between BASE and BSE-C $PrP^{Sc}$ is 0.29 ± 0.12 kDa, corresponding to 2 to 4 amino acid residues. In contrast, the gel mobilities of the PK-resistant $PrP^{Sc}$ species from the BASE strain, BASE strain-infected Tg40 mice, and sCJDMM2, which was used as representative of human $PrP^{Sc}$ of type 2, were indistinguishable. This finding suggests that the PK-resistant $PrP^{Sc}$ electrophoretic heterogeneity between the BASE strain and BSE-C falls well within the 7-amino-acid variability of the N terminus (positions 92 to 99) that is consistently found in PK-resistant $PrP^{Sc}$ of type 2 (16). Therefore, despite their minor but distinct variability in gel mobility, both the BASE strain and BSE-C $PrP^{Sc}$ species appear to belong to the $PrP^{Sc}$ of type 2. However, the $PrP^{Sc}$ glycoform ratios of BASE strain-infected Tg40 mice and the BASE strain inocula display a small but statistically significant difference (Fig. 1). Therefore, $PrP^{Sc}$ in BASE strain-infected human subjects may be expected to display a different glycoform ratio from that of the BASE strain. It is worth noting that the electrophoretic characteristics of the PK-resistant $PrP^{Sc}$ of some human prion strains has been faithfully reproduced by our Tg40 line as well as by other humanized mouse lines (10, 13, 21).

Two distinct histopathological and PrP immunohistochemical phenotypes have been reported following BSE-C inoculation: one reproduced the distinctive features of vCJD with the "florid" plaques that intensely immunostained for PrP, and the other was reminiscent of sCJDMM1, with prominent spongiform degeneration and no plaque PrP immunostaining (1, 23). The brain histopathology, the $PrP^{Sc}$ distribution, and the PrP immunostaining pattern of BASE strain-inoculated Tg40 mice were definitely distinct from such features described above (1, 23), further supporting the notion that BASE and classical BSE are associated with two distinct prion strains (8).

The relatively easy transmission of BASE to humanized Tg mice indicates that effective cattle prion surveillance should be maintained until the extent and origin of this and other atypical forms of BSE are fully understood.

## ACKNOWLEDGMENTS

*This study was supported by Public Health Service grants (AG014359* [P.G.] from the National Institute of Aging and NS052319 [Q.K.] from the National Institute of Neurological Disorders and Stroke) and by an award to P.G. from the Charles S. Britton Fund.

We are grateful to Diane Kofsky, Phyliss Scalzo, Carrie Harris, and Kay Edmonds for their assistance.

