Incongruity between Prion Conversion and Incubation Period following Coinfection

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ABSTRACT
When multiple prion strains are inoculated into the same host, they can interfere with each other. Strains with long incubation periods can suppress conversion of strains with short incubation periods; however, nothing is known about the conversion of the long-incubation-period strain during strain interference. To investigate this, we inoculated hamsters in the sciatic nerve with long-incubation-period strain 139H prior to superinfection with the short-incubation-period hyper (HY) strain of transmissible mink encephalopathy (TME). First, we found that 139H is transported along the same neuroanatomical tracks as HY TME, adding to the growing body of evidence indicating that PrPSc favors retrograde transneuronal transport. In contrast to a previous report, we found that 139H interferes with HY TME infection, which is likely due to both strains targeting the same population of neurons following sciatic nerve inoculation. Under conditions where 139H blocked HY TME from causing disease, the strain-specific properties of PrPSc corresponded with the strain that caused disease, consistent with our previous findings. In the groups of animals where incubation periods were not altered, we found that the animals contained a mixture of 139H and HY TME PrPSc. This finding expands the definition of strain interference to include conditions where PrPSc formation is altered yet disease outcome is unaltered. Overall, these results contradict the premise that prion strains are static entities and instead suggest that strain mixtures are dynamic regardless of incubation period or clinical outcome of disease.

IMPORTANCE
Prions can exist as a mixture of strains in naturally infected animals, where they are able to interfere with the conversion of each other and to extend incubation periods. Little is known, however, about the dynamics of strain conversion under conditions where incubation periods are not affected. We found that inoculation of the same animal with two strains can result in the alteration of conversion of both strains under conditions where the resulting disease was consistent with infection with only a single strain. These data challenge the idea that prion strains are static and suggests that strain mixtures are more dynamic than previously appreciated. This observation has significant implications for prion adaptation.

Prion diseases are transmissible neurodegenerative diseases of animals, including humans, with no known effective treatment. Prion diseases of animals include scrapie of sheep and goats, chronic wasting disease of cervids, bovine spongiform encephalopathy of cattle, and transmissible mink encephalopathy (TME) of ranch–raised mink. Prion diseases of humans include kuru of the Fore people of Papua New Guinea, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome, and fatal familial insomnia (1–8). Human prion diseases are unique in biology in that they can have infectious, familial, or sporadic etiologies. Interestingly, brain material from all three etiologies is infectious and can transmit disease to animals.

The prion agent is comprised mainly, if not entirely, of PrPSc, the pathogenic conformation of the normal host protein PrPC (9–13). Formation of PrPSc occurs when existing PrPSc binds to PrPC, resulting in a conformational change of PrPSc to PrPSc. Fragmentation of the growing PrPSc fibril results in formation of new free ends for PrPSc binding, leading to exponential formation of PrPSc (14–16). In the absence of preexisting PrPSc, PrPC can spontaneously misfold into PrPSc; this process is enhanced by mutations of PrPC, providing a molecular basis for sporadic and familial forms of human prion disease (17).

Prion strains are operationally defined by differences in neuropathology that are observed under controlled experimental conditions. The PrP genotypes of the agent and host, titer of the agent, route of infection, and gender of the host can all influence the outcome of disease (18–25). When these agent and host parameters are controlled, strain-specific differences in disease phenotype such as incubation period, clinical signs, tissue tropism, host range, and neuropathology are observed.

Differences in the conformation of PrPSc may encode prion strain properties. Strain-specific differences in the biochemical properties of PrPSc, such as relative resistance to protease digestion, conformational stability, and relative α-helical and β-sheet contents, are observed (16, 26–29). Recent work suggests that host cofactors may also contribute to strain diversity (30–32). The relationship between the strain-specific biochemical properties of PrPSc and the observed differences in disease outcome are poorly understood.

