Prion Diseases

The transmissible spongiform encephalopathies (TSEs) are invariably fatal, neuro-degenerative diseases that are characterized by a long period of latency followed by a short clinical illness. TSE diseases are found both in animals and man. Scrapie has been known in sheep and goats for more than 250 years but bovine spongiform encephalopathy (BSE) in cattle was first recognized in 1986 (Wells et al., 1987). Diseases apparently related to BSE have been identified in a diverse range of animal species, such as mink, cats, and deer. Chronic wasting disease in elk is a TSE disease that is wide spread in western United States. In man, Creutzfeldt-Jacob Disease (CJD), Gerstmann-Straussler-Scheinker (GSS) and familial fatal insomnia (FFI) syndrome are associated with variations in the PrP gene and occur in families. However, CJD can also occur as a sporadic disease that has a genetic component and most likely environmental triggers that are as yet unidentified. Kuru is a TSE of man that was identified in the Fore tribe in Papua New Guinea and was propagated by cannibalistic rituals (Alpers & Gadjusek, 1965, Matthews et al., 1968).

Scrapie

Offspring of scrapie-affected sheep are at an increased risk of developing disease (Foote et al., 1993), which may reflect either genetic susceptibility or vertical transmission of the infection (Ridley & Baker, 1995). However, unrelated animals in a scrapie-infected flock are also at a high risk of developing the disease. It is not known whether the transmission occurs by direct contact or indirectly from a contaminated environment (Palsson, 1979). Placental material deposited as the afterbirth has been suggested as a potential source of infection.

BSE

BSE was first identified in 1986, and is characterized by neuronal loss, spongiosis and astrogliosis (Wells et al., 1987). The vacuoles in BSE are mainly found in the brain stem, whereas they are more widespread in the brains of scrapie-infected sheep. Following a dramatic increase in the number of BSE cases identified in the UK, feed was identified as the most likely source of infection (Wilesmith et al., 1988). Legislation was introduced in 1988 removing ruminant materials from the ruminant feed chain, which was associated with a decline in the number of cases after 1994, as predicted from the incubation period of about 60 months. A significant number of cases were still being identified in 1996, which were thought to arise from cross contamination with non-ruminant feed. This prompted an extension of the feed ban to prohibiting ruminant material from all animal feedstuffs. Nevertheless, by the end of 2003 there were 75 cases of BSE in cattle born after the 1996 complete ban on feeding ruminant derived material. There have been over 180,000 confirmed BSE cases in the UK and 173 cases in 2003.

The study of calves born to cows that developed BSE suggest that there is some risk of vertical transmission of BSE in calves born within six months of clinical disease in the cow developing disease (Wilesmith et al., 1997; Donnelly et al., 1997a; Donnelly et al., 1997b). It is likely that both genetic susceptibility and the stage of disease in the mother are factors that affect the likelihood of vertical transmission (Ferguson et al., 1997).

VCJD

Several TSE diseases are known in man, which are characterized by dementia, loss of motor neuron control and invariably death. These different types of human TSEs occur at high frequency within families and clearly have a genetic component. Between 1990 and 2003 there were 36 familial cases of CJD and 19 cases of GSS in the UK, however there were 625 sporadic cases over the same period. This suggests that, although susceptibility to TSEs is genetic controlled, there must be other factors, possibly environmental, required to trigger disease. A new variant of Creutzfeldt-Jakob disease (vCJD) was described in 1996 (Will et al., 1996) linked to the consumption of meat from BSE infected cattle (Bruce et al., 1997). By the end of 2003 there were 137 confirmed vCJD cases in the UK. All vCJD cases have
the same PrP genotype with motioning at position 129 of the protein.

Pathology

The characteristic neuropathology of TSE diseases is used to confirm the diagnosis post-mortem: the brains of affected individuals display spongiform (vacuolar) degeneration of synaptic regions of the grey matter, neuronal cell degeneration or loss, a variable level of astrocyte gliosis and the appearance of PrP-containing amyloid plaques (DeArmond & Ionside, 1999). The damage to the brains of TSE victims result in changes in behavior and movement, progressing to ataxia and inevitably death. The reason for vacuole formation and ultimately death are not known, but the disease may induce apoptosis in neurons (Saez-Valero et al., 1999).

