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Preparation of Monoclonal Antibodies Against Prion Proteins With Full-length Hamster PrP[1]

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Objective To prepare the PrP specific monoclonal antibodies (mAbs) that can be used for the detection of mammalian prions and study of pathogenesis of prion diseases. **Methods** Several BALB/c mice were immunized with recombinant hamster prion protein (HaPrP). Three hybridoma cell lines designated as B7, B9, and B10, secreting monoclonal antibodies against HaPrP, were established by hybridoma technique. The mAbs reactivities were evaluated with ELISA, Western blot, and immunohistochemistry. **Results** The mAbs produced by these cell lines reacted well with different recombinant hamster PrP proteins. Western blot analyses showed that mAbs B7 and B9 reacted with PrP^{Sc} from the scrapie-infected animals after proteinase K digestion with three glycosylated forms. The mAbs exhibited cross-reactivity with various PrP^{C} from several other mammalian species, including humans and cattles. Immunohistochemistry assays confirmed that mAbs B7 and B9 could recognize not only extracellular but also intracellular PrP^{Sc}. **Conclusion** The mAbs of prion protein are successfully generated by hybridoma technique and can be applied for the diagnosis of prion associated diseases.

Key words: Prions; Hamster prion protein; Monoclonal antibodies

INTRODUCTION

Prion diseases, or transmissible spongiform encephalopathies (TSEs) are a kind of fatal neurodegenerative conditions afflicting humans and animals and do not provoke any detectable immune response, which are characterized by accumulation of an abnormal form of a cellular prion protein (PrP^{C})[1]. The abnormal form of PrP (PrP^{Sc}) is partially proteinase resistant without any encoding nucleic acid whereas PrP^{C} can be completely degraded by proteinase K[2]. The clinical diagnosis of TSE is usually based on clinical symptoms, slow waves with typical bi/triphasic periodic complexes in electro-encephalogram (EEG) and some changes in neuroradiological images, e.g. CT and MRI. Some proteins in cerebral spinal fluid (CSF) are used as indicators for probable human Creutzfeldt-Jacob disease (CJD), including 14-3-3, S100 and Tau. The diagnosis of definite human or animal TSEs depends on either neuropathological changes in the central nerve system or presence of abnormal PrP^{Sc}. Detection of PrP^{Sc} with immunological methodology in central nerve tissues is the main way to identify TSE cases both in human beings and in animals.

Although it has been confirmed that TSE agents can transfer through various pathways, e. g. intracerebral, intravenous, intraperitoneal, intramuscular, intragastric and subcutaneous infection or challenge, in both natural and experimental TSEs, it lacks either specific humoral or cellular immunoreaction, indicating an immunotolerance probably since PrP^{C} and PrP^{Sc} share the same amino acid sequences[3]. Restriction of infectious agents in the central nerve system during pathogenesis of TSE might be another possibility for blocking the production of specific antibody and cytotoxic lymphocytes. On the other hand, as a house-keep gene, *prnp* gene and PrP protein are widely distributed in many vertebrate animals, sharing high homology (about 90%) in amino acid sequence among the mammalian species[4]. These findings propose a special

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immunological reaction during TSE pathogenesis and exotic PrP immunization, which is significantly distinct from most conventional infectious diseases.

Structural analyses have revealed differences in secondary and possible tertiary structures between PrP^{C} and PrP^{Sc}[3]. The conformational diversity of PrP proteins implies the possibility to produce conformation-specific monoclonal antibody to PrP. Actually, a PrP^{Sc} specific antibody, 15B3, has been described[5], however, not widely used. Although transmissions of TSEs behave as remarkable species barriers, most of them still can overcome the barriers and be induced in different species of animals naturally and experimentally, e. g. bovine spongiform encephalopathy (BSE) to human and cat species, scrapie in goat and sheep to mice and hamsters[6-7]. Species-specific PrP antibodies will help to understand the mechanism of prion conversion and distribution. Currently, several PrP mAbs have been available even commercially[8-9]; however, to understand the biology of PrP and the pathogenesis of prion diseases requires an extensive library of well-characterized antibodies to PrP.