## REFERENCES

1. **Asante, E. A., J. M. Linehan, M. Desbruslais, S. Joiner, I. Gowland, A. L. Wood, J. Welch, A. F. Hill, S. E. Lloyd, J. D. Wadsworth, and J. Collinge.** 2002. BSE prions propagate as either variant CJD-like or sporadic CJD-like prion strains in transgenic mice expressing human prion protein. EMBO J. 21:6358–6366.
2. **Béringue, V., O. Andreoletti, A. Le Dur, R. Essalmani, J. L. Vilotte, C. Lacroux, F. Reine, L. Herzog, A. G. Biacabe, T. Baron, M. Caramelli, C. Casalone, and H. Laude.** 2007. A bovine prion acquires an epidemic bovine spongiform encephalopathy strain-like phenotype on interspecies transmission. J. Neurosci. 27:6965–6971.
3. **Biacabe, A. G., J. L. Laplanche, S. Ryder, and T. Baron.** 2004. Distinct molecular phenotypes in bovine prion diseases. EMBO Rep. 5:110–115.
4. **Bishop, M. T., P. Hart, L. Aitchison, H. N. Baybutt, C. Plinston, V. Thomson, N. L. Tuzi, M. W. Head, J. W. Ironside, R. G. Will, and J. C. Manson.** 2006. Predicting susceptibility and incubation time of human-to-human transmission of vCJD. Lancet Neurol. 5:393–398.
5. **Brown, P., L. M. McShane, G. Zanusso, and L. Detwiler.** 2006. On the question of sporadic or atypical bovine spongiform encephalopathy and Creutzfeldt-Jakob disease. Emerg. Infect. Dis. 12:1816–1821.
6. **Bruce, M. E., I. McConnell, R. G. Will, and J. W. Ironside.** 2001. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 358:208–209.
7. **Buschmann, A., A. Gretzschel, A. G. Biacabe, K. Schiebel, C. Corona, C. Hoffmann, M. Eiden, T. Baron, C. Casalone, and M. H. Groschup.** 2006. Atypical BSE in Germany—proof of transmissibility and biochemical characterization. Vet. Microbiol. 117:103–116.
8. **Casalone, C., G. Zanusso, P. Acutis, S. Ferrari, L. Capucci, F. Tagliavini, S. Monaco, and M. Caramelli.** 2004. Identification of a second bovine amyloidotic spongiform encephalopathy: molecular similarities with sporadic Creutzfeldt-Jakob disease. Proc. Natl. Acad. Sci. USA 101:3065–3070.
9. **Fischer, M., T. Rulicke, A. Raeber, A. Sailer, M. Moser, B. Oesch, S. Brandner, A. Aguzzi, and C. Weissmann.** 1996. Prion protein (PrP) with amino-proximal deletions restoring susceptibility of PrP knockout mice to scrapie. EMBO J. 15:1255–1264.
10. **Hill, A. F., M. Desbruslais, S. Joiner, K. C. Sidle, I. Gowland, J. Collinge, L. J. Doey, and P. Lantos.** 1997. The same prion strain causes vCJD and BSE. Nature 389:448–450.
11. **Hill, A. F., R. J. Butterworth, S. Joiner, G. Jackson, M. N. Rossor, D. J. Thomas, A. Frosh, N. Tolley, J. E. Bell, M. Spencer, A. King, S. Al-Sarraj, J. W. Ironside, P. L. Lantos, and J. Collinge.** 1999. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. Lancet 353:183–189.
12. **Kascsak, R. J., R. Rubenstein, P. A. Merz, M. Tonna-DeMasi, R. Fersko, R. I. Carp, H. M. Wisniewski, and H. Diringer.** 1987. Mouse polyclonal and monoclonal antibody to scrapie-associated fibril proteins. J. Virol. 61:3688–3693.
13. **Kong, Q., S. Huang, W. Zou, D. Vanegas, M. Wang, D. Wu, J. Yuan, H. Bai, M. Zheng, H. Deng, K. Chen, A. L. Jenny, K. O'Rourke, E. D. Belay, L. B. Schonberger, R. B. Petersen, M. S. Sy, S. G. Chen, and P. Gambetti.** 2005. Chronic wasting disease of elk: transmissibility to humans examined by transgenic mouse models. J. Neurosci. 25:7944–7949.
