How Animal Prions Cause Disease in Humans

Although barriers reduce prion movement between species, animal prions pose a threat to human health, making vigilance prudent

Suzette A. Priola

The transmissible spongiform encephalopathies (TSEs) would seem unlikely candidates for animal disease agents that cross species barriers to cause disease in humans. TSEs are a group of rare but fatal neurodegenerative disorders in which neuronal vacuolation and cell loss within the brain lead to the characteristic spongiform changes, namely holes in the brain, which give the diseases their name. Even within a species, they are notoriously difficult to transmit via oral or other peripheral routes. Usually only transmission via direct inoculation of TSE infectivity into the brain, hardly a natural route of exposure, is very efficient at inducing disease. Additionally, even though the prototypic animal TSE, scrapie in sheep, has been recognized since the 1730s, there has never been any evidence to suggest that sheep scrapie could infect humans and induce a human TSE disease such as Creutzfeldt-Jakob disease or Gerstmann-Straussler-Schenker syndrome (Table 1). What, then, has changed to add TSEs to the list of animal diseases which pose a significant threat to human health?

The answer is “bovine spongiform encephalopathy” (BSE), also known more colorfully as “mad cow” disease. The recognition of mad cow disease in cattle in the United Kingdom in the mid-1980s, the excellent epidemiological work tracing its origins to TSE-infected ruminant-derived meat and bone meal being fed back to cattle, and the realization a decade later that BSE was likely responsible for a new form of TSE in humans (variant Creutzfeldt-Jakob disease or vCJD), all combined to propel TSEs onto the list of emerging diseases that can cross species barriers from animals into humans.

TSEs Depend on Proteins Called Prions

Given BSE’s ability to cause disease in humans and uncertainty over whether other animal TSEs may do the same, it is important to understand how barriers to infection control interspecies transmission of TSE diseases. However, since there is no known viral or bacterial component to TSE infectivity, the standard explanations invoking mutation of a nucleic acid genome leading to a population of viruses or bacteria

Summary

- Transmissible spongiform encephalopathies (TSEs) make up a group of rare but fatal neurodegenerative disorders in which neuronal vacuolation and cell loss lead to spongiform changes within the brain.
- The infectious agent in TSEs is a self-replicating protein derived from a normal host protein, called prion protein, or PrP, whose amplification depends on an iterative process that induces a conformational change in those host proteins.
- The key to understanding how species barriers for TSEs are maintained without nucleic acid-containing genomes lies with differences found among amino acid sequences of PrPs.
- Even though PrP amino acid sequences are highly conserved within a species, species barriers to prions are not absolute, meaning other prions such as those that cause chronic wasting disease in elk and deer need careful monitoring.
When Suzette Priola was pondering what to do following her postdoctoral research, her friends warned against pursuing prions, which they considered “a scientific black hole.” “After hearing that, I absolutely had to do prion research,” she says. “Who wouldn’t want to work in a scientific black hole?”

Because Priola’s other research revolved around viral pathogenesis and neurovirology, prions were a comfortable fit. “One of the big things that attracted me to prion research was that, as an intellectual problem, it has very few equals in infectious disease research,” she says. “Most of the regular tools and techniques for studying other infectious diseases simply don’t apply to prion diseases. There is no immune response to infection, no nucleic acid genome to mutate, no defined infectious particle to study, no tried and true rules to follow.

“It really frees you up to think unconventionally and imaginatively about how these diseases work, and to come up with new approaches to address your ideas,” she continues. “They really are an endlessly fascinating group of diseases.”

Priola, 44, is chief of the transmissible spongiform encephalopathy (TSE), prion molecular biology section of the laboratory of persistent viral diseases, at the National Institute of Allergy and Infectious Diseases Rocky Mountain Laboratories (RML) in Hamilton, Mont. This facility has housed a scrapie/TSE research program for more than 40 years. Priola arrived there in 1991, first as a postdoctoral fellow, then later as an investigator, senior investigator, and now head of her own lab.