More than one prion strain can be present in a single host in natural prion disease. Passage of sheep scrapie to wild-type or transgenic mice expressing ovine PrPSc results in the isolation of

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distinct prion strains (33–35). This suggests that more than one strain of sheep scrapie is present in the inoculum. Interspecies transmission can also lead to the generation of new strains (36). More direct evidence for the coexistence of prion strains has been found in humans with CJD. Two major types of PrPSc, type 1 and type 2, have been identified in CJD and are characterized by migration of the unglycosylated PrPSc polypeptide at 21 and 19 kDa, respectively (37, 38). Transmission of type 1 or type 2 CJD to transgenic mice expressing human PrPSc maintains the type-specific PrPSc properties, indicating that these are bona fide human prion strains (39). The cooccurrence of these PrPSc subtypes in natural cases of CJD has been identified based on PrPSc migration and by using anti-PrP antibodies that are type specific (40–44). While the relative percentage of CJD cases where type 1 and type 2 PrPSc coexist is controversial, it is clear that in natural prion disease a mixture of prion strains can occur.

Prion strains in the same host can interfere with each other. Strain interference was first observed in mice in which the long-incubation-period strain 22C was inoculated prior to superinfection with short-incubation-period strain 22A (45). As the interval between inoculation with the blocking strain 22C and superinfection with 22A increased, 22C was able to extend the incubation period of 22A or to completely block 22A from causing disease. Prion strain interference can also occur when the strains are inoculated at the same time, and in this instance the ratio of the strains coinfecting determines the outcome of disease (46, 47). When two prion strains are targeted to infect the same population of neurons, the onset of conversion of each strain is a critical parameter of strain interference (48). Under conditions where the blocking strain extends the incubation period of the superinfecting strain or completely blocks the superinfecting strain from causing disease, the blocking strain can suppress conversion of the superinfecting strain (48). Nothing is known about the conversion of the blocking strain following coinfection or superinfection with a short-incubation-period strain, whether or not there is interference.

MATERIALS AND METHODS

**Ethics statement.** All procedures involving animals were approved by the Creighton University Institutional Animal Care and Use Committee and comply with the Guide for the Care and Use of Laboratory Animals (49).

**Animal inoculations.** Male Syrian golden hamsters (Harlan-Sprague-Dawley, Indianapolis, IN) were used. Animals were inoculated in the sciatic nerve with 2 μl of a 1% (wt/vol) brain homogenate from a hamster at the clinical stage of disease that was infected with the 139H strain of transgenic nerve with 22A (51). The Western blot was developed with Pierce SuperSignal West Femto maximum-sensitivity substrate, according to the manufacturers’ instructions (Pierce, Rockford, IL), and imaged on a Nikon i80 microscope (Nikon, Melville, NY), and images were captured and identically processed for white balance using Adobe Bridge Cs6 (San Jose, CA). Tissue sections were analyzed at sampling intervals of no greater than 126 μm. The rate of PrPSc spread was determined as previously described (51). Briefly, the distance from the site of inoculation in the sciatic nerve to ventral motor neurons (VMNs), lateral vestibular nucleus, red nucleus, and hind limb motor cortex was measured in millimeters. The rate of PrPSc spread to each structure was calculated by dividing the distance in millimeters between the inoculation site and the specific CNS structure by the number of days postinfection (p.i.) when PrPSc immunoreactivity was first detected. Sections were stained for Nissl or with hematoxylin and eosin (H&E) as described previously (51, 52).

**Western blot analysis.** Western blot detection of PrPSc from brain homogenate was performed as described previously (53). Briefly, brain homogenate (5%, wt/vol) was digested with proteinase K (PK) at a final concentration of 2 U/ml (Roche Diagnostics Corporation, Indianapolis, IN) at 37°C for 60 min. PK digestion was terminated by incubating the samples at 100°C for 10 min. The samples were size fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 4 to 12% bis-Tris-acrylamide (NuPAGE; Invitrogen, Carlsbad, CA) and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% nonfat dry milk (Bio-Rad Laboratories, Hercules, CA) for 30 min. Hamster prion protein was detected using the mouse monoclonal anti-PrP antibody 3F4 (1:10,000; Chemicon, Temecula, CA). The Western blot was developed with Pierce SuperSignal West Femto maximum-sensitivity substrate, according to the manufacturer’s instructions (Pierce, Rockford, IL), and imaged on a Kodak 4000R Imaging Station (Kodak, Rochester, NY) as previously described (53).

