Protein Conformation Significantly Influences Immune Responses to Prion Protein

Azadeh Khalili-Shirazi, Sonia Quaratino, Marco Londei, Linda Summers, Mourad Tayebi, Anthony R. Clarke, Simon H. Hawke, Graham S. Jackson and John Collinge

http://www.jimmunol.org/content/174/6/3256

References
This article cites 49 articles, 20 of which you can access for free at:
http://www.jimmunol.org/content/174/6/3256.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Protein Conformation Significantly Influences Immune Responses to Prion Protein

Azadeh Khalili-Shirazi,* Sonia Quaratino,‡ Marco Londei,† Linda Summers,* Mourad Tayebi,* Anthony R. Clarke,* Simon H. Hawke,* Graham S. Jackson,* and John Collinge2*

In prion diseases, such as variant Creutzfeldt-Jakob disease normal cellular prion protein (PrP\textsubscript{C}), a largely α-helical structure is converted to an abnormal conformational isoform (PrP\textsubscript{Sc}) that shows an increase in β-sheet content. Similarly, the recombinant form of PrP\textsubscript{C} (rα-PrP) can be converted to a conformation dominated by β-sheet (rβ-PrP) by reduction and mild acidification in vitro, a process that may mimic in vivo conversion following PrP\textsubscript{C} internalization during recycling. Despite PrP\textsubscript{Sc} accumulation and prion propagation in the lymphoreticular system before detectable neuroinvasion, no Ab response to PrP has been detected, probably due to immune tolerance. To investigate how the immune system may respond to α- and β-PrP, we immunized Prnp\textsubscript{0/0} mice that are not tolerant of PrP with rα-PrP and rβ-PrP. In this study, we show that although T cells stimulated by these differently folded conformers PrP recognize similar immunodominant epitopes (residues 111–130 and 191–210) the cytokine profile in response to rα- and rβ-PrP was different. Challenge with rα-PrP elicited a strong response of IL-5 and IL-10, whereas rβ-PrP led to an early increased production of IFN-γ. In addition, immunization with rα-PrP led to production of predominantly IgG1 isotype Ab in the sera, whereas after immunization with rβ-PrP, IgG2b was significantly produced. Thus, both humoral and cellular responses to these differently folded isoforms of the same protein are different, indicating a possible involvement of Th1 and Th2 pathway activation. These differences may be exploitable diagnostically and therapeutically for prion diseases, such as variant Creutzfeldt-Jakob disease. The Journal of Immunology, 2005, 174: 3256–3263.

Received for publication April 5, 2004. Accepted for publication January 4, 2005.

The prion diseases or transmissible spongiform encephalopathies are a group of fatal, transmissible, neurodegenerative conditions, which include Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and kuru in humans, and scrapie and bovine spongiform encephalopathy in animals. The transmissibility of human prion disease has become an intense public health issue because of evidence that bovine spongiform encephalopathy-like prions have infected humans (1–3). A common feature of all prion diseases is the posttranslational conversion of the normal cellular prion protein (PrP\textsubscript{C}), a host-encoded GPI-anchored sialoglycoprotein (4), α-helical in structure and expressed on most cells including B and T cells (5), into an abnormal isoform termed PrP\textsubscript{Sc} (6), structurally dominated by β-sheet.

Neither amino acid sequencing nor systematic studies of known covalent posttranslational modifications have shown any consistent differences between PrP\textsubscript{C} and PrP\textsubscript{Sc} (7). The abnormal isoform, PrP\textsubscript{Sc}, is isolated from tissue as a highly agglomerated and detergent insoluble polymer, which has markedly increased resistance to proteolysis. PrP\textsubscript{C} can be completely digested by the serine protease, proteinase K (PK), while in marked contrast, PK removes between 90 and 100 amino acids from the N terminus of abnormal isoform PrP\textsubscript{Sc}, depending upon prion strain type, leaving a large protease-resistant C-terminal fragment (8). At low pH, following reduction of the native disulphide bond, the folded C-terminal domain of human prion protein is shown to exist as a soluble material with β-sheet structure (9). It is possible that the in vitro conversion of α-PrP to β-PrP, caused by reduction and mild acidification (10), might occur in vivo, following PrP\textsubscript{Sc} internalization within the cell during recycling (11), and this could underlie prion propagation (9).

