Biological and Biochemical Characteristics of Prion Strains Conserved in Persistently Infected Cell Cultures

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Abnormal prion protein (PrPSc) plays a central role in the transmission of prion diseases, but the molecular basis of prion strains with distinct biological characteristics remains to be elucidated. We analyzed the characteristics of prion disease by using mice inoculated with the Chandler and Fukuoka-1 strains propagated in a cultured mouse neuronal cell line, GT1-7, which is highly permissive to replication of the infectious agents. Strain-specific biological characteristics, including clinical manifestations, incubation period as related to the infectious unit, and pathological profiles, remained unchanged after passages in the cell cultures. We noted some differences in the biochemical aspects of PrPSc between brain tissues and GT1-7 cells which were unlikely to affect the strain phenotypes. On the other hand, the proteinase K-resistant PrP core fragments derived from Fukuoka-1-infected tissues and cells were slightly larger than those from Chandler-infected versions. Moreover, Fukuoka-1 infection, but not Chandler infection, gave an extra fragment with a low molecular weight, ~13 kDa, in both brain tissues and GT1-7 cells. This cell culture model persistently infected with different strains will provide a new insight into the understanding of the molecular basis of prion diversity.

Transmissible spongiform encephalopathies (TSEs) are a series of neurodegenerative disorders that include Creutzfeldt-Jakob disease (CJD), Gerstmann-Strassler-Scheinker syndrome (GSS), and fatal familial insomnia in humans and bovine spongiform encephalopathy and scrapie in animals (23, 25). Human TSEs may have infectious, sporadic, or genetic origins, but the brain tissues from affected individuals always possess an infectious agent, termed prion, capable of transmitting the disease to laboratory animals. The protein-only hypothesis proposes that the abnormal isoform of the prion protein (PrPSc) accumulated via posttranslational modification of the cellular isoform (PrPC) is the sole component of the infectious agent (25). In fact, while the agent is extremely resistant to inactivation by UV and ionizing radiation, protein denaturants can abolish the infectivity, and moreover, no specific genetic materials for infectious agents have been identified. The two PrP isoforms are distinguishable by their biochemical properties. PrPC is readily soluble in nondenaturing detergents and completely digested by proteinase K (PK), whereas PrPSc is detergent insoluble and resistant to proteolysis except for the N-terminal region comprising ~67 residues. Structural studies have suggested that the former is rich in alpha-helical structures with small beta-sheet regions, but the latter has a high beta-sheet content. The central role of PrP in the disease is exemplified by the fact that PrP-null mice are resistant to the disease (6, 27), by the causal linkage of genetic forms of human TSEs with mutation in the PrP gene (25), and by the dependency of the species barriers on the primary PrP sequences (29). The existence of strain variation, however, has challenged the protein-only hypothesis. Individual infectious agents have been shown to maintain their phenotypic characteristics, including the clinical presentation of disease, the length of the incubation period, and the distribution of vacuolar degeneration and PrPSc deposition in the central nervous system (CNS) during serial transmission between same-species animals. In addition to these biological characteristics, biochemical differences in PrPSc have been reported. Transmission of two different inherited human prion diseases, familial Creutzfeldt and familial CJD, to mice resulted in the accumulation of PrPSc with PK-resistant core fragments with molecular masses of 19 and 21 kDa, respectively (35). The difference in the size of PK-resistant PrPSc fragments has been also documented among agents originating from scrapie and mink spongiform encephalopathies (3). The degree of glycosylation is also proposed to be an important signature of some strains. There are two sites of Asn-linked glycosylation at the C-terminal portion, and the degree of glycosylation is thus represented by the ratio of three glycoforms, di-, mono-, and unglycosylated forms. The unique PrPSc glycoform pattern, in which the diglycosylated form dominates, in animals and patients affected with bovine spongiform encephalopathy and variant CJD, respectively, is distinct from those of other known strains (11) with a few exceptions (32). Because diversity in the size of a PK-resistant PrP core fragment and the degree of its Asn-linked glycosylation were thought to be consequences of differences in the conformation, it has been hypothesized that strain-specific conformations of PrPSc could determine the strain phenotype.
However, the strain-specific conformation of PrPSc and, in particular, its causal relationship with strain phenotypes, still remains controversial (13, 21).

