Supporting Information

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SI Methods

Standard methods of animal inoculation, Western blotting, immunocytochemistry, and cell infection were used as previously described in detail. Briefly, for mouse infection, 30 μL of a 1% brain homogenate was inoculated into the left cerebrum with a disposable syringe and 27g needle (4), and when mice expressed clinical signs, animals were anesthetized, and each mouse brain was collected for Western blotting (right half immediately frozen at -70 °C), while the left half was fixed by immersion in freshly made 4% paraformaldehyde in PBS. The fixed brain was embedded in paraffin for standard sectioning. Unmasking of abnormal prion protein (PrP) in histological sections was done by autoclaving in citrate buffer (5), followed by staining with commercially available antibodies to the proteinase-K-resistant carboxy region of PrP (C20 or M20) at a dilution of 1:1000 (3). Antibodies to glial fibrillary acidic protein and keratin sulfate were similarly diluted (5, 6), and secondary antibodies labeled with biotin followed by streptavidin–alkaline phosphatase were developed with Vector Red at pH 8.5. In some cases, especially in Tga20 mice that express high levels of PrP, sections were digested with 0.002–0.004% trypsin for 7–15 min, quenched, and then exposed to antibodies. Similarly, trypsin also was used to expose PrP amyloid in paraffin-embedded sections of GT1 cell pellets. The GT1 cell infection was done as described in ref. 7. Briefly, 10^5 cells were seeded in 6-well costar plates and exposed to 1 mL of <1% test brain homogenate in medium. An additional 1 mL of plain medium was added the next day, and the cells were incubated for 2 more days, washed three times with serum-free medium and once with complete medium, and then incubated with 2 mL of fresh medium for 4 more days (to 90–95% confluence). The cells then were split, and PrP and PrP-res were analyzed at sequential passages on Western blots using the C20 PrP antibody and secondary peroxidase-labeled antibody for chemiluminescent labeling, as described in ref. 2. Digestion for PrP-res was done with proteinase K at 20 μg/μL for 40 min in Nonidet P-40/DOC lysis buffer (5, 8). C. Lasmezas (Scripps, FL) kindly supplied the brain homogenate of a macaque infected with a human kuru brain homogenate (9). The Tga20 mice have been bred at Yale University for >5 years in a pathogen-free facility and were a gift of C. Weissmann (Scripps, FL).

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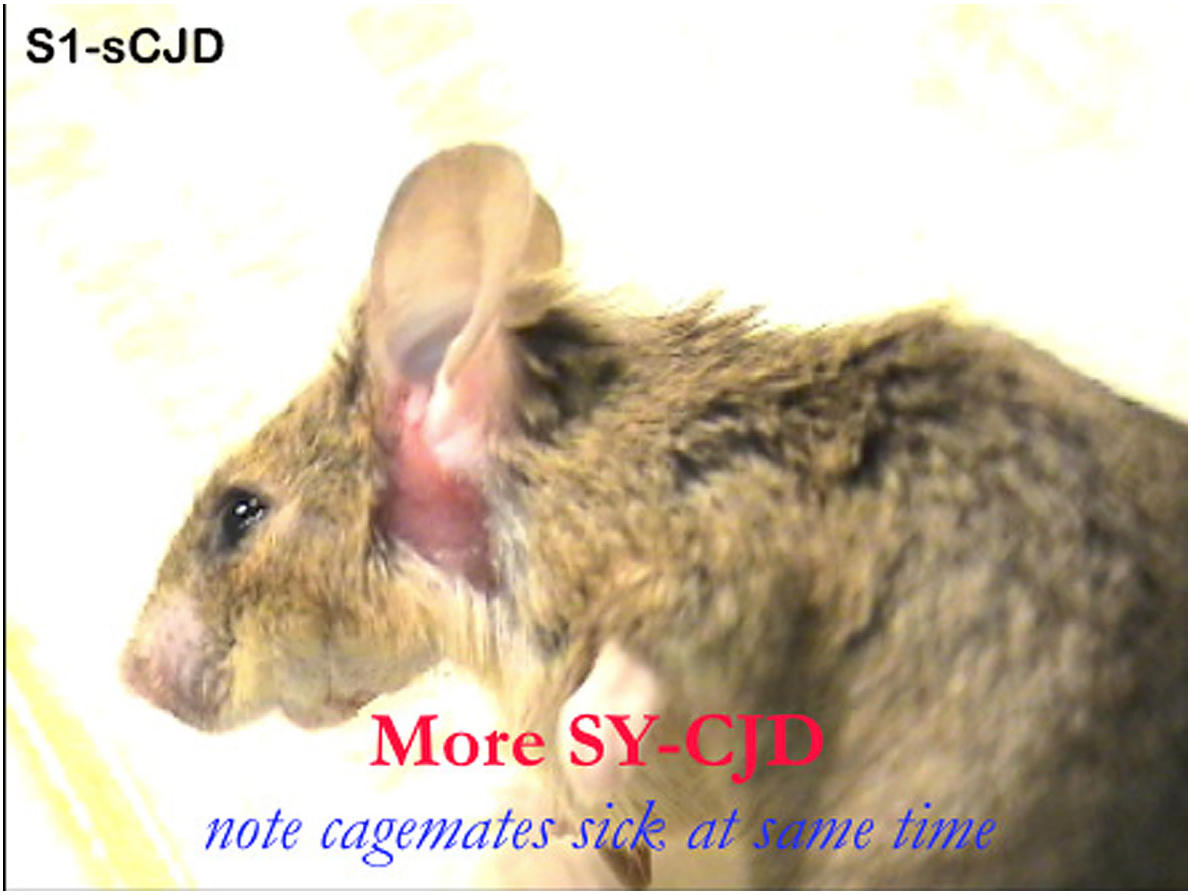

