

Commentary

Oral infection by the bovine spongiform encephalopathy prion

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The route by which prion infection spreads from peripheral tissues to the brain has been the subject of interest and research for decades. The seminal pathogenesis studies by Hadlow and colleagues (1, 2) demonstrated that in natural scrapie in Suffolk sheep infectivity initially was detected at 10–14 months of age in tonsil, lymph nodes, spleen, and intestine, including ileum and upper colon. The tissue distribution of infectivity was consistent with uptake from the alimentary tract and, by implication, oral exposure as the likely portal of entry of infection. By the time clinical disease developed, peripheral tissues continued to exhibit a similar distribution and titer of infectivity, but there was also evidence of infectivity in the central nervous system, with higher titers of infectivity, initially in the medulla and diencephalon. Laboratory studies of oral scrapie infection in rodents have confirmed these findings and suggest that infection spreads from the lymphoreticular system to the spinal cord, presumptively via the autonomic nervous system, and thence rostrally to the brain (3). The importance of peripheral pathogenesis is underlined by the marked increase in incubation time in mice after splenectomy (4).

There is, however, variation in pathogenesis that is determined by factors including the interaction between host genome and agent strain. Some breeds of sheep affected by natural scrapie, for example, Montadales, have no detectable infectivity in peripheral tissues, and the distribution of infectivity in the brain may vary according to the breed of sheep (5). In bovine spongiform encephalopathy (BSE) infectivity has not been detected in peripheral tissues in natural disease, except for dorsal root ganglia and possibly bone marrow, although infectivity has been found in terminal ileum after experimental oral challenge with BSE brain (6). An important implication of this data is that, accepting the limits of the sensitivity of bioassay systems, there may be variation in pathogenesis in different host/agent combinations and extrapolation from data on scrapie, either natural or experimental, to other agent strains in other species, such as primates, may be misleading.

In primates only a small and unpredictable proportion of animals develop disease after oral exposure to tissues containing a high titer of infectivity such as brain (7). There is little information on pathogenesis in nonhuman primates after oral exposure, not least because the relative inefficiency of the oral route makes such experiments difficult to carry out in practice. In kuru, which is presumed to be caused by peripheral, and perhaps oral, exposure to infection through ritual cannibalism, infection has been found by bioassay in lymph nodes, kidney, and spleen (8). Similar experiments in Creutzfeldt-Jakob disease (CJD) have demonstrated infectivity in liver, kidney, lung, and lymph nodes. Examination of peripheral tissues by immunocytochemical techniques, either histologically or by Western blot, in human prion disease has been fairly limited and has not documented evidence of peripheral pathogenesis, although these techniques have a limited sensitivity (9).

More widespread immunocytochemical staining of components of the lymphoreticular system has been found in new variant CJD (nvCJD) (9), raising the possibility of a different

pathogenesis from sporadic CJD. The study by Bons *et al.* (10) in a previous issue of the *Proceedings* provides new data on the pathogenesis of prion disease in one primate species after experimental oral exposure to BSE agent. This paper significantly extends the available information on the tissue distribution of infectivity in the preclinical phase of the incubation period in primates and confirms that primates can be susceptible to oral exposure to the BSE agent. The findings will be of interest and concern to the scientific community and others in view of the hypothesis that nvCJD is causally linked to BSE, presumptively through oral exposure to the BSE agent (11, 12).

Consideration of methodological issues is an important prerequisite to the interpretation of the results. Immunocytochemical methods for the detection of disease associated prion protein (PrP^{sc}) depend on the, presumed, denaturation of normal prion protein (PrP^c), because none of the available antibodies can readily distinguish between the isomers. This differentiation usually involves partial protein digestion using proteinase K, but this methodology was not used in this study (10) because only fixed tissues were examined. Bons *et al.* have addressed this issue by carefully considering a range of criteria for the interpretation of PrP antibody immunocytochemical staining. Although it is highly likely that the positive PrP staining in this report indicates the presence of PrP^{sc}, the findings would be strengthened if they were backed up by other techniques such as Western blotting.

The observations in this paper give some indication as to how prion infection might spread after oral exposure. Of particular interest is the staining of the epithelium of the gut and tonsil, which raises the possibility of a number of mechanisms of onward transmission of infection and subsequent disease. One possibility is that the infectious agent penetrates the epithelial cells and replicates, another that the gut epithelial cells express PrP^c that acts as a receptor down which the agent passes, without necessitating entry to cell cytoplasm. It is not known whether gut epithelial cells express PrP^c, but PrP^{sc} can be detected in gut-associated lymphoid tissue, particularly follicular dendritic cells (13). An important question is how the infectious agent is transmitted from the gut lumen to these cells. Immunocytochemical staining was found in specialized M cells and lymphocytes within the gut epithelium and lymphoreticular system. These interesting findings may stimulate further experiments to address this important issue. It is of note that a recent study has implicated B lymphocytes as necessary for neuroinvasion in one model of experimental scrapie (14).

