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The transmissible spongiform encephalopathies

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Summary

Transmissible spongiform encephalopathies (TSEs) represent a group of neurodegenerative diseases characterised by a very long incubation period in regard to the life expectancy of the host species. The lesions are restricted to the central nervous system, although the pathogenesis of infection implies a primary replication step of TSE agents in the lymphoid organs followed by a neuroinvasive phase. The outcome is always fatal and today there is neither cure nor prophylaxis for these diseases. For years, the causative agents of TSEs have posed a conundrum in terms of current knowledge of microorganisms, and there are still open questions about their exact nature. They are usually called TSE agents or prions because they are thought to be primarily composed of a modified host protein, the prion protein (PrP). A pathological form of the prion protein, called PrP^{Sc} (for scrapie) or PrP^{Res}, an operational definition referring to resistance to proteolytic digestion, accumulates in target organs.

The aim of this introductory chapter is to present the general features of TSEs and a modern understanding of TSE agents and their mode of replication. Notwithstanding the plethora of unsolved questions on these diseases and their aetiology, knowledge of their pathogenesis and recent advances in understanding of the molecular basis of PrP accumulation, together with detection systems, provide the tools to conduct sound TSE risk management.

Keywords

Aetiology – Pathogenesis – Prion protein – Transmissible spongiform encephalopathies.

Human and animal diseases

Animal transmissible spongiform encephalopathies

The oldest known transmissible spongiform encephalopathy (TSE) is scrapie which affects sheep and occasionally goats. This disease already seems to have been an animal health issue of agricultural importance in the 18th Century, as suggested by this observation by Claridge (1795): 'This disorder has been known to be fatal to the greatest part of a flock and is considered as the most calamitous circumstance the sheep owners have to dread' (23).

Scrapie generally affects sheep of two to five years of age. There are several clinical forms and incidence rates in affected flocks (33). There is a strong genetic determinant of scrapie susceptibility in sheep encoded by codons 136, 154 and 171 of the prion protein (PrP) gene (24). The paths of infection are only partially known and probably imply vertical peri-partum transmission via the infectious placenta as well as horizontal transmission (grazing in pastures having hosted scrapie-infected sheep has been incriminated) (7, 36). Scrapie is transmissible experimentally to other ruminants, to primates, cats and a variety of rodents.

Transmissible mink encephalopathy (TME) developed in ranches of the United States of America (USA) as a result of the consumption by the animals of ruminant carcasses (94). In most cases, the mink seem to have been infected by the scrapie agent through sheep carcasses. In one more recent case, however, the mink were seemingly fed only cattle carcasses, raising suspicion about the origin of the contamination in this and other cases. Transmissible mink encephalopathy was experimentally transmitted to sheep, goats and hamsters, but could not be transmitted to mice (93).

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Nowadays, chronic wasting disease (CWD) has become a public health issue in the USA. The disease affects wild and farmed cervids (mule deer [*Odocoileus hemionus*], white-tailed deer [*Odocoileus virginianus*] and elk [*Cervus elaphus*]) in precise geographical areas (determined by the ecology of the affected species), mainly Colorado, Nebraska and Wyoming in the USA and the border regions of Canada. Many aspects of this disease remain to be elucidated, not least whether it is transmissible to primates (133).

Bovine spongiform encephalopathy (BSE) constitutes a major public health problem since the epidemic that affected the United Kingdom (UK) (more than 180,000 contaminated cattle). Cattle were infected through consumption of feed supplemented with meat-and-bone meal (MBM) (130). The disease was first combated by removal of infective material from the ruminant food chain and by a ban on ruminant MBM in ruminant feed (6). More recently, a complete ban on the use of MBMs in animal feed was instituted to contain the epidemic to Europe. Bovine spongiform encephalopathy has contaminated 140 humans, including 129 in the UK, six in France, one in Ireland, one in Italy, one in Hong Kong, one in the USA and one in Canada (the three patients reported outside Europe had spent most of their life in the UK). In humans, the disease is called variant Creutzfeldt-Jakob disease (vCJD) (131). The magnitude of the contamination, which probably occurred by the oral route, is still unknown, but measures have been taken to protect public health such as, for instance, the removal from the human food chain of bovine offal likely to contain the infectious agent (brain, spinal cord, tonsils, spleen, thymus, lymph nodes, intestine). Bovine spongiform encephalopathy has been transmitted experimentally to mice, mink, sheep, pigs and non-human primates (31). Many aspects of vCJD were reproduced in cynomolgus macaques (*Macaca fascicularis*) following experimental transmission of BSE (86).

