The human prion protein residue 129 polymorphism lies within a cluster of epitopes for T cell recognition.


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The Human Prion Protein Residue 129 Polymorphism Lies Within a Cluster of Epitopes for T Cell Recognition

Jeremy D. Isaacs, MRCGP, Rebecca J. Ingram, PhD, John Collinge, FRS, Daniel M. Altmann, PhD, and Graham S. Jackson, PhD

Abstract

T cell immune responses to central nervous system-derived and other self-antigens are commonly described in both healthy and autoimmune individuals. However, in the case of the human prion protein (PrP), it has been argued that immunologic tolerance is uncommonly robust. Although development of an effective vaccine for prion disease requires breaking of tolerance to PrP, the extent of immune tolerance to PrP and the identity of immunodominant regions of the protein have not previously been determined in humans. We analyzed PrP T cell epitopes both by using a predictive algorithm and by measuring functional immune responses from healthy donors. Interestingly, clusters of epitopes were focused around the area of the polymorphic residue 129, previously identified as an indicator of susceptibility to prion disease, and in the C-terminal region. Moreover, responses were seen to PrP peptide 121–134 containing methionine at position 129, whereas PrP 121–134 [129V] was not immunogenic. The residue 129 polymorphism was also associated with distinct patterns of cytokine response: PrP 128–141 [129M] inducing IL-4 and IL-6 production, which was not seen in response to PrP 128–141 [129V]. Our data suggest that the immunogenic regions of human PrP lie between residue 107 and the C-terminus and that, like with many other central nervous system antigens, healthy individuals carry responses to PrP within the T cell repertoire and yet do not experience deleterious autoimmune reactions.

Key Words: Creutzfeldt-Jakob disease, Prion protein, T cell responses.

INTRODUCTION

The prion diseases or transmissible spongiform encephalopathies (TSEs) are fatal neurodegenerative diseases that include Creutzfeldt-Jakob disease (CJD) and kuru in humans, scrapie in sheep, and bovine spongiform encephalopathy (BSE) in cattle (1). The “protein only” hypothesis (2) argues that the infectious agent, the prion (3), is composed of a conformationally altered isomer (PrPSc) of a normal host encoded protein, PrPC. Infection, propagation, and ultimately clinical disease result from the autocatalytic conversion of PrPC to PrPSc to nascent PrPSc and accompanying prion infectivity.

A notable feature of TSE infection is the lack of a classic immune response, either in the brain or in the periphery, where PrPSc may accumulate in the early stages of the disease (4). The “blindness” of the immune system to the pathogenic agent is most likely the result of its inability to distinguish PrPSc from PrPC, which is a ubiquitously expressed cell-surfaceialoglycoprotein (5) of uncertain function (6). The remodeling event, by which PrPSc to PrPSc conversion occurs, involves no change in primary structure producing a pathogenic species consisting entirely of self-sequence (7).

The degree of tolerance to PrP in humans, and whether this might influence susceptibility to prion disease, is not known. PrPSc is expressed in the thymus and animals are tolerant to PrP under normal circumstances, suggesting that most T cells recognizing PrP epitopes undergo thymic deletion. There is no clear HLA association in sporadic CJD, although small patient numbers have precluded large-scale association studies, and an initial report that HLA-DQ7 might protect against variant CJD (vCJD) was not confirmed when repeated on a larger sample (8, 9). Whether some individuals are capable of initiating an immune response against foreign or self-generated PrPSc remains unknown.

The widespread contamination of beef and beef products with BSE suggests that a substantial proportion of the U.K. population has been exposed to bovine prions. Although only approximately 160 people have so far been diagnosed with vCJD in the United Kingdom, the number of individuals who are incubating subclinical or carrier states (10) of the disease remains unknown. The possibility of further peaks of vCJD incidence coupled with the risks of secondary transmission mean that there is an urgent requirement for effective antiprion therapeutics. Indeed, concern that vCJD prions may be transmissible through blood transfusion appears to have been realized (11, 12).