Several years ago, Priola and her collaborators identified a group of cyclic tetrapyroles that act as potent inhibitors of prion diseases. “The chemistry of these compounds is well understood, which opens up the door to rational drug design for prion therapeutics,” she says. Collaborating with Byron Caughey at RML, she has also done work that is “critical to the development of a new and potentially very rapid and sensitive test for prion diseases, the QUIC assay,” she says.

Priola was born in Chicago, but raised in Albuquerque, N.M., where she earned her B.S. in biology at the University of New Mexico (UNM) in 1985. She completed her Ph.D. in microbiology and immunology in 1990 at the University of California, Los Angeles.

Her father was a cardiovascular physiologist and former chair of the department of physiology at UNM. “My brother and sisters and I would hang out at his lab where he would let us play with old oscilloscopes or pieces of non-working scientific equipment with lots of knobs and dials and switches, so I’ve always been pretty comfortable in a laboratory environment,” she says. However, among her siblings, she is the only scientist. One of her sisters is a nurse, the other, a teacher, and her brother is a paralegal.

“By far, the person with the biggest impact on my career was my father, who was always very supportive of my interest in science,” Priola says. “In high school, I was heavily involved in science fairs, and while he would point me to researchers who could help me with my projects, he was very careful to never get involved himself.” However, he did judge science fairs, albeit not his daughter’s section. “I learned a lot about what distinguished a good project from a bad one,” she says. “I gave my first true scientific talk at a science fair competition as a junior, and as a senior won a full four-year scholarship to UNM—so science fairs definitely had a big impact on my decision to choose science as a career.”

In Kim, with whom she worked as an undergraduate summer researcher at Lovelace Medical Center in Albuquerque, was another major influence. “He taught me the importance of actually understanding the techniques you used,” she says. Both Kim and her graduate school mentor, Jack Stevens, encouraged her to work independently. Stevens also was one of the few who suggested she make prions her research focus. His “suggestion was spot on and remains probably the best career advice I have ever gotten,” she says.

She served from 2001 to 2006 on the Food and Drug Administration TSE advisory committee, and chaired the panel from 2003–2006. “It put the real-world consequences of what we do in the lab into perspective,” she says. “I understand far better how the work we do as research scientists can impact everyday life.”

Outside the lab, Priola, who is single, enjoys gardening and playing the guitar. “Both activities are very relaxing after a 12-hour workday,” she says.

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that can efficiently replicate in a new species do not apply. Instead, the infectious agent in the TSEs is believed to be composed primarily of a self-replicating protein derived posttranslationally from a normal host protein called prion protein or PrP. TSE diseases are thus also referred to as prion diseases, and the poorly defined infectious moiety of prion diseases is known as the prion.

All mammalian species express a soluble, protease-sensitive form of prion protein, known as PrP-sen (also called PrP C for PrP cellular). PrP-sen is a glycoprotein which anchors to the cell surface membrane via a glycosylphosphatidylinositol membrane anchor (Fig. 1). PrP-sen is almost ubiquitously expressed throughout the body; its function is unknown, but it is not associated with prion infectivity. Infectivity is associated with an aggregated, protease-resistant form of PrP termed PrP-res or PrP Sc (for PrP scrapie). PrP-res has a much more limited distribution and is present in infected animals at high levels in the brain and other central nervous system tissues and occasionally in peripheral lymphoid tissues such as spleen.

Prion infection begins when infectious tissue is either ingested or inoculated and the PrP-res in this tissue interacts with the normal host PrP-sen. In a process known as seeded polymerization, the exogenous PrP-res binds to the endogenous host PrP-sen and, via a poorly understood process, induces a conformational change that converts the host PrP-sen into PrP-res, which can then repeat the process (Fig. 2). PrP-res thus “replicates” itself and accumulates to high levels in the central nervous system, eventually leading to disease. Although the newly formed PrP-res has a higher beta sheet content and thus is conformationally distinct from PrP-sen, both forms of PrP have the same amino acid sequence.

Amino Acid Differences Help To Explain TSE Species Barriers

The key to understanding how species barriers to infection may be controlled in prion diseases lies in the amino acid sequence of PrP. While highly conserved, it does vary among mammalian species. Since the conversion of PrP-sen to PrP-res is a conformational process, even small differences between the amino acid sequences of the host PrP-sen and the exogenous PrP-res might significantly affect the efficiency of PrP-res formation and thus disease onset.

