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Adaptation and Selection of Prion Protein Strain Conformations following Interspecies Transmission of Transmissible Mink Encephalopathy

JASON C. BARTZ,[1]† RICHARD A. BESSEN,[2]* DEBBIE MCKENZIE,[1] RICHARD F. MARSH,[1]‡ AND JUDD M. AIKEN[1]*

Animal Health and Biomedical Sciences, University of Wisconsin, Madison, Wisconsin 53706,[1] and Medical Microbiology and Immunology, Creighton University, Omaha, Nebraska, 68178[2]

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Interspecies transmission of the transmissible spongiform encephalopathies (TSEs), or prion diseases, can result in the adaptation and selection of TSE strains with an expanded host range and increased virulence such as in the case of bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. To investigate TSE strain adaptation, we serially passaged a biological clone of transmissible mink encephalopathy (TME) into Syrian golden hamsters and examined the selection of distinct strain phenotypes and conformations of the disease-specific isoform of the prion protein (PrP^{Sc}). The long-incubation-period drowsy (DY) TME strain was the predominate strain, based on the presence of its strain-specific PrP^{Sc} following interspecies passage. Additional serial passages in hamsters resulted in the selection of the hyper (HY) TME PrP^{Sc} strain-dependent conformation and its short incubation period phenotype unless the passages were performed with a low-dose inoculum (e.g., 10^{-5} dilution), in which case the DY TME clinical phenotype continued to predominate. For both TME strains, the PrP^{Sc} strain pattern preceded stabilization of the TME strain phenotype. These findings demonstrate that interspecies transmission of a single cloned TSE strain resulted in adaptation of at least two strain-associated PrP^{Sc} conformations that underwent selection until one type of PrP^{Sc} conformation and strain phenotype became predominant. To examine TME strain selection in the absence of host adaptation, hamsters were coinfected with hamster-adapted HY and DY TME. DY TME was able to interfere with the selection of the short-incubation HY TME phenotype. Coinfection could result in the DY TME phenotype and PrP^{Sc} conformation on first passage, but on subsequent passages, the disease pattern converted to HY TME. These findings indicate that during TSE strain adaptation, there is selection of a strain-specific PrP^{Sc} conformation that can determine the TSE strain phenotype.

The transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases of humans and animals. A hallmark characteristic of the TSEs is the conversion of the cellular prion protein (PrP^{C}) to an abnormal conformation (PrP^{Sc}) that progressively accumulates during disease (28). A new phenotype of human Creutzfeldt-Jakob disease (CJD), called variant CJD, that is clinically and pathologically distinct from classic forms of CJD was first diagnosed in 1996 and appears to be a result of interspecies transmission of bovine spongiform encephalopathy (BSE) to humans (12, 31). The BSE strain is highly pathogenic and is also responsible for the BSE-related spongiform encephalopathies found in domestic cats and many species of zoo animals (8). It has been proposed that BSE resulted from interspecies transmission of sheep scrapie, or a previously unrecognized TSE, to cattle via contaminated feed additives. Adaptation and selection of the BSE strain from sheep scrapie or other sources was likely a result of the recycling of BSE-contaminated cattle-derived protein supplements during several cattle-to-cattle passages.

Transmission of a TSE to a new host species is an inefficient process due to a phenomenon known as the species barrier effect (26). This effect refers to an increase in the length of the incubation period upon interspecies transmission compared to the incubation period found in the donor host species. TSE adaptation and selection occur during subsequent serial passages in the new host and lead to a reduction and eventual stabilization of the incubation period (15). For example, the mouse-adapted Chandler strain of scrapie has a 160-day incubation period in C57BL/6 mice, but upon interspecies transmission to Syrian hamsters the incubation period increases to 380 days. Additional serial passages in hamsters results in a reduction and stabilization of the incubation period to 75 days. One factor that can influence the species transmission barrier is the difference in the amino acid sequence between the donor PrP^{Sc} and the host prion protein PrP^{C} (2, 11, 29, 30). Differences in PrP sequence can result in an inefficient propagation of PrP^{Sc} and prolonged incubation periods upon interspecies transmission. On second passage in the same host species, the amino acid sequences of the donor PrP^{Sc} and host PrP^{C} are identical and formation of additional PrP^{Sc} is more efficient, resulting in shorter incubation periods. In some cases, differences in the PrP sequence between the host and donor PrPs can result in a complete interference in PrP^{Sc} formation and development of TSE disease (29, 30).