Recent developments in Alzheimer disease research have demonstrated that immunotherapy may be effective in neurodegenerative diseases characterized by protein misfolding by raising an immune response against the key...
pathogenic protein species (13, 14). The major obstacle to effective immunization against prion infection is tolerance to PrP. Several strategies have been used to counter this, thus far with limited success in experimental models (15–19). Whether such a vaccine should incorporate a T cell epitope or simply stimulate a B cell response against PrP is unknown. Breaking T cell tolerance to self-proteins as a therapeutic strategy may be a prerequisite to an effective response, but can also be hazardous, as demonstrated in a recent Alzheimer disease vaccination trial (20, 21).

Furthermore, full-length recombinant PrP may not be an appropriate immunogen in humans as a result of concerns that de novo prion infectivity may be generated by such molecules (22). Immunogenic peptide fragments of PrP represent a safer alternative for vaccine development. Therefore, rational design of an antiprion vaccine requires knowledge of the dominant linear T and B cell epitopes in PrP, yet these have not previously been studied in humans. Studies in PrP knockout mice, in which PrP is entirely PrP, yet these have not previously been studied in humans.

Therefore, rational design of an antiprion vaccine requires knowledge of the dominant linear T and B cell epitopes in PrP, yet these have not previously been studied in humans. Studies in PrP knockout mice, in which PrP is entirely foreign, indicate that antigenic processing of, and immune responses to, PrP will occur where tolerance is obviated (23–25). Furthermore, wild-type mice will generate T cell responses against ovine (26) and even murine PrP where it is administered with appropriate adjuvants (27, 28).

In this study, we analyzed the T cell response of healthy volunteers to peptides representing the human Prp sequence to identify potential T cell epitopes. We demonstrate that T cell tolerance to PrP is not complete, and we propose that there are distinct immunogenic regions in PrP that could be exploited for vaccine development.

**MATERIALS AND METHODS**

**In silico HLA Binding Prediction**

The TEPITOPE algorithm (Vaccinome, www.vaccinome.com) predicts epitopes that may be able to bind up to 25 HLA-DR molecules offering a wide cross-section of human HLA class II specificities. The amino acid sequence of human PrP 23–231 (129M and 129V) was entered into TEPITOPE using standard protocols (29, 30). Stringency was set at 3% and sequences of 9-mer epitopes were obtained.

**Peptides**

A library of 14-mer peptides overlapping by 7 amino acids spanning residues 23 to 225 of the human PrP sequence, plus a 13-mer consisting of residues 219 to 231, were synthesized by the Advanced Biotechnology Centre (Imperial College, London, UK). The 2 peptides spanning the M/V polymorphism at position 129 were synthesized in both alternate forms: 129-methionine and 129-valine. Peptides were dissolved in phosphate-buffered saline or DMSO (Sigma-Aldrich, Dorset, UK). Peptides were coded 1 (most N-terminal) to 29 (most C-terminal). Peptide codes, positions, and sequences are shown in Table 1.

**Human Volunteers**

Healthy human volunteers (n = 28) were recruited to take part in the study. All participants gave full informed consent. The study was approved by the Hammersmith Hospital Research Ethics Committee (protocol 2003/6663).

**Isolation and Culture of Peripheral Blood Mononuclear Cells**

Peripheral blood mononuclear cells (PBMCs) were isolated from anticoagulated blood by density gradient centrifugation over Histopaque-1077 (Sigma-Aldrich, Dorset, UK). PBMCs were cultured in RPMI (Invitrogen, Paisley, UK) or IMDM (Cambrex, Wokingham, UK) supplemented with 5% nonautologous human AB serum (Cambrex), 1% L-glutamine (Invitrogen), and 1% penicillin–streptomycin (Invitrogen) or in HL-1 serum-free medium (Cambrex) supplemented with 1% L-glutamine (Invitrogen) and 0.5% penicillin–streptomycin (Invitrogen). PBMCs were suspended in flat-bottomed 96-well plates at a density of 5 × 10^5 cells per well in 200 μL culture medium. PrP peptides were added to triplicate wells at a final concentration of 50 μg/mL. Control wells contained cells and tissue culture medium only. When peptides were dissolved in DMSO, triplicate wells containing the same final concentration...
of vehicle were used as controls. In the first round of assays, sufficient PBMCs were harvested from 14 donors for all 31 peptides to be tested. For the remaining 7 donors, PBMCs were cultured with selected peptides. Each peptide was cultured with PBMCs from at least 14 donors (Table 1).

