Minireview

Prion diseases: pathogenesis and public health concerns

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Abstract Transmissible spongiform encephalopathy (TSE) agents or prions induce neurodegenerative fatal diseases in humans and in some mammalian species. Human TSEs include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome, kuru and fatal familial insomnia. In animals, scrapie in sheep and goats, feline spongiform encephalopathy, transmissible mink encephalopathy, chronic wasting disease in wild ruminants, and bovine spongiform encephalopathy (BSE), which appeared in the UK in the mid-1980s [Wells, G.A.H. et al. (1987) Vet. Rec. 121, 419–420], belong to the TSE group. Prions have biological and physicochemical characteristics that differ significantly from those of other microorganisms; for example, they are resistant to inactivation processes that are effective against conventional viruses, including those that alter nucleic acid structure or function. Alternatively, infectivity is highly susceptible to procedures that modify protein conformation. Today, the exact nature of prions remains unknown even though it is likely that they consist of protein only. At the biochemical level, TSEs are characterised by the accumulation, within the central nervous system of the infected individual, of an abnormal isoform of a particular protein from the host, the prion protein [Prusiner, S.B. (1982) Science 216, 136–144]. TSEs are transmissible among their species of origin, but they can also cross the species barrier and induce chronic infection and/or disease in other species. Transmissibility has been proven in natural situations such as the outbreak of CJD among patients treated with pituitary-derived hormones and the appearance of BSE that affected UK cattle in the mid-1980s.

Key words: Transmissible spongiform encephalopathy; Prion; Pathogenesis

1. Prion: a new form of biological information

The nature of transmissible spongiform encephalopathy (TSE) agents is still unknown. However, based on animal model studies in rodents [3], intensive investigation of biochemical and biological properties of prions have been conducted.

The size of TSE agents or prions has been evaluated by ultrafiltration to be between 15 and 40 nm [4], although these agents aggregate easily because of their hydrophobicity; variations in size and density have been reported in the literature. The inactivation processes that are effective against scrapie agents are those that denature or hydrolyse protein components: treatment with high doses of proteinase K, trypsin [5] or sodium dodecyl sulphate. Conversely, procedures that interact with nucleic acids do not modify infectivity titres [2]. Moreover, prions are highly resistant to ionising radiations and their inactivation UV spectrum does not suggest the presence of a coding nucleic acid [6,7]. Therefore, one may hypothesise that TSE agents are only composed of proteins.

Numerous experimental data indicate the absence of specific nucleic acids associated with infectivity, although small nucleic acids can be evidenced in both infected and non-infected control fractions [8]. The theory of a self-propagating proteic agent, or 'prion' (for proteinasceous infectious particle), was proposed at the end of the 1970s [2] after the purification of a 27–30 kDa protein specifically associated with infectivity, the prion protein PrP-sc. This protein is present in infected individuals in proportion to the level of infection. In fact, PrP-sc is an abnormal isoform of a normal component of the host, PrP^c [9]. The mechanism by which PrP-sc accumulates is post-translational and no modulation of PrP gene (*Prnp*) expression has ever been reported during natural or experimental TSEs. PrP-c and PrP-sc do not differ in amino acid sequence: the differences are thought to be at the level of conformation. PrP-sc is insoluble in detergents, although PrP-c is soluble [10]. Moreover, PrP-c is totally degraded by proteinase K concentrations that only partially degrade PrP-sc (Fig. 1). The amount of PrP-c is 50 times greater in brain than in other organs; this may be a critical parameter of the pathogenesis of TSEs [9]. In detergent-treated brain extracts from infected individuals, fibrils composed of polymers of PrP-sc, namely scrapie-associated fibrils or prion rods, can be evidenced by electron microscopy [11].

The normal isoform, PrP-c, is anchored at the cell membrane, in rafts, through a glycosyl phosphatidyl inositol (GPI); its half-life at the cell surface is 5 h, after which the protein is internalised through a caveolae-dependent mechanism and degraded in the endolysosome compartment [12]. Conversion between PrP-c and PrP-sc occurs likely during the internalisation process. In humans, PrP is a 253 amino acid protein, which has a molecular weight of 35–36 kDa. It has two hexapeptides and repeated octapeptides at the N-terminus, a disulphide bond and is associated at the C-terminus with a GPI, which enables it to anchor to the external part of the cell membrane. The fragment of PrP that is resistant to proteinase K digestion (PrP-res) in infected individuals is between amino acid residues 90 and 233 (molecular weight 27–30 kDa). PrP-c has two putative sites of glycosylation; thus,

