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Invited Review

Microglia and prion disease: a review

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Summary. Prion diseases are characterized by the accumulation of PrP^{Sc}, an altered isoform of a normal cellular protein, PrP^{c}. The prion hypothesis holds that the process of conformational change from PrP^{c} to PrP^{Sc} under the influence of PrP^{Sc} constitutes the basic infectious mechanism in prion diseases. It is still unknown whether pathological changes in these diseases, which include spongiform degeneration, nerve cell loss and gliosis, are the result of neurotoxicity of PrP^{Sc}, loss of function of PrP^{c} or some other mechanism. Recent *in vitro* findings using a synthetic peptide of human PrP^{c} implicate microglia as a mediator of pathological changes. The mechanism of the toxicity of this peptide involves activation of microglia, oxidative stress, and direct interactions with PrP^{c}-synthesizing neurones that reduce their ability to cope with oxidative stress. Microglia thus seem to emerge as a mediator of neuronal degeneration and cell death in prion diseases.

Key words: Microglia, Prions, Pathology

Introduction

Prion diseases (transmissible spongiform encephalopathies) are a group of fatal progressive neurological disorders including Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, fatal familial insomnia and kuru in humans as well as bovine spongiform encephalopathy (BSE) and scrapie in animals. Scrapie brings about a wide variety of neurological and systemic changes which have been reviewed by Ye and Carp (1995). Prion diseases are unique, in being both horizontally transmissible as well as sometimes hereditary diseases (Prusiner, 1993).The infectious agent has been termed prion (Prusiner, 1982). The only molecule consistently found in infectious preparations is the scrapie isoform of the prion protein (PrP^{Sc}). This protein derives from a normal cellular isoform of the prion protein (PrP^{c}) which is encoded in the genome of all mammals investigated to date (Prusiner, 1991). PrP^{c} is a glycoprotein of unknown function normally found in neurones (Kretzschmar et al., 1986) and glia (Moser et al., 1995).

The classical pathological features of spongiform encephalopathies include vacuolation of the neuropil, astrogliosis and neuronal loss (Masters and Richardson, 1978). Neuronal degeneration is widespread in many cases of the human prion disease known as Creutzfeldt-Jakob disease (CJD). This may lead to almost complete loss of nerve cells. However, little is known about the mechanisms which underlie neuronal cell death in spongiform encephalopathies.

Cell culture experiments suggest that neuronal cell death in these diseases is due to apoptosis (Forloni et al., 1993; Müller et al., 1993). To test this hypothesis *in vivo* the in situ end-labeling (ISEL) technique and electron microscopy were used to study cell death in the 79A scrapie strain in the mouse. Morphological changes typical of apoptosis and in situ end-labelled nuclei were found to precede massive nerve cell loss in specific areas in this system (Giese et al., 1995).

It is not clear whether apoptotic nerve cell loss is caused by progressive loss of the function of PrP^{c} ("loss of function") or increasing toxicity of PrP^{Sc} ("gain of function hypothesis") in the course of the disease, or some other mechanism.

Büeler et al. (1992) were the first to generate PrP gene-ablated mice ($PrP^{0/0}$ mice) resistant to infection with scrapie (Büeler et al., 1993). These mice, which are devoid of PrP^{c}, develop normally and have apparently only slight behavioural abnormalities although a recent study suggests they have altered circadian rhythms (Tobler et al., 1996). Previous experiments also suggested that $PrP^{0/0}$ mice have altered LTP and synaptic transmission in the hippocampus (Collinge et al., 1994). Another strain of $PrP^{0/0}$ mice (Sakaguchi et al., 1996) differs from the first $PrP^{0/0}$ mice in that the transcript was fully ablated. These animals show abnormalities at 70 weeks of age, with loss of cerebellar Purkinje cells and motor abnormalities. However, the differences between these $PrP^{0/0}$ mouse strains is not understood and will have to be further investigated.

A fragment of human PrP, which consists of amino acid residues 106-126 (KTNAMKHMAGAAAAGA

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VVGGLG) and forms fibrils *in vitro*, has previously been demonstrated to be toxic to cultured hippocampal neurones (Forloni et al., 1993). In cell culture experiments we were able to demonstrate that synthetic PrP106-126 is toxic to cells from the cerebellum but that this toxicity requires the presence of microglia. In response to the presence of PrP106-126, microglia increase production of oxygen radicals. However, the effect of PrP106-126 on microglia, necessary for the toxic effect on normal neurones, is not sufficient to kill neurones from mice not expressing PrP^{c}. These findings are in accord with reports on the resistance of $PrP^{0/0}$ mice to PrP^{Sc} (Brandner et al., 1996). Our results are also indicative of a neurodegenerative mechanism similar to that of *in vitro* models of Alzheimer's disease (Moser et al., 1995).

Thus, one of the basic questions to be answered in the pathogenesis of various neurodegenerative diseases is whether microglia primarily function as scavengers or immunocompetent cells in the CNS or whether they can also be activated to destroy neurons in neurodegenerative diseases.

