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Physicochemical and biological characterizations of distinct strains of the transmissible mink encephalopathy agent

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SUMMARY

Inoculation of the Stetsonville, Wisconsin source of transmissible mink encephalopathy (TME) into Syrian hamsters has identified two strains of the TME agent having distinct biological properties and producing disease-specific prion proteins (PrP^{TME}) having different physicochemical properties. Although several strains of the sheep scrapie agent have been identified in Great Britain, this is the first indication that agents producing transmissible spongiform encephalopathies in the United States also are capable of producing distinct strains.

1. INTRODUCTION

We have previously reported the identification of two strains of the transmissible mink encephalopathy (TME) agent in Syrian hamsters, termed HYPER (HY) and DROWSY (DY) based on the predominant clinical feature of each syndrome (Bessen & Marsh 1992*a*). HY hamster TME has an incubation period of 65 ± 1 days and is characterized by signs of hyperexcitability and cerebellar ataxia. Lethargy and the absence of hyperesthesia and ataxia are representative of DY hamster TME after incubation periods of 168 ± 2 days. Hamsters clinically affected with HY and DY syndromes had brain titers of $10^{9.5}$ LD_{50} g^{-1} and $10^{7.4}$ LD_{50} g^{-1}, respectively. This is the first time that significant differences in titers have been observed between two strains infecting a single outbred species. Hamsters infected with the HY or DY strain of TME produce distinct types of disease-specific prion protein (PrP^{TME}). DY PrP^{TME} sediments more slowly in N-lauroylsarcosine, is more sensitive to digestion with proteinase K, and migrates faster in polyacrylamide gels than HY PrP^{TME} (Bessen & Marsh 1992*b*).

The present paper reports our progress on ongoing experiments to further characterize these strains.

2. PHYSICOCHEMICAL PROPERTIES

(*a*) *N-termini of HY and DY PrP^{TME}*

We have previously reported that after limited proteinase K digestion DY PrP^{TME} migrates 1–2 kDa faster than HY PrP^{TME} on SDS-PAGE (Bessen & Marsh 1992*b*). Differential reactivity with specific PrP peptide antisera suggests that this molecular mass difference maps to the N-terminal end of PrP^{TME}. We have now confirmed this observation by N-terminal sequencing (Bessen & Marsh 1994).

The major N-terminal signal for HY PrP^{TME} begins at Gly_{90} of the hamster prion protein sequence, similar to that reported for proteinase K digested hamster PrP^{SC} from animals infected with scrapie strains Sc237 (Prusiner *et al.* 1984) or 263K (Hope *et al.* 1986). Minor signals for HY PrP^{TME} corresponded to Gly_{74} and Gly_{82}.

Analysis of DY PrP^{TME} also revealed ragged N-termini, but clearly identified a major N-terminus at Gly_{92} with minor fragments at Gln_{98} and Lys_{101}. Therefore, the smallest size difference between proteinase K digested HY and DY PrP^{TME} was 2 amino acid residues (Gly_{90} versus Gly_{92}) while the largest possible difference was 27 residues (Gly_{74} versus Lys_{101}).

(*b*) *Comparison of protease inactivation of infectivity and protease degradation of PrP^{TME}*

We have previously shown that DY PrP^{TME} is significantly more sensitive to degradation by proteinase K than HY PrP^{TME} (Bessen & Marsh 1992*b*). To examine the relationship between PrP^{TME} and infectivity, brain microsomes were prepared as previously described from HY- and DY-affected hamsters (Bessen & Marsh 1992*b*), then treated with 100 μg ml^{-1} of proteinase K at 37°C. Samples were removed at 1, 12, 24 and 48 h and tested for PrP^{TME} by Western blot analysis and bioassayed for infectivity by endpoint dilution. The results showed a $10^{1.0}$ to $10^{1.5}$ LD_{50} loss of infectivity in the HY microsomes after 48 h and a

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corresponding 90% loss in PrP^{TME}. In contrast, the infectivity of DY microsomes was reduced 10^3 to 10^4 LD_{50} after 12 h at which time no PrP^{TME} signal could be detected (R. A. Bessen & R. F. Marsh, unpublished results). We conclude that these experiments suggest an excellent correlation between PrP^{TME} and infectivity.