Interspecies TSE transmission can lead to the maintenance of the original strain phenotype or a change in the TSE strain

* Corresponding author. Mailing address for Richard A. Bessen: Medical Microbiology & Immunology, Creighton University, 2500 California Plaza, Omaha, NE 68178. Phone: (402) 280-3072. Fax: (402) 280-1875. E-mail: rbessen@creighton.edu. Mailing address for Judd M. Aiken: Animal Health & Biomedical Sciences, University of Wisconsin, 1656 Linden Dr., Madison, WI 53706. Phone: (608) 262-7362. Fax: (608) 262-7420. E-mail: aiken@ahabs.wisc.edu.

† Present address: Medical Microbiology & Immunology, Creighton University, Omaha, NE 68178.

‡ Deceased.

phenotype. The selection of TSE strains that have an advantage in the new host is illustrated by the serial transmission of the drowsy goat source of scrapie into rats, mice, and hamsters (20). Early hamster passages identified strain 431K that maintained pathogenicity for mice, but after several additional passages in hamsters, strain 263K was the only strain present. The selection of 263K was accompanied by a shortening of the incubation period and a loss of pathogenicity in mice. This suggested that 431K was the original or dominant murine pathogen, but passage into hamsters selected for, or gave rise to, the variant 263K strain that had a selective advantage in hamsters. The TSE strain with the shortest incubation period in the new host species is the predominate one found between the second and fifth intraspecies serial passage (20, 21). The dose of each TSE strain in the mixture is also likely to influence the outcome of interspecies transmission and subsequent selection upon intraspecies passage (20).

The basis of TSE strain diversity is proposed to be determined by either differences in the conformation of PrP^{Sc} among strains (4, 5, 7, 27) or mutation of the putative TSE nucleic acid genome (9). To investigate the molecular basis of TSE adaptation and selection upon interspecies transmission, we used a model of transmissible mink encephalopathy (TME) in Syrian hamsters (6, 24). Previously, we identified two strains of TME after interspecies passage into hamsters that were called hyper (HY) and drowsy (DY) TME. Phenotypic properties between these TME strains differed with respect to incubation period (e.g., 65 versus 165 days), clinical signs (e.g., hyperexcitability and ataxia versus progressive lethargy), neuropathology, brain distribution of PrP^{Sc}, and TME brain titers (4, 5). PrP^{Sc} isolated from the brains of HY and DY TME-infected hamsters exhibit strain-specific conformations based on differences in sedimentation properties, susceptibility to protease cleavage, N-terminal cleavage sites by proteases, and β-sheet secondary structure (4, 5, 13). Using a PrP cell-free conversion assay, PrP^{C} could be converted into a PrP molecule resembling the HY or DY PrP^{Sc} strain-specific conformation when directly incubated with the respective PrP^{Sc} strains, indicating that a similar PrP^{C} molecule can be folded into multiple conformations via direct PrP^{C}-PrP^{Sc} interactions (3). These findings support the protein-only model on the nature of the TSE agent and TSE strain diversity. We now report that interspecies transmission of a biological clone of TME results in the adaptation of both the HY and DY strain-specific PrP^{Sc} conformations, often present as a mixture, which can undergo selection during subsequent intraspecies hamster passage. The PrP^{Sc} strain pattern was found prior to stabilization of the TME strain phenotype, indicating that the PrP^{Sc} conformation can determine TSE biological characteristics. Furthermore, direct competition studies between HY and DY TME demonstrated that changes in the PrP^{Sc} conformation accompany a switch in TME phenotypes.

MATERIALS AND METHODS

Biological cloning of TME in mink. To produce a biological clone of the Stetsonville TME agent, we performed a series of endpoint dilution experiments as described previously (9). A TME-infected mink brain from the Stetsonville ranch was homogenized to 10% (wt/vol) in phosphate-buffered saline (PBS) using disposable syringes and plasticware. Serial 10-fold dilutions of brain homogenates (10^{-1} to 10^{-8}) were intracerebrally (i.c.) inoculated into weanling mink. Control animals were inoculated with PBS alone. All animals were observed weekly for the onset of neurological disease. Clinically ill animals were sacrificed by CO_2 asphyxiation, and brain tissue was removed with new instruments.

The biological clone of the Stetsonville TME agent (24) was prepared from brain tissue of a TME-positive mink that received the highest dilution of inoculum. The brain was subsequently homogenized in PBS and two additional serial 10-fold dilutions of the brain homogenate, and inoculations were performed in order to obtain TME inocula that had the highest likelihood of containing a single strain of TME agent.

