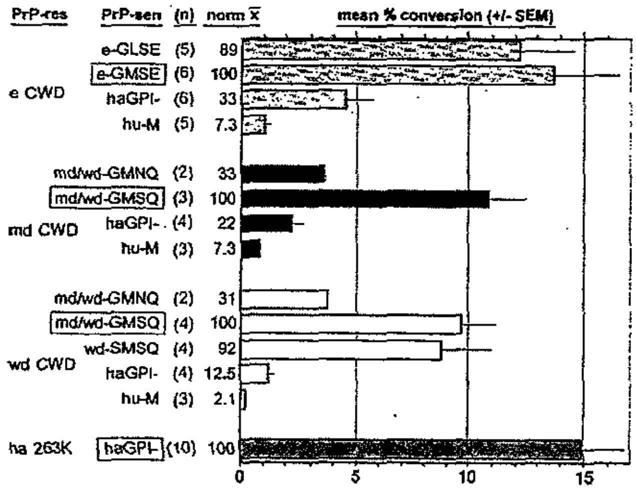

FIG. 2. Relative efficiencies of conversion reactions, examples of which are shown in Fig. 1. The efficiency for each conversion reaction was determined by quantitation of the amount of input radiolabeled ^{35}S-PrP-sen converted to ^{35}S-PrP-res bands, as described in Materials and Methods. Efficiencies are expressed as the mean percent conversion of ^{35}S-PrP-sen to ^{35}S-PrP-res for 2 to 10 replicate experiments (n), with standard errors of the means (SEM) shown by the error bars. For each PrP-res species, the mean efficiency was normalized (norm x) to the mean conversion efficiency of the homologous (boxed) PrP-sen. All data except those using hamster PrP molecules were previously published (20) and are shown here for comparison.

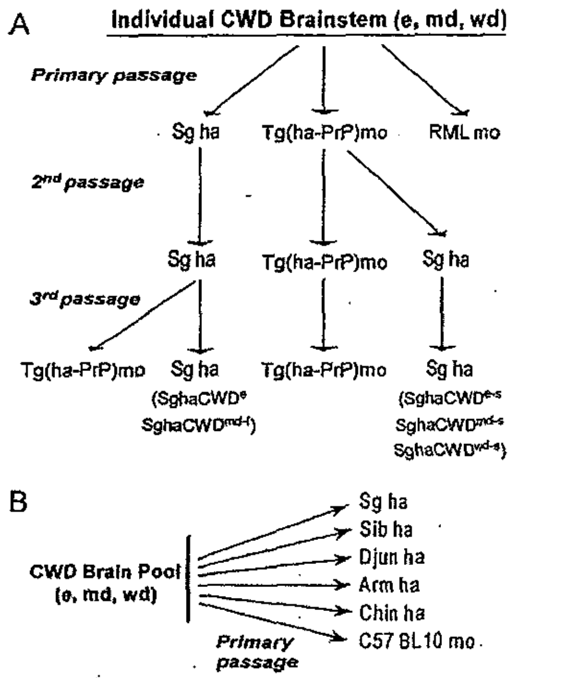

FIG. 3. Passage history of CWD inocula in rodent species. Primary inocula used were brain homogenates of each species (e, elk; md, mule deer; and wd, white-tailed deer) from either individual CWD-positive animals (A) or brain pools from CWD-affected animals of each species (B). The individual brains were passaged serially three times in the rodent species indicated, whereas the pooled brain samples were passaged only one time in the various species shown. Since there were no positive RML mice from the primary passage of the individual positive cervid brains, no additional passes were done. ha, hamster; Sib, Siberian; Djun, Djungarian; Arm, Armenian; Chin, Chinese; mo, mouse.

anti-mouse IgG at 1:250 (Vector Laboratories), Biogenex SS streptavidin (Biogenex), and amino carbazole as the substrate (Ventana). For glial fibrillary acidic protein (GFAP), there was no pretreatment, and the standard avidin-biotin technique was used with anti-GFAP at 1:1,000 (DAKO), biotinylated goat anti-rabbit IgG at 1:250 (Vector), and amino carbazole. Images were magnified at 40× and were captured on an Olympus BX51 light microscope, using MicroSuite software. For the images of whole brain sections, stained microscope slides were scanned using an Epson Expression 1640XL scanner at 1,400 dpi, and the images were processed using Adobe Photoshop software.