**PrPSc conformational stability assay.** The conformational stability of PrPSc was determined as described previously (28). Brain homogenates were combined with 0.04 g/ml SDS or DPBS to a final concentration of 7.5% (wt/vol) homogenate and 1% (wt/vol) SDS or 0% (wt/vol) SDS, respectively. The resulting mixtures were incubated at 70°C for 10 min. Proteinase K (Roche Diagnostics, Indianapolis) was added to a final concentration of 2.0 U/ml, and the samples were incubated at 37°C for 30 min with shaking. Samples were brought to a final volume of 200 μl in DPBS, and 125-μg brain equivalents were analyzed for PrPSc content by 96-well immunoblot as previously described (54) using the monoclonal anti-PrP antibody 3F4 (1:10,000; Chemicon, Temecula, CA). To standardize between samples, the amount of PK-resistant PrPSc in the 1% (wt/vol) SDS group was divided by the amount of PK-resistant PrPSc in the 0% (wt/vol) SDS group (i.e., untreated), resulting in the relative percentage of 1% (wt/vol) SDS PK-resistant PrPSc compared to the total 0% (wt/vol) SDS PK-resistant PrPSc. The value of the standardized 1% (wt/vol) SDS PK-resistant HY TME PrPSc sample was adjusted to 100%, and the remaining sample values were similarly adjusted to the relative percentage of this control. The background was set to the signal intensity of an uninfected brain homogenate treated with 1% (wt/vol) SDS followed by digestion with PK.
RESULTS

Retrograde transsynaptic transport of 139H PrPSc. Sciatic nerve inoculation of the HY TME agent results in retrograde axonal transport along the same four descending motor pathways (51). To investigate whether the 139H strain of hamster-adapted scrapie uses the same pathways, the temporal and spatial spread of PrPSc in the lumbar spinal cord, brain stem, and brain was determined following sciatic nerve inoculation. At 25 days postinfection (p.i.), IHC failed to detect PrPSc in the CNS (Table 1). At 50 days p.i., PrPSc immunoreactivity was detected in lamina IX of the lumbar spinal cord ipsilateral to the side of sciatic nerve inoculation in 2 of the 3 inoculated animals (Table 1; Fig. 1O) and in the magnocellular aspect of the red nucleus contralateral to the side of sciatic nerve inoculation in one of three 139H-inoculated hamsters (Table 1; Fig. 1F). At 75 days p.i., PrPSc immunoreactivity in the lumbar spinal cord and red nucleus was detected in all three animals with a bilateral or contralateral pattern of immunoreactivity, respectively (Table 2). At 75 days p.i., PrPSc was first detected in the reticular formation (Fig. 1L), lateral vestibular nucleus (Fig. 1I), hind limb motor cortex (Fig. 1C), and interposed nucleus and reticular thalamic nucleus (Table 1). At 100 days p.i., increased PrPSc immunoreactivity was observed in the previously listed structures (Table 1) and was also detected in the ventroposterior thalamic nucleus (Table 1). At clinical disease, PrPSc immunoreactivity had become widespread throughout the CNS. In the mock-infected hamsters we failed to detect PrPSc at any time point. Hence, PrPSc immunoreactivity became widespread throughout the CNS in the 139H positive-control-infected group (Table 2). In the 139H positive-control group consisted of 5 hamsters inoculated in the sciatic nerve with the 139H agent and then inoculated 50 days later with uninfected brain homogenate (mock). In this group, all 5 hamsters developed clinical signs consistent with 139H infection (ataxia and weight gain) at 188 ± 3 days p.i. (Table 2).