A further important observation in the study is the immunocytochemical detection of PrP^{sc} accumulation in the ventral and dorsal root ganglia throughout the spinal cord and in the cerebral cortex in the preclinical phase of the incubation period. This finding is consistent with models of scrapie pathogenesis. However, the exact mechanism of transmission from lymphoreticular system to central nervous system has not been precisely defined. Possibilities include transmission via the neuro-immune connection through the autonomic nerves

in organized lymphoid tissue, such as spleen, or via the autonomic nervous system in the gut. Further studies are needed to investigate these interesting possibilities.

From an epidemiological perspective the study by Bons *et al.* (10) raises a number of important issues. First, spongiform change and/or PrP immunostaining were found in the brains of 20 lemurs from three different primate centers in France and a simian in Montpellier zoo. The prevalence of prion infection in the Montpellier primates overall was five of 61 animals, although it is of note that autopsy examination was not carried out in nine of the 26 animals that had died. Although only two of these animals had signs of a neurological disease, 18 lemurs from three other primate facilities in France were apparently healthy and exhibited positive PrP immunocytochemical staining. The proportion of primates that had died with evidence of preclinical or clinical prion disease after presumed oral exposure to BSE was 19%, which may be an underestimate.

On the other hand, requests for information from other zoos in France on neurological disease in primates was largely negative, although the response rate was low. Furthermore, in Montpellier only 8% of primates, including those still living were PrP-positive. Even this figure is a matter for great concern if humans had a similar sensitivity to oral BSE exposure. However, this hypothesis would assume that humans are identical to lemurs in their susceptibility to BSE, an assumption that may not be justifiable. Transmission of BSE to marmosets by intracerebral inoculation was reported in 1993 (15), and this experiment was interpreted by the authors as follows: "Our experiments suggested that there was no specific reason to suppose that the BSE agent was more transmissible to primates than was the scrapie agent" (16). Scrapie is not thought to be a human pathogen.

No primates are reported to have developed a spongiform encephalopathy in British zoos, despite the heightened awareness of these diseases after the identification of a BSE-like illness in a range of potentially exposed zoo species (17) (although only in a minority of such species). It is of interest that BSE is experimentally transmissible to mice but not hamsters, species that are genetically closely related (18).

Second, although two lemurs were PrP-positive after experimental oral exposure to the BSE agent, it is relevant to question the source of infection in the primates with apparently "natural" transmission. Three of the lemurs, which died of spongiform encephalopathy in Montpellier, were almost certainly exposed to oral infection at the zoo, but the source of exposure in the other positive animals in Montpellier and the other three zoos is necessarily uncertain. The animals all were exposed to a food supplement containing cracklings, the "fourth quarter of beef," which might have originated in the United Kingdom. It would be of great interest to determine the constituents of this material and in particular whether it contained central nervous system tissue. Marmosets, which are susceptible to BSE by intracerebral inoculation, were fed in the United Kingdom throughout life with a supplement containing meat and bonemeal potentially contaminated with BSE between 1980 and 1990, but none of more than 100 uninoculated animals developed a spongiform encephalopathy (19). However, Bons *et al.* (10) provide data indicating that the neuropathological profile in the lemurs orally exposed to 0.5–1 g of bovine brain was similar to that in lemurs with a "natural"

spongiform encephalopathy, suggesting that the disease in this group also may have been caused by BSE.

The fact that primates are susceptible to a prion disease through oral exposure to the BSE agent is a finding of great interest. Extrapolating from this finding to human disease is problematic as the sensitivity of one species to a particular prion strain does not necessarily indicate the sensitivity of another species, even if this species is closely related. Because of this constraint, the study by Bons *et al.* (10) may discourage further experiments in other primates if these are aimed at quantifying the risk posed to public health by oral exposure to the BSE agent, as the results of such experiments can never provide hard information on what may happen to the human population after oral exposure to BSE. However, the paper should stimulate further research into pathogenesis of prion diseases in view of the intriguing findings in peripheral tissues, and there is clearly a need for more information on the peripheral pathogenesis of human prion diseases, not least because of possible public health implications.

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