RESULTS

CFC reactions. To test initially for the likelihood that hamsters might be susceptible to CWD, interspecies CFC reactions were done. Purified PrP-res from CWD-affected cervid brain tissue (PrP^{CWD}) was incubated with unglycosylated and immunopurified ^{35}S-labeled PrP-sen from hamsters, humans, and cervids and then digested with PK to detect newly formed ^{35}S-PrP-res (Fig. 1). Although the hamster PrP-sen (Fig. 1A, lanes 1 and 2) used in these reactions lacked the GPI anchor, the other PrP-sen molecules (Fig. 1A, lanes 3 to 9) used did not. Previous studies have shown that this difference does not significantly affect conversion efficiency, at least for hamster PrP (15, 16). Furthermore, glycosylation does not significantly affect conversion efficiencies under these conditions (22).

PK-resistant ^{35}S-PrP bands (Fig. 1B, PrP-res bracket) that were 6 to 8 kDa smaller than the ^{35}S-PrP-sen precursors (Fig. 1A, PrP-sen bracket) were of primary interest because they reflect the 6- to 8-kDa amino-terminal truncation that is observed with the PK digestion of PrP-res isolated from the brains of the TSE-affected species used for these studies (20). These CFC reactions show that elk PrP^{CWD} (eCWD) induced the conversion of cervid, human, and hamster ^{35}S-PrP-sen molecules to ^{35}S-PrP-res (16- to 18-kDa PrP-res [brackets in Fig. 1B]). Cervid and human ^{35}S-PrP-sen molecules were the most and least efficiently converted, respectively (20). Similar results were observed for CFC reactions done using mule deer (mdCWD) and white-tailed deer (wdCWD) PrP^{CWD} (Fig. 2). The hamster ^{35}S-PrP-sen was converted with intermediate efficiency by all PrP^{CWD} isolates (e-, md-, and wdCWD) and with a much higher efficiency by hamster PrP-res (ha 263K) (Fig. 2). For unknown reasons, the conversion products from PrP^{CWD} reaction mixes incubated with hamster ^{35}S-PrP-sen migrated slightly faster by SDS-PAGE than those induced by hamster PrP-res (Fig. 1B, lanes 1 and 2). In the absence of PrP-res, 16- to 18-kDa PK-resistant ^{35}S-PrP bands were not observed (Fig. 1C). The observation that the PrP^{CWD} preparations converted hamster ^{35}S-PrP-sen suggested that hamsters might be susceptible to CWD infection.

Passages of CWD isolates into Sg hamsters. Based on the CFC results, we attempted to transmit different CWD isolates to various species of hamsters as diagrammed in Fig. 3. Immunoblotting showed that PK-treated aliquots of the primary inocula from individual cervid animals (Fig. 4A, lanes e, md, and wd) and brain pools (20) contained PrP^{CWD}, as evidenced by immunoreactivity with antiserum 505, which detects PrP molecules from all of these species (20) (Fig. 4A, left panel). The same samples were not immunoreactive with monoclonal Ab 3F4, which detects PK-treated Sg hamster 263K PrP-res

TABLE 1. Serial passage details of individual CWD-positive cervid animal brain homogenates inoculated into Sg hamsters

CWD inoculum	Primary passage: Species inoculated[a]	Primary passage: No. of animals with TSE signs/no. inoculated[b]	Primary passage: No. of blot-positive animals/no. tested[c]	Primary passage: Incubation time (dpi) of blot-positive samples[f]	Second passage: Donor animal inoculum (dpi)[e]	Second passage: Species inoculated[a]	Second passage: No. of animals with TSE signs/no. inoculated[b]	Second passage: No. of blot-positive animals/no. tested	Second passage: Incubation time of animals with TSE signs (mean dpi ± SD)	Third Passage: Donor animal inoculum[e]	Third Passage: Species inoculated[a]	Third Passage: No. of animals with TSE signs/no. inoculated[b]	Third Passage: Incubation time of animals with TSE signs (mean dpi ± SD)
e	Sg ha	0/7	2/7	381, 668	e 1 (381)	Sg ha	8/8	6/6	427 ± 53	e 1a e 1b e 1b	Sg ha Sg ha Tg (haPrP) mo	6/6 4/4 3/3	463 ± 21 423 ± 28 376 ± 8
md	Sg ha	2/7	4/7	172,* 274, 326,* 393 (291 ± 93)	md 1 (172)	Sg ha	8/8	2/2	85 ± 0	md 1a	Sg ha	6/6	89 ± 0
					md 2 (326)	Sg ha	8/8	2/2	85 ± 0	md 2a	Sg ha	6/6	89 ± 0
wd	Sg ha	0/8	0/8	None[d]	Not done					Not done			