Most of the information regarding strains so far available has been obtained from in vivo experiments using mice or hamsters, a system less advantageous for biochemical approaches to the molecular mechanisms of the strains. Neuronal cell culture models are clearly of greater value for such studies. However, only a few cultured cell lines, including PC12 and mouse neuroblastoma-derived N2a, have been shown to be permissive to scrapie agents (7, 26), and the levels of replication in these cell lines are not satisfactory, at least for quantitative detection of infectivity. Some of us previously demonstrated that cultured mouse neuronal cells expressing a high amount of PrPSc were highly permissive to replication of various mouse-adapted strains (15, 18). Arjona et al. recently compared two CJD strains using GT1-7 and N2aS8 cells (1). The aim of the present study was to confirm the usefulness of the neuronal cell culture models by comparing phenotypes of mice inoculated with two strains, Chandler and Fukuoka-1. We report here that passage through the cell cultures did not change the strain-specific nature of the biological characteristics and discuss the relationship between strain phenotype and biochemical aspects of the PrP.

MATERIALS AND METHODS

Cell cultures. The mouse neuronal cell line GT1-7 (14) was exposed to mouse brain homogenates infected with each prion strain as described previously (15, 16, 18). The cells were cultured in DMEM containing heat-inactivated fetal bovine serum at 10% and penicillin-streptomycin and split every 5 days at a 1:3 ratio. All cultured cells were maintained at 37°C in 5% CO2 in the biohazard prevention area of the authors’ institution.

Mice. ddY mice used in the experiments were fed under specific-pathogen-free conditions. Experiments involving agent inoculation were conducted in the biohazard prevention area (P3) of the Laboratory Animal Center for Biomedical Research of the authors’ institution.

Prion strains. The Fukuoka-1 strain (S1) was passaged three times in the brains of ddY mice. The brains infected with Chandler strains were kindly donated by B. Caughey and R. Carp, respectively. The pooled brains were homogenized to 1% (wt/vol) in cold phosphate-buffered saline (PBS) containing 5% glucose. Cultured cell lysates were prepared by sonication in PBS. All of the homogenates and cell lysates were kept at −80°C until use.

Determination of LD50. Confluent cell cultures in a 100-mm dish (approximately 7.5 × 106 cells) were sonicated in 0.5 ml of PBS. Before use, the cells were cultured for more than 30 passages following the initial ex vivo challenges. The cultured cell lysates and 1% (wt/vol) homogenates of brain tissues were serially diluted 10-fold with PBS, from 106 to 10−6, and 20 μl of each dilution was inoculated into the right brain (five mice for each group). The inoculated mice were observed until 364 days after inoculation. The onset of disease was determined as previously described (28). The 50% lethal dose (LD50) was determined according to the Behrens-Karber formula (10).

Histology. The brains were fixed in 4% paraformaldehyde and sectioned into 7-μm-thick sections at levels 250 and 500, as described by Sidman et al. (30). The tissue sections were stained with hematoxylin and eosin. The pattern of vacuolation was examined in 9 areas, namely the midbrain, hypothalamus, thalamus, hippocampus, paratemporal body, posterior cortex, cerebellar medulla, cerebellar granular layer, and cerebellar molecular layer. The vacuolation score was established based on the pattern, size, and density of the vacuoles using standard criteria from grade 0 for none and grade 5 for maximum vacuolation (9).

Antibodies. The anti-PrP monoclonal mouse antiserum used was described previously (19). The IBL-N rabbit antibody against N-terminal peptides of PrP and M20 goat antibody to C-terminal PrP peptides were purchased from Immuno Biological Laboratories (Gunma, Japan) and Santa-Cruz Biotech (Santa Cruz, CA), respectively. Horseradish peroxidase (HRP)-conjugated anti-mouse and -rabbit immunoglobulin G antibodies were purchased from Amersham. HRP-conjugated anti-goat immunoglobulin G antibodies were purchased from Santa-Cruz Biotech.