Interspecies transmission experiments. Weanling Syrian golden hamsters were i.c. inoculated with 30 μl of a 10% (wt/vol) TME brain homogenate that was biologically cloned in mink by three successive endpoint dilution experiments. Control animals were inoculated with buffer alone. All animals were observed daily for the onset of neurological disease as described previously (6). Animals were sacrificed by CO_2 asphyxiation, and brain tissue was removed using new surgical instruments designated for these studies. Brains from individual animals were homogenized using disposable syringes and plasticware. Serial hamster-to-hamster passages were performed by i.c. inoculation at dilutions of 10^{-1} or 10^{-5}. Up to five additional hamster passages were performed, depending on the time at which incubation period and clinical signs stabilized.

Hamster TME competition experiments. Syrian golden hamsters were i.c. inoculated with either biologically cloned DY TME (10^{-2} brain dilution or $10^{5.4}$ 50 lethal doses per g of tissue) or biologically cloned HY TME (10^{-6} to 10^{-9} brain dilutions or $10^{3.8}$ to $10^{0.8}$ 50% lethal doses per g of tissue) alone or with a mixed inoculum containing DY TME (10^{-2} brain dilutions) and HY TME at either 10^{-6}, 10^{-7}, 10^{-8}, or 10^{-9} dilutions. Brain titers were calculated by endpoint dilution analysis using the Kärber method (25). Animals exhibiting clinical signs of neurological TME disease were sacrificed, and brain material was serially passaged at a 10^{-2} dilution as described above.

Tissue preparations and PrP^{Sc} Western blot analysis. Two protocols were used for the preparation of brain material for PrP^{Sc} analysis. In the abbreviated protocol, a 10% (wt/vol) brain homogenate was digested with proteinase K (50 μg/ml) for 30 min at 37°C. Phenylmethylsulfonyl fluoride was added to 5 mM. For the second method, brain tissue was homogenized to 10% (wt/vol) in 10% sarcosyl in buffer A (10 mM Tris [pH 8.3], 133 mM NaCl, 1 mM EDTA, and 1 mM dithiothreitol). Following ultracentrifugation in a Beckman Instruments TLA-45 rotor at 20,000 rpm (22,000 × *g*) for 20 min in a TL-120 ultracentrifuge, the supernatant was removed and centrifuged in a TLA-45 rotor at 45,000 rpm (125,000 × *g*) for 1 h. The pellets were resuspended in Tris-buffered saline (1 μl per g of tissue), proteinase K was added to 10 μg/ml, and the mixture was incubated at 37°C for 1 h. Phenylmethylsulfonyl fluoride was added to 5 mM, and the preparation was brought to 1% sarcosyl–10% NaCl in buffer A. Following ultracentrifugation in a TLA-45 rotor at 45,000 rpm for 1 h, the pellet was washed and resuspended in sample buffer for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

Brain samples were analyzed by SDS-PAGE (15% gel), transferred to Immobilon P membrane (Millipore), and incubated with monoclonal antibody 3F4 (1:40,000; a gift from Richard Kascsak, Institute for Basic Research in Developmental Disabilities [18]) or rabbit polyclonal R20 (1:3,000; a gift from Byron Caughey, NIH Rocky Mountain Laboratories [14]) for 2 h at room temperature or at 4°C overnight as described previously (5). The secondary antibody used was either anti-mouse immunoglobulin G conjugated to alkaline phosphatase (1:1,000; Sigma, St. Louis, Mo.) or goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (1:1,000; Bio-Rad Laboratories, Hercules, Calif.). Detection of the immunoblot signal was performed using the nitrobluetetrazolium method or chemiluminescence (Amersham).

RESULTS

Interspecies transmission of cloned TME. A biological clone of the Stetsonville TME isolate was produced by 10-fold serial dilutions of a mink brain homogenate and inoculation into pastel mink. The brains from the animals that developed TME after receiving the highest dilution of inocula (e.g., 10^{-5} or 10^{-6}) were collected, and endpoint dilutions and inoculations were repeated two additional times. This approach has been used to isolate a homogenous population of a single TSE strain from a mixture containing more than a single strain (9).

