Three scrapie prion isolates exhibit different accumulation patterns of the prion protein scrapie isoform

(brains mapping/immunostaining/amyloid plaques/prion disease/strain)

STEPHEN J. DEARMOND*, SHU-LIAN YANG*, AUDREY LEE†, RUSSELL BOWLER†, ALBERT TARABOULOS‡, DARLENE GROTH‡, and STANLEY B. PRUSINER§§

Departments of *Pathology, †Neurology, ‡Biochemistry and Biophysics, and §§Medical Students, University of California, San Francisco, CA 94143

Contributed by Stanley B. Prusiner, December 29, 1992

ABSTRACT To investigate the molecular basis of prion diversity, we inoculated transgenic mice expressing the Syrian hamster prion protein (PrP) with three distinct prion isolates. We compared the three isolates designated Sc237, 139H, and Me7 in Tg(SHaPrP)7 mice with clinical signs of scrapie because the incubation times with these mice are considerably shorter than the times found with hamsters. Each prion isolate produced a distinctive pattern of the scrapie isoform of PrP (PrPSc) accumulation, as determined by histoblotting, a technique developed for the regional mapping of PrPSc deposition. The PrPSc pattern with the Me7 isolate was particularly interesting because it appeared to be confined to the hypothalamus and related structures—including the interstitial nucleus of the stria terminalis, the paraventricular nucleus of the thalamus, and periaqueductal grey. Additionally, the regions of PrPSc accumulation remained highly restricted, even though the incubation time for Me7H scrapie was significantly longer than with Sc237 and 139HH isolates. Neuropathological changes characterized by neuronal vacuolation and astrocytic gliosis were confined to those regions where PrPSc accumulated. These findings argue that the cell-specific propagation of prion isolates may be responsible for different properties exhibited by each of the isolates.

The existence of distinct prion isolates ("strains"), each capable of transmitting different scrapie syndromes with specific incubation times and clinical features, was first appreciated in goats (1). In mice, the distribution of vacuolar degeneration in the brain was also found to be distinctive for each prion isolate (2, 3). Genetic studies in mice revealed a single gene that markedly altered scrapie incubation times (4). Subsequent molecular genetic studies showed that the scrapie incubation-time gene is linked to or identical with the prion protein (PrP) gene (5–8).

Finding that the scrapie isoform of PrP (PrPSc) appears to be the sole functional component of the infectious scrapie prion particle (9–13) and that PrPSc and the normal cellular PrP isoform (PrPc) are encoded by a single-copy chromosomal gene (14, 15) and not by a foreign gene carried by the infectious prion particle (16) presents a major conundrum. Specifically biological diversity to date has been found encoded within a nucleic acid genome that provides a template for replication. Recent studies have suggested a cellular mechanism for PrPSc diversity that could account for multiple prion isolates.

Two Syrian hamster-adapted prion isolates (17–19), one causing signs of scrapie in 75 days and the other causing it in 160 days in hamsters, when propagated in a transgenic (Tg) mouse expressing high levels of the Syrian hamster gene [Tg(SHaPrP)7 mice] had similar incubation times of about 50 days (20, 21). The neuroanatomical distribution of PrPSc was markedly different for the two isolates in both hamsters and Tg(SHaPrP)7 mice, suggesting that the pattern of PrPSc accumulation is distinctive for each prion isolate and is independent of scrapie incubation time (22, 23). PrPSc and neuropathological changes were colocalized with both isolates in hamsters and mice, suggesting a causal relationship. Finding that PrPSc accumulation precedes the development of neuropathology is consistent with a cause–effect relationship (22, 24). In hamsters, scrapie incubation time correlated with the rate of PrPSc accumulation and the rate of formation of neuropathology (22, 24). These results indicate that the two clinical features that differentiate prion isolates, scrapie incubation time and distribution of neuropathology, are linked to the pattern and rate or accumulation of PrPSc. PrP gene ablation did not alter normal development or aging in mice, which argues that PrPSc accumulation and not PrPc inhibition is the cause of scrapie (25).

To test further the hypothesis that each prion isolate has a specific pattern of PrPSc accumulation in the brain, we obtained an isolate that was passed in mice and designated Me7 (4) and then passed into Syrian hamsters and designated Me7H (19). We report here that this isolate has a pattern of PrPSc accumulation in Tg(SHaPrP)7 mice that is distinct from the patterns we previously reported for the Sc237 and 139H isolates (22). These results argue that each prion isolate targets a different population of central nervous system cells for PrPSc formation. They raise the possibility that the PrPSc diversity that is proposed to underlie differences among prion isolates is determined by host cells.

