TSE strain variation

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Studies in mice have revealed considerable strain variation in the agents causing transmissible spongiform encephalopathies (TSEs). TSE strains interact with genetic factors in the host (in particular PrP genotype) to influence characteristics of the disease such as incubation period and neuropathology. TSE strains can retain their identity after propagation in different host species or PrP genotypes, showing that these agents carry their own strain-specific information. It is not known whether this information resides in specific self-perpetuating modifications of PrP, or whether a separate informational molecule is required. Strain typing in mice can be used to explore links between TSEs occurring naturally in different species. Such studies have demonstrated that the strain causing BSE in cattle has also infected domestic cats and exotic ungulates. Most importantly, the BSE strain has also been isolated from patients with variant CJD. In contrast, different TSE strains are associated with sporadic CJD and sheep scrapie.

It is well established that TSE agents (otherwise known as prions), like conventional micro-organisms, exhibit strain variation[1]. This has been observed for TSEs in several species, but has been most thoroughly documented for TSEs experimentally isolated in mice. Numerous distinct TSE strains have been identified in mice by serially passaging scrapie, BSE or CJD from a range of sheep, goat, cattle or human sources. The methods used for TSE strain discrimination have traditionally been based on simple observations of disease characteristics. The most useful of these have been the length of the incubation period between initial infection and the development of clinical disease, and the type of pathological changes that are seen in the brains of infected animals[2–4]. TSE strains have also been found to differ in their clinical manifestations, their ease of transmission to new species and their susceptibility to inactivation by heat and chemicals. In recent years the biochemical characteristics of disease-associated forms of the host prion protein, PrP, have provided further criteria for distinguishing between TSE strains. Formal strain typing protocols in mice, based on incubation periods and neuropathology, have been used extensively as research tools. It is now established that these methods can also be used to type the TSE strain present in a naturally infected host. However, it is still not clear what the basis of TSE strain variation is at the

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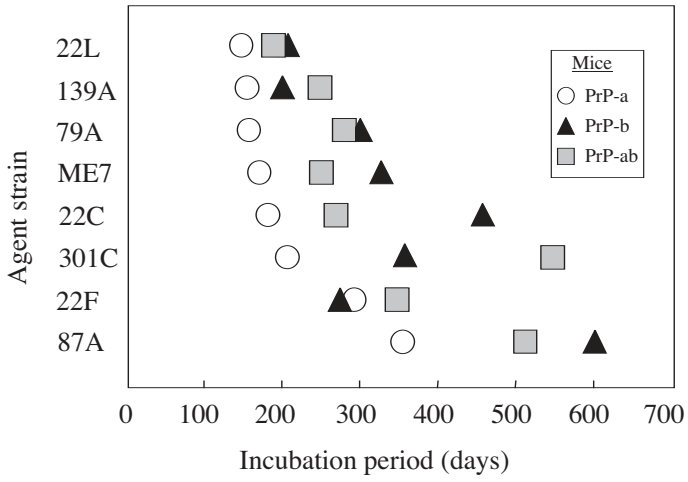

Fig. 1 Incubation periods between intracerebral injection and the development of clinical disease for eight TSE strains in mice of the three possible PrP genotypes. All of these TSE strains had been propagated in mice of the PrP-a genotype.

molecular level. This question is key to the debate concerning the molecular nature of these agents.

Influence of TSE strain on incubation period

TSEs in ordinary non-transgenic mice are characterised by long asymptomatic incubation periods, lasting between about 4 months and the full life-span of the mouse (over 2 years). Following this asymptomatic phase, progressive neurological signs are seen, usually over a period of a few weeks. Despite the length of the interval between exposure to infection and the clinical phase, if all experimental conditions are kept constant the incubation period is remarkably predictable. For example, a single TSE strain injected intracerebrally at high dose into a group of genetically uniform mice will generally give a mean incubation period with a standard error of less than 2% of this mean. The incubation period is also highly repeatable for different groups of genetically uniform mice injected with equivalent doses of the same TSE strain. However, different TSE strains tested in the same mouse strain give markedly different incubation periods (Fig. 1)[4].

