Experimental Physiology

Special Review Series – Gene Manipulation and Integrative Physiology

Neuroinvasion of prions: insights from mouse models

Sebastian Brandner*, Michael A. Klein, Rico Frigg, Valdimir Pekarik, Petra Parizek, Alex Raeber, Markus Glatzel, Petra Schwarz, Thomas Rülicke, Charles Weissmann and Adriano Aguzzi

Institute of Neuropathology, Department of Pathology, Schmelzbergstrasse 12, University Hospital, 8091 Zurich, Switzerland

The prion was defined by Stanley B. Prusiner as the infectious agent that causes transmissible spongiform encephalopathies. A pathological protein accumulating in the brain of scrapie-infected hamsters was isolated in 1982 and termed prion protein (PrP^{Sc}). Its cognate gene *Prnp* was identified more than a decade ago by Charles Weissmann, and shown to encode the host protein PrP^{C}. Since the latter discovery, transgenic mice have contributed many important insights into the field of prion biology, including the understanding of the molecular basis of the species barrier for prions. By disrupting the *Prnp* gene, it was shown that an organism that lacks PrP^{C} is resistant to infection by prions. Introduction of mutant *PrP* genes into PrP-deficient mice was used to investigate the structure–activity relationship of the *PrP* gene with regard to scrapie susceptibility. Ectopic expression of PrP in PrP knockout mice proved a useful tool for the identification of host cells competent for prion replication. Finally, the availability of PrP knockout mice and transgenic mice overexpressing PrP allows selective reconstitution experiments aimed at expressing PrP in neurografts or in specific populations of haemato- and lymphopoietic cells. The latter studies have allowed us to clarify some of the mechanisms of prion spread and disease pathogenesis. *Experimental Physiology* (2000) **85.6**, 705–712.

Prevalence and clinical characteristics of prion diseases

Prion diseases or transmissible spongiform encephalopathies (TSEs) are neurological disorders, characterized by dementia and movement disorder. Histopathological changes of the brain comprise a fine vacuolation, also termed spongiosis, reactive changes of astrocytes (gliosis) and variable loss of neurons. Typical examples of TSEs in humans are Creutzfeldt-Jakob disease (Creutzfeldt, 1920) and Gerstmann-Sträussler-Scheinker syndrome (Gerstmann *et al*. 1936) which were first described in the second and third decade of the 20th century. While the prototype of prion diseases in animals, scrapie in sheep and goats, has been known for more than two centuries, a new form of animal prion disease designated bovine spongiform encephalopathy (BSE) has since its first recognition in 1986 developed into an epizootic (Wilesmith *et al*. 1992; Anderson *et al*. 1996). The emergence of a new variant form of Creutzfeldt-Jakob disease (nvCJD) in young people in the UK has raised the possibility that BSE has spread to humans by dietary exposure (Will *et al*. 1996, 1999). This fearful scenario has recently been supported by experimental evidence claiming that the agent causing BSE is indistinguishable from that causing nvCJD (Hill *et al*. 1997*a*).

The molecular biology of prions

Although there is an increasing body of evidence, that the infectious agent of transmissible spongiform encephalopathies, the prion, partially or entirely consists of an abnormally folded protein (McKinley *et al*. 1983; Prusiner *et al*. 1983; Oesch *et al*. 1985), the nature of this agent is still under debate. The possibility that the agent consists of viral particles can almost certainly be ruled out, since no nucleic acids have been detected. Since prions are distinguished from conventional agents by their physicochemical properties, the protein-only hypothesis has been formulated firstly by Griffith (1967) and later, supported by additional experimental data by Prusiner (1982). He coined the term prion, composed from the words 'proteinaceous infectious agent'. The hypothesis states that the agent is devoid of nucleic acid and consists solely of an abnormal conformer of the cellular prion protein, PrP^{C}. It has been proposed that the partially protease-resistant and detergent-insoluble PrP^{Sc} is congruent with the infectious agent.

Two distinct models have been postulated to explain the mechanism by which a misfolded form of PrP could catalyse the refolding of native PrP molecules into the abnormal

Publication of *The Physiological Society*

* Corresponding author: seb@pathol.unizh.ch

conformation and both models are the subjects of intensive investigation: (i) the template-directed model, and (ii) the nucleation-seeding model (Fig. 1). The first model assumes a PrP^{Sc} monomer promoting the conformational conversion of PrP^{C}, or of a partially destabilized intermediate, into the PrP^{Sc} conformation (Prusiner, 1991). In this model PrP^{Sc} would be intrinsically more stable than PrP^{C}, but kinetically inaccessible. In the second model, formation of PrP^{Sc} would be initiated by a seed of aggregated PrP^{Sc} triggering a nucleation-dependent polymerization process (Jarrett & Lansbury, 1993). In contrast to the template-directed model, the PrP^{Sc} monomer is less stable than PrP^{C} but is stabilized upon joining to the PrP^{Sc} aggregate.

However, both models demand an experimental proof. Consistent with the nucleation model, cell-free conversion studies indicate that PrP^{Sc} aggregates are able to convert PrP^{C} into a protease-resistant PrP isoform. On the other hand, Riesner (Riesner *et al*. 1996) demonstrated that aggregates of PrP^{Sc} do not necessarily contain infectivity.