Microglia

Microglia are thought to be a resident macrophage cell population in the central nervous system (CNS) (Perry and Gordon, 1988; Davis et al., 1994). They arise from circulating monocytes that enter the brain and the retina during embryonic and early postnatal life (Hume et al., 1983; Perry et al., 1985; Ling and Wong, 1993). Yet microglia are functionally different from macrophages (Kettenmann et al., 1990). During brain maturation they phagocytose cellular debris associated with naturally occurring cell death (apoptosis) and remodelling of fiber tracts. After that developmental stage they transform from globose and ameboid, actively phagocytic forms, into the ramified resting microglia of the mature brain (Ling, 1979; Perry et al., 1985; Jordan and Thomas, 1988; Fujimoto et al., 1989; Ling and Wong, 1993; Theele and Streit, 1993). In the mature brain, resting (ramified) microglia constitute 5 to 20% of the neuroglial population, being less numerous in white than in grey matter (Lawson et al., 1990). In the adult, ramified microglia can be upregulated in their activity to activated and/or reactive microglia which then express macrophagic activity (Streit and Kreutzberg, 1987; Graeber et al., 1988; Streit et al., 1988; Davis et al., 1994). Activation of microglia occurs in response to a wide variety of tissue damage (Perry and Gordon, 1988; Streit et al., 1988; Giulian et al., 1993; Giulian and Vaca, 1993; Davis et al., 1994; Dickson et al., 1996). These reactivated microglia phagocytose foreing substances and debris. Microglia upregulation serves as an immune response (Giulian, 1987; Nakajima and Kohsaka, 1993; Davis et al., 1994) for the brain which only occurs under pathological al conditions.

Microglia can be activated by foreign substances introduced into the brain such as trimethyltin (Tschudi et al., 1995), transplanted brain tissue (Poltorak and Freed, 1989; Lawrence et al., 1990) or viruses such as HIV-1 (Watkins et al., 1990; Dickson et al., 1996). These substances, tissues and infectious agents in themselves may not be neurotoxic but may induce microglia to release substances whose accumulation in the CNS initiates neurodegeneration (Giulian et al., 1994). Also, following ischemia, microglia show a cytotoxic effect giving rise to a delayed neuronal death (Giulian et al., 1993; Lees, 1993).

It has been reported that the ß-amyloid protein of Alzheimer s disease also causes activation of microglia (Meda et al., 1995). ß-amyloid stimulates microglia to release tumor necrosis factor-α (TNF-α) and superoxide radicals (Meda et al., 1995) which are known to be neurotoxic at high levels. Microglia have been found to be associated with deposits of ß-amyloid in Alzheimer patients brains (McGeer et al., 1987; Frackowiak et al., 1992; Sheng et al., 1995; Jefferies et al., 1996). Neurodegeneration in Alzheimer s disease may therefore be related to activation of microglia. In addition, upregulation of MHC class II molecules has been observed in a number of neurodegenerative disorders such as Parkinson's disease, progressive supranuclear palsy, Lewy body dementia, multisystem atrophy and amyotrophic lateral sclerosis (Banati and Graeber, 1994).

Microglial activation has been found in human sporadic CJD and in experimental scrapie in the mouse in grey matter and white matter areas and surrounding spongy vacuoles (Williams et al., 1994a; Mühleisen et al., 1995). Microglia have also been observed in association with PrP^{Sc} plaques (Bruce and Fraser, 1975; Miyazono et al., 1991; Ironside et al., 1993; Guiroy et al., 1994).

Prion protein peptide and microglia-induced neurodegeneration

Neuronal loss in scrapie-infected mouse brains involves the activation of apoptotic mechanisms (Giese et al., 1995). The ability of PrP^{Sc} to activate cell suicide mechanisms in cell culture was first demonstrated by Müller et al. (1993) in rat embryonic cortical cell cultures. Demonstration of *in vivo* neurotoxicity of PrP^{Sc} has been difficult to study since PrP^{Sc} is also part of the infectious agent and in *in vivo* is never observed in the absence of infectious disease. Some insights have been gained by using brain tissue transplantation techniques (Brandner et al., 1996). Further insights into the mechanisms of neurotoxicity of PrP^{Sc} will come from studies in cell culture systems.

Cell cultures from embryonic to postnatal mouse or rat brain contain mixtures of cells including oligodendrocytes, astrocytes, microglia and neurones. Nonetheless they are routinely employed to demonstrate neurotoxicity of chemical agents. Forloni et al. (1993) used culture cells from embryonic rat hippocampus to investigate the neurotoxicity of prion protein peptides

based on the N-terminus of the human sequence. Two peptides with the amino-acid residues 106-126 and 127-147 of the human sequence of PrP contained high ß-sheet content but only PrP106-126 reduced cell survival in these cultures. The neurotoxicity of this peptide was confirmed in mixed cerebral cell cultures from the mouse (Brown et al., 1994). Cultures of cortical cells from embryonic mice showed a reduction of the number of MAP2-positive cells. Cell death induced by this peptide is also due to apoptosis and occurs in a concentration-dependent manner (Forloni et al., 1993) with a maximal effect at around 80 μM (Brown et al., 1994). Cell death caused by this peptide was measured using a standard MTT (3,[4,5 dimethyltheazoldyl] 2,5diphenyl tetrazolium bromide) assay and has proved to be an effective method to quantify the degree of cell loss (Table 1). MTT reduction is carried out in mitochondria. Investigations using a peptide based on the ß-amyloid sequence (A 25-35) (Shearman et al., 1994) which is also neurotoxic showed that this peptide inhibited the ability of mitochondria to reduce MTT. However, this loss of MTT-reducing ability which precedes cell death is not induced by PrP106-126 (Hope et al., 1996). Thus loss of MTT reduction is a measure of cell death.

The first insight into the specificity of this peptide's neurotoxicity was the demonstration that cellular expression of PrP^{c} is a requirement (Brown et al., 1994). These experiments were performed with PrP gene ablated mice generated by Büeler et al. (1992). We made cell cultures from the cortex of $PrP^{0/0}$ mice (Büeler et al., 1992) and treated them with PrP106-126 up to 200 μM over a period of ten days. After that time MTT measurements indicated that there was no loss of cells as compared to controls. However, wild type mouse cells thus treated showed a reduction to 65 % of the control value. This result suggests that PrP^{c} expression was necessary for the toxicity of PrP106-126.

Table 1. Toxicity of synthetic PrP peptides to wild-type mouse cells.

