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BSE-associated Prion-Amyloid Cardiomyopathy in Primates

Susanne Krasemann, Giulia Mearini, Elisabeth Krämer, Katja Wagenführ, Walter Schulz-Schaeffer, Melanie Neumann, Walter Bodemer, Franz-Josef Kaup, Michael Beekes, Lucie Carrier, Adriano Aguzzi, and Markus Glatzel

Prion amyloidosis occurred in the heart of 1 of 3 macaques intraperitoneally inoculated with bovine spongiform encephalopathy prions. This macaque had a remarkably long duration of disease and signs of cardiac distress. Variant Creutzfeldt-Jakob disease, caused by transmission of bovine spongiform encephalopathy to humans, may manifest with cardiac symptoms from prion-amyloid cardiomyopathy.

Human prion diseases are progressive neurologic disorders that include sporadic, genetic, and acquired forms of Creutzfeldt-Jakob disease (CJD) (1). A key step in disease initiation is conversion of PrPc into PrPSc, which is partially resistant to proteolytic digestion and an essential part of prion infectivity. Transmission of bovine spongiform encephalopathy (BSE) to humans has led to a novel form of acquired CJD, termed variant CJD (vCJD) (2). The pathogenesis of vCJD differs substantially from sporadic CJD with remarkable colonization of non–central nervous system regions with infectious prions and PrPSc (3).

Although risk reduction measures have been introduced to limit transmission from BSE-diseased cattle to humans, vCJD has occurred in several hundred instances (www.eurocjd.ed.ac.uk). Most clinically affected vCJD patients are homozygous for methionine on polymorphic codon 129 of the gene coding PrP (PRNP), and the clinical presentation of vCJD in these patients is uniform (4). The occurrence of atypical clinical features in persons with vCJD that encodes methionine and valine on PRNP codon 129 and human-to-human transmission of vCJD through blood transfusion have raised concern about atypical clinical features and alternative distribution of PrPSc in vCJD (5). We report on the novel clinicopathologic characteristics of vCJD as prion-amyloid cardiomyopathy in 1 of 3 macaques inoculated with BSE.

The Study

In 2002, three rhesus macaques were inoculated with BSE intraperitoneally (10 mL of a 10% homogenate of brain from BSE-diseased cattle). As controls, 2 rhesus macaques received saline (10 mL) and 1 was untreated. All procedures involving rhesus macaques were performed at the Institute of Neuropathology, University Medical Center Hamburg-Eppendorf (Hamburg, Germany), in accordance with the German Animal Welfare Act and the Council Directive 86/609/EEC (Permit 33.42502/08–08.02 LAVES, Lower Saxony, Germany). Animals were observed for clinical signs of prion disease and, when signs of terminal prion disease became evident, were euthanized and underwent autopsy. In all 3 BSE-challenged macaques and none of the controls a progressive neurologic disease developed 49, 59, and 61 months postinoculation. Examination of brain by using hematoxylin and eosin staining showed typical neuropathologic features of vCJD (data not shown) and abundant deposits of PrPSc in the cortex, basal ganglia, and cerebellum in paraffin-embedded tissue blots performed as described by using 12F10 monoclonal antiprion antibody (6) (Figure 1, panel A). The mobility of the unglycosylated PrPSc band and the glycoform ratio of proteinase K-digested PrPSc were similar to those in BSE when assessed by Western blot analysis using monoclonal POM-1 antiprion antibody as described (7) (Figure 1, panel B).

Besides lymphoreticular tissues, the muscular compartment is targeted by prions (7,8). Thus, we assessed presence of PrPSc in skeletal and heart muscle by Western blot analysis with sodium phosphotungstic acid precipitation for enrichment of PrPSc and protein misfolding cyclic amplification by using published protocols (3). We could not detect substantial amounts of PrPSc in skeletal muscle (Figure 2, panel A). One macaque showed abundant PrPSc (~1/100 of PrPSc found in brain) in heart in Western blot and protein misfolding cyclic amplification (Figure 2, panels A, B). Paraffin-embedded tissue blot analysis of this heart showed PrPSc as amyloid, occupying considerable stretches of heart tissue, mainly in the septum (Figure 2, panel C), whereas no PrPSc could be seen in hearts of other macaques (data not shown). These findings were confirmed by strong Congo red–positive patch-like deposits in cardiomyocytes in the heart of this monkey (Figure 2, panel D). The primate with cardiac PrPSc showed the longest disease duration (4 months, compared with 4 weeks for other BSE-infected monkeys), signs of cardiac affection

1These authors contributed equally to this article.
The most important genetic risk factor for development of vCJD is homozygosity for methionine on PRNP codon 129, and all but 1 patient with clinical vCJD carry this polymorphism (5). Thus, future cases of vCJD with longer incubation times are likely to comprise more patients with alternative codon 129 polymorphisms than methionine homozygosity. Data from rodent experiments indicate that clinical features of vCJD may differ in these patients (9). Thus, the next decades may see a shift in vCJD phenotypes. Further uncertainty for atypical cases in humans results from the possibility of secondary transmission of vCJD through blood products from subclinical carriers, which may lead to development of nonclassical vCJD phenotypes (5).

