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Inhibition of prion propagation in scrapie-infected mouse neuroblastoma cell lines using mouse monoclonal antibodies against prion protein

Kazuyoshi Miyamoto [a], Naoto Nakamura [a], Masayoshi Aosasa [a], Noriyuki Nishida [b], Takashi Yokoyama [c], Hiroyuki Horiuchi [a,d], Shuichi Furusawa [a,d], Haruo Matsuda [a,d,*]

[a] *Laboratory of Immunobiology, Department of Molecular and Applied Bioscience, Graduate School of Biosphere Science, Hiroshima University, 1-4-4 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8528, Japan*
[b] *Department of Molecular Microbiology and Immunology, Graduate School of Medical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan*
[c] *Prion Disease Research Center, National Institute of Animal Health, 3-1-5 Kannondai, Tsukuba, Ibaraki 305-0856, Japan*
[d] *Hiroshima Prefectural Institute of Industrial Science and Technology, 3-10-32 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-0046, Japan*

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Abstract

We screened six mouse monoclonal antibodies (mAbs) against prion protein (PrP), which were previously established in our laboratory, for inhibitory activity against PrP^{Sc}-accumulation in scrapie-infected cell lines and identified two mAbs, 3S9 and 2H9, as possessing this inhibitory activity. mAb 3S9 recognized an epitope including 154th tyrosine in the helix 1 region of PrP, while mAb 2H9 recognized a discontinuous region that included helix 1. In three scrapie-infected cell lines infected with different mouse-adapted scrapie strains, mAb 3S9 strongly inhibited accumulation of PrP^{Sc}, while mAb 2H9 moderately inhibited accumulation of PrP^{Sc}, indicating that inhibition of prion propagation by mAbs may be dependent on PrP^{Sc} characteristics. Furthermore, mAb 3S9 completely excluded PrP^{Sc} from these cell lines. These results suggest that mAbs 3S9 and 2H9 might be useful for clarifying the mechanisms of prion propagation and prevention by PrP-specific antibodies, and for tracing the conversion of PrP^{C} to other PrP^{Sc} isoforms.

Keywords: PrP-specific antibody; Inhibition of prion accumulation; Helix 1 of PrP; 154th tyrosine of PrP; Prion strain

Transmissible spongiform encephalopathies (TSEs) are so-called prion diseases and comprise a group of fatal neurodegenative disorders, including scrapie in sheep and goats, bovine spongiform encephalopathy in cattle, and Creutzfeldt–Jakob disease in humans [1]. These diseases share the accumulation of a pathogenic isoform of prion protein (PrP^{Sc}) in the central nervous system as a common feature and no effective therapy against prion diseases currently exists. The PrP^{Sc} is converted from the host-encoded cellular isoform of PrP (PrP^{C}) by post-translational modifications. Although the two prion isoforms have identical amino acid sequences [2], their biological and biochemical properties differ. PrP^{Sc} is insoluble in detergents and is partially resistant to proteinase K (PK) digestion, whereas PrP^{C} is readily soluble under nondenaturing conditions and is completely digested by PK [3–5]. The mechanism by which PrP^{C} is converted to PrP^{Sc} remains unclear.

PrP-specific antibodies are useful in understanding both the structure of PrP [6,7] and in tracing the conformational changes from PrP^{C} to PrP^{Sc} [8,9]. In addition, PrP-specific antibodies can be used in the diagnosis of prion diseases in experimental and domestic animals as well as therapeutic approaches of Creutzfeldt–Jakob disease in humans [10–13]. Recent reports indicate that

* Corresponding author. Fax: +81 82 424 7968.
E-mail address: hmatsu@hiroshima-u.ac.jp (H. Matsuda).

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some PrP-specific antibodies can inhibit prion propagation and exclude PrP^{Sc} both in vitro and in vivo [14]. Therefore, therapeutic approaches using mAbs are being explored. However, the prevention mechanisms by which PrP-specific antibodies function remain unclear, and few inhibitory mAbs have been reported [8,9,14–18]. The generation and identification of PrP-specific antibodies are thus important in structural studies, diagnosis, and therapeutic approaches.

In this study, we investigated whether six mouse mAbs against PrP established in our laboratory inhibit prion propagation in scrapie-infected cell lines. We report here that two mAbs, 3S9 and 2H9, inhibited the conversion of PrP^{C} to PrP^{Sc} and that one of these mAbs completely excluded PrP^{Sc} from scrapie-infected cells.

Materials and methods

Cell lines. SP2/0-Ag14 (SP2) [19] was used as the myeloma cell line partner in the cell fusion experiment. SP2 was maintained in Iscove's modified Dulbecco's medium (IMDM, Invitrogen, USA) containing 10% fetal bovine serum (FBS, Sigma, USA) in a 5% CO_2 incubator at 37 °C. The scrapie-infected mouse neuroblastoma cell lines N2a/22L [20], N2a/Chandler [20], and N2a/Fukuoka were used to investigate the inhibition of prion propagation by PrP-specific antibodies. These cell lines are persistently infected with three mouse-adapted scrapie strains (22L, Chandler and Fukuoka-1) having the conserved biological and biochemical characteristics of the original prion strain [21]. N2a/22L cells possessed much higher levels of PrP^{Sc} than the other two cell lines. These cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, USA) containing 10% FBS in a 5% CO_2 incubator at 37 °C.

Recombinant PrP. Recombinant Hu122–230 (codons 122–230 of human PrP) was kindly supplied by Dr. Kitamoto (Tohoku University, Japan). Recombinant Ha23–231 (codons 23–230 of hamster PrP) was kindly supplied by Dr. Horiuchi (Hokkaido University, Japan). Recombinant PrPs, Mo121–231 (codons 121–231 of mouse PrP), Sh125–234 (codons 125–234 of sheep PrP), and Bo133–241 (codons 133–241 of bovine PrP), were generated as described previously [22]. Briefly, recombinant PrPs were expressed with pET22b (Novagen, Germany) and then purified by using nickel ion-charged Chelating Sepharose Fast Flow (Amersham Biosciences, USA) and HiPrep Sephacryl S-100 HR (Amersham Biosciences, USA) according to the manufacturer's instructions.

Mouse PrP deletion mutants (mouse PrP: codons 121–231, 131–231, 141–231, 151–231, 161–231, 171–231, 181–231, 191–231, 201–231, 121–221, 121–212, 121–201, 121–191, 121–181, 121–171, 121–163, and 121–151) were generated with pGEX-6P-1 (Amersham Biosciences, USA) in order to synthesize PrP as a glutathione *S*-transferase (GST) fusion protein. Briefly, DNA fragments of deletion mutants were amplified by PCR using primer sets as indicated in Table 1. Amplified fragments were digested with *Bam*HI and *Xho*I, and cloned into the *Bam*HI and *Xho*I sites of pGEX-6P-1. These expression plasmids were transformed into *Escherichia coli* BL21 (DE3) (Novagen, Germany). Protein expression was induced by addition to 0.1 mM isopropylthio-β-D-galactoside. Bacterial cells were collected and sonicated. Deletion mutants were purified with glutathione–Sepharose 4B beads (Amersham Biosciences, USA) according to the manufacturer's instructions.

Concentrations of these recombinant PrPs were measured by BCA Protein Assay Reagent Kit (Pierce, USA) according to the manufacturer's instructions. Hu122–230 and Sh125–234 were used as immunogens to generate mAbs. Recombinant PrPs including in Hu122–230 and Sh125–234 were used as antigens for enzyme-linked immunosorbent assay (ELISA). Deletion mutants were subjected to mAb epitope analysis.

Monoclonal antibodies. Monoclonal antibodies (mAbs) specific to PrP were generated using cell fusion technology. Briefly, 6-week-old $PrP^{0/0}$ mice [23] were immunized intraperitoneally with 100 μg Hu122–230 or Sh125–234 in 0.1 ml phosphate-buffered saline (PBS) together with an equal volume of alum solution. Subsequent immunization was carried out every 3 weeks. Three days after the final injection, spleen cells from the immunized $PrP^{0/0}$ mice were fused with the SP2 cells using 50% (wt/vol) polyethylene glycol 1500 (Roche Diagnostics, Switzerland) and were selected in hypoxanthin aminopterin thymidine (HAT) medium. Hybridoma culture supernatants were screened by ELISA using recombinant PrPs as immunogens. The ELISA procedure is described below. Hybridomas secreting antibodies against PrP were cloned by limiting dilution. The mAbs were purified from mouse ascites and were used in subsequent experiments. Protein concentration was measured with a BCA Protein Assay Reagent Kit (Pierce, USA) according to the manufacturer's instructions. The isotypes of the mAbs were determined using a Mouse monoclonal isotyping kit (Amersham Biosciences, USA) according to the manufacturer's instructions.