Peptide	Cortex	Cerebellum	
	C57BL/6J	F1	F1
No peptide	100± 2	100±3	100±4
PrP 106-126	65±2	63±3	57±4*
PrP 89-106	107±3	106±3	105±4
Scrambled	100±2	98±3	102±3

Quantitative evaluation of the effects of the toxic peptide PrP 106-126. The peptides used were PrP 106-126 (KTNMKHMAGAAAAGAVV GGLG) and PrP 89-106 (WGQGGGTHSQWNKPSKPK). "Scrambled" consists of the same amino acids as PrP 106-126 but in a random order (NGAKALMGGHGATKVMVGAAA). Cells from E16 mouse cortex or P6 mouse cerebellum were cultured in the presence of the peptides for 10 days. After this, survival was determined using an MTT assay. Values are expressed as a percentage of the control value. For each experiment controls treated without peptide were assayed in parallel and the measured value taken to be 100% survival. Shown are the mean and standard error for more than 4 experiments. *: indicates treatments with the peptides that were significantly different to those cultures treated without peptides (Student's t test, $p<0.05$).

Similar results were achieved by Brandner et al. (1996) in an *in vivo* system. When $PrP^{+/+}$ neuronal tissue was transplanted into $PrP^{0/0}$ mice, infection with scrapie prions led to neurodegeneration of the implanted tissue but not in the host, even when PrP^{Sc} plaques were present in the $PrP^{0/0}$ host. Their interpretation was that the availability of Pr^{PC} for some intracellular process was directly linked to spongiosis, gliosis and neuronal death. While graft-produced infectious PrP^{Sc} was present in their system *in vivo*, our system uses a synthetic peptide corresponding to amino acids 106 to 126 of cellular human PrP in a cell culture system.

The PrP106-126 peptide is also toxic to cerebellar granular cells (Brown et al., 1996a) (Table 1). At 80 μM PrP106-126 is more toxic to cerebellar cells than to cortical cells. Nevertheless, $PrP^{0/0}$ cerebellar cells are also resistant to PrP106-126 toxicity (Brown et al., 1996a). This difference may be related to different levels of PrP^{c} expression in the different brain regions. Cell lines with neuronal phenotype show a sensitivity to PrP106-126 toxicity that can be related to their level of PrP^{c} expression (Hope et al., 1996). The higher the expression of PrP^{c} the greater the percentage of cells killed by PrP106-126 treatment.

Treatment of mixed cell cultures from the cerebellum with L-leucine-methylester (LLME) was used to reduce the number of microglia present (Giulian and Baker, 1986). LLME treatment does not significantly affect the survival of other cells in the culture. However, this treatment effectively abolishes the toxicity of PrP106-126 (Table 2). Additionally, co-culturing cerebellar cells with additional microglia increases the number of cells killed by PrP106-126. These results clearly suggest that microglia are involved in the neurotoxic effects of PrP106-126.

Table 2. Effect of PrP 106-126 on survival of mixed cells from cultures of normal mouse cerebellum under various conditions as determined by MTT assay.

	LLME-TREATED			
Co-cultured with	Control	PrP106-126	Control	PrP 106-126
No co-culture	100±2	55±5	98±2	93±4
Normal microglia	98±4	47±4	98±4	55±5
$PrP^{0/0}$ microglia	97±5	53±4	97±4	70±4
Normal astrocytes	99±3	58±4	99±3	100±2

The effect of 80 μM PrP 106-126 on cerebellar cultures was determined by assessing relative survival of treated cells as compared to control cultures not treated with the peptide and expressed as a percentage of control survival. This relative survival was determined by an MTT assay carried out following 10 days' treatment with the peptide. PrP 106-126 treatment produced a significant cell loss (Student's t test, $p<0.05$). L-leucine methyl ester (LLME) treatment of cerebellar cultures did not significantly alter cell survival but blocked the toxic effect of the peptide. Co-culturing LLME-treated cerebellar cells with astrocytes from normal mice did not alter survival of control cultures or restore the toxicity of PrP 106-126. Co-culturing untreated cerebellar cells with normal microglia significantly enhanced the toxicity of PRP 106-126. Co-culturing cerebellar cells with microglia from $PrP^{0/0}$ mice showed a reduced effect. Shown are mean survival + standard error.

Low concentrations (e.g. 20 μM) of PrP106-126 are not toxic to wild type cerebellar cells in culture. However, when wild type cerebellar cells are co-cultured with additional microglia, 20 μM PrP106- 126 becomes toxic to the cerebellar neurones. This toxicity increases in relation to the number of microglia added. Co-culturing cerebellar cells with astrocytes does not have the same effect (Brown et al., 1996a).

Addition of $PrP^{0/0}$ microglia to LLME-treated wild-type cerebellar cells could also restore neurotoxicity of PrP106-126. However, these microglia were less effective than wild-type microglia which suggests that the role of microglia in this neurotoxic mechanism is at least partially independent of PrP^{c} expression of microglia.

If destruction of neurones by PrP106-126 is purely an indirect effect of the peptide on microglia one would expect $PrP^{0/0}$ neurones to be similarly destroyed by the presence of PrP106-126 and wild-type microglia. $PrP^{0/0}$ cortical cells co-cultured with wild-type microglia continued to be resistant to the effects of PrP106-126 even at 200 μM (Fig. 1). This result again emphasizes the importance of PrP^{c} expression to neurotoxic susceptibility. Therefore microglia play a necessary, but not sufficient role in the neurotoxic effect of the peptide. Similarly, PrP^{c} expression is also necessary but at least in a mixed primary cell culture system, not sufficient.