Accumulation of PrP\textsubscript{Sc} has been found in the lymphoreticular system in human variant Creutzfeldt-Jakob disease (12, 13). In scrapie-affected mice, prions are associated with splenic B and T cells, but not (14) or very little (15, 16) with circulating PBMCs. In mice, infectious prions are detectable in the spleen and lymph nodes long before they can be detected in the brain (17) (A. Khalili-Shirazi and S. H. Hawke, unpublished observation), and PrP\textsubscript{Sc} is accumulated in splenic follicular dendritic cells (FDC) (18–20). An intact immune system is essential for prion propagation. FDCs are required for neuroinvasion and the development of clinical scrapie, and in turn, B cells are required for maturation of FDCs and formation of germinal centers (21). In addition, PrP\textsubscript{Sc} has also been reported to be associated with macrophages and dendritic cells (22–26).

Despite the involvement of the immune system in PrP\textsubscript{Sc} replication and neuroinvasion, no Ab response to PrP has been detected in prion disease. The lack of detectable immunity in prion infection is thought to be largely due to immune tolerance because of the widespread expression of PrP\textsubscript{C} in the hemopoietic system (27–29).
Furthermore, attempts to produce PrPSc-specific Abs by immunization of Prnp0/0 mice (that are not tolerant to PrP) with PrPSc have been uniformly unsuccessful, although it has recently been reported that Abs raised to the repeat motif of PrP, tyrosine-tyrosine-arginine, can specifically recognize the pathological isoform of the PrP (30). We had previously immunized Prnp0/0 mice with highly purified human recombinant (r)αr- and rβr-PrP (31) to obtain a panel of mAbs that strongly immunoprecipitate native PrPSc and PrPSc. In this study, we have characterized the T cell-mediated immune responses to αr- and βr-PrP in Prnp0/0 mice and show that the different conformations of the same protein are able to induce a qualitatively different type of response. Such information may provide insights into the biology of prion diseases and the potential use of PrP conformers in therapeutic vaccination strategies.

Materials and Methods

Animals

We used Prnp0/0 mice (27) backcrossed onto an FVB/N mouse background to study the difference between the immune responses of the two different conformers of αr-PrP and βr-PrP. These mice were housed under specific pathogen-free conditions. Animal care conformed to national and institutional guidelines.

Ag-αr-PrP, -rβ-PrP, and -PrP peptides

Human and mouse αr-PrP(91–231) and βr-PrP(91–231) used as Ag were synthesized, as described above (31, 39), αr-PrP(91–231) being a soluble α-helical monomer and PK-sensitive, whereas βr-PrP(91–231) is a soluble monomer, rich in β-sheet, and relatively PK-resistant. We used 91–231 prP, rather than full-length protein because it more readily forms a stable β-sheet. Also, native PrPSc is completely digested by PK, while depending upon (10%) to analyze the T cell response in vitro.

Technologies, BD Biosciences), supplemented with penicillin (100 IU/ml), streptomycin (100 μg/ml), and PBS (pH 7.4)). Blocking solutions (normal goat serum, normal rabbit serum, coating-buffers (carbonate bicarbonate (pH 9.5), Tris (pH 8.5), and PBS (pH 7.4)), and PBS (pH 7.4)), blocking solutions (normal goat serum, normal rabbit serum, coating-buffers (carbonate bicarbonate (pH 9.5), Tris (pH 8.5), and PBS (pH 7.4)), blocking solutions (normal goat serum, normal rabbit serum, coating-buffers (carbonate bicarbonate (pH 9.5), Tris (pH 8.5), and PBS (pH 7.4)) were used as negative control Ag. Cells were maintained in a standard 72-h proliferation assay and pulsed with [3H]thymidine (0.5 μCi/well) (Amersham Biosciences). In the last 18 h prior to harvesting (TomTec cell harvester) and determining cpm, using a beta counter (PerkinElmer).

