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Mouse Polyclonal and Monoclonal Antibody to Scrapie-Associated Fibril Proteins

RICHARD J. KASCSAK,[1]* RICHARD RUBENSTEIN,[1] PATRICIA A. MERZ,[1] MARIA TONNA-DEMASI,[1] REGINA FERSKO,[1] RICHARD I. CARP,[1] HENRYK M. WISNIEWSKI,[1] AND HEINO DIRINGER[2]

New York State Institute for Basic Research in Developmental Disabilities, Staten Island, New York 10314,[1] and Robert Koch Institut des Bundesgesundheitsamtes, D-1000 Berlin 65, Federal Republic of Germany[2]

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Antibody response in mice to scrapie-associated fibril proteins (protease-resistant proteins [PrPs]) was generated to different epitopes depending on the source of antigen. Mice responded differently to PrPs isolated from scrapie-infected animals of homologous (mouse) versus heterologous (hamster) species. An enzyme-linked immunosorbent assay established to monitor this antibody response in mice immunized with PrPs was unable to detect such a response in scrapie-infected mice. A monoclonal antibody (MAb), 263K 3F4, derived from a mouse immunized with hamster 263K PrPs reacted with hamster but not mouse PrPs. MAb 263K 3F4 also recognized normal host protein of 33 to 35 kilodaltons in brain tissue from hamsters and humans but not from bovine, mouse, rat, sheep, or rabbit brains. This is the first demonstration of epitope differences on this host protein in different species. The defining of various epitopes on PrP through the use of MAbs will lead to a better understanding of the relationship of PrPs to their host precursor protein and to the infectious scrapie agent.

Scrapie-associated fibrils (SAF) are abnormal structures uniquely associated with unconventional slow virus diseases (23–25). SAF copurify with infectivity in several isolation procedures (9, 13, 21) and may represent one form of the infectious agent (14, 34). The proteins which form SAF, also known as protease-resistant proteins (PrPs), are antigenically related to a host-coded glycoprotein of 33 to 35 kilodaltons (kDa) (2, 19, 29, 34). Utilizing cDNA clones to the host gene encoding the 33- to 35-kDa protein, it has been shown that mRNAs for this protein are transcribed at equal levels within infected and uninfected animals (18, 29, 33). The normal 33- to 35-kDa protein is not resistant to proteolytic digestion nor does it have the ability to form fibrils in noninfected animals (26). Differences in certain characteristics have been described for SAF isolated from various scrapie strain-host strain combinations (14, 15). These differences appear to be related to specific influences (14) of both host and scrapie strains. Fibril formation and the proteolytic resistance of its component proteins may be related to posttranslational or conformational changes or both in the host 33- to 35-kDa protein occurring as a direct result of the infectious process (14, 22, 26).

One of the hallmarks of unconventional slow virus diseases is the apparent lack of immune response to the infectious agent. Several studies have failed to demonstrate production of neutralizing antibody or immune complex formation during disease (30). Subtle changes in the immune system such as an increase in immunoglobulin levels in mice and sheep (7) and changes in B-cell mitogen activation (10) have been detected but may be indirect responses to the pathological process. Antisera to the protein component of SAF, the scrapie infection-specific marker, have, however, been generated in rabbits by many laboratories (3, 4, 14, 35). These antisera have established the strong antigenic relationship among PrPs isolated from a wide range of species (6, 14). In this study, the antibody response to PrPs in mice was monitored under several conditions of antigen presentation. Unlike rabbits, mice can be readily infected with scrapie and serve as an important experimental model (8). Two previous studies have shown that mice given large doses of antigen can be immunized with hamster 263K PrP (1, 4). In the present study, an antibody response was generated in mice to PrPs isolated from both scrapie-infected mice and hamsters. Our results define some of the parameters necessary for this response and also show that such a response does not occur naturally during the disease process. The antibody response in mice is directed against different epitopes on this protein depending on the source of SAF. These studies suggest the potential biological importance of such epitopes in the scrapie infection process.