Four independent biological clones of mink-passaged TME were i.c. inoculated into a total of 88 Syrian golden hamsters, and the incubation periods, clinical symptoms, and PrP^{Sc} patterns were determined. For TME clone 4, disease was observed in 12 out of 21 hamsters with an incubation period ranging from 219 to 522 days ($\bar{x}$ = 450 ± 83 days [standard error of the mean]; Table 1). Analysis of PrP^{Sc} after limited proteinase K digestion (of 7 out of 12 clinically affected hamsters) revealed that six (incubation period = 473 ± 24 days) had a polypeptide banding pattern characteristic of the DY TME strain in hamsters. This pattern was characterized by three polypeptide bands (i.e., unglycosylated, monoglycosylated, and diglycosylated isoforms of PrP) ranging from 19 to 29 kDa (i.e., 19-kDa pattern; Fig. 1B, lane DY) (6). The one remaining PrP^{Sc} pattern (incubation period = 219 days) was

TABLE 1. Interspecies transmission of biologically cloned TME to Syrian hamsters

Animal no.	PrP^{Sc} pattern (kDa)	Incubation period (days)
1	19	446
2	ND[a]	501
3	ND	522
4	19	473
5	ND	473
6	21	219
7	ND	383
8	19	501
9	ND	501
10	19	453
11	19	501
12	19	461
13–21	ND	>600

[a] ND, not determined.

similar to that of the HY TME strain, which has a molecular size ranging from 21 to 31 kDa and will be referred to as the 21-kDa pattern (Fig. 1B, lane HY). The clinical symptoms upon interspecies transmission to hamsters were not characteristic of the hamster-adapted HY and DY TME strains described previously but were variable in type and duration. The results from TME clone 4 were similar to those obtained for the other three biological clones of TME (data not shown).

Two additional criteria were used in discriminating the HY and DY PrP^{Sc} 21- and 19-kDa banding patterns. The migration distance of the top edge of the diglycosylated PrP^{Sc} band at approximately 30 kDa also differs by 1 to 2 kDa between the TME strains (Fig. 2). In addition, rabbit polyclonal antibody R20, which is directed to the C-terminal portion (i.e., amino acids 218 to 232) of the murine prion protein, has stronger immunoreactivity in the 6- to 8-kDa molecular size range of DY PrP^{Sc} compared to HY PrP^{Sc} (Fig. 2).

Intraspecies transmission of TME. Additional serial passages of the hamster-passaged cloned TME agent into Syrian hamsters resulted in a reduction of the incubation period and the selection of distinct TME strain phenotypes. Two individual hamsters inoculated with TME clone 4 were further passaged at a 10^{-2} dilution into six hamsters. The most common outcome of second passage of the TME clones into hamsters is illustrated in passage line A (Fig. 1A). Passage of animal 11, which had an incubation of 501 days and the 19-kDa PrP^{Sc} pattern, resulted in an incubation period of 167 ± 10 days ($n = 6$), the 19-kDa PrP^{Sc} pattern, and clinical signs predominantly of lethargy but with some evidence of hyperexcitability. Since these animals did not exhibit the classical HY or DY clinical signs, they were considered to represent a syndrome sharing features of both phenotypes. At the next passage, the dilution of the brain inocula was an important determinant for the outcome of TME strain selection. Third hamster passage with a 10^{-1} brain dilution inoculum resulted in an incubation period of 129 ± 4 days, no change in the clinical symptoms, and a mixture of the 19- and 21-kDa PrP^{Sc} patterns (Fig. 1C, lane 3). The transition from DY to HY TME continued at fourth hamster passage, where the incubation period (i.e., 59 ± 2 days), clinical symptoms, and 21-kDa PrP^{Sc} band pattern characteristic of HY TME were found. However, third hamster passage at a 10^{-5} brain dilution from the same animal having a 159-day incubation period resulted in an increase in the incubation period to 193 ± 11 days, clinical disease characterized by a progressive lethargy, and the 19-kDa PrP^{Sc} pattern (Fig. 1A, passage line A). This DY TME phenotype was maintained on additional passages at low dilutions (e.g., 10^{-2}). Similar results were obtained from the other TME clones.