For proliferation and cytokine assays

For proliferation assays, cells derived from the lymph nodes of mice immunized with either αr- or βr-PrP Ag or control mice were used. The study with αr- and βr-PrP as Ag were repeated four times and with PBS twice. The T cells were derived from a pool of lymph nodes from three mice and tested in triplicate, presenting the results as stimulation index (SI) ± SE. The cells proliferated to ~9000 cpm and a SI of 2.5–4 was considered significant. For proliferation assays, cells were plated in round-bottom 96-well plates (Falcon), at 4 × 103 cells per 0.2-ml well, and for cytokine assays in 24-well plates, at 1–2 × 105 cells per 2-ml well. Human αr- and βr-PrP were sterile filtered through a 0.2-μm filter (Nalgene), titrated in culture for T cell proliferation, and 50 μg/ml was found to be the optimal concentration. Human PrP peptides were also sterile filtered and used at 10 μg/ml. Where appropriate, OVA at 50 μg/ml was used as negative control Ag. Cells were maintained in a standard 72-h proliferation assay and pulsed with [3H]thymidine (0.5 μCi/well) (Amersham Biosciences). In the last 18 h prior to harvesting (TomTec cell harvester) and determining cpm, using a beta counter (PerkinElmer).

The production of cytokines from cells in lymph nodes were measured in vitro after 24, 48, and 72 h. IL-2, IL-4, IL-5, IL-10, TGF-β, and IFN-γ were analyzed by sandwich ELISA, using standard protocols, including a standard curve for each plate (BD Biosciences, BD Pharmingen).

Characterization of anti-PrP mAbs

As previously described, mAbs ICSM4, -10, and -18 were obtained upon immunization with αr-PrP(91–231) while ICSM35 and -37 with βr-PrP(91–231) were a normal or scrapie-affected brain homogenate was added as Ag, and PrPSc was detected by direct ELISA, where the peptides were used to inhibit binding to recombinant αr- and βr-PrP. These mice were housed under specific pathogen-free conditions. Animal care conformed to national and institutional guidelines.

ELISA

Direct ELISA method was used to detect binding of ICSM18 and -35 to αr-PrP(91–231) at 20 μg/ml, using anti-mouse IgG (Fab-specific) HRP (Sigma-Aldrich) as secondary Ab and o-phenylenediamine (Sigma-Aldrich) as chromogen. Direct ELISA for detection of native PrP proved difficult, despite using different brain homogenate concentrations, plate manufactures, coating-buffers (carbonate bicarbonate (pH 9.5), Tris (pH 8.5), and PBS (pH 7.4)), blocking solutions (normal goat serum, normal mouse serum, FCS, BSA, and soya milk), and temperatures (4°C, 23°C, and 37°C), so an indirect sandwich ELISA technique was devise. ICSM18 and ICSM35 were individually used to coat the ELISA plate and after washing, normal or scrapie-affected brain homogenate was added as Ag, and PrPSc was detected with biotinylated (B) mAb ICSM18B or ICSM35B, using a different Abs for capture and detection. Finally streptavidin-HRP was added and o-phenylenediamine was used as chromogen. The brain homogenates were tested at 0.5–0.1%, the mAbs used for PrP capture coating the ELISA plate was tested at IgG concentrations of 2–10 μg/ml and the biotinylated mAb used for PrP detection was tested at IgG concentrations of 1 and 0.5 μg/ml. Both ICSM18 and 35 worked well for capturing and for detecting native PrPSc.

The isotypes of these mAbs were determined using a panel of anti-mouse Ig subclass-specific mAbs (Pierce) and their immunodominant epitopes were defined by direct ELISA, using 20-mer synthetic peptides (10–20 μg/ml), spanning the PrP sequence 91–231 overlapping by 10 residues. These epitopes were further refined by direct peptide ELISA, using 15-mer peptides overlapping by 13 residues. The accuracy of the immunodominant epitope found was confirmed by competition by peptide ELISA, where the peptides were used to inhibit binding to recombinant αr- and βr-PrP. For these studies, ELISA plates were coated with αr- and βr-PrP and the mAbs were coated on the plate either by preincubating them with the peptides to inhibit binding to αr- and βr-PrP, or the peptides were added simultaneously as the mAb to ELISA plates, to compete for the binding of the mAbs to αr- and βr-PrP. Similar results were obtained, and the latter

method was mainly conducted. ELISA plates were coated with α- or β-PrP (20–10 μg/ml) in carbonate/bicarbonate buffer (pH 9.5), to which each mAb IgG (1 μg/ml) and peptide (10–50 μg/ml) were added. The ELISA was then conducted by conventional methods as described above.