Human transmissible spongiform encephalopathies

Human TSEs are rare diseases, Creutzfeldt-Jakob disease (CJD) being the most common. The disease was first described by two German neuropathologists in 1920 and 1921 (29, 74). Several clinical variants of the disease exist, each of which is characterised by the predominance of one of the CJD-characteristic clinical features (dementia, cerebellar ataxia, pyramidal motor impairment, amaurosis, akinetic mutism) and by a particular neuropathological pattern.

Creutzfeldt-Jakob disease can be sporadic (85% to 90% of the cases), familial (10% to 15% of the cases) or iatrogenic (<1%). The incidence of the disease does not differ significantly in countries in which epidemiological surveillance is performed (132) and is of the order of one per million inhabitants per year. To date, no link has been described with animal TSE except for the new variant of CJD. Several clusters of CJD have been described in the past, especially in Slovakia and Israel (77). Progress in molecular genetics has demonstrated that these clusters correspond to inherited forms of CJD (61, 62). It should be noted that all forms of human TSEs are transmissible, including the genetic diseases (20).

Several iatrogenic contaminations have been reported in the literature (11). All of these contaminations implicated the central nervous system (CNS) as the 'donor tissue' of prions. They occurred mainly after treatment with extracted pituitary hormones (growth hormone, gonadotrophins), dura mater transplantation and the use of surgical instruments contaminated with prions. In several of the cases, transmission was precisely documented.

The existence of TSEs has a number of consequences for safety regarding the use of biological products in therapy. Such biological products must be evaluated for potential risk of transmission of TSE agents, i.e. safety will depend on screening of the donor or animal source, the purification process, which may include several steps that can inactivate or eliminate TSE agents, the dose and the administration route of the final product with the intracerebral route being the most risky, and the indication for which the product is used. The replacement of biological products with synthetic/recombinant molecules is desirable whenever possible. For biological and synthetic molecules, a precise evaluation of the therapeutic risk/benefit ratio should be performed.

The appearance of vCJD raised an additional risk of human contamination by the BSE prion as disease-associated prion protein (PrP^{Res}) and infectivity was detected in the lymphoid organs of vCJD patients (tonsils, lymph nodes, spleen) in all cases, whereas infectivity seems to be restricted to the central nervous system in the case of sporadic CJD (sCJD) (13, 127).

These problems are currently being evaluated and the public health consequences of vCJD will depend upon the future number of cases in and beyond the UK (60, 125). Nevertheless, precautionary measures have been taken for blood transfusions and the use of plasma-derived products: most European countries have introduced a systematic leucodepletion of blood donations. In several countries (such as the USA, Canada and Japan), individuals who spent more than six months in the UK (this sometimes also applies to continental Europe) between 1980 and now are excluded from blood donation and plasma from Great Britain is no longer used for plasma derivative product purification.

Another human TSE was discovered in the 1950s in Papua-New-Guinea by Gajdusek and Zigas. They elucidated the epidemiology of this strange disease confined to a number of adjacent valleys in the mountainous interior of Papua-New-Guinea and affecting mostly people from the Fore cultural and linguistic group (54). Individuals contracted the disease during cannibalistic rites during which the corpses of deceased kinsmen were prepared and consumed. Infection occurred by

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ingestion or through cuts and abrasions of the skin or close contact with the eyes or mucosa. In 1976, Gajdusek was awarded the Nobel Prize for elucidation of the epidemiology of kuru and experimental transmission studies (he transmitted kuru, CJD, scrapie, TME to non-human primates, rodents and cats) that better defined this and other human and animal TSEs (55).

The biology of transmissible spongiform encephalopathy agents

Neuropathology of transmissible spongiform encephalopathies

The neuropathology of TSEs involves neuronal death, spongiosis and gliosis with hyperastrocytosis (3, 51). The PrP accumulates in the protease-resistant form (PrP^{Res}) in the brain of affected individuals. In some cases, this leads to the formation of amyloid plaques. The precise mechanisms that culminate in brain cell damage are not known and *in vitro* studies using primary cultures of neurons have revealed the complexity of the mechanisms leading to apoptotic neuronal death. Several model peptides encompassing a region of the PrP referred to as 'neurotoxic' and spanning residues 106-126 have been used and induce the death of primary neurons (46). While there is converging evidence that peptide 106-126 acts by the intermediary of glial cells, mainly microglial cells, and requires the presence of cellular prion protein (PrP^{C}) at the cell surface (8), other longer peptides are able to directly induce neuronal death and also exert their effect on neurons devoid of PrP. Prion protein-induced neurotoxicity is probably mediated by secretion of cytokines by microglial cells and astrocytic activation, but other mechanisms also occur, such as membrane destabilisation by trans-membrane forms of PrP (69, 102). These mechanisms of neuronal death are probably non-exclusive and reflect the diversity of the diseases belonging to the TSE group, some of which involve exogenous infection while others are triggered by mutated PrP harbouring abnormal topology at the cell membrane.