Four mAbs, 22L/2H9 (2H9), 22L/2H12 (2H12), 22L/1A3 (1A3), and 22L/8H12 (8H12), specific for PrP generated previously [22], were used in epitope analysis and inhibition assay for prion propagation.

ELISA. ELISA plates (Nunc, USA) were coated with 50 μl/well of 2.5 μg/ml of recombinant PrPs (Hu122–230, Mo121–231, Ha23–231, Sh125–234, and Bo133–241) in PBS at 4 °C overnight. Plates were blocked with 380 μl/well of 25% (vol/vol) BlockAce (Yukijirushi, Japan) in PBS at 37 °C for 1 h. After washing with PBS containing 0.05% (vol/vol) Tween 20 (PBS-T), hybridoma culture supernatants were added (50 μl/well) and plates were incubated at 37 °C for 1 h. After washing with PBS-T, HRP-labeled goat anti-mouse IgA + IgG + IgM (H + L) (Kirkegaard and Perry Laboratories, USA) was added

Table 1
Primers for generation of deletion mutants

Sense primer	Anti-sense primer
MoPrP121-F: 5′CGGGATCCGTGGGGGGCCTTGG3′	MoPrP231-R: 5′CCCTCGAGGCTGGATCTTCTCC3′
MoPrP131-F: 5′CGGGATCCAGCGCCGTGAGCAG3′	MoPrP221-R: 5′CCCTCGAGGGACTC CTTCTGG3′
MoPrP141-F: 5′CGGGATCCGGCAACGACTGGGAGGA3′	MoPrP212-R: 5′CCCTCGAGCATCTG CTCCACCA3′
MoPrP151-F: 5′CGGGATCCGAAAACATGTACCGCTAC3′	MoPrP201-R: 5′CCCTCGAGATCGGT CTCGGTGA3′
MoPrP161-F: 5′CGGGATCCTACTACAGGCCAGTGG3′	MoPrP191-R: 5′CCCTCGAGGGTGGTGGTGACC3′
MoPrP171-F: 5′CGGGATCCCAGAACAACTTCGTG3′	MoPrP181-R: 5′CCCTCGAGGATACTTACGCAGTCGT3′
MoPrP181-F: 5′CGGGATCCATCACCATCAAGCAG3′	MoPrP171-R: 5′CCCTCGAGCTGGTTGCTGTACTGATCC3′
MoPrP191-F: 5′CGGGATCCACCACCAAGGGGGAGA3′	MoPrP163-R: 5′CCCTCGAGCCTGTAGTACACTTGGTTAGG3′
MoPrP201-F: 5′CGGGATCCGATGTGAAGATGATGG3′	MoPrP151-R: 5′CCCTCGAGTTCACGGTAGTAGCGGTCCT3′

Primer sets of deletion mutants on N-terminal: sense primers + MoPrP231-R. Primer sets of deletion mutants on C-terminal: MoPrP121-F + anti-sense primers.

(50 μl/well) as a detection antibody and plates were incubated at 37 °C for 1 h. After washing with PBS-T, *o*-phenylenediamine sulfate (Sigma, USA) was added as a substrate and optical density was then measured at 490 nm.

Preparation of PrP^C and PrP^{Sc} for Western blotting. Brain tissues from different animals (mouse, sheep, and cow) were homogenized in 9 volumes of lysis buffer [10 mM Tris (pH 7.5), 100 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 0.5% sodium deoxycholate] containing Complete Protease Inhibitor Cocktail Set (Roche Diagnostics, Switzerland). Homogenates were centrifuged at 800*g* for 5 min at 4 °C and the supernatants were used as a source of PrP^C. Protein concentration of the supernatant was then measured using a BCA Protein Assay Reagent Kit (Pierce, USA). PK-treated materials were prepared using brain tissues from scrapie-infected mice and the scrapie-infected mouse neuroblastoma cell lines N2a/22L, N2a/Chandler, and N2a/Fukuoka [20,23]. These brain homogenates and cell lysates were treated with 20 μg/ml PK for 40 min at 37 °C. Digestion was stopped with 1 mM Pefabloc Sc (Roche Diagnostics, Switzerland) and the materials were centrifuged at 1,00,000*g* for 1 h at 25 °C. After removing the supernatant, the pellet was resuspended in 30 μl Laemmli buffer, followed by incubation at 55 °C and boiling for 10 min. PrP^C and PrP^{Sc} were then subjected to Western blotting.

Western blotting. PrP^C and PrP^{Sc} were separated by SDS–PAGE on 13.5% polyacrylamide gel and were then transferred to an Immunblot PVDF membrane (Bio-Rad, USA) by electroblotting at 300 mA for 2 h. Membranes were blocked with blocking buffer (8% (wt/vol) skim milk, 0.2% (vol/vol) Tween 20, and 2.5 mM EDTA in PBS) at room temperature for 1 h and then incubated for 1 h at room temperature with the anti-PrP mAbs in washing buffer (1% (wt/vol) skim milk, 0.2% (vol/vol) Tween 20, and 2.5 mM EDTA in PBS). Membranes were washed in washing buffer with agitation, and then incubated at room temperature for 1 h with HRP-labeled Goat Anti-Mouse IgA + IgG + IgM (H + L) (Kirkegaard and Perry Laboratories, USA) in washing buffer. After membranes were washed, the blots were developed with ECL plus Western blotting detection reagents (Amersham Biosciences, USA) and detected by LAS-3000 lumino image analyzer (Fujifilm, Japan).

Epitope analysis. The PrP epitopes recognized by mAbs were determined by Western blotting using mouse PrP deletion mutants (mouse PrP: codons 121–231, 131–231, 141–231, 151–231, 161–231, 171–231, 181–231, 191–231, 201–231, 121–221, 121–212, 121–201, 121–191, 121–181, 121–171, 121–163, and 121–151). Western blotting was as described above. As primary antibodies, six mAbs were used; 17H5, 3S9, 2H9, 2H12, 1A3, and 8H12. Anti-GST Antibody (Amersham Biosciences, USA) was used for detection of GST fusion PrPs as a control. As second antibodies, HRP-labeled goat anti-mouse IgA + IgG + IgM (H + L) (Kirkegaard and Perry Laboratories, USA) and HRP-labeled rabbit anti-goat IgG (H + L) (Kirkegaard and Perry Laboratories, USA) were used.

Inhibition analysis of PrP^{Sc} accumulation in prion-infected cell lines using mAbs. The scrapie-infected mouse neuroblastoma cell lines, N2a/22L, N2a/Chandler, and N2a/Fukuoka, were each seeded (2×10^5 cells/dish) in 60-mm dishes containing 3 ml of 10% FBS–DMEM. Cells were cultured for 4 days in the absence or presence of 10 μg/ml mAbs. After culture, cells were lysed according to the procedure described above. Western blotting analysis was performed as described above, with the following exceptions. Detection of PrPs (PK-treated and -untreated PrPs) was performed using HUC2-13 specific for the N-terminal of PrP [24] or HUNN1 specific for the PK cleavage site [25]. As a detection antibody, HRP-labeled goat anti-chicken IgG (H + L) (Kirkegaard and Perry Laboratories, USA) was used.

In order to determine the dose-dependency of mAbs in the inhibition of PrP propagation, N2a/Chandler cells were seeded (2×10^5 cells/dish) in 60-mm dishes containing 3 ml of 10% FBS–DMEM. Cells were cultured for 4 days in the presence or absence of 0.0032–10 μg/ml mAbs. After culture, cells were lysed and analyzed by Western blotting according to the procedure described above. The intensity of immunostained bands on Western blotting was quantified using Science Lab 2001 Image Gauge software (Fujifilm, Japan).

