in infected tissue, and co-purifies with infectivity. Definitive diagnosis of TSE disease can only be obtained by transmission studies to mice or other mammals. However these experiments are expensive and time consuming. Current diagnostic methods are therefore based on the detection of PK-resistant PrP^{Sc} in post mortem brain tissue, and its identification is taken as indicative of the presence of TSE infectivity. The relationship between PrP^{Sc} and TSE infectivity is however still unclear, and questions the reliability of such diagnostic methods. We have identified two mouse models of TSE disease in which high titres of infectivity are associated with low or undetectable levels of PrP^{Sc} in brain tissue, as measured by immunoblot and DELFIA. PrP in these mice appears to display the same detergent solubility and PK sensitivity as normal PrP (PrP^{C}), despite the presence of high levels of the infectious agent. We aim to utilise these models to investigate the true nature of the TSE infectious agent, and assess current commercial TSE diagnostic assays.