

Cellular pathogenesis in prion diseases

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Abstract – Prion diseases are characterised by neuronal loss, vacuolation (spongiosis), reactive astrocytosis, microgliosis and in most cases by the accumulation in the central nervous system of the abnormal prion protein, named PrP^{Sc}. In this review on the “cellular pathogenesis in prion diseases”, we have chosen to highlight the main mechanisms underlying the impact of PrP^C/PrP^{Sc} on neurons: the neuronal dysfunction, the neuronal cell death and its relation with PrP^{Sc} accumulation, as well as the role of PrP^{Sc} in the microglial and astrocytic reaction.

Prion / neurodegeneration / cell death / neuronal dysfunction / gliosis

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1. INTRODUCTION

Prion diseases are characterised by neuronal loss, vacuolation (spongiosis), reactive astrocytosis, and microgliosis. They are associated in most cases with the accumulation in the central nervous system (CNS) of the abnormal protease resistant prion protein, named

PrP^{Sc} resulting from the post-translational conversion of the normal host-encoded PrP^C protein [96, 97]. These few general sentences that often, if not always, start reviews and prion articles are particularly relevant in the context of this review that sets out to address the vast issue of ‘cellular pathogenesis in prion diseases’. They also prompt us to focus on the impact of disease associated PrP species production/accumulation on neurons

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(neuronal dysfunction, neuronal cell death) and on astrocytes and microglia.

2. NEURONAL IMPAIRMENT

Although severe cognitive deficits observed in transmissible spongiform encephalopathies (TSE) may result from neuronal loss, early behavioural impairments are probably related to primary neuronal dysfunction. This has been exemplified in a recent study by Mallucci et al. [73, 74] who have shown that behavioural and synaptic impairments, as well as spongiosis, recover when neuronal PrP^C is depleted. These results suggest that early functional impairments precede neuronal loss in prion disease. Moreover, this rescue occurred before extensive PrP^{Sc} deposition, supporting the concept that a PrP transient species, distinct from aggregated PrP^{Sc} may be involved. Little is known about the cellular mechanisms underlying prion disease neuronal dysfunctions. Cellular models are poorly representative of the complexity of neuronal circuitry and their susceptibility to prion strains is limited [122]. Therefore, neuropathological data of the different prion strains, which are able to induce different but specific lesions in the infected brain [13, 36], result mainly from histopathological or biochemical examination of human brain or animal models.

2.1. Alteration of neurotransmitter systems

Several neurotransmitter systems (acetylcholine, GABA (Gamma-aminobutyric acid), dopamine, etc.) are damaged in TSE. Loss of GABAergic neurons appeared to be the first detectable neuropathological change. Presynaptic bouton loss (and alteration with presence of abnormally aggregate vesicles) is also observed, but only at the terminal stages of disease. This is associated with a significant decrease of the stimulated [3H]-GABA release from synaptosomes and with the accumulation of PrP^{Sc}. Functional abnormalities could thus be linked to PrP^{Sc} deposition [7]. Alterations may be also attributed to PrP^C loss of function since hippocampal slices from PrP null mice have weakened GABA receptor-mediated fast inhibition and impaired long-term potentiation [23]. This impaired synaptic inhibition

could be involved in the epileptiform activity seen in Creutzfeldt-Jakob disease (CJD) [23]. Some studies performed during the late stages of TSE have also indicated altered concentrations of dopamine, norepinephrine and serotonin in scrapie-infected animal models [3, 48]. Additional studies have reported the results of behavioural tests and monoamine concentrations in hamsters at different times after infection with the scrapie 263K strain. They have shown significant changes in spontaneous motor activity later after infection correlated with a marked alteration in serotonergic function. Ten days before any evident clinical signs and motor disturbances, memory deficit related to alteration in the dopaminergic system (assessed by metabolite/dopamine ratios measures) was also found in the infected animals [2]. The serotonergic system has also been analysed in human TSE by measuring the size and number of tryptophan hydroxylase (TPH) positive cells in the dorsal raphe. While there were no differences in the number of TPH-positive cells, the mean TPH-positive cell size and TPH staining intensity was significantly greater in CJD compared to controls, suggesting a pathological over-activity of the brain serotonergic systems in CJD. This may result in excessive release of 5HT (5-hydroxytryptamine or serotonin) within the brain, leading to clinical symptoms such as fluctuating attention and arousal [44]. Other changes in neuropeptide expression in mice infected with prion have also been reported [31]. Neuropeptide Y (NPY), enkephalin and dynorphin-like immunoreactivities increased in mossy fibers of infected mice, whereas cholecystokinin-like immunoreactivity decreased compared to non infected mice [31]. These modifications in neuropeptides ratio may trigger abnormal response in the infected brain.