The use of anti-oxidants, such as vitamin E and N-acetyl-cysteine, allowed us to demonstrate that oxidative stress was also involved in the toxic mechanism of PrP106-126 (Brown et al., 1996a). Both these agents blocked the neurotoxicity of the peptide on wild-type cerebellar cultures (Fig. 2). The implication of this result is that the peptide PrP106-126 induces the generation of oxidative substances by cells present in the mixed cell culture. Alternatively or in addition there may be a lowering of neuronal resistance to the effects of reactive oxygen species (ROS) or inhibition of defensive enzymes.

Microglia already implicated in the toxic mechanism of PrP106-126 are a major source of ROS when activated (Giulian, 1987). Substances released from activated microglia such as ROS are often short-lived in solution and therefore are difficult to measure. However, superoxide and nitric oxide, which can be both released from microglia and have destructive effects can react to form nitrite, a more stable compound (Beckman et al., 1990). Microglia isolated from cerebral cortex of wild-type mice were exposed to PrP106-126 for four days and the supernatant was assayed for nitrite. First, the supernatant was treated with hydroxyammonium chloride to react all superoxide present to nitrite. Measurement of nitrite production showed that PrP106-126 stimulated microglia to a high degree (Fig. 3). In the presence of PrP106-126, nitrite production was greatest from wild-type mouse microglia but microglia from $PrP^{0/0}$ mice could also be stimulated to release significant levels. This indicates that microglia release ROS upon activation by PrP106-126. The role of microglia within the toxic mechanism of PrP106-126 is

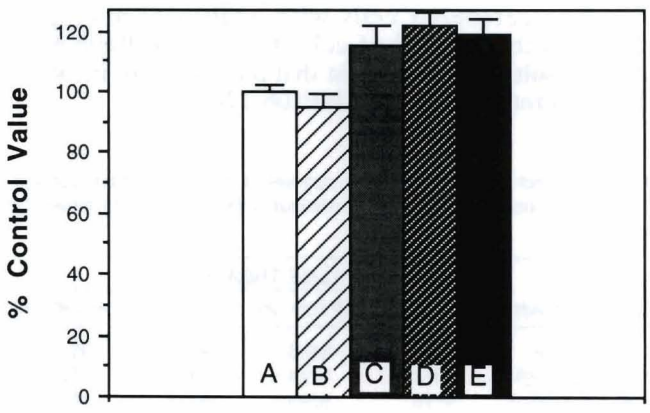

Fig. 1. Effects of microglia co-culture and treatment with PrP106-126 on survival of $PrP^{0/0}$ cerebellar cells. $PrP^{0/0}$ cerebellar cells were prepared from 6-day-old mice and plated on coverslips. The effect of a ten-day treatment was determined by MTT assay. Values are expressed as percentage of the untreated control value **(A)**. Co-cultured cerebellar cells **(B)** in the absence of PrP106-126 show the same degree of survival as cells without co-culturing. Treatment with PrP106-126 alone **(C)** causes a slight increase in observed cell survival above control levels, but this effect is unaltered (no significant difference $p>0.05$) by co-culturing the cerebellar cells with microglia **(D)** even if the concentration of PrP106-126 is increased to 200 μM **(E)**. Taken together with the findings in Table 1 this result suggests that expression of PrPC is necessary for microglia to induce neuronal destruction in the presence of PrP106-126. Shown are mean ± standard error for 5 experiments with 3 determinations each.

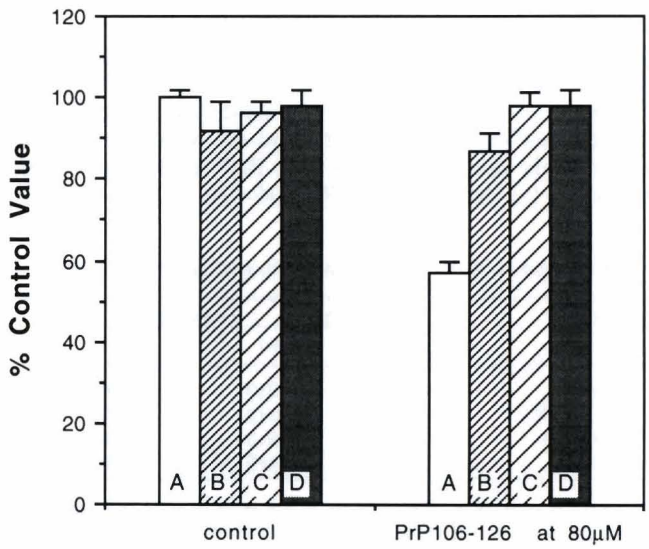

Fig. 2. Histograms show the survival of wild-type cerebellar cultures treated with anti-oxidants **(B-D)** in the presence or absence of PrP106-126. Cultures treated with antioxidants are compared to untreated cultures **(A)**. PrP106-126 and antioxidants were applied every second day. Antioxidants used were Vitamin E at 60 mM **(B)** and at 120 mM **(C)** concentrations and N-acetyl-cysteine (**D**, 10 μM). After ten days, survival was determined by MTT assay. The ability of anti-oxidants to abolish the toxicity of PrP106-126 suggests that the mechanism of toxicity of this peptide involves oxidative stress. Shown are the mean ± standard error of 5 experiments with 3 determinations each.

thus likely to be one that induces oxidative stress on neurones by the release of neurotoxic substances.

PrP106-126 stimulates microglia to release oxidative substances regardless of whether they express PrP^c or not. Activation of $PrP^{0/0}$ microglia to release ROS, albeit to a lesser extent, suggests that they should also be able to kill neurones. As indicated above, $PrP^{0/0}$ microglia can restore the toxic effect of PrP106-126 to wild-type cerebellar cell cultures pretreated with LLME to remove their resident microglia. This indicates that these $PrP^{0/0}$ microglia, activated by PrP106-126, can also be neurodestructive. PrP^c expression by neurones may therefore make them more sensitive to oxidative stress. We investigated this by subjecting cerebellar cultures from wild type and $PrP^{0/0}$ mice to oxidative stress from an alternative source of oxygen radicals, i.e. xanthine oxidase-produced oxygen radicals in the presence of xanthine. We showed that, in fact, $PrP^{0/0}$ microglia-reduced cerebellar cells are more sensitive to oxidative stress than wild type cells. Therefore PrP^c expression does not make cells more sensitive to oxidative stress but more likely the opposite (Fig. 4).

