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Age-related alterations affect the susceptibility of mice to prion infection

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Abstract

The sporadic and familial forms of Creutzfeldt-Jacob disease (sCJD and fCJD) usually appear at older ages (60–70 years and ∼50, respectively). Nevertheless, infectious forms such as Kuru and variant CJD (vCJD) present mostly at a much earlier age. To study the effect of age on the pathogenesis of infectious prion disease, we inoculated young and aged mice intraperitoneally with RML prions, followed them to disease end point and studied their disease characteristics. We now show that mice infected at older age present a significantly longer incubation time then mice infected at young age. Additionally, brains of mice infected at older age present significantly less disease-specific pathological markers such as gliosis, vacuolation and PrP^{Sc} accumulation. Concomitantly, gene expression analysis revealed that the upregulation of disease-associated inflammatory and stress-response genes, was significantly less pronounced in the brains of mice infected at older age. Based on this data, we suggest that alterations associated with aging, are accountable for the delay in the disease onset and the milder pathology in prion-infected aged mice.
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1. Introduction

Creutzfeldt-Jakob disease (CJD), a fatal neurodegenerative disorder, is the most common form of human prion diseases also denominated transmissible spongiform encephalopathies (TSEs). The ultimate marker of prion infection is the accumulation of PrP^{Sc}, an aberrant conformer of the membrane glycoprotein PrP^{C} (Prusiner, 1998). During the disease incubation period, PrP^{Sc} accumulates primarily in lymphoreticular organs such as the spleen (Fraser and Dickinson, 1978; Hilton et al., 1998; Kimberlin and Walker, 1979) and thereafter reaches the central nervous system (CNS) through peripheral nerves (Beekes et al., 1998; Bencsik et al., 2001; Glatzel et al., 2001).

CJD can manifest at three different etiologies—sporadic, familial and infectious. Both the sporadic CJD (sCJD) and the familial form of CJD (fCJD; linked to mutations in the PrP gene) are considered as late onset neurodegenerative diseases with an average appearance age of 60–70 for sCJD, and age 50 for fCJD (Brown et al., 1994). In contrast, the transmissible prion diseases, such as Kuru and most iatrogenic CJD, often appear at early age (Gajdusek and Zigas, 1957). In addition, variant CJD (vCJD), which is related to the consumption of meat contaminated with bovine spongiform encephalopathy (BSE), also show an early age of onset of 26 on average (Spencer et al., 2002; Will et al., 1996). The early age of onset typical to acquired prion diseases could be attributed to the characteristics of each specific prion strain, as defined by parameters such as incubation time and vacuolar pathology following transmission to wild-type mice (Collinge et al., 1996; McLean et al., 1998; Ritchie et al., 2009). As for Kuru and iatrogenic CJD, additional epidemiological factors were found that affect the age at onset (Gibbs et al., 1985; Koch et al., 1985; Will, 2003). However, regarding vCJD and despite many efforts, no age-related epidemiological factor was found that could explain this bias (Boelle et al., 2004;

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Ghani et al., 2003). Thus, young age may constitute a risk factor for infectious prion diseases following exposure to the prion agent.

Normal aging is associated with alterations in various organs and functional pathways. In the brain, such variations include changes in neuron morphology, genomes instability (Bohr et al., 2007), production of reactive oxygen species (Sohal and Weindruch, 1996), glial cells activation, inflammation and neuronal damage (Block et al., 2007; Blumenthal, 1997; Nakanishi, 2003). Several genes whose expression levels change during aging were identified in several tissues including brain (Lee et al., 1999, 2000; Lu et al., 2004). Among those, genes involved in stress and inflammatory responses were shown to be upregulated. Interestingly, several of these genes were also shown to be upregulated in the brains of prion-infected animals and of human sporadic CJD patients (Booth et al., 2004; Riemer et al., 2004; Xiang et al., 2005, 2004).

