**Prion infectious diseases: History and molecular pathology of this heretical hypothesis**

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are a set of rare, fatal neurodegenerative diseases affecting humans and other mammals. The protein-only hypothesis relating to these diseases proposes that infectious particles made only of protein material, and which arise from the misfolding of the normal host protein (PrPc) to become an abnormal isoform (PrPSc), propagate in the absence of nucleic acid [25]. Once present, the infectious protein recruits more of the normal protein causing a chain reaction of aggregation. Although now widely accepted, the prion hypothesis has challenged some of the most established scientific thinking.

Scrapie was one of the first prion diseases to be investigated due to an outbreak in Europe in the late 18th century. Various causes were suggested, but the lack of scientific understanding in the early 19th century, when people were still asking questions such as: “If you can catch a disease, why can’t you catch a cure?”(Max, 2008, p.33), limited the ability to investigate Scrapie further. Baron Von Richthofen, an agricultural expert, believed the disease was caused by a mite, which could be transferred to other sheep through nasal mucus or through copulation. Although he did not understand the difference between inherited disease and sexually transmitted disease, he had unknowingly accounted for two different methods of spreading [15].

Transmissibility of TSEs was accidentally demonstrated in 1937 when around 10% of a sheep flock developed scrapie two years after being inoculated against a common virus using a formalin extract of brain tissue unknowingly derived from a scrapie infected animal [25]. By this time, bacteria and viruses had been discovered and a causal link established between microbes and disease, and it was assumed that scrapie was caused by a virus.

In the 1950s, scientists noticed that large numbers of women and children were dying of kuru in the villages of the Fore tribespeople in Papua New Guinea. Vincent Zigas, a Lithuanian doctor, and researcher, Carleton Gajdusek, suspected an infectious route of the disease. On examination of victims’ brains, however, there was no evidence of a viral or bacterial infection, or of an immune response. The disease appeared similar to Creutzfeldt-Jakob Disease (CJD), discovered in the 1920s, and to scrapie. In the 1960s, a correlation between ritualistic cannibalism in the villages and kuru was suggested by anthropologists Robert and Shirley Glasse [15]. Gajdusek had previously been close to this solution, having written that: “Women and children, particularly, partake of the human flesh.” (Max, 2008, p.83). In 1966, collaborative efforts succeeded in transmitting kuru and, subsequently, CJD and Gerstmann-Straussler-Scheinker syndrome (GSS) to primates, demonstrating an infectious route.

Because of their long incubation period, and considering the scientific knowledge at the time, TSEs were initially considered to be a ‘slow virus’ but, in 1967, Tikvah Alper discovered that infectivity survived harsh chemicals, high temperatures, UV light and radiation [25] - the agent was indestructible. There were also no signs of DNA or RNA. It was clearly not a virus. The idea that a protein-only agent could cause a disease had previously not been considered, one reason being the underlying assumption that infectious diseases must be a war between the victim and the infectious agent; this view of a competitive system did not fit with the way the body seems to destroy itself in TSEs.

It was the mathematician J.S. Griffith who, in 1967, first suggested that a protein could convert to different forms, acting as a catalyst for its own replication. This would allow for replication without DNA and explain the lack of immune response. It was not until 1982 that Stanley Prusiner isolated, cloned and sequenced the infectious protein, naming it the prion (proteinaceous infectious particle). Prusiner’s discovery challenged the accepted central dogma of biology – provided by Crick and Watson in 1953 when they deciphered the genetic code – that nucleic acid is the carrier of genetic information and essential to replication [32].

In 1985, an mRNA transcript for the prion particle and the gene encoding PrPc were identified. The PrPSc did not have its own gene but was coded for by the same gene as the normal isoform [32].

Since then, significant advances have been made that support the prion hypothesis, including the development of PMCA, a technique to accelerate *in vitro* replication, and spontaneous generation of PrPSc infectivity *in vitro* and *in vivo*.

The function of a protein depends on its folding into a precise 3D conformation. The function of the normal, correctly folded PrPc protein – essential for prion disease to develop – is unclear. Experimental evidence suggests cytoprotection against pro-apoptotic agents such as Bax (Bcl2 associated X protein). PrPc reduces the programmed death induced by these agents, possibly by inhibiting the change caused by the agent or by binding to the agent, hindering its ability to affect the cells [31]. PrPc may also aid uptake of copper ions, transmembrane signalling and the formation of synapses [8]. In the presence of PrPc, neurites grow rapidly and re-orientate themselves towards higher concentrations of the protein, allowing neural cell adhesion molecules to bind to its N-terminus (the free end), promoting further growth [18].