The existence of several strains

Transmissible spongiform encephalopathies are characterised by the existence of numerous different strains of infectious agents. A single species can be infected by various strains. These induce specific clinical and neuropathological patterns. In natural animal diseases, the strains are often referred to as 'isolates' and may contain one or more TSE strains. In sheep, the variability of clinical signs is illustrated by the existence of 'drowsy' or 'scrapie' forms of the disease (33, 47, 66, 73).

Transmissible spongiform encephalopathy strains can be characterised biologically and biochemically. Biological identification involves inoculation of syngeneic mice. The read-out is the length of the incubation period and the distribution of vacuolation (12, 52). The severity of spongiform changes in about ten different brain regions can be plotted in a 'lesion profile'. These two parameters are unique for a given mouse line and a single agent strain. Two distinct genotypes exist in mice and determine the length of the incubation period and the lesion profile for a given strain of agent. The susceptibility gene was called *Sinc* s7 or p7 in Scotland (reflecting whether the incubation period was short or prolonged after inoculation with the ME7 murine strain of scrapie) (34). After the role of PrP in TSEs had been identified, it was suspected that *Sinc* corresponded to PrP (in the USA, the two mouse genotypes were denominated PrP A and B [17]). In 1998, Manson definitively demonstrated that these two genes were one and the same (96). In most cases, a TSE strain is identified by inoculation of one mouse line from each PrP genotype and the F1 cross.

Transmissible spongiform encephalopathy strains also differ in resistance to chemical and heat inactivation, pathogenicity and distribution in the infected organism (41). In kuru or CJD in humans, the infectious agent occurs at levels below detection in organs other than those of the central nervous system. In vCJD, on the other hand, infectivity and PrP^{Res} accumulation have been demonstrated in various lymphoid tissues including the tonsils, the appendix, lymph nodes and the spleen (127).

Similarly to conventional viruses, TSE agents can compete for replication in a single host. In such cases, inoculation with a 'slow' strain can protect against subsequent infection with a 'rapid' strain (35, 37).

The strain phenomenon has been the largest outstanding challenger to the 'protein-only' hypothesis with regard to the nature of TSE agents. The question was raised as to how different strains can replicate independently in a single host producing only one type of PrP. For a long time, the sole answer was that the PrP probably associated with an as yet unidentified informational molecule, probably nucleic acid in nature, to fit in with current virology dogma.

Propagation in the organism

Since the late 1970s, the long incubation period of TSE has been known to conceal primary propagation and amplification of the agent in the lymphoid tissues and the secondary neuroinvasive phase. Analysis of the infectivity of different organs in animals infected experimentally and culled at different time-points post-infection has shown that neuroinvasion occurs at about half the incubation period (44, 65, 81). Irrespective of the route of contamination, the infectious agent is rapidly taken up by the loco-regional lymphoid organs and transmitted to other lymphoid tissues along lymphatic and systemic paths. The lymphoid organs, instead of combating infection, provide a substrate for replication. Long-lived, non-dividing cells are known from

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irradiation experiments to be the key to the sustaining of infection (53). Today, many lines of evidence point to follicular dendritic cells (89, 92, 95). Their long processes would retain and accumulate PrP^{Res}, whereas other cells, as yet unidentified, are thought to play a role in the propagation of infectivity within and between organs.

Neuroinvasion occurs from nerve endings in lymphoid tissues and follows neuroanatomical paths to the spinal cord and brain (50, 82).

The pathogenesis of TSEs involves complex interactions of the infectious agent with different target cells and extracellular environments. This is readily comprehensible with conventional viruses, but difficult to explain for a protein which poses one more challenge to the prion hypothesis. Efforts to find possible cellular receptors of the PrP might provide partial answers, but much research is still required before this matter is elucidated.