2.2. Synaptic alteration

In experimental scrapie, loss of synapses is also an early abnormality. In ME7 infected mice, there is a significant reduction in synaptophysin in the CA1, 13 weeks after ME7 hippocampic injection, indicating loss of presynaptic terminals [29]. In addition, in

sporadic CJD brains, it has been reported a decreased expression of crucial proteins (synaptophysin, p38, synaptosomal-associated protein (SNAP-25), syntaxin-1, synapsin-1, α - and β -synuclein and Rab3a). Moreover, synaptic protein accumulation in the soma and axonal torpedoes of Purkinje cells could result in the impairment of axonal transport [41, 114]. Defects in axonal transport and synaptic dysfunctions have also been challenged by testing the effect of full-length mammalian prion protein rPrP fibrils on human embryonic teratocarcinoma NTERA2 cells (NT2) that were terminally differentiated into neuronal and glial cells and co-cultured. The authors have observed degeneration of neuronal processes accompanied by a collapse of microtubules and aggregation of cytoskeletal proteins, formation of neuritic beads, and a dramatic change in localization of synaptophysin [88]. Whether these changes are linked to abnormal PrP metabolism is not known. However, in retina and optic nerves, it has been shown that like its normal counterpart PrP^C, most PrP^{Sc} remained associated with lipid rafts while usual PrP-interacting proteins caveolin-1 and synaptophysin shifted to non-detergent-resistant membrane (non-DRM) fractions during the course of scrapie infection. This suggests that the conversion of PrP^C into PrP^{Sc} could modify the PrP^C/caveolin-1/synaptophysin complex [107].

2.3. Dendritic atrophy

Dendritic atrophy is also a prominent feature of prion diseases [63]. A recent report has shown that Notch activation (which is implicated in both dendrite growth and maturation) may be involved in this atrophy. Indeed, the released cleaved intracellular fragment of Notch-1 (NICD) increased concomitantly with PrP^{Sc} levels in the neocortex of infected mice. In addition, increased levels of *Notch-1* mRNA and translocation of NICD to the nucleus correlated well with regressive dendritic changes. Furthermore, compared to uninfected control (N2a) cells, the level of NICD was elevated in scrapie-infected neuroblastoma (ScN2a) cells. ScN2a cells exhibit short dendritic processes whereas non infected cells have long processes

suggesting thus that PrP^{Sc} activates Notch-1 cleavage in ScN2a cells [61, 62]. Whether these abnormalities are the consequence of abnormal PrP metabolism remains to be proven. Despite the numerous uncertainties regarding the physiological function of PrP^C, the consequences of its conversion into PrP^{Sc} could explain the synaptic dysfunctions occurring in prion diseases. Indeed, through recruitment and interactions with NCAM (Neural Cell Adhesion Molecule) at the neuronal surface, PrP is implicated in neurite outgrowth via signal transduction pathways including the activation of Fyn kinases [17, 35, 109]. In another study it has been shown that the coupling of PrP^C to Fyn is dependent on caveolin-1 and is restricted to the fully 1C11 differentiated serotonergic or noradrenergic cells and occurs mainly at neuritis [83]. Taken together, alteration of these specific protein-protein interactions with receptors, ion channels and transporters, following the conversion of PrP^C into PrP^{Sc}, may trigger the related cell-death cascade resulting in neuronal loss.

3. NEURONAL LOSS

The premature cell death of neurons is a typical feature of TSE. Nevertheless, the cellular pathways contributing to neuronal loss are far from being elucidated. Presence of cells bearing nuclear DNA fragments is a common feature in the brains of humans affected by prion diseases [40, 41]. This is reminiscent of apoptosis described in rodent experimental scrapie. Even if autophagic cell death may be also incriminated (see below), there is no doubt that apoptosis is responsible for cerebral damage in prion infected brains, as exemplified by the succession of caspases activation (mainly caspase 3) occurring in the brains of mice with experimental scrapie and in the cerebellum of patients with sporadic CJD [41, 114]. Since PrP^{Sc} accumulates in the CNS early during the course of the disease, this abnormal protein is supposed to be the toxic entity involved in the molecular mechanism leading to neurodegeneration. However, whether this misfolded protein is directly toxic is a matter of controversy. Other PrP species might be involved, and it is interesting to note

that when the molecule is targeted to different cellular compartments, including the cytosol, nucleus, and mitochondria, it can adopt a misfolded and partially proteinase K-resistant conformation [50, 51, 71]. In addition, since some of PrP^C functions are associated with anti-oxidant and/or anti-apoptotic activity, the neurological dysfunction observed in prion diseases could be due to an impairment of endogenous PrP^C metabolism. Microglial activation and the associated neuroinflammation may also be part of the mechanisms by which neurons die in prion disease but it is still poorly understood. We will try to address these different points in the following sections.

3.1. Is PrP^{Sc} neurotoxic?

The involvement of PrP^{Sc} in neurodegeneration is still an important and controversial issue. Some data indicate that neuronal cell death might be related to modifications of several cellular processes, including proteasome dysfunction, alterations in PrP maturation pathway and the unfolded protein response (see below). On the other hand, some studies suggest that PrP^{Sc} is not neurotoxic *per se*, since neurodegeneration is not always associated with the presence of PrP^{Sc} [60, 66]. Other studies showed that the pro-apoptotic markers Fas and caspase 3 were upregulated early in the incubation period in the brain of prion infected mice prior to PrP^{Sc} deposition and clinical signs [63]. As some cell death events may occur before extensive PrP^{Sc} accumulation, the existence of a transient neurotoxic species, distinct from aggregated PrP^{Sc} can be envisioned [74]. In cellular models, the role of PrP^{Sc} in cell death is also difficult to analyze because in most cases, prion infection has little or no apparent effect on the cell viability or phenotype. It is probably linked to the establishment of a fine balance between prion propagation, prion synthesis and prion degradation (for review see [124]). Moreover, it appears difficult to recapitulate completely *in vivo* models of prion disease with *in vitro* cellular models. Nevertheless, recombinant human PrP⁹⁰⁻²³¹ folded in a structural 'PrP^{Sc}-like' conformation was shown to

