Generation of Antibodies against Bovine Recombinant Prion Protein in Various Strains of Mice

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Transmissible spongiform encephalopathies (TSEs), also known as prion diseases, belong to a group of neurodegenerative disorders affecting humans and animals. To date, definite diagnosis of prion disease can only be made by analysis of tissue samples for the presence of protease-resistant misfolded prion protein (PrPSc). Monoclonal antibodies (MAbs) to the prion protein provide valuable tools for TSE diagnosis, as well as for basic research on these diseases. In this communication, the development of antibodies against recombinant bovine prion protein (brecPrP) in four strains of mice (BALB/c, ND4, SJL, and NZB/NZW F1) is described. Immunization of autoimmunity-prone NZB/NZW F1 and SJL mice with brecPrP was applied to overcome self-tolerance against the prion protein. ND4 and SJL mice did not develop an immune response to brecPrP. BALB/c mice produced antibody titers of 1:1,000 to 1:1,500 in an enzyme-linked immunosorbent assay (ELISA), while NZB/NZW F1 mice responded with titers of 1:7,000 to 1:11,000. A panel of 71 anti-brecPrP MAbs recognizing continuous and discontinuous epitopes was established from BALB/c and NZB/NZW F1 mice. Seven anti-brecPrP MAbs reacted with both the cellular form of PrP and protease K-resistant PrPSc from sheep brain in Western blot assays. The epitope specificity of these MAbs was determined, and applicability to immunohistochemical detection of prions was studied. The MAbs generated will be useful tools in the development of TSE immunochemical diagnosis and for research. This is the first report of the development of anti-PrP MAbs by use of autoimmune NZB/NZW F1 mice as an alternative approach for the generation of PrP-specific MAbs.

Material and Methods

Mice. All of the mice used in the present study were purchased from Harlan and kept in a specific animal facility. All procedures involving experimental animals were carried out according to Canadian Council on Animal Care guidelines.

Plasmid construction. The bovine prp DNA fragment that translates 218 amino acids (aa; from aa 25 to aa 242) was amplified by PCR from bovine genomic DNA with primers P13 (forward sequence; 5′-GGG AAT TCC ATA TGA AGA AGC GAC CAA AAC CTG-3′) and P17 (reverse sequence; 5′-CGG ATC CGA ACT TGC CCC TCG TTG GTA-3′). The PCR products were inserted into cloning vector pET-30a-c(-+) (Novagen). Proofreading of the inserted DNA sequence was performed by dideoxy sequencing. Transformation of Escherichia coli BL21(DE3)pLysS (Novagen) was performed by conventional

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Expression and purification of His-tagged brecPrP. An overnight culture of E. coli BL21(DE3) containing plasmid pET-30a(+) -bovPrP was transferred to 5 liters of Luria-Bertami medium containing kanamycin (50 μg/ml) and grown at 37°C until the optical density at 600 nm reached 0.8 to 1.0. Expression was induced with isopropyl-β-d-thiogalactopyranoside (IPTG) at a final concentration of 1 mM, and the culture was grown for another 4 h. The cells were harvested by centrifugation at 8,000 rpm for 15 min and suspended in phosphate-buffered saline (PBS; pH 7.5) supplemented with 1 mM phenylmethylsulfonyl fluoride. The bacteria were disrupted in a French pressure cell (Thermo IEC), and the lysate was centrifuged at 16,000 rpm for 30 min at 4°C. The insoluble inclusion bodies were washed with the above buffer and solubilized in 30 ml of solubilization buffer (6 M guanidine hydrochloride, 100 mM NaH2PO4, 10 mM Tris-HCl, pH 8.0) overnight at 4°C. brecPrP was further purified by Ni2+-immobilized metal affinity chromatography (Ni-NTA Superflow; QIAGEN). The brecPrP in 15 ml of solubilization buffer was loaded onto Ni2+ resin (4 ml) and equilibrated with buffer D (8 M urea, 100 mM Na2HPO4, 10 mM Tris, pH 8.0 [adjusted with HCl]). The column was washed with 30 ml of buffer D and then with 30 ml of buffer A (8 M urea, 100 mM NaH2PO4, 10 mM Tris, 0.5 M NaCl, 5 mM imidazole, pH 7.0 [adjusted with HCl]). On-column refolding of brecPrP was performed by applying 100 ml of a 0 to 100% linear gradient of buffers A and B (PBS [10 mM Tris-HCl, 150 mM NaCl], 15% glycerol, pH 7.4). Finally, the column was washed with 30 ml of buffer B. Recombinant bovine PrP was eluted with 30 ml of buffer E (0.25 M imidazole, TBS, 15% glycerol, pH 7.4), dialyzed against water, and concentrated with an Amicon Ultra-15 10,000 MWCO Centrifugal Device (Millipore). The protein concentration was determined spectrophotometrically at 280 nm with an extinction coefficient of 58,886 M-1 cm-1. The calculated molecular mass of brecPrP was 25.75 kDa. For long-term storage, brecPrP was lyophilized and stored at −20°C. The quality of expressed and purified brecPrP was monitored by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) under reducing and nonreducing conditions.