Statistical analysis

Data were analyzed for statistical significance using ANOVA and two-tailed t test.

Results

Both α- and β-PrP are recognized by T cells

Immunization with either α- or β-PrP evoked clear proliferative T cell responses to both α- or β-PrP Ags (Fig. 1), unlike T cells from immunizations with PBS-CFA (Fig. 1) or OVA-CFA (data not shown). There was no significant difference between the in vitro recognition of α- and β-PrP by T cells derived from either α- or β-PrP immunization (p = 0.316, p = 0.154, two-tailed t test). The viability and Ag-recognition pathway of all the T cells was tested by their response to stimulation with anti-CD3 Ab, where a mean SI of 7–12 was observed (data not shown).

T cell epitope recognition of α- and β-PrP is similar

T cells from α- and β-PrP immunizations showed no significant difference in their recognition of PrP peptides (Fig. 2, A and B). T cells from lymph nodes of both α-PrP (Fig. 2A) and β-PrP (Fig. 2B) immunized mice recognized N-terminal residues within the sequence 111–130 and C-terminal residues within 191–210 (p = 0.005, ANOVA). In keeping with the previous result, T cells from PBS (or OVA)-immunized mice did not proliferate in response to PrP peptides (Fig. 2C and data not shown). PrP residues 111–130 are thought to be within the structured part of PrP while residues 191–220 are just C-terminal to the glycosylation sites of PrP.

The intracellular proteolytic degradation of Ag by APC might generate different peptide ligands for interaction with MHC class II molecules. Because for both α- and β-PrP immunizations we used Prnp<sup>-/-</sup> mice of FVB/N background with identical MHC class II molecules, it is not surprising that we found a similar linear sequence of peptides being recognized by T cells from both groups.

The cytokines produced by α- and β-PrP-derived T cells are different

The overall proliferation of T cells from Prnp<sup>-/-</sup> mice, and in particular proliferation to PrP peptides was weak. Therefore, we checked for differences in cytokine profiles of lymph node cells from α- and β-PrP-immunized mice. Measuring cytokines, in cultures of lymph node cells challenged in vitro with rPrP, was most suitable after 24 h and 48 h. Although there was some overlap of the cytokines produced, T cells from lymph nodes of β-PrP-immunized mice were skewed more toward Th1 (producing more IFN-γ and sooner, and less IL-5 and IL-10), whereas those from α-PrP-immunized mice were skewed more toward Th2 (producing more IL-5 and IL-10, and less or later IFN-γ) (Fig. 3).

The cytokine levels were clearly affected by the rPrP isoform used for in vitro challenge, as well as for immunization. After 24-h stimulation with α-PrP in vitro, T cells from α-PrP-immunized
mice produced only 340 pg/ml IFN-γ, whereas T cells from rβ-PrP immunization produced >2000 pg/ml IFN-γ (Fig. 3A). After 48-h in vitro stimulation of cells, T cells from both α- and β-PrP immunizations produced a large amount of IFN-γ (>2000 pg/ml). A small amount of IFN-γ was also produced after 48 h, by un-stimulated T cells from rβ-PrP immunization, and from PBS immunization stimulated in vitro with rβ-PrP (Fig. 3A).

