ISNV Symposium Session V

Prion detection and transmissable encephalopathies

I.1

Molecular pathology of prions

Adriano Aguzzi
Universitatsspital Zurich, Institute of Neuropathology, Department of Pathology, Schmelzbergstrasse 12, Zurich, CH-8091, Switzerland

Prions have been responsible for an entire century of tragic episodes. Fifty years ago, Kuru decimated the population of Papua New Guinea. Then, iatrogenic transmission of prions caused more than 250 cases of Creutzfeldt-Jakob disease. More recently, transmission of bovine spongiform encephalopathy to humans caused a widespread health scare. On the other hand, the biology of prions represents a fascinating and poorly understood phenomenon, which may account for more than just diseases, and may represent a fundamental mechanism of cross-talk between proteins. The two decades since Stanley Prusiner's formulation of the protein-only hypothesis have witnessed spectacular advances, and yet some of the most basic questions in prion science have remained unanswered.

I.84

How does host PrP control TSE disease?

Jean Manson,[1] R Barron,[1] N Tuzi,[1] H Baybutt,[1] M Bishop,[1] Enrico Cancellotti,[1] P Hart,[1] L Jamieson,[1] L Aitchison,[1] E Gall,[1] B Bradford,[1] D King,[1] R Moore,[2] D Melton,[2] J Ironside,[3] and R Will[3]
[1]*Institute for Animal Health, Neuropathogenesis Unit, Edinburgh;* [2]*University of Edinburgh; and* [3]*CJD Surveillance Unit, Edinburgh*

PrP is central to the TSE disease process and has been hypothesised to be the infectious agent. Polymorphisms in the PrP gene of a number of species are associated with different incubation times of disease following exposure to an infectious agent and mutations in the human PrP gene can apparently lead to spontaneous genetic disease. Strains of TSE agent are proposed to be generated and maintained through differences in glycosylation or conformation of PrP and the barrier to infection between species is thought to be due to the differences in the sequence of PrP between different species.

In order to test these hypotheses, we have introduced specific modifications into the endogenous mouse *Prnp* gene by gene targeting. The mutated PrP gene is in the correct location under the control of the endogenous *Prnp* regulatory sequences and thus expressed in the same tissues and amounts as the wild type *Prnp* gene. This strategy therefore allows the effect of specific mutations in the PrP gene to be assessed.

We have introduced mutations into the *Prnp* gene which prevent glycosylation at each or both of the two N-linked glycosylation sites of PrP and have produced mice with altered PrP glycosylation. We have infected these mice with TSE agents to define the role of PrP glycosylation in strain targeting and strain determination. We have investigated the role of the sequence of the host PrP gene in determining susceptibility to TSE agents by inserting point mutations or replacing the murine PrP gene with that of human or bovine PrP and infecting these lines of mice with TSE agents from different species. We have produced a model of TSE disease which contains high levels of infectivity in the absence of PrSc and we are using this model to determine the nature of the infectious agent.

We have thus established that the gene targeting approach can produce models for TSE disease which address fundamental questions associated with these diseases. We aim to use these models to address central issues including the origin of strains, the species barrier and the nature of the infectious agent.

I.12

Poliovirus infection of prion-deficient neuronal cells

Andreina Baj,[1] Alessia Bettaccini,[1] Takuya Nishimura,[2] Takashi Onodera[2] and Antonio Toniolo[1]
[1]*Department of Clinical and Biological Sciences, University of Insubria, Varese, Italy and* [2]*Department of Molecular Immunology, University of Tokyo, Japan*

Transfection of the prion protein gene (Prnp) into prion-deficient mouse cells has been shown to reduce the replication of coxsackievirus B3. We analyzed the susceptibility to poliovirus-1 (PV-1) of a panel of murine hippocampal cell lines differing in their ability to express the Prnp gene: prionless HpL3.4 cells, HpL3.4 cells transfected with the Prnp gene, control HpL3.4 cells transfected with a void vector, wild type Hw3.5 Prnp+/+ cells. The four cell lines expressed the murine homologue (Tage4; Ravens et al, 2003) of the human poliovirus receptor (CD155/hPVR). Mice are susceptible to PV infection by parenteral routes and virus replication is associated with an apoptotic response. PV-1 infection of Prnp−/− HpL3.4 cells resulted in the production of high viral

titers, although viral antigens could be detected in only 0.5–2% of cells. Wild type Prnp+/+ cells and prion-deficient cells transfected with the Prnp gene were not permissive to PV-1. Results of viral titration and immunofluorescence were confirmed by conventional PCR and quantitative real-time PCR. Exposure to PV-1 had no influence on the gene expression profile of Prnp+/+ cells, but modified that of permissive Prnp−/− cells. Several genes were upregulated in permissive cells: type I IFN genes, IFNRD1, TNFSF13b, IL7, granulocyte/macrophage CSFs, HGF, VEGF-A, TGFb1 and b3 as well as a variety of bone morphogenetic proteins that also have neuroprotective activity. Distinction of permissive from non-permissive neuronal cells on the basis of Prnp expression suggests that infection of prion-deficient mice with poliovirus may represent a model for investigating pathogenetic events.