affect survival of SH-SY5Y cells, inducing a caspase-3 and p38-kinase dependent apoptosis [25]. Mature amyloid fibrils produced from full-length recombinant mammalian prion protein (rPrP) were also highly toxic to cultured cells and primary hippocampal and cerebella neurons [87]. In GT1 cells a pathological phenotype was observed with an increase in the number of apoptotic cells in the infected cultures [85, 110] and recently Cronier et al. reported that prion infection of primary cultured neurons and astrocytes induced neuronal cell death [27]. In several studies although it is controversial, PrP^C expression seemed to be crucial for intracellular intra-neuronal toxicity. This has been demonstrated by Malucci et al. in their study showing that glial cells accumulate PrP^{Sc} but, this did not cause neurotoxicity if the neurons did not express PrP^C [73]. Moreover, prion intracerebral injection into PrP knock-out mice grafted with neural tissue overexpressing PrP, induced specific TSE lesions only in the grafted area [8]. This suggests that only neurons expressing PrP^C would be damaged by exogenous PrP^{Sc}.

3.2. Transmembrane PrP, CtmPrP and NtmPrP

PrP can be produced experimentally under three topological conformers: the regular plasma membrane glycosylphosphatidylinositol-anchored PrP (GPI-anchored PrP), the transmembrane C-trans form (CtmPrP), for which the C-terminus part is in the endoplasmic reticulum (ER) lumen and the N-trans transmembrane (NtmPrP) for which the NH₂-terminus is in the ER lumen. In both of the latter forms, the transmembrane domain is represented by the hydrophobic domain between residues 110 and 135. No study has been published linking NtmPrP to TSE pathology. Conversely, CtmPrP was detected in inherited Gerstmann Sträussler Scheinker syndrome (GSS) carrying the A117V mutation. The disease was linked to a change in structure of the A117V mutated PrP [9, 55] resulting in an increase of the CtmPrP expression. CtmPrP is thus supposed to be an intermediate form involved in the neurodegenerative pathways, in the absence of PrP^{Sc} entities. Besides, it has also been shown

that PrP^{Sc} accumulation can promote CtmPrP production suggesting that this isoform is a major determinant of the prion cell death pathway [56] (for review see [54]). The toxic effect of CtmPrP has also been studied using peptides (PrP peptides 118-135 and 105-132) whose sequences are encompassing the transmembrane hydrophobic domain. These peptides are able to induce the death of pure cortical neurons in vitro [52] as well as the death of retinal neurons in vivo [16] independently of PrP expression. This 118-135 PrP peptide, exhibited membrane fusogenic properties and its toxicity has been correlated with its high propensity to insert stably into and to destabilize cell membranes [94, 95]. This mechanism is independent of fibril formation and could reflect the effect of the transmembrane insertion of CtmPrP [52, 94]. A recent study confirmed these findings and described apoptotic events induced by CtmPrP, as well as by the 118-135 PrP peptides, in oligodendroglial cells, in vitro [116]. The fact that antioxidant molecules, such as probucol, propylgallate, and promethazine can inhibit this apoptosis suggests that oxidative injuries contribute to the 118-135 PrP cytotoxicity [116] (see below for oxidative stress). Finally, in a recent report using cultured neurons from transgenic mice expressing CtmPrP (L9R-3AV), the CtmPrP was shown to localize to the Golgi and not to the ER as described above [118], suggesting that a re-examination of the previous data is necessary.

3.3. Cytosolic PrP and Nuclear PrP

Using high-resolution cryoimmunogold electron microscopy in the mouse hippocampus, PrP^C is detected in the standard biosynthetic trafficking pathway with a preferential localization in late endosomal compartments and on the plasma membrane of neurons and neuronal processes [81]. Unexpectedly, under normal conditions, PrP is also present in the cytosol in subpopulations of neurons in the hippocampus, neocortex and thalamus but not the cerebellum. This cytosolic PrP may have altered susceptibility to aggregation, suggesting also

that these neurons might play a significant role in the prion pathogenesis [81]. These results emphasise the largely documented but fully controversial field of the toxic/non toxic/anti-apoptotic/cytosolic/nuclear PrP protein. Indeed, wild-type PrP^C molecules have been reported to be processed by the endoplasmic reticulum-associated degradation (ERAD) proteasome pathway [70] in the course of which they can be mislocated in the cytosol [125]. Dysfunction of the ER quality control machinery may result in an accumulation of PrP in the cytosol thus triggering a neuronal death cascade activation in the cytoplasm [70]. In addition, treatment of cultured cells with proteasome inhibitors induces the accumulation of an aggregated and non-glycosylated form of PrP in the cytoplasm. Interestingly, both CtmPrP and NtmPrP exhibit an important cytosolic region which may induce a potential toxic pathway. Moreover, it has recently been shown that the disease-associated prion protein specifically inhibits the proteolytic beta subunits of the 26S proteasome and that there is an impairment of the ubiquitin-proteasome system (UPS) in prion-infected UPS-reporter mice [65]. This may in turn lead to an autostimulation of the cytosolic PrP accumulation. By contrast, other studies observed that neither wild-type or mutant PrP proteins were major substrates for retranslocation from the ER prior to proteasome degradation and that mutant PrP was only delayed in its exit from the ER [32]. However, artificial expression of a PrP construct lacking the N-terminal signal sequence and the GPI anchor (23-230 PrP), therefore supposedly cytosolic, was found to be toxic to cultured cells [72]. Transgenic mice expressing the only 23-230 PrP, develop cerebellar degeneration, severe gliosis, and a neurological dysfunction resulting in a severe ataxia, characteristic of prion diseases [72]. It has therefore been suggested that mis-localization of PrP in the cytoplasm could be responsible for the pathogenesis of prion diseases. The toxic potential may be linked to the 115-156 internal domain of PrP and involves co-aggregation of cytosolic PrP with the cytosolic anti-apoptotic Bcl-2. This co-aggregation can be prevented