The role of PrP in prion disease

According to the protein-only hypothesis, PrP^{C} is a substrate for the PrP^{Sc}-mediated conversion of PrP^{C} into new PrP^{Sc} molecules. An important corollary to this hypothesis is that an organism lacking PrP^{C} should be resistant to scrapie and unable to propagate the infectious agent. To this end, Büeler and colleagues generated mice with a targeted disruption of the *Prnp* gene (Büeler *et al*. 1992). Although the ubiquitous expression of PrP in the developing and adult central nervous system (CNS) suggested a significant role in development and function of the CNS, the resulting mice showed no overt phenotype. In particular, they show no developmental defects, no behavioural abnormalities and have a normal life expectancy. Intracerebral inoculation of these mice with prions revealed that they were resistant and did not propagate the agent,

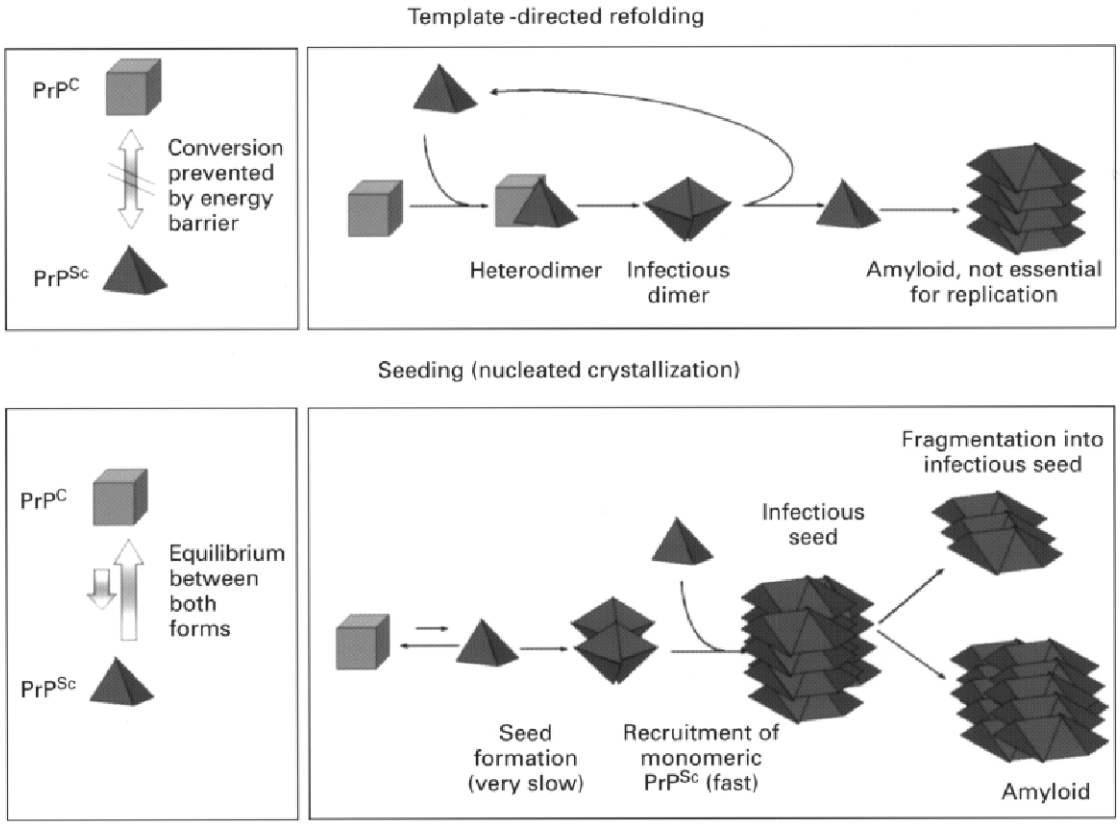

Figure 1

Models for the conformational conversion of PrP^{C} into PrP^{Sc}. Upper panel, the template-directed refolding model postulates an interaction between exogenously produced PrP^{Sc} and endogenous PrP^{C}, which is induced to transform itself into further PrP^{Sc}. A high energy barrier may prevent spontaneous conversion of PrP^{C} into PrP^{Sc}. Sporadic CJD may arise when spontaneous mutations in single cells lead to the conversion from PrP^{C} to PrP^{Sc} and give rise to manifold subsequent conversions. Lower panel, the seeding or nucleation model proposes that PrP^{C} and Prp^{Sc} are in a reversible thermodynamic equilibrium. Only if several monomeric PrP^{Sc} molecules are mounted up to a highly ordered seed, can further monomeric PrP^{Sc} be recruited and eventually aggregated to amyloid. The likelihood of spontaneous formation of a seed is a function of the local PrP^{Sc} concentration (which may be modulated by PrP^{Sc}-binding proteins) and is inversely dependent upon the number of monomers needed to form a proto-seed. Within such a crystal-like seed, PrP^{Sc} becomes stabilized. Fragmentataion of PrP^{Sc} aggregates increases the number of nuclei which can recruit further PrP^{Sc} and therefore results in apparent replication of the agent.

thereby verifying the prediction of the protein-only hypothesis (Büeler *et al*. 1993; Sailer *et al*. 1994). Heterozygous mice with only one disrupted allele ($Prnp^{+/0}$) also showed partial resistance to scrapie infection as manifested by prolonged incubation times of about 290 days compared with about 160 days in $Prnp^{+/+}$ mice (Büeler *et al*. 1994). This suggests that the amount of PrP^{C} protein in the brain is a rate-limiting step in the development of the disease may point to therapeutic efforts aimed at reducing the production of the normal PrP^{C} isoform.