Immunoblotting. Confluent cultures were lysed for 30 min at 4°C in Triton-DOC lysis buffer (50 mM Tris-HCl [pH 7.5] containing 150 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, and 2 mM EDTA). After 1 min of centrifugation at 5000 × g, the supernatant was collected and its total protein concentration was measured using the Bio-Rad protein assay. To detect PrPSc, the brain homogenates and cell lysates, with the protein concentration adjusted to 10 mg/ml, were treated with 100 μg/ml of proteinase K (Sigma) at 37°C for 30 min. To remove N-linked glycosylation, PNGase F was used according to the manufacturer’s protocol (New England Biolabs) before PK digestion. The samples were boiled for 5 min in sodium dodecyl sulfate (SDS) loading buffer (50 mM Tris-HCl, pH 6.8, containing 5% glycerol, 1.6% SDS, and 100 mM dithiothreitol) and subjected to SDS–12% polyacrylamide gel electrophoresis. The proteins were transferred onto an Immobilon-P membrane (Millipore) in transfer buffer containing 15% methanol at 400 mA for 60 min, and the membrane was blocked with 5% nonfat dry milk in TBST (10 mM Tris-HCl [pH 7.8], 100 mM NaCl, 0.1% Tween 20) for 1 h at room temperature and reacted with anti-PrP antibodies. Immunoreactive bands were visualized by HRP-conjugated secondary antibodies using an enhanced chemiluminescence system (ECL; Amersham Pharmacia Biotech).

RESULTS

Biological characteristics of prion strains in cultured cells: clinical signs in inoculated mice. To examine the biological characteristics of the Chandler and Fukuoka-1 prion strains, GT1-7 cells independently infected with the two strains as well as infected mouse brain homogenates were inoculated into the brains of ddY mice. As shown in Table 1, all of the homogenates produced neurological symptoms and subsequent death in the inoculated mice. They exhibited some common clinical signs, such as weight loss, ruffled and greasy yellowish hair, tremor, hypersensitivity to sound and touch, and locomotor disturbance. However, all of the Chandler-infected mice were hyporeactive at the early stages of the disease, in contrast to the progressive hypoactivity of Fukuoka-1-infected mice. When the mice at the terminal stages were sacrificed, a markedly extended bladder, due to urination disturbance, was observed in Fukuoka-1-inoculated, but not Chandler-inoculated, mice. These strain-specific symptoms were reproduced without exception in all of the mice, irrespective of whether brain homogenates or cultured cell lysates were used.

Incubation periods in inoculated mice. As shown in Table 1, the incubation periods of the mice inoculated with 1% brain homogenates of Fukuoka-1 and Chandler were 128.6 ± 9.9 days (mean ± standard deviation) and 149.8 ± 4.4 days, respectively. Interestingly, GT1-7 cells infected with Fukuoka-1 also exhibited a shorter incubation period than Chandler-infected cells: 139.7 ± 12.5 versus 150.2 ± 5.9 days. To analyze more quantitatively, the brain homogenates and GT1-7 cell lysates infected with Fukuoka-1 and Chandler, designated Fukuoka-1/brain, Chandler/brain, Fukuoka-1/GT1-7, and Chandler/GT1-7, respectively, were subjected to the end-point 10-fold dilution assay (Table 1). According to Behrens and Korber’s formula, the infectious titers were estimated to be 108.1 and 108.8 LD50 units/g of the brain tissues and 105.3 and 105.5 LD50 units/107 GT1-7 cells infected with Fukuoka-1 and Chandler, respectively. After each dilution was converted to its infectious titer, the relationships between infectious titers and incubation periods in the four materials were analyzed. As shown in Fig. 1, plots of Fukuoka-1/brain and Fukuoka-1/GT1-7 clustered in the same region, and those of Chandler/brain and Chandler/GT1-7 formed another cluster located at the region representing much longer incubation periods. The linear relationships between infectious titers and incubation periods in brain homogenates and cell lysates in-
fected with each strain overlapped but were distinct between the strains (Fig. 1).

**Pathological findings in inoculated mice.** Brain sections including the hippocampus, thalamus, and cerebellum from inoculated mice at the terminal stage were stained with hematoxylin and eosin. As shown in Fig. 2, although spongiform change, neuronal loss, and gliosis are common characteristics of prion diseases, the severity and distribution of histological abnormalities differed between the brain tissues of Fukuoka-1- and Chandler-infected mice. In the Fukuoka-1-infected brains, large empty vacuoles were prominent mainly in the white matter, and a microcystic structure measuring up to 100 μm in diameter was observed in the cerebellar medulla (Fig. 2a and e). The grey matter was also affected in advanced cases, while the cerebellar granular and molecular layers were not damaged. The vacuoles in Chandler strain-infected brains were distributed equally in the grey and white matter. However, the number of vacuoles was fewer, and the size, an average of 27 μm in diameter, was obviously smaller than that of those from Fukuoka-1 strain-infected brains (Fig. 2b and f). In general, histopathological changes were much more severe in Fukuoka-1-infected tissues compared with those infected with the Chandler strain. These strain-specific pathological profiles were reproduced by inoculation of GT1-7 cell lysates infected with either strain (Fig. 2c, d, g, and h). A semiquantitative evaluation of the number and size of vacuoles (vacuolation score) in selected areas of brain tissues confirmed that the lesion profiles were strain specific (Fig. 3).