by over-expression of the chaperones Hsp70 and Hsp40 thus interfering with PrP-induced apoptosis [102]. PrP is also subject to retrograde transport from the plasma membrane towards the ER compartment which has been shown to play a significant role in PrP^{Sc} conversion [5]. Therefore, the presence of PrP^{Sc} in the ER may lead to an accumulation of misfolded PrP molecules which could be subsequently mislocated in the cytoplasm.

Conflicting results have recently been reported; they showed that although proteasome inhibition resulted in accumulation of misfolded cytosolic PrP^C, as well as neurotoxicity, the level of proteasome inhibition, which was the one used by Ma et al., is too far from physiological levels that could occur during prion pathogenesis [64]. In another independent study, quantification of N2a viability in the presence of proteasome inhibitors showed that accumulation of aggregated forms was not toxic [42]. Finally, in contrast to its supposed pro-apoptotic role, cytosolic PrP was not toxic but rather protects against Bax-mediated cell death [104]. To add to these conflicting results, we have recently shown that the 23-230 PrP can be localized in the nucleus [28]. For this study, we have established several inducible cell lines expressing either wild-type PrP, 23-230 PrP, or a tagged 23-230 PrP using hippocampal Np11 *Prnp*^{-/-} cells [86] and neuroblastoma N2a cells. We found that in both cell lines, 23-230 PrP was mostly localized in the nuclei independently of the presence of nuclear localization signals [28, 50]. There were no significant differences in the viability of the cells expressing 23-230 PrP raising again questions about the neurodegenerative phenomena observed in transgenic mice over-expressing 23-230 PrP [72]. This nuclear localization was also reminiscent to what we had previously observed in prion infected N2a cells where 10% of the PrP^{Sc} localized in the nucleus independently of proteasome inactivation [75]. Since small proteins can freely pass through the nuclear membrane and because 23-230 PrP has a molecular weight of 23 kDa, it could enter and exit the nucleus by passive diffusion or during cell division. As described for recombinant PrP [45] and for PrP^{Sc} [75],

23-230 PrP binds to chromatin. This DNA binding capacity could explain the nuclear accumulation of 23-230 PrP. This is reminiscent of a truncated PrP mutant, PrP Q160^{Stop}, associated to a familial early onset dementia, which is almost exclusively localized in the nucleus of transfected cells [69]. We were also surprised that overexpression of a 23-230 PrP in the nucleus induces formation of multinucleated cells. This was the first demonstration that mis-localization of the prion protein can be associated to some extent with altered cytokinesis. The underlying mechanism responsible for 23-230 PrP-induced generation of multinucleated cells remains to be determined. Nevertheless, in a culture system, such multinucleated cells could result from multiple rounds of nuclear division without cytokinesis. Neurons in the adult brain are traditionally viewed as having permanently exited the cell cycle and entered a differentiated quiescent state. However, recent evidence shows that mature neurons can abnormally re-enter the cell cycle, which may be an important mechanism of cell death [24]. Recent studies of brain injuries or human neurodegenerative disorders such as Alzheimer's disease (AD) have discovered that *bona fide* cell cycle regulators were expressed in post mitotic neurons of affected brain regions, leading to successful DNA replication, but unsuccessful mitosis (for a review, see [123]). The accumulation of tri- or tetraploid nuclei in AD neurons indicates that degenerating neurons synthesize DNA but fail to undergo mitotic division, which leads to a critical sequence of events that could provoke neurodegeneration in AD. Consequently, 23-230 PrP expression might cause abnormal cell-cycle re-entry in post mitotic neurons, a mechanism which could explain the toxic effects of 23-230 PrP expression that has been observed in transgenic animals [72].

3.4. PrP in aggresomes

In a recent study Kritiansen et al., have shown that PrP^{Sc} entities form aggresomes in neuronal cells resulting in caspase activation and apoptosis [64]. This was observed in prion infected N2a and GT-1 cells treated with mild levels of proteasome inhibitors to