The incubation period is also profoundly influenced by genetic factors in the mouse[2]. In mice, only two alleles of the PrP gene have been recognised (designated a and b), encoding proteins that differ by two amino acids at codons 108 and 189[5]. When mice are infected with a

single TSE strain, the PrP genotype can make a difference of hundreds of days to the incubation period. This effect is related to the rate of progression of the disease, rather than to differing susceptibilities to infection. The effects of PrP genotype on disease progression were identified many years ago, long before the protein itself was discovered[6]. The gene, later found to encode PrP[7,8], was called *Sinc* (acronym for scrapie incubation), with the two *Sinc* alleles s7 and p7 corresponding to the a and b alleles of the PrP gene.

The strain of TSE agent interacts with the PrP gene in a complex and curious way, with each TSE strain producing a characteristic and highly reproducible pattern of incubation periods in the three possible PrP mouse genotypes (the two homozygotes and the heterozygote F_1 cross; Fig. 1)[2,4]. The mouse genotype showing the shortest incubation period is PrP-a for some TSE strains and PrP-b for other strains. Although there is a tendency for the incubation period to be shortest in the genotype matching that in which the TSE strain has been propagated, this is not always the case. The incubation period in the F_1 cross (PrP-ab) lies sometimes between those of PrP-a and PrP-b mice and sometimes beyond the longer of the two, but is never shorter than both. The molecular basis of these effects is not known, but transgenic mouse models now provide opportunities for investigating the influence of single amino acid substitutions on incubation periods with different TSE strains[9,10]. Genes other than the PrP gene influence incubation period, but usually to a lesser extent. An exception is the dramatic non-PrP genetic effect seen in some primary transmissions of natural TSEs to mice[11].

Influence of TSE strain on neuropathology

TSE strains also show dramatic and reproducible differences in the type, severity and distribution of pathological changes they produce in the brains of infected mice[12]. In routine histological sections, TSE-specific vacuolation can be seen to be targeted to particular parts of the brain, which depend mainly on the TSE strain, but also to some extent on PrP and other genetic factors. This is the basis of a semiquantitative method of strain discrimination in which the severity of vacuolation is scored from coded sections in nine grey matter and three white matter brain areas to construct a 'lesion profile' that is characteristic for each combination of TSE strain and mouse genotype[3,4].

The targeting of neuropathology can be demonstrated clearly in sections immunostained with PrP-specific antisera. With most TSE strains, pathological accumulations of PrP can readily be demonstrated in the brain, in the form of diffuse deposits in areas of vacuolation and, more focally, as amyloid plaques. As with vacuolation, there are clear

and reproducible differences between TSE strains in the distribution and severity of these changes[13]. Some TSE strains target PrP pathology precisely to particular groups of neurons, leaving the surrounding brain substance unaffected. Other strains produce a more generalised pathology, albeit with a preference for particular brain areas. Some TSE strains produce many amyloid plaques while others produce few or none. These observations suggest that a fundamental difference between TSE strains is their ability to recognise and replicate in different neuronal populations.

Isolation and stability of TSE strains

On transmission of a TSE to mice from another species, the incubation period is usually very long and there may be survivors. In subsequent serial mouse-to-mouse transmissions, the incubation period shortens and stabilises after a few passages. The lesion profile and other neuropathological features also stabilise in the course of these first few passages. Thereafter, the incubation period and neuropathological characteristics are stable indefinitely on further mouse-to-mouse passage, as long as the conditions of passage, particularly PrP genotype of the mice in which infection is propagated, remain constant. A TSE strain is defined from this set of stable properties, rather than its origin. Over 20 distinct TSE strains have been isolated in mice.