In this study, a recombinant hamster PrP protein (HaPrP), PrP23-231, was purified and immunized into BALB/c mice. After cell fusion and selection, three strains of hybridomas that produced PrP antibody were obtained. We confirmed that these antibodies reacted with recombinant PrP proteins, native PrP^{C} and PrP^{Sc}. The immunohistochemical patterns and features used with newly prepared antibodies were distinct from those used with the commonly employed PrP mAb 3F4.

MATERIALS AND METHODS

Cell Culture

Sp2/0 cells were maintained in 1640 medium containing 10% fetal calf serum (FCS), 50 U/mL penicillin and 50 mg/mL streptomycin and incubated in a 5% CO_2 incubator at 37℃. This cell line was deficient in hypoxanthineguanine phosporibosyl transferase (HGPRT) activity and resistant to 8-azaguanine (20 μg/mL), being not able to grow in HAT (5×10^{-3} mol/L hypoxanthine, 2×10^{-5} mol/L aminopterin, 8×10^{-4} mol/L thymidine) medium.

Plasmid Construction

Hamster PrP gene sequence was amplified by polymerase chain reaction (PCR) with primer Haprp-F (5′GGATCCATGAAGAAGCGGCCAAA-GCCTGG 3′, with a BamH I site underlined) and Haprp-B (5′GTCGACTCATGGATCTTCTTCCGT-CGTAGT 3′, with a Sal I site underlined) at the cycle condition of 94℃ for 30 s, 56℃ for 30 s, 72℃ for 50 s, using the recombinant plasmid pGEMT-HaPrP[10] as the template. The 630 bp PCR product was ligated with a commercially supplied T-vector (Promega), to generate plasmid pT-HaPrP. After having been verified with sequence assay, the insert was released from pT-HaPrP with BamH I and Sal I, and cloned into an expression vector pQE-30, generating plasmid pQE-HaPrP.

Expression and Purification of Recombinant PrP Protein

Hamster PrP (HaPrP) was expressed in *E. coli* strain M15. Briefly, plasmid pQE-HaPrP transformed bacteria were grown to an OD_{600} of 0.5-0.6 and induced with 0.5 mmol/L isopropyl-D-thiogalactoside (IPTG) at a final concentration. Cells were harvested by centrifugation and resuspended in 0.01 mol/L PBS, pH 7.4, with 1 mmol/L phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor. Lysozyme was added to a final concentration of 2 mg/mL, and cells were lysed by incubation for 30 min and treated with sonication 24 times in power of 400 W at 10 s intervals. For purification of the proteins with histidine tag, the soluble cell lysate was incubated with nickel-NTA agarose (Pharmacia Biotech) and stirred at 4℃ for 30 min. HIS-fusion proteins were eluted according to the manufacturer's guidelines. Protein concentrations were determined by using the BCA method (Pierce, USA).

Animal Immunization

Five eight-month-old female BALB/c mice were employed for the immunization. One hundred μg purified HaPrP was injected into each mouse subcutaneously, after mixing with an equal volume of Freund's complete adjuvant (FCA) thoroughly. A same quantity of HaPrP mixed with Freund's incomplete adjuvant (FIA) was administrated into mice 3, 5, and 7 weeks after the first challenge. After the third round of immunization, blood was taken for monitoring the inducement of the specific antibody. Mice showing the strongest PrP-reactive titer in ELISA were utilized for the generation of mAbs. Three days before cell fusion, 50 μg purified HaPrP was injected into spleen. The mice were sacrificed under anesthesia with ether and the spleens were taken.

Production of Hybridomas

Cell fusion of Sp2/0 cells and spleen lymphocytes was conducted by 50% (w/v) PEG4000, with a ratio of 1:5 of Sp2/0 and spleen cells. The cells were

maintained in HAT medium and the formation of hybridomas was monitored by light microscopy. The secretion of PrP specific antibody of cloned hybridomas was measured by a PrP protein based ELISA. The positive hybridomas were subsequently cloned three times with a limiting dilution procedure.

Chromosome Staining

The cloned hybridomas producing PrP specific antibody were cultured with 1640 medium containing 400 mg/mL colchicine in a 5% CO_2 incubator at 37℃ for 4 h. The hybridoma cells were treated briefly with 0.075% KCl (w/v) for 30 min, and then with methyl alcohol and glacial acetic acid with a ratio of 3:1(v/v) for 30 min or overnight. The cells were spread onto the slices and dyed with Giemsa staining after having baked at 80℃ for a few seconds.