14. **Korth, C., B. Stierli, P. Streit, M. Moser, O. Schaller, R. Fischer, W. Schulz-Schaeffer, H. Kretzschmar, A. Raeber, U. Braun, F. Ehrensperger, S. Hornemann, R. Glockshuber, R. Riek, M. Billeter, K. Wüthrich, and B. Oesch.** 1997. Prion (PrPSc)-specific epitope defined by a monoclonal antibody. Nature 390:74–77.
15. **Parchi, P., A. Giese, S. Capellari, P. Brown, W. Schulz-Schaeffer, O. Windl, I. Zerr, H. Budka, N. Kopp, P. Piccardo, S. Poser, A. Rojiani, N. Streichemberger, J. Julien, C. Vital, B. Ghetti, P. Gambetti, and H. Kretzschmar.** 1999. Classification of sporadic Creutzfeldt-Jakob disease based on molecular and phenotypic analysis of 300 subjects. Ann. Neurol. 46:224–233.
16. **Parchi, P., W. Zou, W. Wang, P. Brown, S. Capellari, B. Ghetti, N. Kopp, W. J. Schulz-Schaeffer, H. A. Kretzschmar, M. W. Head, J. W. Ironside, P. Gambetti, and S. G. Chen.** 2000. Genetic influence on the structural variations of the abnormal prion protein. Proc. Natl. Acad. Sci. USA 97:10168–10172.
17. **Prusiner, S. B., M. Scott, D. Foster, K. M. Pan, D. Groth, C. Mirenda, M. Torchia, S. L. Yang, D. Serban, G. A. Carlson, P. C. Hoppe, D. Westaway, and S. J. DeArmond.** 1990. Transgenetic studies implicate interactions between homologous PrP isoforms in scrapie prion replication. Cell 63:673–686.
18. **Safar, J., H. Wille, V. Itri, D. Groth, H. Serban, M. Torchia, F. E. Cohen, and S. B. Prusiner.** 1998. Eight prion strains have PrP(Sc) molecules with different conformations. Nat. Med. 4:1157–1165.
19. **Seitz, R., F. von Auer, J. Blumel, R. Burger, A. Buschmann, K. Dietz, M. Heiden, W. E. Hitzler, H. Klamm, T. Kreil, H. Kretzschmar, M. Nübling, R. Offergeld, G. Pauli, V. Schottstedt, P. Volkers, and I. Zerr.** 2007. Impact of vCJD on blood supply. Biologicals 35:79–97.
20. **Taraboulos, A., K. Jendroska, D. Serban, S. L. Yang, S. J. DeArmond, and S. B. Prusiner.** 1992. Regional mapping of prion proteins in brain. Proc. Natl. Acad. Sci. USA 89:7620–7624.
21. **Telling, G. C., M. Scott, J. Mastrianni, R. Gabizon, M. Torchia, F. E. Cohen, S. J. DeArmond, and S. B. Prusiner.** 1995. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 83:79–90.
22. **Tremblay, P., Z. Meiner, M. Galou, C. Heinrich, C. Petromilli, T. Lisse, J. Cayetano, M. Torchia, W. Mobley, H. Bujard, S. J. DeArmond, and S. B. Prusiner.** *1998. Doxycyline control of prion protein transgene expression* modulates prion disease in mice. Proc. Natl. Acad. Sci. USA 95:12580–12585.
23. **Wadsworth, J. D., E. A. Asante, M. Desbruslais, J. M. Linehan, S. Joiner, I. Gowland, J. Welch, L. Stone, S. E. Lloyd, A. F. Hill, S. Brandner, and J. Collinge.** 2004. Human prion protein with valine 129 prevents expression of variant CJD phenotype. Science 306:1793–1796.
24. **Ward, H. J. T., D. Everington, S. N. Cousens, B. Smith-Bathgate, M. Leitch, S. Cooper, C. Heath, R. S. G. Knight, P. G. Smith, and R. G. Will.** 2006. Risk factors for variant Creutzfeldt-Jakob disease: a case-control study. Ann. Neurol. 59:111–120.
25. **Zanusso, G., D. Liu, S. Ferrari, L. Hegyi, X. Yin, A. Aguzzi, S. Hornemann, S. Liemann, R. Glockshuber, J. C. Manson, P. Brown, R. B. Petersen, P. Gambetti, and M. S. Sy.** *1998. Prion protein expression in different species:* analysis with a panel of new MAbs. Proc. Natl. Acad. Sci. USA 95:8812–8816.

