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Microglia and Prion Disease

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ABSTRACT Gliosis is one of the hallmarks of the prion diseases. Prion diseases are fatal neurodegenerative conditions of low incidence made famous by both the hypothesis that a protein acts as the infectious agent without involvement of nucleic acid and the speculative idea that a disease of cattle, BSE, has spread to humans from the ingestion of prion-infected beef. Despite these unproved hypotheses, the aetiology of the prion diseases remains unsolved. The rapid degenerative course of the disease is preceded by a long incubation period with little or no symptoms. The rapid neurodegeneration in the disease follows from increased deposition of an abnormal isoform of a normal neuronal protein. Co-incident with the appearance of this abnormal protein is the activation of large numbers of microglia. Studies in cell culture with both the abnormal prion protein and a peptide-mimic suggest that neuronal degeneration occurs because of two concurrent effects. First, there is a reduction in neuronal resistance to toxic insults and, second, there is an increase in the production of toxic substances such as reactive oxygen species by microglia and a decrease in glutamate clearance by astrocytes. Microglia activated by the abnormal form of the prion protein also release cytokines, which stimulate changes in astrocytes such as proliferation. The implication of this is that microglia may play a major role in initiating the pathological changes in prion disease. This review discusses the role of microglia in these changes. *Microsc. Res. Tech. 54:71–80, 2001.*

PRION DISEASE

Prion diseases are characterised by neuronal death or neurodegeneration. However, prion diseases are more widely known because of the theory that infectivity is carried by a protein only (Prusiner, 1982), namely the abnormal isoform of the prion protein. Despite this controversial and interesting claim it is probably only relevant to a small proportion of human sufferers of these diseases. The vast majority of human prion disease called Creutzfeldt-Jakob disease (CJD) occur spontaneously with no known cause (Prusiner, 1991). There are also inherited forms of prion disease that include Gerstman-Sträussler-Scheinker Syndrome (GSS), Fatal Familial Insomnia, and inherited CJD (Prusiner, 1991, Ghetti et al., 1996a). The number of cases where transmission of disease has occurred by "infection" is quite limited. The only confirmed cases are those of iatogenic transmission resulting from transplantation of human tissue such as dura mata or central nervous system products (Jaegly et al., 1995). The disease Kuru is believed to have been spread by eating of human brains. Although there are quite a number of unequivocal similarities between Bovine Spongiform Encephalopathy (BSE) and the recently described variant CJD (vCJD) (Bruce et al., 1997; Collinge et al., 1996), a causal connection between the two disease has not been demonstrated.

The human diseases are linked together collectively with several animal diseases, which include BSE of cattle (Hope et al., 1988), scrapie of sheep (Prusiner, 1982), Chronic Wasting Disease of deer (Guiroy et al., 1991), and transmissible mink encephalopathy (Marsh et al., 1969) because large amounts of an abnormally folded isoform of the prion protein (PrP^{Sc}) can be detected within the brains of affected individuals (Prusiner, 1991). PrP^{Sc} is a derivative of a normal extracellular glycoprotein termed cellular prion protein (PrP^{c}). Although widely accepted as being the "prion" or infectious agent of prion disease PrP^{Sc} accumulation, generated by the host, may not be the only component of the infectious agent. Despite this uncertainty there is little doubt that accumulation of host generated PrP^{Sc} is the cause of neurodegeneration in these diseases. Despite this, the mechanism by which PrP^{Sc} is causative to the neurodegeneration remains unresolved.

PrP^{c} has been shown to be a copper-binding protein (Brown et al., 1997a) that influences uptake of copper (Brown, 1999a) and also functions as an antioxidant (Brown et al., 1999) that protects cells from oxidative stress. Mice deficient in expression of PrP^{c} have reduced activity of the cytoplasmic superoxide dismutase (SOD-1) (Brown et al., 1997b) probably because of reduced incorporation of copper into SOD-1 (Brown and Besinger, 1998). Given that the protein has a rather general function, it is not surprising that it is expressed by a number of different cell types including neurones (Salès et al., 1998), astrocytes (Brown, 1999b; Moser et al., 1995), microglia (Brown et al., 1998a), muscle cells (Brown et al., 1998c), keratinocytes (Pammer et al., 1998), and various cells of the blood (Dodelet and Cashman, 1998). Nevertheless, the highest level of expression is within the nervous system at endplates and more particularly at synapses in the CNS. Despite PrP^{Sc} accumulation in other tissues in prion disease (Ye and Carp 1995), it is probably because of this neuronal expression that most PrP^{Sc} accumulates in the central nervous system.

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IN VITRO MODEL OF NEURODEGENERATION

Analysis of the mechanism by which PrP^{Sc} causes neurodegeneration in prion disease has been limited by the complexity of the system. It has proven impossible to separate the neurodegenerative effects of PrP^{Sc} from its possible infective qualities. Secondly, it has not been possible to induce acute neurodegeneration in brain tissue by injection of PrP^{Sc} (Betmouni and Perry, 1999) as the experiment procedure produces a severe response either masking or more significant than the local neurodegeneration caused by PrP^{Sc}. One implication of this is that host-generated PrP^{Sc} is necessary to observe neurodegeneration in vivo. This implies that infection with prion disease must occur before neurodegeneration can be initiated. Transplantation studies have provided one of the few clear insights into the mechanism involved as host-generated PrP^{Sc} is not neurotoxic to brain tissue that is genetically modified to lack expression of PrP^{c} (Brandner et al., 1996). This implies that PrP^{c} is involved in the way in which PrP^{Sc} kills neurones. However, extending the analysis beyond this point *in vivo* has not provided insights of significance.