MATERIALS AND METHODS

Animals and scrapie agents. Mouse scrapie strains ME7 and 87V were kindly provided by Alan G. Dickinson (ARC and MRC Neuropathogenesis Unit, Edinburgh, Scotland). Hamster scrapie strain 263K and mouse scrapie strain 139A were kindly provided by Richard H. Kimberlin (ARC and MRC Neuropathogenesis Unit). Strains ME7 and 139A were used to infect C57BL/6J mice (Jackson Laboratory, Bar Harbor, Maine), strains ME7 and 87V were used to infect IM/Dk mice (obtained from Alan G. Dickinson and maintained in our animal colony), and strain 263K was used to infect LVG/LAK hamsters (Charles River Breeding Laboratories, Inc., Wilmington, Mass.) The preparation of the inoculum, injection, scoring, and sacrificing of animals were performed as previously described (14).

Purification of SAF. SAF were isolated from brains of infected animals at the clinical stage of disease by a modification of the procedure of Hilmert and Diringer (12) as previously described (14, 34). The procedure involves the use of detergent extraction, differential centrifugation, and treatment with proteolytic enzymes. The final pellet obtained from 12 hamster brains or 24 mouse brains contained

* Corresponding author.

100 to 500 μg of SAF proteins (PrPs) and 1 to 10% of the infectivity found in the original homogenate (14).

Immunization of animals. Mice receiving 139A and ME7 SAF antigen were immunized by a modification of the procedure of Vaitukarlis et al. (36). Because of the availability of only small amounts of antigen, only two mice were used per immunization group. For mice receiving 139A SAF antigen, SAF were purified as described above and used without further treatment. For mice receiving ME7 SAF antigen, PrPs were further purified by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and gel elution as previously described (14). Antigen was emulsified in the presence of complete Freund adjuvent (1:1, vol/vol; Difco Laboratories, Detroit, Mich.) with the minichanger of an Omnimixer (Omni Corporation International, Waterbury, Conn.) at 4°C. BALB/cJ mice (7 weeks old; Jackson Laboratory) were inoculated intradermally and subcutaneously in a shaven area of the back with approximately 25 μg of antigen in 400 μl. Each mouse also received 100 μl of *Bordetella pertussis* antigen (Difco) intramuscularly in their hind legs. One week later, animals were reinoculated with 25 μg of antigen in complete Freund adjuvant into the granulomas formed as a result of the first immunization. Two weeks later, animals were boosted with 25 μg of antigen in incomplete Freund adjuvent (Difco). This antigen was given both intramuscularly and intraperitoneally. One week later, the animals were bled retro-orbitally and monitored for antibody response by the enzyme-linked immunosorbent assay (ELISA) procedure. Mice received five additional immunizations of either 139A or ME7 PrPs in incomplete Freund adjuvent at 2-week intervals.

Antigen for immunization with 263K PrPs was extracted with formic acid. Purified SAF preparations were treated in 77% formic acid (Sigma Chemical Co., St. Louis, Mo.) for 2 h at 4°C. The sample was microcentrifuged for 5 min at 4°C, and the supernatant was dried in a Speed-Vac (Savant Instruments, Inc., Hicksdale, N.Y.). The sample was suspended in 150 mM sodium phosphate buffer containing 0.1% SDS. Mice received approximately 30 to 40 μg of SAF protein per immunization as previously described (4).

ELISA. Antigen for the ELISA was prepared by three different methods. Purified SAF obtained as described above were used untreated at a concentration of 1 μg/ml. Antigen was also used after solubilization of SAF in 1% SDS at 37°C for 15 min and dilution in phosphate-buffered saline to 1 μg/ml (0.001% SDS). Antigen was also extracted with formic acid as described above and diluted in phosphate-buffered saline to 1 μg/ml. Bovine serum albumin (BSA) at 5 μg/ml served as a control antigen. Antigen (50 μl) was bound to Falcon Microtest III ELISA trays (Becton Dickinson Labware, Oxnard, Calif.) for 1 h at 37°C and at 4°C overnight. The ELISA was done as described by Nowinski et al. (28). All primary and secondary (either goat anti-mouse immunoglobulin G plus immunoglobulin M or goat anti-rabbit immunoglobulin G conjugated with alkaline phosphatase [Tago, Burlingame, Calif.]) antibodies were diluted in phosphate-buffered saline containing 1% normal goat serum and 0.2% Tween 20. Optical density readings at 405 nm were obtained with a Dynatech automatic ELISA reader (model MR580).