Disappearance of PrP^{Sc} from prion-infected cells by anti-PrP mAbs. N2a/Chandler or N2a/22L cells were seeded (2×10^5 cells/dish) in 60-mm dishes containing 3 ml of 10% FBS–DMEM. Cells were cultured for 4 days in the presence of 10 μg/ml of mAbs. After cultivation for 4 days, cells were passaged into two dishes at 2×10^5 cells/dish. One group was used for Western blotting, the other was serially treated with identical mAbs. Cells were treated with 10 μg/ml of mAbs for 4–16 days. Every 4 days, the cells were passaged. After each treatment with mAbs, cells were further incubated in the absence of mAbs for 4–16 days. Cells were then lysed and analyzed according to the procedure described above.

Results

Monoclonal antibodies

The six mouse mAbs (17H5, 3S9, 2H9, 2H12, 1A3, and 8H12) used in this study were generated by immunizing $PrP^{0/0}$ mice with recombinant PrPs or scrapie-infected mouse neuroblastoma cell lines [22]. Of the mAbs, 17H5 and 3S9 were newly generated in this study. We further examined the PrP epitopes recognized by the mAbs, and Western blotting was performed using mouse PrP deletion mutants (Fig. 1). mAb 17H5 reacted with mutants lacking 171 N-terminal residues and 221 C-terminal residues. mAb 3S9 reacted with mutants lacking 141 N-terminal residues and 161 C-terminal residues. mAbs 2H9 and 2H12 reacted with mutants lacking 151 N-terminal residues and 221 C-terminal residues. mAbs 1A3 and 8H12 reacted with mutants lacking 141 N-terminal residues and 221 C-terminal residues. These results indicate that the epitopes recognized by mAbs 17H5 and 3S9 were located at residues 171–221 and 141–161, respectively. The epitopes recognized by mAbs 2H9 and 2H12 were located at residues 151–221, and those recognized by mAbs 1A3 and 8H12 were located at residues 141–221. The characteristics of the six mAbs are shown in Table 2.

Inhibition of PrP-propagation by mAbs

In order to identify mAbs that inhibit accumulation of PrP^{Sc} in cultured cells, inhibition studies of prion propagation were performed using scrapie-infected neuroblastoma cell lines N2a/22L, N2a/Chandler, and N2a/Fukuoka. mAbs 3S9 and 2H9 induced decreases in the amount of PK-treated PrP^{Sc} in these cell lines, whereas mAb 17H5 had no effect on the amount of PK-treated PrP^{Sc} (Fig. 2). The other mAbs, 2H12, 1A3, and 8H12, had no effect on the amount of PK-treated PrP^{Sc}, as observed with mAb 17H5 (data not shown). Furthermore, the amounts of PK-untreated PrP were not affected by treatment with mAbs. mAbs 3S9 and 2H9 inhibited the accumulation of PrP^{Sc} in these cell lines. Interestingly, mAb 3S9 was particularly effective at

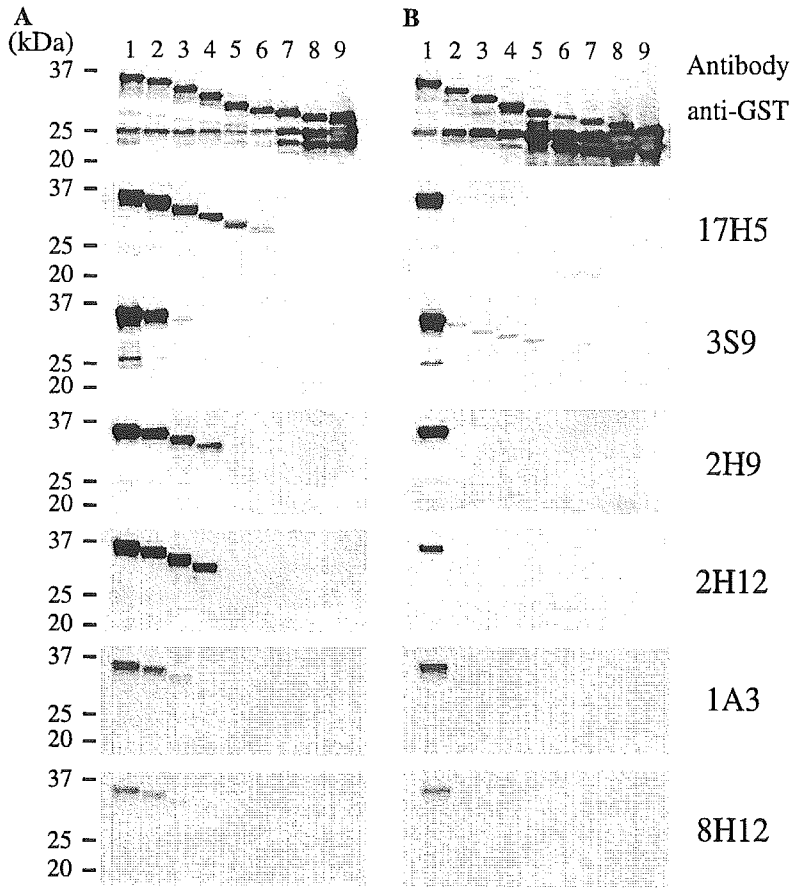

Fig. 1. Epitope mapping of PrP recognized by PrP-specific antibodies. (A) Western blotting profiles using N-terminal deletion mutants. Lanes 1–9 show amino acid residues of PrP, lane 1; 121–231, 2; 131–231, 3; 141–231, 4; 151–231, 5; 161–231, 6; 171–231, 7; 181–231, 8; 191–231, and 9; 201–231. (B) Western blotting profiles using C-terminal deletion mutants. Lanes 1–8 show amino acid residues of PrP, lane 1; 121–221, 2; 121–212, 3; 121–201, 4; 121–191, 5; 121–181, 6; 121–171, 7; 121–163, and 8; 121–151. Lane 9 shows GST alone. Anti-GST antibody was used for detection of deletion mutants (GST fusion proteins) as a control. Molecular masses are indicated on the left.

Table 2
Characteristics of mouse monoclonal antibodies against PrP

mAbs	Isotype	Immunogen	Epitope	Reference
3S9	IgG_1	Recombinant sheep PrP	141–161	—
17H5	IgG_1	Recombinant human PrP	171–221	—
22L/2H9	IgG_1	Scrapie-infected mouse	151–221	Nakamura et al. [22]
22L/1A3	IgG_1	Neuroblastoma cell lines	141–221	
22L/2H12	IgG_1		151–221	
22L/8H12	IgM		141–221	

inhibiting accumulation of PrP^{Sc} in all cell lines, whereas mAb 2H9 markedly inhibited accumulation of PrP^{Sc} in N2a/Chandler and N2a/Fukuoka cells, but did so slightly in N2a/22L.

In order to compare inhibition activity, the dose-dependency of the mAbs was examined. N2a/Chandler cells were incubated for 4 days with various concentrations of mAbs ranging from 0.0032 to 10 μg/ml. Dose-dependent inhibition was determined using the mAb concentration at which 50% inhibition of PrP^{Sc} levels was seen (50% inhibitory concentration (IC_{50})). When compared with PrP^{Sc} in untreated cells, PrP^{Sc} levels in treated cells was reduced in a dose-dependent manner. The IC_{50} values obtained for mAbs 3S9 and 2H9 were 0.08 μg/ml (0.6 nM) and 1.2 μg/ml (8.4 nM), respectively (Fig. 3). The inhibition activity of mAb 3S9 was stronger than the activity of mAb 2H9.

In order to investigate whether it is possible to completely exclude PrP^{Sc} from N2a/Chandler and N2a/22L cells using mAbs, these cell lines were treated with

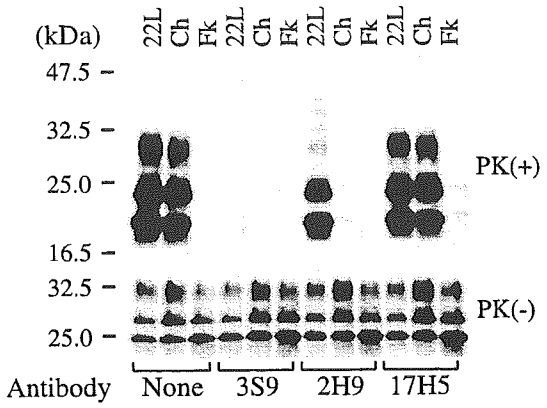

Fig. 2. Inhibition of prion propagation in scrapie-infected cell lines N2a/22L (22L), N2a/Chandler (Ch), and N2a/Fukuoka (Fk) by PrP-specific antibodies. PrP treated with or without PK was detected by Western blotting using HUC2-13 specific for the N-terminal of PrP and HUNN1 specific for the PK cleavage site of PrP. Molecular masses are indicated on the left.