As a result of these difficulties, analysis of the mechanism of neurodegeneration caused by PrP^{Sc} has turned to cell culture models. PrP^{Sc} was shown to be toxic to cultured neurones by Müller et al. (1993). However, PrP^{Sc} as such is purified from mouse brain by proteinase digestion and as such is a relatively impure and ill-defined agent. Consequently, many studies have turned to synthetic peptides to extend the analysis. Studies of peptides related to PrP^{Sc} were carried out and Forloni et al. (1993) identified one peptide as containing the neurotoxic region of the full molecule. This peptide termed PrP106-126 (Fig. 1) after the human prion protein sequence it is derived from has become the standard peptide for the analysis of the in vitro toxicity of PrP^{Sc} being used by a large number of individual groups. A recent report (Kunz et al., 1999) suggesting that PrP106-126 is not neurotoxic is ill-founded and fundamentally flawed and can be dismissed as the result of poorly controlled experimentation (for analysis see Brown, 1999b). Furthermore, recent well-controlled experiments have also demonstrated the toxicity of PrP106-126 in vivo using the retina as a model (Ettaiche et al., 2000).

PrP106-126 has many of the qualities of PrP^{Sc}. It is protease resistant, forms fibrils readily, and aggregates to form a structure rich in β-sheet (De Gioia et al., 1994; Tagliavini et al., 1993). Analysis of the peptide in detail indicates that the toxicity is related to the hydrophobic region of the peptide (amino residues 113–126) and fibril formation is endowed by the eight amino acid residues with the sequence AGAAAAGA (Brown, 2000a). Indeed, peptides based on AGAAAAGA can form fibrils spontaneously, making this peptide the shortest fibrillar protein yet known (Brown, 2000a). Deletion of the region AGAAAAGA from the prion protein sequence prevents formation of PrP^{Sc} (Chabry et al., 1998) and causing a dominant negative effect abolishing infection from scrapie-infected cultured cells (Hölscher et al., 1998).

Analysis of both PrP^{Sc} and PrP106-126 has identified a basic model mechanism relevant to both and providing evidence that PrP106-126 is an adequate neurotoxic mimic of PrP^{Sc}. First, the neurones present in cerebellar culture must express PrP^{c} (Brown et al., 1994). Neither PrP106-126 nor PrP^{Sc} is toxic to cerebellar cells from mice lacking PrP^{c}-expression (Brown et al., 1996a; Giese et al., 1998) Second, the presence of microglia in the cerebellar cultures is necessary for the toxicity of the peptide and PrP^{Sc} (Brown et al., 1996a; Giese et al., 1998). Reduction of microglia in cerebellar culture prevents the toxicity of PrP106-126 whereas additional microglia enhance toxicity. Analysis of the effect on the microglia indicates that PrP106-126 activates microglia inducing release of higher levels of superoxide, which can react to form other intermediates (Brown et al., 1996a). Antioxidants inhibit the toxicity of PrP106-126, implying that oxidative stress arising from microglia forms a component of the toxicity of PrP106-126 to cultured cells (Brown et al., 1996a).

This basic mechanism has been studied further and naturally it appears that the system is far more complex than this simplified description. PrP106-126 was found to be more toxic to cerebellar cells from mice transgenically modified to overexpress PrP^{c} (Tg35 mice) supporting the hypothesis that PrP106-126 toxicity is related to the level of PrP^{c} expression (Brown, 1998). Thus, as regards susceptibility to PrP106-126 neurotoxicity: Tg35 > wild-type >> PrP-deficient cells. However, cerebellar cells from another mouse strain overexpressing PrP^{c} (Tg20), which have higher PrP^{c} expression than Tg35 mice (Fischer et al., 1996), were no more sensitive to PrP106-126 toxicity than wild-type cells (Brown, 1998a). This implies that PrP106-126 toxicity is not only related to the level of neuronal PrP^{c} expression. Increasing neuronal PrP^{c} expression above a certain level may have no further consequence as regards the toxicity of PrP106-126. Other factors must be implied to explain the differences between the effects on Tg20 and Tg35 cultures.

Further analysis of cells from PrP^{c}-overexpressing mice have shown that the greater toxicity of PrP106-126 to Tg35 cerebellar cells can be related to differences in microglia. Microglia have been shown to express PrP^{c} (Brown et al., 1998a). Tg35 microglia express more PrP^{c} than wild-type or Tg20 microglia (Brown, 1998). LLME blocked the toxicity of PrP106-126 to Tg35 cerebellar cells, confirming that microglia are necessary for the toxicity of the peptide to these cells. Co-culture of LLME-treated Tg35 cerebellar cells with microglia restored susceptibility to PrP106-126 toxicity. However, the toxic effect was greater if the microglia used for co-culturing were also derived from Tg35 mice rather than wild-type mice (Brown, 1998). This implies that the level of PrP^{c} expression by microglia can also influence the toxicity of PrP106-126. Microglia from Tg20 mice could not enhance the toxicity of the peptide above that of wild-type microglia. Tg35 microglia cannot be induced to proliferate by PrP106-126 whereas proliferation of wild-type or PrP^{c}-deficient microglia is greatly enhanced by PrP106-126 treatment (Brown et al., 1996a, 1998a). Tg35 microglia were found to be more readily activated by LPS and concavalin A than wild-type microglia (Brown et al.,