Implications for health issues

These outstanding questions should not overshadow the extent of the knowledge that has been gained on infection in different organs by experiments on transmission to ruminants and to laboratory rodents. Sheep scrapie was the first TSE for which the Office International des Epizooties (OIE: World organisation for animal health) classified organs and tissues as a function of infectivity. Risk assessment of BSE and TSEs takes into account the time-course of the disease as well as the differences between sheep scrapie and BSE. In field cases of BSE, infectivity in 'peripheral' (i.e. non-CNS) tissues is lower than in scrapie to the extent of being undetectable in the mouse bioassay (129). In cattle experimentally infected by the oral route, infectivity was found in the ileum (128). The exact degree of infectivity in the lymphoid tissues of BSE-infected cattle has been impossible to determine due to lack of a sufficiently sensitive infectivity test. Responses may arise from ongoing cattle-to-cattle inoculation experiments as well as from novel, more sensitive biochemical tests (114). However, the fact that with BSE, the CNS-peripheral tissue differential is higher than that found in sheep scrapie is important for decision takers who apply the worst-case scenario rule.

The aetiology of transmissible spongiform encephalopathies

The search for the culprit

The transmissibility of scrapie was demonstrated by Cuillé and Chelle in 1938 (30). Human TSEs were shown to be transmissible by Gajdusek in the 1960s and 1970s (56, 57). The pathogenesis of scrapie in ruminants and rodent models had been studied by Hadlow and Scottish researchers working with Dickinson (65, 67) and in the early 1980s, the existence of genetic susceptibility to these diseases was demonstrated which, combined with an infectious component, was shown to determine the characteristics of the disease (32, 38). In the past, TSEs were classified as unconventional slow virus diseases because of their lengthy progression and the unusual properties of the aetiological agent (55). Like viruses, TSE agents pass through filters below the micrometre range and are, in this respect, comparable to the agent responsible for other slow infections of ruminants such as maedi-visna. Similarly to other slow viruses, these agents show a tropism for the reticuloendothelial system, adapt to new hosts, comprise several strains of varying virulence and pathogenicity, and slow-growing strains have been shown to interfere with replication of fast-growing strains. However, TSE agents also harbour several non-canonical properties: no viral particles could be observed by electron microscopy in highly infectious brains containing as much as 10^8 lethal dose 50 (LD_{50}) and no non-host nucleic acid or protein could be detected. Furthermore, TSE agents are resistant to most of virus-inactivating treatments (including formaldehyde, nucleases, heating at 80°C) and they provoke no systemic inflammatory response nor antigenicity. These agents show an atypical ultraviolet inactivation spectrum with a six-fold increase in sensitivity at 237 nm over that at 254 nm and their ionising radiation data point to a target of 150,000 daltons (55). These inactivation data are more compatible with TSE agents being related to the recently discovered plant viroids comprising small, single-stranded naked ribonucleic acids (RNAs) than to lentiviruses (42).

The hypotheses that TSE agents were solely self-replicating proteins (64), or small nucleic acids associated with a host component (39), or replication defective viruses, were formulated (111). Then, in 1981, Prusiner discovered that infectious fractions reliably contained a hydrophobic protein that co-purified with infectivity (103). He hypothesised that this protein was the infectious agent and named it prion, which is the anagram of 'proteinaceous infectious particle' (104).

The prion protein

Partial sequencing enabled the encoding nucleic acid of the protein to be cloned (21, 99). This led to the surprising finding that this apparently infection-associated protein was encoded by a host gene. Subsequently, the use of antisera to the protein and RNA detection methods demonstrated that this host protein was expressed in many body tissues, the highest levels being found in the brain (21, 110). The primary structures of PrP of most mammalian species have been derived from complementary deoxyribonucleic acid (cDNA). They are highly conserved between species. In hamsters (124), the PrP consists of 254 amino acid residues, of which the N-terminal 22 amino acids constitute a signal sequence which directs the protein into the lumen of the endoplasmic reticulum where it is then cleaved off. Amino acids 53 to 93 are composed of five octapeptide repeats (five or six in the case of bovine PrP [63]). A contiguous stretch of 20 hydrophobic residues (commencing

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approximately at amino acid 106) meets the criteria for a stop-transfer effector, which, however, could not be shown to be functional. Other features of the PrP are the presence of a disulphide bridge between cysteines 179 and 214 and two N-glycosylation sites at residues 181 and 197. The N-glycans are so diverse that calculations show that 401 different isoforms of PrP can exist when both asparagine residues are glycosylated (45). A glycophosphatidylinositol (GPI) anchor is attached concomitantly with the removal of 23 amino acid residues from the C-terminus. The GPI anchor is attached in a classical way to serine residue 231 through a phosphoethanolamine. As a consequence, PrP is attached at the outer leaflet of the plasma membrane through the GPI and, in cultured cells and lymphocytes, is mainly associated with membrane microdomains called rafts (126). The mechanism of internalisation of the PrP is not yet known. This may occur through caveolae-like domains but has also been associated with clathrin. The recent discovery of cell-surface receptors for PrP may soon help to elucidate the endocytic pathway of this protein (59, 134). The secondary structure of PrP was resolved by nuclear magnetic resonance (NMR) spectroscopy, first from amino acids 121 to 231, which forms what is now denominated the 'structured core' of the PrP (108). The N-terminal region of the PrP was then shown to be unstructured and is now referred to as the 'flexible tail' (88, 109) (Fig. 1).