Western blot immunoblot analysis. SAF antigens (approximately 200 ng) were solubilized in 2% SDS–0.5% β-mercapthoethanol and electrophoresed on 12% Laemmli gels as previously described (15). Proteins were electrophoretically transferred to nitrocellulose and processed for reactivity with various antisera as previously described (14).

Production of hybridomas. One of the mice immunized with hamster 263K PrP was used to produce hybridomas. The mouse received a final immunization of antigen (30 μg) by the intravenous route in phosphate-buffered saline 4 days before fusion. Spleen cells were fused to myeloma cell line X63-Ag8.653 as previously described (28). Initial screening of hybridoma supernatants from 96-well plates was performed by the ELISA. One of the hybridomas (263K 3F4) was cloned three times by limiting dilution and used to produce ascites in pristane (Sigma)-primed mice (31). The immunoglobulin subtype of this clone was determined with a mouse immunoglobulin subtype identification kit (Boehringer Mannheim Biochemicals, Indianapolis, Ind.).

Protein determinations. Protein concentrations in purified SAF preparations were determined by a modified Bradford (5) protein assay. Reaction mixtures in 96-well plates (Falcon Microtest III) contained 200 μl of Bradford reagent (0.125 mg of Coomassie blue G250, 6% ethanol, 10.6% phosphoric acid) and 50 μl of diluted protein. The change in optical density at 595 nm was detected with an automatic ELISA reader (Flow Laboratories, Inc., McLean, Va.). This assay, developed by David Soifer (Institute for Basic Research, Staten Island, N.Y.; personal communication) was sensitive to 500 ng of protein in the reaction mixture.

Tissue homogenates. Brain homogenates (10%) were prepared by Dounce homogenization in 1 mM $NaHCO_2$–1 mM $MgCl_2$–0.5 mM KCl–0.32 M sucrose containing 5 μg each of phenylmethylsulfonyl fluoride, pepstatin A, antipain, leupeptin, and benzimedine hydrochloride per ml as previously described (34). Homogenates were clarified at 500 × *g* for 10 min and extracted with 1% SDS before use on polyacrylamide gels.

RESULTS

The ELISA was established initially with polyclonal rabbit antisera to ME7 PrPs which had previously been shown to react to SAF protein by immunodecoration (22) and Western blot (WB) analysis (14). Antigen applied to the ELISA trays was prepared by three different procedures as described in Materials and Methods. The highest immunoreactivity was seen with the formic acid-extracted antigen (data not shown). The SDS-solubilized preparation was three- to fourfold less reactive. Untreated SAF were approximately 10-fold less immunoreactive than the formic acid-extracted sample. In these studies, BSA was used as a nonspecific control antigen and was processed in the same manner as SAF, i.e., either untreated or extracted with SDS or formic acid. Anti-PrP antisera did not react with BSA under these conditions. WB analysis did not reveal any differences in the protein-banding pattern between SDS-treated and formic acid-treated SAF (data not shown).

Mice were immunized with various SAF preparations in an attempt to induce a response to PrPs in this species. Mouse-derived SAF were initially used as an immunogen since most of our studies involve the use of mouse scrapie model systems. Mice immunized with purified intact infectious SAF isolated from 139A-infected mice did not exhibit an immune response to PrPs (data not shown) and eventually exhibited clinical signs indicative of scrapie infection despite the immunization protocol. In a further attempt to induce an immune response in mice to PrPs, an immunization schedule was used which was identical to that previously used successfully to immunize rabbits with mouse ME7 PrPs (14). Mice immunized with purified gel-eluted (i.e., noninfectious) mouse ME7 PrPs exhibited a weak immune response to PrPs