The biochemical events associated with TSEs are less well-characterized than the pathology, however in all species a normal host-encoded prion protein (PrP\(^\alpha\)) becomes post-translationally-modified to a protease-resistant disease form (PrP\(^\delta\)) during the course of disease (Prusiner, 1993). Attempts to identify the infectious agent have consistently found infectivity associated with this modified form of the PrP protein (Prusiner, 1982). In addition, mice deficient in PrP are resistant to disease, pointing to the central role of this protein in establishment and progression of the disease. So far, all attempts to find any nucleic acid associated with infectivity have failed. The normal PrP protein is about 250 amino acids long, depending on the species, and possesses two potential glycosylation sites. The normal form of the protein has an alpha-helix whereas during the disease process the three dimensional structure changes so the protein adopts a primarily \(\beta\)-sheet conformation (Reik et al., 1996), which is consistent with the observed increased resistance to proteinase digestion. The mechanism whereby the normal form of the protein is changed to the disease form is not known, but it is thought that this is a self-catalyzing process. The conversion process is thought initially to be slow, but becomes more rapid as the concentration of the disease form increases, which may, in part, explain the long incubation period.

The extent of the pathology observed is not directly related to the concentration of PrP\(^\delta\) present (Manson et al., 1999). Therefore, although it is assumed that PrP\(^\delta\) accumulation is the cause of disease progression, which is supported by the fact that PrP peptides are directly neurotoxic (Ma et al., 2002), the actual cause of vacuole formation in the brain, and ultimately death, is not known.

Prion Protein Gene

One of the key features of TSE disease is the appearance of a disease form of a normal cellular protein, prion related protein (PrP). The normal form of the protein has an alphahelical structure and is readily digested by proteinase. During the disease process the protein is converted to a proteinase resistant form that has a beta-sheet conformation. This disease form of the protein aggregates and is neurotoxic, and form the scrapie associated fibrils that are seen in the brain of TSE cases.

Human PrP

Several mutations in the human prion protein gene are known to be associated with inherited TSE diseases e.g. a proline to leucine change at codon 102 of the human PrP gene (i.e. P102L) is the most common mutation associated with GSS. Classical FFI is associated with the D178N mutation accompanied by 129M, whereas D178N and 129V result in a form of CJD (Young et al., 1999). The neuropathology of the two diseases associated with these variations is distinct. The majority of sporadic CJD cases are homozygous for either methionine or valine at PrP codon 129 (Collinge & Palmer 1994).

Susceptibility of Sheep to Scrapie

Incidence of scrapie sheep is related to variations in the PrP gene (Goldmann et al., 1994). There have been 14 polymorphisms reported in the open reading frame of the ovine PrP gene (Hunter et al., 1991; Goldmann et al., 1994; Hills et al., 2001; Hills et al., 2003). However, codons 136, 154 and 171 have been shown to be associated with incidence of clinical scrapie (Hunter et al., 1996). Sheep that are homozygous for VRQ respectively at these codons are generally susceptible to scrapie, however, animals with this genotype can remain healthy (Hunter, 1997). Sheep Glutamine (Q) at position 171 have highest incidence of disease, whereas individuals that are arginine (R) at codon 171 are more resistant (Goldmann et al., 1994). The difference in susceptibility of genotypes is not known, one possibility is that the residues present at the three critical codons affect rate of conversion of the normal host protein PrP\(^\alpha\) to disease form PrP\(^\delta\).

PrP in Cattle

The coding region of the bovine PrP gene is much less variable than that of sheep. Two polymorphisms have been reported: a silent change that affects a Hind II restriction site and a difference in the number of G-C rich octa-repeat elements, alleles with either 5, 6 or 7 copies of the octa-repeat are known (Hunter et al., 1994; Schläpfer et al., 1999). It is possible that animals with genotypes containing the 6 and 7 octa-repeat PrP alleles are susceptible to disease, while the 5/5 genotype is associated with increased resistance to BSE, as no cases of BSE have been reported that have the 5/5 genotype (Hunter, 1997). However, two case-control studies (Hunter et al., 1994; Neibergs et al., 1994) found no association between the octapeptide-repeat and incidence of BSE. The frequencies of the different octa-repeat PrP genotypes varies between populations and countries, in the UK the version of the gene with 6 repeats is at more than 90% frequency (Neibergs et al., 1994). The only suggestive evidence for an influence of PrP genotype on incidence of BSE in cattle is that the sequence variation that give rise to the Hind II RFLP has been found at significantly high fre-
frequency in BSE cases and their families (Neibergs et al., 1994).

The DNA sequence of the bovine Prnp gene shows 90% similarity to that of sheep (Hills et al., 2001). Sequence analysis of the gene has revealed nine polymorphisms in the PrP coding region, including the two mentioned above, and more than 40 SNPs and insertions in non-coding regions (Hills et al., 2003). However, to date, there is no strong evidence to date for polymorphisms within the bovine Prnp coding region, which affect susceptibility to BSE.