Most, but not all, TSE sources have given rise to two different strains when serially passaged in PrP-a and PrP-b mice. Clearly, these differences are not simply imposed by the host as numerous strains have been isolated in the same mouse PrP genotype and the same strain has occasionally been isolated in both genotypes. Rather, most TSE sources behave like mixtures of strains. The resolution of an isolate into two distinct stable strains is consistent with the selection, from a mixture, of strains that replicate more rapidly in the particular mouse genotype used for passage.

In view of current assumptions that modified forms of PrP are integral to TSE agent structure, an important question is whether there are any 'donor' effects on TSE strain characteristics. In fact, the characteristics of several TSE strains have been found to be unchanged when the genotype of the mice in which they are propagated is changed from PrP-a to PrP-b[1]. In contrast, some isolates change their properties when propagated in the alternative PrP genotype, in a manner consistent with the selection of strains with shorter incubation periods under the new passaging conditions. Similarly, some TSE strains remain unchanged on switching species between mouse and hamster, while others give rise to new strains[14,15]. Because such changes can occur in isolates that have

been previously cloned (*i.e.* serially passaged several times at the minimum infective dose, to remove minor strains from the isolate), these results have been interpreted in terms of the generation of variant strains, analogous to mutational events in conventional micro-organisms. This is followed by a host-permitted selection of shorter incubation period variants. There is no clear evidence that the PrP genotype of the host can actively modify the properties of a strain.

Strain typing of natural TSEs

Animal TSEs

The strain typing approaches described above were developed originally for fundamental studies of rodent-passaged, laboratory TSE isolates. As many of the mouse-passaged strains that have been characterised were derived from experimentally scrapie-infected sheep or goats and may subsequently have had a complicated passage history in rodents, it is unclear whether such extensive strain diversity exists in the naturally occurring diseases. These studies have now been extended to explore the extent of strain variation in natural TSEs and the links between TSEs occurring naturally in different species. These questions became particularly pressing in the late 1980s with the emergence of BSE in cattle and novel TSEs in domestic cats and several feline and ungulate species in zoological collections in the UK.

A plausible explanation for the origin of BSE is that it was derived from rendered scrapie-infected sheep tissues included in feed supplements. Thereafter, the BSE epidemic was almost certainly fuelled by the recycling of rendered cattle tissues in these supplements. As sheep were fed the same supplements until at least the late 1980s and are experimentally susceptible to orally delivered BSE, there is a theoretical possibility that some sheep became accidentally infected with BSE at that time. Transmission to mice provides an opportunity to characterise and compare the TSE strains derived from the above range of species.

When BSE is transmitted to mice from cattle brain, it consistently produces a characteristic pattern of incubation periods and neuropathology in mice that serves as a 'signature' for the BSE strain (Figs 2 & 3)[16,17]. As non-PrP genetic effects are often seen in transmissions between species[11], two different PrP-a mouse strains are included, as well as PrP-b and PrP-ab mice. The same strain signature has been seen in primary transmissions of novel TSEs from domestic cats (Fig. 2) and two exotic ungulate species, confirming the suspected link with BSE and providing the first clear evidence for the accidental spread of a TSE between species[17]. The BSE signature has also been seen in transmissions