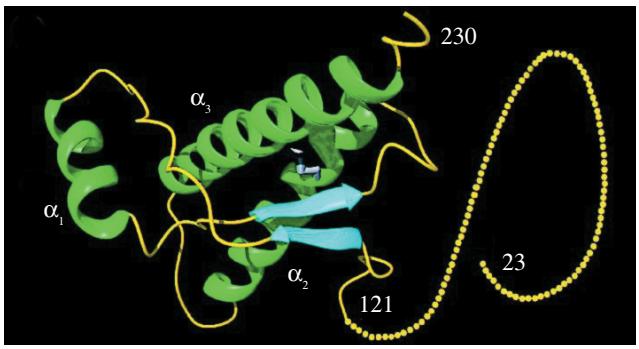

Fig. 1
Three-dimensional structure of the intact bovine prion protein (23-230) showing the helices, β-strands, segments with non-regular secondary structures and the flexibly disordered 'tail' of residues 23-121 represented by dots
(88)

Over the last ten years, a wealth of data has emerged on the role of the PrP in the pathogenesis of TSE. Relatively early, mutations within the human PrP gene were found to be responsible for the development of the hereditary forms of TSEs. Experiments with transgenic mice expressing various levels of homologous or heterologous PrPs, have shown that PrP expression is positively correlated with disease susceptibility and that the PrP sequence is in part responsible for the species barrier (105, 117). The latter phenomenon is complicated by the occurrence of different TSE agent strains which vary in their capacity to cross the species barrier. In this respect, the BSE agent is a 'spoilsport', in that the agent is able to replicate with undetectable amounts of PrP^{Res} at the first passage into mice (87) and transgenic mice expressing bovine PrP have not reduced the species barrier between cattle and mice as much as was hoped, at least with regard to the incubation periods (118). The advent of PrP knock-out mice ($PrP^{0/0}$) and inoculation experiments in these hosts have supported the concept that the PrP is indispensable for disease induction by TSE agents. However, they were shown to harbour small amounts of infectivity for up to twenty weeks post-inoculation (15). The most probable explanation of these findings is the persistence of the inoculum (115). This, however, has never been formally demonstrated and in some instances, infectivity was surprisingly significant. Notwithstanding the latter observations, the PrP has been shown to play a major role in the pathogenesis of TSEs.

The role of cellular prion protein in the absence of a transmissible spongiform encephalopathy

Researchers are beginning to understand the function of PrP^{C}, but answers in terms of one or several defined function(s) cannot be expected too rapidly.

Much hope was attached to the use of PrP knock-out mice to unveil the function of PrP^{C}, but no obvious phenotype was observed (14). Thus, most proposed functions for the ubiquitous protein have been inferred from data on localisation, molecular interactions or on the observed effects of its absence.

In non-neuronal cells and in cultured cell lines, PrP^{C} is generally found at the cell membrane. There are some exceptions such as the stomach, where PrP^{C} is observed in secretion granules of epithelial cells (49). In neuronal cells, PrP^{C} is present in presynaptic nerve terminals (48, 68, 70, 116). Biochemical studies have demonstrated that PrP^{C} is a membrane-bound protein, thus a likely localisation would be at the plasma membrane of a synapse. However, PrP^{C} is also associated with synaptic vesicles. This would suggest that the protein plays a role in the recycling of vesicles or a more direct role in synaptic transmission (83). The latter hypothesis has been substantiated by electrophysiological studies in mice devoid of or expressing various levels of PrP^{C} (16, 26, 90). In addition, aberrant sleep patterns were observed in PrP knock-out mice (123).

The fact that the PrP^{C} binds copper with an affinity highly relevant to a potentially related activity makes it a metal ion-binding protein (9). As such, the protein could regulate the copper concentration in the synaptic region of neurons. Alternatively, PrP^{C} has been shown to harbour the activity of a copper-dependent superoxide dismutase (10).