TABLE 1. ELISA for mouse anti-PrP antibody

Plasma	Plasma dilution	Antigen[a]				
		ME7 SAF		263K SAF		BSA
		SDS	FA	SDS	FA	
Preimmune	1×10^3	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Anti-ME7	1×10^3	0.62 ± 0.01	1.38 ± 0.05	0.60 ± 0.02	1.01 ± 0.03	0.09 ± 0.01
M-PrPs	2×10^3	0.44 ± 0.02	0.93 ± 0.02	0.38 ± 0.02	0.88 ± 0.02	0.04 ± 0.02
	5×10^3	0.04 ± 0.01	0.10 ± 0.01	0.03 ± 0.01	0.08 ± 0.01	0.02 ± 0.01
Anti-263K	1×10^4	0.02 ± 0.01	0.02 ± 0.01	1.42 ± 0.07	>1.50	0.03 ± 0.01
PrP	1×10^5	0.02 ± 0.01	0.04 ± 0.01	0.83 ± 0.03	1.50 ± 0.04	0.02 ± 0.02
	1×10^6	0.02 ± 0.01	0.02 ± 0.01	0.13 ± 0.02	0.28 ± 0.02	0.02 ± 0.01

[a] Purified SAF (1 μg/ml) were applied to the ELISA trays either treated with 1% SDS or extracted with 77% formic acid (FA) as described in Materials and Methods. BSA was used as a nonspecific control antigen at 5 μg/ml. Data are shown as the optical density reading ± standard error for triplicate samples.

with an endpoint ELISA titer of 1:5,000 (Table 1). This contrasts with an endpoint ELISA titer of approximately 10^5 for rabbit antisera produced to ME7 PrPs under the same immunization conditions.

The inability to induce a strong immune response in mice to mouse-derived PrPs suggested a comparison with mice immunized with PrPs isolated from a heterologous species. Mice immunized with formic acid-extracted (noninfectious) PrPs isolated from 263K-infected hamsters showed a strong response to 263K PrPs, with an endpoint titer of $>10^5$ (Table 1). This response was specific to hamster 263K PrPs in that no reactivity to similarly prepared mouse PrPs was evident. PrPs purified from mice infected with scrapie agents 139A, 22L, and 87V also did not react in the ELISA with this antisera (data not shown). Mouse antisera to ME7 PrPs and 263K PrPs reacted more strongly in the ELISA with formic acid-extracted antigen than with SDS-solubilized antigen (Table 1), and this result further supports the use of formic acid-extracted antigen for the ELISA.

Comparisons were made by WB analysis between polyclonal rabbit sera raised to ME7 and 263K PrPs and polyclonal mouse sera raised to these same antigens (Fig. 1). As shown previously, both rabbit sera reacted with SAF proteins (PrPs) isolated from mice infected with scrapie agents 139A and ME7 and from hamsters infected with 263K (14). Profiles were specific to each type of SAF but were the same regardless of which antiserum was used. Antisera to mouse anti-ME7 PrPs also reacted with PrPs isolated from mice infected with ME7 and 139A and from hamsters infected with 263K. The staining pattern was similar to that seen with the polyclonal rabbit anti-ME7 PrP sera, but was much less intense. In addition, reactivity was seen to a protein of about 7 kDa that was not stained with either of the polyclonal rabbit sera. This 7-kDa protein was also present in normal brain material processed in the same manner. This protein appeared to be antigenically distinct from PrPs and unrelated to a 7-kDa protein derived from PrPs after either formic acid or hydrofluoric acid extraction (27; R. Rubenstein, manuscript in preparation). Mouse anti-263K PrP sera reacted only with PrPs isolated from 263K-infected hamsters and not with PrPs from ME7- or 139A-infected mice. The profile was similar to that seen with the polyclonal rabbit sera with bands at 26 to 28, 23 to 24, and 19 to 20 kDa. This is the first demonstration of differences in epitopes on PrPs isolated from different species.