Prion neurotoxicity: the neurografting approach

What is the pathogenetic event that impairs the central nervous system? It is a common belief, that deposits of pathological prion protein are to blame by inducing neuronal cell death (Fairbairn *et al*. 1994; Giese *et al*. 1995), and among several possible factors are elevated cytokine levels in the brain (Campbell *et al*. 1994; Williams *et al*. 1994). To clarify, whether or to what extent prions are directly neurotoxic, we introduced a transplant which continuously delivered PrP^{Sc} into the brains of $Prnp^{0/0}$ mice. This was accomplished by grafting neural tissue overexpressing PrP into the brain of PrP-deficient mice (Fig. 2). After intracerebral inoculation with scrapie prions, these grafts not only developed severe signs of neurodegeneration (Fig. 3) (Brandner *et al*. 1996, 1998) but also accumulated high levels of PrP^{Sc} and infectivity. Substantial amounts of graft-derived PrP^{Sc} migrated even into distant areas of the host brain which then contained substantial amounts of infectivity. But surprisingly, these brain areas did not show pathological changes, not even in the immediate vicinity of the grafts or the PrP deposits (Fig. 3) (Brandner *et al*. 1996). We concluded that PrP^{Sc} is inherently non-toxic and PrP^{Sc} plaques found in spongiform encephalopathies may be an epiphenomenon rather than a cause of neuronal damage. It is conceivable that PrP^{Sc} is only toxic when it is formed and accumulated within the cell but not when presented from outside. Results from other researchers suggest that microglia may play a certain role in mediating the toxic effect from PrP^{Sc} on PrP^{C}-expressing neurons.

The neuroinvasion of prions

Although the intracerebral application is by far the most effective route for prion infection, oral uptake is the epidemiologically more relevant. This route is thought to be responsible for the transmission not only of BSE but also of new variant CJD (Hill *et al*. 1997*b*). Since prion diseases primarily manifest as a disease of the CNS, invasion of prions from the periphery must be of crucial importance and, considering the precision of incubation times even after intraperitoneal inoculation, are tightly controlled. We therefore set out to elucidate the mechanisms of prion spread from periphery to the CNS. In a first set of experiments, we asked whether prions can migrate from the periphery to a graft in the CNS of a $Prnp^{0/0}$ mouse: intraperitoneal inoculation of such grafted

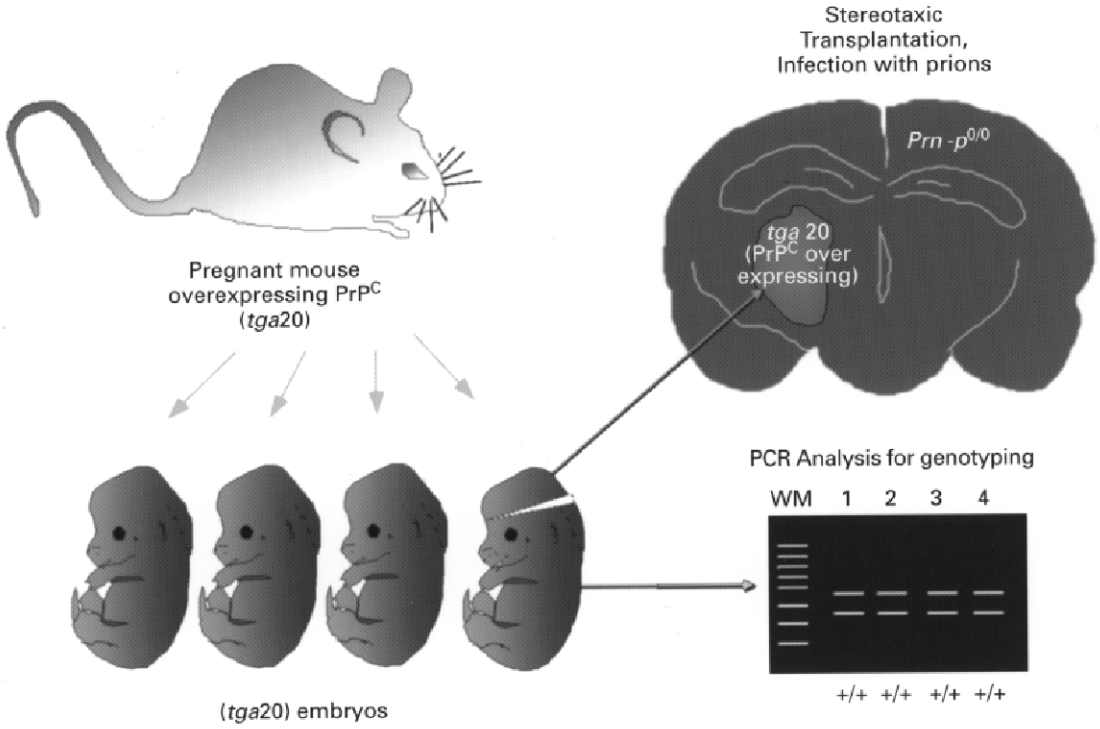

Figure 2

Schematic drawing of the transplantation procedure to obtain PrP-expressing grafts within the brain of $Prnp^{0/0}$ mice. Transgenic mice overexpressing PrP (termed *tga*20) were mated and embryos were removed at day 13.5. The neuroectodermal brain anlage was removed and transplanted into the brain of an adult recipient ($Prnp^{0/0}$) mouse, where it differentiates into mature neuroectoderm. Inoculation with prions was carried out at various time points after transplantation.

mice did not provoke any disease in the graft and did not result in accumulation of prions in the spleen (Blättler *et al.* 1997). In contrast, wild-type mice develop scrapie and accumulate high titres of infectivity in the lymphoreticular system, shortly after intraperitoneal or intracerebral inoculation. Then we asked, whether transfer of wild-type bone marrow to into *prnp*$^{0/0}$ mice would restore prion replication. First, *Prnp*$^{0/0}$ mice received intracerebral grafts of Prnp overexpressing cells which eventually serves as an indicator for infectivity brought into the CNS. Then, these mice were irradiated and received bone marrow from wild-type mice. After peripheral prion infection, there was infectivity detectable in the spleen, but surprisingly no disease in the CNS graft. We concluded, that the transport from the spleen to the CNS requires another tissue compartment, which is not restored by bone marrow reconstitution (Blättler *et al.* 1997). A possible candidate is the peripheral nervous system, as suggested by indirect findings.