Two additional but less frequent outcomes were found in passage line A during second passage. In one group, the HY TME strain emerged similar to that observed in passage line B on second and third passages (see below), while in a third group, the DY TME clinical symptoms and 19-kDa PrP^{Sc} pattern stabilized by the third passage (data not shown). In passage line B, brain inocula from animal 6, which had an incubation period of 219 days and a 21-kDa PrP^{Sc} pattern, resulted in an incubation period of 67 ± 3 days, the HY TME clinical phenotype characterized by hyperexcitability, tremor, and ataxia, and the 21-kDa PrP^{Sc} banding pattern (Fig. 1A and B, passage line B). Third passage using an individual animal from this group (incubation period of 66 days) resulted in stabilization of the HY TME PrP^{Sc} banding pattern and clinical phenotype. The results from intraspecies transmission using TME clone 4 as the inoculum were similar to those obtained for the other three biological clones of TME (data not shown).

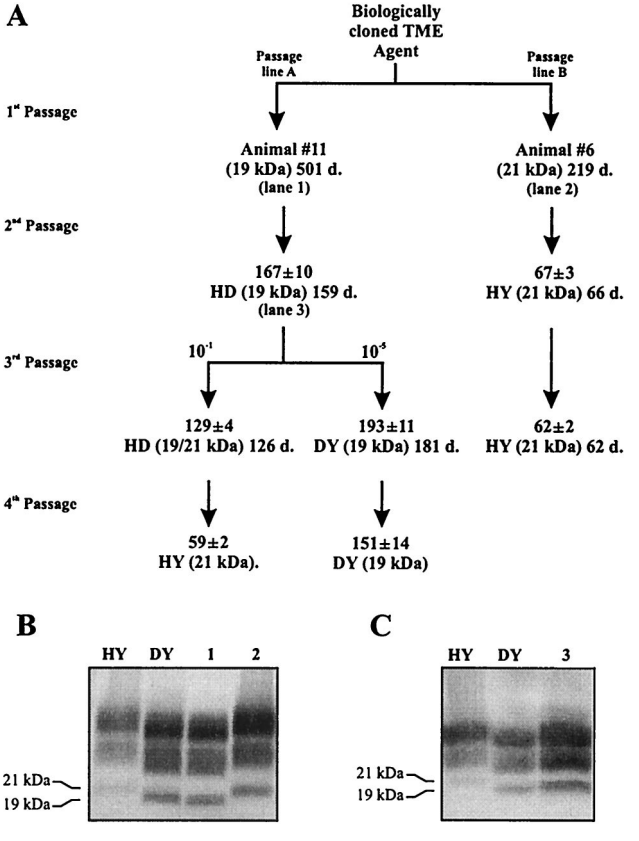

FIG. 1. Transmission of a biological clone of TME to hamsters. (A) Serial transmission of TME into hamsters is indicated in passage lines A and B. On first passage, the animal numbers refer to Table 1, with incubation period in days (d.) ± standard error of the mean and clinical phenotype, when apparent, noted as either HY, DY, or HD, a combination of both. Incubation periods without a standard error of the mean refer to individual animals that were used for both PrP^{Sc} analysis and subsequent passages. The PrP^{Sc} polypeptide migration pattern (19 or 21 kDa) is indicated; numbers 1, 2, and 3 refer to animals whose PrP^{Sc} patterns were analyzed in panels B and C. (B and C) Western blot analysis of PrP^{Sc} from first interspecies passage (lanes 1 and 2) and second intraspecies hamster passage (lane 3). Brain samples were resolved by SDS-PAGE (15% gel), and PrP was detected with monoclonal antibody 3F4 as described in Materials and Methods. Lanes HY and DY are the control 21- and 19-kDa patterns, respectively.