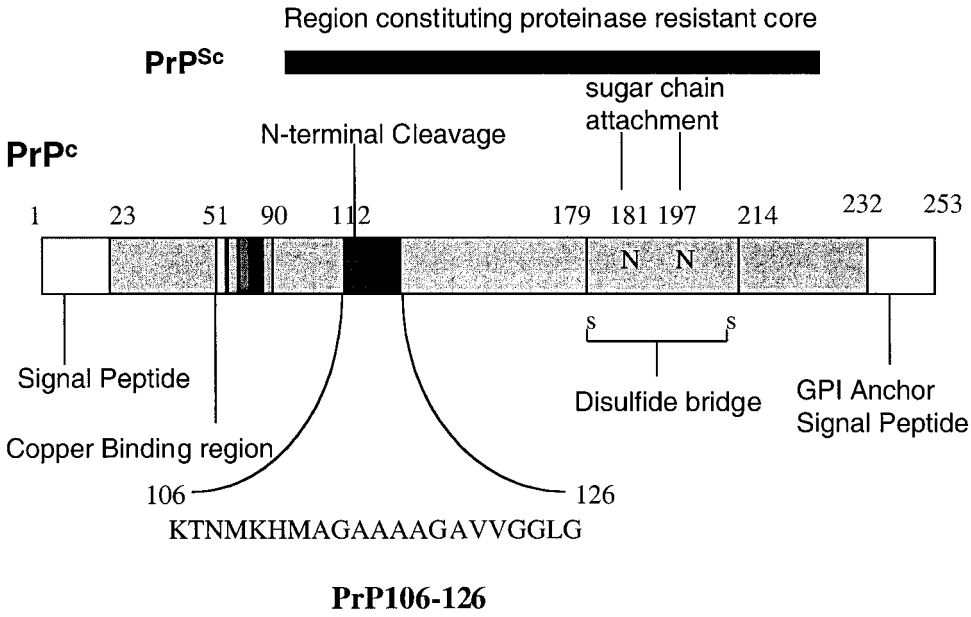

Fig. 1. An illustration relating the normal cellular prion protein (PrP^{c}), the abnormal isoform (PrP^{Sc}), and the neurotoxic peptide (PrP106-126). The number of the sequence represents the human sequence. This protein is anchored to the cell membrane by a GPI anchor. The signal peptides for entry into the endoplasmic reticulum and the GPI signal peptide are cleaved off before the protein reaches the cell surface. Glycosylation can occur on one, two, or none of the asparagine residues indicated. A hydrophobic region envelops a cleavage point where the protein is cleaved during normal metabolic breakdown. This hydrophobic region includes the sequence of the neurotoxic peptide, PrP106-126. A disulphide bond links two regions of the protein that form separate alpha-helices in the three-dimensional structure of the protein. The complete octarepeats can bind up to four copper atoms. Most mammals also have an incomplete repeat prior to this. Shown for comparison is the proteinase-resistant core PrP^{Sc} that co-purifies with the infectious agent. The sequence of this protein lacks the N- and C-terminus of PrP^{c}.

1998a). These differences were not observed in Tg20 microglia. Also, Tg35 microglia proliferate more rapidly in the presence of astrocytes than wild-type microglia (Brown, 1998). Tg35 microglia also showed a higher basal rate of superoxide release than wild-type microglia when freshly isolated. These differences disappeared after continued separation from astrocytes. This also implies that factors released by astrocytes stimulating microglia proliferation were more effective at stimulating the proliferation of Tg35 microglia. This was not due to an increase in the amount of factors released by Tg35 astrocytes as wild-type astrocytes stimulated Tg35 microglia proliferation more than Tg35 astrocytes. These results suggest that increased PrP^{c} expression by microglia increases their sensitivity to various stimuli leading to activation and also to proliferation induced by astrocyte secreted cytokines. In support of this, it has also been observed that there is a greater number of Mac-1 positive microglia in the cerebellum of Tg35 mice than in wild-type mice (Fig. 2).

As indicated above, PrP106-126 is not toxic to neurones that lack the expression of PrP^{c} (Brown et al., 1994). When cerebellar cells from PrP^{c}-deficient mice are co-cultured with wild-type or Tg35 microglia, PrP106-126 treatment does not lead to increased neuronal death. This may at first seem contradictory. However, PrP106-126 effects on PrP^{c}-expressing neurones are necessary to make them more sensitive to the toxic products of microglia (Brown et al., 1996a). It has recently been shown that PrP106-126 requires direct interaction between PrP106-126 and PrP^{c} on cells (Brown, 2000c). This interaction is mediated by the palendromic sequence of the protein. The result of the interaction is inhibition of PrP^{c} activity (Brown, 2000c). The effect of PrP106-126 on neurones via interaction with PrP^{c} is to increase neuronal sensitivity to oxidative stress by indirectly inhibiting the activity of anti-oxidant enzymes such as superoxide dismutase (Brown et al., 1997b; Brown and Besinger, 1998) and enzymes related to glutathione metabolism (Perovic et al., 1997; White et al., 1999). Once again these effects are dependent on the expression of PrP^{c} possibly because the peptide can prevent copper transport mediated by PrP^{c} (Brown, 1999a, 2001). Additionally, PrP106-126 inhibits neuronal resistance to oxidative stress by directly binding to and inactivating PrP^{c}, which has recently been shown to be an anti-oxidant protein (Brown et al., 1999; 2000, Brown, 2001). It has been suggested that activation of microglia in the brain has little effect on healthy neurones. Microglia activation by PrP106-126 may only be sufficient to kill "sick" neurones. PrP106-126 impairs the metabolism of PrP^{c}-expression neurones, which may therefore be "sick." PrP106-126 can be taken up by neurones lacking PrP^{c} expression (McHattie et al., 1999) and may even alter their calcium metabolism by binding to tubulin (Brown et al., 1998d). However, PrP^{c}-deficient neurones may compensate by increasing levels of other anti-oxidants such as manganese-superoxide dismutase (Brown et al., 1997b).

Interaction of PrP106-126 with neurones and microglia has been observed by the use of biotin-labelled PrP106-126 (McHattie et al., 1999). This work confirms that neurones, microglia, and astrocytes take up the