WB studies employing formic acid-extracted antigen did not reveal differences in the profile or intensity of staining compared with SDS-solubilized PrPs regardless of which antiserum was used (data not shown).

With the ELISA established to monitor antibody response to PrPs in mice, plasma from mice infected with several different scrapie agents was examined before and during clinical disease (data not shown). Plasma was obtained from C57BL/6J mice infected with ME7 or 139A scrapie agents at four time points between 50 to 135 days postinfection and from IM/Dk mice infected with 87V at four time points from 123 to 350 days postinfection. An antibody response to PrPs was not detected at any of these points. There was a slight increase in ELISA reaction in animals with clinical signs of disease (130 days for 139A, 135 days for ME7, 350 days for 87V). This reaction was shown not to be specific for PrPs and may be related to the increase in immunoglobulin levels

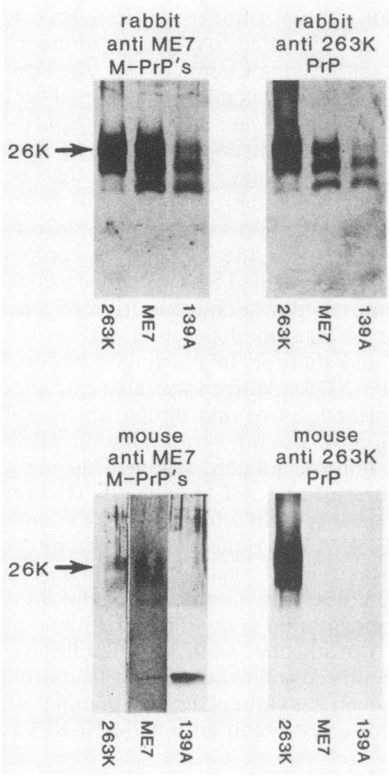

FIG. 1. Comparison of rabbit and mouse polyclonal antisera to PrPs. Purified SAF from hamsters infected with 263K and mice infected with ME7 and 139A were examined by WB analysis for reactivity with rabbit anti-263K PrP (1:10,000) and anti-ME7 PrP (1:5,000) and with mouse anti-263K PrP (1:10,000) and anti-ME7 PrP (1:1,000) antisera. K, Kilodaltons.

seen previously in mice and sheep showing clinical signs of disease (7).

One of the rationales for immunizing mice with PrPs is the production of monoclonal antibodies (MAbs). Lymphocytes isolated from the spleen of the mouse immunized with 263K PrPs were fused to X63-Ag8.653 myeloma cells, and hybridomas were formed. Over 400 wells containing hybridomas were screened, and only 3 wells yielded ELISA readings indicative of a positive response to PrPs. A positive response was defined as an optical density reading of >1.0 and a PrP/BSA antigen index ratio of >10 (37). Only one of these clones was characterized. This clone, designated 263K 3F4, produced immunoglobulin G2A, kappa chain. Ascites was produced which had a titer of greater than 10^6 by ELISA and 10^5 by WB analysis.

263K 3F4 ascites was analyzed by WB analysis for reactivity to various purified SAF preparations (Fig. 2). The results were similar to those shown in Fig. 1 for the polyclonal mouse anti-263K sera; the 263K 3F4 ascites reacted only with hamster 263K PrPs and not with PrPs isolated from mice infected with 139A, ME7, or 87V scrapie agents. PrPs isolated from IM/Dk mice infected with 87V exhibited a WB profile very similar to that of PrPs isolated from hamsters infected with 263K. Despite this similarity, 3F4 MAb did not react with 87V PrPs.