[a] Sg ha, Sg hamster; Tg (haPrP) mo, Tg mouse with mouse PrP gene knocked out and Sg hamster PrP gene inserted (18).
[b] For each group, 12 animals were inoculated for the first passage, 8 animals were inoculated for the second passage, and six Sg hamsters or four Tg (haPrP) mice were inoculated for the third passage. Animals lost due to intercurrent deaths were not included in these data. Intercurrent deaths are defined as animals that died without neurological signs prior to the date that the first animal in the group was determined to be TSE positive by either neurological signs or brain PrP-res by immunoblot analysis.
[c] During the first passage, all animals with neurological signs were PrP-res positive, but not all brain PrP-res immunoblot-positive animals had neurological signs.
[d] The animals in this group did not have neurologic signs and were not positive for brain PrP-res during their life span (range, 351 to 553 dpi; mean, 451 ± 62 dpi).
[e] The code designates the individual Tg (haPrP) mouse or Sg hamster that was used as a source of inoculum in the passage specified. For example, "e 1" designates an individual TSE-positive Sg hamster that had been inoculated with eCWD and was used as the source of inoculum for a second passage; "e 1a" designates an individual Sg hamster from the second passage that had been inoculated from the "e1" Sg hamster.
[f] The days to death for the individual animals displaying neurological signs consistent with TSE disease are indicated by asterisks. Brain homogenates from each of these were inoculated into groups of eight Sg hamsters for the second passage.

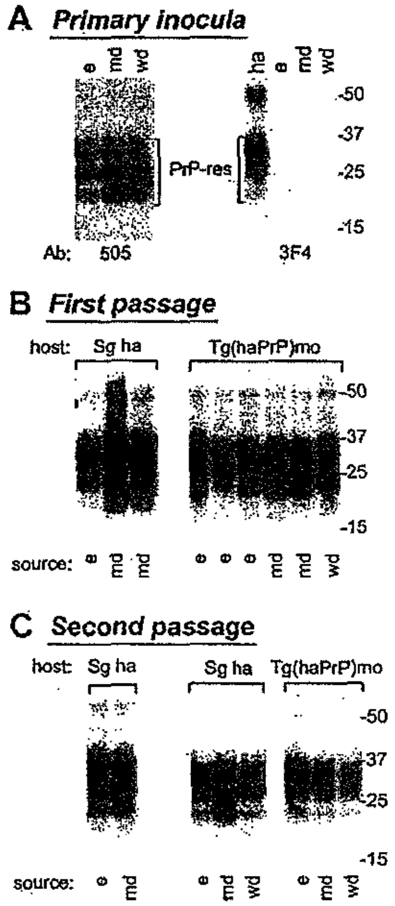

FIG. 4. Analysis of PrP-res from sequential passages of CWD inocula in Sg hamsters and Tg (haPrP) mice. (A) Fluorescent immunoblots of SDS-PAGE gels show PK-resistant PrP in CWD primary inocula from individual elk (e), mule deer (md), and white-tailed deer (wd). Equal aliquots of the primary inocula were immunoblotted and analyzed with either 505 antiserum for the left blot or 3F4 Ab for the right blot. The 3F4 Ab detected 263K PrP-res from Sg hamster brain (ha), but it did not detect the PrP^{CWD} in any of the primary inocula. (B and C) Immunoblots of representative examples of first and second serial passages, respectively, into either Sg hamsters or Tg (haPrP) mice, analyzed using 3F4. The sources indicated in panels B and C identify the primary CWD passage inocula. The migration of molecular mass standards, in kilodaltons, is shown to the right of each panel.

(Fig. 4A, right panel). Thus, the 3F4 Ab was used to detect newly formed PrP-res in the host and to discriminate it from the primary inocula. Since 3F4 does not detect mouse or Chinese hamster PrP, PrP-res from these species was detected on immunoblots using R30 (data not shown), which, like 3F4, does not react with cervid PrP.

Upon primary passage of the various individual cervid inocula into Sg hamsters, only two hamsters inoculated with mule deer CWD showed clinical signs of TSE disease during an observation period of up to 2 years (Table 1, primary passage column, 172 and 326 days to death postinoculation [dpi]). Although they did not show evident clinical signs of TSE disease, two additional Sg hamsters for each of the md- and eCWD inoculations showed disease-associated PrP-res-positive immunoblots, indicating subclinical infections. None of the

Sg hamsters inoculated with wdCWD showed any signs of neurological disease or tested positive for PrP-res on immunoblots.