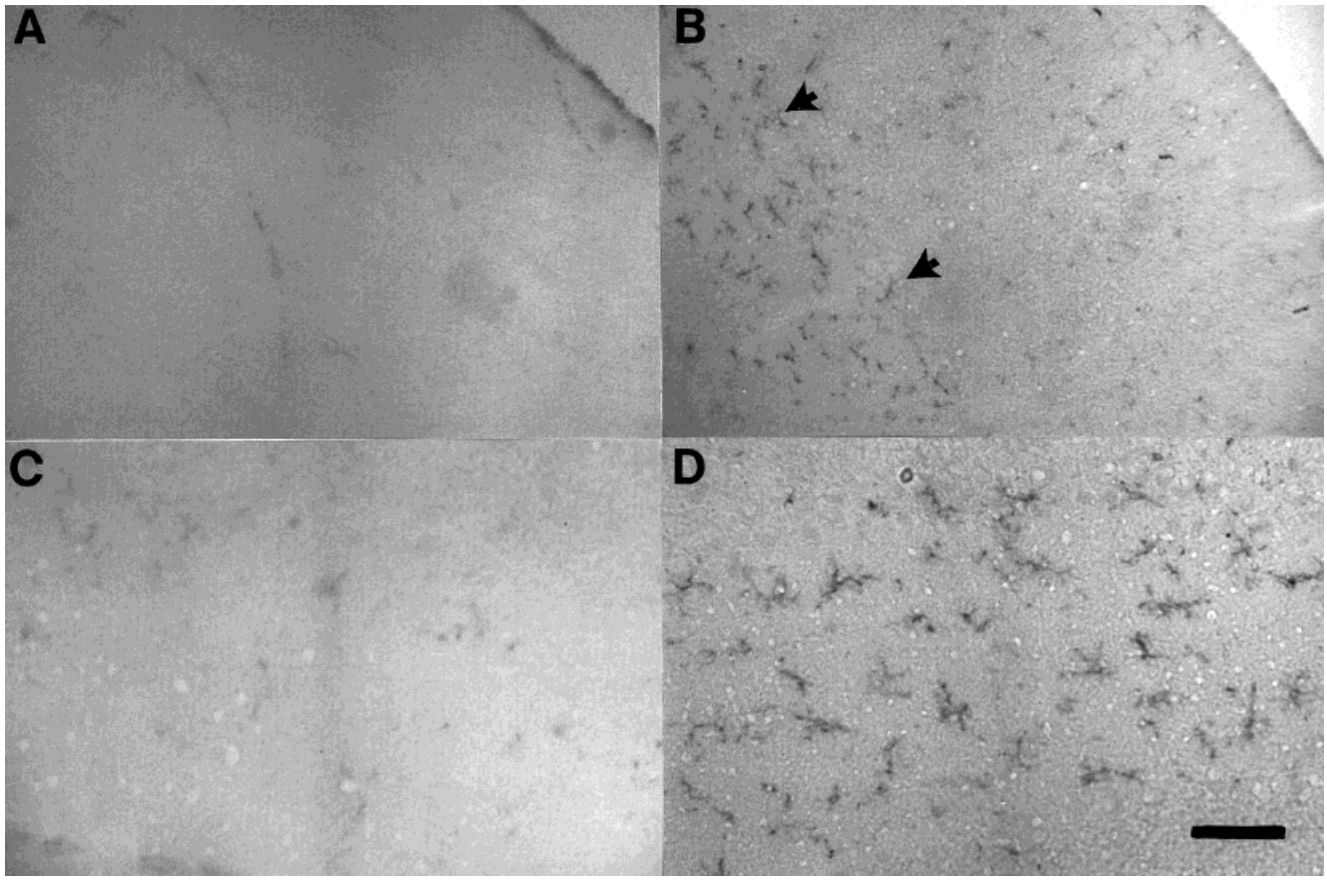

Fig. 2. Photomicrographs of sections from adult mouse cerebellum (4–5 months of age). Wild-type (**A,C**) and Tg35 (**B,D**) were stained with an antibody to Mac-1. Microglia stained with Mac-1 appear darkly coloured (arrows). No staining was observed in wild-type sections but large numbers of microglia are present in Tg35 sections. Section stained in A is enlarged in C and that of B is enlarged in D. Scale bar = 400 μM in A,B and 100 μM in C,D.

peptide. However, further experiments have shown that different forms of the peptide induce the different effects on microglia and neurones. Storing PrP106-126 in solution and thus "ageing" the peptide before applying it to cultures enhances its β-sheet content and probably its aggregation but this reduces its toxicity (Brown et al., 1998e). This aged peptide causes a greater activation of microglia than does less aggregated peptide. Filtration preventing aggregated peptide interacting with neuronal cultures does not abolish the toxicity of PrP106-126 as long as the aggregated PrP106-126 can interact with microglia placed in their vicinity (Brown et al., 1998e). These results suggest that the neurone-specific effects of PrP106-126 require PrP106-126 to be non-aggregated or even monomeric. Therefore, PrP106-126 containing a mixed population of aggregates and free peptide represents the most toxic form of the peptide.

The toxicity of PrP106-126 is altered by modification of the sequence. Alteration of the alanine at amino-residue 117 to a valine as in the human inherited disease Gerstmann-Sträussler-Scheinker syndrome (GSS) increases the toxicity of the peptide (Brown, 2000b). The toxicity is unlike that of wild-type PrP106-126 in that it cannot be abolished by ablation of microglia from the cultures and is not entirely dependent on PrP^c expression by the cells. Blockers of calcium uptake through L-type voltage-dependent calcium channels can inhibit the toxicity. The mutant peptide enhances displacement of tau from microtubules by binding to tubulin. Stabilisation of microtubules has been suggested to activate calcium uptake (Johnson and Byerly, 1993). Binding of PrP106-126 to tubulin has been reported but probably isn't involved in the toxic process of the wild-type protein (Brown et al., 1998d). This finding is interesting as some cases of GSS show increased deposition of tau in paired helical filaments, a pathological marker normally associated with Alzheimer's disease (Ghetti et al., 1996b). The finding that PrP106-126 carrying a mutation has a different mechanism of toxicity suggests that the mechanism of toxicity of PrP^{Sc} might vary with changes to its sequence.