Cerebellar cells treated with LLME to reduce microglia are resistant to PrP106-126 toxicity, but when a source of oxidative stress is added to the culture at the same time as PrP106-126 enhanced cell destruction is seen greater than that induced by that oxidative stress alone. Xanthine oxidase toxicity to wild type cells is thus enhanced by addition of 80 μM PrP106-126. Toxicity of xanthine oxidase-produced free radicals on $PrP^{0/0}$ cerebellar cells is not enhanced by PrP106-126. This result therefore ties together the two previous findings. PrP106-126 is neurotoxic as a consequence of at least two factors. Firstly, oxidative stress is induced in the culture by the effect of PrP106-126 on microglia. Secondly, PrP106-126 is neurotoxic only to PrP^c-expressing cells. This second effect of PrP106-126 results in reduced cellular resistance to the oxidative substances released by the activated microglia.

These results clearly implicate PrP^c in cellular resistance to oxidative stress. In our *in vitro* system PrP106-126 both increases oxidative stress by activation of microglia and by interfering directly with cellular resistance to the toxic effect of ROS. PrP^c-deficient cells have an impaired resistance to oxidative stress but the level of ROS release by PrP106-126-activated microglia alone is not sufficient to be significantly neurotoxic to PrP^c deficient cells. PrP^c expression therefore increases cellular resistance to oxidative stress, but nevertheless makes those cells prone to inhibition of their resistance by PrP106- 126. PrP^c-deficient cells cannot be inhibited in this way.

Prion protein peptides, microglia and gliosis

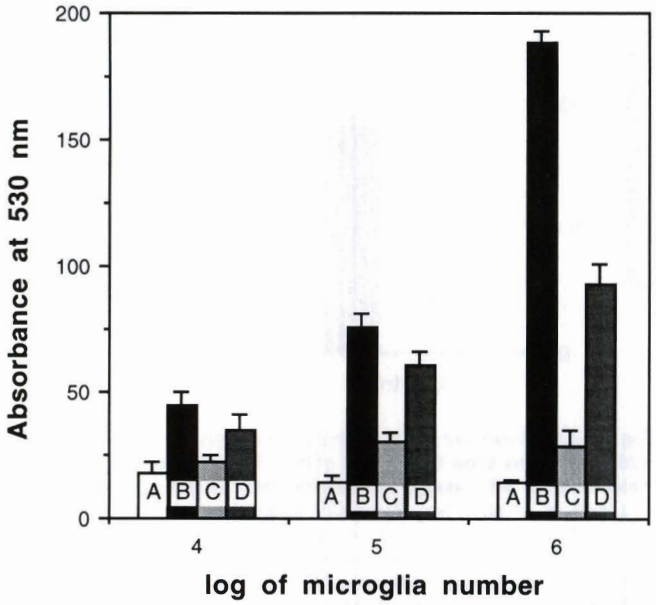

Fig. 3. Nitrite production by varying concentrations of microglia is measured by a colorimetric assay. Absorbance values at 530 nm correspond to the presence of nitrite in the media of cultured cells. Microglia exposed to 80 μM PrP106-126 (**B**: wild type, **D**: $PrP^{0/0}$) show significantly more nitrite production than controls (A: wild type, **C**: $PrP^{0/0}$). Enhanced nitrite production is proportional to the log number of microglia. Enhanced nitrite production is greater in normal microglia. Shown are the mean and standard error for 12 determinations.

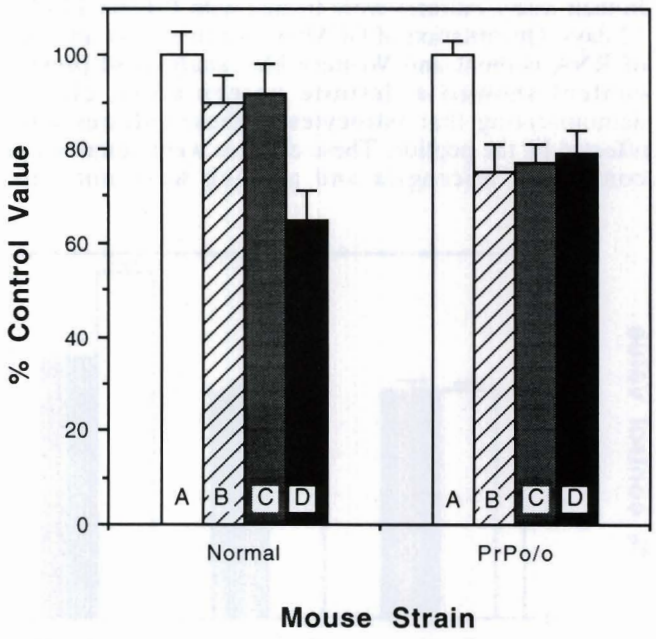

Fig. 4. PrP106-126 treatment enhances susceptibility of PrPC expressing cells to oxidative stress. Wild-type or $PrP^{0/0}$ cerebellar cells are treated with LLME to destroy microglia. Compared to controls **(A)** 5 μUnits of xanthine oxidase (an oxygen radical producer) is slightly toxic to normal LLME-treated cells but significantly more toxic (Student s t test, $p<0.05$) to $PrP^{0/0}$ cerebellar cells, suggesting they are less resistant to oxidative stress **(B)**. 80 μM PrP106-126 alone did not kill LLME-treated cerebellar cells (see Table 2) and enhanced the effect of xanthine oxidase only on wild-type LLME-treated cells **(D)**. 80 μM scrambled peptide applied with xanthine oxidase did not enhance the toxicity of xanthine oxidase **(C)**.