mimic what could be found during senescence or prion pathogenesis [65]. In this context, cells were susceptible to cell death (activation of caspases 3 and 8) whereas the non-treated cells were not susceptible. Moreover, the cells accumulated large cytoplasmic perinuclear aggresomes structures containing PrP^{Sc}, Hsp70, ubiquitin, proteasome subunits and vimentin that were temporally associated with caspase 3 and 8 activation and subsequent apoptosis. A possible retranslocation of PrP^{Sc} from the ER may be incriminated to explain the presence of PrP^{Sc} in those structures [5]. However, and contrary to what was published by Ma et al. previously [72], cell death was neither observed in non infected cells nor in cells overexpressing high levels of cytoplasmic aggregating PrP^C. Interestingly, it had been previously shown that misfolded cytosolic PrP as well as pathogenic prion protein mutants were able to form aggresome in response to cyclosporin A and proteasome inhibition respectively [22, 82]. In another study, immunofluorescence and electron microscopy have revealed that cytoplasmic PrP was localized in juxtannuclear aggregates reminiscent of aggresomes in human embryonic kidney 293 cells, human neuroblastoma BE2-M17 cells and mouse neuroblastoma N2a cells [49]. Time course studies revealed that discrete aggregates form first throughout the cytoplasm, and then coalesce to form an aggresome. In parallel, cytoplasmic PrP aggregates induced mitochondrial clustering, reorganization of intermediate filaments, preventing wild-type PrP secretion and promoting cell death [49]. It is likely that any event leading to accumulation of PrP in the cytoplasm is likely to result in cell death. On the other hand, a new emerging concept proposes that in proteinopathies, protein aggregates are not toxic *per se* and might even serve as a protective role by sequestering misfolded proteins in aggresomes. Specifically, experimental models of polyglutamine diseases, Alzheimer's disease, and Parkinson's disease revealed that the appearance of aggregates can be dissociated from neuronal toxicity. Misfolded monomers or oligomeric intermediates appear to be the more probable toxic species. This is consistent

with recent data indicating that smaller subfibrillar particles with a mass equivalent to 14-28 PrP molecules are the most infectious moiety and may be much more toxic than larger amyloid fibrils or plaques [113].

3.5. Anchorless-PrP

It was believed for a long time that expression of a GPI anchored PrP^C was necessary for PrP function [115] or infection [103]. Amazingly, transgenic mice expressing only GPI anchorless-PrP could be infected with prions and accumulated high levels of PrP^{Sc} and amyloid plaques associated with high prion titers, but they do not suffer neurodegeneration [18]. This result fits with the idea that PrP^{Sc} is involved in a neurotoxic pathway that could be activated in endocytic compartment and/or at the plasma membrane and involve PrP^C, caveolin, Fyn kinases [111] and other partners that remain to be identified.

3.6. Analysis of prion neurotoxicity using PrP peptides

It is not clear whether the use of peptides such as PrP106–126 to mimic the physiopathology of TSE is really pertinent. However, the latter peptide shares many physicochemical properties with PrP^{Sc}, is neurotoxic *in vitro* and has been largely used to explore the neurotoxic mechanisms underlying TSE. Many types of PrP peptides have also been studied under multiple conditions and in many different cellular models. This complex situation is summarized in Table I where we have listed several studies in relation with cell death and PrP peptides.

Some results obtained with peptides were similar to those obtained with prion infection suggesting that peptides were able to activate pathways targeted when PrP^{Sc} itself was present. A demonstrative example is the study of the apoptotic events induced by exposure to HuPrP106-126 peptide in primary culture of murine cortical neurons. The aggregated peptide indeed induced a massive neuronal death within 24 h while reactive oxygen species were detected after 3 h and were followed by a peak of phosphorylated c-Jun-N-terminal

Table I. Summary of studies linking PrP peptides and neurotoxicity.

Peptide	Cellular model	Results/Observation/Concluding remarks	Ref.
PrP 106-126	SH-SY5Y human neuroblastoma	Cell death through p38 MAPK activation	[119]
Recombinant PrP90-231	Gliaial cells	ERK1/2 and p38 MAP kinases control prion protein fragment 90-231-induced astrocyte proliferation and microglia activation	[120]
PrP 106-126	Cortical neurons	Cell death through release of Ca ²⁺ and ROS, caspase activation	[39]
PrP 106-126	Murine cortical neurons	The early decision phase of the apoptotic event involves JNK pathway and activation of c-Jun	[14]
Sheep scrapie	Primary Tg338 cortical neurons	Gradual apoptosis that involves JNK and c-Jun	[14]
PrP 106-126	Mixed porcine retinal cell culture	61% loss of rod receptor / cone receptor not affected / 47% loss of amacrine cells / microglial cell proliferation (X5)	[47]
PrP 106-126	Primary cerebellar granule neurons	Upregulation of arachidonic acid showing that the 5-lipoxygenase pathway plays a central role in apoptosis	[117]
PrP 106-126	Cortical cell cultures	NMDA receptor involve in the cell death signaling pathway	[11]
PrP 106-126	SH-SY5Y human neuroblastoma	Rapid depolarization of mitochondrial membrane and release of cytochrome c and caspase activation demonstrating that mitochondria constitutes the primary site of proapoptotic pathway	[89]
PrP 106-126	Retinal neurons (in vivo)	Neurotoxicity / less toxicity when the peptide has a relaxed structure demonstrating the role of the structure in the neurotoxicity pathway	[6]
PrP 106-126	Primary culture of rat cortical neurons/cerebellar granule cells and astrocytes	Neuronal cell death and astrocyte proliferation Decrease of PrP gene expression in neurons and increase of its expression in astrocytes	[84]
PrP 106-126	IC11/GT1-7	The decrease of PrP expression may lead to apoptosis by loosing PrP defensive function The peptide triggers ROS overflow and activation of Kinases (ERK1/2, P38, JNK1/2) and apoptotic signals in the IC11 derived serotonergic and noradrenergic neuronal cells but not in precursor	[93]
PrP 106-126/PrP ^{Sc}	Neuronal rat brain culture	Decrease of phagocytosis microgliale activity	[21]
PrP 106-126	Cortical neurons + PrP-KO cortical neurons	Activation of a subset of intracellular kinases followed by caspase 3 activation suggesting that PrP ^C should not be necessary	[46]
PrP 106-126	Human neuroblastoma lacking neurotrophine receptor and expressing p75 ^{NTR}	Binding of the peptide to the extracellular region of p75 ^{NTR} followed by signaling activation leading to caspase 8 activation + oxidant species production	[30]
PrP 106-126	Rat cortical neurons	Activation of caspase-3 activity (CCP32) but: – applying CCP32, specific inhibitor inhibits the activation of caspase-3 but not the cell death – applying a non selective caspase inhibitor (z-VAD-fmk) inhibits caspase-3 activation and attenuates partially the cell death	[108]
PrP 106-126	Normal human neuroblastoma SH-SY5Y SH-SY5Y overexpressing murine PrP	The neurotoxic effect of the PrP106-126 peptide is independent to the caspase-3 activation that occurs Both cells are susceptible to the toxic effect of the peptide. PrP106-126 cell alteration is independent of PrP ^C expression, but rather dependant of the lipid components of the plasma membrane of neuronal and non-neuronal cell lines that significantly differ by their membrane fatty acid composition	[35]
PrP 106-126	PrP-Doxycycline inducible Rabbit kidney epithelial A74 cells	The peptide is not toxic, either the cells produce PrP or not	[35]