IN VITRO MODEL OF ASTROGLIOSIS

Astrogliosis is one of the hallmarks of prion disease. This in itself is not indicative of specific changes in the CNS peculiar to prion disease as most neurodegenera-

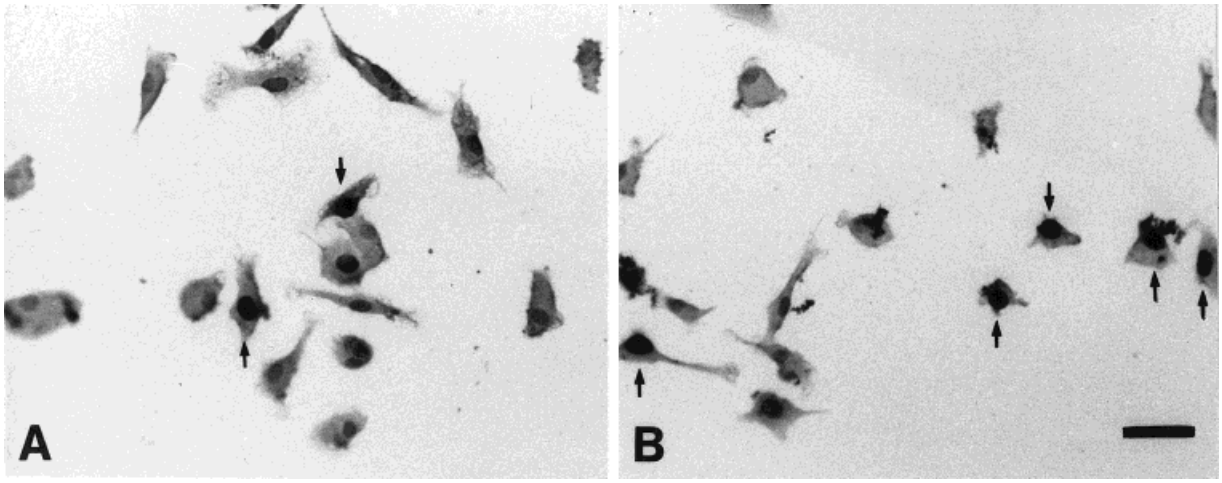

Fig. 3. Proliferation of microglia induced by PrP106-126. After treatment of isolated mouse microglia with 80 μM PrP106-126 and bromodioxiuridine (BrdU) for 4 days, the microglia were stained for BrdU labelling. Cells with dark nuclei (arrows) are indicative of proliferating cells. Although labelled cells could be seen in untreated cultures (**A**), there were approximately three times as many labelled cells in the treated cultures (**B**). Scale bar = 25 μm.

tive changes are accompanied by proliferation of astrocytes. These changes are usually a result of damage to or death of neurones. However, in prion disease there is strong evidence that gliosis occurs before neurodegeneration (Jendroska et al., 1991; DeArmond et al., 1992; Giese et al., 1998). Furthermore, there is evidence that astrocytes are the cell type in which the abnormal form of the prion protein, PrP^{Sc}, is first replicated in the central nervous system (Diedrich et al., 1991). Indeed, animals that only express PrP in astrocytes are susceptible to prion disease (Raeber et al., 1997). A recent in vitro study also indicates that the presence of large numbers of astrocytes can accelerate the rate at which neurones are killed by peptide PrP106-126 (Brown, 1999c). This finding does not contradict what has been described above. The toxicity of PrP106-126 is not enhanced by astrocytes (Brown et al., 1996a); instead the toxicity of glutamate to neurones can be enhanced by the effect of PrP106-126 on PrP^{c}-expressing astrocytes. A cell culture system is by design a simplification of the situation in the brain. The basic mechanism of PrP106-126 to cerebellar cells as described above requires microglia. However, when increased number of astrocytes are added into this system to mimic the astrogliosis seen in prion disease, then increased toxicity is observed in this system that can be directly attributed to effects of PrP106-126 on astrocytes. This system, therefore, represents a more accurate model of the situation in the brain when the most rapid neurodegeneration is occurring. Thus, a model of the end stage pathology must also account for the presence of large numbers of astrocytes and their likely contribution.

Gliosis (of both microglia and astrocytes) is often believed to be a response to damaged neurones. However, this does not imply that the gliosis in prion disease is a response to neuronal damage. As indicated above, there are indications that gliosis occurs before neurodegeneration in prion disease begins but does coincide with deposition of PrP^{Sc}. Therefore, there is the possibility that PrP^{Sc} can stimulate gliosis. It has been demonstrated previously that PrP106-126 can induce astrocyte proliferation in culture (Forloni et al., 1994). PrP106-126 can also induce microglia to proliferate (Brown et al., 1996b) (Fig. 3). The initial studies of astrocyte proliferation were on mixed glial preparations from rat cortex (Forloni et al., 1994). However, when astrocytes were purified free of contaminating cells it was found that PrP106-126 could not induce their proliferation (Brown et al., 1996a, 1998b). It was found that, like the neurotoxic effect seen in cerebellar cell cultures, the induction of astrocyte proliferation was also dependent on the presence of microglia (Brown et al., 1996b). However, microglia on their own are not sufficient. In order for PrP106-126 to induce astrocyte proliferation, the astrocytes must express PrP^{c}. PrP106-126 cannot induce proliferation of astrocytes in mixed glial cultures from PrP^{c}-deficient mice (Brown et al., 1996b) and addition of wild-type microglia is insufficient for PrP106-126 to induce PrP^{c}-deficient astrocyte proliferation (Brown et al., 1998b). Furthermore, changes induced directly on the astrocyte, by PrP106-126, are necessary for the astrocytes to respond to the mitogenic factors released by PrP106-126 stimulated microglia (Brown et al., 1998b).

This model of astroglial proliferation is similar to the model of neurotoxicity as it requires (1) a direct involvement of microglia, (2) the indirect effect of microglial released substances, and (3) a direct effect on the target cell (neurone or astrocytes), which requires PrP^{c} expression.