Gliosis, and particularly astrogliosis, is one of the major pathological changes of the spongiform encephalopathies and occurs early in the course of the diseases (DeArmond et al., 1987; Liberski, 1993). It is not clear at present if this high proliferation of glia occurs as a response to the presence of PrP^{Sc} or as a response to damage to neurones or some other stimulus. Close analysis has shown that the sites of astrogliosis coincide with distribution of PrP^{Sc} accumulation (DeArmond et al., 1987). Also, sites of astrogliosis and of high glial fibrillary acid protein (GFAP) mRNA have proved to be good indicators of the presence of PrP^{Sc} (Mackenzie, 1983; Manuelidis et al., 1987). Some reports indicate that astrocytes upregulate a number of specific proteins as a result of scrapie infection including GFAP and glutamine synthase (Andres-Barquin et al., 1994; Lazarini et al., 1994; Lefrancois et al., 1994). However, hypertrophy, proliferation and associated histological and metabolic changes are characteristic responses of astrocytes to injury and neurodegeneration other than those induced by PrP^{Sc} (Eng et al., 1987; Malhotra et al., 1990).

Forloni et al. (1994) showed that the peptide PrP106-126 induces astrocytic proliferation and upregulation of GFAP expression in mixed glial cultures. In their assay, cultures were treated with PrP106-126 for 12 days. Quantitation of GFAP by Northern blot analysis of RNA content and Western blot analysis of protein content showed a definite upregulation, clearly demonstrating that astrocytes in these cultures were affected by the peptide. These cultures were described as containing microglia and as such were not pure astrocytic cultures. In mixed glial cultures cytokines released by microglia can stimulate astroglial proliferation (Giulian and Baker, 1986).

This issue was reinvestigated (Brown et al., 1996b) by applying PrP106-126 to both mixed glial cultures and cultures of almost pure astroglia and microglia isolated by stringent conditions (Giulian and Baker, 1986; Levison and McCarthy, 1991), taking advantage of the greater adhesiveness of microglia in culture.

Under controlled culture conditions PrP106-126 induces astrocytic proliferation in mixed glial cultures (Fig. 5) but does not stimulate proliferation of purified type 1 or type 2 astrocytes (Fig. 7). And, PrP106-126 did not increase the level of GFAP expression in purified astrocytes (Brown et al., 1996b). When mixed glial cultures were treated with LLME to destroy microglia, PrP106-126-induced proliferation of astrocytes was abolished. Addition of extra microglia to LLME-treated astrocytes, however, restored the proliferative effect (Fig. 6). This implies that PrP106-126-induced astrocytic proliferation is dependent on the presence of microglia under our experimental conditions. As cytokines are secreted by activated microglia and are known to induce astroglial proliferation (Giulian et al., 1988a,b) they may also be involved in this mechanism in our cell culture system.

The experiments described above were also performed on mixed glial and astrocytic cultures derived

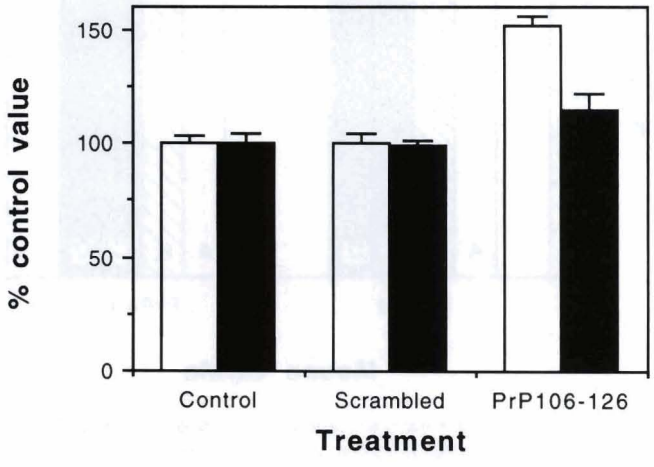

Fig. 5. Treatment of mixed glial cultures with PrP106-126. Mixed glial cultures from either wild-type (open bars) or $PrP^{0/0}$ (black bars) mice are treated with either PrP106-126 or the scrambled peptide for 12 days. After this time cell survival was determined using an MTT assay. A highly increased cell number is only observed for PrP106-126 treatment of mixed glia of wild-type mice. All values are expressed as a percentage of the control absorbance at 570 nm. Shown are mean ± standard error.