Ninety-six-well plates were incubated for 6 days at 37°C, 5% CO2 and were then pulsed overnight with 1 µCi ([3H]-thymidine (Amershams Biosciences, Little Chalfont, UK) per well. Plates were harvested and counted with a β-counter (Wallac, Turku, Finland). Stimulation indices (SI) were obtained by dividing the mean counts per minute in the peptide-treated triplicate wells by the mean counts per minute in the relevant control triplicates. A response was considered positive when the SI was >2 and the counts per minute in at least 2 of the 3 peptide-treated wells were >2× the mean of the control wells.

Cytokine Quantification

For the initial cytokine analysis, unused PBMCs were frozen at −80°C until required. After thawing, cells were washed and cultured in supplemented IMDM as previously described with or without PrP peptides at 50 µg/mL. In the extended cytokine analysis, fresh ex vivo PBMCs were used. On day 6, 100 µL of culture medium was removed from each well and replaced with 100 K containing 1 µCi [3H]-thymidine. Aspirated 100 µL culture medium samples from triplicate control and peptide treated wells were pooled and stored at −20°C. After overnight incubation, plates were harvested and peptides eliciting positive responses identified as described previously. Cytokine levels were then quantified in culture medium from control and positive peptide treated wells using the Cytometric Bead Array Human Th1/Th2 cytokine kit II (BD Biosciences, Oxford, UK) according to the manufacturer’s instructions.

**PRNP Codon 129 Allele Discrimination and HLA Typing**

DNA was extracted from whole EDTA-anticoagulated blood using the Nucleon kit (Amershams Biosciences). HLA typing was performed by the Oxford Radcliffe Hospitals Transplant Immunology Laboratory by polymerase chain reaction with sequence-specific primers (PCR-SSP) using a thermal cycler (Applied Biosystems, Foster City, CA) using standard conditions. Amplification primers were as follows: forward 5’tca tga gaa caa gcc gag taa g-3; reverse 5’cat agt cac tgc cga aat gta tga t-3 and allele discrimination probes were 129M 5'-GAGGCGGGTAACATTGTTCTACAC-3' and 129V 5'-GAGGCGGGTAACATTGTTCTACAC-3'.

**Statistical Analysis**

Results were compared as 2 × 2 contingency tables using Fisher exact test in GraphPad InStat (GraphPad Software, San Diego, CA).

**RESULTS**

In silico Epitope Prediction

When the full-length human PrP (residues 23–231) sequence with either methionine or valine at position 129 is analyzed by TEPITOPE at a stringency of 3%, the program generates 13 predicted 9-mer epitopes (Table 2). Interestingly, a number of these span the position 129 polymorphism. In contrast, the N-terminal region of PrP from positions 23 to 109 is not predicted to generate any HLA-binding epitopes.

**T Cell Proliferation Assay**

PBMCs from 21 healthy donors were then cultured with up to 29 peptides spanning the full length of human PrP 129M. Proliferative responses were considered positive when the SI was >2 (Fig. 1A). Positive responses were seen to peptides spanning residues 23–36 (peptide 1), 107–120 (peptide 13), 114–127 (peptide 14), 121–134 (peptide 15), 128–141 (peptide 16), 170–183 (peptide 22), 177–190

**TABLE 2. 9-mer Epitopes Predicted by TEPITOPE for Available HLA-DRB Alleles From the Sequence of Full-Length Human PrP**