Three experimental groups were generated by first inoculating the animals in the sciatic nerve with the 139H agent and then superinfecting the same sciatic nerve with HY TME at 25, 50, and 75 days after 139H infection. In the 25- and 50-day interval groups, all 5 animals developed clinical signs of hyperexcitability and ataxia at 78 ± 3 days p.i. (Table 2). In the 75-day interval group, 1 hamster developed clinical signs of hyperexcitability and ataxia at 87 ± 6 days postinfection, respectively, which does not significantly differ (P > 0.05) from the results for the HY TME positive-control-infected group (Table 2). In the 75-day interval group, 1 hamster developed clinical signs of hyperexcitability and ataxia at 87 days postinfection, consistent with HY TME infection. The remaining 4 hamsters developed clinical signs of ataxia and weight gain at 188 ± 3 days p.i., which does not significantly differ (P > 0.05) from the results for the positive-control 139H-infected group (Table 2). Western blot analysis of 250-μg brain equivalents of PK-digested hamster homogenate from all clinically affected animals contained PrPSc, confirming the clinical diagnosis of prion disease (Fig. 2). PK-digested PrPSc from

139H and HY TME strain interference. To investigate whether 139H could interfere with HY TME, hamsters were infected in the sciatic nerve with 139H agent at 25, 50, or 75 days prior to superinfection with HY TME agent (Table 2). The sciatic nerve route was chosen since both HY TME and 139H are transported by the same descending motor tracks (Table 1; Fig. 1); therefore, both of these strains will be targeted to the same population of neurons in each brain area. In this study, all three intervals were based on the temporal and spatial spread of PrPSc in hamsters infected in the sciatic nerve with 139H (Table 1).

Five hamsters were inoculated in the sciatic nerve with uninfected brain homogenate (mock) 50 days prior to infection with HY TME to serve as HY TME positive controls. All 5 positive-control animals developed clinical signs of HY TME (hyperexcitability and ataxia) at 82 ± 3 days p.i. (Table 2). The 139H positive-control group consisted of 5 hamsters inoculated in the sciatic nerve with the 139H agent and then inoculated 50 days later with uninfected brain homogenate (mock). In this group, all 5 hamsters developed clinical signs consistent with 139H infection (ataxia and weight gain) at 188 ± 3 days p.i. (Table 2).
both HY TME- and 139H-infected hamsters had similar glycoform ratios and an unglycosylated PrP Sc polypeptide that migrated at 21 kDa; therefore, it was not possible to confirm which strain caused disease by using migration or glycoform ratio (Fig. 2). Overall, we conclude that 139H can interfere with HY TME.

To determine whether 139H or HY TME caused disease in the coinfected hamsters, we utilized a modified PrPSc conformational stability assay. HY PrPSc has a higher conformational stability in SDS than 139H PrPSc, with $[SDS]_{1/2}$ values of 1.14% ± 0.03% (wt/vol) versus 0.50% ± 0.01%, respectively (28). Brain homogenates are treated with or without 1% (wt/vol) SDS, followed by treatment with PK. The remaining PrPSc in the 1% (wt/vol) SDS-treated sample was represented as percentage of that for the 0% (wt/vol) SDS sample. Since each sample was normalized to its respective 0% (wt/vol) SDS control, reductions in PrPSc concentration in the 1% (wt/vol) SDS group were due to increased sensitivity to PK digestion under these conditions and not to differences in the starting amount of PrPSc. Setting the relative PrPSc concentration in the 1% (wt/vol) SDS-treated HY TME control as 100% allows us to determine if HY or 139H PrPSc is present in the CNS of the coinfected hamsters.

**FIG 1** Distribution of PrPSc in spinal cord, brain stem, and brain of hamsters infected in the sciatic nerve with the 139H agent. (A to C) Nissl staining (A) and PrPSc immunostaining (B and C) of the contralateral hind limb motor cortex from mock-infected (B) or 139H-infected (C) hamsters at 75 days postinfection. (D) Nissl staining of the mesencephalon containing the red nuclei (dashed outline). (E and F) PrPSc immunoreactivity was detected in the ventrolateral portion of the contralateral red nucleus at 75 days postinfection (F) but was not detected in mock-infected animals (E). (G) Hematoxylin and eosin (H&E) staining of the pons ipsilateral to the side of inoculation that contains the lateral vestibular nucleus. (H and I) At 100 days postinfection, PrPSc immunoreactivity is detected in the 139H-infected tissue (I) but is not detected in mock-infected animals (H). (J to L) H&E-stained section of the pons (J) containing the reticular formation contains PrPSc immunoreactivity in 139H-infected (L) but not in mock-infected (K) hamsters. (M) Nissl-stained section of lumbar spinal cord that contains ventral motor neurons (VMNs) whose axons are contained in the sciatic nerve. (N and O) PrPSc immunoreactivity of lumbar spinal cord from uninfected animals failed to detect PrPSc (N), while in 139H-infected hamsters at 50 days postinfection, PrPSc deposits were associated with VMNs ipsilateral to the side of inoculation (O). Scale bars, 200 μm in main panels and 100 μm in insets.
TABLE 2 Incubation periods and clinical signs in hamsters inoculated with the 139H strain prior to superinfection with the HY TME agent