kinase (JNK) translocation into the nucleus after 8 h, along with the activation of the nuclear c-Jun transcription factor [14]. The involvement of JNK in the early decision phase was confirmed in a more physiological model of prion infected primary tg338 cortical neurons [27] but with a slight shift in time [14]. In this model the apoptosis gradually increased with time and induced a 40% cell death after two weeks exposure and the early c-Jun activation was detected after seven days [14].

3.7. ER stress/ER apoptotic pathway

Endoplasmic reticulum (ER) stress is caused by disturbances in the structure and function of the ER and is associated with the accumulation of misfolded proteins and alterations in the calcium homeostasis. The ER response is characterized by changes in specific proteins, causing translational attenuation, induction of ER chaperones and degradation of misfolded proteins. In case of prolonged or aggravated ER stress, cellular signals leading to cell death are activated. Castilla et al. [15] have proposed a general pathogenesis model of prion disorders based on the alteration of ER-homeostasis. This alteration could be due to drastic modifications of the biophysical properties of PrP, leading to the activation of ER-dependent signalling pathways that controls cellular survival. Indeed, prion replication has been shown to trigger ER stress as reported by Hetz et al. [59]. In this study, purified PrP^{Sc} extracted from mouse scrapie brain induced apoptosis of N2a cells mediated by an increase of calcium release from ER and caspase 12 activation. The infected cells were in turn more susceptible to ER stress. The correlation between caspase 12 activation and neuronal loss was confirmed in models of prion infected mice as well as in CJD patients [57]. Moreover, Ferreiro et al. have recently shown that ER stress cell death induced by PrP peptides was also associated with calcium homeostasis perturbation [39]. They suggested that the early PrP-induced perturbation of ER Ca²⁺ homeostasis is a death message that leads to neuronal loss [39]. In addition, ER stress led to the generation of a misfolded PrP isoform, which was detergent-insoluble but protease-

sensitive [58] suggesting that ER-damaged cells appear more susceptible to prion replication. This is emphasised also by results showing that ER stress conditions weaken PrP co-translational translocation, favouring accumulation of cytosolic PrP [91]. Finally, the PrP isoform generated after ER stress seemed more efficiently converted into PrP^{Sc} in *in vitro* replication systems compared with the protein extracted from non ER damaged cells [58]. Since PrP^{Sc} induces ER stress, there may be thus a positive feedback loop that could accelerate prion replication.

Finally, all these results should be considered with caution since no evidence of ER stress was found in histopathological studies of post-mortem brains of CJD patients [121].

3.8. Autophagy

While apoptosis may play a major role in the events leading to neuronal loss in TSE, autophagy was also identified in experimentally induced scrapie a long time ago, and was recently re-evaluated as a possible cell death program in prion diseases. Indeed, autophagy represents a common ultrastructural feature of TSE and autophagic vacuoles are frequently present in experimentally induced scrapie, CJD and GSS [112]. At the ultrastructural level, autophagy is represented by membrane sequestration of neuronal cytoplasm by concentric arrays of double membranes, membrane proliferation and the formation of autophagic vacuoles of various sizes in all parts of the neurons including synaptic endings. Finally, a large area of the cytoplasm was transformed into a collection of autophagic vacuoles of different sizes. On the basis of these studies, Liberski et al. have suggested that autophagy plays a major role in TSE and may even participate in the spongiform changes [68]. At the molecular level, Dron et al. [33, 34], have shown that the *Scrg1* gene was upregulated in TSE. This gene encodes a protein associated with the Golgi apparatus as well as with autophagic vacuoles of degenerative neurons. The authors suggest that *Scrg1* is involved in the host response to stress and/or the death of neurons and could be used to follow autophagic