<table>
<thead>
<tr>
<th>Predicted Epitope</th>
<th>Position</th>
<th>HLA DR Allele(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mkhmagaaa</td>
<td>109–117</td>
<td>B1*0101</td>
</tr>
<tr>
<td>vvglggyym</td>
<td>121–129 [129 M]</td>
<td>B1*0301</td>
</tr>
<tr>
<td>vvglggyml</td>
<td>122–130 [129 M]</td>
<td>B1*0101</td>
</tr>
<tr>
<td>vvglggyvl</td>
<td>122–130 [129 V]</td>
<td>B1*0101</td>
</tr>
<tr>
<td>lggymigsa</td>
<td>125–133 [129 M]</td>
<td>B1*0802</td>
</tr>
<tr>
<td>lggylvgsa</td>
<td>125–133 [129 V]</td>
<td>B1*0802</td>
</tr>
<tr>
<td>lgsamsrpi</td>
<td>130–138</td>
<td>B1*0102</td>
</tr>
<tr>
<td>mhrynqavy</td>
<td>154–162</td>
<td>B1*0402</td>
</tr>
<tr>
<td>ysnqmnfvh</td>
<td>169–177</td>
<td>B1*0405</td>
</tr>
<tr>
<td>fhdcvnit</td>
<td>175–183</td>
<td>B1*0301</td>
</tr>
<tr>
<td>vkmnvverve</td>
<td>203–211</td>
<td>B1*0421</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B1*1305</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B1*1321</td>
</tr>
</tbody>
</table>

Altogether there were 22 positive responses out of 513 peptide assays elicited by 13 of the 29 peptides (44.8%). The most immunogenic peptides were 14 and 15, to which, respectively, 4 of 20 (20%) and 4 of 21 (19%) donors made responses (Fig. 1B). Overall, 21 of the positive responses (95.5%) were clustered in 2 regions of the protein: 107–141 (peptides 13–16) and 170–231 (peptides 22–29) (Fig. 1B, C). These peptides accounted for 236 of the 513 total assays (46%), of which 8.9% were positive, whereas in the remaining 277 stimulations using peptides outside these 2 regions, there was only one positive response, to peptide 1. Thus, peptides within regions PrP 107–141 or 170–231 were significantly more immunogenic than those spanning the rest of the protein sequence (Fisher exact test p < 0.0001). Indeed, no responses at all were generated by peptides 2 to 12, covering approximately 40% of the PrP sequence (Fig. 1A–C).

Interestingly, 8 of the 10 epitopes predicted by TEPITOPE from the human PrP 129 M sequence were
represented among the 13 peptides that elicited responses in vitro. Thus, putative epitopes generated in silico had high predictive value for in vitro responses. In agreement with the TEPITOPE predictive algorithm, the N-terminal region of PrP was not immunogenic with the exception of a single response to PrP 23–36. Overall, peptides spanning epitopes generated by TEPITOPE accounted for 9 of the 22 responses to PrP 129M sequence (40.9%), although none of our synthesized peptides completely spanned PrP 169–177.

Responses to one or more peptide were seen in 9 of 21 donors. However, positive responses were not evenly distributed among donors (Fig. 2). The majority of responding individuals generated responses to between 5.0% and 14.9% of the peptides with which their PBMCs were challenged, whereas 2 responded to >15%.

In addition, PBMCs from 20 of the donors were cultured with peptides spanning PrP 121–134 and 128–141 with Valine at position 129. Only one response was positive out of 40 individual peptide assays. This was to peptide 16V (Fig. 3A, B). Positive responses were generated to peptide 15 by 4 of 21 donors compared with zero of 20 donors challenged with peptide 15V. Thus, the epitope PrP 121–134 appeared to be more immunogenic with methionine at position 129 compared with valine, although this observation did not achieve statistical significance (Fisher exact test p = 0.11).

Role of PRNP Codon 129 Genotype

Four of the 10MM homozygotes and 5 of the 8 MV heterozygotes responded to one or more peptide (Table 3). No responses were made by any of the 3VV individuals. Responses to 129M spanning peptides were seen in 3/8 129 MV heterozygotes and one of 10 129MM homozygotes (Fisher exact test p = 0.27). The single response to a 129V-spanning peptide was in an MM donor.

Role of HLA Type

Responses to peptides were seen in individuals with a range of HLA genotypes (Table 3). The sample size was too small to allow an association with any particular genotype to emerge. However, responses to peptide 114–127 were only seen in individuals expressing HLA-DR52 (4 of 15 DR52+, zero of 6 DR52−, Fisher exact test p = 0.28).