<table>
<thead>
<tr>
<th>First inoculation</th>
<th>Interval (days) between inoculations</th>
<th>Second inoculation</th>
<th>Clinical signs</th>
<th>PrPSc</th>
<th>No. affected/no. inoculated</th>
<th>Onset of clinical symptoms (avg days ± SD) after:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mock</td>
<td>50</td>
<td>HY TME</td>
<td>HY TME</td>
<td>HY</td>
<td>5/5</td>
<td>NAa</td>
</tr>
<tr>
<td>139H</td>
<td>25</td>
<td>HY TME</td>
<td>HY TME</td>
<td>HY/139H</td>
<td>5/5</td>
<td>78 ± 3b</td>
</tr>
<tr>
<td>139H</td>
<td>75</td>
<td>HY TME</td>
<td>HY TME</td>
<td>HY/139H</td>
<td>5/5</td>
<td>87 ± 6b</td>
</tr>
<tr>
<td>139H</td>
<td>50</td>
<td>Mock</td>
<td>139H</td>
<td>139H</td>
<td>4/5</td>
<td>113 ± 3</td>
</tr>
<tr>
<td>139H</td>
<td>50</td>
<td>HY TME</td>
<td>139H</td>
<td>139H</td>
<td>5/5</td>
<td>NAa</td>
</tr>
</tbody>
</table>

a NA, not applicable.
b Incubation period similar to that for control animals inoculated with the HY TME agent alone (P > 0.05).
c Incubation period similar to that for control animals inoculated with the 139H agent alone (P > 0.05).

(Fig. 3A). The conformational stability assay can identify mixtures of 139H and HY PrPSc. We compared the relative 1% (wt/vol) SDS-resistant PrPSc of a mixture of 139H- and HY TME-infected brain homogenates (50% 139H and 50% HY) to that of homogenates with 139H (100% 139H) or HY TME (100% HY) alone (Fig. 3A). Due to the presence of PrPSc from the two different strains, the relative 1% (wt/vol) SDS-resistant PrPSc of the 139H/HY homogenate mixture was not similar to that of either the homogenate with HY TME alone (P < 0.05) or the homogenate with 139H alone (P < 0.05).

In the 75-day strain interference interval group, 1 animal had clinical signs and an incubation period consistent with HY TME infection (Table 2) that contained high-stability PrPSc similar (P > 0.05) to that in the HY TME control (Fig. 3B, lane 11). The remaining 4 hamsters in the 75-day group had clinical signs and incubation periods that were consistent with 139H infection (Table 2), and they contained low-stability PrPSc which was similar (P > 0.05) to that in the HY TME control (Fig. 3B, lanes 12 to 15). In the 50-day interval group, all 3 animals tested had clinical signs and incubation periods that were consistent with HY TME infection (Table 2). In the 50-day interval group, two animals contained low-stability PrPSc similar (P > 0.05) to that in the 139H control (Fig. 3B, lanes 8 and 10), and the third tested animal had relative 1% (wt/vol) SDS-resistant PrPSc that was not similar to that in either the HY TME (P < 0.05) or 139H (P < 0.05) control (Fig. 3B, lane 9). Frozen tissue samples were unavailable for the remaining 2 animals in the 50-day group. In the 25-day interval group, all 5 animals had incubation periods and clinical signs consistent with HY TME infection (Table 2). In this group
one animal contained high-stability PrPSc similar ($P > 0.05$) to that in the HY TME control (Fig. 3B, lane 4), three animals contained relative 1% (wt/vol) SDS-resistant PrPSc that was not similar to that in either the HY TME ($< 0.05$) or 139H ($< 0.05$) control (Fig. 3B, lanes 3, 5, and 6), and one animal (Fig. 3B, lane 7) had a high variance that precluded meaningful statistical analysis.