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Several experimental findings suggest that the PrP may play a major role in cell survival: the protein can bind to the anti-apoptotic factor Bcl2 (84) and induce neuroprotective signals (5, 22). Moreover, primary neurons taken from $PrP^{0/0}$ are less resistant to serum deprivation than their PrP-expressing counterparts (85).

Recent efforts to identify PrP-binding molecules have led to the discovery of several receptor candidates for the PrP among which the 37/67 kDa laminin receptor (LRP/LR) (59) and murine stress inducible protein I (mSTI) (134). The LRP/LR mediates PrP internalisation, suggesting a role in the endocytic pathway of the protein. However, whether the LRP/LR acts as the cellular receptor for recycling or catabolism of endogenous PrP, whether it acts as the receptor for PrP^{Res}, hence promoting cell-to-cell propagation of infectivity, or whether it interacts with PrP molecules present at the surface of other cells, thus contributing to cell communication and survival (maybe via the activation of the tyrosine kinase fyn [97]) is unknown.

Modern understanding of prion replication

The disease-associated isoform of the protein is referred to as PrP^{Sc} (for scrapie) or PrP^{Res}, which is an operational definition due to the fact that when submitted to proteolytic digestion, a protease-resistant core is yielded that can be typically recognised by electrophoresis (named PrP27-30 because of the molecular weight of the bands corresponding to di- and mono-glycosylated PrP) (4). The PrP^{Res} represents an abnormal structural conformation of the cellular prion protein PrP^{C} (19, 58, 100). The protein has a higher β-sheet content than its normal counterpart (Table I) but at present, assigning additional β-sheets to specific regions of the protein is risky.

Table I
Secondary structure of the cellular prion protein, the scrapie-associated prion protein and the prion protein 27-30 (after partial proteolytic digestion of the scrapie-associated prion protein)
(100)

Percentage of secondary structure	β-sheet	α-helices	β turns	Random coils
PrP^{C} (a)	3	42	32	23
PrP^{Sc} (b)	43	30	11	16
PrP 27-30 (c)	54	21	9	16

(a) Cellular prion protein (PrP^{C})
(b) Scrapie-associated prion protein (PrP^{Sc})
(c) Prion protein (PrP)

Within the framework of the prion hypothesis, the infectious agent is composed entirely of PrP^{Res}. In the heterodimerisation model, one PrP^{Res} molecule associates with a native, host-synthesised PrP molecule to which the agent imprints the same abnormal conformation, which can then be transmitted to a further molecule (25). This model implies that a high energy barrier is overcome at each new structural transconformation. The PrP^{Res} molecules would eventually aggregate.

This process could be assisted by a molecular chaperone or another unknown host factor, referred to as factor X, since experimental findings have provided clues to the existence thereof (121).

Formation of PrP^{Res} may follow a nucleation process where conversion of PrP^{C} into PrP^{Res} is a slow and rate-limiting step resulting in a polymerisation nucleus composed of PrP^{Res} oligomers. Addition of PrP^{C} to the nucleus generates large amyloid polymers of PrP^{Res} (76). Analysis of the structural states of PrP using decreasing amounts of detergent showed that PrP forms an alpha-helical dimer, then an oligomer rich in β-sheets, which then aggregates into large polymers (75).

Recent findings have reconciled the existence of several TSE strains with the PrP-only concept. The strain specificity of TSE agents may be enciphered by the multiplicity of structural conformations possibly harboured by a PrP molecule. In 1985, Kascsak and co-workers described several aspects of PrP^{Res} after electrophoretic separation and immunodetection (78). From 1996, these biochemical analyses of PrP received new impetus and showed that PrP^{Res} could display different electrophoretic patterns characteristic of various strains (28, 101). The differences lie in the degree of glycosylation of the PrP^{Res} and in the gel mobility of the protein, which, in turn, depends on the size of the protease-resistant fragment. The protease cleavage site varies depending on the strain; this has been attributed to a distinct conformation of the abnormally folded, pathogenic, PrP (122). Although the current consensus is that the variety of TSE strains is not exactly reproduced by that of the electrophoretic patterns of PrP (72), these findings have provided an alternative explanation for the TSE strain phenomenon. The strains all correspond to a different fold of the abnormal protein, which provides a specific template for the faithful conversion of the host protein into the disease-inducing conformer. From this point on, 'TSE strains' can be referred to as 'prion strains'. Another PrP-based method able to discriminate between different strains is called the conformation-dependent immunoassay (CDI). The assay exploits the fact that discrete PrP epitopes can be differentially recognised by the antibodies used in the assay depending on the conformation of the abnormally folded protein (113).