別紙様式第2-1

No. 24

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2008. 2. 18 | 該当なし | |

| 一般的名称 | 新鮮凍結人血漿 | 研究報告の公表状況 | Tsukui K, Takata M, Tadokoro K. Microbiol Immunol. 2007;51(12):1221-31. | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 新鮮凍結血漿「日赤」(日本赤十字社)<br>新鮮凍結血漿-LR「日赤」(日本赤十字社) | | | 日本 |

**研究報告の概要**

○スクレイピー感染ハムスター血漿中の$PrP^{res}$様タンパクの糖鎖依存性凝集検出による伝達性海綿状脳症血液検査の可能性

感染組織に多量の$PrP^{res}$を含有することが知られている感染動物モデルにおいても、血中の$PrP^{res}$は(白血球を除き)めったに検出されない。血中$PrP^{res}$の検出が困難なのは、血中感染価が低いことを反映していると見られる。ここでは、新規酸性SDS沈殿法と高感度化学発光法を組み合わせて、プロテイナーゼK耐性3F4反応性タンパクが、スクレイピー感染ハムスターの血漿中からは検出されるが、疑似感染ハムスターでは検出されないことを示す。高感度化学発光法では、$1.4x10^{-9}$gの脳ホモジネート、及び$1.5x10^{-12}$g ($6.5x10^{-17}$mol)のrPrPを従来型のウエスタンブロットで検出した。スクレイピー感染ハムスターの血漿中の3F4反応性タンパクは複数の分子量からなるタンパクバンドとなり、二糖鎖PrP分子のバンドよりも高い位置に検出された。スクレイピー感染ハムスター脳ホモジネートと疑似感染ハムスター血漿を混合することにより、3F4反応性タンパクと類似する分子量の位置にバンドが形成された。混合前に、血漿または脳ホモジネート中のタンパクから予め糖鎖を除去することにより、上記の複数の3F4反応性タンパクは検出できなくなった。これらの結果から、血漿中において$PrP^{res}$は他の血漿タンパクと糖鎖を通じて凝集しており、スクレイピー感染ハムスター血漿において検出可能となったことが示唆される。スクレイピー感染ハムスターの血漿中で$PrP^{res}$様タンパクと凝集している相手の血漿タンパクが何であるかはまだ不明であるが、それはプロテイナーゼKに抵抗性を持っていると思われる。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 新規酸性SDS沈殿法と高感度化学発光法を組み合わせて、スクレイピー感染ハムスターの血漿中からプロテイナーゼK耐性3F4反応性タンパクを検出したとの報告である。 | 今後も引き続き、検査法の研究を進めるとともに、プリオン病に関する新たな知見及び情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Microbiol. Immunol., 51(12), 1221–1231, 2007

Editor-Communicated Paper

# A Potential Blood Test for Transmissible Spongiform Encephalopathies by Detecting Carbohydrate-Dependent Aggregates of PrPres-Like Proteins in Scrapie-Infected Hamster Plasma

**Kazuo Tsukui*[,1], Masuhiro Takata[2], and Kenji Tadokoro[1]**

[1]*Central Blood Institute, The Japanese Red Cross Society, Koto-ku, Tokyo 135–8521, Japan, and* [2]*Research Center for Prion Diseases, National Institute of Animal Health, Japan, Tsukuba-shi, Ibaraki 305–0856, Japan*

Communicated by Dr. Takashi Onodera: Received June 13, 2007. Accepted October 17, 2007

*Abstract*: **PrPres has rarely been detected in blood (except in leukocytes) even in diseased animal models that are known to contain a large amount of PrPres in infected tissues. It seems likely that PrPres detection in blood is difficult because of the low titer of infectious material within the blood. Here, we demonstrate the detection of proteinase K-resistant 3F4-reactive protein in the plasma of scrapie-infected hamsters but not in the plasma of mock-infected hamsters by partial purification using a novel method termed "acidic SDS precipitation," in conjunction with a highly sensitive chemiluminescence detection system used to show the presence of PrP at a concentration equivalent to $1.4\times10^{-9}$ g of brain homogenate or $1.5\times10^{-12}$ g ($6.5\times10^{-17}$ mol) of rPrP by conventional Western blotting. The 3F4-reactive proteins in scrapie-infected hamster plasma often resulted in multiple Mw protein bands occurring at higher Mw positions than the position of the di-glycosyl PrP molecule. Mixing scrapie-infected hamster brain homogenate with mock-infected hamster plasma resulted in the formation of similar Mw positions for multiple 3F4-reactive proteins. Predigestion of carbohydrate side chains from the proteins in the plasma or brain homogenate before mixing resulted in failure to obtain these multiple 3F4-reactive proteins. These observations indicate that PrPres aggregated with other proteins in the plasma through carbohydrate side chains and was successfully detected in the plasma of scrapie-infected hamsters. Counterparts in these aggregates with PrPres-like proteins in scHaPl are not known but any that exist should resist the PK digestion.**