**Biochemical aspects of PrP.** Biochemical characteristics of PrP in the noninfected brain tissues and GT1-7 cells were

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**TABLE 1.** Mortality and incubation periods of mice inoculated with prion strains

<table>
<thead>
<tr>
<th>Tissue or cell type</th>
<th>Fukuoka-1 strain</th>
<th>Chandler strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dilution (no. dead/total)</td>
<td>Incubation period (days ± SD)</td>
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<tr>
<td>Brain homogenate</td>
<td>10⁻² 5/5 128.6 ± 9.9</td>
<td>10⁻² 5/5 149.8 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>10⁻³ 5/5 139.2 ± 8.9</td>
<td>10⁻³ 5/5 155.4 ± 7.1</td>
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<tr>
<td></td>
<td>10⁻⁴ 5/5 145.6 ± 11.4</td>
<td>10⁻⁴ 5/5 183.4 ± 8.0</td>
</tr>
<tr>
<td></td>
<td>10⁻⁵ 4/5 184.7 ± 25.7</td>
<td>10⁻⁵ 5/5 193.4 ± 16.8</td>
</tr>
<tr>
<td></td>
<td>10⁻⁶ 4/5 250.5 ± 57.4</td>
<td>10⁻⁶ 3/5 204.0 ± 27.7</td>
</tr>
<tr>
<td></td>
<td>10⁻⁷ 1/5 230.0</td>
<td>10⁻⁷ 4/5 248.0 ± 37.4</td>
</tr>
<tr>
<td></td>
<td>10⁻⁸ 0/5</td>
<td>10⁻⁸ 0/5</td>
</tr>
<tr>
<td></td>
<td>10⁻⁹ ND</td>
<td>10⁻⁹ 0/5</td>
</tr>
<tr>
<td>GT1-7 cell lysate</td>
<td>10⁻⁰ 4/4 139.7 ± 12.5</td>
<td>10⁻⁰ 4/4 150.2 ± 5.9</td>
</tr>
<tr>
<td></td>
<td>10⁻¹ 5/5 137.2 ± 12.6</td>
<td>10⁻¹ 4/4 167.3 ± 11.8</td>
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<tr>
<td></td>
<td>10⁻⁷ 0/5</td>
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</table>

* Twenty-microliter aliquots of serial 10-fold dilutions of GT1-7 cell lysates and brain homogenates (10⁻², 1% [wt/vol] homogenate) were inoculated into the brain of a mouse. Inoculated cells were passaged 35 times before use.

† Infectious titers of the brain tissues infected with Fukuoka-1 and Chandler were 10⁸.¹ and 10⁸.⁸ LD₅₀ units/g tissue, respectively.

‡ Infectious titers of Fukuoka-1 and Chandler-infected GT1-7 cells were 10⁶.³ and 10⁶.⁵ LD₅₀ units/10⁷ cells, respectively.

§ ND, not defined.

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![Fig. 1](http://jvi.asm.org) Linear relationship between infectious titers and incubation periods. Each dilution used in the end-point assay shown in Table 1 was converted to its infectious titer, and the relationships between infectious titers (LD₅₀) and incubation periods (days) in Fukuoka-1/brain (closed circles), Chandler/brain (open circles), Fukuoka-1/GT1-7 (closed squares), and Chandler/GT1-7 (open squares) were analyzed. Horizontal bars indicate standard deviations.
compared by immunoblotting. As shown in Fig. 4a, without PK treatment, the IBL-N antibody raised against N-terminal peptides of PrP visualized PrPs both in the noninfected brain and in GT1-7 cells. However, glycosylated components, a diglycosylated band in particular, of the latter migrated much more slowly, indicating that PrP in GT1-7 cells was more heavily glycosylated. Moreover, migration patterns of unglycosylated PrPs from the two sources also looked different: that in GT1-7 cells migrated a little faster. In the tissues and cells infected with Chandler and Fukuoka-1 strains, these host-specific char-