Preparation of Ascites in Mice

The cloned hybridomas producing PrP antibody were intraperitoneally injected into BALB/c mice, one week after treatment with 0.5 mL pristane (Sigma) per mouse according to the protocol described previously[11]. The animals were killed two to three weeks after the injection when transplant tumors were formed and ascites was collected for antibody detection.

Immunoglobulin G (IgG) Subtyping

The IgG subtypes of the prepared antibodies were determined with a mouse immunoglobulin subtype identification kit (Roche). Briefly, ascites was freshly diluted to 0.5 μg/mL with PBS and incubated at room temperature for 30 s. One hundred and fifty μL diluted ascites was transferred into a testing tube and an isotyping strip was inserted in the development tube for 10 min according to the protocol provided by the manufacturer.

ELISA

HaPrP (1 μg/mL) in 0.1 mol/L sodium carbonate buffer, pH 9.6, was coated overnight onto 96-well plates at 4℃. The plates were blocked with 5% (w/v) defatted milk powder in PBS containing 0.05% (v/v) Tween-20 (PBST) for 2 h at 37℃. One hundred μL tested supernatants of cloned hybridoma or ascites were added into the PrP-coated wells and incubated at 37℃ for 2 h, while PrP specific monoclonal antibody 3F4 diluted at 1:2 000 in PBST was used as a positive control. After an excessive rinse with PBST three times, the possibly captured mouse antibodies were further identified with horseradish peroxidase (HRP) conjugated anti-mouse IgG (Santa Cruz) in PBST for 2 h. Color was developed by adding TMB (3,3′,5,5′-tetramethyl benzidine) substrate solution per well containing 0.03% H_2O_2. Absorbance was measured at 450 nm after quenching the wells with 50 μL of 2 mol/L H_2SO_4.

Preparation of Hamster PrP^{Sc}

Brain tissues from the hamsters infected with Scrapie strain 263K[12] were used to prepare PrP^{Sc} extracts. In short, 10% homogenates of the infected brain samples were prepared in lysis buffer (100 mmol/L NaCl, 10 mmol/L EDTA, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mmol/L Tris, pH 7.5). The tissue debris were removed with low speed centrifugation at 2000 g for 10 min, and the supernatants were further centrifuged at 20 000 g for 90 min at 4℃. The pellets were resuspended in 40 μL deionized and distilled water and stored at -70℃ until they wre used.

Preparation of PrP^{C} Extracts From Non TSE-infected Mammalian Species

Various PrP^{C} extracts were prepared from non-CJD cattles, as well as from non TSE-infected hamsters, cattles, mice, rats and rabbits based on a modified protocol as described by Merz *et al.*[13]. Brain tissues (200 mg) were homogenized in 2 mL lysis buffer as described above. The tissue debris were removed with low speed centrifugation at 2000 g for 10 min and the supernatants were stored as PrP^{C} extracts for Western blot assays.

Proteinase K Digestion and Western Blot Analysis

For the detection of recombinant PrP proteins and PrP^{C}, 2 μg individual recombinant proteins and 20 μL PrP^{C} extracts (yield equivalent roughly to 20 mg brain tissues) were separated in 15% SDS-PAGE. For the detection of PrP^{Sc}, 20 μL PrP^{Sc} extracts (yield equivalent to 500 mg brain tissues) were mixed with 50 μg/mL proteinase K at 37℃ for 1 h, prior to Western blot. Digestion was terminated by adding an equal volume of 2 × loading buffer (125 mmol/L Tris hydrochloric acid, 20% (v/v) glycerol, pH 6.8, 4% (w/v) sodium dodecylsulphate, 4% (v/v) 2-mercaptoethanol, 8 mmol/L 4-(2-aminoethyl)-benzene sulfonyl fluoride, and 0.02% (w/v) bromo-phenol blue) and all samples were separated in 15% polyacrylamide gels. The gels were electroblotted onto nitrocellulose membranes and blocked for 2 h in 5% (w/v) defatted milk powder in PBST. The blotting membranes were incubated with prepared antibodies diluted at 1:100 to 1:500 and PrP-specific monoclonal antibody 3F4 diluted at 1:1000 in PBST for 2 h at 37℃. After washing with PBST, blots were

incubated with HRP conjugated anti-mouse IgG in PBST for 2 h, followed by development in DAB substrates.