When the TSE-positive Sg hamsters from the primary passage of eCWD were passaged a second and then a third time, all recipients showed clinical signs after prolonged mean incubation periods of ≥423 dpi (Table 1, second- and third-passage columns), and all of the brains tested were PrP-res positive on immunoblots. Passage of an inoculum from one of the second-passage eCWD-inoculated positive hamsters into Tg (haPrP) mice gave a slightly shorter mean incubation period (Table 1, third-passage column). Second and third passages of mdCWD into Sg hamsters gave much shorter incubation periods, averaging 85 to 89 dpi, suggesting that the mule deer-derived CWD isolate was a different and much faster (f) isolate (SghaCWD$^{md\text{-}f}$) than that obtained from the eCWD inoculum (SghaCWD^{e}). The TSE neurological signs of infection with this fast isolate differed from those for the SghaCWD^{e} isolate, with affected animals presenting initially with a waddling gait, head bobbing, and unkempt appearance that developed into severe ataxia to the point of repeated falling and loss of the righting reflex. Approximately 2 to 3 weeks after the first neurological signs were observed, these animals became recumbent and were euthanized. In contrast, SghaCWD^{e}-infected animals presented with a more subtle neurologic disorder that progressed at a much lower rate. After a period of 1.5 to 3 months, the disease typically progressed to hind leg paralysis, increased ataxia, tremors, and eventually, wasting.

Passages into "Sg-hamsterized" Tg (haPrP) mice. Tg (haPrP) mice overexpressing Sg hamster PrP on a mouse PrP null background have shorter incubation periods than do Sg hamsters when inoculated with 263K hamster-adapted scrapie (18). Based on this observation, we suspected that Tg (haPrP) mice may have a more rapid disease response to CWD infection than that of Sg hamsters. Therefore, the same cervid inocula used for the Sg hamsters were used for the Tg (haPrP) mice (Fig. 3A, primary passage). After inoculation with brain homogenates from each of the CWD-affected cervid species, approximately one-third of the Tg (haPrP) mice showed clinical signs of TSE disease after extended mean incubation periods ranging from 585 to 668 dpi (Table 2, primary passage column), and a majority (62 to 88%) of these mice were positive for brain PrP-res by immunoblot analysis (examples are shown in Fig. 4B). Second and third serial passages into Tg (haPrP) mice caused clinical disease in all of the recipients and reductions in average incubation periods of the various groups to 185 to 282 dpi. Sg hamsters receiving second and third passages from clinically affected Tg (haPrP) mice were much slower (s) to develop disease (408 to 544 dpi) with elk (SghaCWD$^{e\text{-}s}$), mule deer (SghaCWD$^{md\text{-}s}$), or white-tailed deer (SghaCWD$^{wd\text{-}s}$)-derived CWD isolates than when Tg (haPrP) mice were inoculated with the same material. The longer incubation periods observed for the Sg hamsters compared to those for the Tg (haPrP) mice remained stable upon additional passages (Table 2, second- and third-passage columns). These hamsters displayed neurological signs and disease courses that were similar, if not identical, to those seen with the SghaCWD^{e} isolate described above. The brains of all mice and hamsters analyzed for PrP-res on immunoblots were positive (examples are given in Fig. 4B and C). The clinical presentation of all affected Tg (haPrP) mice was similar to that for the SghaCWD^{e} isolate, with subtle neurological disturbances and a prolonged disease course lasting at least 1 to 2 months. The Tg (haPrP) mice also exhibited kyphosis, a tiptoed gait, hind leg clasp when suspended by the tail, and eventually hind leg paralysis in most of the animals. This disease course contrasted with that seen with 263K scrapie-inoculated Tg (haPrP) mice, in which subtle neurological symptoms are followed rapidly by death, within 2 to 4 days (18).

Immunohistological analyses of different isolates. Immunohistological analyses of brain sections from Sg hamsters clinically affected with the faster isolate, SghaCWD$^{md\text{-}f}$, and with the slower isolates first passaged through the Tg (haPrP) mice, i.e., SghaCWD$^{e\text{-}s}$, SghaCWD$^{md\text{-}s}$, and SghaCWD$^{wd\text{-}s}$, revealed different patterns of PrP-res accumulation and gliosis (Fig. 5, PrP-res and GFAP panels, respectively). PrP-res was detected using 3F4 Ab, and gliosis was detected with anti-GFAP Ab as described in Materials and Methods. Immunohistological data are shown for sagittal sections of brains of an uninfected (normal) Sg hamster and a terminally affected 263K Sg hamster, SghaCWD$^{md\text{-}f}$, which was generated by serial passage of mdCWD through two Sg hamsters, and SghaCWD$^{md\text{-}s}$, which was generated by sequential passages, first into Tg (haPrP) mice and then into Sg hamster. Both PrP-res deposition and gliosis were widespread in the brains of 263K- and SghaCWD$^{md\text{-}f}$-affected hamsters, whereas in the SghaCWD$^{md\text{-}s}$-affected hamster they were not. The hippocampus, cerebellum, and caudal colliculus regions are also shown at higher magnification for more detailed analysis. SghaCWD$^{e\text{-}s}$ and SghaCWD$^{wd\text{-}s}$ showed patterns of PrP-res deposition and gliosis indistinguishable from those of SghaCWD$^{md\text{-}s}$ shown in Fig. 5 (data not shown).