FIG. 2. Histological features of brain tissues infected with prion strains. Sections of the cerebrum (A to D) and cerebellum (E to H) of mice inoculated with Fukuoka-1/brain (A and E), Chandler/brain (B and F), Fukuoka-1/GT1-7 (C and G), and Chandler/GT1-7 (D and H) were stained with hematoxylin and eosin.
The characteristics of the PrP structure were essentially preserved. To confirm the difference in the migration patterns between unglycosylated PrPs from brain tissues and GT1-7 cells, Asn-linked glycosylation was completely removed by PNGase F treatment before immunoblotting. As shown in Fig. 4b, regardless of the presence or absence of prion infection, unglycosylated PrP from GT1-7 cells always migrated faster than that from brain tissues.

**FIG. 3.** Region profiles of vacuolation scores in infected mouse brain tissues. Scores were established based on the pattern, size, and density of vacuoles in the tissue using standard criteria with zero for none and five for maximum vacuolation. The pattern of vacuolation was examined in 9 areas, namely the midbrain (1), hypothalamus (2), thalamus (3), hippocampus (4), paraterminal body (5), posterior cortex (6), cerebellar medulla (7), cerebellar granular layer (8), and cerebellar molecular layer (9). Closed and open symbols indicate brain sections infected with brain homogenates and GT1-7 cell lysates, respectively. Each plot indicates the average score of sections from two mice.

**FIG. 4.** Detection of PrP in brain homogenates and GT1-7 lysates without PK treatment. (A) Noninfected (N) brain homogenates and GT1-7 cell lysates and those infected with Fukuoka-1 (F) or Chandler (C) prions were subjected to immunoblotting with the IBL-N antibody against N-terminal PrP peptides. (B) The mobilities of nonglycosylated PrPs (arrow) from the brain tissues (Br) and GT1-7 cells (GT) were compared on an immunoblot with the IBL-N antibody after deglycosylation by PNGase F treatment. MW, molecular weight.
Strain-dependent differences in the biochemical aspects of PrP<sub>Sc</sub>. PK completely digested PrP in the noninfected tissues and cells, while the resistant components (PrP<sub>Sc</sub>) in the infected tissues and cells were visualized by polyclonal antiserum raised against a recombinant PrP (Fig. 5). Again, diglycosylated PrP<sub>Sc</sub> in GT1-7 cells migrated to a much higher molecular mass, and its unglycosylated component migrated faster than PrP<sub>Sc</sub> in the brain tissues. Of importance, PrP<sub>Sc</sub> developed in the brain tissues of mice inoculated with the lysates of GT1-7 cells infected with Fukuoka-1 or Chandler prions (Br/GT) were also analyzed. MW, molecular weight.

![Image](image1.png)

**FIG. 5.** Immunodetection of PK-resistant PrP in brain homogenates and GT1-7 lysates infected with prion strains. After treatment with PK, noninfected brain homogenates (Br) and GT1-7 cell lysates (GT) and those infected with Fukuoka-1 or Chandler prions were subjected to immunoblotting with polyclonal mouse antisera against PrP. The brain homogenates from terminal-stage mice inoculated with the lysates of GT1-7 cells infected with Fukuoka-1 or Chandler prions (Br/GT) were also analyzed. MW, molecular weight.

![Image](image2.png)

**FIG. 6.** Differences in the mobility of PK-resistant PrP between hosts and between strains. (A) After successive treatments with PK and PNGase F, mobilities in a gel of nonglycosylated PrP<sub>Sc</sub> from the brain tissues (Br) and GT1-7 cells (GT) were compared on an immunoblot with the M20 antibody against C-terminal PrP peptides. (B) Mobilities of nonglycosylated PrP<sub>Sc</sub> generated by Fukuoka-1 (F) and Chandler (C) strains are directly compared by immunoblotting with the M20 antibody. PrP in noninfected tissues and cells (N) is completely digested by PK. The arrowhead indicates the 13-kDa PrP fragment specifically found in the Fukuoka-1-infected tissues and cells. MW, molecular weight.
the mobility of PK-resistant unglycosylated PrPSc in gel. Interestingly, in this immunoblotting, the M20 anti-C-terminal PrP antibody clearly visualized at a low molecular size, ~13 kDa, a component of PrPSc in Fukuoka-1-infected, but not Chandler-infected, GT1-7 cells (Fig. 5b). A faint but significant band of the 13-kDa fragments was also detectable in the Fukuoka-1-infected brain tissue. Since this fragment was similarly detectable even before the PK treatment and recognized by C-terminal (M20) but not N-terminal (IBL-N) PrP antibodies (Fig. 7), it is likely to be a C-terminal PrP fragment lacking a PK cleavage site.