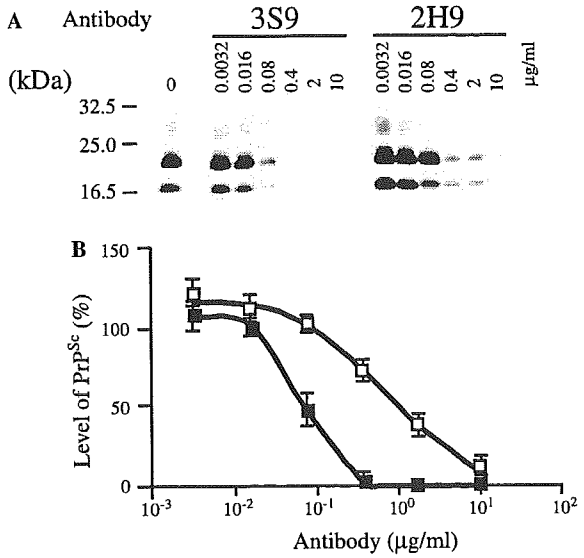

Fig. 3. Dose-dependency of mAbs 3S9 and 2H9 on the inhibition of PrP^{Sc} accumulation in N2a/Chandler cells. (A) Western blotting profiles of PrP^{Sc} from N2a/Chandler cells using mAbs 3S9 and 2H9. N2a/Chandler cells were cultured for 4 days with various concentrations of mAb. Cells were lysed and then digested with PK. Levels of PrP^{Sc} in the cells were determined by Western blotting using mAb HUNN1, which is specific for the PK cleavage site of PrP. Molecular masses are indicated on the left. (B) Inhibition curve for mAbs 3S9 and 2H9. Levels of PrP^{Sc} given as 100% correspond to the intensity of PrP^{Sc} bands in the absence of mAbs, while 0% represents undetectable levels of PrP^{Sc}. Each square (closed squares, 3S9; open squares, 2H9) represents means ± SD from at least three independent experiments.

10 μg/ml mAbs for 4, 8, 12 or 16 days. After treatment, cells were further incubated in the absence of mAbs for 4, 8, 12 or 16 days. PrP^{Sc} in N2a/Chandler cells treated with mAb 3S9 for 4 days was reduced to non-detectable levels, but gradually recovered on incubation in the absence of mAb (Fig. 4A). However, PrP^{Sc} in cells treated with mAb 3S9 for 8 days remained at non-detectable levels after 12 days in the absence of mAb. mAb 2H9 was also able to reduce and exclude PrP^{Sc} from N2a/Chandler cells after 16 days of treatment with mAb. These two mAbs reduced accumulation of PrP^{Sc} in N2a/22L cells, and mAb 3S9 was able to continue excluding PrP^{Sc} from cells for 12 days after incubation (Fig. 4B). In addition, cells treated with mAb 3S9 for 8 days did not express PrP^{Sc} in the absence of mAb, even after 1 year of culture (data not shown). These results indicate that mAbs enable complete exclusion of PrP^{Sc} from infected cell lines following continuous treatment with mAb.

Reactivity of mAbs against PrP

The reactivity of inhibitory mAbs, 3S9 and 2H9, against PrP^{C} and PrP^{Sc} was investigated by Western blotting. mAb 3S9 recognized the mouse and sheep PrPs, while mAb 2H9 recognized mouse PrP. mAbs 3S9 and 2H9 recognized the PK-treated PrP^{Sc} (Fig. 5). mAb 3S9 strongly recognized three glycoforms of PrP from scrapie-infected mouse brain and N2a/22L cells, whereas mAb 2H9 failed to recognize the di-glycosylated form of PrP^{Sc} from N2a/22L cells.

The reactivity of inhibitory mAbs was also confirmed by ELISA using recombinant human, mouse, hamster, sheep, and bovine PrPs. mAb 3S9 recognized mouse and sheep PrPs, while mAb 2H9 recognized mouse and hamster PrPs (Table 3).

Discussion

Antibodies against PrP are indispensable in the diagnosis of prion diseases in humans and animals. The results of recent reports [8,9,14–18] have shown that certain PrP-specific mAbs may be useful in therapeutic approaches for prion diseases. Research into mAbs against PrP is thus important in the diagnosis and treatment of prion diseases.

To identify mAbs that prevent the PrP^{Sc} accumulation in prion-infected cell cultivation, we screened various mAbs against the C-terminal portion of PrP. We found that two mAbs, 3S9 and 2H9, inhibited conversion of PrP^{C} to PrP^{Sc} (Fig. 2–4). These mAbs recognized different epitopes; the epitopes recognized by 3S9 and 2H9 were located in residues 141–161 and 151–221, respectively (Fig. 1). Epitopes recognized by mAbs that inhibit prion propagation have been classified into four regions; 59–89, 90–109, 144–156, and 225–231 [8,9,14–18]. It is thought that residues 144–156 (helix 1 region) are particularly important in the conversion of PrP^{C} to PrP^{Sc} [8,9,26,27].

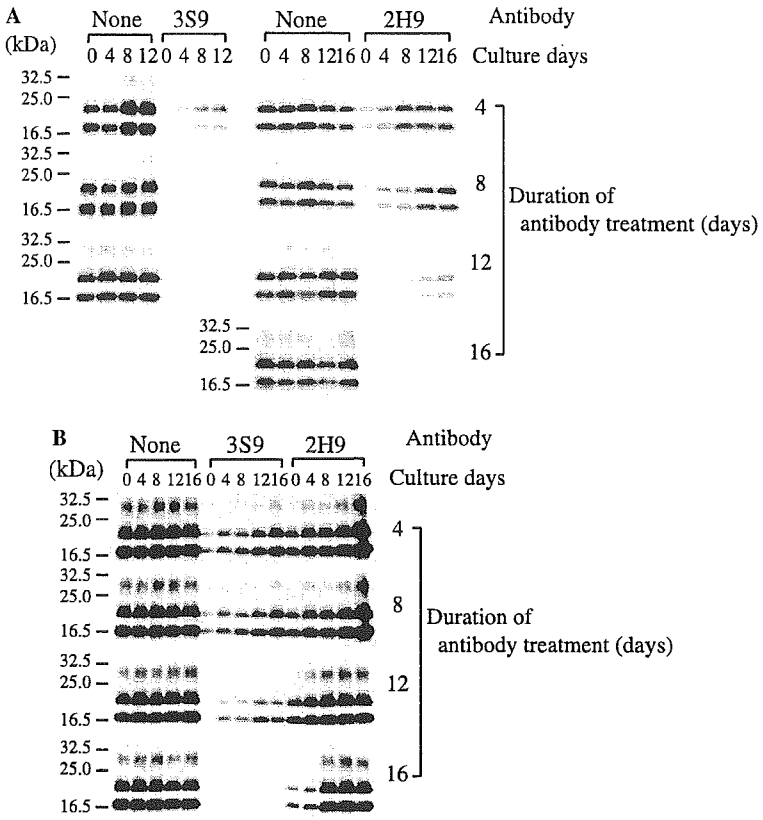

Fig. 4. Disappearance of PrP^{Sc} from prion-infected cells treated with mAbs 3S9 and 2H9. (A) N2a/Chandler cells were cultured for 4, 8, and 12 (and 16) days with or without 10 μg/ml of 3S9 or 2H9. (B) N2a/22L cells were cultured for 4, 8, 12, and 16 days with or without 10 μg/ml of 3S9 or 2H9. Cells were lysed and then digested with PK. Levels of PrP^{Sc} in cells were determined by Western blotting immediately after antibody treatment was initiated (day 0) or after cell cultivation for 4, 8 or 12 (or 16) days (day 4, 8, 12 or 16) in the absence of antibody. PrP^{Sc} was detected by mAb HUNN1, which is specific for the PK cleavage site of PrP. Molecular masses are indicated on the left.