In this study, we used a mouse model for infectious prion disease to examine the effects of age on the susceptibility to prion infection and the pathology of the disease. To this end, we infected young (1 month) and aged (16 months) C57BL/6 mice intraperitoneally with Rocky Mountain Laboratory (RML) strain mouse prions. As controls we used mock-inoculated age-match mice (referred here as 'naïve'). We followed the mice for clinical signs of prion disease and measured disease-specific pathological markers such as gliosis, vacuolation and accumulation of PrP^{Sc} in the spleen and brain, and the expression profile of age and disease-related genes. Our results demonstrate that younger mice are more susceptible to prion infection, and that this differential susceptibility correlates with the age-related response of pro-inflammatory and stress-response genes to prion infection. Whether the expression of these genes plays a causal role in prion infection remains to be established.

2. Materials and methods

2.1. Animal study

All groups of mice comprised of 5–10 animals. 1- and 16-month-old C57BL/6 female mice (Harlan, Israel) were inoculated intraperitoneally (IP) with 85–100 μl of 1% Rocky Mountain Laboratory (RML) strain of prion-infected brain homogenate. The inoculum volume was adjusted according to the mice weight, which was 15% lower in the young mice. However, we found that such changes in the amount of inoculum were insignificant, as previously shown (Prusiner et al., 1982). Age-matched control mice (naïve) were inoculated with sterile PBS not containing infectious material. Inoculated mice were followed twice weekly until the appearance of clinical signs of prion disease such as rigidity, ataxia, and lethargy and from then on daily. The mice were sacrificed at the end point of the bioassay, when severe clinical signs were apparent. Throughout the experiment, mice were kept in specific pathogen-free conditions with food and water *ad libitum*. All animal experiments were done according to our institutional guidelines and National Institute of Health regulations. Mice were sacrificed at the terminal stage of disease or at early sampling point and their brains and spleens were removed and frozen for future analyses. For histopathology applications, samples were immersed-fixed in 4% formalin buffer and embedded in paraffin. For preparation of RNA, brain samples were kept in RNA later solution (Biological Industries, Israel) for future isolation of total RNA. Spleen samples were immediately processed for RNA isolation using RNA isolation kit (Promega, Madison, WI).

2.2. Histology and immunohistochemistry

To visualize vacuolation, paraffin-embedded fixed mouse brains were sectioned at 5 μm, deparaffinized and counterstained with hematoxylin and eosin. For detection of activated astrocytes, sections were stained for glial fibrillary acidic protein (GFAP). Briefly, deparaffinized sections were pretreated by steaming for 60 min in citric acid buffer at pH 6.0 to reveal antigens, rinsed in PBS, blocked and incubated with anti-GFAP at a dilution of 1:1000 (Dako), following incubation with anti-mouse FITC (1:250, Jackson Immunoresearch, West Grove, PA) secondary antibody.

2.3. Preparation of brain homogenates

Prion-infected mouse brains (RML strain) were homogenized as described earlier (Avrahami et al., 2008) and frozen in aliquots at −80 °C for future use.

2.4. Immunoblotting

Brain homogenate postnuclear supernatants were assayed for total protein using a BCA assay (Pierce, Rockford, IL) to ensure that all samples loaded on the gel correspond to equal amounts of original homogenate. For detection of PrP^{Sc}, samples were digested with 40 μg/ml proteinase K (PK) for 30 min, boiled and applied to 12% SDS-PAGE. For detection of PrP^{C}, the PK digestion step was omitted, and the samples were separated on 12% SDS-PAGE and electro-transferred to nitrocellulose membrane in a Tris/glycine buffer. Membranes were blocked with 5% milk in TBST (10 mM Tris–HCl, pH 7.4, 150 mM NaCl, 0.05% Tween 20), incubated overnight at 4 °C with anti-PrP mouse IPC1 (sigma, Israel) primary antibody and then with AP-conjugated secondary antibodies (Promega, Madison, WI). The relative intensities of the bands were measured in NIH Image J analysis software.

2.5. Real-time PCR

Total RNA from mice brains was isolated using TRI reagent (Sigma, Israel). Total RNA from spleens was prepared using a SV total RNA kit (Promega, Madison, WI). cDNA was prepared from 2 μg of total RNA using

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