**The Infection Process**

Pathology associated with TSE infection is primarily observed in the brain; however, the lympho-reticular system is involved in the establishment of disease (McBride et al., 1992). Cells of the immune system, and in particular the B-lymphocytes and dendritic cells of the spleen, play an important role in the replication of the scrapie agent in some species (Brown et al., 1999; Klein et al., 1997). In contrast the spleen of BSE infected cattle does not appear to harbor infectivity, although the bovine spleen is known to express PrP (Horiuchi et al., 1997).

The most likely route of BSE, scrapie and vCJD infection from the environment is via the alimentary tract, or possibly the nasal mucosal membranes. Recent findings have shown that that M cells of the Peyer’s Patches are responsible for uptake of PrPSc from the gut lumen (Heppner et al., 2001) and that follicular dendritic cells, which trap antigens, have a role in transporting PrPSc from the gut to the lympho-reticular system (Huang et al., 2002). The presence of B lymphocytes is essential for infectivity as B cell deficient mice are resistant to disease (Klein et al., 1997), however the role of the B cells seems to be indirect as they produce lymphotxin necessary for the maturation of the dendritic cells (Montrasio et al., 2000). The route of infection from the lympho-reticular system to the CNS is unclear, but is presumable via neurons innervating peripheral lymphoid tissues, such as lymph nodes, tonsil and spleen.

**Control of TSE Diseases**

The control of TSE disease in livestock could be achieved through genetic selection, or elimination of infected animals. In both approaches the goal is to remove potentially infected animals and hence the source of disease.

*Genetic control*

In sheep three codons, at positions 136, 154 and 171, of the PrP gene are associated with differences in the incidence of scrapie, as discussed above. This information has been used to develop the “National Scrapie Plan” in the UK, under which males with genotypes showing lower incidence of disease are used exclusively for breeding. However it should be noted that variations in the PrP gene have been associated with variations in incubation period in both sheep and mouse models of scrapie, rather than resistance per se (Carlson et al., 1994). Disease has also recently been observed in sheep with the resistant genotypes following experimental infection via a cerebral route, demonstrating that they can harbor the infectious agent. Thus, apparently healthy animals harboring disease are retained for breeding and may infect other individuals. A further problem is that the susceptibility of particular genotypes differs between sheep breeds: the most resistant genotype in all sheep breeds is AA136/RR154/QQ171 (Goldmann et al., 1994) whereas susceptible genotypes are breed-dependent with VV136/RR154/QQ171 being the most susceptible in Cheviots, Swaledales and Shetlands while AA136/RR154/QQ171 is most susceptible in Suffolk (Hunter et al., 1996). This suggests that genes in addition to the PrP locus may be involved in scrapie susceptibility.

Only a very small proportion of cattle in the UK developed BSE, in all less than 3% of the cattle population. This may reflect genetic resistance, a low level of exposure to the infectious agent or that other environmental factors are required to trigger disease. There are some suggestions that variations in the PrP gene are associated with BSE susceptibility (Hunter et al., 1994; Neibergs et al., 1994), but the evidence is weak. A genome wide scan for loci associated with incidence of BSE did not identify any association between loci on BTA 13, where the PrP gene is located, and disease status (Hernandez-Sanchez et al., 2001). However, the higher incidence of disease in progeny of BSE affected dams implies a certain level of genetic susceptibility (Ferguson et al., 1997). These two observations suggest that there may be loci, other than PrP, that may be involved in development of TSE diseases.

Examination of loci in a genome-wide scan of BSE affected and unaffected cattle from paternal half-sib groups detected 3 loci that were tentatively associated with incidence of BSE (Hernandez-Sanchez et al., 2002). The loci identified were, INRA107 (chromosome 10), INRA36 (chromosome 20) and BM315 (chromosome 5). Work with mouse models of TSE disease has also identified several loci associated with incidence of disease that do not include the PrP locus (Stephenson et al., 2000; Lloyd et al., 2001). The marker INRA107 on bovine chromosome 10 is close to the cytochrome P450 gene that maps in the same interval on chromosome 9 in mice as the TSE associated QTL reported by Stephenson et al., (2000). This region of mouse chromosome 9 also contains the gene coding for the enzyme hexosaminidase A (HEX A), which is involved in Tay-Sachs disease in man, a progressive and lethal neurodegenerative disorder (Bach et al., 2001). HEX A may contribute to variations in development of BSE.

**Diagnosis**

Tests for TSE infection have primarily focused on the detection of disease specific PrPSc, for example in tonsil biopsies (Ghani et al., 2000) or blood (Schmerr et al., 1997). Such assays are far from ideal; firstly because PrPSc is not detected in the tonsils of BSE infected cattle, while the amounts of PrPSc found in blood of animals, even at late
stages of infection, are at the limit of detection. Attempts to
derive highly sensitive tests for the disease form of the pro-
tein risk increasing the number of false positive results.