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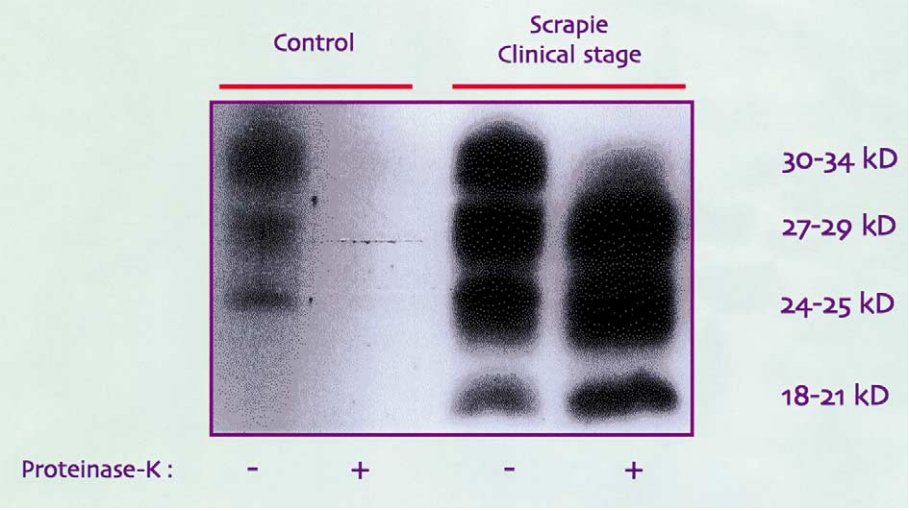

Fig. 1. Differential susceptibility of PrP-c and PrP-sc to proteinase K digestion.

three glycoforms of PrP can be described. The relative proportions of these glycoforms and the size of the unglycosylated PrP-res fragment are dependent on the strain of prion [13]. Infrared spectroscopy and circular dichroism have shown that the secondary structure of PrP-c is mainly composed of α-helices, whereas PrP-sc is mainly β-sheets [14]: transconformation of α-helices into β-sheets has been proposed as the structural basis by which PrP acquires pathogenicity in TSEs. The three-dimensional structures of a normal human, murine, bovine, and hamster PrP have been published [15–17]: the protein is made of a globular domain (a.a. 121–231) which includes three α-helices and two small antiparallel β-sheet structures, and a long flexible tail whose conformation depends on the biophysical parameters of the environment

