Heterogeneity of Abnormal Prion Protein (PrPSc) in Murine Scrapie Prions Determined by PrPSc-Specific Monoclonal Antibodies

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NOTE

Transmissible spongiform encephalopathy (TSE) is a neurodegenerative disorder in humans and animals, such as scrapie in sheep and goats. An abnormal isoform of prion protein, PrPSc, which is generated by the posttranslational modification of cellular prion protein (PrPc), accumulates in affected animals. PrPSc is believed to be the major, or the only, component of the infectious agent, that is, the prion [18]. Unlike PrPc, PrPSc has many β-sheets [15], and this structure is considered responsible for the aggregation of PrPSc. These characteristics contribute to the relative resistance to proteinase K (PK) digestion [17]. Biochemical detection of the moieties remaining after PK digestion, designated as PrPres, has generally been utilized as a criterion for diagnosing TSE.

Accumulating evidence indicates that PrPSc-specific monoclonal antibodies (mAbs) can be employed to detect conformations of PrPSc [6, 9, 12, 14, 16, 21]. Although their deduced epitopes vary, almost all mAbs seemed to detect PrPSc irrespective of the species-based differences. The mAb 3B7 and 3H6 possessed different reactivities to the PrPSc of several species; this represents their unique characteristics. This differential reactivity enabled us to trace the conformational transition of mouse PrPSc during adaptation in the sheep-to-mice transmission of scrapie [21]. This also indicated the co-existence of heterogeneous PrPSc in early-passaged mice in inter-species transmission of prion. These unique characteristics of mAbs are expected to provide an advantage in the conformational analysis of PrPSc.

TSE can be transmitted across species, and during this transmission, the incidence of different strains exhibiting different disease phenotypes have often been found [7]. The “protein-only” hypothesis suggests that conformational differences in PrPSc determine the strain phenotype [20]. It has been proposed that PrPSc has several conformations and this concept adequately explains the variation in the susceptibility and the emergence of new strains during interspecies transmission [3, 4]. However, direct evidence based on a biochemical approach is limited. The conformational differences in PrPSc have been estimated by the biochemical properties of PrPSc during immunoblotting [5, 20]. These analyses are effective in the comparison of PrPSc conformations among strains. However, distinguishing a particular PrPSc from a mixture of PrPSc is difficult, except in case of clearly distinguishing characteristics [1, 2]. Therefore, simple procedures for conformational discrimination of PrPSc in non-denatured condition would be of great value.

In this study, we aimed to determine whether heterogeneous PrPSc exists within mouse-adapted prion strains, by using PrPSc-specific mAbs.

Initially, we examined their reactivity to the scrapie Ohbï [19]-affected mouse brain homogenate by the immunoprecipitation (IP) assay. The mAbs 3B7 and 3H6 were conjugated to Dynabeads M-280 Tosylactivated (Invitrogen, Carlsbad, CA, U.S.A.), in accordance with the manufacturer’s instructions and used in an IP assay as described earlier [21]. In short, 200 µl of 0.025% brain homogenate (equivalent to 50 µg of brain tissue) in 2% Triton X-100 in PBS (Triton/PBS) and 5 µl of mAb-conjugated beads were rotated for 1 hr at room temperature and then washed 4 times with Triton/PBS. The mAb-bound PrPSc was directly eluted into
sodium dodecyl sulfate (SDS) sample buffer by heating. The unbound PrP remaining in the supernatant was precipitated using a 2-butanol/methanol solution [11] and resuspended in SDS sample buffer [21]. Both bound and unbound PrP<sub>Sc</sub> were detected by immunoblotting as described previously [21]. Preliminary IP analysis showed that the quantity of PrP<sub>Sc</sub> detected by mAb 3H6 was lower than that detected by mAb 3B7. Additionally, preparatory repetitive IP indicated that most of the mAb 3B7/3H6-precipitated PrP<sub>Sc</sub> was detected by 1st round-IP. However, the supernatant retained 47% of the total PrP after the 4th round of repetitive IP using mAb 3H6 (Fig. 1A). Therefore, we assessed whether the PrP<sub>Sc</sub> remaining in the supernatant after the IP with mAb 3H6 could be detected by mAb 3B7. As shown in Fig. 1B,
most of the PrPSc was precipitated with mAb 3B7, and only a small amount remained in the supernatant. In contrast, after IP with mAb 3H6, the supernatant contained a large amount of PrPSc, while most of the remaining PrPSc could be precipitated with mAb 3B7. These results suggested that the mAb 3H6 selectively precipitated a portion of the PrPSc present in the brain homogenate of the Obihiro strain, leaving a substantial amount of PrPSc that could be precipitated by mAb 3B7.