Overall, in the 75-day interval group, the strain-specific PrPSc stability properties corresponded with the clinical signs and incubation period of disease (Table 2; Fig. 3). In the 25- and 50-day interval groups, all of the hamsters exhibited incubation periods and clinical signs indistinguishable from those in HY TME-infected control animals (Table 2), but a subset of these animals contained 1% (wt/vol) SDS-resistant PrPSc that was intermediate between those in HY TME- and 139H-infected controls (Fig. 3), suggesting that they contained a mixture of both 139H and HY PrPSc.

DISCUSSION

Transsynaptic neuronal transport of PrPSc along known neuro-anatomical pathways has been reported following peripheral inoculation into several different targets and appears to be a characteristic aspect of prion neuroinvasion and transport. Ocular inoculation of prions results in a sequential spread of spongiform degeneration that is consistent with anterograde transport along well-defined neuroanatomical tracts (55, 56). Intradural inoculation of prions resulted in transport to the agent to the ipsilateral trigeminal ganglia, consistent with transport along the mandibular branch of the trigeminal nerve (57). Moreover, prion inoculation into the tongue results in retrograde axonal transport via the hypoglossal nerve ipsilateral to the hypoglossal nucleus in the medulla, followed by subsequent transport to brain areas that project axons to this nucleus, providing further evidence for transsynaptic axonal transport along both peripheral nerves and CNS tracts (58). Our group and others have shown that sciatic nerve inoculation results in direct neuronal spread of prions in rats, mice, and hamsters (51, 59–61). Detailed analysis of the temporal and spatial spread of PrPSc in the peripheral nervous system and CNS of hamsters inoculated with either the HY or DY TME strain indicated that both of these strains were retrogradely transported along the same 4 descending neuroanatomical pathways. In the current study, the initial deposition of 139H PrPSc in the lumbar cord was associated with ventral motor neurons in lamina IX, similar to the case for HY and DY TME-infected hamsters (Fig. 1; Table 1) (51). The temporal and spatial spread of 139H PrPSc followed the same 4 descending motor pathways: the reticulospinal, vestibulospinal, rubrospinal, and corticospinal tracks (Fig. 1; Table 1). These data are consistent with the hypothesis that PrPSc is able to cross the synaptic cleft between synaptically connected neurons within a pathway.

There are strain specific differences in the rate of prion spread in the nervous system. The rate of HY PrPSc spread in the nervous system appears faster compared to DY PrPSc (51). The rate of HY PrPSc formation is greater than DY PrPSc, therefore, it is unclear if the observed higher rate of HY PrPSc spread along neuroanatomical pathways is due to faster axonal transport, or due to the faster formation of HY PrPSc. Interestingly, the rate of PrPSc formation is similar between 139H and DY TME, yet 139H has a higher rate of spread (28). This observation suggests that factors in addition to the rate of PrPSc formation are involved in strain specific differences in prion transport.

Our results showed that the 139H strain can interfere with, or completely block, the emergence of HY TME. A previous study determined that intracerebral inoculation of 139H up to 63 days prior to superinfection with Sc237 failed to extend the incubation period of Sc237 (62). This result indicated that 139H was unable to interfere with the superinfecting strain. The authors hypothesized that the failure of 139H to interfere with Sc237 was due to conversion of 139H and Sc237 in different populations of cells (62). In the current study, the sciatic nerve route of inoculation was found to direct both 139H and HY TME to the same population of neurons (Fig. 1; Table 1) (51) and to result in 139H interfering with HY TME. These data indicate that the failure of 139H to interfere with Sc237 was not due to an intrinsic inability of 139H to act as a blocking strain but is consistent with the hypothesis that prion strains must infect common populations of neurons for interference to occur (48). We cannot exclude the possibility, however, that differences between HY TME and Sc237 could account for the contradictory interference results, although this is unlikely since Sc237 and HY TME are similar, if not identical, strains.