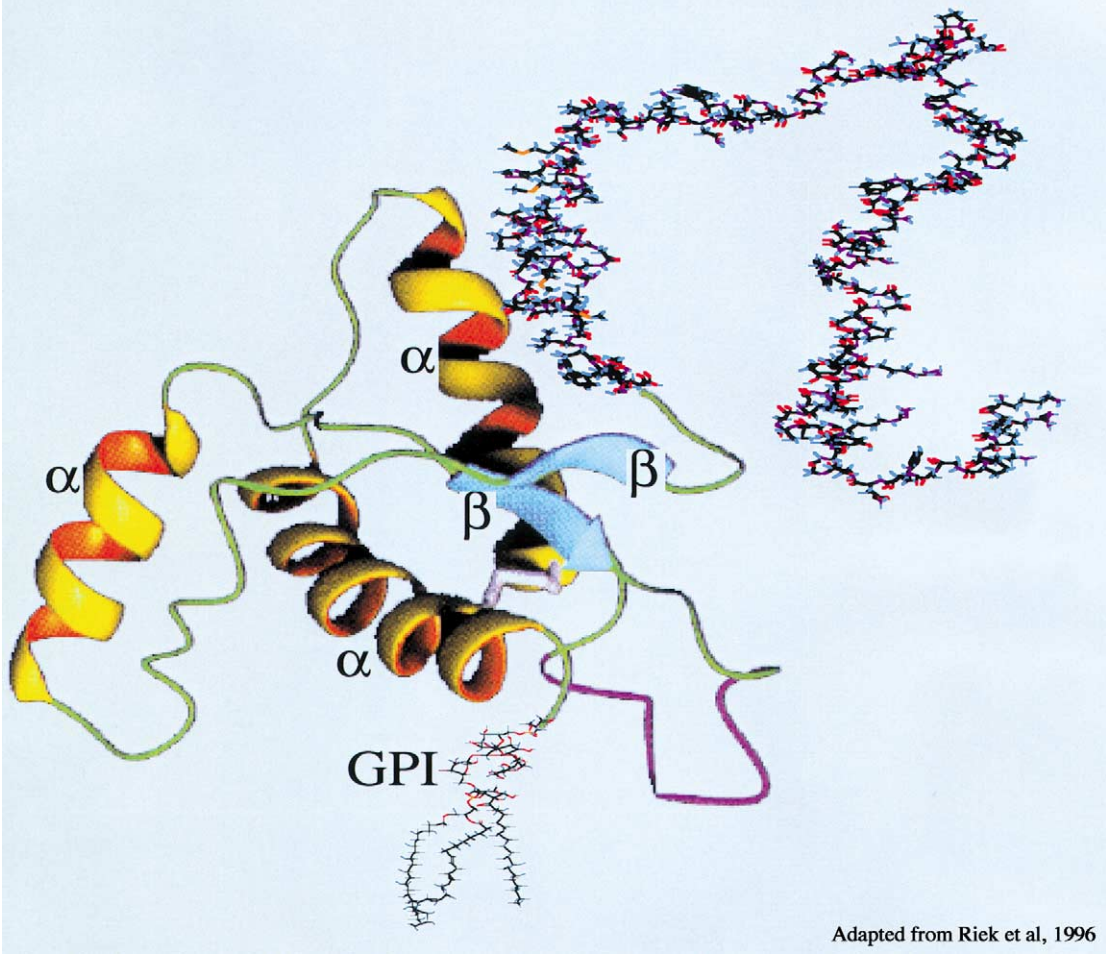

Fig. 2. Three-dimensional structure of the cellular PrP.

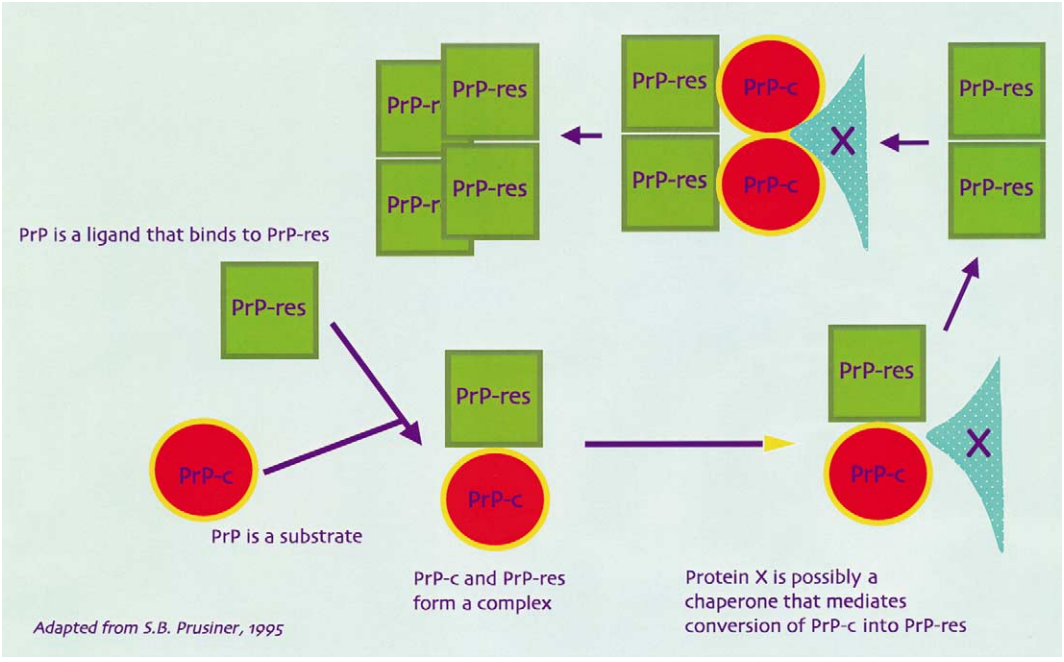

Fig. 3. The prion hypothesis.

(Fig. 2). Crystals of the globular domain of PrP have recently been obtained; their analysis suggests a possible dimerisation of the protein through the three-dimensional swapping of the C-terminal helix 3 and rearrangement of the disulphide bond [18]. In vitro conversion of PrP-c into a protease-resistant isoform is possible in an acellular experimental system [19].