Passages of CWD into various hamster species. In order to determine whether other hamster species may be susceptible to CWD and result in a useful rodent-adapted CWD animal model, brain homogenates isolated from elk, mule deer, or white-tailed deer brain pools were inoculated into several other hamster species (Table 3). In contrast to the transmission experiments using CWD isolates from individual elk, mule, and white-tailed deer (Table 1), none of the Sg hamsters inoculated with the CWD brain pools developed clinical disease within their life span, and no PrP-res was detected by immunoblot analysis of their brains. There was also no evidence of transmission to Djungarian hamsters. In contrast, there were obvious clinical signs in a majority of Chinese hamsters inoculated with the e- and mdCWD brain pools, though not with the wdCWD pool. Most of the clinically positive Chinese hamster brains tested were PrP-res positive by immunoblot analysis. With Siberian hamsters, there was one animal that received the mdCWD pool inoculum that was positive for both neurological signs of TSE disease and PrP-res. Another Siberian hamster was confirmed to be positive from the white-tailed deer pool inoculum. A single Armenian hamster was clinically suspect and immunoblot positive for the eCWD pool inoculum. All of the clinically infected animals initially presented with rapid tremors and ataxia that progressively worsened for 2 to 4 weeks until the animals became recumbent and were euthanized.

Passages into wild-type mice. The inocula used for the RML outbred mice and C57BL10 inbred mice were from the CWD-affected individual animal brains and the CWD brain pools,

TABLE 2. Serial passage details of individual CWD-positive cervid animal brain homogenates inoculated into Tg (haPrP) mice

	Primary passage					Second passage					Third passage			
CWD inoculum	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time (dpi) of TSE-positive sample (mean ± SD)	Time (dpi) of blot-positive samples (mean ± SD)	Donor animal inoculum[c]	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time of animals with TSE signs (mean dpi ± SD)	Donor animal inoculum[c]	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	Incubation time of animals with TSE signs (mean dpi ± SD)
e	Tg (haPrP) mo	3/9	8/9	632 ± 89	585 ± 142	e 1	Tg (haPrP) mo	7/7	3/3	282 ± 39	e 1a	Tg (haPrP) mo	4/4	203 ± 9
											e 1b	Tg (haPrP) mo	5/5	202 ± 7
						e 1	Sg ha	5/6	4/5[d]	509 ± 70	e 1c	Sg ha	5/5	543 ± 39
											e 1d	Sg ha	5/5	526 ± 90
						e 2	Tg (haPrP) mo	8/8	3/3	215 ± 9	e 2a	Tg (haPrP) mo	4/4	210 ± 10
											e 2b	Tg (haPrP) mo	4/4	185 ± 16
						e 3	Tg (haPrP) mo	7/7	3/3	212 ± 10	e 3a	Tg (haPrP) mo	6/6	247 ± 36
											e 3b	Tg (haPrP) mo	3/3	204 ± 9
md	Tg (haPrP) mo	3/8	5/8	726 ± 47	668 ± 105	md 1	Tg (haPrP) mo	8/8	3/3	202 ± 8	md 1a	Tg (haPrP) mo	4/4	213 ± 5
											md 1b	Tg (haPrP) mo	4/4	259 ± 33
						md 1	Sg ha	7/7	7/7	485 ± 44	md 1c	Sg ha	4/4	544 ± 110
											md 1d	Sg ha	2/2	540 ± 614
						md 2	Tg (haPrP) mo	8/8	4/4	209 ± 28	md 2a	Tg (haPrP) mo	3/3	228 ± 15
											md 2b	Tg (haPrP) mo	4/4	212 ± 14
wd	Tg (haPrP) mo	4/9	8/9	632 ± 73	621 ± 80	wd 1	Tg (haPrP) mo	7/7	4/4	272 ± 62	wd 1a	Tg (haPrP) mo	4/4	212 ± 32
											wd 1b	Tg (haPrP) mo	4/4	237 ± 13
						wd 1	Sg ha	5/5	5/5	462 ± 3	wd 1c	Sg ha	3/3	408 ± 42
											wd 1d	Sg ha	4/4	436 ± 15

[a] Abbreviations used are the same as those in Table 1.