events [33, 34]. However, increased numbers of autophagosomes/autophagic vacuoles are seen in a variety of physiological and pathological states in the nervous system. It is unclear if this phenomenon is the result of increased autophagic activity or decreased autophagosome-lysosome fusion. The functional significance of autophagy and its relationship to cell death in the nervous system is also poorly understood. While many issues remain unresolved, it raises the possibility that autophagy can have either deleterious or protective effects depending on the specific situation and stage in the pathological process [106]. It is also unclear whether the aggregated PrP enhances the autophagic activity as it has been proposed for the misfolded huntingtin protein [78, 106]. In this particular case, autophagy constitutes an alternative route for the intracytosolic protein degradation or clearance of aggregated proteins when the ubiquitin-proteasome system is altered. This system could thus rescue toxicity mediated by proteasome inhibition and provide a protective effect [78, 106]. Whether autophagy may first play a protective role in prion disease therefore needs to be analysed in more detail.

3.9. Loss of PrP^C function/interference with PrP^C function

Other hypotheses for prion induced cell death involved PrP^C loss of function. This would occur when PrP^C is converted into PrP^{Sc}, or if its normal function is subverted by its association with PrP^{Sc}. Even if the role of PrP^C is not totally elucidated, there are many evidences that PrP^C can exert a cytoprotective function through an anti-oxidant, as well as an anti-apoptotic activity. It is therefore easy to imagine that loss of these functions could directly increase susceptibility to oxidative stress and result in cell death. This is exemplified by studies reporting an increased susceptibility of PrP knock-out neuronal cells to oxidative stress [80]. Moreover, PrP exerts an anti-apoptotic function via its anti-Bax activity. As the Bax pro-apoptotic pathway seems to be implicated in neuronal death, as shown by Chiesa et al. in the inherited prion disease transgenic mouse model

Tg(PG14) [20, 67], it is therefore tempting to propose that loss of this function will be a determinant for neuronal cell death. However, the Bax pathway is not an obligatory phenomenon [67] since in another study Couplier et al. have reported that *Bax*^{-/-} mice challenged with the BSE (Bovine spongiform encephalopathy) agent developed an unaltered prion disease in terms of neuronal loss [26]. To reconcile these data, a third study using transgenic mice showed that N-terminally deleted forms of the prion protein activated both Bax-dependent and Bax-independent neurotoxic pathways. *Bax* deletion in Tg(PrPΔ32-134) mice delayed the development of clinical illness and slowed apoptosis of cerebellar granule cells but had no effect on white matter degeneration [67]. *Bax* deletion had also no effect in Tg(PrPΔ105-125) mice [67] suggesting that the PrP deleted domains are important for the interaction with Bax cell-death signalling molecules. Altogether these studies suggest the existence of multiple molecular death pathways in prion diseases and that specific domains of PrP are essential for its neuroprotective activity. The anti-Bax function of PrP, could be linked to PrP^{Sc} conformation or at least to PrP sequence. For example, the ectopic expression of PrP protects primary neurons against Bax mediated cell death [105]. This activity is lost when PrP contains the familial mutation (D178N) associated with the genetic Fatal Familial Insomnia prion disease, suggesting again that the normal structure of the PrP protein is necessary to promote an anti-Bax function [105]. These results suggest that loss of anti-Bax activity could be deleterious in some prion diseases.

Another putative function of PrP^C that could be lost during the development of the disease consists in copper binding and metabolism. In scrapie infected mice, levels of copper are modified and PrP copper binding decrease. Using radioactive copper (⁶⁴Cu) at physiological concentrations, Rachidi et al., have shown that prion infected cells display a marked reduction in copper binding [100]. These modifications of copper homeostasis may play an essential role in the pathogenesis process [101]. PrP^C is also supposed to

promote copper uptake into cells and to enhance copper incorporation into superoxide dismutase to regulate intracellular Cu/Zn superoxide dismutase activity [12]. PrP^C itself also exerts a superoxide dismutase (SOD) role [12] and appears to serve as a stress sensor that is sensitive to copper and is able to initiate, following copper binding, a signal transduction process acting on the anti-oxidant systems to improve cell defences [101]. Conversion of PrP^C into PrP^{Sc} could alter this function and result in the loss of anti-oxidant defences [100]. In addition as abnormal protein accumulates in the injured brain, there is an increase of oxidative substances such as reactive oxygen species (ROS) (for review see [80]). The loss of the anti-oxidant defences will increase cell susceptibility to oxidative substances and therefore promotes cell death. This is in accordance with data reporting that prion infection renders neuronal cells more susceptible to oxidative stress and impairs their free radical metabolism promoting neuronal damages [14, 79]. However, PrP^{Sc} could affect the putative role of PrP in the defence against oxidative stress in many different ways: PrP^{Sc} could compete with PrP^C by binding to PrP^C or PrP^C putative co-receptors involved in PrP-dependent signalling and could lead to the opposite effect that was expected. The inhibition of the signalling pathway may result in the down regulation of the different defences (SOD, etc) against oxidative stress. As one illustration, it has been shown that the neurotoxic action of PrP106-126 peptide relies on cell surface expression of PrP^C, recruitment of a PrP^C-Caveolin-Fyn signalling pathway, and over stimulation of NADPH-oxidase activity causing oxidative injury of bioaminergic neuronal cells [93, 111]. PrP106-126-induced neuronal injury appears to be caused by an aberrant amplification of PrP^C-associated signalling responses, which in turn promote oxidative stress conditions [93]. In the same idea, PrP^{Sc} production might subvert PrP^C function by cross-linking PrP^C at the cell surface thus promoting cell death [115]. PrP^{Sc} 'gain of function' could also lead, by itself and independently of PrP^C function, to an increase of intracellular ROS. ROS would impair the