Several hypotheses have been proposed to explain the nature of the TSE agents and the pathogenesis of TSE:

- The virino hypothesis [20], which suggests that the agent consists of a nucleic acid, which codes only for its own replication surrounded by host-encoded PrP (thus accounting for the lack of an immune response);
- The unknown conventional virus hypothesis; current concepts of microbiology indicate that this is most unlikely; and
- The protein-only hypotheses that include the seeding model, the chaperone-disease model and the prion theory.

The protein-only hypotheses are supported by the most recent results from transgenic experiments and molecular biology. In these theories, PrP-sc is the agent, or a major component of infectivity [2]. The prion theory postulates that pathogenicity is enciphered into the tertiary structure of PrP-sc. Propagation of the abnormal conformation results from the ability of PrP-sc to form dimers with PrP-c; this heterodimerisation induces a transconformation of PrP-c into PrP-sc, and, therefore, the propagation of this abnormal isoform of PrP (i.e. PrP-sc). Recently, it has been suggested that PrP-c transconformation into PrP-sc requires a host-encoded cellular factor, factor X [21], which is thought to be a chaperone (Fig. 3).

Other theories involving chaperoning molecules [22] or nucleation have also been proposed [23]; in this last hypothesis, the transformation of PrP-c into PrP-sc is reversible, PrP-sc being stable only when aggregated. Then, binding of PrP-c to PrP-sc aggregates results in PrP-c transconformation. The size of the aggregate then increases until the limit of cohesion of the aggregate, above which a dissociation occurs, giving birth to small seeds efficient for PrP-c transconformation.

Wüthrich et al. have proposed that the excess of β-sheet measured in PrP-sc could be the consequence of aggregation through the globular domains which could trap a part of the flexible tail inside the aggregate and therefore induce a β-sheet conformation of a part of the molecule that was not structured before; if demonstrated, this mechanism would not require any transconformation process of PrP.

2. The role of the prion protein

The precise function of the normal PrP isoform in healthy individuals remains unknown. Several results, mainly obtained in transgenic animals, indicate that PrP-c might play a role in long-term potentiation, in sleep physiology, in oxidative burst compensation (PrP can fix four Cu^{2+} through its octarepeat domain) [24], in interactions with the extracellular matrix (PrP-c can bind to the precursor of the laminin receptor, LRP) [25], in apoptosis and in signal transduction (co-stimulation of PrP-c induces a modulation of Fyn kinase phosphorylation) [26]. Recently, interactions between PrP and retroviral nucleic acids have been reported [27,28].

In TSE-affected individuals, PrP has a determinant role in the incubation time and in the species barrier [29] and its role in pathogenesis is now established. Transgenic mice lacking *Prnp* expression (i.e. knockout mice that do not express any PrP) are not susceptible to TSE agent or prion infection, demonstrating the key role of PrP in TSEs [29]. Susceptibility to prions thus depends upon the presence of PrP-c on the cell membrane of the host; prions do not propagate in brains that lack PrP-c [30]. Moreover, in transgenic animals that express large numbers of PrP gene (*Prnp*) copies, incubation time is inversely correlated with PrP-c expression; that is, susceptibility to infection and prion propagation depend on the amount of PrP-c available in the host. Finally, transgenic animals with

a PrP gene mutation equivalent to one described in human familial disease spontaneously exhibit a spongiform encephalopathy that is transmissible under certain conditions [31].

3. Prion disease pathogenesis

Natural and experimental TSEs are characterised by a long incubation phase without clinical symptoms: this silent phase may last as long as 40 years in humans with kuru or infected through extracted growth hormone treatment. Once started, the clinical course of the disease evolves slowly without remission. No inflammatory process is identifiable in blood or CSF; none of the usual immunological stigma or specific signs of chronic viral infections are observed in infected individuals. No virus-like or micro-organism-like structure is identifiable in the brains of infected patients, regardless of which microscopic technique is used.

The intracerebral route is the most effective for prion contamination and the oral route is the least effective (1 intracerebral route infectious unit = 125 000 oral route infectious units) [32]; this has been demonstrated in experimental models, but natural disease-associated agents, especially the bovine spongiform encephalopathy (BSE) agent [1], may behave differently and be more infectious by a peripheral route (including oral exposure). In vivo, there are no alterations of the B, T, and non-T-non-B cells (quantitative or functional) and no antibody against PrP has been detected in natural or experimental diseases; therefore, there is no test available for screening asymptomatic infected individuals.