The above results demonstrated the strong influence of the host species in determining the antigenic response to PrPs and the existence of epitopes on PrPs which are species specific. To investigate both the reactivity of MAb 263K 3F4 with the non-protease-resistant host glycoprotein of 33- to 35-kDa protein and to further analyze its species specificity, we prepared homogenates of normal brain tissue from several animal species. These homogenates were prepared in the presence of protease inhibitors to protect the 33- to 35-kDa host protein from degradation. WB analysis showed that MAb 263K 3F4 reacted with hamster 33- to 35-kDa protein and with an analogous protein in human brain tissue homogenates (Fig. 3). Homogenates from two human autopsy cases, a 56-year-old male and a 23-year-old male, both exhibited reactivity with MAb 263K 3F4. Similar preparations derived from mouse, rat, rabbit, bovine, and sheep brain tissue failed to show this reactivity. The band at approximately 25 kDa in the mouse lane is the result of

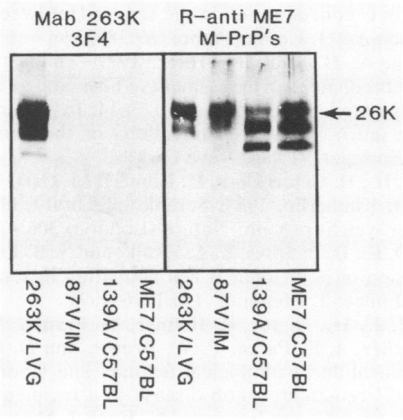

FIG. 2. Comparison of immunoreactivity of rabbit anti-ME7 PrP antisera with MAb 263K 3F4. SAF were purified from mice infected with ME7, 139A, and 87V and from hamsters infected with 263K. WB analysis was done as described in the legend to Fig. 1 with rabbit anti-ME7 PrPs (1:5,000) and MAb 263K 3F4 (1:50,000). K, Kilodaltons.

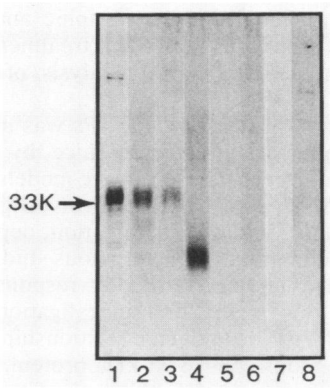

FIG. 3. Reactivity of MAb 263K 3F4 with homogenates of brain prepared from a variety of species. Homogenates were prepared from uninfected brain of hamster (lane 1), human (two cases; lanes 2 and 3, respectively), mouse (lane 4), sheep (lane 5), rat (lane 6), bovine (lane 7), and rabbit (lane 8). Reactivity was determined by WB analysis as described in the legend to Fig. 2. Band staining in the lane containing mouse brain homogenate (lane 4) is immunoglobulin reactive with the secondary antibody.

reactivity of mouse immunoglobulin with the secondary antibody. Analysis of the proteins present in these homogenates by both silver staining and lectin binding (data not shown) indicated the presence of 50 to 70 visible bands, many of which were glycosylated. Thus, despite the presence of a large amount of protein (approximately 50 μg per lane), reactivity was specific for the 33- to 35-kDa protein band and for this protein in only hamsters and humans.

DISCUSSION

This study demonstrated that the antibody response to scrapie-specific PrPs in mice is primarily influenced by the source of antigen. The ability to respond differed from that previously seen in rabbits in the intensity or epitope specificity of this immune response. Difficulties have not been encountered in producing rabbit antisera to mouse PrPs (14, 35). Mice did not respond to immunization with intact 139A SAF. This is in contrast to the response in rabbits to intact hamster-derived 263K fibrils (26) and in goats to mouse-derived 22L SAF (R. Sommerville, personnel communication). After an immunization regimen even more vigorous than that used in rabbits, only a weak response to mouse ME7 PrPs could be obtained in BALB/cJ mice. Mice immunized with hamster 263K PrPs produced a strong response to an epitope present on hamster but not mouse PrPs. This is in contrast to antiserum produced in rabbits to 263K PrPs which cross-reacts with PrPs derived from many species. This difference in response may be related to the mouse reacting most strongly to epitopes seen as nonself (32). This is the first time epitope differences have been demonstrated on PrPs isolated from different species.