PrP Deglycosylation

After the treatment with PK at 37℃ for 1 h, proteolytic digestion of brain homogenates from infected hamsters was terminated by 5 mmol/L PMSF. Fifteen μL-aliquot of PK-treated solution was boiled for 10 min in denaturing buffer (0.5% (w/v) SDS, 1% (v/v) β-mercaptoethanol), and deglycosylated with PNGase F (Biolab, 1500 U in 1% Nonidet P-40, 50 mmol/L sodium phosphate, pH 7.5) at 37℃ for 12 h. Proteins were precipitated with 4 volumes of cold methanol at -20℃ for 6 h, and centrifuged at 15 000 g for 30 min. The pellets were resuspended in sample loading buffer and separated in 15% SDS-PAGE. Western blot assays were done as described above.

Immunohistochemistry

Before histological processes, all the fixed tissues with 10% formalin were immersed in ⩾88% (v/v) formic acid for at least 1 h for inactivation. After deparaffinization, the slices were treated with: microwave irradiation (Galanz, WP1000L30-2) for 30 min, 3% (v/v) H_2O_2-methanol for 15 min, 0.1% (w/v) trypsin for 15 min and proteinase K (5 μg/mL) for 3-5 min. The slices were immersed in 1% (v/v) normal horse serum for 20 min before incubated with the prepared antibodies at the dilution from 1:100 to 1:500, while using antibody 3F4 as a positive control. The employment of the secondary antibody and visualization of the immunostainings were performed with a commercially supplied ABC (avidin-biotin-complex) kit and a DAB kit (Vector Laboratories, Inc.).

RESULTS

Purification HaPrP Protein in E. coli

Hamster *prp* gene encoding the residues 23-231 was amplified from recombinant plasmid pGEMT-HaPrP[10] and cloned into vector pQE-30. After inducement with IPTG, the lysates of the transformed *E. coli* strain M15 were run through the affinity chromatography of Ni-NTA agarose. SDS-PAGE revealed a 24 kD band in the eluted fraction (Fig. 1a). Western Blot assay with PrP antibody 3F4 demonstrated a specific reactive fragment at the expected mobilizing position (Fig. 1b). The yield of purified PrP in this system was about 3 mg per 100 mL cultured bacteria.

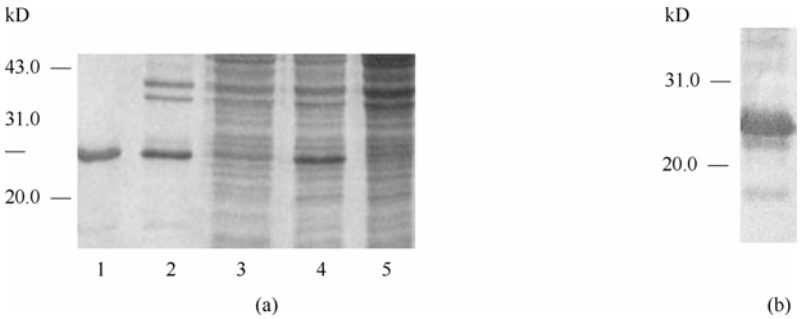

FIG. 1. Purification and Western blot analysis of HaPrP in *E. coli*. (a) The expressed proteins were purified with Ni-NTA agarose affinity chromatography. Lane 1: purified HaPrP, lane 2: pellet after sonication, lane 3: supernatant after sonication, lane 4: lysate of *E. coli* transformed with pQE-HaPrP after inducement, lane 5: lysate of *E. coli* transformed with pQE-HaPrP before inducement. (b) Western blot analysis of purified HaPrP proteins with 3F4. Molecular mass markers are indicated on sides of the picture.