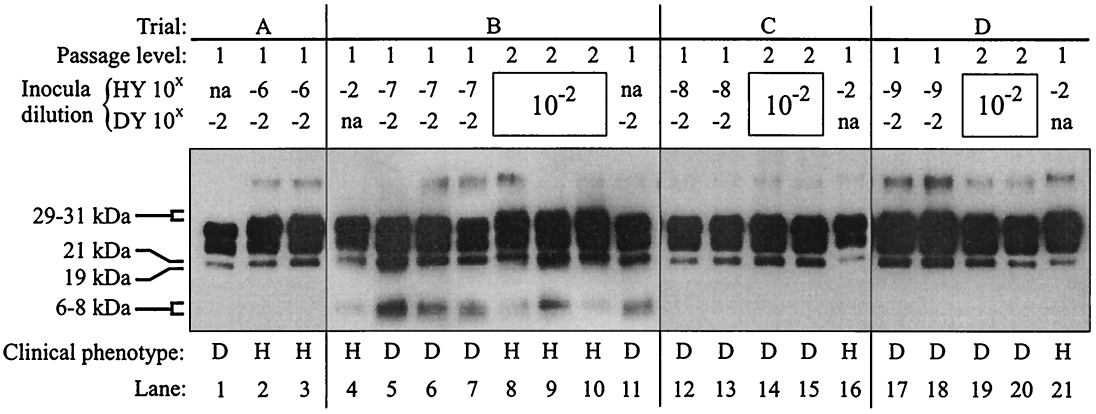

FIG. 2. PrP^{Sc} polypeptide patterns from Syrian hamsters infected with the HY and DY strains of TME. Animals were infected with different combinations and dilutions of HY and DY TME as indicated in each of the trials reported in Table 2 and also illustrated above each lane. Each lane contains an enriched PrP^{Sc} brain fraction digested with proteinase K from an individual animal and analyzed by SDS-PAGE and Western blot analysis using monoclonal antibody 3F4 (lanes 1 to 3 and 12 to 21) or rabbit polyclonal antibody R20 (lanes 4 to 11). R20 is immunoreactive to a synthetic peptide containing the C-terminal end of the mature prion protein; in addition to recognizing similar-molecular-weight PrP polypeptides as 3F4, it can also detect a 6- to 8-kDa band. Boxed areas refer to the second serial passage, inoculated at a 10^{-2} brain dilution, of individual brains from the first passage. HY and DY TME control PrP^{Sc} banding patterns are indicated in lanes 1, 4, 11, 16, and 21 and in lanes 1 and 11, respectively.

Intraspecies competition of HY and DY TME. To test the hypothesis that low levels of HY TME can emerge from a combined infection of HY and DY TME that initially exhibits a DY TME phenotype, hamsters were coinfected with biologically cloned HY and DY TME. Clinical disease, incubation period, and PrP^{Sc} pattern were investigated during several serial passages.

In the intraspecies competition experiment, hamsters were coinfected with a 10^{-2} brain homogenate of DY TME and increasing brain dilutions (e.g., 10^{-6} to 10^{-9}) of HY TME-infected brain homogenates. All passages after the initial coinfection experiment were performed at a brain inoculum dilution of 10^{-2}. Three patterns of TME disease were found, depending on the dose of each strain: (i) the short incubation HY TME phenotype and strain-specific PrP^{Sc} pattern was established and maintained (Table 2, trial A; Fig. 2, lanes 1 to 3); (ii) the DY TME or mixed pattern found on first passage reverted to the HY TME phenotype after additional passages (Table 2, trial B; Fig. 2, lanes 4 to 11); and (iii) the DY TME strain was able to block the selection of the short incubation HY TME strain (Table 2, trial C; Fig. 2, lanes 12 to 16).

The selection of HY TME from animals exhibiting the DY TME phenotype was found when hamsters were coinfected with brain dilutions of HY TME at 10^{-7} and DY TME at 10^{-2} (Table 2, trial B). At first passage, one animal with an incubation period of 126 days exhibited a HY TME clinical phenotype and a 21-kDa PrP^{Sc} pattern that was maintained on second serial passage in which the incubation period shortened to 68 ± 2 days. The remaining four animals had incubation periods ranging from 148 to 160 days and exhibited clinical symptoms of DY TME. Two had the 19-kDa PrP^{Sc} banding pattern (Fig. 2, lanes 6 and 7), while the remaining two had an intermediate size banding pattern that was between the 19- and 21-kDa patterns and could represent a mixture of HY and DY