The role of the lymphoreticular system in the neuroinvasion of prions

Several studies indicate an important role of lymphoid organs in prion replication, but there is little knowledge about which cells support prion propagation in the lymphoreticular system. Whole-body ionizing radiation studies in mice after intraperitoneal infection have suggested that the critical cells are long lived (Fraser & Farquhar, 1987). Follicular dendritic cells (FDCs) are possible candidates (Kitamoto *et al.* 1991), and

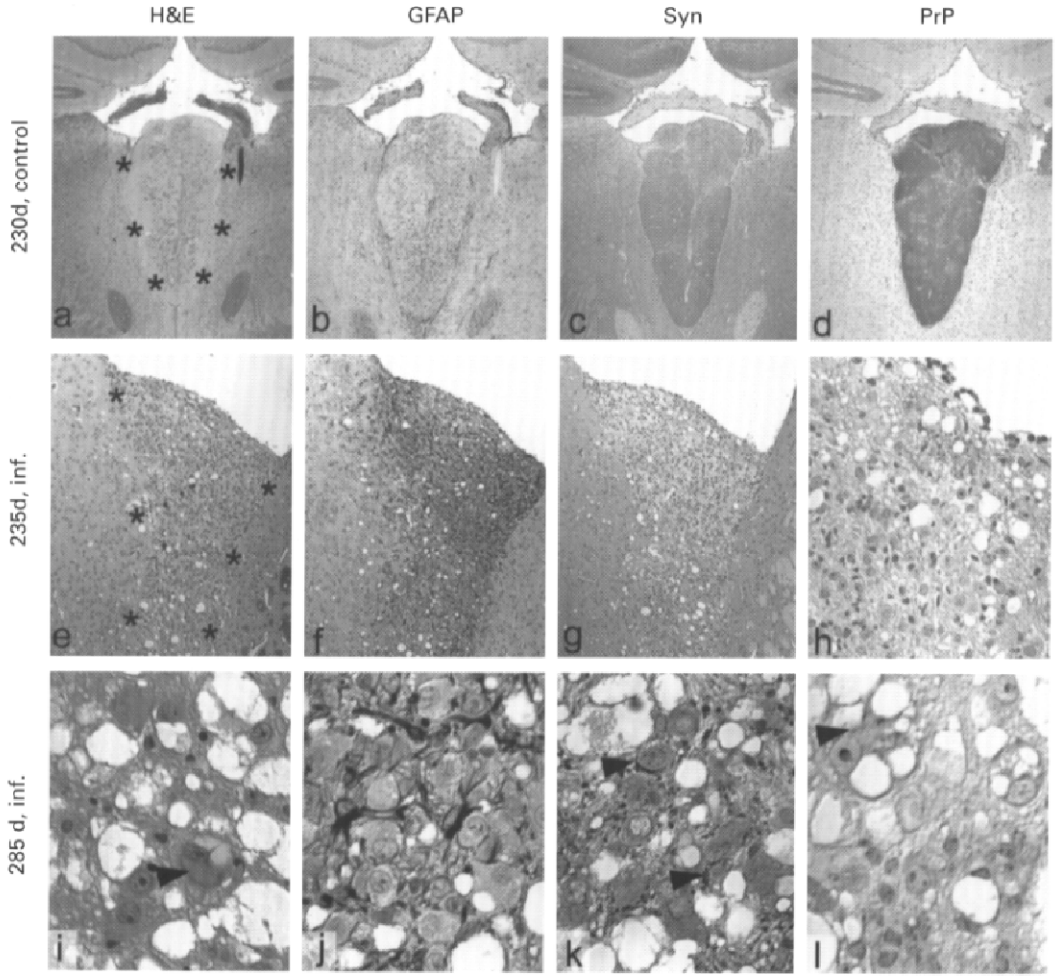

Figure 3

Non-infected neural grafts in brains of *Prnp*$^{0/0}$ mice. Upper row (a–d), healthy control graft 230 days after mock inoculation. The graft is located in the third ventricle of the recipient mouse (a, see asterisks, haematoxylin–eosin), and shows no spongiform change, little gliosis (b, immunostain for glial fibrillary acidic protein (GFAP)), and strong expression of synaptophysin (c) and of PrP^{C} (d). Middle row (e–h), scrapie infected graft 235 days after inoculation with increased cellularity (e), brisk gliosis (f) and a significant loss of synaptophysin (g) and PrP (h) staining intensity is shown. Bottom row, high magnification of a similar chronically infected graft shows characteristic pathological changes. i, appearance of large vacuoles and ballooned neurons (arrow). In the GFAP immunostain (j), astrocytes appear wrapped around densely packed neurons. Glandular deposits and intracytoplasmic accumulation of synaptophysin (k) and PrP immunoreactivity (l) in the cytoplasm of neurons.

