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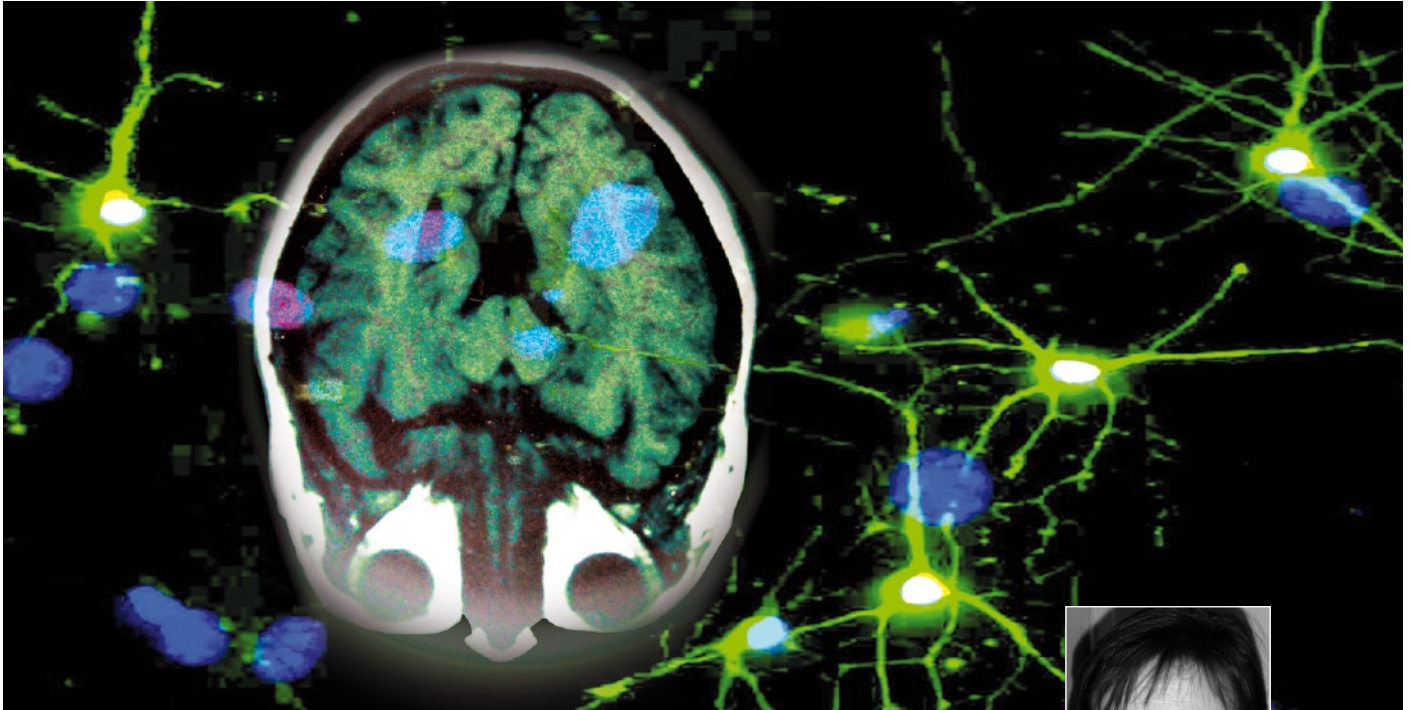
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TSE Infectivity

The Nature of the Prion



The abnormal prion protein (PrP^{Sc}) is currently the only biomarker used for the rapid diagnosis of Transmissible Spongiform Encephalopathy (TSE) disease (or Prion disease). PrP^{Sc} is deposited in infected tissue and co-purifies with infectivity, and is thought to be the sole component of the infectious agent. However, evidence now exists of TSE disease in the absence of this marker protein, raising questions about the nature of the infectious agent and the future of TSE diagnosis.