The misfolding of the normal host protein involves a conformational change whereby the normal protein’s α-helix content decreases and its β-sheet content increases.The difference in function between PrPc and PrPSc seems to be due only to these conformational differences as the amino acid sequences are usually identical. Once the incorrectly folded isoform is present, it recruits more PrPc, causing a chain reaction of aggregation by conformational influence [26]. The prion polymers break into smaller chains, increasing the number of PrPSc ‘seeds’ that can convert PrPc. The exponential replication of prions affects the incubation period of prion disease and depends on the PrPc concentration [14].

As well as being self-catalysing, prions are generally protease resistant, so they cannot be broken down as easily as other proteins and so accumulate. The conversion of PrPc to PrPSc results in neuronal degeneration by an unknown mechanism, possibly loss-of-function of PrPc or toxic gain-of-function of PrPSc.

Prion diseases arise through different mechanisms, and are classed into three categories: sporadic, acquired and inherited.

Over 80% of all human prion disease is considered sporadic and appears to be caused by spontaneous or random misfolding of PrPc due to an unknown trigger – possibly a somatic mutation in the gene coding for the protein [30]. Acquired prion disease occurs when prions are introduced inadvertently into the body. This includes iatrogenic CJD (iCJD), related to medical procedures such as injection of growth hormones taken from prion-infected pituitary glands of corpses, implantation of cadaver-sourced tissue, or contaminated surgical instruments [9].

Probably the best known acquired prion disease is vCJD (variant CJD), caused by the ingestion of BSE (bovine spongiform encephalopathy) infected foods, primarily beef, which raises questions about the transmissibility of the prion: how can the prion protein be absorbed without breaking down into amino acids? One suggestion is that it attaches itself to ferritin, which can be absorbed by the intestines. The prion appears to remain attached after passing through the intestinal wall, but it is not yet clear why or whether this is, in fact, the mechanism by which the prion transfers to lymph tissue [3].

It is likely that animals acquire infection mainly through ingestion. Recent research shows that small quantities of PrPSc deposited in the environment in the remains of dead animals or body fluids and faeces can bind to grass roots and be taken up into the leaves and stems. When hamsters were fed prion-contaminated plants, they became infected with the disease. This supports earlier observations that, even after periods as long as 16 years, animals can contract TSEs when reintroduced to pastures previously exposed to prion diseased animals [19]. There are far-reaching implications for agriculture and food production if prions could be progressively increasing in the environment.

Inherited prion disease accounts for around 15% of prion diseases and includes CJD, GSS and fatal familial insomnia (FFI). It is passed on as a genetic mutation on the gene PRNP, which codes for PrPc, with over 30 known mutations that can lead to misfolding [8]. The mutated gene is autosomal dominant [27], so if the mutated gene is in the genotype, it will be expressed in the phenotype and the person will have the disease.

Clinical presentation of prion diseases varies greatly. In humans, symptoms include progressive dementia, personality changes, confusion, hallucinations, ataxia, muscle stiffness and fatigue. In scrapie-infected sheep, intense skin irritation, ataxia, convulsions, abnormal posture, excitability, nervousness and aggression are observed [16], with similar symptoms in BSE-infected cows. All prion diseases are fatal, but the time from onset to death varies widely. CJD, for example, tends to last no more than a few months, with the average onset age around 60 [9], while GSS normally occurs between the ages of 35 and 55, and the average time before death is 2-10 years.

Although pathology develops in the brain and neural tissue, PrPSc is also present in lymphoid tissue, including the spleen, tonsils and appendix, and in muscle tissue. In vCJD, the tonsils, in particular, are involved. A feature of prion diseases is the lack of lymphocytic inflammatory response because the agent is the body’s own protein (misfolded), but PrPSc can be detected in the diseased brain after death by Western blotting [30], a technique that separates different sized proteins by gel electrophoresis, transfers them into a solid support, and detects the target protein by matched antibodies [1].

