Immunization Delays the Onset of Prion Disease in Mice

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The outbreak of new variant Creutzfeldt-Jakob disease has raised the specter of a potentially large population being at risk to develop this prionosis. None of the prionoses currently have an effective treatment. Recently, vaccination has been shown to be effective in mouse models of another neurodegenerative condition, namely Alzheimer’s disease. Here we report that vaccination with recombinant mouse prion protein delays the onset of prion disease in mice. Vaccination was performed both before peripheral prion exposure and after exposure. A delay in disease onset was seen in both groups, but was more prolonged in animals immunized before exposure. The increase in the incubation period closely correlated with the anti-prion protein antibody titer. This promising finding suggests that a similar approach may work in humans or other mammalian species at risk for prion disease. (Am J Pathol 2002, 161:13–17)

Prions are very unusual infectious agents. Current evidence suggests that they lack nucleic acid and their pathogenic potential is dependent on the conformation of prion protein (PrPSc). The normal mammalian prion protein is known as PrPC. The disease-associated protein, PrPSc, has the same amino acid sequence but the conformation is altered, having a higher β-sheet content. Experimental treatment approaches that have been reported for prion diseases include the use of amphotericin B, Congo Red, sulfated polyanions, anthracyclines, β-sheet breaker peptides, porphyrin, and phthalocyanine compounds. Some of these compounds delay the incubation time of animals infected with PrPSc but all have limitations in terms of toxicity and/or pharmacokinetics. Prion infections do not elicit a classical immune response; however, transport of prions from the periphery to the central nervous system is critically dependent on the lymphoreticular system. The immune system appears to assist rather than impair the propagation of prions and their access to the central nervous system, which is required for pathogenicity. In the current study, we sought to determine how overcoming the natural immunological tolerance to PrP by active immunization would influence progression of the disease.

Materials and Methods

Prophylactic Treatment Group

Twenty female CD-1 mice, 2 to 3 months of age, were immunized with mouse recombinant prion protein (recPrP). The recPrP was prepared as previously described. For the first injection, the recPrP (1 mg/ml in 0.5 mol/L urea) was mixed with an equal volume of complete Freund’s adjuvant immediately before subcutaneous administration (50 μg recPrP/100 μl). Twenty control mice received the adjuvant plus vehicle. Subsequent immunizations were performed at 2-week intervals in incomplete Freund’s adjuvant. Fourteen weeks after the first vaccination, the mice were bled and the anti-recPrP antibody titer was determined by enzyme-linked immunosorbent assay (see below). The mice were subsequently divided into two groups matched for their titer to recPrP and were inoculated intraperitoneally with a brain homogenate of the mouse-adapted scrapie strain 139A at a 10-fold or a 1000-fold dilution. The control mice were also divided into two groups that received either the
10-fold or 1000-fold dilution of the same 139A inoculum. This represents a well-established model of prion disease in mice, which leads to central nervous system scrapie infection and death in all cases, if the disease is allowed to progress. The immunization was continued thereafter at monthly intervals until the first mice showed clinical symptoms of scrapie. The mice were bled again at 14 weeks after the intraperitoneal PrPSc inoculation, a few weeks before they were expected to show clinical signs of the disease. Final bleeding was performed at the time of sacrifice, which occurred when the mice scored signs of the disease. Final bleeding was performed at the time of sacrifice, which occurred when the mice scored signs of the disease. The mice were bled again few weeks before they were expected to show clinical signs of the disease. Final bleeding was performed at the time of sacrifice, which occurred when the mice scored signs of the disease.

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**Antibody Titters**

Antibody titters to recPrP were determined by serial dilutions of plasma, in which mouse recPrP at 100 ng/well is coated overnight onto microtiter wells. The titer, defined as the dilution yielding 50% of the maximum signal, was detected by a goat anti-mouse IgG linked to horseradish peroxidase (Amersham Pharmacia Biotech, Piscataway, NJ) and tetramethyl benzene (Pierce, Rockford, IL) was used as the substrate.

To determine the correlation between PrPSc antibody recognition and disease incubation time, enzyme-linked immunosorbent assay plates were coated overnight with PrPSc at 50 ng/well. The PrPSc was prepared by the purification of ME7 strain PrPSc and reverting the conformation and proteinase K sensitivity to a protease-sensitive PrPSc-like state by solubilization in 99% formic acid, as previously described. Mouse plasma was used at a 1000-fold dilution in duplicate and the signal was detected by goat anti-mouse alkaline phosphatase (Bio-source International, Camarillo, CA) and p-nitrophenyl phosphate (Sigma Diagnostics, St. Louis, MO) was the substrate.