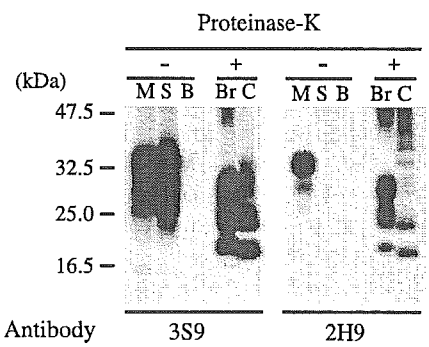

Fig. 5. Western blotting profiles of mammalian PrP^{C} and PK-treated PrP^{Sc} using inhibitory mAbs identified in this study. Mammalian PrP^{C} are brain homogenates from normal mouse (M), sheep (S), and cow (B), respectively. PK-treated PrP^{Sc} are from scrapie infected-mouse brain (Br) and N2a/22L cells (C). The designation, + or −, indicates whether PK was applied to the sample. Molecular masses are indicated on the left.

Table 3
Reactivity of anti-PrP mAbs against mammalian PrPs

mAb (Isotype)	Mouse	Hamster	Human	Sheep	Cow
3S9 (IgG1)	+	−	−	+	−
2H9 (IgG1)	+	+	−	−	−

+, reactive (OD value: >0.1); −, non-reactive (OD value: <0.1).

mouse	141	GNDWEDRYYRENMYRYPNQVY	161
hamster	141	GNDWEDRYYRENMNRYPNQVY	161
human	141	GSDYEDRYYRENMHRYPNQVY	161
sheep	141	GNDYEDRYYRENMYRYPNQVY	161
cow	141	GSDYEDRYYRENMHRYPNQVY	161

Fig. 6. Alignment of amino acids of the PrP epitope recognized by mAb 3S9. Residue numbers are indicated on the left and right. The helix 1 region of PrP is underlined, and the arrowheads indicate non-conserved amino acids in this region.

Most of the inhibitory mAbs reported previously, 6H4 [8], D18 [9], ICSM18 [14], ICSM17 [15], SAF61 [16,18], and SAF53 [18], recognized epitopes in the helix 1 region. This region is highly conserved among mammalian PrPs, and many inhibitory mAbs recognize epitopes common to all mammalian PrPs in the helix 1 region. The epitope recognized by mAb 3S9 is also located on helix 1 of PrP^{C} as well as other mAbs that

prevent PrP^{Sc} propagation. However, species specificity of PrP recognition by this mAb was different from other inhibitory mAbs.

On Western blot and ELISA, mAb 3S9 recognized mouse and sheep PrPs (Fig. 5 and Table 3). Three amino acid residues between 141 and 161 (142, 144, and 154) are not conserved among mammalian PrPs (Fig. 6). Amino acid 154 (Tyr) is the only residue that is common to mouse and sheep PrPs in this region. The results indicate that the epitope of mouse and sheep PrPs recognized by mAb 3S9 includes residue 154 (Tyr), and that mAb 3S9 recognizes different residues than other mAbs [8,9,14,16–18] having epitopes in the helix 1 region. These facts suggest that many amino acids important to prion propagation are present in the helix 1 region, that common amino acids recognized by these mAbs may be significant for prion propagation, and that residue 154 (Tyr) is required for the strong inhibition of prion propagation by mAb 3S9.

The fact that the epitope recognized by mAb 2H9 is located in residues 151–221 suggests that mAb 2H9 may recognize a discontinuous epitope. Inhibition of prion propagation by mAb 2H9 may be dependent on the recognition of amino acids in the helix 1 region or another region of PrP.

Two inhibitory mAbs identified in this study, 3S9 and 2H9, inhibited conversion of PrP^{C} to PrP^{Sc} in both N2a/Chandler and N2a/22L cell lines (Fig. 4). One of these, 3S9, effectively inhibited prion propagation in N2a/22L cells expressing PrP^{Sc} at high levels. Furthermore, mAb 3S9 was able to completely exclude PrP^{Sc} from N2a/22L cells with continuous treatment. The effects of inhibitory antibodies reported previously were determined in ScN2a cells expressing low levels of PrP^{Sc}. Because the levels of PrP^{Sc} are much higher in N2a/22L cells than in ScN2a cells [20], the results obtained here show that the inhibitory activity of mAb 3S9 was high. Interestingly, mAb 2H9 markedly inhibited accumulation of PrP^{Sc} in N2a/Chandler, but did so slightly in N2a/22L cells (Fig. 2). Recent reports [21] have shown that the characteristics of prion strains were conserved in persistently infected cell lines and that the characteristics of PrP^{Sc} depend on both the host cell type and the strains used for infection. Indeed, the band patterns of PrP^{Sc} were slightly different among the cell lines (Fig. 2). Therefore, the results suggest that differences in inhibition efficiency by mAb 2H9 may be due to the expression levels or characteristics of PrP^{Sc} in the two cell lines.

A recent paper has suggested that the inhibition of prion propagation by mAbs was caused by degradation of PrP^{C} [16]. However, the fact that levels of PK-untreated PrP were not affected by treatment with mAbs (Fig. 2) indicates that inhibition of prion propagation by mAbs 3S9 and 2H9 is unrelated to degradation of PrP^{C}. Inhibition of prion propagation by mAbs 3S9 and 2H9 was probably due to inhibition of the formation of molecular complexes between PrP^{C} and PrP^{Sc}, as suggested in previous studies [8,9]. Although the amount of PrP^{Sc} in N2a/22L cells treated with mAb for 4 days decreased slightly, the amount did not increase for 4 days after treatment with mAb 2H9 (Fig. 4B). This result suggests that the conversion of PrP^{Sc} from PrP^{C} occurs very slowly.

The inhibitory mAbs, 3S9 and 2H9, identified in this study may be useful for clarifying the mechanisms by which prion propagation is inhibited by PrP-specific antibodies and may be valuable in the search for variations in PrP^{Sc}. At present, the reasons for differences in inhibition activity between the two mAbs are not clear. Further experiments to clarify these differences are underway.

Acknowledgments

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特集・プリオン病とBSE

7. プリオン株をめぐる謎

岐阜大学人獣感染制御センター・プリオン病研究部門助教授
/米国Yale大学医学部神経病理学講座客員教授　西田　教行

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〒101-0061 東京都千代田区三崎町3丁目
3番1号TKiビル
電話03(3265)7681(代) FAX03(3265)8369

(株) 医薬ジャーナル社

〒541-0047 大阪市中央区淡路町3丁目
1番5号淡路町ビル21
電話06(6202)7280(代) FAX06(6202)5295

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特集 プリオン病とBSE

7. プリオン株をめぐる謎

西田 教行*

プリオンとは何？と専門外の人によく聞かれる質問であるが，それこそが我々の命題である。定義上は「蛋白質だけからなる感染性粒子」と説明できるが，病原体そのものが果たしてプリオンであるのか，真実はまだ明らかではない。株すなわち病原体の多様性の存在とその挙動は，病原体が遺伝子を持つことを強く示唆する。株の存在そのものは謎ではなく観察結果であるが，病原体をプリオンであると仮定すると謎めいてくるわけである。プリオン株とは何か，性質の違いがどう認識されてきたのか，文献的な解説と最新の研究結果を紹介する。

Key Words：CJD／scrapie／BSE／prion／strain diversity

I はじめに：プリオン病とその病原体

ヒトのクロイツフェルト・ヤコブ病（CJD），ゲルストマン・ストライスラー・シュラインカー病（GSS）・家族性致死性不眠（FFI），kuru（カーニバリズムによる経口感染），および動物では羊のスクレイピー(scrapie)，ミンクの伝達性ミンク海綿状脳症（TME），牛海綿状脳症（BSE），エルク等の野生シカに見られる慢性消耗症(CWD)は，その感染性と病理学的特徴に基づき，伝達性海綿状脳症（transmissible spongiform encephalopathies：TSE）として一つのカテゴリーに分類される疾患群である。いずれも実験的に罹患脳の乳剤を動物に接種すると，長い潜伏期間の後に疾患に特徴的な神経の脱落変性，海綿状変性，グリア細胞の増生が起こり高次脳機能障害を来たし確実に死に至る。罹患動物からヒトへの感染は通常認められないが，BSEは例外的にヒトを含めた広い宿主域をもつことが知られている。ヒトから動物への実験的感染は容易ではないが，類人猿のほかギニアピッグ，ラット，マウス，ヤギ，ネコにて成功例が報告されている。ヒトからヒトへの感染は，kuru（カーニバリズムによる経口感染）と医原性（硬膜移植など）のものが知られている。