In the early 1990s, a series of studies took advantage of the very strong species barrier between mice and hamsters. Mice can be infected with mouse-adapted strains of sheep scrapie but are resistant to infection with hamster scrapie. To test whether the primary sequence of PrP influences the mouse/hamster species barrier to prion infection, Stanley Prusiner and collaborators at the University of California, San Francisco, generated transgenic mice that overexpressed normal hamster PrP-sen and infected both these mice and wild-type mice with hamster scrapie. While the wild-type mice did not get sick, the transgenic mice developed disease with incubation times that were inversely related to the level of hamster PrP-sen expression. Thus, the higher the expression level, the shorter the incubation time. These experiments convincingly show that the amino acid sequence of PrP is a major factor in determining whether prions cross a species barrier to cause disease. If the
primary sequence of the host PrP-sen and foreign PrP-res molecules are homologous, new PrP-res can be made and an infection established. If those primary sequences differ, then presumably the species barrier will be maintained and infection prevented.

The major region of PrP involved in species barriers to PrP-res formation and infection resides within the middle third of the PrP molecule (Fig. 3). The conversion of PrP-sen to PrP-res is exquisitely sensitive; even a single amino acid change within this region can determine whether exogenous PrP-res can convert endogenous host PrP-sen. Predicting whether a given species would be susceptible to prions from another species would seem to be a simple matter of comparing the amino acid sequences within this part of PrP and finding the mismatches.

Not unexpectedly, the reality is not that simple. The critical residues involved in interspecies formation of PrP-res differ among species (Fig. 3). For example, codon 142 in goat PrP is associated with resistance to infection with BSE. The homologous position in mice (codon 138) is associated with resistance to hamster scrapie. However, in sheep it is codon 171 that is associated with resistance to BSE infection. In humans it is a naturally occurring polymorphism at codon 129—all of the clinical cases of human vCJD have been homozygous for methionine at codon 129. The fact that the amino acid residues critical to the species-specific formation of PrP-res differ among species suggests that differences in the overall conformation of the PrP molecules are important rather than direct interactions between homologous amino acid sequences.

To further complicate predicting prion disease species barriers based upon the primary sequence of PrP alone, posttranslational modifications to PrP, such as glycosylation (Fig. 1), can also affect PrP-res formation at a molecular level. The conformational conversion of PrP-sen to PrP-res can be divided into two general steps, (i) binding of PrP-sen and PrP-res, and (ii) conversion of PrP-sen to new PrP-res (Fig. 2). Work in my laboratory shows that glycosylation affects the binding step of the conversion process in a sequence-dependent (and thus species-dependent) manner. We suspect that the sugars obscure a normally accessible PrP-sen/PrP-res binding site. By contrast, PrP amino acid sequence homology appears to be most critical for the subsequent conversion step.

PrP amino acid sequences, although not predictive of infection, may be the basis of a mechanism for how species-specific barriers to prion infection are controlled at the molecular level. In intraspecies transmission where both the host PrP-sen and the incoming PrP-res have the same amino acid sequence, new PrP-res is formed, accumulates, and causes disease. If the host PrP-sen and exogenous PrP-res are from different species but have amino acid sequences that match at certain critical residues, then PrP-res can efficiently “replicate” itself and trigger an infection, i.e., a species barrier is crossed.
However, when the endogenous host PrP-sen and the exogenous PrP-res do not match at the amino acid residues that are critical for PrP-res formation, new PrP-res is not made at levels sufficient to cause disease and there is a true species barrier to TSE infection.

**Prion Species Barriers Are Not Absolute**

Even though PrP amino acid sequences are so highly conserved within a species, prion species barriers are not absolute, according to Richard Race and Bruce Chesebro at the National Institutes of Health Rocky Mountain Laboratories (Fig. 4). Taking advantage of the strong species barrier to infection between mice and hamsters, they inoculated wild-type mice with hamster scrapie. Over a year later brain material from some of these mice, none of which had any sign of clinical scrapie, was inoculated into a second group of wild-type mice. Of these second-passage mice, one group became clinically sick with scrapie (Fig. 4, group C), while two groups were subclinically infected as judged by the presence of PrP-res in the brain (Fig. 4, Groups A and B).