Th2-induced cytokines like IL-5 and IL-10 were also investigated. IL-5 production was substantially higher after in vitro stimulation with rα-PrP compared with rβ-PrP, by T cells from both rα-PrP (p = 0.001, t test) and rβ-PrP (p = 0.0108, t test) immunizations. T cells from rα-PrP-immunized mice produced 227 pg/ml IL-5 in response to rα-PrP but only 90 pg/ml in response to rβ-PrP, and T cells from rβ-PrP-immunized mice produced 122 pg/ml in response to rα-PrP and only 16 pg/ml in response to rβ-PrP (Fig. 3B). The T cells from rα-PrP-immunized mice also produced significantly more IL-5 in response to in vitro stimulation with rα-PrP, than those from rβ-PrP immunization (p = 0.0147, t test). No IL-5 was produced by T cells from PBS-immunized mice, regardless of using rα- or rβ-PrP for the in vitro challenges (Fig. 3B). The IL-10 responses showed that it was produced, slightly more by T cells from rα-PrP than rβ-PrP-immunized mice (Fig. 3C).

Not surprisingly, in view of the results shown in Figs. 1 and 2, there was no statistical difference in the production of IL-2 in response to either rα- or rβ-PrP from both control groups (Fig. 3D), and small amounts of IL-2 was also produced by T cells from PBS-immunized mice, after in vitro challenge with rα- or rβ-PrP (Fig. 3D). Production of IL-4 and TGF-β are not shown, as they could not be convincingly measured.

**PrP conformation influences the Ab Ig isotypes produced in the sera**

Total IgG Ab levels were similar in mice immunized with rα- and rβ-PrP and their sera Abs were positive to rα- and rβ-PrP Ags by ELISA, while no Ab to rα- and rβ-PrP was present in the serum of the unimmunized mouse (Fig. 4A). However, a significant difference was observed in the Ig isotypes produced in the sera of mice immunized with rα-PrP, as compared with those immunized with rβ-PrP. Immunization with rα-PrP evoked predominantly Th2 isotype Abs of IgG1 isotype, whereas rβ-PrP evoked mainly Th1 IgG2b in the serum (Fig. 4B).

Significantly higher levels of IgG1 Abs were produced in the sera of rα-PrP-immunized mice, compared with those produced in sera of mice immunized with rβ-PrP (p < 0.005, two-tailed t test). In contrast, IgG2b Ab was produced at higher levels in the sera of rβ-PrP-immunized mice, when compared with rα-PrP-immunized mice (p < 0.005, t test). There was no significant difference between the levels of the other Ig isotypes in the sera of these two groups of mice. Because the mice were bred under specific pathogen-free conditions, it is not surprising that no Ab IgG isotypes and only very low levels of IgM were detected in the sera of the unimmunized mice (Fig. 4B). In addition, different anti-PrP Ab IgG isotypes were found in the sera of mice immunized with rα-PrP and rβ-PrP and tested by ELISA against both rα-PrP (data not shown) or rβ-PrP (Fig. 4C). IgG1 anti-PrP Ab was produced in sera of mice immunized with rα-PrP and IgG2b Ab produced after rβ-PrP immunization (Fig. 4C). These findings confirm that despite the weak T cell proliferation responses, the α- and β-isoforms of PrP are recognized differently by the immune system.

**PrP conformation affects the IgG isotype and the specificity of the mAbs produced**

A significant difference was also observed in the specificity and IgG isotype of the mAbs raised against α- and β-isoforms of PrP. The results obtained for the mAbs produced to rα- and rβ-PrP further demonstrate that PrP in the different conformations (α or β) give rise to a different type of cellular response, skewed more toward Th1 for rβ-PrP and Th2 for rα-PrP. This reconfirms the
data already obtained for T cells and the sera Ab Ig isotypes. ICSM4, -10, -18, -35, and -37 mAbs, raised to rH9251- and rH9252-PrP, had different specificities and IgG isotypes. Those produced against rH9251-PrP (ICSM4, -10, and -18) were of IgG1 isotype, whereas rH9252-PrP-derived mAbs (ICSM35 and -37) were IgG2b and IgG2a. Furthermore, IgG isotypes were also different in the 40 mAbs we have produced to rH9251-PrP and 41 mAbs to rH9252-PrP. The isotypes of the mAbs produced from rH9251-PrP immunizations were 92.5% IgG1 and 7.5% IgG2 (2.5% IgG2a and 5% IgG2b), whereas the isotypes of those produced to rH9252-PrP immunizations were 53.6% IgG1, 41.5% IgG2 (12.2% IgG2a, 29.3% IgG2b), and 4.9% IgM (manuscript in preparation).