**Results**

**Prophylactic Treatment Group**

The anti-recPrP titer in the mice just before receiving the 1:10 and 1:1000 dilution of the mouse-adapted scrapie strain 139A intraperitoneally was 20,135 ± 17,916 (±SEM) and 9645 ± 8063, respectively. When the mice were bled at 14 weeks after the intraperitoneal PrPSc inoculation the group inoculated with the higher dose (1:10 dilution) had a titer of 6618 ± 2481 and the group inoculated with the lower dose had a titer of 1562 ± 621 (P = 0.09 by unpaired t-test). As depicted in Figure 1A, the mice immunized with the recPrP had a statistically significant delay in the onset of scrapie symptoms. The treatment effect was more pronounced at the 10-fold dilution [days to sacrifice, 173 ± 2 days (control) versus 189 ± 4 days; P = 0.002, two-tailed t-test], than at the 1000-fold dilution [days to sacrifice, 197 ± 3 (control) versus 205 ± 3; P = 0.040, one-tailed t-test]. This may be related to the trend for a higher titer of anti-PrP antibodies in the 10-fold PrPSc dilution group (see above), although titer alone may not be the sole determining factor. The epitope specificity of this response is also likely to influence progression of disease. A higher anti-PrPSc antibody level in vaccinated animals correlated with a longer incubation time (Figure 1, B and C) in both PrPSc-inoculated mouse groups (lower dilution group: r² = 0.453, P < 0.005; higher dilution group: r² = 0.744, P < 0.0001).

**Rescue Treatment Group**

As expected, the effects of the treatment (Figure 2A) were not as pronounced in the rescue mouse group, compared to the prophylactically treated mice (Figure 1A). No significant group difference was observed in
disease onset in mice receiving the 10-fold dilution of the brain inoculum [days to sacrifice, 192 ± 5 days (control) vs. 190 ± 5 days], although the levels of antibodies against PrPSc correlated with disease onset ($r^2 = 0.279, P < 0.017$, data not shown). However, at the 1000-fold dilution, a delay in symptoms was observed in the vaccinated mice [days to sacrifice, 210 ± 3 days (control) vs. 222 ± 4 days; $P = 0.018$, t-test, one-tailed]. As with the prophylactic treatment, the anti-PrP C antibody levels in the immunized mice correlated with a longer incubation time (Figure 2B; $r^2 = 0.772, P < 0.0001$).

Histological and Western blot evaluations of all of the brains of the treated and control groups did not reveal any apparent differences in the degree of spongiform change or PrPSc levels at the time of sacrifice (Figure 3) in either the prophylactic or rescue treatment mouse experiments. Hence, immunization delayed PrPSc propagation, but ultimately similar pathology and levels of PrPSc were obtained in treatment and control groups.

**Discussion**

The prionoses belong to the category of conformational disorders, which are all characterized by the accumulation of a constitutively expressed protein in an abnormal, toxic conformation. Alzheimer’s disease also falls into this category, in which the disease state is characterized by the conformational change of normal soluble amyloid β (Aβ) peptide to aggregated/fibrillar Aβ. A number of recent reports have shown that immunization with Aβ peptides is highly successful at reducing cerebral amyloid accumulation, a key neuropathological feature of Alzheimer’s disease, in transgenic mouse models of this disease. Passive immunization studies in the Alzheimer’s disease mouse model suggest that an antibody-mediated clearance of Aβ is critical for a therapeutic response. In this report we extend this immunological approach to prion disease and suggest that it may be applied to all members of this extended category of conformational diseases. Although neither of our treatment paradigms prevented prion disease, the close correlation between antibody levels and incubation time shows the promise of vaccination therapy for this untreatable and fatal neurodegenerative disease. Overall, the vaccination-mediated delay in the onset of prion disease is highly reproducible, correlates well with antibody titer, with the best therapeutic effect being obtained in mice preimmunized before infection.

It is not clear how the immunization delays the onset of prion disease in these mice. Our preliminary studies of passive immunization using anti-PrP antibodies suggest
that humoral immunity is critical for a therapeutic response. It is possible that antibody binding to PrPC and/or PrPSc may interfere with PrPSc-mediated conversion of PrPC to PrPSc and thereby delay the onset of clinical symptoms. Recent in vitro studies support this view, and immunization with prion peptides of 20 amino acids has been shown to reduce the levels of PrPSc in scrapie-infected mouse tumors without affecting PrPC levels. Hence, epitope mapping of the anti-PrP antibodies produced by immunization may provide insights on which portions of the prion molecule are important for prion replication. The ultimate goal of such immunological approaches is for human testing; although the recently reported problems with the Aβ42 vaccine for Alzheimer’s disease highlight the difficulties of translating successful approaches in mice to humans. There are a number of potential toxic side effects of vaccine-based approaches in humans that will require further animal and in vitro experimentation. One source of toxicity is from the immunogen that is used. In our Alzheimer’s disease vaccine development studies we altered the Aβ sequence making it nonfibrillogenic and nontoxic, while maintaining or increasing its immunogenicity, reducing the potential of this toxicity. Similar types of alterations are underway to limit any potential toxicity from using the native PrP sequence as an immunogen. Our in vivo findings serve as a starting point for the development of vaccine-based approaches for the prion diseases and suggest that prion-based immunization is promising as a potential therapy.

References