Transmissibility is easy inside the same species, but is also possible between different species. The strength of the species barrier is variable; for example, BSE is not transmissible to hamsters although it can be easily transmitted to mice. The major molecular determinant of the species barrier is the homology between the PrP gene of the donor and the PrP gene of the recipient; other genes play minor roles in TSE agent or prion susceptibility, for example major histocompatibility genes. In humans, the PrP gene exhibits a polymorphism at codon 129; either valine or methionine can be encoded, 50% of the general population being homozygous. Homozygosity at codon 129 has been associated with susceptibility to sporadic iatrogenic and variant Creutzfeldt–Jakob disease (CJD) [33].

The kinetics of PrP-res accumulation has been studied in a number of experimental animal models. Results have confirmed that accumulation follows the increase in infection. Infectivity distribution depends upon the route of inoculation, the strain of prion, the dose of inoculum and the genetic background of the host. For example, after peripheral exposure, the TSE agent is detectable in the lymphoid system of infected animals soon after inoculation; it then appears in the central nervous system during the second half of the total duration of the experimental disease.

These facts illustrate two main characteristics of the transmissible spongiform encephalopathies:

- Certain organs of the infected individual, particularly the brain, spinal cord and retina, are heavily infected before clinical signs appear [34], and
- These diseases develop without interruption, that is, without any latency of the infectious agent during the asymptomatic phase.

The immune system plays a role during the pathogenesis of peripheral infection with TSE agents. Immune cells (most probably dendritic cells and macrophages [35,36]) can be the first site of replication of these agents. In secondary lymphoid structures, infectivity is associated with follicular dendritic cells (FDCs); FDCs are critical in the neuroinvasion processes [37]. Neuroinvasion then occurs through the nerve endings that are present in lymphoid organs. It is not known if PrP-sc propagates in the peripheral nervous system (PNS) via a retroaxonal transport or through a propagation of abnormal conformation following a 'domino model' at the surface of the axon or of the Schwann cells [38]; the presence of PrP-c is required for agent propagation in the PNS [39].

Neuropathology of TSEs consists of neuronal death, spongiosis and gliosis with hyperastrocytosis. The precise mechanisms that lead to brain cell damage are not known. Nevertheless, several experimental results indicate that neuronal death can occur in two ways: by accumulation of PrP-sc in the cytoplasm through a toxic mechanism; and by apoptosis of non-infected neurones induced by PrP. Indeed, exposure of primary neuronal cell cultures to peptides derived from the 106–126 domain of the PrP molecule induces apoptosis [40]. This apoptosis requires the presence of microglial cells and the presence of PrP-c at the cell surface; the apoptotic pathway requires N-methyl-D-aspartate receptor activation. Microglial cells exposed to PrP 106–126 or to PrP-sc secrete IL-1 β, IL-6 and other neurotoxic mediators that could participate in neuronal death observed during TSEs. On the other hand, exposure of primary astrocyte cell culture to PrP-sc in liposomes induces astrocyte activation and hyperexpression of the glial fibrillary acidic protein gene, which is a biochemical hallmark of natural and experimental TSE (unpublished data).

4. Conclusion

Human TSEs are rare diseases, CJD being the most common. CJD incidence does not differ significantly among countries [41]; incidence ranges between 1 and 1.7 per million inhabitants per year. To date, no link has been described with animal TSE except for the new variant of CJD (vCJD) [42], which occurred mainly in the UK and is thought to be caused by the BSE agent [43–45]. Several clusters of CJD have been described in the past, especially in Slovakia and Israel. Progress in molecular genetics has demonstrated that these clusters were in fact inherited forms of CJD; it should be noted that all forms of human TSEs are transmissible, including the genetic diseases. Several iatrogenic accidents have been reported in the literature [46], underlying the absolute need of prevention in daily medical and surgical practice; one has to keep in mind that no treatment exists today for TSEs, although a few drugs have proved relatively effective when administered at the time of the experimental infection (Congo red, dextran sulphate, tungstoantimoniate, amphothericin B and its derivatives). [47,48]

Since 1996, 125 cases of a new variant of CJD have been reported in young individuals in the UK [42]. In this disease, PrP-sc and infectivity can be detected in lymphoid organs of the affected patients (tonsils, lymph nodes, appendix) [49,50]; this demonstrates the role of the lymphoid system in peripheral oral infection in humans exposed to the BSE agent and this distribution of infectivity in periphery has numerous public health consequences, the size of which will depend on the number of future cases of vCJD.

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