*Key words*: **PrPres-like protein, Carbohydrate, Scrapie infection, Discrimination**

Transmissible spongiform encephalopathy (TSE) is a fatal infectious neurodegenerative disease. It is characterized pathologically by spongy deterioration of the central nervous system (CNS) and by the deposition of amyloid plaques composed of an abnormal isoform of the prion protein ($PrP^{sc}$) in infected tissues (1, 2, 19). An important biochemical property of $PrP^{sc}$ is its partial resistance to protease digestion, which results in the formation of a β-sheet-rich isoform. This molecule has therefore also been called PrPres, and it has been considered a disease-specific entity associated with TSE (1, 2, 9). Although the vCJD epidemic in the U.K. is declining, expansion of the disease throughout continental Europe and in many other countries has raised concern all over the world (9, 10, 28). After the appearance of three cases of transfusion-related vCJD infection

**Address correspondence to* Dr. Kazuo Tsukui, 2–1–67 Tatsumi, Koto-ku, Tokyo 135–8521, Japan. Fax: +81–3–5534–7588. E-mail: k-tsukui@bs.jrc.or.jp

*Abbreviations*: 2× acidic saline, 0.02 M acetic acid containing 0.15 M NaCl and 10 mM EDTA-2Na; Brh, brain homogenates; CNS, central nervous system; KLH, keyhole limpet hemocyanin; mAb, monoclonal antibody; mc, mock-infected; Pl, plasma; PMCA, protein misfolding cyclic amplification; $PrP^{c}$, normal prion protein; PrPres, proteinase K-resistant prion protein; $PrP^{sc}$, disease-associated prion protein; PTA, phosphotungstic acid; PVDF, polyvinylidene fluoride; RES, reticuloendothelial system; rPrP, recombinant hamster PrP(25-233); SB, super block; sc, scrapie-infected; TBST, Tris Buffered Saline containing 0.05% Tw20; TSE, transmissible spongiform encephalopathy.

in the U.K., transfusion-related iatrogenic expansion of vCJD between the asymptomatically infected donor and blood recipients has caused growing concern (13, 21, 26). In addition, the U.K. government has recently reported a fourth case of vCJD associated with a blood transfusion (HPA Press statement; 18 Jan. 2007, abbreviated in 13). For this reason, the aim of research into developing an antemortem test has changed from detecting infected persons in an endemic area to estimating the population size of infected persons within a more global area in order to prevent the iatrogenic infection by tainted blood (4, 8, 28). The most useful tissues for the diagnostic confirmation of vCJD in humans are CNS and reticulo-endothelial system (RES) tissue as well as the tonsils and appendix (14–16). The tonsils and appendix have been used successfully for the histopathological detection of PrPres in epidemiological studies of vCJD infection in the U.K.: an extremely high frequency of infection was detected compared to the frequency of so-called classical CJD (16, 17, 22). However, it is difficult to sample the tonsils and the appendix in living subjects. Therefore, the pathological diagnosis of TSE is currently made principally on the basis of postmortem preparations of CNS tissues, highlighting the need for the development of a more rapid diagnostic method using body fluids, especially blood (6, 27). For this purpose, several methods have been proposed and examined for prophylactic use (23, 30, 32). However, none of these methods has proved to be sufficient for the purposes (5, 7, 20, 29). To achieve this goal, several problems must be solved; solutions include using preparations with minimally invasive sampling techniques and establishing an effective and specific method for detecting the disease marker with sufficient sensitivity (5). The first problem can be solved by using body fluids such as blood or urine as test specimens. Therefore, the key is to develop a system with sufficient sensitivity to detect PrPres in blood or urine (5, 6, 20, 22, 29). The presence of PrPres in the urine of TSE-infected animals and humans has been reported previously (33). However, it has been suggested that this $uPrP^{sc}$ may be contaminated bacterial components in infected animal urine and not a marker of TSE (12). Blood has not been considered a highly infective source of classical CJD. The same was true for vCJD until the first victim of vCJD resulting from a blood transfusion was reported (21). This report was followed by reports of three more cases of possible transfusion-related transmission of vCJD (18, HPA Press statement; 18, Jan. 2007, 13). The development of a testing method using blood has therefore become a major goal of TSE research.