Preparations of Mouse mAbs Against HaPrP

After cell fusion and selection with HAT medium culture, there were 113 wells containing growing cell clones in three pieces of 96-well plates. Detections of PrP antibody in the culture supernatant of hybridoma cells with established PrP-coated ELISA revealed that 15 wells had a positive reaction. The hybridoma cells producing PrP antibody were subsequently cloned three times with a limiting dilution procedure and three hybridoma cell lines were obtained, thus producing a relatively high titer of PrP antibody based on ELISA testing (data not shown), which was nominated as B7, B9, and B10 respectively. Giemsa staining indicated the chromosome number of hybridoma cells producing mAbs B7, B9 and B10 was 95, 94, and 96. Transplant tumors and ascites were identified in the abdominal cavity of BALB/c mice injected with these three hybridoma cell lines. ELISA showed that the collected ascites contained a high titer of PrP antibody, which reacted well with coated HaPrP, reaching 1:640 000 for antibody B7, 1:640 000 for

B9 and 1:20 000 for B10 when *P/N* value ⩾2.1 as the cut-off value (Fig. 2). Compared with the reactive titers of the individually cultured supernatants of hybridomas measured by the same ELISA protocol, the reactive intensities of the respective ascites were 100-folds increased. IgG subtyping analyses showed that B7 and B10 belonged to IgG_1 with a kappa light chain, and B9 was IgG_3 with a kappa light chain.

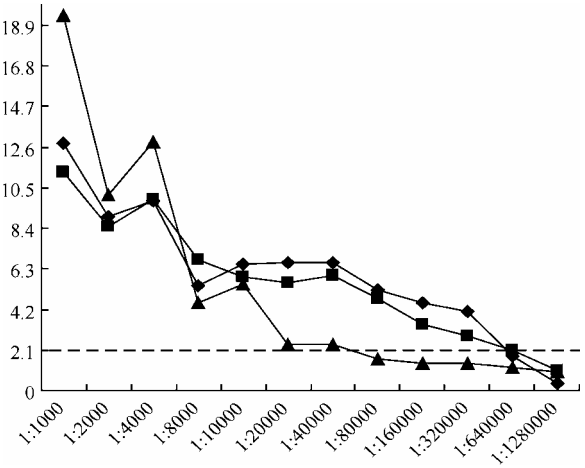

FIG. 2. Analyses of PrP specific antibodies in ascites with ELISA. The *P/N* value was calculated by comparing the OD value of individual testings with that of negative controls. Triangle represents B7, square represents B9 and arris indicates B10. The broken line signs that *P/N* value is 2.1. The dilutions of the tested ascites are shown in the bottom.

Recognition of Different Length Recombinant PrP Proteins

To see the ability of the prepared mAbs to recognize the recombinant PrP proteins, various *E.coli*-expressed recombinant PrP proteins, including hamster HaPrP90-231, HaPrP120-231 and HaPrP23-231, human PrP proteins HuPrP120-231 and HuPrP23-231, hamster HaPrP23-90 in GST fusion form, as well as GST protein, were purified as the protocol described before[14]. Meanwhile, HaPrP23-90 expressed in GST fusion form was purified with affinity chromatography of glutathione sepharose 4B (Han J. *et al*., in preparation). At the dilution of 1:500 mAb B7, the full-length PrP proteins HuPrP23-231 and HaPrP23-231, as well as the N-terminal truncated PrP proteins HuPrP90-231, HuPrP120-231 and HaPrP120-231, were visible at the expected mobilizing positions after immunoblotting (Fig. 3a), while similar blotting patterns were observed with mAb B9 and B10. In contrast, only HuPrP90-231, HuPrP23-231 and HaPrP23-231 reacted with PrP mAb 3F4 (Fig. 3b).

Recognition of Native PrP^C From Different Mammalian Species

As a membrane protein, PrP^C was expressed ubiquitously in brain tissues of various species of mammalian animals. To see the immunoreactivity of