3. BIOLOGICAL PROPERTIES

(*a*) *Mink infectivity*

We have previously reported that the HY strain of TME loses its pathogenicity for mink after three passages in hamsters whereas the DY strain retains mink pathogenicity for at least six passages (Bessen & Marsh 1992*a*). From these results we speculated that the DY strain of TME may be the mink pathogen in the original Stetsonville source.

We have now further examined mink pathogenicity of the DY strain using inocula cloned by limiting dilution of fifth and sixth DY passaged hamster brain. Two cloned DY inocula produced no disease in mink after observation periods of two years, while one cloned inoculum produced progressive neurologic disease in only three of ten intracerebrally inoculated animals and the histopathology in these affected mink was atypical of TME.

These findings suggest that the DY strain of TME is not the mink pathogen. Perhaps the longer incubation periods of 26–30 weeks with DY TME allowed the mink agent to persist and amplify to a higher level than the 10–12 week course of disease with HY TME. Cloning the DY inoculum removed the mink pathogen. We are presently attempting to clone the mink agent by limiting dilution of the Stetsonville TME source and will inoculate hamsters with the highest dilution of mink brain producing disease in mink.

(*b*) *Timecourse study*

Experiments are in progress to measure infectivity and PrP^{TME} concentrations in brain at weekly intervals after intracerebral inoculation of HY and DY strains of TME. Studies on HY-inoculated hamsters are now complete and the results are similar to those seen with scrapie strain Sc237 (263K). After four weeks, there is a weekly 1 $\log_{10}$ increase in titer reaching 10^8 LD_{50} by week 8 at which time the hamsters begin to show clinical signs of disease. Detection and concentration of HY PrP^{TME} coincided with infectivity. Results with DY inoculated hamsters are not yet complete but several interesting observations have been made. DY infectivity concentrations plateau at 10^7 LD_{50} g^{-1} of brain tissue at 90 days into the 160 day incubation period. DY PrP^{TME}, first detectable 5 weeks postinfection, increases 100-fold until week 13 and ten-fold over the next 13 weeks until the onset of clinical signs of disease. It has previously been thought that these neuropathic agents attain a threshold titer causing neuronal damage producing clinical signs of disease. These results suggest that there may be an equilibrium established in DY TME between PrP^{TME} accumulation and neuronal damage.

4. ADDITIONAL REMARKS

The identification of distinct strains of TME in outbred Syrian hamsters emphasizes the importance of the transmissible agent in determining the outcome of infection by these proteinaceous pathogens (prions). The different biologic expressions of disease can not be mediated by the primary amino acid sequence of the prion protein as seen in heritable human spongiform encephalopathies, but must be conveyed by some as yet unknown property of the proteins themselves. Studies on HY and DY PrP^{TME} may reveal the post-translational mechanism that allows these proteins to acquire their neurodegenerative effect.

It is now generally accepted that the study of prion diseases is relevant to our understanding of Alzheimer's disease (AD). Both seem to be caused by the failure to completely degrade a precursor protein causing accumulation of amyloid that produces neuronal degeneration. Although the proteins are quite different, the methods of processing and accumulation may be similar. The identification of a disease-specific PrP^{TME} having an intermediate protease sensitivity between the normal protein and those producing very short incubation periods may have important implications for the pathogenesis of AD. The most simple interpretation of our findings is that the long incubation periods produced by the DY strain of TME are due to the increased protease sensitivity of the PrP^{TME} causing a slower accumulation of amyloid. The new observations that DY PrP^{TME} and infectivity reach a plateau months before the onset of clinical disease suggest that some form of equilibrium is established between degradation and accumulation of PrP^{TME}. This feature of the disease is likely a result of the properties of the DY PrP^{TME} rather than protein degradation pathways of the host. The implication of this for AD may be that more emphasis should be placed on characterization of the Alzheimer precursor protein, or its protease-resistant β-amyloid. Changes within these peptides, even in subtle carbon-to-carbon orientations, may cause profound alterations in protease sensitivity or aggregation.

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