MATERIALS AND METHODS

Sources of Scrapie Prions. A prion isolate passed in an inbred Syrian hamster was provided by Richard Marsh (University of Wisconsin, Madison, WI) (26) and subsequently designated Sc237 after repeated passage in randomly bred Syrian hamsters (Lak:LVG) purchased from Charles River Breeding Laboratories. The 139H and Me7H isolates were provided by Richard Kimberlin (Scrapie Advisory Service, Edinburgh) and Richard Carp (Institute for Basic Research, Staten Island, NY), respectively. 139H originated as an isolate designated 139A after >20 passages of the Chandler isolate in mice (27). Passage of mouse 139A isolate in Syrian hamsters (Lak:LVG) produced the 139H isolate (18). Me7H isolates were originally isolated from a natural case of scrapie in a Suffolk sheep by intragastric injection of spleen into random-bred Moredum mice (28). It was subsequently passaged...

Abbreviations: PrP, prion protein; PrPSc, scrapie isoform of prion protein; PrPc, cellular isoform of prion protein; Tg, transgenic; mAb, monoclonal antibody.

†To whom reprint requests should be addressed at: Department of Neurology, Health Sciences East-781, University of California, San Francisco, CA 94143-0518.
Fig. 1. Distribution of PrPSc revealed by histoblot for three prion isolates in four different regions of the brains of Tg(SHaPrP)7 mice with scrapie: level of the caudate nucleus and septum; level of the hippocampus and thalamus; the midbrain, at the level of the inferior colliculus; and the level of rostral pons. For the Sc237 and 139H prion isolates, the animals developed clinical signs between 49 and 55 days after inoculation. For the Me7H isolate, animals developed clinical signs between 180 and 200 days. Am, amygdala; As, accumbens septi; Cd, caudate nucleus; dB, diagonal band of Broca; Hp, hippocampus; Hy, hypothalamus; NC, neocortex; S, septal nuclei; st, interstitial nucleus of the stria terminalis; ZI, zona incerta. Thalamic nuclei in italics: Hb, habenula (epithalamus); L, lateral; ML, medial, pars lateralis; MM, medial, pars medialis; Pf, paraventricular; VPL, ventral posterior lateral; VPM, ventral posterior medial; Cst, corticospinal tract; IC, inferior colliculus; LC, locus coeruleus; mlf, medial longitudinal fasciculus; N Sp tr V, nucleus of the spinal trigeminal tract; N d raphe, dorsal nucleus of the raphe; PAG, periaqueductal grey.
passaged by intracerebral injection of brain extracts into random-bred Moredum mice (29) followed by passage in C57BL mice (30) and subsequently in Syrian hamsters, where it was designated Me7H isolate (19).

**Inoculation of Mice.** Weaning Tg(SHAPrP)7 mice were produced as described (20, 21) and inoculated in the thalamus with 30 μl of a 1:10 dilution of prion-infected brain homogenate. The Sc237 and 139H inocula were derived from Syrian hamsters after multiple passages. The Me7H inoculum was derived from a single passage in Tg(SHAPrP)7 mice after passage in Syrian hamsters. By endpoint titration, the Sc237 and 139H inocula contained ×10⁷ ID₅₀ units of scrapie infectivity (22); the titer of Me7H has not been determined.

**Histoblot.** Animals were sacrificed by asphyxiation with CO₂. The brain was removed and frozen in powdered dry ice. Ten-micrometer-thick cryostat sections were prepared and processed as described (23).

Three PrP-specific antibodies were used. R073 is a rabbit antiserum raised against purified SHAPrP 27–30, the proteinase K digestion product of PrPSc (31). The 13A5 monoclonal antibody (mAb) was also raised against SHAPrP 27–30 (32). The 3F4 mAb was raised against SHAPrP 27–30 (33) and obtained from R. Kascak (Institute for Basic Research, Staten Island, NY); it recognizes the epitope Met-Lys-His-Val (34). The dilutions used were as follows: R073, 1:1000–1:5000; 13A5 mAb supernatant, 1:2; 3F4 mAb ascitic fluid, 1:1000; and 3F4 mAb cultured cell supernatant, 1:1. Similar results were obtained for each of the prion isolates with the three antibodies.

An estimate of the regional concentration of PrPSc was made by measuring the average density of immunostaining in a brain region using video-assisted morphometric analysis as described (23).

Eight-micrometer-thick sections of formalin-fixed brain were stained with hematoxylin/eosin for evaluation of spongiform degeneration. Peroxidase immunohistochemistry with antibodies to glial fibrillary acidic protein was used to evaluate the degree of reactive astrocytic gliosis.