Next, we determined whether the difference in discrimination of mAb 3H6 was commonly found in other mouse-adapted scrapie strains. The brains of scrapie 22L-, Chandler-, 79A- and ME7-infected mice [10, 22] were examined. We confirmed that all scrapie-affected brains contained approximately similar amounts of PrPres (34–52% of total PrP) by immunoblotting (Fig. 1C and Table 1). Then, IP was performed with both mAbs 3B7 and 3H6, and the ratio of the precipitated PrPSc was calculated as a percentage of total PrP (sum of intensities on the immunoblot of mAb-precipitated PrPSc and that of PrP in the supernatant). We found that mAb 3B7 precipitated approximately 93–96% of the PrPSc from the 22L, Chandler, 79A and ME7 homogenates (Table 1), similar to the results obtained for Obihiro (Fig. 1B), and almost no PrPSc was detected in the remaining supernatant (Fig. 1D). In contrast, the ratio of mAb 3H6-precipitated PrPSc to total PrP was lower: 55% for Chandler, 51% for 79A, 47% for ME7 and 15% for 22L (Table 1). This finding suggested that the partial recognition of PrPSc by mAb 3H6 was not specific to the Obihiro strain, but was also observed in all the prion strains examined. These data suggested that the mAb 3H6 precipitated and unprecipitated PrPSc coexisted in the scrapie-affected mice.

It should be noted that the partial detection of PrPSc by mAb 3H6 was possibly due to its low binding affinity. However, low affinity does not adequately explain the reason for lesser binding to 22L (Fig. 1D and Table 1). Interestingly, these mAbs possessed different species specificity. The mAb 3H6 was considered mouse specific, while mAb 3B7 reacted with the PrPSc of mice, hamsters and deer [21]. This difference in the species specificity of these two mAbs would have an effect on the ratio of mAb-precipitated PrPSc to total PrP. Detailed kinetic analyses of these mAbs need to be carried out in future. Understanding the mechanism underlying the differential reactivity of mAb 3H6 would enable us to clarify the species-specific conformational characteristics of PrPSc.

The findings of this study indicated that the PrPSc of the five strains examined by us consisted of at least two types of PrPSc, viz., the mAb 3H6-precipitated PrPSc and the other PrPSc, even in the strains stabilized by sufficient adaptation. To date, some models of PrPSc conformation at the molecular level have been proposed, which suggest that the PrPSc of an individual strain could be represented as a mixture of several conformations, including intermediate forms [3, 4]. Our data present the direct evidence of heterogeneity of PrPSc in prion-affected mice. It is necessary to compare the conformational characteristics of mAb 3H6-precipitated PrPSc and the other PrPSc in future studies.

In conclusion, our study demonstrated the conformational heterogeneity of PrPSc in mouse-adapted scrapie prions by utilizing the unique specificity of mAb 3H6. A panel of mAbs capable of delineating specific PrPSc conformation could be a powerful tool for further investigation of conformational variation of PrPSc. If other biochemical approaches that focus on the heterogeneity of PrPSc conformation within a strain were to be combined with our mAb-based strategy, it could shed light on the mechanism by which PrPSc conformations may generate various strain phenotypes.

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REFERENCES

1. Baron, T. G. and Biacabe, A. G. 2001. Molecular analysis of the abnormal prion protein during coinfection of mice by bovine spongiform encephalopathy and a scrapie agent. J. Virol. 75: 107–114. [Medline] [CrossRef]

2. Bartz, J. C., Bessen, R. A., Mckenzie, D., Marsh, R. F. and Aiken, J. M. 2000. Adaptation and selection of prion protein strain conformations following interspecies transmission of transmissible mink encephalopathy. J. Virol. 74: 5542–5547. [Medline] [CrossRef]

3. Collinge, J. 2010. Prion strain mutation and selection. Science 328: 1111–1112. [Medline] [CrossRef]

4. Collinge, J. and Clarke, A. R. 2007. A general model of prion strains and their pathogenicity. Science 318: 930–936. [Medline] [CrossRef]

5. Collinge, J., Sidle, K. C., Meads, J., Ironside, J. and Hill, A. F. 1996. Molecular analysis of prion strain variation and the aetiology of ‘new variant’ CJD. Nature 383: 685–690. [Medline] [CrossRef]

6. Curin Serbec, V., Bresjanac, M., Popovic, M., Prenat Hartman, K., Galvani, V., Rupreht, R., Cernilec, M., Vranac, T., Hafner, I. and Jerala, R. 2004. Monoclonal antibody against a peptide of human prion protein discriminates between Creutzfeldt-Jacob’s disease and scrapie.
7. Groschup, M., Gretzschel, A. and Kucziius, T. 2009. Prion Strains, 1st ed., Walter de Gruyter GmbH & Co., Berlin.

8. Hayashi, H. K., Yokoyama, T., Takata, M., Iwamaru, Y., Imamura, M., Ushiki, Y. K. and Shinagawa, M. 2005. The N-terminal cleavage site of PrPSc from BSE differs from that of PrPSc from scrapie. Biochem. Biophys. Res. Commun. 328: 1024–1027. [Medline] [CrossRef]

9. Iwamaru, Y., Takenouchi, T., Ogihara, K., Hoshino, M., Takata, M., Imamura, M., Shimizu, Y., Okada, H., Shinagawa, M., Kitani, H. and Yokoyama, T. 2007. Microglial cell line established from prion protein-overexpressing mice is susceptible to various murine prion strains. J. Virol. 81: 1524–1527. [Medline] [CrossRef]