indeed PrP^{Sc} accumulates in these cells of wild-type mice and in mice with a selective T cell defect. Further evidence for the importance of follicular dendritic cells came from experiments with severe combined immunodeficiency (SCID) mice whose FDCs are thought to be functionally impaired. In these mice, intraperitoneal infection does not lead to replication of prions in the spleen nor in the CNS. Reconstitution of SCID mice with wild-type spleen cells restores susceptibility to scrapie after peripheral infection (Fraser *et al*. 1996). These findings suggest that components of the immune system are required for efficient transfer of prions from the periphery to the CNS. Thus, we analysed the role of other components of the immune system which may contribute to neuroinvasion of prions. We used mice with genetically engineered selective inactivation of specific component of the immune system. We used mice with defects of B and T cells (RAG-$2^{-/-}$, RAG-$1^{-/-}$ and SCID mice) and $AGR^{-/-}$ mice with an additional defect of interferon $\alpha\beta$ and interferon-γ. Defects of B cells alone were assessed with $\mu MT^{-/-}$ mice which not produce any immunoglobulins but have complete and functional

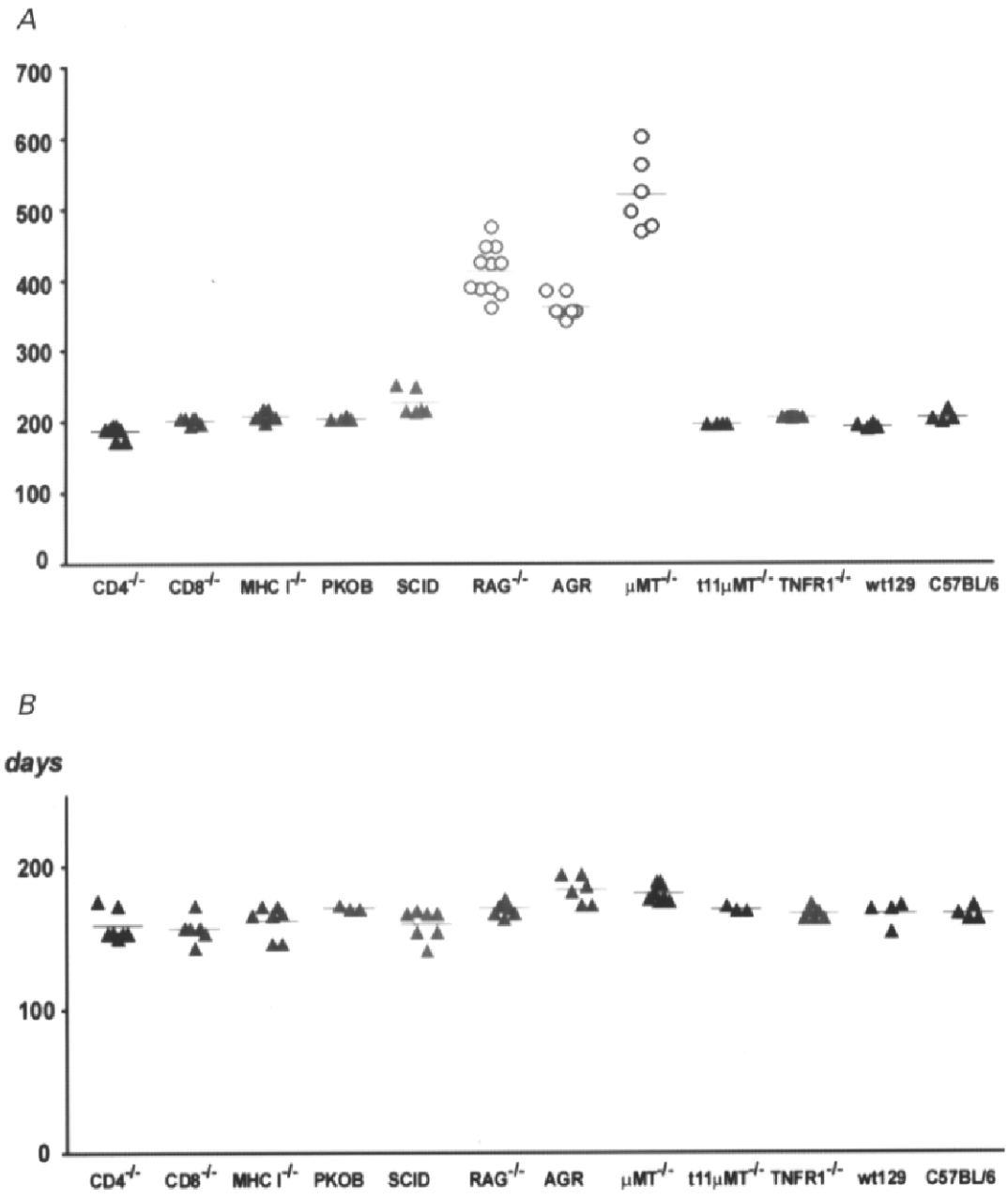

Figure 4

Latency of scrapie in various strains of immunodeficient mice. B cell-deficient mice remained healthy after I.P. inoculation with the Rocky Mountain Laboratory (RML) scrapie prion strain as 100 μl of a 10^{-1} or a 10^{-4} dilution of a scrapie-infected brain homogenate (*A*; open circles). In contrast, all mice developed spongiform encephalography after I.C. inoculation (*B*), regardless of their immune status. The horizontal lines represent mean values.