### RESULTS

Tg(SHAPrP)7 mice either heterozygous or homozygous for the SHAPrP transgene array were inoculated in the thalamus with the Me7H isolate. The mean scrapie incubation time for the first Me7H passage in heterozygous Tg(SHAPrP)7 mice was 144 ± 5.2 (SD) days (n = 7). For the second passage to heterozygous Tg(SHAPrP)7 mice, the mean incubation time was 201 ± 17 (SD) days (n = 21), and for homozygous transgenics, the mean time was 177 ± 21 (SD) days (n = 15).

Although the mean incubation times differed significantly for heterozygous and homozygous Tg(SHAPrP)7 mice (Student’s t test, P < 0.01), the distribution and intensity of the PrPSc signals in histoblots were indistinguishable when six heterozygotes were compared with four homoyzgotes (data not shown).

For Sc237 isolate, the mean incubation time was 48 ± 3.4 (SD) days (n = 26). For 139H isolate, the mean incubation time was 42 ± 7.2 (SD) days (n = 34). Histoblots made throughout the course of Sc237 and 139H scrapie showed that the fastest rate of PrPSc accumulation occurred after post-inoculation day 42 (data not shown) and, after 49 days, strong PrPSc signals were detected in multiple brain regions. For these reasons, we chose histoblots from Tg(SHAPrP)7 mice infected with either Sc237 or 139H prions, which presented with clinical signs of scrapie after 49 days for comparison with Me7H-inoculated animals. For all three prion isolates, mice were sacrificed within 1–2 days of the onset of clinical signs.

Differences in the distribution of PrPSc as a function of prion isolate were readily seen throughout the neuraxis in

<table>
<thead>
<tr>
<th>Location</th>
<th>PrPSc level, mean of arbitrary units ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc237</td>
<td>139H*</td>
</tr>
<tr>
<td>Neocortex</td>
<td>(9, 7)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>(9, 4)</td>
</tr>
<tr>
<td>Thalamus (all)</td>
<td>(6, 5)</td>
</tr>
</tbody>
</table>

* Values are indicated in parentheses; the first value represents n for selected brain regions, whereas the second represents an r value for selected nuclei of the thalamus.
† Statistically significant (Student’s t test, P < 0.05).
‡ Ventral posterior lateral nucleus.

histoblots from individual animals (Fig. 1). In Table 1, the mean values of PrPSc levels from four to nine animals are presented. Variations among these PrPSc levels presumably reflect biological variation of scrapie among animals and variations in the histoblot technique; nevertheless, the SDs were often sufficiently small to achieve statistically significant differences.

PrP amyloid plaques were found in Tg(SHAPrP)7 mice as symmetrical subcallosal deposits beginning 2–3 weeks after inoculation with both Sc237 and 139H prions (22), but none were found with Me7H prions. The PrP plaques were most readily seen in the histoblots of the subcallosal region with Sc237 because there was little or no immunostaining of the hippocampus to obscure them, in contrast to the brains of mice inoculated with 139H prions (Fig. 1).

Spongiform degeneration and reactive astrocytic gliosis were found to follow the accumulation of PrPSc in each brain region in Syrian hamsters and Tg(SHAPrP)7 mice inoculated with Sc237 or 139H prions (22, 24, 35). Similarly, there was an excellent correlation between PrPSc deposition and neuropathologic changes in Tg(SHAPrP)7 mice inoculated with Me7H prions (Fig. 2). No PrPSc accumulated in the hippocampus or most of the thalamus, and no spongiform degeneration or reactive astrocytic gliosis was found in these locations. In contrast, PrPSc accumulation in the hypothalamus, paraventricular nucleus of the thalamus, and interstitial nucleus of the stria terminals was accompanied by spongiform degeneration in the neuropil between nerve cell bodies and reactive astrocytic gliosis. The most intense neuropathologic changes occurred in the hypothalamus and the reticular formation of the brainstem.