DISCUSSION

Passage through the neuronal cell cultures of two prion strains, Chandler and Fukuoka-1, did not affect the biological characteristics, including clinical signs, incubation periods, and pathological findings, in the inoculated mice. Carryovers of the original strains into the cultures were unlikely, since the infected cells were cultured for more than 30 passages since the initial ex vivo challenges, assuming that residuals of original brain homogenates in the cultures would be diluted far greater than 10 orders of magnitude. We used a mouse neuronal cell line, GT1-7, which expresses a large amount of PrPSc and is highly permissive for replication of the agent (15, 18). Infected GT1-7 cells persistently produced PrPSc for more than 30 passages without subcloning and maintained high infectious titers of Fukuoka-1 and Chandler at the levels of $10^{5.3}$ and $10^{6.5}$ LD50 units/107 cells, respectively. The high degree of competence in prion replication allowed us to quantify infectious titers in the cultured cells by end-point assay. Although data are not shown, we have found that the biological characteristics of the two strains, including clinical signs and incubation periods in the inoculated mice, are also conserved in other two neuronal cell lines, N2a58 (18) and 1C11 (17), which are permissive to various prion strains (16, 18; N. Nishida, unpublished). These findings are consistent with those of previous reports indicating that strain phenotypes did not change during several passages in cultured cells (1, 5).

Western blotting identified some differences in the biochemical features of PrPSc between the brain tissue and GT1-7 cells. The degrees of glycosylation of PrPSc derived from the two strains in GT1-7 cells were clearly higher than those in the brain tissues. A similar difference was observed before PK treatment even between the noninfected cells and tissues, suggesting the involvement of host cell factors rather than the strains. In some conditions, the degree of glycosylation (a ratio of glycoforms) of PrPSc is an important signature of the prion strain (5, 8). However, in our experimental models, it is largely determined by the hosts, presumably due to differences in the enzymatic activities involved in glycosylation or the trafficking pathway of de novo-synthesized PrPC. Host cell- or tissue-determined PrPSc glycoforms have also been reported by others (31, 36). The mobility of unglycosylated PrPSc fragments in gel was also distinguishable between the brain tissues and GT1-7. This possibly reflected PK cleavage site heterogeneities due to the difference in the conformation of PrPSc or an artifact of experimental conditions such as pH (20). However, it is noteworthy that the size difference was also the case for PrP without PK treatment even between the noninfected tissues and cells, arguing against a difference in the PK cleavage sites. Sequencing of PrP cDNAs amplified by reverse transcription-PCR from the brain tissues and GT1-7 cells confirmed that their primary structures were identical (data not shown). A previous study identified, by use of mass spectrometry, six different glycosylphosphatidylinositol (GPI) glycoforms with molecular masses ranging from 2,670 to 3,285 Da in PrPSc purified from infected hamster brains as well as partially purified PrPC (33). The presence of tissue-specific differences in the GPI composition was also suggested (12). Although the involvement of some difference in the PrP conformation preserved even in a denatured condition cannot be ruled out, a more likely explanation is that it is due to heterogeneity in the composition of GPI moieties. Precise mechanisms for the diversity in PrP structures among the hosts await elucidation, but these structural features are unlikely to affect the strain phenotype, which is shared by the hosts.