TABLE 2. HY and DY TME competition experiment

Trial	Inoculum dilution		1st passage			2nd passage		
	HY	DY	Mean incubation period (days) ± SEM	Clinical symptoms	PrP^{Sc} pattern (kDa)	Mean incubation period (days) ± SEM	Clinical symptoms	PrP^{Sc} pattern (kDa)
A	10^{-6}		107 ± 1 (5/5[a])	HY	21	61 ± 1 (4/4)	HY	21
		10^{-2}	158 ± 2 (6/6)	DY	19	157 ± 3 (4/4)	DY	19
	10^{-6}	10^{-2}	104 ± 1 (5/5)	HY	21	55 ± 2 (4/4)	HY	21
B	10^{-7}		154 ± 22 (5/5)	HY	21	ND[b]	ND	ND
	10^{-7}	10^{-2}	126	HY	21	68 ± 2 (5/5)	HY	21
	10^{-7}	10^{-2}	148	DY	19	61 ± 0 (6/6)	HY	21
	10^{-7}	10^{-2}	155	DY	19	75 ± 0 (6/6)	HY	21
	10^{-7}	10^{-2}	155	DY	19/21	75 ± 0 (5/5)	HY	21
	10^{-7}	10^{-2}	160	DY	19/21	81 ± 3 (5/5)	HY	19/21
C	10^{-8}		190 ± 22 (5/5)	HY	21	ND	ND	ND
	10^{-8}	10^{-2}	122	HY	21	66 ± 0 (5/5)	HY	21
	10^{-8}	10^{-2}	150	DY	19	143 ± 2 (6/6)	DY	19
	10^{-8}	10^{-2}	150	DY	19	147 ± 2 (5/5)	DY	19
	10^{-8}	10^{-2}	155	DY	19	154 ± 7 (6/6)	DY	19
D	10^{-9}		>400 (5/5)	None	ND	ND	ND	ND
	10^{-9}	10^{-2}	147	DY	19	133 ± 0 (4/4)	DY	19
	10^{-9}	10^{-2}	154	DY	19	137 ± 0 (4/4)	DY	19

[a] Number affected/number inoculated.
[b] ND, not determined.

PrP^{Sc} (Fig. 2, lane 5, and data not shown). In the second passage performed at a 10^{-2} brain dilution, all of the DY TME animals reverted to a HY TME clinical phenotype and the incubation periods shortened to between 61 and 81 days. The PrP^{Sc} banding pattern switched to the 21-kDa pattern (Fig. 2, lanes 8 and 10); in one case, an intermediate PrP^{Sc} pattern between the 19- and 21-kDa patterns was found (Fig. 2, lane 9). Rabbit polyclonal antibody R20, directed to a synthetic peptide corresponding to the C-terminal portion of the prion protein, was used to aid in identification of the HY and DY TME banding patterns. Antiserum R20 immunoreacts with a 6- to 8-kDa PrP^{Sc} band present in control DY TME hamster brain (Fig. 2, lane 11) but only weakly reacts with this PrP^{Sc} band from the control HY TME brain (Fig. 2, lane 4).

DISCUSSION

The interspecies and intraspecies transmission of a biological clone of TME was investigated by monitoring the adaptation and selection of TME strains through evaluation of the PrP^{Sc} banding patterns, incubation periods, and clinical symptoms. Two distinct PrP^{Sc} banding patterns were detected prior to the stabilization of the HY and DY TME clinical phenotypes. In some hosts a mixture of the 19- and 21-kDa PrP^{Sc} patterns and clinical symptoms characteristic of both strains were found; in these cases, the shorter incubation HY TME strain was predominant on subsequent serial passages indicating that strain selection determines the emergence of strain phenotypes. Direct evidence for TME strain selection was provided by coinfection with predetermined amounts of each strain. High doses of DY TME were able to interfere with low titers of HY TME and cause partial or complete interference of the HY PrP^{Sc} banding pattern and clinical phenotype. A mixture of PrP^{Sc} banding patterns could be found in hosts that switched from a DY to HY phenotype in a single passage, indicating the selection of the HY TME strain. These findings provide the first evidence for selection of TSE strains at the molecular level.

In a previous study, transmission of the Stetsonville TME field isolate to hamsters resulted in the identification of the HY and DY TME strains (6). In the present study, the Stetsonville TME field isolate was cloned by limiting dilution in mink in three consecutive passage experiments in order to isolate the single predominant mink strain. The TME biological mink clone had a similar transmission history in hamsters as the Stetsonville field isolate, suggesting that upon interspecies transmission, two hamster-adapted TME strains emerged from a single mink TME strain. Although it cannot be ruled out that more than a single pathogenic PrP^{Sc} conformation exists in the biologically cloned inoculum, we propose that the ability of the donor mink PrP^{Sc} to convert the host hamster PrP^{C} into more than one stable hamster PrP^{Sc} conformation can give rise to multiple TME strains.

An alternative explanation for the emergence of TSE strains from a biological clone is a mutation in the putative TSE nucleic acid genome, despite a lack of a candidate molecule (1, 9, 10). The current findings provide molecular evidence that two distinct PrP^{Sc} patterns were found upon interspecies passage of a biological clone into a new host species. Furthermore, these PrP^{Sc} conformations could determine, or be used to predict, which strain will emerge upon additional passage. The strain-specific PrP^{Sc} patterns also were consistently found prior to stabilization of the TME phenotype in hamsters and therefore could be the molecular determinant of strain phenotype and preclude the requirement for a nucleic acid genome.