[b] For each group, 12 animals were inoculated for the first passage, 8 animals were inoculated for the second passage, and 4 to 6 animals were inoculated for the third passage. Intercurrent deaths, as defined in Table 1, are not included in these data.

[c] The code designates the individual Tg (haPrP) mouse or Sg hamster that was used as a source of inoculum in the passage specified. For example, "e 1" designates an individual TSE-positive Tg (haPrP) mouse that was inoculated with eCWD and was used as the source of inoculum for a second passage; "e 1a" designates an individual Tg (haPrP) mouse from the second passage that had been inoculated from the e 1 Tg (haPrP) mouse.

[d] The blot-negative animal (second passage, e 1 inoculated into Sg hamster) was also negative for neurological signs and likely died due to another undetermined cause.

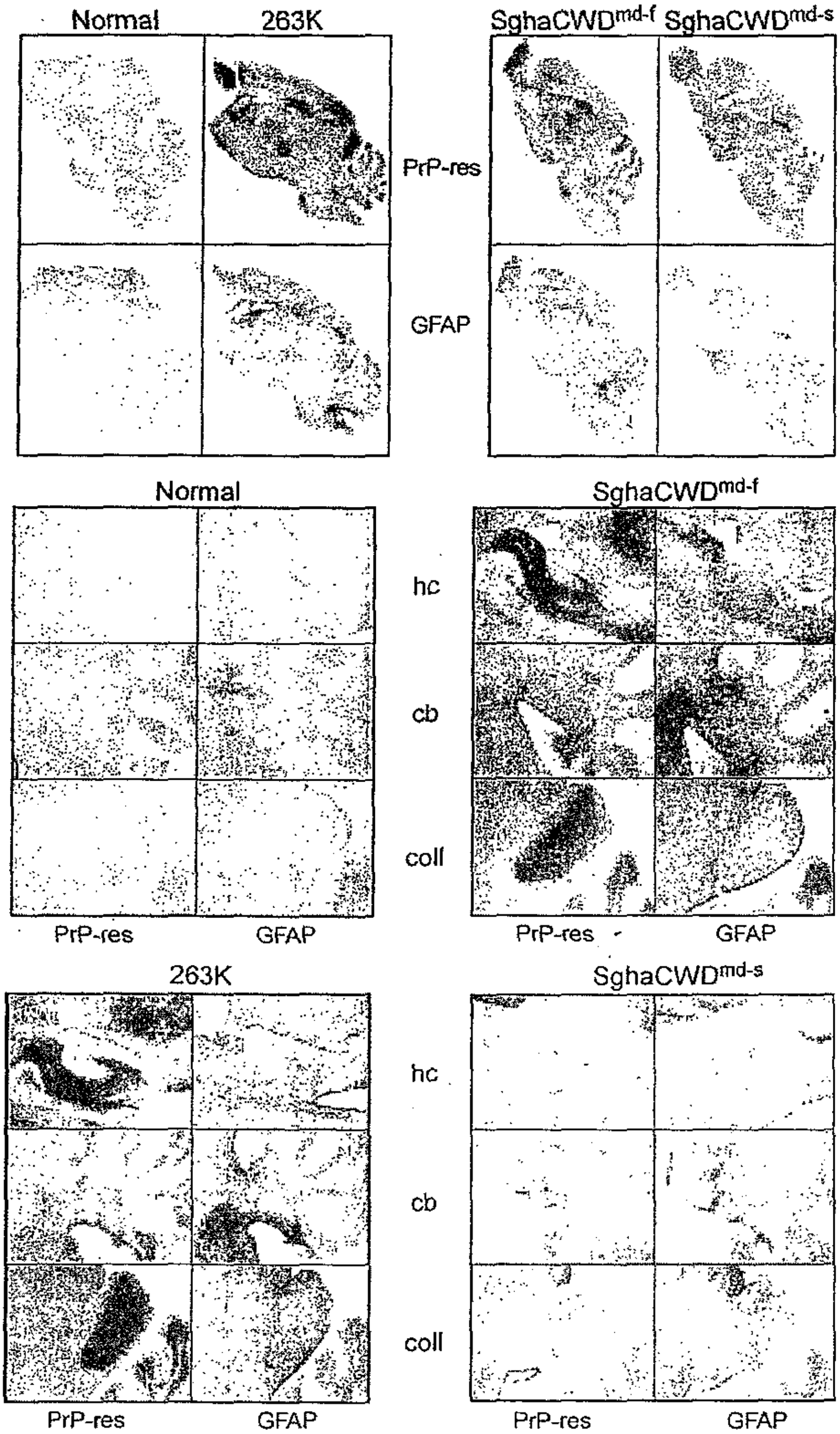

FIG. 5. Immunohistochemical analysis of brain sections from slow (SghaCWD^{md-s}) and fast (SghaCWD^{md-f}) isolates compared to uninfected (normal) and 263K-infected Sg hamster brains. PrP-res deposition was visualized in sagittal sections of the various brains, using 3F4 Ab ("PrP-res" panels). Using adjacent brain sections, the extent of gliosis was visualized using anti-GFAP Ab ("GFAP" panels). Scanned whole-brain images are shown in the upper panels. For the middle and lower panels, hippocampus (hc), cerebellum (cb), and caudal colliculus (coll) regions were magnified at ×40 for more detailed analysis. The images shown are representative of the following numbers of brains analyzed: two normal brains, two 263K-infected brains, three each of second- and third-passage SghaCWD^{md-f}-infected brains, and two each of third-passage SghaCWD^{md-s}-, SghaCWD^{e-s}-, and SghaCWD^{wd-s}-infected brains.

TABLE 3. Passage details of CWD-positive cervid brain homogenates inoculated into various hamster and wild-type mouse species

CWD inoculum	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time of animals with TSE signs (mean dpi ± SD)[c]	Life span (dpi, mean ± SD)	Life span range (dpi)