T cell subsets. Isolated T cell defects were investigated with $CD4^{-/-}$, $CD8^{-/-}$, β_2-microglobulin- or Perforin-deficient mice. We found that defects affecting T cells had no apparent effect on the incubation time after I.P. inoculation while all mutations that disrupted the differentiation of B cells prevented the development of clinical scrapie in I.P. inoculated mice (Fig. 4*A*). Intracerebral inoculation of all these mice, however, did not reveal any difference between the different groups (Fig. 4*B*). These results seem to indicate a crucial role for B cells in the propagation of scrapie after peripheral infection (Klein *et al*. 1997). However, since mice devoid of B cells fail to produce antibodies, and FDCs fail to develop in their lymphoid organs, we asked which of these three factors might be responsible for prion pathogenesis. Thus two further mouse strains were investigated. To elucidate the role of immunoglobulins we analysed mice producing antibody exclusively of the IgM subclass (t11 μMT), which had no detectable specificity for PrP^C. The role of FDCs was addressed using mice that lacked functional FDCs ($TNFR1^{-/-}$) but have differentiated B cells. Both strains developed scrapie after peripheral inoculation and again supported the notion of a crucial role for differentiated B cells in neuroinvasive scrapie (Klein *et al*. 1997).

PrP expression in B cells is not necessary for prion neuroinvasion

Replication of prions and their transport from the periphery to the CNS relies on the expression of PrP^C on lymphocytes. Thus we further asked, whether expression of PrP^C on B cells was a prerequisite for neuroinvasion of prions. Again, mice with various immune defects (SCID, RAG $1^{-/-}$ and $\mu MT^{-/-}$) were used for adoptive transfer of fetal liver cells either from wild-type or from $Prnp^{0/0}$ mice. This reconstitution induced the formation of germinal centres in spleens of all mice, including differentiation of FDCs. As control, we used B and T cell-deficient mice reconstituted with FLCs from (B cell-deficient) μMT embryos, and found no follicular dendritic cells in accordance with the notion that B cells or products thereof are required for FDC maturation. Reconstituted mice were challenged I.P. with scrapie prions. Surprisingly, all mice that received FLCs of either genotype, $Prnp^{+/+}$ or $Prnp^{0/0}$, from immunocompetent donors developed scrapie after inoculation with a high dose of prions, and most mice after a low dose. Transfer of FLCs from μMT donors, as well as omission of the adoptive transfer procedure, did not restore susceptibility to disease in any of the immunodeficient mice challenged with the low dose. In a further step we asked whether spleen PrP^{Sc}

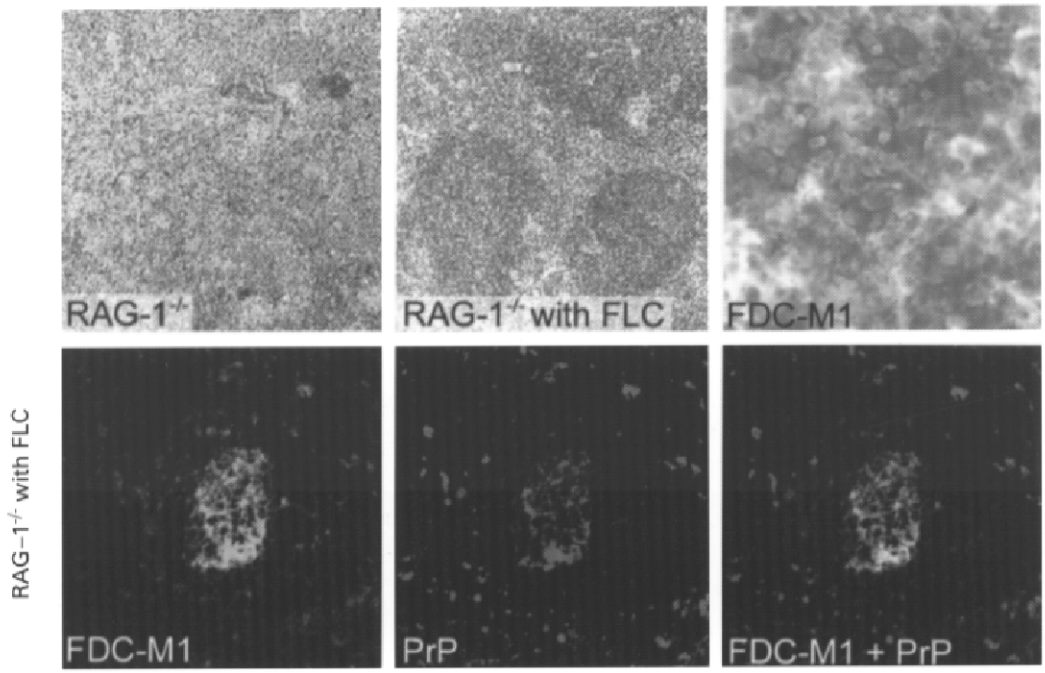

Figure 5

Spleen history of Rag-$1^{-/-}$ mice reconstituted with $Prnp^{0/0}$ fetal liver cells. Upper row, paraffin sections stained with hemalaun before FLC transfer (left). No B cell follicles and germinal centres were discernible in *Rag-*$1^{-/-}$ mice. Restoration of organized B cell follicles and germinal centres after FLC reconstruction (middle, magnification × 200). Frozen sections immunostained with antibody FDC-M1 revealed formation of prominent FDC clusters with germinal centres after FLC transfer (right, magnification, × 250). Lower row, confocal double-colour immunofluorescence analysis of splenic germinal centres in *Rag-*$1^{-/-}$ mice reconstituted with $Prnp^{0/0}$ FLCs after I.P. inoculation with RML prions. Sections were stained with antibody FDC-M1 to follicular dendritic cells (left) and with antiserum R340 to PrP (middle). Regions in which both signals are detectable appear as a bright network within the follicle centre (right, magnification × 250). Most of the PrP signal in germinal centres appeared to co-localize with the FDC-network.

was associated with FDCs in FLC reconstituted mice. With double-colour immunofluorescence confocal microscopy we detected deposits of PrP-immunoreactive material in germinal centres in spleens of reconstituted mice which largely co-localized with the follicular dendritic network (Fig. 5) (Klein *et al*. 1998).