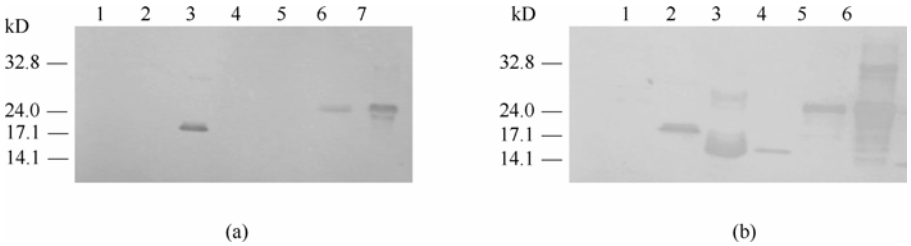

FIG. 3. Comparative analyses of the immunoreactivities of mAb 3F4 and B7 with different length recombinant PrP proteins in Western blot. (a) mAb 3F4, (b) mAb B7. Lane 1: purified GST, lane 2: purified HuPrP23-90 in GST fusion form, lane 3: purified HaPrP90- 231, lane 4: purified HuPrP120-231, lane 5: purified HaPrP120-231, lane 6: purified HuPrP23-231, lane 7: purified HaPrP23-231. The molecular mass marker is shown on the left.

the prepared mAbs to native PrP^C from humans as well as various species animals, brains tissues from non-CJD postmortem and healthy mice, rats, cattles, rabbits and hamsters were collected and PrP^C extracts were prepared. Western blot analysis showed that mAb B7 reacted with the PrP^C from humans, hamsters, mice, rats and cattles, which migrated at 25-35 kD, but failed to recognize PrP^C prepared from rabbit brain (Fig. 4a). MAbs B9 and B10 could recognize the PrP^C from hamsters, mice, rats, and cattles, except for that from humans and rabbits.

Recognition of PrP^{Sc} From the Hamsters Infected With Scrapie Strain 263K

To address the reactivity of the prepared mAbs with pathological PrP^{Sc}, brain homogenates were prepared from the hamsters infected with scrapie strain 263K, which developed typical TSE clinical manifestations about 66 days after the infection intracerebrally[15-16]. The presence of PrP^{Sc} in the brain tissue was verified by special PK-treated Western blot and immunohistochemistry, with PrP specific antibody 3F4[17-18]. Using mAb B7, three immuno-reactive bands were observed in the

preparation without treatment of PK, which were mobilized at the positions from 25 to 35 kD, representing diglycosylated, monoglycosylated and aglycosylated forms (Fig. 4b), while in the PK treated preparation, clear PK-resistant bands were identified that were mobilized at the positions from 19 to 27 kD, in which the diglycosylated and monoglycosylated forms predominated. Similar immunoblotting patterns were identified in the preparation reacting with mAb B9. However, only PrP bands were observed in the preparation without treatment of PK, but not in that with PK, when mAb B10 was employed in the test.

To see whether the prepared mAb could recognize the deglycosylated PrP^{Sc}, PNGase F was introduced into the PrP^{Sc} preparation after digestion with PK. A single immunoblotting band at the position of 19 kD was detected in the reaction with mAb B7, which showed the same electrophoresis position as the aglycosylated form of PrP^{Sc} (Fig. 5a). The same electrophoresis pattern was seen in the reaction with antibody 3F4 used as the positive control (Fig. 5b).

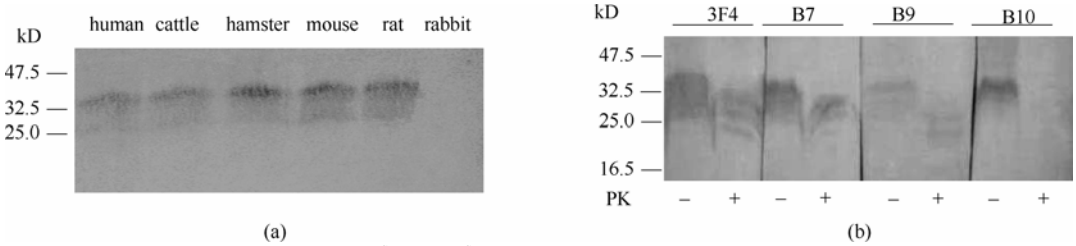

FIG. 4. Reactivities of mAbs with native PrP^{C} and PrP^{Sc} from various brain tissues in Western blot analyses. (a) Western blot analyses of the recognition activity of mAb B7 with the PrP^{C} prepared from the brain tissues of non-infected humans, cattles, hamsters, mice, rats and rabbits (shown at the top of the figure). (b) Western blot analyses of the recognition activities of mAbs with PrP^{Sc} from the brain tissues of hamsters infected with scrapie strain 263K. +: treatment with PK, -: treatment without PK. Molecular mass markers are indicated on the chart.