e	Sg ha	0/12	0/2	None positive	628 ± 98	480–751
md	Sg ha	0/12	0/2	None positive	591 ± 98	469–693
wd	Sg ha	0/12	0/3	None positive	510 ± 95	430–644
e	Djun ha	0/4	Not done	None positive	500 ± 95	384–615
md	Djun ha	0/10	Not done	None positive	507 ± 97	399–659
wd	Djun ha	0/1	Not done	None positive	476	476
e	Chin ha	6/8	4/5[e]	626 ± 80	730 ± 220	555–1,133
md	Chin ha	6/8	6/7[e]	648 ± 58	690 ± 166	563–1,133
wd	Chin ha	0/6	Not done	None positive	722 ± 239	448–1,133
e	Sib ha	0/2	Not done	None positive	532 ± 69	483–581
md	Sib ha	1/3	1/3[e]	464	482 ± 23	464–509
wd	Sib ha	1/4	1/4[e]	735	638 ± 118	499–739
e	Arm ha	1/4	1/2[e]	745	669 ± 121	490–745
md	Arm ha	0/15	0/1	None positive	625 ± 96	348–704
wd	Arm ha	0/8	0/8	None positive	587 ± 40	532–649
e[d]	RML mo	0/9	0/9	None positive	592 ± 80	488–736
md[d]	RML mo	0/6	0/6	None positive	679 ± 93	488–805
wd[d]	RML mo	0/6	0/6	None positive	730 ± 101	562–893
e	C57BL10 mo	0/15	0/2	None positive	724 ± 107	588–837
md	C57BL10 mo	0/16	0/2	None positive	657 ± 169	368–837
wd	C57BL10 mo	0/14	0/2	None positive	772 ± 125	368–837

[a] Abbreviations are the same as those in Table 1. Djun, Djungarian; Chin, Chinese; Sib, Siberian; Arm, Armenian; RML mo, Rocky Mountain Laboratory mouse.
[b] For each group, 12 to 16 animals were inoculated. Animals lost due to intercurrent deaths (as defined in Table 1) are not included in these data.
[c] None positive, none of the animals in the group were TSE positive by either neurological signs or immunoblot analysis for brain PrP-res during their life span.
[d] RML mice were inoculated with the individual CWD-positive cervid brain homogenates used for both the Sg hamsters in Table 1 and the Tg (haPrP) mice in Table 2.
[e] The blot-positive animals also showed neurological signs consistent with TSE disease, while the blot-negative animals did not.

respectively (Table 3). None of the animals of either mouse species developed any neurological signs within their life span (ranging from 488 to 893 dpi), and none were immunoblot positive for brain PrP-res.