これらの感染性疾患の病原体はいまだ不明である。非通常型ウイルスによるスローウイルス感染症として扱われてきたが，ウイルスの同定には至っておらず，一方，罹患脳に宿主蛋白のアミロイド変性体凝集（蓄積）を認め（これをプリオン蛋白と呼ぶ），この異常型プリオン蛋白が病原体の本体であるとの仮説に基づきプリオン病とも呼ばれる[1]。この謎の多い病原体の多様性（株の存在）について概説することが本稿の目的であるが，そのためにまずは用語の定義をしておきたい。

疾患名は総称としてプリオン病と呼ばれることが多いが，個別の疾患名をできうる限り用いる。病原体はそれぞれの疾患ごとに，“CJD病原体”や“BSE病原体”とその由来に基づいて名称を用いる。『プリオン』とは『蛋白性感染粒子』と定義されるがその存在の最終証明がなされていないた

Mystery of diversity among TSE agents

* Noriyuki Nishida　岐阜大学人獣感染防御センター・プリオン病研究部門 助教授／米国Yale大学医学部神経病理学講座 客員教授

め，『病原体＝プリオン』との使用はあえて行わない。またウイルスの同定もされていないのでCJウイルスとは呼ばず上記の表現にとどめ，総称はTSE病原体とする。プリオン蛋白は宿主の蛋白質で分子量25～35 KDaの膜蛋白のことであり，正常細胞あるいは正常脳に発現しているものを正常型プリオン蛋白(PrP^C)と呼び，疾患特異的な蛋白分解酵素に部分抵抗性の異常型をPrPresと呼ぶ。異常型はその由来にちなんでPrP^{Sc}，PrP^{CJD}，PrP^{BSE}と呼ばれることがあるが本稿ではPrPresと統一して記載する。

Ⅱ　株多様性とは

TSE病原体はその遺伝子が同定されていないため分子レベルでの病原体の同定，記述はできない。しかし現象論的に異なる表現型が存在するため，ウイルスや細菌などの病原微生物と同様に「病原体株」として認識されている。つまり遺伝子背景が均一な実験動物を宿主として用いた場合に，固有の表現型（潜伏期間，臨床像，病理像）が再現されるため，その表現型は病原体の生物学的活性の違いに由来すると解釈され，異なる“病原体株”として認識されるわけである。実際に実験を行う上では限界希釈した罹患脳を感受性動物に接種し，単一病原体による表現型の再現をもって株を定義・記述することが可能になる。ただ単に由来の違いだけでは厳密にはそれが単一のものか，複数の病原体によるものかわからない。またやっかいな問題は，異なる由来を持つ「分離株」が非常によく似た表現型である場合，それは同一株といっていいのか，異なる株として記載するかという問題である。表現型の記載はどうしても曖昧さを払拭できない部分があるので，この問題はいまのところ解決不可能と言うよりほかない。

プリオン病における病原体株の存在は，その分子機序がいかなるものであるかという科学的興味と，その性質の違いから生じる不活化方法，安全性の確立といった社会的(公衆衛生学的)問題にかかわるやっかいな側面とをもたらす古くて新しいテーマである。では，具体的に株多様性についての報告をまとめ，最後に最近の培養細胞系を用いた株に関する知見と深まる謎について述べていきたい。

Ⅲ　スクレイピーについて

スクレイピー（scrapie）はイギリスを中心に欧米諸国および日本や他のアジア，アフリカ地域（オーストラリアとニュージーランドには存在しない）の羊に発生する海綿状脳症で，18世紀にすでにその記載があり[2]，遺伝性疾患であるとか毒物の蓄積によるであろうとか諸説が提唱されたが，その感染性が1936年，フランスのCuilleとChelleによって初めて実験的に証明された[3]。その後Wilsonらの感染実験によってフィルター通過性でウイルス様であること，熱抵抗性が高いこと，フォルマリン抵抗性であることなどが報告されている[4]。これらの羊を用いたスクレイピー感染実験やフィールド調査では株として記載されていないが，症状の違い(hyperexcitable〔易興奮性〕なものとsleepy〔嗜眠性〕なもの)があることが観察されている。またPattisonらはヤギへの感染実験において，「drowsy type(嗜眠型)」と「scratchy type（掻痒型)」を記述し，異なる株が存在する可能性を提案した[5]。このdrowsy goat scrapieを用いて，1961年，Chandlerはマウスへの感染に成功し[6]これがブレークスルーとなってスクレイピー研究が実験室にて行えるようになり，その後マウスを用いた研究が主流になってscrapie株の研究が始まったといっていいだろう。スクレイピー羊からのマウスへの感染と病原体分離はさかんに行われ，主にDickinsonらのグループによって株多様性の記載がなされてきた[7]。彼らは系統の異なる2種類のマウスおよびそのF1ヘテロへの感染で分離株の固有の潜伏期間と臨床症状，病理変化(おもに空胞の脳内分布)パターンを詳細に比較検討し，多様なscrapie株が分離されることを示した[8]。

Chandlerによってマウスへの感染継代されたdrowsy goat scrapieをChandler株（別名RML株，あるいは139A株，ハムスターに順化したものを263K株）と呼び，Dickinsonらのscratchy type-sheep scrapieからの分離株をそれぞれ22A，22C，22L，などの名称で呼んでいる。さらにいろんな研究室で独自に分離されたscrapie株

が報告されているがその詳細は省く。それらのすべてが羊において存在する病原体株であるかどうかは結論がでておらずDickinsonらの分離した株が実験室での人為的なものと批判するグループもあるが，表現型の再現性からして病原体としての株多様性の存在は明らかにされたといっていいだろう。マウスから羊へあるいはヤギに戻した場合には，典型的な症状と病理変化を呈することが報告されている。TMEおよびCWDについては他稿を参照されたい。

Ⅳ CJDおよびその他のヒトTSEについて

ヒトのプリオン病は病因論的に5つに分類される。散発性CJD，家族性疾患(家族性CJD, GSS, FFI)，kuru，変異型CJD(vCJD)，医原性CJD。表現型はkuruとvCJDがほぼ単一であるのに対し，そのほかはヴァリエーションが多い。kuruはパプアニューギニアに限局した疾患であり，初めて動物への感染が報告されたヒトTSEであり，一方vCJDはBSE病原体の経口感染によるものと考えられ，他のヒトTSEとは病原体株が異なると思われるが，その他の疾患がすべて単一の病原体の異なる表現型であるのか，いくつかの異なる病原体が存在するのかは，臨床および病理データからは結論できない。家族性のものはいまではPrP遺伝子に変異があることが明らかになっているが，この変異が直接病気の原因であるのか，あるタイプの病原体に感受性が高くなっているのかも結論が出されていない。羊の系統ごとに調べられたPrP遺伝子多型とスクレイピーに対する感受性の違いの相関データを参考にすると，家族性疾患にリンクしたPrPの変異は，疾患感受性と関係があると考えるのが自然であろう。

実験的にヒトからマウスへの感染はTateishiらが初めて成功した[9, 10]。興味を引かれるのは，家族性であるGSSのマウスへの初代感染は，ほぼ3分の1のケースで成功する一方，散発性CJDでは100%，ただし潜伏期を比較するとGSSよりも長い[11]。このことは一部のGSSは異なる病原体株である可能性が高い。そしてそのようなGSSの報告は日本に限定されている。こうした状況証拠に加え，マウスを用いた感染継代実験からは，スクレイピーと同様，単一系統の宿主マウスを使う限り，その表現型が再現され継代を経ても安定で異なる病原体株として認識されている。現在ヒト由来病原体では，Fukuoka-1株(GSS102L Japan)，Fukuoka-2株（CJD-Japan)，SY株（CJD-US)が分離され詳細に報告されている株である。CJD-US由来のSY株は，マウスでは300日以上の潜伏期をもって発症し，病変は視床に比較的限局する傾向がある。本邦で分離されたFukuoka-1株は120～140日で発症し，び漫性の病変を呈するアグレッシブなタイプである。Fukuoka-2株はマウス脳でプラーク形成が認められるCJD株である。