Disease progression depends on route of entry, disease strain and PrP genotype of the host. PRNP, at its 129th codon, can code for valine homozygous (VV), methionine homozygous (MM) or heterozygous (MV). Being homozygous appears to be a risk factor for developing human prion diseases: in most countries, while around 40% of the normal population is MM, all confirmed cases of vCJD and 70% of sCJD had this genotype; however, it is unknown whether the MM genotype increases risk of misfolding, or whether it simply decreases the incubation period. Of four cases where vCJD was acquired by blood transfusion, three recipients developed the disease and had the MM genotype. The fourth, with MV genotype, did not develop the disease, although the prion was found in his body – it is impossible to know whether he would have contracted vCJD at a later stage [2].

Diseased brain tissue shows neuronal vacuolation and degeneration, which cause a spongiform appearance of the infected tissue. Gliosis and brain atrophy are typical of advanced cases and, in certain prion diseases, such as kuru and GSS, there is also deposition of amyloid plaques, although the plaques differ between the two diseases [30].

One feature of prions that has been difficult to explain using the protein-only model is how the agent encodes information needed for multiple disease phenotypes to develop and, linked to this, how the species barrier is overcome. It appears that different folding patterns in the prion protein result in varying strains. Evidence supports the idea that the ability to transmit within and between species depends on the compatibility of the particular conformation state with the new host [26, 30]. In a recent experiment, MM2, the agent for a subtype of human CJD, was introduced into transgenic mice. Mice with natural levels of human PrP (low) did not contract the disease, while those with high levels of PrP developed a new unique strain of CJD; in mice containing ovine PrP, two new subtypes of the disease were created [5].

In another recent study, scientists created a mutant scrapie prion, which was able to replicate *in vitro*, but the protein did not cause disease when injected into bank voles, even though the un-mutated form of the prion did successfully infect the animals [24]. This suggests that the inability of the mutated form to infect them was not due to the species barrier – although, to confirm this it would have been ideal to inject the mutated form into sheep as well – but because the mutated strain was incompatible with the host.

It is confirmed that a single protein acts as the infectious agent in prion diseases, but the exact mechanism is unknown and continues to be a challenge.It is likely that, as well as known risk factors, such as codon 129 of PRNP, there are a number of unidentified factors that contribute to infectivity of prion diseases – for example, it is unclear whether PrPSc is the only component of the infectious agent; recent research has shown that purified hamster PrPc is not converted when mixed with purified PrPSc, but conversion occurs when complete brain homogenate is added [26], suggesting that a cofactor might be needed. Lipids have been found to accelerate *in vitro* replication, but it is unknown whether they are a cofactor or if they might mimic an unknown cofactor in the brain [13, 26].

It is possible that prions might also operate in other diseases such as Alzheimer’s and Parkinson’s, which show tissue damage associated with misfolded proteins. Although these other misfolding disorders were not thought to be transmissible, recent research has shown that misfolding of tau proteins, associated with a number of neurodegenerative disorders, follows a prion-like mechanism, with a ‘seed’ and templating pattern, suggesting that these diseases might have a level of infectivity [26].

In Alzheimer’s, protein aggregation forms amyloid plaques, made up of β-amyloid, in the brain. Research has shown a correlation between deaths of acquired CJD and the presence of β-amyloid in the brain, while in deaths of non-acquired (inherited or sporadic) CJD there was no β-amyloid. This suggests that Alzheimer’s disease could be transmissible if amyloid is introduced into the body. Amyloid plaques can form in response to microbial infection. β-amyloid was found to be 100 times more lethal than penicillin - it could serve a purpose in the body to help defend against other diseases. This raises the possibility that, if Alzheimer’s disease and prion diseases are both a result of misfolded proteins, microbial infections may also have a role in the development of prion diseases [29].

Environmental factors may also affect the expression of the genes that code for misfolding. The fact that there are so many variables makes it difficult, yet important, to isolate these factors during research. Understanding the normal function of PrPc, the structure of PrPSc, how PrPSc causes neuronal death, and the time frame of the progression of prion and other neurodegenerative diseases may help prevent, or allow the development of treatments that slow down the progress of, these diseases sufficiently for them to not become clinically apparent - possibly a more successful route than attempting to completely eliminate the prion protein.

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