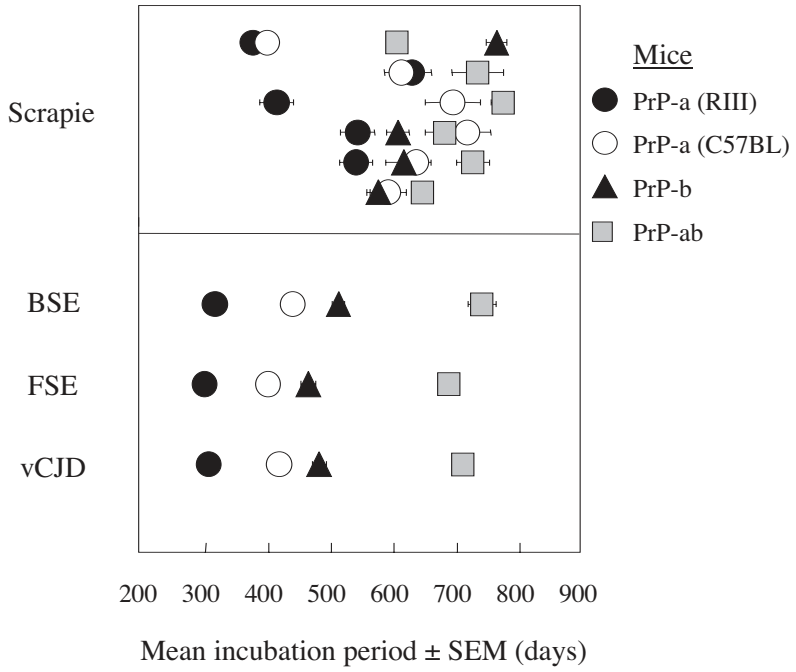

Fig. 2 Incubation periods in mice injected with TSEs from naturally infected hosts. Results are shown for transmissions from six individual scrapie sheep. Pooled data from several transmissions are shown for cattle BSE, feline spongiform encephalopathy (FSE) and human vCJD.

to mice from experimentally BSE-infected sheep, goats and pigs[17]. BSE isolates have been further characterised by setting up separate serial passage lines in PrP-a and PrP-b mice. This results in the isolation of a pair of distinct mouse-passaged strains, as explained above. The same pair of strains has been isolated from three different BSE cattle[18].

In contrast, primary transmissions of natural sheep scrapie to mice have varied in the proportion of mice showing clinical signs, and in the incubation periods and neuropathology seen in affected mice[17–19]. So far, no transmission to mice of natural scrapie has shown the BSE signature. Furthermore, the mouse-passaged strains isolated from natural scrapie have shown no overlap with those isolated from BSE[18]. However, only a very small sample of the total number of scrapie cases in the UK has been tested. Also, studies in rodents have shown that variant strains may be selected when TSE infections are transmitted to a new species (see above). Therefore, although these results provide no evidence that BSE was derived from sheep scrapie or that BSE has infected some sheep, they certainly do not rule out these possibilities.

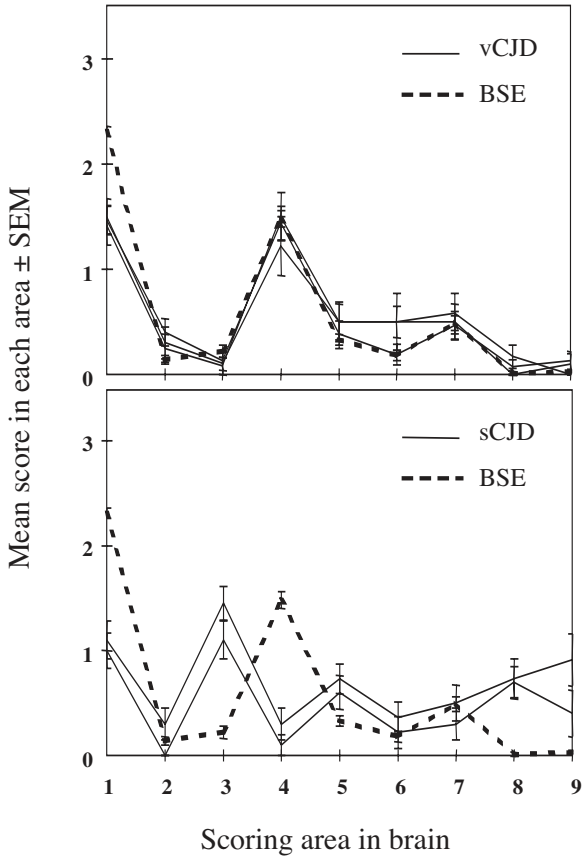

Fig. 3 Lesion profiles for PrP-a (RIII) mice, infected with vCJD from three patients (above) and sCJD from two patients (below). In each case these are compared with the lesion profile for BSE in the same mouse strain. Lesion profiles were constructed from the mean vacuolation scores in nine areas if brain.