Immunoassays. Supernatant tissue culture fluids were screened by ELISA, and positive cell lines were cloned by limiting dilution. The identity of the MAbs was determined by capture ELISA with goat anti-mouse immunoglobulin isotype-specific antibodies.

ELISA. Recombinant bovine PrP or recombinant mouse PrP (moPrP; Alcion AG) at 2.5 μg/ml in PBS was used to coat 96-well flat-bottom plates overnight at 4°C. Excess protein was removed, and wells were blocked with 5% nonfat milk in PBS-Tween 80 (PBST) for 60 min. Plates were washed with PBST three times. Appropriate dilutions of serum or hybridoma tissue culture fluid in PBST containing 3% bovine serum albumin (BSA) were added, and the plates were incubated for 60 min at 25°C, followed by three washes with PBST. Anti-mouse IgG-HRP conjugate (Jackson ImmunoResearch Laboratories Inc.) at a 1:2,000 dilution in PBST containing 3% BSA was added, and the mixture was incubated for 60 min at 25°C and then washed three times with PBST. After the final washing, 100 μl of a substrate solution containing 5 mM citrate buffer (pH 4.5), 4 mM 2.2‘-azinobis-(3-ethylbenzthiazoline-6-sulfonic acid) diionium salt (ABTS), and 1 mM H2O2 was added to each well. After 10 min of incubation, the optical density at 414 nm of each well was measured with a Labsystems Multiskan RC spectrophotometer.

Extraction of PrPSc. Infected brain tissue was obtained from sheep with confirmed scrapie and negative controls were immunostained with primary antibodies M2182, M2197, and M2199. Sections were cut at 5 μm and mounted on positively charged glass slides. Tissue sections were immunostained with MAbs for detection of PrPSc as previously described (20). Briefly, sections were dewaxed, rehydrated, and treated with 88% formic acid for 5 min, followed by hydrated autoclaving in antigen retrieval buffer (pH 6.1; DAKO) at 120°C for 20 min. Primary antibody treatment (diluted 1:1,000) was performed by AEC (Ventana Medical System), and counterstaining with hematoxylin were performed on an automated immunostainer (NeXES; Ventana Medical Systems).

RESULTS

Expression and on-column refolding and purification of recombinant bovine PrP. Expression plasmid pET-30a(-)-bovPrP encoding fragment 25 to 242 of bovine PrP was constructed. The brecPrP sequence was linked to a 20-aa C-terminal extension containing a pentahistidine to enable purification of the protein by immobilized Ni2+ chromatography. Nucleotide sequencing of pET-30a(-)-bovPrP verified its identity with the bovine PrP cDNA sequence of Goldman et al. (6).

Bovine PrP with a C-terminal histidine tail was expressed as inclusion bodies in the cytoplasm of E. coli. The purification procedure was based on the method of Zahn et al. (24), with variations. After cell lysis and inclusion body solubilization in guanidine-HCl buffer, denatured proteins were loaded onto Ni2+-NTA agarose resin, which has a high affinity for polypeptides containing four to six consecutive histidine residues at the N or C terminus. brecPrP was purified under the conditions shown on Fig. 1A. Proteins not containing a histidine tag were removed from the column by washing the resin with a buffer
containing 8 M urea, 0.5 M NaCl, and 5 mM imidazole. Refolding of brecPrP was performed on the column via a 100% to 0% gradient of the denaturing agent (urea) and a 0 to 15% glycerol gradient. Glycerol was used to reduce the aggregation of brecPrP during renaturation. The yield of recovered brecPrP was two to three times higher when 15% glycerol was added to the buffers during the refolding and elution steps. brecPrP was eluted at an imidazole concentration of 0.25 mM, dialyzed, and concentrated. Oligomeric/aggregated brecPrP remained adsorbed on the Ni\(^{2+}\)/H\(^{10}{\text{NO}}\)1 column and could be eluted with 0.25 M imidazole in the solubilization buffer. After one-step purification and refolding on the Ni\(^{2+}\)/H\(^{10}{\text{NO}}\)1 column, monomeric brecPrP with a purity of more than 95%, as determined by nonreducing SDS-PAGE, was obtained (Fig. 1B). The total yield of purified brecPrP was 15 to 17 mg/liter of cell culture.