Both ICSM18 and ICSM35 (raised against rα- and rβ-PrP respectively), were equally suitable for capturing or detecting native PrP from normal and RML-infected scrapie-affected mouse brain homogenates by sandwich ELISA (Fig. 5A). As previously described ICSM4, -10, and -18 (raised to rα-PrP) and ICSM35 and

#### FIGURE 4.

**A**, Serum Abs are produced in mice immunized with α-PrP and β-PrP. Sera from Prnp0/0 mice immunized with rα-PrP ( ), rβ-PrP ( ), or not immunized ( ) were tested at 1/100 dilution against rα-PrP or rβ-PrP (20 μg/ml) by ELISA. Anti-mouse Ig (Fab-specific)-HRP (Sigma-Aldrich) was used as secondary Ab. 

**B**, Different serum Ab Ig isotypes are produced after immunization with α-PrP and β-PrP. The ELISA results of the total Ig isotypes, as the mean OD ± SEM, are shown for sera of mice immunized with rα-PrP ( ), rβ-PrP ( ), and not immunized ( ). The sera from three mice immunized with rα-PrP, three mice immunized with β-PrP, and three unimmunized mice were tested in duplicate at 1/100 dilution. 

**C**, Different serum anti-PrP Ab Ig isotypes are produced after immunization with α-PrP and β-PrP. Sera from three mice immunized with rα-PrP ( ) and three mice immunized with rβ-PrP ( ) were tested in duplicates at 1/100 dilution in an rβ-PrP ELISA, using different anti-mouse IgG isotype-specific HRP and IgM HRP (DakoCytomation) secondary Abs. The rβ-PrP ELISA data are shown as mean OD ± SEM.

**FIGURE 5.** Detection of PrP by ELISA (**A**) and Western blotting (**B**), using α-PrP- and β-PrP-derived mAbs. A, ICSM18 and -35 were tested for their ability to capture (at IgG 10 μg/ml) and detect (at IgG 0.5 μg/ml) PrP in an indirect sandwich ELISA, using normal wild-type (Wt) mouse brain homogenate, RML-infected scrapie-affected (Sc) brain homogenate and PBS as Ag. For the ELISA, either ICSM18 was used for PrP capture and ICSM35B for detection ( ) or ICSM35 was used for PrP capture and ICSM18B for detection ( ). B, ICSM18 and ICSM35 recognize all three PrP bands by Western blot, representing unglycosylated (29 kDa), monoglycosylated (33 kDa), and diglycosylated PrP (36 kDa), in murine PrPSc. ICSM4 labeled the full-length unglycosylated PrP band (~29 kDa), but not the mono- and diglycosylated bands in normal (N) brain homogenate. After PK treatment (+PK), all the truncated and the PrP bands were digested from scrapie-affected (Scrapie) brain homogenate and only PrPSc bands were recognized at reduced molecular weights of diglycosylated (~33 kDa), monoglycosylated (~33 kDa), and unglycosylated (~28 kDa) PrP. No binding to PrP was observed, when isotype control-negative mAbs were used and the above mAbs were negative, when tested against Prnp0/0 mouse brain (data not shown).
37 (raised to rβ-PrP) were all positive to denatured PrP\(^c\) and PK-treated PrP\(^sc\) in a Western blot, but they recognized different PrP glycoforms.\(^4\) The Western blots of ICSM18 and ICSM35 showed that they both recognize all the PrP glycoforms (Fig. 5B). In contrast, ICSM4 only recognized unglycosylated PrP\(^c\) and PrP\(^sc\); while ICSM18 recognized di-, mono-, and unglycosylated PrP\(^c\) and PrP\(^sc\), by Western blot using aliquots of the same normal and scrapie-affected mouse brain homogenates (Fig. 5B). ICSM4 and -10 also differentially immunoprecipitated PrP glycoforms from PrP\(^c\), while anti-β-PrP mAbs ICSM35 and -37 did not.\(^4\) ICSM18 and ICSM35 were chosen, as strongly binding representatives of PrP-specific Th2 response for rβ-PrP and Th1 response for β-PrP.