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The TSE Infectious Agent

TSE diseases (also known as Prion diseases) are fatal infectious neurodegenerative diseases of animals which include Creutzfeldt-Jakob Disease (CJD) in humans, scrapie in sheep and goats, BSE in cattle, and Chronic Wasting disease (CWD) in deer. The true nature of the TSE infectious agent is unknown but it has been proposed that the agent is composed solely of a misfolded form of the host prion protein, PrP. This abnormal form (PrP^{Sc}) is resistant to digestion with proteinase K (PK), is deposited in infected tissue, and co-purifies with infectivity. For these reasons, all current Rapid TSE Diagnostic assays are based on the detection of PrP^{Sc} either by its PK resistance, its altered conformation, or its affinity for specific ligands. Current tests can only be performed on post-mortem tissue, but new pre-mortem tests in de-

velopment are also based on the detection of PrP^{Sc} in accessible tissues and body fluids (such as blood) by amplifying or concentrating the low levels of PrP^{Sc} which may be present.

All of these assays rely on the assumption that the correlation between TSE infectivity and PrP^{Sc} is always 100%. However if disease can exist in the absence of large amounts of PrP^{Sc}, reliance on PK-resistant PrP as a sole measure of infectivity may in some instances underestimate the biological properties of diagnostic samples, and undermine current efforts to contain or eradicate TSEs.

Does PK-resistant PrP Correlate with Infectivity?

In our laboratory, transgenic mice which have a single amino acid mutation in the endogenous PrP gene (P101L) were inoculated intracerebrally with TSE infectivity

from a case of human familial prion disease (Gerstmann-Straussler-Scheinker disease; GSS) or a strain of scrapie propagated in hamsters (263K). These mice (Tg 101LL) developed clinical signs of TSE disease and also showed classical TSE associated spongiform degeneration of the brain on post-mortem examination. However they contained extremely low or undetectable levels of PK-resistant PrP in the brain [1]. The lack of PrP^{Sc} would suggest that these tissues were not infectious, however disease could be transmitted very efficiently from these tissues to Tg 101LL and wild type mice. We performed further analyses of these tissues to determine the exact levels of the infectious agent and PrP^{Sc} that were present, and compared these with a well characterised laboratory strain of TSE agent, ME7. In 4 of 5 tissues examined the titres of infectivity were equivalent to or higher than ME7, but the levels of PK-resistant PrP were undetect-

able by standard assay, and therefore estimated to be less than 1% of that seen in ME7. These data implied that either the number of PrP^{Sc} molecules per unit of infectivity varies by over 1000-fold between strains, or that most PrP^{Sc} seen in end stage disease is not infectious.

Is PK-sensitive PrP^{Sc} the Infectious Agent?

Analyses of other PrP transgenic models with the 101L mutation have revealed the presence of an abnormal, disease associated conformer of PrP which appears to be sensitive to PK [2, 3]. It has been suggested that such PK-sensitive PrP^{Sc} (sPrP^{Sc}) conformers may be responsible for the observed high levels of TSE infectivity in tissues containing no PK-resistant PrP. We analysed tissues from our mice showing high titres of infectivity and low levels of PrP^{Sc} for the presence of sPrP^{Sc} by limited PK digestion studies, and immunoprecipitation studies using monoclonal antibodies specific for abnormal conformers of PrP. We found no evidence of sPrP^{Sc} in any of the tissues [1]. We also analysed these tissues using a Conformation Dependant Immunoassay (CDI) [4] which does not require PK digestion of samples to identify PrP^{Sc}. This assay measures the change in antibody binding following denaturation in guanidine. An increase in the denatured/native ratio due to increased availability of an antibody epitope indicates the presence of abnormal PrP conformers. Again, this assay revealed no evidence of any abnormal PrP conformers in the high infectivity/low PrP^{Sc} samples.

The 101L-PrP transgenic models in which sPrP^{Sc} is observed were created by a method which results in overexpression of the PrP transgene, and it is possible that the abnormal sPrP^{Sc} observed in these mice is produced in response to the 8–16 fold overexpression of PrP in the mice. Our Tg 101LL line was produced by gene targeting, which introduces the mutation into the endogenous murine PrP gene resulting in wild type expression of the mutated gene. The disease in Tg 101LL mice is therefore due to infection of the mice with an exogenous TSE agent, implying that the conformers of abnormal PrP produced due to overexpression of PrP and TSE infection must be different.

