

Exploring the susceptibility of humans to animal prion diseases

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Introduction

Prion diseases are fatal, infectious diseases of cattle (bovine spongiform encephalopathy, BSE), sheep (scrapie), deer and elk (chronic wasting disease, CWD) and humans (Creutzfeldt Jakob disease). Disease is caused by the misfolding of a normal protein, PrP, into a pathogenic form called a prion. Prions then coerce normal PrP to misfold, resulting in propagation of the pathogenic form in the brain and elsewhere, where it is toxic to neurons. Over 200 people were infected with BSE and 4 million cattle were culled during an outbreak in the United Kingdom in the 1980s. The fallout led to constraints on the use of animal by-products, organ donation and cattle culling, which have effectively ended the BSE outbreak. Nevertheless, an atypical form of BSE has been identified in older cattle in several countries, including the USA. These cases are disruptive to the beef supply chain and raise concerns for another outbreak. CWD is spreading through deer and elk in the United States and, increasingly, abroad. CWD infection of humans has not been documented, but we do not know the risk of CWD transmission to humans, how BSE and CWD may be different in their host ranges, or what defines this difference. Using an *in vitro* model of prion conversion, we have demonstrated that CWD prions adapt more readily to a new host than do BSE prions, which remain largely unchanged. These observations prompted us to investigate the role of specific regions of the PrP molecule in defining species barriers.

Materials and Methods

To test the susceptibility of humans to BSE and CWD, we used an *in vitro* system called real-time, quaking-induced conversion (RT-QuIC). RT-QuIC relies on the property of prions to cause PrP to adopt a prion conformation as well. When prions are formed, their structure is dramatically different than the starting PrP, and the new conformation binds a dye and causes its fluorescence, which we can record in real-time. We added recombinant PrP from a given species, then added prions from another species, and measured how quickly new

prions were formed in the reaction. We then recorded the time at which the normal prion protein substrate was converted to the abnormal form and compared the lag phases of these reactions (nonparametric Wilcoxon-Mann-Whitney test) to determine which prion/PrP combinations caused the most efficient conversion of PrP to prions.

Results

We observed an unexpected susceptibility of human PrP to conversion by CWD prions. In fact, CWD was almost as efficient as CJD at causing human PrP to convert to the abnormal form. We decided to capitalize on the flexibility of the RT-QuIC system to test regions of the PrP protein for their role in susceptibility to conversion *in vitro*. Specifically, we tested an amino-terminal region of the PrP protein, the absence of which leads to faster prion conversion. We hypothesized that the amino-terminal domain (NTD) may be protective against: a) all prion conversion or b) against trans-species prion conversion. We demonstrated that the NTD is indeed protective against all prion conversion, but that its protective role is not species-specific. We discovered that the NTD of the human PrP is different from that in the cattle and deer PrP. Without the NTD, the human PrP is only converted by native, human CJD prions. We swapped the NTD of bovine and human PrP to test the effect of the NTD on another species' susceptibility, but this made no difference in trans-species susceptibility.

Significance

Our results reveal a surprising susceptibility of human PrP to conversion by CWD prions. Mechanistically, our data suggest a role for the NTD of the human PrP protein in humans' susceptibility to animal prion disease. The public health threat of CWD is undefined and little is known about BSE transmission to humans, despite years of research. Without a better understanding of the mechanism behind trans-species prion infection, we are ill-equipped to characterize threats and monitor for new species-crossing events.