These results support our notion that cells whose maturation depends on B cells are responsible for accumulation of prions in spleen. The most likely candidates are FDCs, since their maturation correlates with the presence of B cells and the formation of immune complexes.

On the other hand, it is equally possible that the follicular dendritic network serves as a reservoir for the accumulation and multiplication of prions. Other B cell-dependent processes could enable the transfer of prions to autonomic nerve terminals or the agent may be transported on or within B cells directly within peripheral lymphoid tissue. Alternatively, antibodies or other factors may bind prions, especially since PrP can be detected by immunohistochemical stains in the germinal centre area where immune complexing occurs, and this formation may be of importance for mediating neuroinvasion and facilitating the access to peripheral nervous system terminals.

Current threats and future perspectives

What are the implications of the above findings in the framework of the current epidemics of BSE and the occurrence of nvCJD? For one thing, there is some evidence that nvCJD is more lymphotropic than sporadic CJD, since nvCJD but not sporadic CJD prions have been detected in tonsils and appendix. Therefore understanding the peripheral pathogenesis is of immediate importance in assessing risks of iatrogenic transmission of human BSE via exposure to blood or tissues from preclinical cases, and possibly from contaminated surgical instruments, or even blood and blood products. It is unfortunate that little is known about the distribution of preclinical disease in the UK, leaving the actual persistence of the agent in the population entirely obscure. What is particularly unsettling about this lack of knowledge is the fact that sensitive and reliable assays are now available for detection of PrP^{Sc} in lymphatic tissues of cadavers, and these assays have been shown to constitute reliable indicators of nvCJD. Given that the most problematic aspect in public health decision-making is the uncertainty in prevalence of subclinical disease, the use of such assays for unlinked anonymous surveys of post-mortem material would seem to be logical, imperative and extremely urgent for planning measures aimed at limiting the spread of nvCJD among humans.

Our results indicate that follicular dendritic cells are most probably the prion reservoir in lymphatic organs but also splenic lymphocytes of experimentally inoculated mice can be infected with prions. This finding raises an alert, because blood lymphocytes might well be in equilibrium with their splenic counterparts and may be relevant if blood donors are subclinical carriers of the nvCJD agent (Aguzzi, 1997). However, there are still controversies as to whether depletion of leucocytes in blood products is necessary and/or sufficient to effectively prevent any possible risk.

Another consideration applies to secondary prophylaxis. As mentioned above, there might be a considerable number of preclinically infected people (Aguzzi & Collinge, 1997) who may have acquired infectious BSE material via the human food chain. It is a pressing issue to develop approaches that will help control spread of the agent and that will hopefully prevent the clinical outbreak of symptoms in these persons. In light of the above findings, possible targets for the interference with neuroinvasion are rate-limiting processes that control prion replication within the infected individual. In light of the knowledge discussed above, treatments that target the neuro-immune interface of prion replication and neuroinvasion seem a promising area for research aimed at post-exposure prophylaxis.

Aguzzi, A. (1997). Neuro-immune connection in spread of prions in the body? *Lancet* **349**, 742–743.

Aguzzi, A. & Collinge, J. (1997). Post-exposure prophylaxis after accidental prion inoculation. *Lancet* **350**, 1519–1520.

Blättler, T., Brandner, S., Raeber, A. J., Klein, M. A., Voigtländer, T., Weissmann, C. & Aguzzi, A. (1997). PrP-expressing tissue required for transfer of scrapie infectivity from spleen to brain. *Nature* **389**, 69–73.

Brandner, S., Isenmann, S., Kuhne, G. & Aguzzi, A. (1998). Identification of the end stage of scrapie using infected neural grafts. *Brain Pathology* **8**, 19–27.

Brandner, S., Isenmann, S., Raeber, A., Fischer, M., Sailer, A., Kobayashi, Y., Marino, S., Weissmann, C. & Aguzzi, A. (1996). Normal host prion protein necessary for scrapie-induced neurotoxicity. *Nature* **379**, 339–343.

Büeler, H., Raeber, A., Sailer, A., Fischer, M., Aguzzi, A. & Weissmann, C. (1994). High prion and PrPSc levels but delayed onset of disease in scrapie-inoculated mice heterozygous for a disrupted PrP gene. *Molecular Medicine* **1**, 19–30.

Büeler, H. R., Aguzzi, A., Sailer, A., Greiner, R. A., Autenried, P., Aguet, M. & Weissmann, C. (1993). Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339–1347.

Büeler, H. R., Fischer, M., Lang, Y., Bluethmann, H., Lipp, H. P., Dearmond, S. J., Prusiner, S. B., Aguet, M. & Weissmann, C. (1992). Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* **356**, 577–582.

Campbell, I. L., Eddleston, M., Kemper, P., Oldstone, M. B. & Hobbs, M. V. (1994). Activation of cerebral cytokine gene expression and its correlation with onset of reactive astrocyte and acute-phase response gene expression in scrapie. *Journal of Virology* **68**, 2383–2387.