Immunization of mice with formic acid-extracted 263K SAF protein resulted in a strong response to PrP antigen. In ELISAs, formic acid-treated PrPs were 3- to 4-fold more reactive than SDS-treated antigen and 10-fold more reactive than untreated SAF. Such treatment would appear to make the protein more reactive with antibody, perhaps by more efficient unfolding and exposure of antigenic sites. This increased reactivity and perhaps antigenicity is obtained without alteration in the one-dimensional gel banding pattern of these proteins. Formic acid solubilization has been used

for high-pressure liquid chromatographic analysis of SAF (13) and related structures (20). Such treatment may also be valuable for the immunological analysis of SAF protein components.

A natural antibody response to PrPs was not detected in the plasma from scrapie-infected mice by the sensitive ELISA. Three different mouse scrapie models, with scrapie strains 139A, ME7, and 87V, were investigated at times ranging from 50 to 350 days postinfection, depending on the agent-strain combination. While previous studies have failed to demonstrate a humoral antibody response in scrapie-infected mice, this is the first investigation of response specifically to PrPs. The antigenic relationship between PrPs and the normal mouse 33- to 35-kDa protein, as mentioned earlier, would seem to explain this lack of response.

An MAb was derived from a mouse immunized with 263K PrPs. 263K 3F4 MAb specifically reacts with PrPs from hamsters infected with 263K and the 33- to 35-kDa protein present in both 263K-infected and uninfected hamster brain. The sharing of this epitope between these proteins and no other proteins in hamster brain adds strong support to the concept previously proposed that the PrPs are derived by proteolytic breakdown from the precursor host 33- to 35-kDa protein (19, 29, 34). Similar results have been obtained with another MAb, 13A5, raised to scrapie-hamster PrPs (1). Immunodecoration of 263K SAF with MAb 263K 3F4 antibody (P. Merz, manuscript in preparation) also further supports PrP as the major component of SAF (22).

MAb 263K 3F4 recognizes a species-specific epitope present on hamster and human 33- to 35-kDa brain protein which is absent from analogous proteins from rabbits, bovines, mice, rats, and sheep. This result was unexpected in light of the high degree of species cross-reactivity of previous antisera raised to PrPs (14, 19) and the strong homology (89%) in the coding region of the genes encoding the 33- to 35-kDa protein in mice, humans, and hamsters (2, 17, 18, 33). Recently, antibody derived to mouse PrPs in hamsters displayed an analogous species specificity. This antibody reacted with the 33- to 35-kDa protein in mice and rats but not in hamsters or humans (R. Kascsak, manuscript in preparation). These results suggest that minor differences in this protein at the level of either amino acid sequence or posttranslational processing are highly antigenic when placed into a heterologous species. Comparison of the protein sequence of 27- to 30-kDa PrPs from mice, humans, and hamsters revealed a potential epitope in which sites (positions 108 and 111) are identical in the human and hamster protein but different in the mouse protein. Both glycosylation sites (positions 179 to 181 and 195 to 197) on this protein are conserved in these three species and represent another possible site for species-specific epitopes (27) involving host-directed posttranslational modification. Such epitopes may be of biological significance if PrPs represent the protein components of the scrapie infectious agent. These sites could influence both the host processing and recognition of the agent.

The strong reactivity and high specificity of MAb 263K 3F4 for human 33- to 35-kDa protein indicates the potential role of 263K 3F4 in detecting human PrPs. At present, the only definitive diagnosis for human unconventional slow virus diseases is the assay for infectivity, which is very time consuming and costly. Previously, studies have demonstrated the presence of SAF and PrPs in human as well as all other unconventional slow virus diseases and have documented the value of these markers as diagnostic tools (6, 11). Assay for PrPs with 263K 3F4 MAb may also prove to be very useful in the diagnosis of human unconventional slow virus diseases.

The availability of large quantities of highly specific antibody to PrPs should aid in answering several questions related to these proteins. The antibody can be used to purify the related 33- to 35-kDa host protein and to study its function and the conditions necessary for fibril formation. Anti-idiotype antibody (16) could be generated to this antibody and could be used as an immunogen or blocking agent. This MAb and other similar antibodies will help to establish whether PrPs represent merely a specific pathological marker or a vital component of the infectious agent in unconventional slow virus diseases.

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