Three distinct TME strain patterns were observed upon interspecies transmission of cloned TME to hamsters: establishment of either the HY or DY TME phenotype during second or third hamster passage without evidence of mixed infection, and an indirect route in which a mixture of HY and DY TME was initially observed and led to the selection of either one of the TME strains upon additional passage. It appears that the DY TME strain was responsible for clinical disease upon interspecies transmission since the 19-kDa PrP^{Sc} pattern was most often found. DY TME likely represents the pathogenic strain present in the cloned mink inocula. Selection of the HY TME strain occurred after additional passage because it has a shorter incubation period than DY TME even though it was initially present at lower doses. Once the HY TME phenotype and PrP^{Sc} pattern were found, they never reverted to the DY TME strain. It is difficult to resolve whether the HY TME strain was generated de novo upon interspecies transmission or was present at low levels in the cloned TME inoculum. However, the findings that the HY TME agent could be eliminated after a single 10^{-5} dilution of second hamster passage TME indicates that it was unlikely that the biologically cloned TME agent contained a minor population of HY TME. Furthermore, previous studies demonstrated that hamster-adapted HY TME loses its pathogenicity in mink (6). These observations strongly support the concept of adaptation of the HY strain-specific PrP^{Sc} conformation upon interspecies transmission and the selection of HY TME upon additional hamster passages.

TME strain selection during coinfection with biological clones of HY and DY TME support the interspecies transmission findings that in a mixed TME infection, small doses of the short-incubation-period HY TME require additional animal passages in order to outcompete the long-incubation-period DY TME. The transition from the DY to HY TME phenotype corresponded with a switch in the PrP^{Sc} banding pattern. This was observed when DY TME titer was inoculated with a 100- and 1,000-fold excess with respect to HY TME and was followed by an additional passage. Complete interference of HY TME could be achieved in a mixed TME infection when the titer of the DY TME was greater than 1,000-fold excess over HY TME in the inoculum. In this case, the DY TME phenotype was maintained upon two additional serial passages; therefore, the HY TME agent could not be recovered when provided the opportunity to replicate. These findings were confirmed at the molecular level by the propagation of the 21-kDa DY TME banding pattern. Our results have similarities to a competition study between mouse-adapted strains of scrapie upon intraspecies infection (16, 17). In that study, the long-incubation (i.e., 550 days) 22A strain interfered with the short-incubation (i.e., 230 days) scrapie strain 22C when 22A was inoculated at least 100 days prior to 22C. Both the incubation period and brain neuropathology were characteristic of 22A despite the delayed coinfection with the faster-replicating 22C strain and indicated that the long-incubation pathogen blocked the replication sites of the short-incubation agent. An additional passage from animals coinfected with 22A and 22C was not performed, but we speculate that the shorter incubation 22C strain would not have emerged since a mixture of the 22A and 22C neuropathological lesion profiles or incubation periods was not observed. This is in contrast to the present study, in which a mixture of the HY and DY PrP^{Sc} patterns could give rise to either TME strain phenotype or PrP^{Sc} banding pattern, depending on the dose of inoculum.

A model for TSE strain adaptation and selection at the molecular level following interspecies transmission is proposed based on both the current findings of TME transmission to hamsters and previous transmission studies (5, 6, 19, 20–23,

29). Two events likely occur upon interspecies TSE transmission: (i) TSE strain adaptation involves the conversion of host PrP^{C} to PrP^{Sc} by a donor PrP^{Sc} molecule having a different primary structure (i.e., responsible for the species barrier effect) and (ii) the formation of multiple PrP^{Sc} conformations from a single donor PrP^{Sc} conformation that is responsible for generation of TSE strains. During intraspecies transmission, the selection of TSE strain phenotypes results from competition among the multiple PrP^{Sc} conformations. The outcome of TSE strain selection is dependent on the dose of each strain and kinetics of agent replication. This can result in propagation of several PrP^{Sc} strain-specific conformations or in interference with the formation of a particular PrP^{Sc} conformation. In the case of a mixture of strain-specific PrP^{Sc} conformations, the more rapidly propagating PrP^{Sc} strain in the new host species will predominate after additional passages. However, if the PrP^{Sc} conformation of a long incubation TSE strain is present at high doses, then it could interfere with the manifestation of a rapid onset TSE strain PrP^{Sc} conformation and phenotype.

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