DISCUSSION

We have shown that CWD from one or more cervid species can be transmitted to Sg, Chinese, Siberian, and Armenian hamsters and to Tg mice that express Sg hamster prion protein. Transmission of CWD to Sg hamsters was attempted previously without generating disease, most likely because the animals in that study were incubated for only 1 year (2). In the present study, inoculated animals were observed for periods exceeding 2 years for some animals. We found CWD transmission, with the highest attack rates in Chinese hamsters and Tg (haPrP) mice. The other rodent species had much lower attack rates or were not susceptible. The incomplete attack rates for the hamster species and Tg (haPrP) mice indicated that the cervid CWD inocula contained an average of only roughly 1 ID_{50} (the dose that would infect 50% of the animals) for these species. However, these inocula produced disease in Tg mice expressing deer PrP at a 100% attack rate, indicating that the titer for mice with homologous PrP is greater (R. E. Race, K. Meade-White, and B. Chesebro, unpublished data). The lack of transmission of some of the cervid CWD inocula to the other rodent species could be due to small differences in inoculum titers or to heterogeneity in the PrP sequences in the pooled inocula rather than to fundamental differences in host susceptibility. The amounts of normal host PrP expressed in the different hamster species are similar (K. Meade-White and R. E. Race, unpublished data) and are not likely an explanation for the different susceptibilities. Due to the low attack rates and long incubation periods seen with primary passages from cervids, none of these rodent species would be practical for use in direct bioassays for cervid CWD.

Nonetheless, the rodent-adapted CWD models we have developed may be useful to experimentally analyze TSE species and strain differences. Despite the low initial attack rates for the first passage of CWD into Sg hamsters, CWD isolates derived initially from elk and mule deer readily adapted to hamsters, as evidenced by the 100% infection rate on second and third passages. The average incubation periods were similar for the second and third passages but considerably shorter than that for the first passage for the Sg hamsters, suggesting that any species barrier to infection (formally, the shortening of the incubation period between the first and subsequent passages in a new species) was overcome quickly.

When mdCWD was serially passaged in Sg hamsters, an isolate, $SghaCWD^{md\text{-}f}$, was obtained that had a relatively short incubation period. When the same inoculum was passaged first into Tg (haPrP) mice followed by serial passage in Sg hamsters, an isolate with a fivefold longer incubation period developed, namely, SghaCWD^{md-s}. The CWD inocula from elk and white-tailed deer led only to the slow isolates SghaCWD^{e-s} and SghaCWD^{wd-s}, which were indistinguishable from the slow mule deer isolate SghaCWD^{md-s}. The markedly different incubation periods of these two isolates from the mdCWD inoculum, as well as the distinct clinical signs and patterns of brain pathology and PrP-res deposition, raise the possibility that different strains of CWD isolates may exist, at least in mule deer, which in turn can lead to distinct CWD strains in Sg hamsters. Another possibility is that the strains diverged upon introduction into Sg hamsters, as suggested for the HY and DY Sg hamster strains from TME inoculum-infected mink brain homogenates (1).

Differences in PrP-res glycoform patterns analyzed from several CWD-affected deer and elk also suggested that CWD strains in mule deer may be more heterogeneous than those in elk (19). Others have also found evidence of CWD strains (16a). Curiously, however, this apparent strain difference was not manifested when the identical mdCWD inoculum was serially passaged through only one recipient species. Serial passage in Sg hamsters yielded only the fast-growing isolate (Table 1 and Fig. 3), while passage first through Tg (haPrP) mice and then into Sg hamsters yielded only the slow-growing isolate (Table 2 and Fig. 3). With this in mind, it is important to consider other possible explanations for these results. One possibility is that the CWD isolate might be able to undergo a stochastic change into a more rapid and aggressive strain in Sg hamsters and that this happened to occur after the mdCWD inoculations. This would be similar to the emergence of fast (HY) and slow (DY) strains upon inoculation of TME isolates into Sg hamsters (5). These strains developed even when a clonal isolate of the TME inoculum was used, suggesting that they arose in the recipient Sg hamsters rather than in the mink source (1).

Finally, although extensive precautions were taken, we cannot formally prove that inadvertent contamination of the mdCWD inoculum with the hamster-derived 263K strain did not occur, which potentially could yield short incubation period passages in Sg hamsters (Table 1). However, the incubation periods observed with the CWD passages (85 to 89 days) were significantly longer than the 263K incubation periods observed in our lab (70 to 75 days), and no mock-infected controls became sick during their life span. Also, we saw no 263K-like infectivity develop in the highly susceptible Tg (haPrP) mice, even though we used the identical primary inoculum for both recipient species. Interestingly, the similarity of the Sg hamster-adapted CWD fast-growing isolate and 263K might be due to a common origin, since there is circumstantial evidence that CWD arose from cervid exposure to sheep scrapie, which was also the origin of the 263K strain in hamsters (14). Furthermore, the Hyper strain derived from TME inoculations has 263K-like strain characteristics in Sg hamsters (5). Thus, it would appear that both CWD and TME transmissions into Sg hamsters can result in divergent fast and slow strains.

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