Here we show the successful detection (using a highly sensitive chemiluminescence immunoblotting system) of a PrPres-like protein molecule in plasma collected from scrapie-infected (sc) but not from mock-infected (mc) hamsters. Although the infectivity of this molecule has not been tested, and the immunoblot pattern of the anti-PrP reactive protein in plasma (scHaPl) was somewhat different from that of the brain homogenate (scHaBrh) in scrapie-infected hamsters, the specific reactivity of these proteins to anti-PrP mAb, the demonstration of carbohydrate side chain-mediated association between PrPres and plasma proteins, and the removal of the carbohydrate chain resulted in the appearance of similar Mw proteins in scBrh and scPl firmly support the conjecture that the extra Mw proteins observed in the trial were the aggregates of PrPres and some plasma proteins.

## Materials and Methods

*Enzymes, monoclonal antibodies (mAb) and recombinant hamster PrP peptide.* Proteinase K (PK: 40.0 mAnson units/mg protein) was purchased from Merck Co. (Rahway, N.J., U.S.A.). Peptide *N*-glycosidase F (PNGaseF, 25,000 units/mg protein) was purchased from Roche Diagnostics Co., Ltd.

The anti-PrP mAbs 3F4 (Signet, Mass., U.S.A.) and 6H4 (Prionics AG, Zürich, Switzerland) were stored in aliquots at −80 C until use. mAb 5C8-113 was prepared by immunizing PrP knockout mice with bovine recombinant PrP (Prionics AG); screening was conducted using the same molecule. TA180 and TA181 were provided by Medical Biological Laboratory (MBL) and were prepared by immunizing conventional Balb/c mice with synthetic peptides of the hamster PrP sequence CERYYRE or CAVVGGLGGYML conjugated with keyhole limpet hemocyanin (KLH), respectively, then screened by the same peptides without KLH and conjugated with an ELISA plate. The epitope sites of the mAbs were 150–152 and 163–165 for TA180, and 129–131 for TA181. The epitope site of 5C8-113 has not yet been determined but is possibly an unknown conformation-dependent site. Anti-HIV P24 mAb (7A8.1; CHEMICON) was kindly donated by Dr. Iwakura of the Institute of Medical Sciences, Tokyo University and was used as a negative control for anti-PrP mAb reactions. Hamster recombinant PrP(25-233) (abbreviated rPrP hereafter) was purchased from Alicon AG (Switzerland).

*Material from scrapie-infected and mock-infected hamsters.* Twelve Syrian golden hamsters were inoculated with scrapie (Sc237)-infected hamster brain homogenate intra-cerebrally. Six hamsters were similarly inoculated with uninfected normal hamster brain

homogenate and were used as mock-infected hamsters. Hamsters from the two groups were anesthetized with ether at the terminal stage of disease among animals in the scrapie-infected group (approximately 50–70 days after inoculation) and after the same time interval among animals in the mock-infected group. Blood was collected from the animals with ACD containing 10 mM EDTA as an anticoagulant. Blood samples from scrapie-infected and mock-infected hamsters was centrifuged at low speed and the plasma fractions were collected (scPl and mcPl, respectively). Both scPl and mcPl were processed similarly thereafter. Brains were removed from the terminal-stage infected hamsters or the mock-infected hamsters and homogenized in TBS containing 0.5% NP40, 0.5% DOC and a protease inhibitor cocktail (Sigma) using a closed system homogenizer. These brain homogenates were then adjusted to a concentration of 10% with the above-mentioned buffer ($scBrh^{crude}$ or $mcBrh^{crude}$, respectively). $scBrh^{crude}$ or $mcBrh^{crude}$ were centrifuged at low speed to remove insoluble materials, and the supernatant fractions (scBrh or mcBrh) were processed as described below.