It has been hypothesized that the strain-specific conformation of PrPSc determines the pathological features and func-

FIG. 7. The 13-kDa PrP fragment is detectable in Fukuoka-1-infected GT1-7 cells even without PK treatment. The cell lysates from noninfected (N) GT1-7 cells and those infected with the Fukuoka-1 (F) or Chandler (C) strain, with (±) or without (−) PK treatment, were subjected to immunoblotting using M20 (A) or IBL-N (B) anti-PrP antibodies. MW, molecular weight.
tions as a template during pathogenic structural conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup> in affected brain tissues (25). The present study also revealed evidence that some strain-specific features of the PrP<sup>Sc</sup> band pattern on an immunoblot were conserved in the cultured cells. The unglycosylated PK-resistant PrP<sup>Sc</sup> fragment derived from Fukuoka-1 always migrated more slowly than the Chandler-derived version accumulating in the same host, either in brain tissues or GT1-7 cells. So far, many prion strains, including those of human, sheep, and mink origin, have been characterized by the size of the PrP<sup>Sc</sup> core fragment generated by PK (2–4, 24). In most cases, the size difference due to the diverse cleavage sites of PK is presumed to be a consequence of the extent of the β-sheet structure. However, Arjona et al. reported that identical PrP<sup>Sc</sup> band patterns could be observed in GT1-7 cells infected with distinct CJD strains, FU and SY, but were different from those in brain tissues and N2a cells (1). This indicated that the conformational divergence of PrP<sup>Sc</sup><sup>C</sup> does not necessarily alter strain characteristics. We also demonstrated host-determined divergence, such as glycosylation patterns, which did not affect the biological characteristics of prion strains. Furthermore, the possible involvement of a putative agent or agent-induced factors other than the PrP<sup>Sc</sup> conformation itself is not precluded. It would be of value to search for such factors that affect the mobility of PrP<sup>Sc</sup> core fragment by using the cell culture model.

Strikingly, the small 13-kDa PrP<sup>Sc</sup> fragment detected in Fukuoka-1-infected tissues and cells was not seen in Chandler-infected materials. It is likely that the 13-kDa Fragment. Fragment is strain-specific, since Fukuoka-1 but not Chandler resulted in the band not only in GT1-7 cells but also in N2a58 and 1C11 cells (N. Nishida, unpublished). It was PK resistant but detectable before PK treatment, suggesting a lack of PK cleavage sites and the involvement of endogenous proteolytic processes. It is possible that certain environmental factors, for instance, pH and metal ion concentration, influenced by the strain may alter the catalytic activities, but it is also possible that the Fukuoka-1-specific PrP<sup>Sc</sup> conformation could allow endogenous proteases to access and catalyze the full-length PrP<sup>Sc</sup>. Although both the Chandler and Fukuoka-1 strains are mouse-adapted ones, the former is of scrapie origin and the latter was derived from a GSS patient carrying the P102L mutation. One group previously demonstrated a similar 13-kDa fragment in the brain tissues from five of seven P102L GSS patients, and this fragment was immunoreactive to a C-terminal PrP antibody but not to 3F4 monoclonal antibody, indicating that it was N-terminally truncated beyond residue 112 (22). It would be intriguing to see whether the specific PrP<sup>Sc</sup> conformation determined by a particular genetic mutation in the human brain tissues has been conserved during successive transmission to mouse brains and cultured cells lacking such a mutation. Some other reports demonstrated that PrP<sup>Sc</sup> derived from F198S GSS and CJD also displayed patterns of endogenous proteolytic characteristic of each disorder, leading to distinct sets of PrP<sup>Sc</sup> fragments (12). It is conceivable that different PrP<sup>Sc</sup> fragments may exhibit unique biological and pathological consequences in the CNS. The most important pathological consequence of prion strains is the difference in the distribution of vacuolar degeneration among the CNS regions of affected animals. A possible explanation is that each prion strain possesses its own cell tropism. The investigation of cell tropism requires the stable infection of a single cell type which is permissive to more than one strain. In our preliminary experiments using the cell culture models, some strains revealed differential tropism among the cell types examined (N. Nishida, unpublished).

In conclusion, we demonstrated here that the prion strains tested conserved their biological characteristics following cell culture, and the accumulated PrP<sup>Sc</sup> reproduced some specific features of its band pattern on an immunoblot. However, the molecular basis for conformational divergence of PrP<sup>Sc</sup> is still enigmatic, and whether or not there is a causal relationship between the PrP<sup>Sc</sup> conformation and strain phenotype remains to be concluded. Our cell culture models allow the analysis of trafficking and metabolism of PrP, i.e., posttranslational cleavage, glycosylation, recycling, and degradation, etc., which will provide a new insight into the understanding of the molecular basis of prion strains.

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