Interleukin-1 and interleukin-6 were identified as the factors present in the medium of microglia and astrocyte co-cultures necessary for the proliferation of astrocytes induced by PrP106-126 (Hafiz and Brown, 2000). Previous studies have shown that PrP106-126 induces release of both cytokines from microglia (Peyrin et al., 1999). Treatment with antibodies against IL-1 and IL-6 inhibits the proliferation of astrocytes

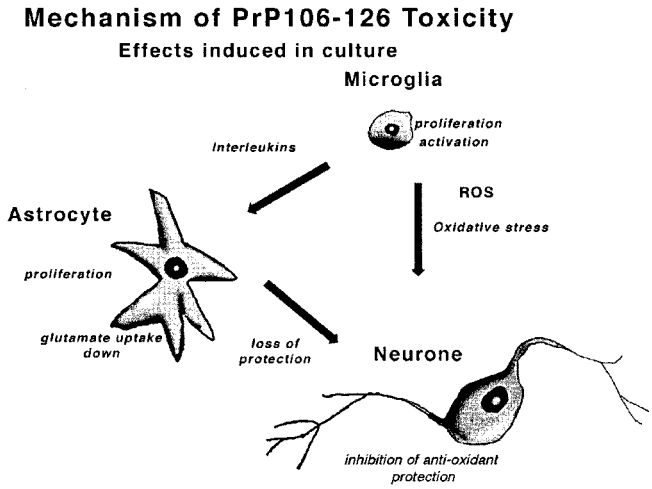

Fig. 4. Summary figure showing the details of the theoretical toxic mechanism of PrP106-126 to neurones as described in the text.

mediated by PrP106-126-treated microglia (Hafiz and Brown, 2000). PrP106-126 treatment enhances the level of expression of cytokine receptor for IL-1 and IL-6. However, the application of these cytokines in combination with PrP106-126 was insufficient to induce astroglia proliferation, implying that these factors do not completely replace the effect of microglia (Hafiz and Brown, 2000). The effects of IL-1 and IL-6 were potentiated with superoxide generated from xanthine oxidase and the proliferation of the astrocytes in co-culture with microglia was inhibited by superoxide dismutase, suggesting that superoxide or its products are somehow necessary for the proliferative effect of PrP106-126. Therefore, the signal coming from microglia inducing astrocyte proliferation in the presence of PrP106-126 is a multifactorial signal (Hafiz and Brown, 2000). The role of reactive oxygen species in enhancing the proliferation appears to be novel as there appears to be no other system so far described that includes reactive oxygen species in the induction of proliferation. This signal does not appear to include oxidative stress per se as the anti-oxidant N-acetyl cysteine does not inhibit astrocyte proliferation induced by PrP106-126 treated microglia (Hafiz and Brown, 2000).

As indicated above, the multifactorial signal coming from microglia just described is insufficient to induce proliferation of astrocytes equivalent to that induced by PrP106-126 in the astrocyte/microglia co-culture system. Astrocytes expressing PrP^c are primed by PrP106-126 to proliferate when the appropriate signal is received from microglia (Brown et al., 1998b). In other words, PrP106-126 enhances the proliferation signal from microglia to induce significant proliferation. The dependence of this astrocyte-specific effect on PrP^c expression suggests that PrP106-126 may directly interact with astrocytes via PrP^c or that PrP^c expression changes astrocytes to respond differently when PrP106-126 is in the environment. Understanding the direct effects of PrP106-126 on astrocytes that primes them to respond more to proliferation signals is more interesting, as identifying pathways involved in these changes may lead to ways to intervene and prevent astrocyte proliferation in prion disease.

Inhibitors of the MAP kinase/ERK pathway in astrocytes prevented the proliferation of astrocytes due to a direct effect of PrP106-126 on astrocytes (Hafiz and Brown, 2000). The proportion of the ERK proteins that were phosphorylated in astrocytes was increased after 1-day exposure of pure astrocyte cultures to PrP106-126. Increased phosphorylation of ERKs is, however, not a specific effect of PrP106-126 as increased phosphorylation of ERKs occurs under many conditions including treatment with βA25-35 (Hafiz and Brown, 2000).

PrP106-126 treatment of pure astrocytes greatly enhances cyclin E levels (Hafiz and Brown, 2000). This in itself is suggestive that PrP106-126 treated astrocytes are more likely to progress through to the late stages of the G1 phase of the cell cycle that proceeds DNA replication in S phase. However, PrP106-126 does not increase astrocytic expression of PCNA, a marker of entry into S phase, indicating that PrP106-126 does not induce increased entry into this part of the cycle (Hafiz and Brown, 2000). Therefore, in terms of "priming" astrocytes to respond more rapidly to proliferation signals, PrP106-126 induces progression through the cell cycle.

PrP106-126 induces other direct effects on astrocytes other than ones related to cell cycle. PrP106-126 inhibits glutamate uptake (Brown and Mohn, 1999) and increases the susceptibility of astrocytes to oxidative stress (Brown et al., 1998f). There are similarities between the phenotype of astrocytes deficient in PrP^c expression and the susceptibility of astrocytes to various insults in the presence of PrP106-126. PrP^c-deficient astrocytes also show increased susceptibility to the toxicity of oxidative stress (Brown et al., 1998b) and take up less glutamate than wild-type cells (Brown and Mohn, 1999). These similarities as well as similarities between PrP106-126 treated neurones and PrP^c-deficient neurones suggest that PrP106-126 may have the effect of inhibiting PrP^c function. This may once again relate to the recent discovery that PrP^c is an antioxidant (Brown et al., 1999) and the changes in astrocytes due to PrP106-126 may be a response to oxidative stress. However, PrP^c-deficient astrocytes do not proliferate more rapidly than wild-type cells (Brown et al., 1996b) and there is no indication that these cells respond more rapidly to mitogenic factors. Therefore, it is more likely that the changes induced in astrocytes by PrP106-126 leading to proliferation are more likely to be linked to the "stress" induced on the cells by the peptide. Astrocytes treated with PrP106-126 but prevented from proliferating by cytosine arabinoside show increased cell death (Brown et al., 1998c). In this case, the astrocytes probably die by a p53-mediated pathway as is known to be the case for cytosine arabinoside-induced cell death (Anderson and Tolkovsky, 1999) especially as PrP106-126 treatment enhances p53 expression (Hafiz and Brown, 2000). Therefore, increased progression through the cell cycle by PrP106-126 is possibly a way by which astrocytes try to escape commitment to cell death, an option neurones treated with the neurotoxic peptide do not have. Evidence for this also comes from studies with PC12 cells and myoblasts