Movie S1. Typical repetitive motions and scratching in mice injected with human-derived sporadic Creutzfeldt–Jakob disease (sCJD). Note the otherwise normal appearance and good grooming of these mice. Scratching after prolonged incubation of >300 days was seen in all genotypes of mice with WT PrP (outbred, inbred, and Tga20). The same behavior results were observed from many different human sCJD isolates originating from the United States and Europe. Prior passage through guinea pigs and hamsters and directly from humans to mice by intracerebral inoculation also show the same agent-specific clinical signs. Stereotypic repetitive scratching leads to severe skin abrasions within 24 h. Hyperventilation and muscle fasciculations (twitching) also can be observed. If not killed at this time, these sCJD mice typically dropped dead without further signs. Some sCJD-infected mice did not develop such severe scratching but displayed very subtle or questionable signs at the same incubation time and, if not killed, also would die suddenly. In contrast to mice, hamsters with sCJD have a fast 125-day incubation time on secondary and later passages and do not show either scratching behavior or very limited medial thalamic lesions. Instead, they have widespread spongiform lesions throughout the cerebrum and, like humans, do not develop PrP plaques as previously demonstrated in hamsters with >15 independent human sCJD isolates. The sCJD infectious titer in hamsters is 8.3 logs (1), >3 logs higher than that in mice (2).

Movie S1 (MOV)

Movie S2. In contrast to sporadic Creutzfeldt–Jakob disease (sCJD), only one kuru infected (kCJD) mouse, and only at passage 1, displayed the same extreme scratching syndrome with lesions of the neck. A few other passage 1 mice showed rubbing of the nose and other parts of the body but no neck lesions. The passage 1 kCJD mice also showed far more diverse behavioral changes, including head shaking and difficulty walking. Scratching behavior was lost after passage 1, suggesting selection of an adapted agent. Mice at passages 2 and 3 continued to have difficulty walking and often developed abnormal splaying of the legs when picked up. They also showed extremely poor grooming, unsteady balance, slowness, loss of body mass, and severe kyphosis (hunched neck and back), as shown. The kCJD mice can be distinguished readily from sCJD mice simply on the basis of behavior.

Movie S2 (MOV)

Movie S3. The variant Creutzfeldt–Jakob disease (vCJD)-infected mice were hyperactive until terminal disease and displayed an extreme and diagnostic twisting motion when picked up, as shown. Some kyphosis was seen terminally in these well-groomed animals, and body mass remained normal or somewhat increased. The Japanese CJD isolates were also distinct from all other CJD agent strains, as previously shown in still photographs, with distinctive hind limb signs (3).

Movie S3 (MOV)

Movie S4. Extreme obesity was seen only in 263K-sc-infected mice, as shown. Mice infected with this highly cloned mutant scrapie developed slowness and rough fur. In summary, there were obvious behavioral differences with each of these CJD and scrapie agents, and these differences were further substantiated by the distinctive brain lesion profiles that each agent induced.

Movie S4 (MOV)