brecPrP was able to bind with commercial anti-PrP MAb F89/160.1.5 (recognizes conserved epitope LIHF) and F99/97.6.1 (recognizes conserved epitope QYQRES) (VMRD Inc.), as demonstrated by ELISA (data not shown) and Western blot analysis (Fig. 1C).

Protease sensitivity of His-tagged brecPrP. Incubation of brecPrP with proteinase K resulted in complete digestion of prion protein, indicating that the brecPrP had the protease-sensitive conformation (data not shown).

Generation of humoral responses to brecPrP in various lines of mice. Immunization with brecPrP-KLH conjugate induced a low anti-brecPrP antibody response in BALB/c mice (titers, 1:1,000 to 1:1,500) and an almost undetectable response in ND4 mice (Fig. 2A and B). Autoimmunity-prone NZB/NZW F\(_1\) mice gave strong immune responses to brecPrP, with antibody titers of 1:7,000 to 1:11,000. In contrast, SJL mice did not develop anti-brecPrP antibodies (Fig. 2C and D). No mice showed any clinical abnormalities throughout the entire period of immunization with brecPrP-KLH conjugate.

Polyclonal anti-brecPrP antibodies derived from BALB/c and NZB/NZW F\(_1\) mice after immunization with brecPrP have been analyzed for reactivity with recombinant mouse PrP (moPrP) by ELISA. Anti-brecPrP sera from NZB/NZW F\(_1\) mice weakly reacted with moPrP, and a negligible reaction with moPrP was observed in BALB/c mice (Fig. 2E and F).

Sera obtained after immunization of BALB/c and NZB/NZW F\(_1\) mice were tested by Western blot analysis with protease K-treated extracts from scrapie-affected sheep brain homogenate. Both BALB/c and NZB/NZW F\(_1\) antisera reacted with sheep PrP\(^{Sc}\), but the staining pattern obtained with BALB/c antisera was less intense than that obtained with NZB/NZW F\(_1\) antisera (Fig. 3). NZB/NZW F\(_1\) antisera preferentially recognized the diglycosylated form of sheep PrP\(^{Sc}\), whereas BALB/c antisera recognized the di- and monoglycosylated forms equally. Both types of antisera reacted weakly with the unglycosylated isoform of PrP\(^{Sc}\) (Fig. 3).

Epitope analyses of anti-recPrP polyclonal antibodies. To further characterize the polyclonal antisera, epitope mapping was performed with a biotinylated synthetic peptide library covering the brecPrP sequence. BALB/c and NZB/NZW F\(_1\) mice responded with similar epitope patterns (Fig. 4). NZB/NZW F\(_1\) mice produced antibodies to a wider spectrum of brecPrP epitopes than BALB/c mice did, and the intensity of the ELISA signal was higher (not shown). NZB/NZW F\(_1\) mouse antisera reacted with seven to nine antigen sites (positions 28 to 43, 52 to 61, 119 to 134, 143 to 155, 167 to 178, and 215 to 229). Of note, conformational epitopes could not be determined in the assay.