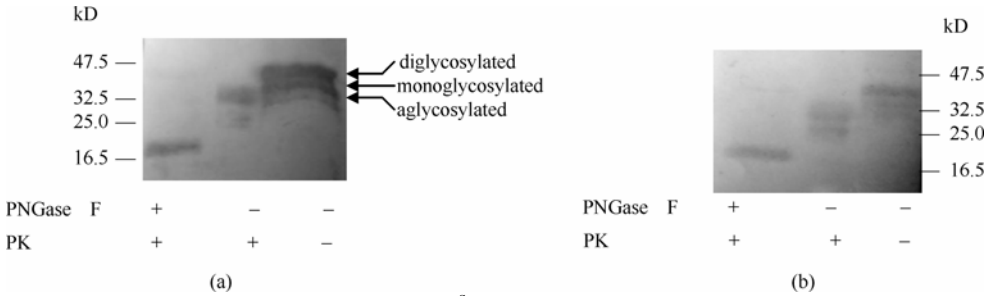

FIG. 5. MAb B7 recognizes the deglycosylation form of PrP^{Sc} in Western blot assays. (a) mAb 3F4, (b) mAb B7. The individual preparations with (+) or (-) PK and/or PNGase F are indicated at the bottom. Molecular mass markers are indicated on the chart.

To determine the potential usage of the prepared antibodies in neuropathological assays, mAb B7 was employed in immunohistochemical tests. The brain slices were prepared from healthy hamsters and the hamsters infected with scrapie strain 263K. As expected, with mAb B7, none of immunoblots was detected in the brain tissues from healthy hamster, whereas PrP^{Sc} deposits were repeatedly seen in various brain regions of infected animals. Interestingly, compared with those observed with antibody 3F4, the distribution and pattern of PrP^{Sc} deposits with antibody B7 showed distinct features. In line with the previous observations[15-16], brain stems were identified by B7 as the most severely affected regions with a mass of PrP^{Sc} deposits. In contrast to the remarkable extracellular deposits of PrP^{Sc} with plaques in the preparation stained with 3F4 (Fig. 6 b1), a large number of positively stained neuronal cells were observed besides the notable extracellular deposits in the preparation of B7 (Fig. 6 a1). Significantly immunostained neuronal cells were observed with B7 in cerebral cortex (Fig. 6 a2), but few deposits or labeled cells with 3F4 (Fig. 6 b2). In cerebellum, the deposits stained with 3F4 were mainly located in the white matter and distributed along with nervous fibrils (Fig. 6 b3), whereas only a few deposits were found in the region of cortex. However, PrP^{Sc} was mainly identified in cerebellum cortex in the slices stained by B7, with a large number of labeled neurons in the layer of Purkinje cells (Fig. 6 a3). These data suggested that antibody 3F4 mainly recognized the extracellular PrP^{Sc} deposits, while B7 stained intracellular PrP^{Sc}.