Alternative hypotheses

In recent years there have been claims that BSE is not induced by prions or even so-called 'TSE agents', but that it, like other diseases of the same family, is due, similarly to multiple sclerosis, to acinetobacter (43) or organophosphate poisoning (106, 107). Had these assertions been well founded, they would have had a major impact on BSE risk analysis and management. However, they do not withstand thorough scientific analysis. In the first case, none of the postulates of Koch incriminating acinetobacter as the aetiological agent of TSEs are fulfilled. The obvious reason for dismissing the second hypothesis is the transmissibility of TSEs.

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Today, alternatives to the 'protein-only' hypothesis rely mainly on a few facts, i.e. the virus-like pathogenesis of infection, the existence of several strains of the agent and the fact that infectivity is not strictly associated with PrP^{Res} in some situations. Under certain experimental conditions, inoculated animals develop a TSE-like disease although no PrP^{Res} can be detected. This has been observed in wild-type mice inoculated with BSE (87) as well as in transgenic mice inoculated with human TSEs (27, 91, 121). A widely preferred interpretation of these observations is that the pathogenic, misfolded PrP species is not necessarily protease-resistant and that PrP^{Sc} or PrP* would therefore be a better designation of the infectious protein than PrP^{Res}. The alternative interpretation, that the infectious agent comprises a hitherto undiscovered molecule (presumably but not necessarily a nucleic acid) in addition to PrP, cannot yet be discounted.

The viral hypothesis advances that TSE agents are composed of an amyloidogenic virus probably defective in capsid proteins (hence no viral particles are seen by electron microscopy). This hypothesis has been substantiated by the fact that nucleic acids co-purify with the PrP (1, 2). Moreover, infectivity associated with the brain of CJD-infected hamsters sediments at 120 S, corresponding to ribonucleoprotein complexes (119). Treatment of similar fractions with ribo- and micrococcal nucleases did not lead to significant decreases in infectivity (120). This shows that the observed resistance of TSE infectivity to nucleases should not be taken as evidence against nucleic acid participation in the infectious particle. Indeed, packaging into a protein, as in conventional viruses, would render the nucleic acid inaccessible to the action of nucleases.

However, attempts to identify nucleic acids correlated with infectivity have not always met with success. Applying return refocusing gel electrophoresis (RRGE) techniques to nuclease-treated infectious fractions from scrapie-infected hamsters has shown that, if at least one molecule of nucleic acid is to be associated with one infectious unit, the maximum size is in the range of 76 nucleotides. The size could reach about 240 nucleotides if the nucleic acid population were allowed to be heterogeneous (79).

The virino hypothesis was first formulated by Dickinson and Outram in 1979 (39). The original hypothesis was based on the observation of the absence of immune reaction in the infected host, as well as the existence of several strains of agent and their capacity to undergo mutations as an indicator of an independent genome. The virino was seen as an informational molecule (probably a nucleic acid) and a host protein or multimeric complex that would account for the immunological neutrality of the virino and resistance to host defences. This hypothesis has since been readjusted based on data on the molecular biology of the PrP (40, 41, 80). Today, the 'modern' virino should be envisaged as a complex between a nucleic acid, too small to encode any protein, and host PrP to which the infective agent would imprint an abnormal conformation through interference with normal metabolism.

Much remains to be discovered

The ultimate proof that misfolded PrP is the infectious agent responsible for TSEs could be obtained by expressing recombinant PrP and manipulating the resultant protein in a test tube in such a way that misfolding occurs, then to prove by bioassay that the PrP has become infectious and reproduces a disease that has all the characteristics of a TSE in the recipient animal. This demonstration has long been hampered by the fact that the only existing *in vitro* conversion assay involved the addition to the test tube of excess PrP^{Res} extracted from infectious brain (18). In this experimental set-up, the high input of infectivity obviated the possibility of reliably detecting *de novo* infectivity arising from normal PrP being converted to PrP^{Res}. However, experiments using heterologous hamster PrP to convert chimaeric mouse-hamster PrP and mice as recipient animals to detect infectivity did not show any evidence of *de novo* generation of infectivity (71). Recent tools such as protein misfolding cyclic amplification (PMCA), which combine the use of crude brain homogenate in a reaction tube with that of sonication and iterative cycles of *in vitro* conversion, may be more suitable for demonstrating new infectivity arising from the conversion of PrP into PrP^{Res} (112). Results using such techniques are, however, still pending, and whether an undisputable answer to the question of the molecular basis of infectivity will be provided in the near future is increasingly uncertain. The need for some co-factors of conversion present in brain homogenates may support the concept of the 'factor X' or even an infectivity-related nucleic acid. Although much research is still required before forging this link, it certainly seems that the PrP may not be the only player in TSEs.