### DISCUSSION

The results of this and an earlier study (22) indicate that the pattern of PrPSc in the brain is specific for and determined by each prion isolate. The differences and similarities can be grouped together into four categories (Table 2). From this analysis, different patterns of PrPSc accumulation were found to be reproducible for and characteristic of each prion isolate. The Me7H pattern was particularly interesting because it appeared to be confined to the hypothalamus and related structures—including the interstitial nucleus of the stria

---

**Table 1. Relative level of PrPSc in selected brain regions and thalamic nuclei as a function of the prion isolate after clinical signs of scrapie were evident in Tg(SHAPrP)7 mice**

<table>
<thead>
<tr>
<th>Location</th>
<th>PrPSc level, mean of arbitrary units ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc237</td>
<td>139H*</td>
</tr>
<tr>
<td>Neocortex</td>
<td>(9, 7)</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>(9, 4)</td>
</tr>
<tr>
<td>Thalamus (all)</td>
<td>(6, 5)</td>
</tr>
</tbody>
</table>

* Day 49 and 56 histoblots for selected brain regions but only day 56 histoblots for selected nuclei of the thalamus.
† Values are indicated in parentheses; the first value represents n for selected brain regions, whereas the second represents an r value for selected nuclei of the thalamus.
‡ Statistically significant (Student’s t test, P < 0.05).
Whether the posterior lateral nucleus.
Differential patterns of PrPSc accumulation for three distinct scrapie prion isolates in Tg(SHaPrP)7 mice

<table>
<thead>
<tr>
<th>Brain regions of PrPSc accumulation</th>
<th>Sc237</th>
<th>139H</th>
<th>M37H</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific to a single isolate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accumens septi</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebellar nuclei</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermittial nucleus, stria terminalis</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lateral septal nuclei</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neocortex</td>
<td></td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Periaqueductal grey</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Two isolates with similar deposition levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brainstem reticular formation</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalamus</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vestibular nuclei</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All three isolates with similar deposition levels</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Habenula</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Diagonal band of Broca</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Locus coeruleus</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial septal nucleus</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raphe nuclei of brainstem</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Region of brain that exhibits PrPSc accumulation for a particular isolate is indicated by +.

Although PrPSc was present in some of these regions with Sc237 and 139H prions, the deposition of PrPSc with Me7H Isole was significantly greater than that found with either of the other two isolates (see Table 1).

PrPSc/PrPC complexes (21, 36). Nascent PrPSc results from posttranslational modification of PrPC, probably in the endocytic pathway (37–40). In the brain, the conversion of PrPC to PrPSc may occur largely in neurons because they express the highest levels of PrP mRNA (41, 42). In mouse expressing two different PrPSc molecules, prion isolates interacted only with PrPC, which had a structure homologous with the PrPSc of the infecting prion (20, 21). The different patterns of prion isolate–determined PrPSc accumulation may reflect, therefore, neuron–determined differences in the secondary and/or tertiary structure of PrPC.

It has been suggested that the asparagine–linked carbohydrates of PrPSc might target infectious prions to specific sets of neuronal cells which express lectins on their surfaces that recognize complex type oligosaccharides (22). Such cells would have to synthetize PrPC with the same asparagine–linked carbohydrates to produce more of the same PrPSc and, thus, propagate a given isolate. Although PrPSc formation has been shown to occur in the absence of asparagine–linked glycosylation (43), it is unknown whether infectious prions can be formed with unglycosylated PrPSc or, more relevant to this discussion, whether different prion strains can be formed. Alternatively, isolate–specific information might be encoded within the tertiary and quaternary structure of PrPSc, but such a hypothesis must consider that the number of different conformations that PrPSc might assume is probably quite limited (44, 45). Whether either of these proposals is correct remains to be established.

We thank Mr. J. McCulloch for photographic prints and Mrs. J. Cayetano–Canlas for neurohistology. This work was supported by research grants from the National Institutes of Health (AG02132, AG08967, NS14069, and NS22786), the American Health Assistance Foundation, and the Science Foundation of Ireland. We also acknowledge the kind assistance of Mr. J. McCulloch. We also acknowledge the kind assistance of Mr. J. McCulloch. We also acknowledge the kind assistance of Mr. J. McCulloch.

Fig. 2. Spongiform degeneration of gray matter colocalizes with PrPSc deposition in Tg(SHaPrP)7 mice inoculated with Me7H prions. The approximate location of each photomicrograph is indicated in the diagram at top. Two regions in Me7H–infected animals in which little or no PrPSc was deposited (see Fig. 1), the CA1 region of the hippocampus (A) and the ventral posterior lateral (VPL) nucleus of the thalamus (B), were without vacuoles. Two regions with an intense PrPSc signal, the hypothalamus (C) and the paraventricular nucleus of the thalamus (D), were severely vacuolated. See Fig. 1 for definition of abbreviations. Hematoxylin/eosin–stained histological sections. (Bar in A = 50 μm and applies to all photomicrographs.)
Medical Sciences: DeArmond et al.
Foundation, as well as by gifts from Sherman Fairchild Foundation and National Medical Enterprises.