response of the cells to oxidative stress, inhibiting or destroying important anti-oxidant enzymes [1,90]. In addition this scenario could be a consequence of the two previous possibilities and could even be amplified by the activation of microglial cells by PrP^{Sc} and the release by these cells of significant amounts of ROS. These changes create a paradox because the loss of activity is not accompanied by a loss of PrP expression and PrP knock-out mice have no pathologic phenotype. However, activation of microglial cells by PrP^{Sc} and the associated increase of ROS production could explain the lack of this strong phenotype for PrP knock-out mice. For a revue, see Harris and True [53].

3.10. Inflammation and microglia

The contribution of inflammation to the progression of prion diseases is poorly understood. The inflammatory response is mediated by the activated microglia, the resident immune cells of the CNS, which normally respond to neuronal damage and remove the damaged cells by phagocytosis. However, the chronic activation of microglia may cause neuronal damage through the release of potentially cytotoxic molecules such as pro-inflammatory cytokines, ROS, proteases, and complement proteins [19]. Both in experimental and natural TSE it has been shown that activation of microglial cells and astrocytes is a common and early physiopathogenic event during the course of the disease [98]. These features have been modelled in vitro upon exposure of neuronal and glial cultures to PrP^{Sc} or to PrP peptides. For example the PrP82-146 peptide was shown to exert a gliotrophic activity by inducing astrocyte proliferation [43]. The PrP106-126 peptide also elicited a specific over-production of pro-inflammatory cytokines IL-1 β and IL-6 in microglial cells (but not an increased expression of TNF- α , IL-10, or TGF- β 1) [92]. These effects were strictly dependent on the ability of the peptide to form amyloid fibrils. The microglial reaction also appears to be related to the biochemical type and deposition pattern of PrP^{Sc}. In Human, strong microglial activation was shown to be associated with type 1 PrP^{Sc} and diffuse PrP

immunoreactivity, whereas type 2 PrP^{Sc} and focal PrP deposits were accompanied by mild microglial reaction [99]. In another study, the toxic effect induced by PrP106-126 peptide on neuronal cultures can be amplified in the presence of microglial cells which responds to the peptide by producing ROS [10]. It was also shown that this peptide triggered the expression of the inducible form of NOS (Nitric oxide synthase) in a TNF- α -dependent manner involving p38 MAPK (Mitogen-Activated Protein Kinases) [37] in microglia and that the nitric oxide release was responsible, at least in part, to neuronal cell death [10]. To respond to these cytokines and oxidative species, several surface receptors need to be activated. One of them, the neuronal p75 neurotrophin receptor (p75^{NTR}) has been already identified [30]. This receptor responds to pro-inflammatory cytokines such as TNF- α and IL-1 β released from PrP106-126 treated microglia and kills SK-N-BE human neuroblastoma cells via its intracellular 'death' domain [30]. On the other hand, a bidirectional neuron-microglia cross-talk is necessary since microglial cells were shown to kill neurons in vitro through a microglial CD14-dependent process in reaction to a neuron specific phenotype arising only following PrP106-126 treatment [4]. Because reactive microglia are frequently present in the vicinity of PrPres aggregates [76], the recruitment of microglia is supposed to be one of the early steps of TSE pathogenesis. This recruitment appears to be under the control of chemokines acting through the activation of specific G-protein-coupled receptors. It has been shown in vitro and in vivo, that PrPres exposure induced the up-regulation of the chemokine RANTES through MAPkinase/ERK1/2 pathways inducing Elk-1 phosphorylation and RANTES transcription factor Egr-1 expression [76, 77]. This led to a rapid microglial migration as a result of chemokine receptor-5 (CCR-5) activation. When recruited in the vicinity of prion infected neurons, activated microglia may therefore cause neuronal cell damages [76, 77].

In addition, as 'macrophage' of the central nervous system, the phagocytic capacity of microglia is well recognized, and involvement

of microglia in the removal and processing of amyloid fibrils was thought possible, thus preventing the harmful effect of the amyloid fibrils. Supporting this concept, recent data is demonstrating that 'pharmacogenetic' ablation of microglia from infected organotypic brain slices provoked an increase of PrP^{Sc} levels, as well as an increase in susceptibility to infection [38]. However, fibrillar prion peptide (106-126) and scrapie prion protein were shown to hamper phagocytosis in microglia [21]. Altogether, this suggests that the extensive microglial activation accompanying prion diseases represents an efficient defensive reaction at least in a particular time frame.

4. CONCLUSION

Many questions concerning the role of PrP in the neurodegeneration pathways remain to be answered. The situation is complex as many interconnected pathways may be involved and interfere with each other: PrP^C loss of function and PrP^{Sc} acquisition of new toxic properties, proteasome alteration, ER-stress activation, autophagy, microglial activation in response to PrP^{Sc} or the release of ROS, etc. It is possible that infected cells try to set up different means to overcome or fight PrP^{Sc} accumulation but each of them seems to be limited and can in turn exert a deleterious effect contributing finally to cell death.

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