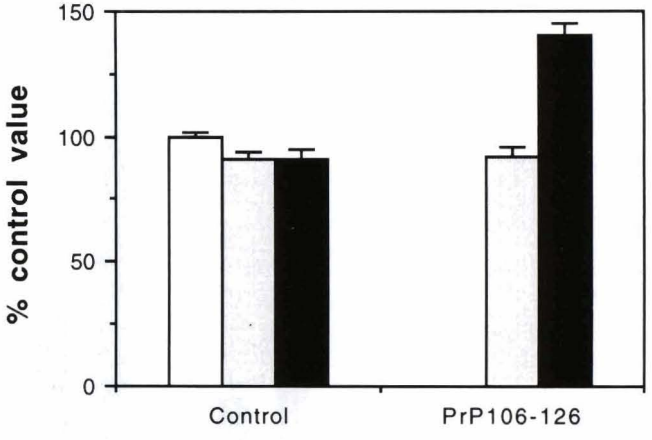

Fig. 6. LLME treatment of mixed glial cultures and the effect of PrP106-126. Histograms show the survival of mixed glia from wild type mice as determined by MTT assay. The determination for the untreated control is shown as an open bar. Mixed glial cultures were treated with LLME for 2 hours (grey bars). This treatment results in approximately 8% reduction in MTT assay absorbance after 12 days. PrP106-126 application for 12 days following LLME treatment resulted in no further reduction in the MTT assay absorbance. LLME-treated mixed glia were also co-cultured with normal microglia for 12 days (black bars). Co-culturing with microglia does not alter the MTT assay absorbance of LLME-treated mixed glia. This suggests that no cross-contamination of microglia from co-culturing occurrs. However, co-cultured LLME- treated mixed glia, after application of PrP106-126 for 12 days, increase substantially in number, which indicates that PrP106-126-treated microglia stimulate astroglial proliferation. All values are expressed as a percentage of the absorbance at 570 nm of the untreated control. Shown are mean ± standard error.

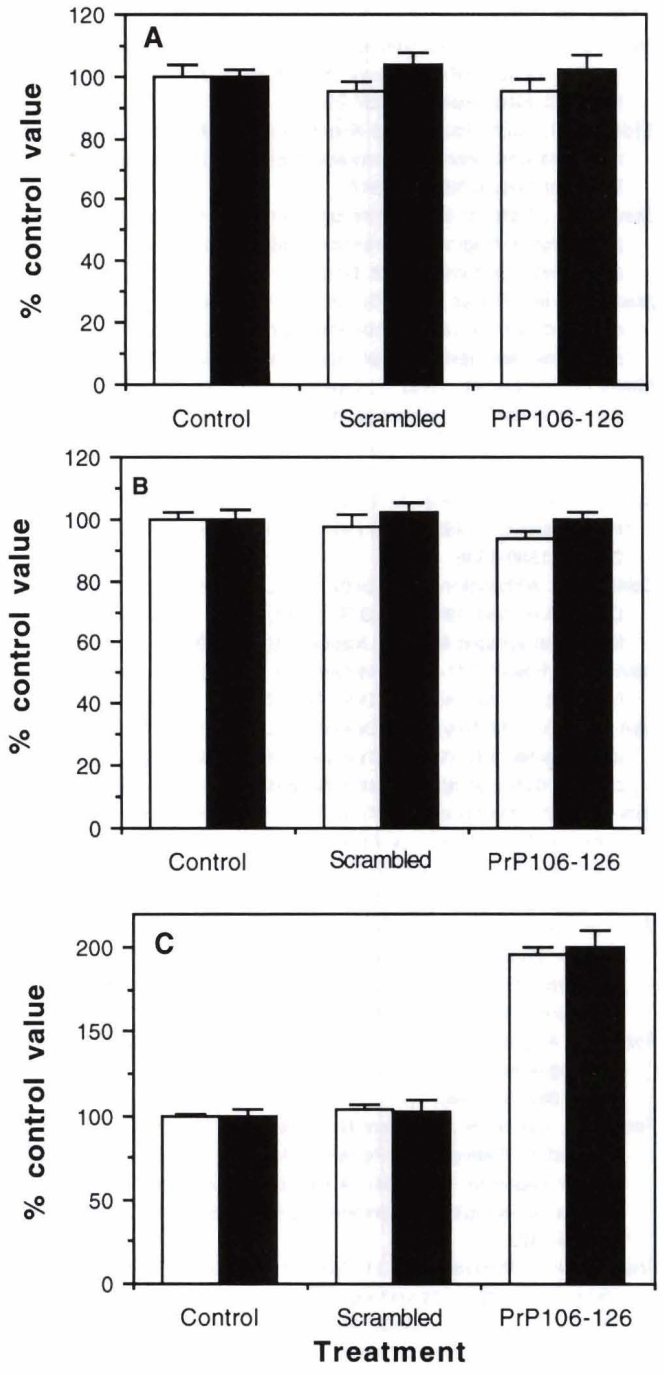

Fig. 7. Treatment of glial subtypes with PrP106-126. Separation of type 1 astrocytes **(A)** type 2 astrocytes **(B)** and microglia **(C)** from mixed cultures from wild-type (open bars) or $PrP^{0/0}$ (black bars) mice is carried out and treatment with PrP106-126 and the scrambled peptide performed. MTT assays after 12 days demonstrate increased proliferation of microglia only. The proliferation is similar for wild-type and $PrP^{0/0}$ microglia. All values are expressed as a percentage of the absorbance at 570 nm for untreated cells (control). Shown are mean ± standard error.

from $PrP^{0/0}$ mouse cortex. Mixed glial cultures from $PrP^{0/0}$ mice only showed a slight (10%) (Fig. 5) but significant increase in number of cells present as compared to cultures from wild type mice (50% increase). This suggests that astrocytic proliferation as induced by PrP106-126 is also dependent on the expression of PrP^{cC}.