Cytokine Profiles

The highest number of positive responses to PrP peptides was made by PBMCs from donor D. Cryopreserved PBMCs from this donor were cultured with peptides 24, 25, 28, and 16V to which this individual had previously made a response, as well as peptides 15, 16, and 15V. Positive responses, as judged by SI >2, were seen to peptides 16, 24, 25, 28, and 16V with negative responses to 15 and 15V. Despite similar stimulation indices, cytokine production elicited by in vitro culture differed between PrP peptides. Whereas peptides 16 and 28 elicited very strong IL-4 and IL-6 responses, peptides 24, 25, and 16V did not induce strong cytokine production (Fig. 4Ai, iii, iv). These responses were associated with modest IFN-γ secretion (Fig. 4Ai) and there was no significant TNF-α, IL-2, or IL-10 response to any of the peptides (data not shown). Interestingly, peptide 16 elicited a distinct cytokine profile compared with its 129V polymorph, 16V.

Further cytokine analysis of responses was then undertaken to obtain a more comprehensive understanding of the nature and functionality of responses to PrP epitopes. Responses to the peptide panel were analyzed by repeat culture of PBMCs from 3 of the individuals shown in Table 3 (donors A, N, and T) as well as an additional 7 donors. Positive responses to one or more peptides were observed in 5 of these additional assays. In accordance with our previous observations, there were no positive responses to N-terminal peptides other than PrP 23–36, confirming the lack of immunogenicity within this region. Again, only modest IFN-γ production was observed (Fig. 4Bi) and no significant quantities of TNF-α or IL-2 were detected (data not shown). There was once again considerable heterogeneity in cytokine levels produced in response to different peptides. Peptides 16 and 28 induced high levels of IL-6 and, to a lesser extent...

**FIGURE 2.** Percentage of positive peptide responses. Most responders generated responses to 5.0% to 14.9% of peptides, whereas 2 of 21 responded to >15.0%.

**FIGURE 3.** (A) Stimulation indices for peptides spanning position 129V elicited by 7-day culture with fresh ex vivo peripheral blood mononuclear cells from 20 donors. Each point represents the stimulation indices (SI) for an individual donor for that peptide. SI <2 (gray) is considered negative; >2 (black) is considered positive. (B) Percentage of donors responding to 129V-spanning peptides. There was one positive response to peptide 16V.
IL-4, as well as low levels of IL-10 (Fig. 4Bii–iv). A similar pattern of IL-6 and IL-4 release was seen in response to peptide 27, although this peptide did not stimulate any IL-10 production. Peptide 16V was again divergent from its PrP129M variant in being IL-4 and IL-6 silent (Fig. 4Bii–iv). The majority of the other peptides were associated with production of IFN-γ, IL-4, IL-6, and IL-10 at low levels.

**DISCUSSION**

A notable feature of vCJD is the accumulation of PrPSc in lymphoreticular tissues, including tonsil, appendix, and terminal ileum (33, 34). Extraneural PrPSc has also been detected, albeit at lower titers, in some cases of sporadic CJD (35). Despite this, no systemic acute phase or immune response has been detected in any human prion disease or experimental model (4, 36). Although T cells have been detected in prion-infected brain, lymphocytes from scrapie-infected mice have not been demonstrated to possess anti-PrP cytolytic activity or secrete cytokines on stimulation with PrP peptides (37). Thus, the atypical inflammation (38) that ensues in the prion-infected central nervous system is largely secondary to rapid neurodegeneration. The failure of adaptive immunity against prions during the lymphoreticular and neural phases of the disease has been ascribed at least partly to T cell tolerance to PrPSc, a ubiquitously expressed self-protein with which PrPSc has 100% sequence identity. However, the extent of tolerance to PrP in humans has not previously been studied.

This is the first report in which human autoreactive T cell responses to PrP have been examined. We found that a significant minority of individuals made responses to PrP peptides and that these reproducibly cluster between residue 107 and the C-terminus. The peptides that elicited responses were remarkably close to those identified using an epitope prediction algorithm. This combined in silico and in vitro approach suggests that despite being a self-protein to which tolerance is assumed to be robust, human PrP is an antigen that is tractable to epitope discovery techniques and hence rational vaccine design.