In stark contrast to the first two passages, a third passage into mice led to clinical disease in all groups. However, one inoculum (Fig. 4, group A) was unusual in that it caused disease in only 25% of the recipient mice and was still more virulent for hamsters, all of which succumbed to disease. Continued passage of this inoculum led to the generation of a prion agent that could infect both hamsters and mice (Fig. 4). Remarkably, after four or five serial passages in mice, prion agents that were mouse tropic, hamster tropic, or dually tropic (i.e., infected both mice and hamsters) could be isolated at varying passages. Thus, not only did hamster scrapie cross a very strong species barrier to cause disease in mice, but in doing so it also led to prion agents with species specificities different from that of the original inoculum.

Such experiments reaffirm something that has been known for decades. Like an ordinary viral or bacterial inoculum, a prion inoculum consists of a pool of infectious agents with different tropisms which, within the host, are selected for or against by a variety of pressures. In the case of prion agents, however, diversity may be more related to the conformation and/or biophysical properties of PrP-res (such as size and relative protease-resistance) than to the mutation of a nucleic acid genome.

Thus, it is likely that during the first passage of hamster scrapie into mice, some conformationally divergent fraction of the input hamster PrP-res was able to efficiently convert endogenous mouse PrP-sen to PrP-res (Fig. 4). Over subsequent passages, this newly derived mouse PrP-res was more efficiently amplified because of its homology to the host PrP-sen. Slight differences in the conformation of the pool of newly formed mouse PrP-res would then account for the eventual emergence of prion agents with different tropisms (Fig. 4).

**Other Prions Might Breech Species Barriers**

In fact, if sheep were substituted for hamsters and cattle for mice, the emergence of BSE in the UK would be much like the data summarized in Fig. 4. This is how a prion agent can change tropism by passage through another species. In
the case of BSE, changes in the process by which ruminants were rendered into meat and bone meal (MBM) likely led to incomplete inactivation of an animal prion disease such as sheep scrapie. Under such a scenario, sheep scrapie PrP-res in the contaminated MBM ingested by cattle would have converted some bovine PrP-sen to PrP-res, which then accumulated to subclinical levels. Then, in a situation analogous to the experiment shown in Fig. 4, clinically normal but PrP-res-positive cattle were rendered into MBM and fed back to cattle. This cycle continued unnoticed for several years until the recognition of BSE in the mid-1980s and the determination in the mid-1990s that vCJD in humans was the result of BSE infection. Thus, passage of this prion agent with no history of infecting humans through a different species led to an animal prion that infects people.

The threat of animal prion diseases crossing species barriers to cause disease in humans is quite real. In the United States, only two BSE-positive cattle have been found (versus over 184,000 in the United Kingdom), and the handful of vCJD cases have occurred in individuals who had originally moved from higher-risk countries. These low numbers suggest that the relative risk of BSE emerging as a significant threat to human health in the United States is comparatively small, a conclusion also reached in a study by Joshua T. Cohen and colleagues at the Harvard Center for Risk Analysis. However, another animal prion disease in North America needs to be considered—namely, chronic wasting disease (CWD) in deer and elk.

CWD was first recognized as a TSE disease 30 years ago by the late Elizabeth Williams at Colorado State University. CWD differs from both BSE and scrapie in several important ways. First, it is found both in controlled captive populations of deer and elk and in wild cervid populations. In endemic areas up to 20% of wild deer are CWD positive. Second, unlike BSE, CWD infectivity is present in excreta such as saliva, according to Edward Hoover at Colorado State University. Furthermore, experiments by Elizabeth Williams and colleagues have shown that excreta from CWD-infected animals can contaminate pastures. Third, whereas spread of BSE within a herd has not been documented and the spread of scrapie within a flock of sheep is restricted to about 40% of the animals, introduction of a single CWD-infected animal into a captive herd can lead to the spread of CWD to over 80% of the animals. Thus, CWD appears to spread much more efficiently than other prion diseases. Finally, CWD is present in wild populations of deer and elk, which often share range land with livestock such as cattle and sheep. These factors all support the possibility of cross-species transmission of CWD.