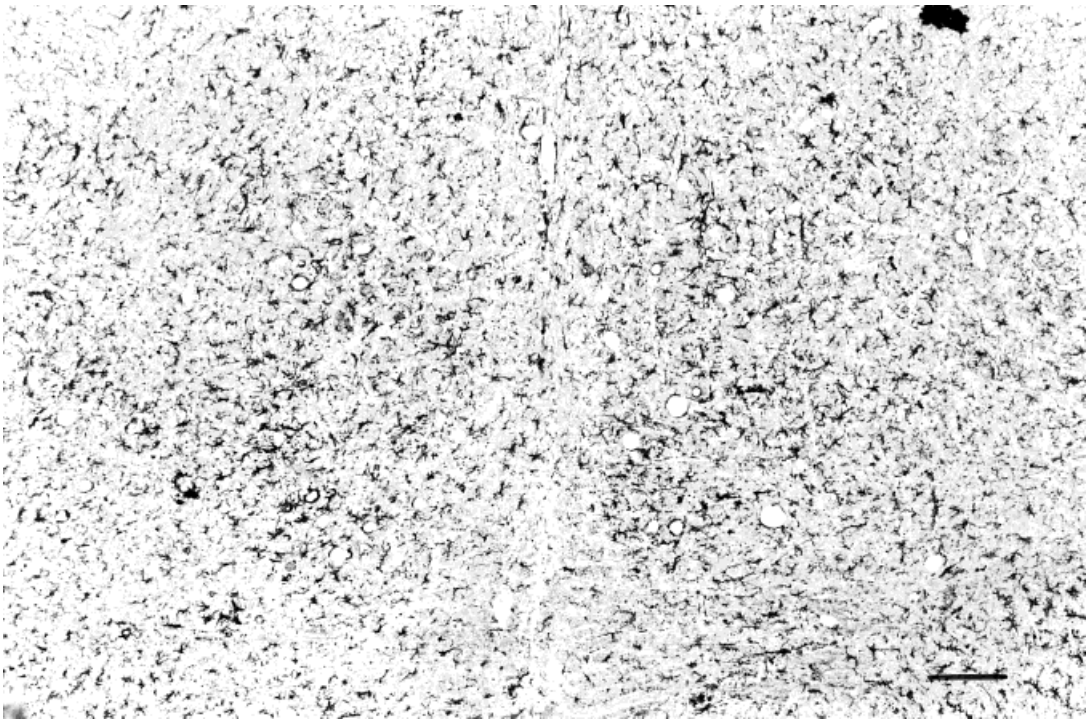

Fig. 5. A transverse section through the thalamus of an adult mouse infected with the 22A/VM strain of scrapie. Microglia were labelled with an antibody to the F4/80 epitope. Labelled cells are present throughout the section. These changes are present with the onset of clinical signs. Scale bar = 500 μm. Figure kindly provided by Dr. Alun Williams of the University of Glasgow.

as PrP106-126 is not toxic to undifferentiated PC12 cells or myoblasts that can divide but is toxic to differentiated PC12 cells and myotubes that cannot divide (Brown et al., 1997c, 1998c).

The result of these studies is a cell culture model of the late stages of prion disease pathology that emulates deposition of PrP^{Sc} in the form of PrP106-126 added to the culture, induction of gliosis, and subsequent neurodegeneration. Microglial proliferation and activation by PrP106-126 may lead to increased superoxide and release of cytokines. In the presence of PrP106-126, astrocytes then proliferate. However, there is possibly a decrease in glutamate clearance by the astrocytes leading to toxic levels of glutamate. PrP106-126 inhibits the functions of PrP^{c} as an antioxidant and neurones metabolically impaired by PrP106-126 then die from a mixture of oxidative stress either induced either directly or via glutamate. Although this model is detailed and complex (Fig. 4), its relevance hangs on evidence supporting it from animal models of the disease.

EVIDENCE FOR MICROGLIAL INVOLVEMENT IN VIVO

Unfortunately, at present a clear picture of the role of microglia in prion disease has not emerged. Changes in microglia were first noted in prion disease models by Williams et al. (1994a). There continues to be an increasing number of studies of microglia in prion disease. Nevertheless, evidence of microglia involvement in human disease is limited. There is evidence that microglia may be associated with PrP^{Sc} plaques (Guiroy et al., 1994; Ironside et al., 1993; Miyazono et al., 1991). A clearer picture comes from an animal model of prion disease, scrapie-infected mice (Figure 5). Mice infected with scrapie show significant activation of microglia (Betmouni et al., 1996). There is also evidence that this microglial activation precedes neuronal death. Studies of apoptosis using in situ end-labelling with terminal transferase have lead to evidence for the time course of apoptosis in mice infected with several different strains of scrapie (Giese et al., 1995). This apoptosis was found to follow activation of microglia sequentially as indicated by immunohistochemistry for markers of activated microglia (Giese et al., 1998). There is other evidence that microglia activation precedes neuronal death in general and especially precedes clinical symptoms (Betmouni et al., 1996) and that large numbers of microglia are present in the scrapie-infected mouse brain throughout the disease (Williams et al., 1994a).This suggests that microglial activation precedes neuronal death in mouse scrapie (Figure 6). Unfortunately, there is no proof that all the neuronal death in prion disease is apoptotic and similarly there remains no proof that microglial activation is causative as regards apoptosis. It is possible (as suggested above) that although microglia may play a role in neuronal death, they may also enhance astrocytic proliferation (gliosis) resulting in neuronal death by indirect means.