Generation of mouse anti-recPrP MAbs. SP2/0-Ag14 myeloma cells were hybridized with spleen cells from mice immunized with brecPrP-KLH conjugate. Hybridomas positive for brecPrP by ELISA were selected. A number of positive hybridoma cell lines were unstable or stopped producing antibodies after recloning. After anti-recPrP hybridoma cell line stabilization, the isotypes of all MAbs were determined. The data

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**FIG. 1.** Recombinant bovine prion protein purification and verification. (A) Profile of purification of His-tagged brecPrP by Ni\(^{2+}\) affinity chromatography. OD 280, optical density at 280 nm; Im, imidazole. (B) SDS-PAGE of purified bovine recPrP under nonreducing conditions. The single band indicates that the purified protein does not contain intermolecular disulfide bonds. MWM, molecular weight markers. (C) Verification of the sequence of purified brecPrP by Western blotting with MAbs VMRD F89/160.1.5 and VMRD F99/97.6.1. The values between panels B and C are molecular masses.
obtained are presented in Table 1. BALB/c mice produced MAbs predominantly of the IgM and IgG1 classes (IgM, 26; IgG1, 29; IgG2b, 1), whereas autoimmune NZB/NZW F1 mice produced anti-recPrP antibodies of all IgG subclasses, with fewer IgM isotype antibodies than BALB/c mice (IgM, 7; IgG1, 11; IgG2a, 12; IgG2b, 16; IgG3, 2). The result was that 71 (31 from BALB/c mice and 40 from NZB/NZW F1 mice) anti-recPrP hybridoma cell lines producing MAbs of the IgG class were established. These MAbs were further analyzed for anti-PrP properties as described below.

Characterization and reactivity of MAbs. MAbs against brecPrP were examined for binding with moPrP by ELISA. Three of 30 BALB/c-derived anti-recPrP IgG MAbs and 11 of 41 NZB/NZW F1-derived anti-recPrP IgG MAbs reacted with moPrP (Table 1).

To determine the reactivity of anti-recPrP MAbs against linear epitopes, their binding to brecPrP was examined by Western blotting. Twenty-one of 30 BALB/c-derived IgG MAbs and 29 of 41 NZB/NZW F1-derived IgG MAbs reacted with denatured brecPrP in Western blot assays. To determine if anti-recPrP MAbs detect sheep PrPSc and PrPSc, Western blot analysis was performed on normal and scrapie-infected sheep brain homogenate. Seventeen of 21 anti-recPrP MAbs from BALB/c mice and 29 of 41 MAbs from NZB/NZW F1,

FIG. 2. ELISA evaluation of mouse sera after immunization with brecPrP. (A to D) Reactivity of anti-brecPrP sera from different strains of mice against brecPrP. Note the pronounced immune responses of BALB/c and NZB/NZW F1 mice. (E and F) Reactivity of anti-brecPrP sera from NZB/NZW F1 and BALB/c mice against moPrP. Note the lack of cross-reaction of BALB/c anti-brecPrP sera with moPrP and the presence of anti-moPrP reactivity in anti-brecPrP NZB/NZW F1 sera. Antibody titers were evaluated 1 week after the last immunization with brecPrP conjugated with KLH. Control sera were obtained 7 days before the first immunization and did not contain the anti-brecPrP and anti-moPrP antibodies, as determined by ELISA (data are not presented). OD414, optical density at 414 nm.
mice cross-reacted with sheep prion protein. The results obtained are presented in Table 1. Seven MAbs reactive with sheep PrPSc recognized linear epitopes of the protease-resistant part of PrPSc from scrapie-affected sheep brain (Fig. 5A and B). Five of them were derived from BALB/c mice (M2182, M2188, M2197, M2199, and M2200), and two were derived from NZB/NZW F1 mice (M2281 and M2283). These antibodies showed binding patterns similar to that of commercial F89/160.5.1.

**Epitope mapping of anti-PrP MAbs.** To identify the epitopes recognized by anti-brecPrP MAbs, an ELISA was performed on an adjacent overlapping bovine PrP peptide library (Table 2). MAbs M2182, M2197, M2200 (all IgG1), and M2281 (IgG2a) recognized segment HVAGAAAAGAVVG (positions 123 to 135 of bovine prion protein); MAbs M2188 (IgG2b) and M2199 (IgG1) recognized segment SRPLIHFG (positions 146 to 153) and segment KMMERVVEQMCITQY (positions 215 to 229), respectively; and M2283 (IgG2b) bound to segment GENFTETDI (positions 206 to 214).

**Immunohistochemistry of PrPSc in fixed tissues.** Fixed medulla oblongata and lymph node tissue sections of control and scrapie-infected sheep were immunostained with MAbs M2182, M2188, and M2197. All three MAbs had disease-specific reactivity and identified prion depositions in infected tissues, but M2197 produced the most intense staining among the MAbs tested (Fig. 6).