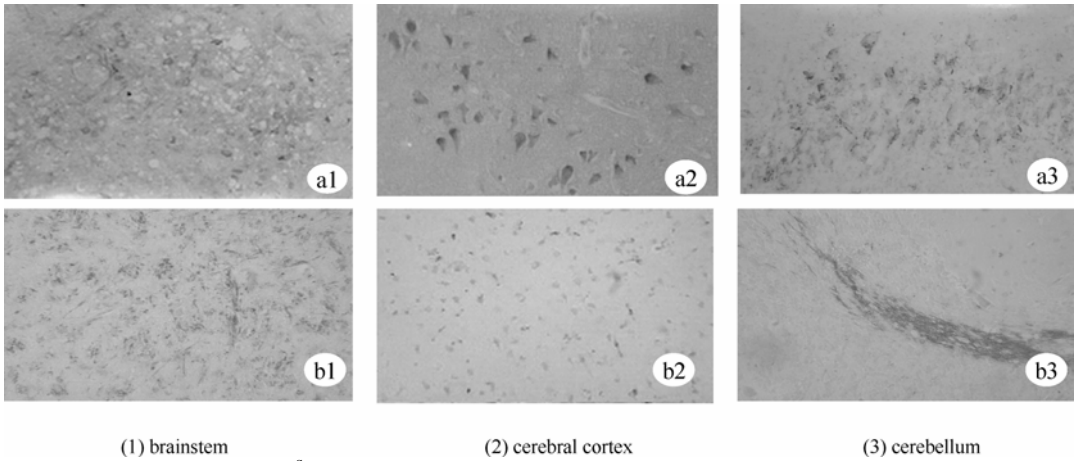

FIG. 6. Different patterns of PrP^{Sc} deposits in brain regions from the hamsters infected with scrapie strain 263K in immunohistochemical stainings with mAbs B7 (a) and 3F4 (b). The observed regions of brain stem, cerebral cortex and cerebellum are indicated as (1), (2), and (3). (×280).

DISCUSSION

Three cell lines of hybridomas that secreted PrP antibody were obtained using recombinant hamster PrP23-231 as the immunogen. It was confirmed that mAbs B7 and B9 could recognize the recombinant PrP proteins, native PrP^{C} and PrP^{Sc} using ELISA, Western blot and immunohistochemical assays, while B10 failed to react with PrP^{Sc}. Successful preparaons of mAbs to PrP imply that recombinant hamster PrP can overcome the potential immunotolerance, although PrP proteins of hamsters and mice shared 95% homology in amino acid sequences[4]. Mostly, PrP mAbs were prepared with PrP-null mice, in which the *prnp* gene was knocked out in embryo-stage[19]. The mAbs in this study were obtained by immunizing ordinary BALB/c mice many times. Repeated irritations of extragenetic protein might help to break through the potential immunotolerance. Nevertheless, the recombinant protein expressed in *E. coli* might differ from the native one in the modified procedures at post-translation, possibly in the tertiary structure of protein as well. In fact, native PrP^{C} and recombinant PrP have been identified to contain different contents of secondary structure[17], which might be another explanation for awaking immunological lymphocytes. Remarkable reactions of mAbs B7 and B9 with PrP^{Sc} from scrapie-infected hamsters indicate wide usages of human and other animal TSEs in experiments and clinical practice.

Western blot assays of mAbs with different deletions of PrP proteins revealed that the prepared mAb B7 and B9 were able to recognize PrP peptides started with the 120th amino acid residue, which varied remarkably from mAb 3F4 that did not recognize PrP120-231 peptide. These data are consistent with previous reports that the recognizing epitope of mAb 3F4 could be likely restricted within 109 to 112 amino acid residues. Theoretically, mAb could react with only specific epitopes or antigenic determinants. Establishment of a bank of PrP mAbs with various immuno-reactivities may provide more possibilities in prion research and TSE diagnosis. Synthesized PrP peptides will help to map the exact reacting region in PrP protein of mAbs B7, B9, and B10.

Although electrophoresis and glycosylated patterns of native PrP^{Sc} and PK-treated PrP^{Sc} in the Western blot tests with mAb 3F4 and the prepared mAbs failed to show any detectable difference, the PrP^{Sc} deposit patterns in immunohistochemical assays with 3F4, B7 and B9 looked differently (B9's data not shown). Slices stained with B7 and B9 revealed more vivid morphology of neurons in addition to dispersive extracellular staining, whereas 3F4 exhibited more extensive coarse granular extracellular deposits. Different strains of PrP mAbs may interact with their antigens in different pathways, leading to stabilization, refolding, and suppression of aggregation. MAbs against the peptides 106-126 of human prion protein could prevent PrP 106-126 fibrillar aggregation, disaggregate already formed aggregates, and inhibit the neurotoxic effect of peptide on the PC12 cell system, while mAb 3F4 had no protective effect[20]. Different immunohis-tochemical staining was observed in the sections of scrapie with mAbs KG9 and 3F4, but almost the same picture was seen in the sections of BSE[21]. Different binding activities of various PrP specific mAbs for scrapie PrP^{Sc} from extra- or intra-cellular origins and from cortex or medulla of cerebrum and cerebellum may help to understand the pathogenesis of these diseases and raise the possibility to use different or mixed mAbs in the diagnosis.

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