Ⅴ BSEにおける株の存在について

BSEにおける病原体の多様性があるかどうか，現在ホットな話題の一つである。BSE罹患ウシの異常PrPの体内分布パターンおよび生化学的性状を比較検討した日本とイタリアのグループがイギリスや他のヨーロッパに見られるBSEの所見と異なる特徴があるBSEの存在を報告し，株の違いであるかどうかが議論されている。しかし，生物学的な特徴をもって株を記載するには同一宿主を用いた詳細な感染実験を行い，その表現型をあらためて比較検討する必要があり，現在得られる情報だけでは判断できない。ラボでのクロスコンタミネーションを注意深くさけ，さらに再現性を確認するまでにはかなりの時間がかかるであろう。

だが，BSEに異なる株が分離されても驚くことではない。そもそもBSE病原体の由来についても，スクレイピーだとする見方とウシにもともと存在したのではないかという見方とがあり（ヒト由来との説もある)，スクレイピー由来だとしたらスクレイピーには異なる病原体株が存在しているので，レンダリングによる汚染が起ったときに複数の株が食物連鎖に入った可能性は容易に想像できる。問題は異なる株が存在するとしたら，BSEスクリーニングの方法，判定基準，病原体の不活化方法など現実に基準をもうけている事柄の，その基準の妥当性を科学的に再検討する必要が生じることである。ヒトへの感染性も株ごとに

異なるかもしれない。BSEに感受性が高く早く発症する遺伝子改変マウスを用いた感染実験が始まっているが，BSE株の存在についての詳細な検討はまだこれからである。

Ⅵ 株多様性とPrPresについて

Collingeらは，若年発症者が多いこと，病理像が特徴的であること，異常PrP(PrPres)のSDS-PAGE(SDSアクリルアミドゲル電気泳動)での泳動パターンが他の疾患のものと異なりBSEのものと類似していることなどから，イギリスにて見いだされたCJD亜型を変異型CJD（vCJD）と呼び，おそらくBSE感染牛の経口摂取によるものであろうとした[12]。このようにPrPresの泳動パターンが病原体株特異的であるとする見方は，若干問題を含んでいる。マウスに感染継代したさまざまなscrapie株やヒトTSE株のPrPresの比較を行うと相対的に違いを見いだすことは可能であるが，異なる株が同一パターンを示すこともある。相違に注目して異常PrPが病原体株に特異的異常構造を取っているであろうとする考えが提示されているが，異常構造が直接観察できないので想像の域を出ない。この泳動パターンによる株の同定は，あくまで実験室での限られた条件でのみ可能なことである。

プリオン仮説を支持する立場の考えに立つと，これらの病原体の性質はPrPresの構造に由来すると考えるよりほかに説明しようがない。つまり，PrPresは正常型PrPとは異なるβ sheet richな3次構造を取っていると思われるが，その構造が株ごとにユニークで，構造の違いが細胞指向性(cell tropism）や増殖速度に関係していると考えるわけである。我々はスローウイルス説の立場をとる。つまり病原体に特異的遺伝子情報があって遺伝子変異によって株多様性が生じると考えると，これはウイルスでのよく知られた現象であり株の存在は理解しやすい。病原体の物理化学的性質についての比較検討は紙面の制約上割愛する。

Ⅶ 培養細胞系を用いた病原体株の検討

では，TSE病原体株とは何であろうか。病原体の本体が明らかになるまで，それは想像するよりほかにないが，潜伏期の違いは単純に解釈すると病原体の増殖速度を反映すると思われる。症状および病理像は，病原体が脳内のどの領域に感染増殖し細胞障害を起こす傾向にあるのか，つまり細胞指向性を反映していると考えていいだろう。次に我々が近年行ってきた培養細胞を用いた病原体株の解析について具体的に述べ，株の存在する意義について考えてみたい。病原体の本質を理解するには，均一な培養細胞を宿主に用いた持続感染系を作出し，病原体の感染様式，増殖メカニズムを解析するのが王道であるが，TSE病原体は持続感染系の作成が長い間極めて困難とされていた。それでもマウスおよびハムスターの感染脳から不死化細胞が分離され持続感染が確認され[13, 14]，またマウス神経芽細胞腫（Neuro2a）[15]とラット褐色細胞腫(PC-12)[16]が *in vitro* で低いレベルながらも持続感染が成立すると報告された。我々はこのNeuro2aにマウスPrPを過剰発現させることで比較的容易に感染する細胞を得ることに成功した[17]。またマウスの視床下部神経細胞由来の不死化細胞株(GT1)と奇形種から分離された神経前駆細胞（1C11）への感染を試み，GT1が多くのマウス順化株に感受性であること，1C11はscrapie株よりもヒトTSE株に感受性であることを見いだした（Nishida，未発表)。ここでいう感染成立とは，異常PrPの産生と実験動物への細胞接種による病原性の伝搬確認のことである。これらの培養細胞では細胞毒性が見られないため，感染の指標としては上記の方法以外にいまのところ検出方法がない。マウス順化株(表1)のChandler株，22L株，Fukuoka-1株(FU-GSS)を用いて，Neuro2a，GT1および1C11細胞への感染効率を比較すると株ごとに細胞指向性があることが明らかになった。その分子レベルでのメカニズムは不明であるが，PrPだけでは説明が困難であり，宿主の別の分子（因子）が関与しているものと思われる。

我々はこれらの持続感染細胞において，病原体がそれぞれの株としての性質を長期培養後も保持していることを確認した[18, 19]。非常に興味深いのは，これらの異なるTSE病原体株間の相互作用についての報告である。Dickinsonらはスクレイピー由来の22Aと22C株を用いて，一定期間をあ

表1　我々が実験に用いている分離株

マウス順化 TSE 株	由来（病名 / 国）	種
SY	CJD sporadic/USA	Human
Fukuoka-1 (FU)	GSS P102L/Japan	Human
Chandler (Ch)	drowsy scrapie/UK	Goat
22L	scratchy scrapie/UK	Sheep

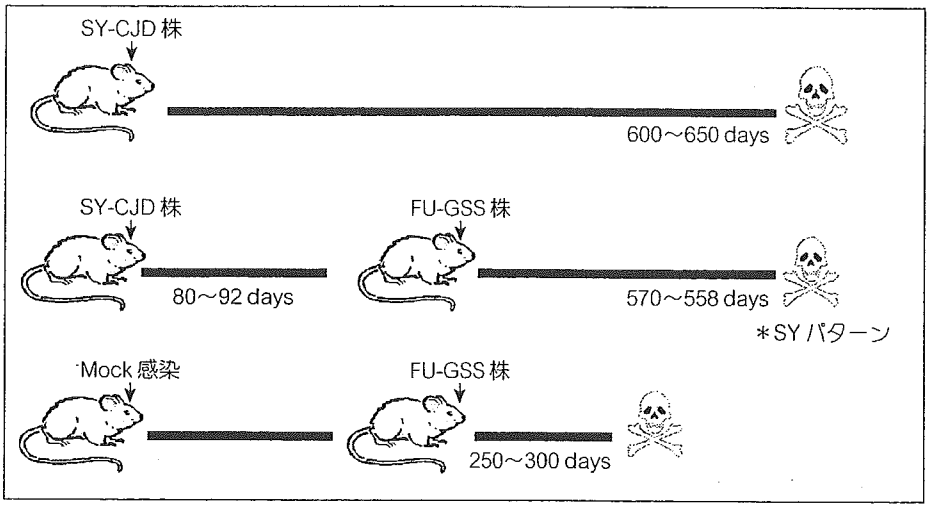

図1　弱毒株による干渉（*in vivo*）

ヒト由来の SY 株と FU 株の重感染実験（Manuelidis, et al.：PNAS 1998）。潜伏期が 600 日以上の SY 株を接種したのち FU 株を接種したところ，FU の感染は干渉を受け，先行感染する SY による発症しか認められなかった。