We have labeled PrP on RML-infected N2a cells with ICSM18 and ICSM35 by immunofluorescence (Fig. 6). Although both ICSM18 and ICSM35 specifically labeled PrP on these cells, staining of PrP with ICSM18 proved to be stronger and more uniform (Fig. 6A) than ICSM35 (Fig. 6B). ICSM18 has a high affinity for native PrP\(^c\) and a weak affinity for native PrP\(^sc\), while ICSM35 has a higher affinity for native PrP\(^sc\) than for native PrP\(^c\) by immunoprecipitation.\(^4\) This may explain the strong immunofluorescence detected by ICSM18 and the weaker immunostaining detected by ICSM35. The weak staining of ICSM35 may also indicate that native PrP\(^sc\) is mainly intracellular. No nonspecific binding of PrP was observed using negative control mAb (Fig. 6C), or no primary Ab, second layer alone (Fig. 6D).

Detailed epitope mapping, showed striking differences for the ra- and rβ-PrP-generated mAbs. Approximately similar epitopes for rβ-PrP-derived mAbs ICSM35 and -37 were found that were different from the ra-PrP-derived mAb ICSM18 (Fig. 7). ICSM18 was specific for the linear sequence 143–153 (Fig. 7A); ICSM35 and ICSM37 for sequences 93–105 and 97–105, respectively (Fig. 7B). ICSM4 and -10 were negative in the peptide ELISA and could not be epitope mapped. The immunodominant epitopes of ICSM18 (Fig. 7C) and ICSM35 (Fig. 7D) were reconfirmed by peptide competition ELISA, using the peptides as competitors for binding of the mAbs to ra- and rβ-PrP Ags.

These data show that ra- and rβ-PrP generate diverse immune responses, cellular and humoral, and mAbs could be raised with high affinity for native PrP\(^c\) (like ICSM18) or for native PrP\(^sc\) (like ICSM35).

**Discussion**

In this study, we have demonstrated that immunization of mice with either α- or β-PrP evokes a different cytokine milieu and Ab isotype profile. Usually CD4\(^+\) T cells recognize linear processed peptides presented in the groove of MHC class II molecules, whereas B cells recognize both conformational and/or linear epitopes of an Ag. When we immunized PrP-deficient mice (of FVB/N MHC H-2\(^b\) haplotype) with either ra- or rβ-PrP, comparable T cell proliferation against similar immunodominant epitopes was observed. Although α- and β-PrP do not differ in amino acid sequence, they elicited qualitatively different cellular and humoral response. Although T cells from ra- and rβ-PrP immunizations recognized both ra- and rβ-PrP and peptide residues 111–130 and 191–210; by contrast the mAbs reflecting the activity of B cells could partially discriminate between PrP\(^c\) and PrP\(^sc\), and their immunodominant peptides were different: 93–105 for rβ-PrP immunizations (ICSM35 and -37) and 143–153 for ra-PrP (ICSM18).

We have observed that the T cell proliferation, particularly against PrP peptides, was weak. One possible explanation for this could be the absence of PrP in the cells of Prnp\(^0/0\) mice. Lack of PrP\(^c\) on T lymphocytes could lead to generally low activation levels, as we found that even the response to soluble anti-CD3 Ab was very modest. Indeed, PrP\(^c\) may have an important role in T cell activation. PrP\(^c\) is significantly up-regulated on mouse T cells upon challenge with concanavalin A (Con-A), but in Prnp\(^0/0\) mice, T cell response to Con-A is significantly reduced to 50–80%, suggesting that PrP\(^c\) is a lymphocyte surface molecule that participates in T cell activation (35). In human T lymphocytes, PrP\(^c\) expression is also increased upon challenge with Con-A and reduced by the addition of anti-PrP polyclonal Ab (36). PrP\(^c\) expression on normal human T lymphocytes is also increased after challenge with either Con-A or anti-CD3 Ab and inhibited by addition of anti-PrP mAbs, and in addition, CD45RO\(^+\) memory T cells consistently expressed more PrP\(^c\) than CD45RA\(^+\) naıve T lymphocytes (37). A recent report suggests PrP\(^c\) to be a component of the multimolecular signaling complex, within microdomains involved in T cell activation (38). The absence of PrP\(^c\) on T lymphocytes of Prnp\(^0/0\) mice may therefore be responsible for the weak proliferative response we have observed in this study, but