**DISCUSSION**

Immunohistochemical detection is currently the most efficient and reliable tool for the diagnosis of prion diseases, and therefore MAbs to different epitopes of PrP are valuable reagents for the development of diagnostic tests and for basic research. The goal of this study was to generate MAbs to various regions of bovine prion protein.

Due to the high homology of PrP among mammalian species, anti-PrP antibodies are not readily developed in laboratory mice; however, the problem can be overcome by using (i) immunization of transgenic PrP0/0 mice (4, 14, 16, 23, 25), (ii) immunization with PrP of distant species (11, 18), and (iii) extensive immunization with synthetic fragments of prion protein (1, 3, 13, 17). In this study, four strains of mice (BALB/c, ND4, SJL, and NZB/NZW F1) were immunized with recombinant bovine PrP conjugated with KLH to enhance the probability of a humoral immune response to PrP. The strains of mice had different haplotypes of the major histocompatibility complex (H-2d [BALB/c], H-2s [SJL], and heterozygote H-2d/H-2z [NZB/NZW F1]); ND4 is an outbred Swiss Webster strain) (2, 7). Assuming that autoimmune mice could develop an immune response to autoantigens, NZB/NZW F1 and SJL mice were immunized with recombinant bovine PrP to overcome the autotolerance associated with PrP and probably allow access to a wider spectrum of PrP-specific MAb isotypes.

Immunization resulted in very low and variable antibody titers in the ND4 and SJL mouse strains, while a reasonable brecPrP-specific immune response was obtained in BALB/c.
ELISA titers, 1:1,000 to 1:1,500) and NZB/NZW F1 mice (ELISA titers, 1:7,000 to 1:11,000, which is 7 to 10 times higher than that of BALB/c mice). Interestingly, both strains of mice had the H-2d major histocompatibility complex gene. Determination of the most antigenic sites on bovine prion protein was based on epitope mapping of polyclonal antisera. Thus, BALB/c antibodies recognized the same epitopes as NZB/NZW F1 antibodies but sera from autoimmunity-prone mice were able to recognize the wider spectrum of PrP epitopes and had higher ELISA titers. NZB/NZW F1 antisera reacted with seven to nine antigen sites (positions 28 to 43, 52 to 63, 106 to 115, 119 to 134, 143 to 155, 159 to 170, 187 to 201, 215 to 226, and 228 to 241 of the bovine prion protein), while BALB/c antisera reacted with four to six sites (positions 28 to 43, 52 to 61, 119 to 134, 143 to 155, 167 to 178, and 215 to 229). Antibodies were directed mainly to domains with bovine-mouse interspecies amino acid sequence variations (G33-, G58T, G59-, G108N, M120L, L149M, S154N, Y156W, H166Y, V195I, E197Q, I214V, I226V, R231K, and G240R [bovine PrP sequence numbering]). The lack of antibody response to the murine amino acid sequence could be due to host tolerance to endogenously expressed PrPC. Nevertheless, in contrast to those of BALB/c mice, anti-brecPrP sera derived from NZB/NZW F1 mice reacted with recombinant mouse PrP in ELISA.

| Mouse strain | Reciprocal titer of anti-brecPrP serum | Total no. of anti-brecPrP MAbs (ELISA) | Isotype (no. of MAbs) | No. of MAbs reactive with: |
|--------------|----------------------------------------|----------------------------------------|-----------------------|---------------------------|
|              |                                        |                                        |                       | noPrP in ELISA (IgG only) | breccPrP in Western blot assay (IgG only) | Sheep PrPSc in Western blot assay (IgG only) | Sheep PrPSc in Western blot assay (IgG only) |
| BALB/c (10)  | 1,000–1,500                            | 56 (30 IgG)                            | IgG1 (29)             | 3             | 21          | 17          | 5          |
|              |                                        |                                        | IgG2a (0)             |               |             |             |             |
|              |                                        |                                        | IgG2b (1)             |               |             |             |             |
|              |                                        |                                        | IgG3 (0)              |               |             |             |             |
|              |                                        |                                        | IgM (26)              |               |             |             |             |
| NZB/NZW F1 (5) | 7,000–11,000                           | 48 (41 IgG)                            | IgG1 (11)             | 11            | 29          | 23          | 2          |
|              |                                        |                                        | IgG2a (12)            |               |             |             |             |
|              |                                        |                                        | IgG2b (16)            |               |             |             |             |
|              |                                        |                                        | IgG3 (2)              |               |             |             |             |
|              |                                        |                                        | IgM (7)               |               |             |             |             |