Are All Abnormal Forms of PrP Infectious?

Our data indicate that the PrP^{Sc} observed in terminal disease tissues may be a pathological by-product of the infection rather than the infectious agent. Other

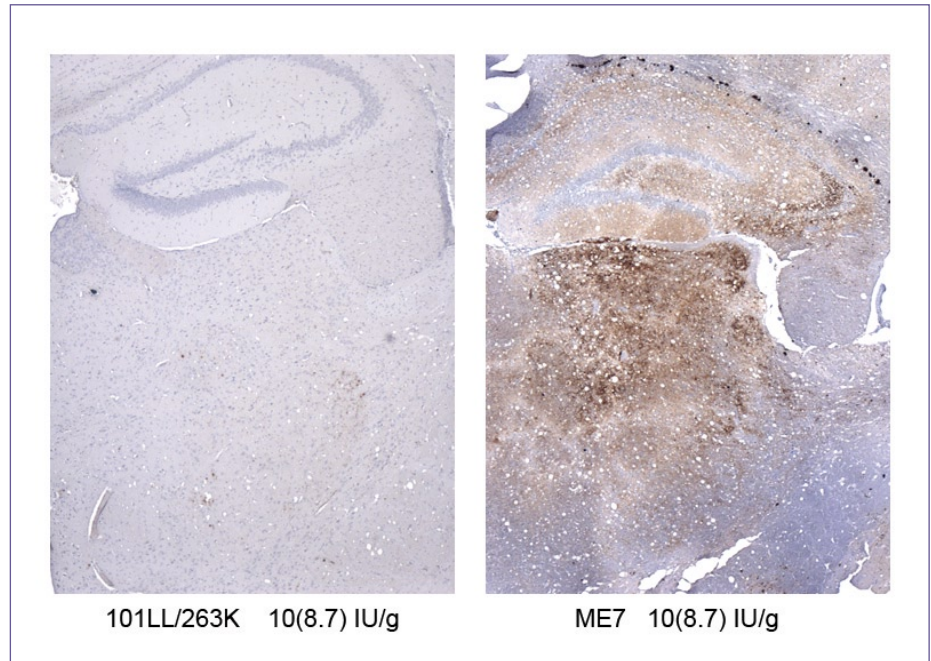


Fig. 1 Immunohistochemistry of sections from 101LL/263K infected brain, and control ME7 infected brain. Both tissues contain similar titres of the infectious agent (108.7 IU/g tissue), but 101LL/263K infected tissue contains < 1% of the amount of PrP^{Sc} found in ME7 tissue.

studies have also reported transmission of disease from tissues containing no PK-resistant PrP [5], indicating that this observation is not unique to the Tg 101LL line. In addition, following inoculation with an isolate of atypical human GSS we have shown the presence of PrP amyloid plaques in the brains of Tg 101LL mice which show no clinical signs of disease or spongiform degeneration in the brain [6]. The presence of PrP amyloid plaques in these apparently healthy mice implies that these amyloid deposits also do not contain the TSE infectious agent.

The true nature of the TSE infectious agent is still the subject of much debate. Our data suggest that PK-resistant PrP, sPrP^{Sc} and PrP amyloid are not the infectious agent, but it is possible that an as yet unidentified specific conformer of abnormal PrP holds the key to TSE infectivity. However we cannot yet rule out the possible involvement of other molecules.

Although our data suggest that PrP^{Sc} is not a marker of the TSE infectious agent, it is currently the best marker for diagnosis of TSE disease, and current testing is identifying and removing infected animals from the food chain. However, it is vital that markers of TSE infectivity other than PrP^{Sc} are identified and validated in models such as those we have described and characterised here. We anticipate that such research will lead to the development of more robust diagnostic assays for TSE disease which will have important implications for both animal and human health.

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