![FIGURE 6](https://example.com/fig6.png)

**FIGURE 6.** Immunofluorescence labeling of native PrP on RML-infected N2a cells by anti-α- and β-PrP mAbs. ICSM18 (A) and ICSM35 (B) successfully labeled PrP on RML-infected N2a cells. The cells were grown on multichamber glass slides, and when semi-confluent, they were directly incubated with the mAbs. An irrelevant mAb (C) and no primary Ab, second layer alone (D) were used as negative control.
apparently it does not affect their cytokine production. The proliferation response was particularly weak after in vitro challenge with the peptides, but in a preliminary study we have found that splenocyte T cells from rPrP-immunized mice produce IL-5 in response to in vitro challenge with peptide sequence 191–210, and T cells from both α- and β-PrP immunizations produce IFN-γ in response to this peptide (S. H. Hawke and Z. Sattar, unpublished data). Sera Abs were also positive to this epitope after immunization (39). T cell activation leads to a CD4+ T cell response, characterized by production of a series of different cytokines and IgG isotypes (40–42). Th1 type response leads to activation of macrophages and is characterized by production of IFN-γ, whereas Th2 leads to activation of different APCs, and production of IL-4, IL-5, and IL-10 (43, 44). This dichotomy might be interpreted as the result of different T cell activation. Indeed, it has been reported that Th2 cells provide B cell help and stimulate the production of noncomplement fixing IgG1 Abs, while Th1 cells induce B cells to switch to IgG2 and IgG3 (45).

In this study, we have shown that despite some overlap, the immune response to α-PrP was skewed toward Th2, inducing T cells to produce increased amounts of IL-5 and IL-10, and B cells to produce polyclonal IgG1 Abs in the sera, and IgG1 mAbs such as ICSM4, -10, and -18. In contrast, the immune response to β-PrP was skewed more toward Th1, with a high and early production of IFN-γ, less IL-5 and IL-10 cytokines, and production of serum IgG2b isotype Abs and IgG2b and IgG2a mAbs, like ICSM35 and -37. These data indicate that different APCs may be involved in processing of α- and β-PrP. When α-PrP is used as the immunogen, its greater sensitivity to proteolytic cleavage might mean that it is more easily processed for presentation to B cells and may involve different APCs from those that process β-PrP. In comparison with α-PrP, β-PrP is rich in β-sheet and partially PK-resistant (9). It is possible that α-PrP is mainly processed by B cells and dendritic cells and much less by macrophages, whereas β-PrP is mainly processed by macrophages and less by B cells or dendritic cells. Immune tolerance to PrP in Prnp+/−+ mice can reportedly be overcome by improved immunization techniques, using mouse rPrP (46) and dimeric mouse rPrP (47), leading to production of anti-PrP polyclonal Abs. In the course of prion inoculation, activated T cells have been detected in the brain and spleen of mice affected with scrapie (48). Macrophages appear to have an important role in neutralizing infectivity (23) and an association between dendritic cells and PrPSc has been reported (26, 49). Our findings may provide an insight into how PrPSc and PrPSc might be differentially processed by APCs, such as dendritic cells and macrophages. The data presented in this study on the immune responses to α- and β-PrP might reflect those to PrPSc and PrPSc, if there was no tolerance to PrP or if it could be overcome by vaccination. A transient, but undetected, immune response may also occur early in the course of prion infection, when conversion of PrPSc to PrPSc has just begun and before formation of large PrPSc deposits and onset of clinical signs. These findings may therefore be relevant to the understanding of the molecular mechanisms underlying prion diseases in humans and help in the design of an effective therapeutic immunization or vaccination approach.

Acknowledgments

We thank Daljit Bhelt for technical help in making the rPrP, the Central Biomedical Services staff of Imperial College London at Charing Cross campus, and Ray Young for graphics.

Disclosures

J. Collinge and G. S. Jackson are consultants and shareholders in D-Gen Ltd., and J. Collinge is also a Director.

References