There is more evidence for the production of microglia products and especially cytokines. Increased

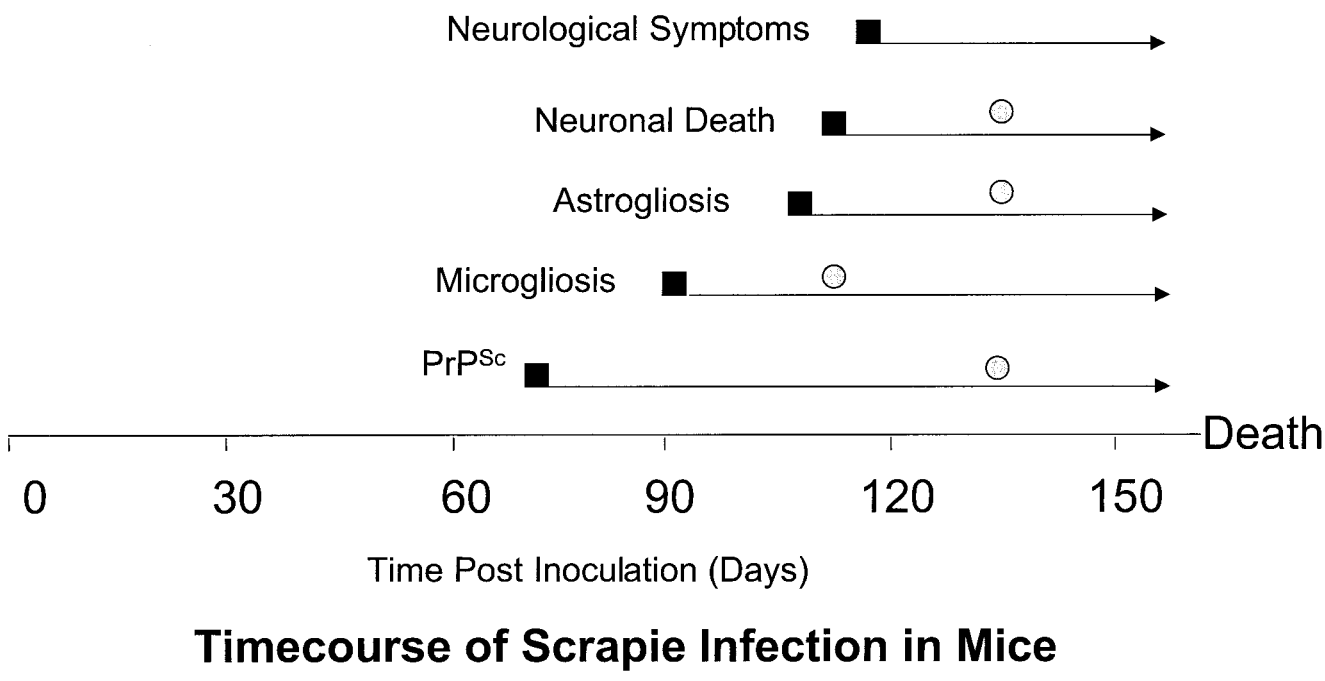

Fig. 6. This is a summary showing the relevant cellular changes in mice that have been experimentally inoculated with scrapie. This summary is based on approximation relating a number of similar strains such as RML and ME7, rather than a description of the effects of any particular scrapie strain. Black square, onset of the change (microglial proliferation/activation, neuronal death). Circle, point in time when the change reaches its maximal level.

expression of interleukin-1 and interleukin-6 as well as tumor necrosis factor α has been detected in the brains of hamsters (Kim et al., 1999) and mice (Williams et al., 1994b). As indicated above, the expression of both these cytokines is increased when microglia are treated with PrP106-126. Also, these cytokines are involved in PrP106-126 induced proliferation of astrocytes. Astrocytes in scrapie-infected hamsters show increased levels of the transcription factor NF-κB (Kim et al., 1999). It is known that interleukin causes increased nuclear entry of NF-κB in astrocytes (Moynagh et al., 1993). Increased levels of NF-κB can lead to increased expression of cytokines by astrocytes.

Several studies have now identified markers of oxidative stress in the brains of rodents with prion disease. There are increased levels of oxidised lipids in the brains of scrapie-infected hamsters (Guan et al., 1996). A recent study (Guentchev et al., 2000) has shown increased levels of nitrotyrosine and hemeoxygenase-1 in the brains of scrapie-infected mice. These observations imply that significant free radical damage is being generated in the brains of scrapie-infected mice. Additionally, there is evidence for mitochondrial damage in cells from brains of scrapie-infected hamsters and mice (Choi et al., 1998; Lee et al., 1999). These changes include reduction in the activity of mitochondrial enzymes and structural abnormalities in the mitochondria. Other enzymes known to be associated with resistance to oxidative stress such as catalase and glutathione-S-transferase show increased expression (Lee et al., 1999). Together, these results suggest that oxidative stress is involved in the pathology of prion diseases. It is possible that the oxidative damage to the brain in scrapie might be a result of the damage to mitochondria, which can generate superoxide but the measured level of oxygen radicals detected with dichlorofluorescein in mitochondrial fractions from the brains of scrapie-infected mice was not greatly increased above that of controls (Kim et al., 1999). Reactive oxygen species such as superoxide are generated by microglia and the implication of this is that microglia cause damage in the brain of scrapie-infected mice. However, microglia activation has also been postulated to be a response to neuronal damage rather than the cause of it. As suggested in the description of the culture model described above, microglia are unable to kill neurones in culture unless their metabolism has been deleteriously affected by the neurotoxic peptide. Therefore, it is possible that microglia are activated because of "damage" and/or PrP^{Sc} and the result of this is toxic only for impaired or sick neurones in the vicinity.

In summary, microglia are emerging as a causative component in prion disease. Abnormal activation of microglia as a result of accumulation of aggregated molecules of PrP^{Sc} may lead to neuronal death. Although cell culture studies have provided insights into the possible mechanism of neuronal death in these diseases, new in vivo models of prion disease are necessary to determine what parts of these insights hold true in the disease. Nevertheless, abnormal microglia behaviour has been noted in prion disease, implying that the cell culture model may not be far from the truth. Therefore, understanding and controlling the

microglial response may be important in treating such diseases as CJD.

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