Microglia and prion disease pathology

Our results from experiments with neuronal and glial cultures focus attention on microglia. Activation of microglia by PrP106-126 leads to increased production of reactive oxygen (ROS) and possibly cytokines by these cells which then have secondary effects on neurons and astrocytes present in the culture (Fig. 8). In addition, PrP106-126 treatment of purified microglia culture stimulates microglial proliferation. Treatment with 80 μM PrP106-126 for 12 days almost doubled the microglia number in cultures, as determined by MTT assay (Fig. 7). Interestingly, this proliferation was not dependent on PrP^{c} expression, as microglial proliferation was observed even in microglia isolated from $PrP^{0/0}$ mice. An effect on $PrP^{0/0}$ microglia is consistent with our previous results, suggesting that $PrP^{0/0}$ microglia increase ROS production in the presence of PrP106-126. It should also be pointed out that activated microglia do not proliferate. However, both activation and proliferation of microglia may be occurring in different subgroups of microglia present in our cultures at different times. The small increase in cell number seen in PrP106-126-treated $PrP^{0/0}$ mixed glial cultures may therefore be due to proliferating microglia

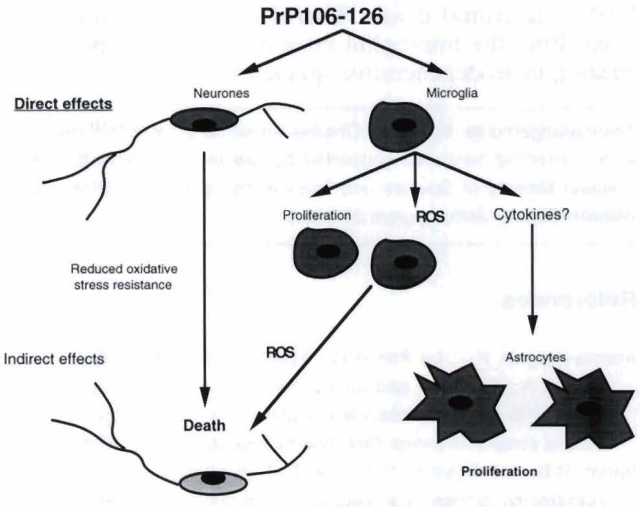

Fig. 8. Summary of the effects of PrP106-126 on cells in culture. PrP106-126 has direct effects on neurones and microglia. The direct effect on neurones reduces their resistance to oxidative stress and is dependent on PrPC expression. The direct effects on microglia cause them to release ROS and possibly cytokines. The effects on microglia then cause indirect effects on neurones and astroglia. The result is neuronal death and astroglial proliferation.

and not astrocytes.

The conclusion that both astrocytes and neurones are dependent on PrP^c expression for their susceptibility to the direct or indirect effects of PrP106-126 may reflect their common developmental origin. Microglia do not share this origin and are believed to be derived from monocytes that invade the brain during embryonic development.

The results from investigations in our system therefore suggest that a significant role in the pathogenesis of prion diseases may be played by microglia. It is important to bear in mind that our findings derive from cell culture experiments and that are only a small number of investigations dealing with microglia in prion diseases *in vivo*. Some publications suggest that microglia may be associated with PrP^{Sc} plaques (Bruce and Fraser, 1975; Miyazono et al., 1991; Ironside et al., 1993; Guiroy et al., 1994). A pronounced microglial activation was found in mice infected with various scrapie strains. This activation was felt to represent a modified inflammatory response (Williams et al., 1994a). It is unknown whether microglia are involved in processing of PrP^c or PrP^{Sc}.

At present there is some evidence for cytokine production in scrapie (Williams et al., 1994b), but there is no direct indication as yet for increased generation of reactive oxygen species in vivo. It will be interesting to see whether microglial activation precedes neuronal death in experimental models of scrapie in the mouse.

In summary, microglia are emerging as a mediating component in neurodegenerative disorders, such as Alzheimer's disease, amyotrophic lateral sclerosis and prion disease. Activation of microglia and production of reactive oxygen species as a result of interaction with neurotoxic molecules, such as PrP^{Sc} or ß-amyloid, may lead to neuronal death. Therefore, understanding and controlling the microglial response will be important in treating neurodegenerative species.

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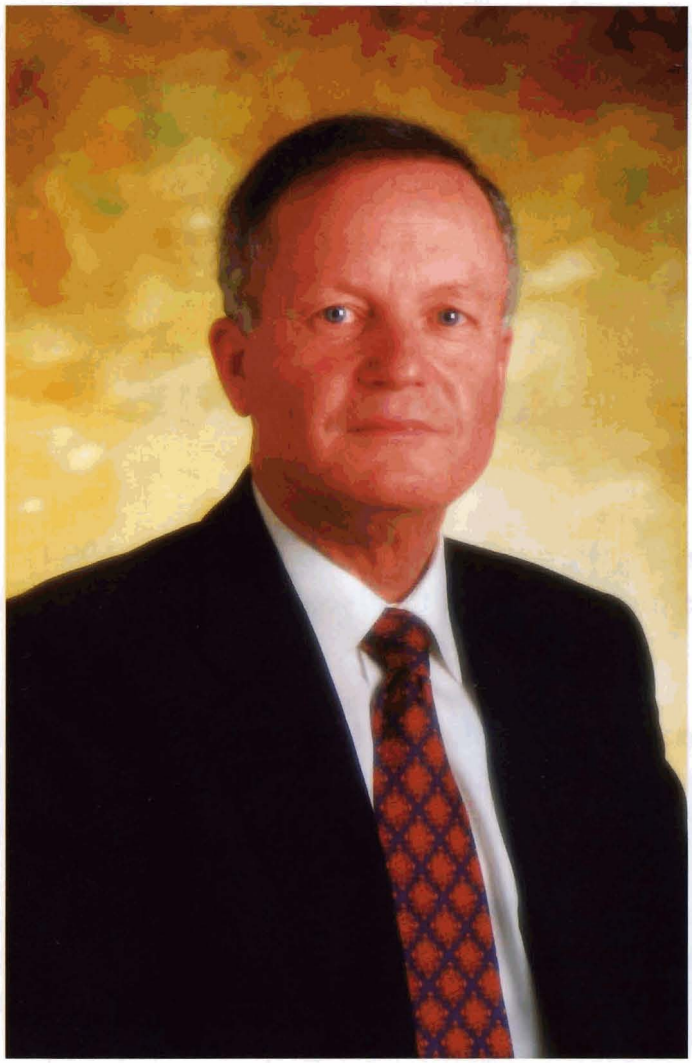

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