The blocking strain can inhibit conversion of the superinfecting strain PrPSc. Lesion profile, clinical signs, incubation period of disease, and strain-specific differences in PrPSc Western blot profiles can be used to determine the predominant strain in the infected animals at terminal disease. Under conditions where the blocking strain extends the incubation period of the superinfecting strain, there is evidence that the blocking strain inhibits the rate of accumulation of superinfecting PrPSc (48). In situations where the blocking strain completely inhibited the superinfecting strain from causing disease, the blocking strain suppressed conversion of the superinfecting strain (48, 63). The results for the 75-day interval group in the current study are consistent with these previous findings. In the 75-day interval group, 139H was able to completely block HY TME from causing disease and suppressed HY PrPSc accumulation in 4 of the 5 animals (Table 2; Fig. 3). The fact that one animal in this group had the HY PrPSc and incubation period may be due to an incomplete inhibition by 139H infection. In previous studies in hamsters coinfected with the DY and HY TME strains under conditions where DY TME was able to completely block HY TME emergence, protein misfolding cyclic amplification (PMCA) of brain homogenate from these animals indicated that a small amount of HY TME persisted in these animals (63). While we did not determine whether low levels of HY TME persist in this study, taken together, these results suggest that the blocking strain can inhibit, but not extinguish, superinfecting strain conversion. In this model, altering the conditions of prion formation can lead to the emergence of different strains (64–68).

Strain interference can alter the accumulation of the blocking prion strain. Using a modification of the PrPSc conformational stability assay, we were able to determine whether the brain contained HY TME, 139H, or a mixture of PrPSc from each strain. It is important to emphasize that under the assay conditions, we can only conclude whether a mixture of HY and 139H PrPSc is present and cannot quantify the amount of PrPSc from each strain. With this limitation in mind, we found evidence of a mixed population of HY and 139H PrPSc in the 25- and 50-day interval groups (Fig. 3). In both of these interval groups, the clinical signs and incubation period of disease did not differ from those for animals inoculated with HY TME alone, indicating that 139H was not able to extend the incubation period of HY TME under these conditions. Previous strain interference studies have indicated that the presence of PrPSc in the lumbar spinal cord corresponds with the ability of the blocking strain DY TME to interfere with or completely
block superinfection with HY TME (48). At 25 and 50 days 139H infection, PrPSc is not detected or is weakly positive in the lumbar spinal cord (Table 1). In coinfected animals, in the 25- and 50-day interval groups, 139H does not extend the incubation period of HY TME, consistent with our previous work (Table 2) (48). Interestingly, in the 25- and 50-day interval groups, where 139H was not able to extend the incubation period of HY TME, a mixture of both 139H and HY PrPSc was present in these animals at terminal disease (Fig. 3). These data indicate that in coinfected animals where the blocking strain does not affect the incubation period or clinical signs of disease, interference between the blocking and superinfecting strains can still occur. In this scenario, 139H is able to interfere with HY TME conversion, but is unable to delay HY TME from reaching and affecting the HY TME clinical target areas.

We interpret the intermediate PrPSc stability profile as a mixture of PrPSc from both the HY TME and 139H strains; however, we cannot exclude the possibility that the intermediate PrPSc stability profile is the result of a strain other than 139H or HY TME. In this scenario, it is possible that the presence of both 139H and HY PrPSc favors the de novo formation of a new strain-specific conformation of PrPSc or that interactions between 139H and HY TME allow for the emergence of a preexisting strain (64, 69). Future serial passage experiments will differentiate between these possibilities. Finally, the mixed-strain PrPSc profile in hamsters with a single-strain phenotype may provide a basis for the observation in human prion disease where more than one PrPSc strain profile is observed with the clinical presentation of disease (40, 70).

Overall, these studies demonstrate that in mixed-strain infections, the dynamics of PrPSc formation of each strain are more complex than previously appreciated. This work adds to the growing body of literature that suggests that prions are a dynamic mixture of substrains (64). The ratio of strains can be altered depending on the environment and, as demonstrated here, the initial ratio of strains present. Importantly, in coinfect ed animals, each strain can persist regardless of the outcome of infection.

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