Surrogate markers for TSE infection

An alternative way to identify TSE infected individuals
would be to use markers that may not be associated with
disease itself, but that are changed as a result of infection,
so called surrogate markers. The search for surrogate mark-
ers has been undertaken using a variety of approaches, with
limited success to date. Northern blotting, RT-PCR and im-
munohistology studies of specific target genes have revealed
up-regulated expression of MHC class I and class II genes
(Duguid & Trzepacz 1993). Variations have also been de-
scribed for the expression of cathepsin S and aspartyl prote-
ase, but changes in expression are only seen late in in-
fec tion and following appearance of PrP Sc (Diedrich et al.,
1991). Differential display studies using brain from scrapie-
infected mice have revealed changes in expression of sev-
eral genes (Dandoy-Dron et al., 1998), however it is im-
portant to differentiate between TSE specific variations and
non-specific changes that occur in response to the disease.
The increase in the expression of eg GFAP and F4/80 ob-
served following TSE infection is also associated with
trauma to the brain and is explained by the gliosis. Simi-
larly, changes in expression of apolipoprotein E, cathep-
sin D, sulphated glycoprotein 2, transferrin and HSP70 have
been reported in other neurodegenerative diseases that are
associated with abnormal protein deposition, such as Alz-
heimer’s disease, and therefore are not specific to prion
diseases. Two previously unidentified scrapie-response
genes (ScRG-1 and ScRG-2) were also found to be over-
expressed in scrapie-infected mouse brain. ScRG-1 is
mainly expressed by glial cells, while ScRG-2 is only up-
regulated during the terminal stages of the disease. Several
molecular markers have been tested for diagnosing BSE by
ante-mortem biopsy. For example, the cerebrospinal fluid
of BSE-affected animals has been found to have an elevated
concentration of apolipoprotein E (Hochstrasser et al., 1997)
while CSF of CJD patients have elevated levels of 14.3.3
protein (Giraud et al., 2002). However, these proteins are
not TSE specific and so do not provide the basis for a spe-
cific diagnosis.

Differential display analysis of spleen from scrapie in-
fected mice revealed an erythroid cell-specific marker
(EDRF) that is down-regulated during the course of disease
(Miele et al., 2001). This gene is also expressed in blood
born cells and changed expression is observed during the
later stages of both sheep scrapie and bovine BSE. Unfortu-
nately significant variations in EDRF expression only occur
close to onset of clinical symptoms. Nevertheless, this value
of this gene is that it is a blood born marker, and is currently
being evaluated for identifying blood donated from indi-
viduals incubating TSE diseases.

Macro and micro-array approaches allow the expression
of many thousands of genes to be examined simultaneously.
The application of this technology in scrapie research using
macro-arrays to assess the expression of 597 genes showed
changes in the expression of 42 genes between PrP Sc−
and PrP Sc+ murine skin fibroblasts (Satoh et al., 2000). The
expression of these genes in control and TSE infected whole
animals has yet to be investigated. A bovine non-redundant
set of ESTs developed from a cDNA library representing
several regions of normal bovine brain (Jantz et al., 2003) has
been used to create a micro-array with 23,000 gene
probes to investigate the expression profiles in cattle. Com-
parisons of brainstems from normal cattle and BSE infected
cattle just prior to the onset of clinical symptoms have re-
vealed about 150 genes with variations in expression at a
statistically significant level. Work is underway to character-
ize these clones and examine their expression at different
stages of the disease. The next phase of the study will be to
extend the work to examine expression in immune cells
from blood of control and infected cattle.

Conclusion

We also do not yet understand the mechanisms involved
in the TSE infection process or transmission of the TSE dis-
eases. We do know that in some species polymorphisms
within the PrP gene are associated with TSE incidence;
however cattle variations in the gene are not convincingly
associated incidence of BSE. Genome wide studies have
uncovered other loci that are potentially involved in suscep-
tibility or progression of the TSEs, but as yet the genes hav-
ing an effect at these loci are unknown. Thus, selection of
cattle resistant to BSE is currently not possible.

The search for surrogate markers that could be used to
identify individuals incubating TSE disease is focusing on
genotes that show altered expression levels. Most genes with
reported so far altered levels of expression are involved in
the infection process. However, with the advances in the
methods available the next few years should see significant
progress towards identifying genes with expression patterns
that are influenced by the infection process, which will
allow important questions related to the biology and control
of TSE disease to be addressed.

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