*Enzyme treatment.* The plasma or brain homogenates were diluted 4-fold with TBS containing 10 mM EDTA and digested with PK (50 μg/ml) at 37 C for 60 min. These reactions were stopped by adding 1 mM Pefablock. The digestion step was omitted in a set of controls. The samples were then treated with 3% SDS and 50 mM DTT in TBS before being inactivated at 100 C for 10 min and stored at −80 C in small aliquots.

*Acidic SDS precipitation.* Stored preparations were inoculated with equal volumes of 0.02 M acetic acid containing 0.15 M NaCl and 10 mM EDTA-2Na (2× acidic saline) at 10 C, followed by centrifugation at 15,000 rpm for 10 min. The resulting precipitates were dispersed in Tris Buffered Saline (TBS) with 5 mM EDTA and inoculated with equal amounts of 2× acidic saline again. After further centrifugation, the resulting precipitates were rinsed with a 5-fold volume of methanol, then dissolved in Laemmli's SDS sample buffer and analyzed thereafter.

*Immunoblot detection of PrP-like proteins.* SDS-PAGE was carried out on a 15% gel using Laemmli's conventional buffer system. The electrophoresed proteins were transferred onto polyvinylidene fluoride (PVDF) membranes using a semidry system. After Western blotting, the membranes were blocked with Super Block (SB; PIERCE, Rockford, Ill., U.S.A.) for 1 hr at room temperature then overnight at 4 C. The blocked membranes were first washed three times with TBS containing 0.05% Tween 20 (TBST), then incubated with an anti-PrP monoclonal antibody (mAb; 3F4, 6H4 or similar), in SB containing 10% Block Ace (Dainippon Pharmaceutical Co., Ltd.) and 0.01% BSA for 1 hr at room temperature then overnight at 4 C thereafter. For maximum detection of protein signals, the blotted membranes were incubated overnight at 4 C. After incubation, the membranes were washed five times with TBST, incubated with horseradish peroxidase-conjugated goat anti-mouse IgG (HRPGAM) in TBST cotaining 10% Block Ace and 0.1% BSA for 1 hr as a secondary antibody, washed five more times with TBST and incubated with a chemiluminescence substrate (Super Signal West Femto Maximum Sensitivity Substrates: SSWF; PIERCE). To obtain optimum chemiluminescence signals, HRPGAM was used at a concentration of 2 ng/ml according to the manufacturers instruction manual and chemiluminescence signals for antibody-reactive bands were detected using an LAS3000 image analyzer (Fuji Film, Tokyo).

## Results

*Sensitivity of Detection Systems (Fig. 1)*

To determine the limits of the detection system, rPrP, 10% homogenates of sc- or mcBrh (crude or low-speed centrifugation supernatant) and PK predigested sc- or mcBrh were used. They were abbreviated as sc- or $mcBrh^{crude}$, sc- or $mcBrh^{sup}$ and sc- or mc $BrhPK_{50}$, respectively. In these experiments, the amount of PrP was indicated as brain equivalent (panel A) or brain protein (panel B) to enable convenient comparison between the equivalence to brain amount and brain protein. Protein amounts in the brain were determined before PK digestion. In panel A, $1.5\times10^{-12}$ g ($6.5\times10^{-17}$ mol) for rPrP and PrPres in $1.4\times10^{-9}$ g brain equivalent were detected. About 1/3 (equivalent to $2.25\times10^{-6}$ g brain protein) of the PrP molecule in scBrh, and none of those in mcBrh looked like the PK-resistant molecule (PrPres; panel B). PrPres in scBrh was shifted from 30–32 kDa and 27–28 kDa before PK treatment to 25 and 20 kDa positions after PK treatment, respectively. As the total amount of PrPres plus PrPc in scBrh looked 3-fold larger than the amount of PrP in mcBrh, synthesis of the PrP was enhanced by scrapie infection in hamsters.

*Discrimination of Scrapie Infection from Mock Infection by Plasma*

When scrapie-infected or mock-infected Brh and plasma were pretreated with PK and subjected to immunoblot analysis, sc and mcBrh were easily discriminated by the PK treatment but sc and mcPl were not discriminated by the enzyme treatment (panel A). In mcPl, similar 3F4-reactive proteins were also