We showed that BSE infection of primates may occur as prion-amyloid cardiomyopathy. Because prion-amyloid cardiomyopathy developed in only 1 of 3 macaques, host-encoded factors, such as genetic makeup, probably influence development of this cardiac phenotype. All macaques are homozygous for methionine on PRNP codon 129; thus, prion-amyloid cardiomyopathy cannot be related to polymorphic codon 129 in our study (10). Cardiac involvement has been observed in a patient with sporadic CJD and is prominent in prion-diseased mice expressing PrPSc lacking its membrane anchor (11,12). We considered the possibility that preexisting pathology, such as spontaneous cardiomyopathy or inflammation of the heart, might have contributed to cardiac PrPSc, and the fact that we did not find any evidence for toxic cardiomyopathy or inflammation in the primate does not exclude this possibility. Because the macaque with abundant PrPSc deposition in heart had longer disease duration, it is also possible that longer disease duration, which favors centrifugal spread of prions to peripheral tissues, contributed to cardiac affection in this primate (7). Peripheral deposition of PrPSc in vCJD is well studied (3). We were surprised by the amount and deposition type of PrPSc in heart, reaching 1/100 of the amount seen in brain and deposited as amyloid across large stretches of heart tissue. Skeletal muscle of prion-diseased patients and nonhuman primates routinely harbor minimal amounts of PrPSc (<1/1000 that found in brain), and PrPSc in muscle is virtually impossible to detect by in situ methods (6,8,13).

To our knowledge, PrPSc has not been detected in heart of vCJD-diseased persons or in patients with systemic amyloidosis, although primates orally exposed to BSE show

Table. Characteristics of 3 rhesus macaques in study of BSE-associated prion-amyloid cardiomyopathy*

<table>
<thead>
<tr>
<th>Primate</th>
<th>Age at inoculation</th>
<th>Time to clinical disease, mo</th>
<th>Disease duration, wk</th>
<th>Cardiac PrPSc</th>
<th>Signs of cardiac distress at autopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSE inoculated</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 y</td>
<td>49</td>
<td>4</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>5 y</td>
<td>59</td>
<td>18</td>
<td>Pos</td>
<td>Pos</td>
<td></td>
</tr>
<tr>
<td>1 y</td>
<td>61</td>
<td>4</td>
<td>Neg</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 mo</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>17 y</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Neg</td>
<td></td>
</tr>
<tr>
<td>19 y</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>Neg</td>
<td></td>
</tr>
</tbody>
</table>

*BSE, bovine spongiform encephalopathy; Neg, negative; Pos, positive; NA, not applicable.
very low amounts of cardiac PrPSc (8,14,15). The lack of cardiac PrPSc in vCJD may result from small cohorts investigated. Because the spectrum of vCJD is likely to change, broad application of current clinical criteria for vCJD in clinical practice may lead to underreporting of vCJD, missing atypical cases of vCJD.

In conclusion, we showed that BSE-infection of primates may lead to prion-amyloid cardiomyopathy. These data should be considered when vCJD surveillance is conducted.

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The overall study was conceived and designed by M.G., A.A., F.J.K., and S.K. Animal care, housing, and observation were conducted by F.J.K., W.B., and W.S.S. Experiments were performed by S.K., G.M., E.K., W.S.S., and M.N. Data were analyzed by S.K., G.M., M.B., A.A., and M.G. S.K. and M.G. wrote the paper with substantial contributions from G.M. and A.A.

Dr Krasemann is a research scientist at the Institute of Neuropathology of the University of Hamburg working on prion spread. Her primary research interests are factors involved in spread and clearance of prions.

References

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etymologia

Shewanella haliotis

[shoo"nələ hāilətis]

From the Greek halios (marine) and ōtos (ear), abalones, genus Haliotis, were first mentioned ≈2,500 years ago by Aristotle, who wrote of “the wild limpet (called by some the ‘sea ear’).” In D’Arcy Thompson’s translation of Aristotle, he notes that “wild limpet” is “commonly attributed to Fissurella graecea ... and conceals a forgotten name for Haliotis.” The “sea ear” was familiar to the Greeks and was named oitia (little ear) by Pliny.

Shewanella haliotis, a species of rod-shaped, gram-negative, facultatively anaerobic bacteria, was first isolated from the gut microflora of abalones collected from the ocean near Yeosu, South Korea, by Kim et al. in 2007. The genus Shewanella had been previously named in 1985 by MacDonell and Colwell in honor of Scottish microbiologist James M. Shewan, for his work in fisheries microbiology.

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