Chazot, G., Broussolle, E., Lapras, C., Blättler, T., Aguzzi, A. & Kopp, N. (1996). New variant of Creutzfeldt-Jakob disease in a 26-year-old French man [letter]. *Lancet* **347**, 1181.

Creutzfeldt, H. G. (1920). Über eine eigenartige herdförmige Erkrankung des Zentralnervensystems. *Zeitschrift der Gesellschaft für Neurologie und Psychiatrie* **57**, 1–19.

Fairbairn, D. W., Carnahan, K. G., Thwaits, R. N., Grigsby, R. V., Holyoak, G. R. & O'Neill, K. L. (1994). Detection of apoptosis induced DNA cleavage in scrapie-infected sheep brain. *FEMS Microbiology Letters* **115**, 341–346.

Fraser, H., Brown, K. L., Stewart, K., McConnell, I., McBride, P. & Williams, A. (1996). Replication of scrapie in spleens of scid mice follows reconstitution with wild-type mouse bone marrow. *Journal of General Virology* **77**, 1935–1940.

Fraser, H. & Farquhar, C. F. (1987). Ionising radiation has no influence on scrapie incubation period in mice. *Veterinary Microbiology* **13**, 211–223.

Gerstmann, J., Sträussler, E. & Scheinker, I. (1936). Über eine eigenartige hereditär-familiäre Erkrankung des Zentralnervensystems. Zugleich ein Beitrag zur Frage des vorzeitigen lokalen Alterns. *Zeitschrift fur Neurologie* **154**, 736–762.

Giese, A., Groschup, M. H., Hess, B. & Kretzschmar, H. A. (1995). Neuronal cell death in scrapie-infected mice is due to apoptosis. *Brain Pathology* **5**, 213–221.

Griffith, J. S. (1967). Self-replication and scrapie. *Nature* **215**, 1043–1044.

Hill, A. F., Desbruslais, M., Joiner, S., Sidle, K. C., Gowland, I., Collinge, J., Doey, L. J. & Lantos, P. (1997*a*). The same prion strain causes vCJD and BSE [letter] [see comments]. *Nature* **389**, 448–450.

Hill, A. F., Zeidler, M., Ironside, J. & Collinge, J. (1997*b*). Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. *Lancet* **349**, 99.

Jarrett, J. T. & Lansbury, P. T. Jr (1993). Seeding 'one-dimensional crystallization' of amyloid: a pathogenic mechanism in Alzheimer's disease and scrapie? *Cell* **73**, 1055–1058.

Kitamoto, T., Muramoto, T., Mohri, S., Dohura, K. & Tateishi, J. (1991). Abnormal isoform of prion protein accumulates in follicular dendritic cells in mice with Creutzfeldt-Jakob disease. *Journal of Virology* **65**, 6292–6295.

Klein, M. A., Frigg, R., Flechsig, E., Raeber, A. J., Kalinke, U., Bluethmann, H., Bootz, F., Suter, M., Zinkernagel, R. M. & Aguzzi, A. (1997). A crucial role for B cells in neuroinvasive scrapie. *Nature* **390**, 687–690.

Klein, M. A., Frigg, R., Raeber, A. J., Flechsig, E., Hegyi, I., Zinkernagel, R. M., Weissmann, C. & Aguzzi, A. (1998). PrP expression in B lymphocytes is not required for prion neuroinvasion. *Nature Medicine* **4**, 1429–1433.

McKinley, M. P., Bolton, D. C. & Prusiner, S. B. (1983). A protease-resistant protein is a structural component of the scrapie prion. *Cell* **35**, 57–62.

Oesch, B., Westaway, D., Walchli, M., McKinley, M. P., Kent, S. B., Aebersold, R., Barry, R. A., Tempst, P., Teplow, D. B., Hood, L. E. & Weissmann, C. (1985). A cellular gene encodes scrapie PrP 27–30 protein. *Cell* **40**, 735–746.

Prusiner, S. B. (1982). Novel proteinaceous infectious particles cause scrapie. *Science* **216**, 136–144.

Prusiner, S. B. (1991). Molecular biology of prion diseases. *Science* **252**, 1515–1522.

Prusiner, S. B., McKinley, M. P., Bowman, K. A., Bolton, D. C., Bendheim, P. E., Groth, D. F. & Glenner, G. G. (1983). Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* **35**, 349–358.

Riesner, D., Kellings, K., Post, K., Wille, H., Serban, H., Groth, D., Baldwin, M. A. & Prusiner, S. B. (1996). Disruption of prion rods generates 10-nm spherical particles having high α-helical content and lacking scrapie infectivity. *Journal of Virology* **70**, 1714–1722.

Sailer, A., Büeler, H., Fischer, M., Aguzzi, A. & Weissmann, C. (1994). No propagation of prions in mice devoid of PrP. *Cell* **77**, 967–968.

Will, R., Cousens, S., Farrington, C., Smith, P., Knight, R. & Ironside, J. (1999). Deaths from variant Creutzfeldt-Jakob disease. *Lancet* **353**, 9157–9158.

Will, R., Ironside, J. W., Zeidler, M., Cousens, S. N., Estibeiro, K., Alperovitch, A., Poser, S., Pocchiari, M., Hofman, A. & Smith, P. G. (1996). A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* **347**, 921–925.

Williams, A. E., van Dam, A. M., Man, A. H. W. K., Berkenbosch, F., Eikelenboom, P. & Fraser, H. (1994). Cytokines, prostaglandins and lipocortin-1 are present in the brains of scrapie-infected mice. *Brain Research* **654**, 200–206.