Human TSEs

In 1996, the recognition of a new variant of CJD (vCJD), occurring predominantly in young adults in the UK, raised serious concerns that BSE had spread to humans. To investigate the possible link between vCJD and BSE, transmissions to mice were set up from brain tissue of three patients with vCJD, using an identical protocol to that used in the series of animal TSE transmissions described above. Transmissions to mice were also set up from six cases of sporadic CJD (sCJD), with no unusual clinical or neuropathological features. The sCJD patients included two dairy farmers who may have been exposed to BSE-infected cattle or contaminated animal feed, two 'contemporary' cases with no known occupational exposure to BSE and two 'historical' cases from before the onset of the BSE outbreak. All nine individuals had the same

PrP genotype, methionine at codon 129 with none of the mutations associated with familial TSEs.

The primary transmission results (incubation periods and lesion profiles) for all three vCJD sources were closely similar to those seen in transmissions to mice of BSE from cattle and other species, showing that these vCJD patients were infected with the BSE strain (Figs 2 & 3)[19]. Furthermore, the pair of strains isolated from vCJD sources by passage in PrP-a and PrP-b mice were closely similar in their properties to the pair of mouse-passaged strains isolated previously from cattle with BSE. This set of observations provides the strongest available evidence of a link between vCJD and BSE.

In contrast to the results for vCJD, no clinical neurological disease was seen in mice injected with sCJD tissues within their life-span[19]. However, in all six sCJD transmissions, vacuolar degeneration typical of TSE infection was seen in the brains of most mice dying with intercurrent disease, from about 400 days following challenge. Transmission was confirmed by the presence of disease-associated PrP in these mouse brains. The lesion profiles in mice infected with sCJD were similar for the six sources and strikingly different from those seen in mice with BSE or vCJD (Fig. 3). Serial mouse-to-mouse passage of sCJD isolates has yielded a unique strain that is distinct from the pair of strains isolated from BSE. These results indicate that sCJD, even in dairy farmers potentially exposed to BSE, is associated with a different TSE strain from that causing vCJD or BSE.

Alternative strain typing methods

Although strain typing by transmission to mice has been very informative, it is slow, unwieldy and expensive. It is, therefore, unsuited to large-scale surveys of strain variation in the natural diseases. In recent years, biochemical strain typing approaches have been developed, based on the molecular characteristics of disease-associated forms of PrP. These are discussed in more detail elsewhere in this volume, but, briefly, two features have been considered. First, TSE strains can be characterised by the relative prominence of the three differently glycosylated PrP bands seen in Western blots of proteinase K treated brain samples[20,21]. Second, the conformation of abnormally folded PrP varies according to the TSE strain[22,23]. At present, it is not clear how these approaches relate to full strain typing in mice; nevertheless, they provide more practical methods for screening large numbers of samples, to identify which should be investigated in greater detail.

Implications of strain variation in TSEs

The studies described above indicate that TSE agents carry some form of strain-specific information that is independent of the PrP amino acid

sequence of the host. For example, the BSE strain has retained its identity when propagated in at least seven different species with differing PrP sequences. This has to be taken into account when proposing molecular models for the structure of these agents. According to protein-only models, infectious TSE agents consist only of conformationally modified forms of PrP, that can impose the same modification on new host PrP molecules. It has been suggested that each TSE strain represents a specific self-propagating PrP conformation[22,23], in which case there would have to be as many of these specific abnormal conformations as there are strains. Another requirement would be that the strain-specific conformation can reproduce itself faithfully in PrP molecules with differing amino acid sequences. A crucial, as yet unanswered, question is whether a host protein alone can carry strain-specific information in this way, or whether a separate informational molecule is required, as suggested in the 'virino' hypothesis[24]. However, even without a full understanding of the molecular basis of TSE strain variation, strain-typing in conventional mice can be used effectively to answer pressing questions about the naturally occurring diseases. Most importantly, these methods have shown that the BSE strain has accidentally infected several different species, including humans.

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