Conclusion

Research in the field of TSEs has made major advances in the last decade. Data on TSE pathogenesis that already existed prior to the outbreak of BSE have been fundamental to implementing measures for the protection of public health. Modern tools of biology have since further explored the role of the PrP in these diseases with success, even if many questions still remain unanswered. In addition, technological advances and the use of the PrP as the hallmark for the presence of infectivity in the central nervous system have led to the development of diagnostic tests suitable for large-scale use (98). These tests have further contributed to BSE risk management. In this respect, the next step forward will be the development of practical ante-mortem diagnostic procedures. If TSE research continues at the same pace, new developments that can be foreseen in immunological and therapeutic approaches of TSEs will also have an impact on the management of animal and human TSEs.

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Les encéphalopathies subaiguës spongiformes transmissibles

C.I. Lasmézas

Résumé
Les encéphalopathies subaiguës spongiformes transmissibles (ESST) constituent un groupe d'affections neurodégénératives qui, par rapport à l'espérance de vie de l'espèce hôte, se caractérisent par une très longue durée d'incubation. Les lésions se confinent au système nerveux central, même si la pathogenèse de l'infection comprend une première phase de réplication des agents infectieux dans les organes lymphoïdes, suivie d'une phase de neuro-invasion. Ces maladies ont toujours une issue fatale. On ne dispose actuellement d'aucun remède ou prophylaxie contre ces affections. Après avoir constitué une énigme par rapport aux connaissances scientifiques des microorganismes pendant plusieurs années, les agents responsables des ESST continuent à susciter des questions, notamment en ce qui concerne leur nature exacte. Ils sont généralement appelés agents des ESST ou prions, dans la mesure où ils se composeraient essentiellement de la forme modifiée d'une protéine de l'hôte, la protéine du prion (PrP). Une forme pathologique de la protéine du prion, appelée PrP^{Sc} (Sc signifiant scrapie, la dénomination anglo-saxonne de la tremblante) ou PrP^{Res} – une définition opérationnelle découlant de sa résistance à la digestion protéolytique – s'accumule dans les organes cibles.
Ce chapitre introductif a pour objet de présenter les caractéristiques générales des ESST ainsi que nos dernières connaissances sur leurs agents et leur mode de réplication. Malgré les multiples questions restées sans réponse concernant ces maladies et leur étiologie, l'étude de leur pathogenèse, les dernières avancées dans la compréhension de la base moléculaire de l'accumulation du PrP et les dispositifs de dépistage sont autant d'outils permettant une gestion rationnelle du risque lié aux ESST.

Mots-clés
Encéphalopathie subaiguë spongiforme transmissible – Étiologie – Pathogenèse – Protéine du prion.

■

Las encefalopatías espongiformes transmisibles

C.I. Lasmézas

Resumen
Las encefalopatías espongiformes transmisibles (EET) constituyen un grupo de enfermedades neurodegenerativas caracterizadas por un período de incubación muy largo con respecto a la esperanza de vida de las especies afectadas. Las lesiones se limitan al sistema nervioso central, pero la patogenia de la infección implica una etapa inicial de replicación de los agentes patógenos en los órganos linfoides, seguida de una fase de invasión del sistema nervioso. El desenlace es siempre mortal y en la actualidad no existe ningún medio de curación ni de prevención de estas enfermedades. Los agentes causantes de las EET constituyen desde hace años un enigma para nuestros conocimientos de los microorganismos, y todavía persisten varias incógnitas sobre su naturaleza

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exacta. Son denominados habitualmente agentes de EET o priones, porque se piensa que derivan de una proteína huésped modificada, la proteína prion (PrP). Una forma patológica de la proteína prion, denominada PrP^{Sc} (en referencia al prurigo lumbar, *scrapie* en inglés) o PrP^{Res} (definición que hace referencia a la resistencia a la digestión proteolítica) se acumula en los tejidos diana.
El objeto de este capítulo de introducción es presentar las características generales de las EET y los últimos conocimientos sobre sus agentes causantes, así como sobre la forma en que éstos se replican. Pese a las numerosas incógnitas sobre estas enfermedades y su etiología, el conocimiento de su patogenia y los últimos descubrimientos sobre la base molecular de la acumulación de la PrP, así como los adelantos en la creación de sistemas de detección, permiten una gestión satisfactoria del riesgo de EET.

Palabras clave
Encefalopatías espongiformes transmisibles – Etiología – Patogenia – Proteína prion.

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