（文献 21 より引用）

けて両者をマウスに接種した場合，先行感染株がチャレンジ株の感染を妨げると報告した[7]。また Manuelidis らは，SY-CJD 株が FU-GSS 株の重感染を阻止することを報告している（図1）[20, 21]。この現象は生体内での免疫系の関与があるのか（抗体産生は否定されている），ウイルス干渉現象と類似の病原体間の作用であるのか明らかではなかった。そこで GT1 細胞が複数の病原体株に感受性があることを利用して，異なる株の重感染あるいは株間の干渉現象の有無について検討した（図2）。詳細は省くが，結論だけを述べると SY-CJD 株持続感染細胞は FU-GSS 株，scrapie 株（22L, Ch）に抵抗性を示した（図3）。また scrapie 株感染細胞は GSS 株の感染を完全に干渉する場合もあれば（22L），干渉せず重感染を許すこともある（Ch）[22]。この実験結果の我々の現時点での“拡大解釈”は，「TSE 病原体にはいろんな性質の異なる株が存在し，ひょっとすると CJD 病原体は広く人間界に存在する毒性が低い株で，scrapie 株や毒性の強い GSS 株の感染をある程度防いでいるのかもしれない」というものである。これまで疫学調査でスクレイピーのヒトへの感染はないとされてきたことや，ヨーロッパでの BSE 暴露人口に対する vCJD の発生数の低さ（この 10 年間で 180 人程度）は，「種の壁」の存在に加え，ウイルス様干渉が関与しているのではないかと考えている。

まとめると（1）TSE 病原体には性質の異なる「株」が存在し，（2）株の性質は培養細胞にて長期継代しても安定で，（3）株ごとに異なる細胞指向性あり，（4）異なる株の重複感染は起る場合もあれば干渉される場合もある（表2）。このような生物現象は単純に「異常 PrP が感染性の蛋白質で，感染とは異常 PrP が正常 PrP を異常型へと変換すること」との考えでは非常に説明が困難であ

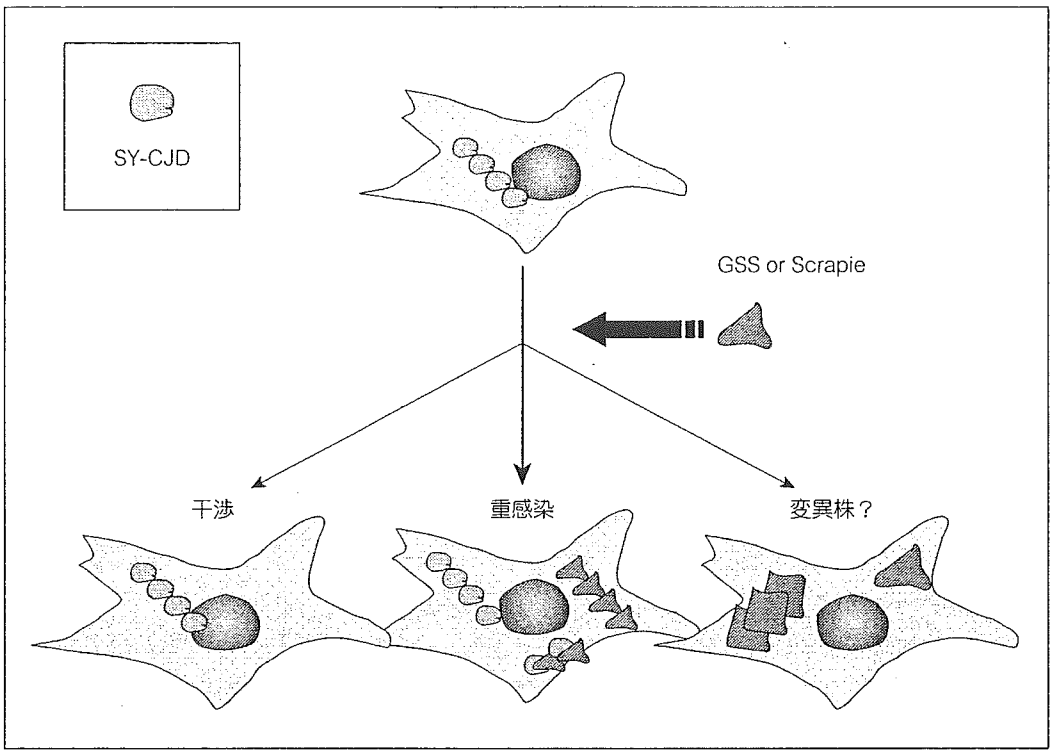

図2　培養細胞GT1を用いた病原体株間の重感染のイメージ

A株による先行感染が成立している細胞にB株をチャレンジした場合，図に示す3つのパターンが期待される。ただし変異株の産生に関しては現在まだ特定方法がない。

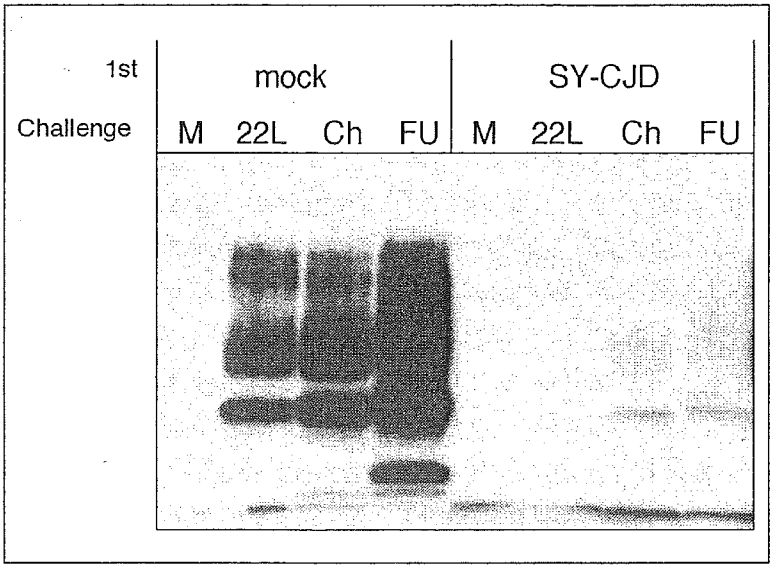

図3　GT1細胞を用いた干渉現象の検討

非感染細胞（mock）とSY-CJD感染細胞を用い，スクレイピー株（22L，Ch）およびGSS株（FU）をチャレンジした。継代培養5回目にて細胞から蛋白を抽出しプロテネースK処理後，PrPのウェスタンブロッキング法にて異常PrPの存在を確かめた。mock細胞では，22L，Ch，FUの感染が成立しているのに対し，SY細胞は22Lを完全に，ChとFUを部分的に干渉している。　（文献22より引用）

表2　プリオンにおける株とは（まとめ）

1．固有の症状，潜伏期間，脳組織の病理変化で特徴づけられる。継代によってその性質は変化しない。
2．何が株を規定しているのか，つまり情報を担う物質は何であるのかは不明。
3．株固有の細胞指向性がある。
4．ウイルス様の株間での干渉現象が見られることがある。

り，ウイルスの存在を強く示唆するものである。ウイルス説を支持するその他の根拠とウイルス同定の困難な理由については別の機会に考察したい。

Ⅷ　最後に

限られた紙面の都合上，詳細な実験データの説明は省かせてもらって概要をつかんでもらうことを念頭に記述したが，興味のある方はぜひ参考文献を精読してほしい。Prusinerをはじめとして複数の研究者がプリオン仮説の最終証明に取り組んでおり，証明できたとする論文も近年発表されているが，現実に自然界で起っていることをこの説ですべて説明できるわけではなく，実験データもこれから検証されないことには真実はまだわからないということを理解してほしい。BSEスクリーニングの問題や米国産牛肉の輸入解禁の問題にしても，我々が如何に頼りない限られた科学的知識に基づいて議論を繰り返しているか，結局それは病原体の本質が不明で物事の中心がぼけているためである。さらに株多様性の存在はBSE対策を考える上で，これまでに得られてきた膨大なスクレイピーやCJDの研究データがそのまま適応できないという問題を引き起す。この謎の病原体の研究が始まってほぼ70年になるが，我々の探求はまだその本質には届いていない。若い才能のある研究者の参画を期待する。

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