TABLE 2. Summary of anti-brecPrP MAbs specific for linear epitopes of protease K-resistant sheep PrPSc

| MAb     | Isotype | Mouse strain | Epitope*                        |
|---------|---------|--------------|---------------------------------|
| M2182   | IgG1    | BALB/c       | HVAGAAAGAVVG (aa 122–134)       |
| M2197   | IgG1    | BALB/c       |                                 |
| M2200   | IgG1    | BALB/c       |                                 |
| M2281   | IgG2a   | NZB/NZW F1   |                                 |
| M2188   | IgG2b   | BALB/c       | SRPLIHFG (aa 146–153)           |
| M2199   | IgG1    | BALB/c       | KMMERVVEQMCTTQY (aa 215–229)    |
| M2283   | IgG2b   | NZB/NZW F1   | GENFTETDI (aa 206–214)          |

* Position numbers represent the bovine amino acid sequence.
which could be explained by particular breakage of tolerance to PrP in these autoimmunity-prone mice.

A panel of 71 anti-brecPrP antibodies of the IgG isotype was developed by conventional hybridoma technology. The observation that 3 of 30 BALB/c anti-brecPrP IgG MAbs and 11 of 41 NZB/NZW F1 anti-brecPrP IgG MAbs reacted with mouse recombinant PrP supports the rationale of the use of autoimmunity-prone mouse strains to circumvent the tolerance to PrP.

MAbs to linear epitopes of brecPrP (50 MAbs) were tested for the ability to recognize ovine PrP. The ability of anti-bovine PrP antibodies to cross-react with ovine PrP was expected because of the high PrP homology between the two species. Forty anti-brecPrP MAbs cross-reacted with linear epitopes of denatured ovine PrPSc in Western blot assays (17 BALB/c and 23 NZB/NZW F1 mice), and 7 MAbs (5 BALB/c and 2 NZB/NZW F1 mice) out of those 40 produced consistent results with proteinase K-resistant PrPSc from different scrapie sheep brain homogenates.

Despite the high titer of anti-brecPrP antibodies in NZB/NZW F1 mice and the effective binding of polyclonal antibodies with protease K-digested sheep PrPSc in Western blot assays, not many MAbs specific for protease-treated PrPSc resulted from autoimmune NZB/NZW F1 mice. This may be a
consequence of the high level of instability of anti-brecPrP hybridoma cell lines observed. The synthesis of highly effective antibodies to the physiologically important epitopes of the cellular surface PrP probably caused hybridoma cells to be unstable, as previously reported by Williamson et al. (23).

Epitope specificity of MAbs reacted with ovine PrPSc was determined: MAb M2182, M2197, M2200 (all IgG1), and M2281 (IgG2b) recognized segment SRPLIHFG (positions 146 to 153); M2199 (IgG1) recognized segment KMMERVQEOMC ITQY (positions 215 to 229); and M2283 (IgG2b) bound with GENFETETD (positions 206 to 214). Region 122 to 134 of PrP is highly conserved in many different species, including humans (22), and antibodies against these epitopes probably could react with pathological PrPSc from other mammals. One of the MAbs against region 123 to 135, M2197, is suitable for immuno-histochemistry of PrPSc on fixed tissues. Regions 146 to 153, 206 to 214, and 215 to 229 of PrP have sequence variability between species, and applicability of MAbs against these epitopes has to be further investigated.

In this study, a number of anti-PrP MAbs able to react with distinct epitopes of brecPrP, sheep PrPSc, and proteinase K-resistant PrPSc were developed. These antibodies will be used for diagnostic and research purposes. Although numerous stable hybridoma cell lines producing MAbs to PrPSc were not obtained from NZB/NZW F1 mice, it still appears that the use of autoimmune mice can be an alternative way to generate antibodies to a broad spectrum of mammalian PrP epitopes.

Upgrading of the procedure for development of autoimmune anti-PrP antibodies by improved immunization methods and the phage display technique may help to obtain a variety of antibodies for prion research and diagnosis of TSEs.

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