The Risk from CWD Crossing Species Barriers

Can CWD jump species barriers to cause disease in humans? Human and cervid PrP amino acid sequences differ by as many as 11 residues within the region of PrP that appears to influence species barriers to infection (Fig. 3). Trans-
Cross-species transmission of prion infectivity can lead to prion agents with altered species tropisms. At the level of PrP-res formation, the ability of hamster scrapie to cross species barriers into mice is likely due to the presence of PrP-res molecules with different species tropisms within the original hamster scrapie inoculum. These different tropisms are represented by green squares (hamster tropic), red squares (mouse tropic), or yellow squares (mouse and hamster tropic). On first passage into mice selective pressures determine which, if any, of these PrP-res molecules will be able to convert mouse PrP-sen (red circles) to PrP-res. Since this process is inefficient, very little PrP-res will be produced on first passage into mice and the mice will not get sick. On subsequent passages this low amount of PrP-res is amplified until, by the third passage, all of the animals show signs of clinical scrapie. In the original experiments, one group of mice (Group A) accumulated prion infectivity which was initially more efficient at infecting hamsters than mice but upon subsequent passage was able to infect both species. In a second group (Group B), the prion agent that emerged was only able to infect mice while in a third group (Group C) a prion agent that was able to infect both mice and hamsters was isolated. The experiment illustrates not only that species barriers to prion infection are not absolute but also that it is virtually impossible to predict the species specificity of the prion agents that eventually emerge. The passage history shown is a simplified version of the original data in Race et al. J. Infect. Dis. 186(Suppl 2): S166 (2002). Circles=PrP-sen, Squares=PrP-res. Open circles and squares represent mouse PrP sequence while crossed-out circles and squares represent hamster PrP sequence.

CWD can be transmitted to nonhuman primates, report Jason Bartz and colleagues at Creighton University. Their data raise the possibility that humans are susceptible to CWD. However, since the in vivo results are mixed, caution should be used when extrapolating these data to humans. Perhaps the best evidence suggesting that there is a very strong species barrier to CWD infection of humans is that there are no epidemiological data linking any human cases of prion disease to ingestion of venison.

If there is a strong barrier to direct infection of humans with CWD, its potential threat to human health is reduced, but not eliminated. We do not know whether CWD in wild populations of deer and elk can spread to other animal species and thus lead to new prion agents with the potential to infect humans. Experimentally at least, CWD can infect a variety of species. CWD can be transmitted into cattle, sheep, and even raccoons following intracerebral inoculation, according to a series of studies by Amir Hamir and colleagues at the U.S. Department of Agriculture National Animal Disease Center. Material from CWD-inoculated ferrets was, unlike the original CWD isolate, able to infect hamsters, according to Judd Aiken at the University of Wisconsin.

Thus, passage of CWD through a different species can change the tropism of the original prion agent. All of these experiments suggest that CWD has the potential to infect different species and produce prion agents which can cause disease in previously resistant species. Fortunately, a recent survey conducted by Daniel Gould and colleagues at Colorado State University showed that cattle in areas where CWD is endemic had no signs of TSE disease, suggesting that it is difficult for CWD to infect other species under natural conditions.

At the molecular level, our knowledge of how prions cross species barriers has increased significantly over the last decade. However, it remains impossible to predict what will happen when a prion agent begins to bounce among species.
Although the current data would suggest that the risk from CWD is low, it would be a mistake not to remain vigilant. Prion diseases are very slow to manifest, and if CWD were to cross a species barrier, it may not be recognized until years or even decades later. After all, BSE was recognized as a new animal TSE disease 10–15 years after the change in the rendering process which led to its emergence. Given such uncertainties, it seems likely that prion diseases will remain on the list of animal diseases that pose a threat to human health for some time to come.

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SUGGESTED READING