The major predisposing factor for vCJD is methionine homozygosity at codon 129 of PRNP. However, because this genotype accounts for 40% of the U.K. population and to date, approximately 160 people have been affected by vCJD in the United Kingdom, other susceptibility factors must be involved. An initial report postulating that HLA-DQ7 might confer protection against vCJD (8) was not confirmed when repeated on a larger but not completely overlapping sample (9). With the current small number of affected cases, firm conclusions about HLA association will be difficult to draw unless any protective effect is extremely strong. In this study, T cell responses to PrP peptides were seen in individuals with a variety of HLA genotypes, suggesting that the ability to generate protective immunity may not be restricted to particular HLA types, although only individuals expressing HLA-DR52 responded to PrP 114–127.

Of particular interest, however, is our finding that the polymorphic residue 129 resides within a major human T cell epitope with nearly 20% of donors making a response to PrP 121–134. Moreover, our data are highly suggestive of PrP 121–134 with methionine at position 129 being more immunogenic than PrP 121–134 with 129-valine. Whether the ability to induce an immune response against this region of PrP might be protective against prion disease remains unclear. Our data suggest that PRNP 129 MV or VV individuals are preferentially able to induce a response to
129M-containing sequence although the current study was not sufficiently powered to demonstrate this conclusively. If confirmed, this might provide a basis for differential susceptibility to infectious BSE prions. Furthermore, even among MM homozygotes, there must be additional susceptibility factors. The possibility that some individuals are able to mount an effective immune response against invading PrP© by HLA-restricted presentation of peptidic fragments corresponding to the immunogenic epitopes identified in this study cannot be excluded.

Incomplete tolerance to neuronal proteins is not an unexpected finding. Healthy individuals without multiple sclerosis routinely have T cell responses to myelin antigens (39). Furthermore, a number of studies have demonstrated T cell reactivity to amyloid precursor protein (APP) or Aβ peptides (40–43). However, unlike APP, PrP is highly expressed within the immune system itself, where it has been proposed to play a role in T cell activation (44, 45).

Although the mechanisms underlying tolerance to PrP have not been fully elucidated, in PrP©/© mice in which PrP expression is directed to specific organs by insertion of PrP transgenes under specific promoters, tolerance is readily induced by targeted expression in lymphocytes and other extraneural tissues (46). Expression of PrP© is higher in

![FIGURE 4](image-url)
human lymphocytes compared with those of rodents (47). Consequently, we anticipated that T cell tolerance to PrP in humans would be tighter than to other neural proteins, most likely as a result of PrPSc expression in the thymus and other lymphoid organs and repeated exposure to animal PrP in food. However, we found T cell responses to PrP peptides in a significant proportion of the subjects in our study, suggesting that auto-PrP-reactive clonal deletion may not be complete. Despite this, there is no evidence for autoimmune disease in these individuals as a result of spontaneous breakdown of tolerance to PrP analogous to the presence of antimyelin T cell responses in healthy individuals. Thus, for certain epitopes in some individuals, peripheral suppressor mechanisms such as regulatory T cells or low-affinity antigen presentation may help maintain tolerance to PrP. Indeed, Polymenidou and colleagues found no relationship between the ability of mice to produce antibodies to PrP and thymic PrPSc expression level, implying a role for peripheral tolerance mechanisms (46).

Although tolerance to PrP has been successfully broken in rodents, this has generally required use of powerful adjuvants or novel immunization strategies (48). T cell responses have been demonstrated to a variety of self-PrP sequence epitopes in rodents (Table 4). Systematic studies by Aucouturier et al suggest that the only significantly immunogenic epitope of self-PrP in C57BL/6 mice resides within PrP 158–172 (24, 27, 49). However, peptides spanning 158–172 did not induce a significant antibody response in vivo. In this model, the major B cell epitope was 98–127. In contrast, another group used a variety of PrP sequence peptides selected on the basis of putative MHC binding motifs to elicit both strong T cell and IgG responses in rats and C57BL/6, NOD, and A/J mice (28, 50).

The relevance for humans of epitopes revealed by rodent studies is constrained by the considerable sequence diversity between human and rodent MHC class II. Our data suggest that human PrP 158–172 does not contain a T cell epitope, although the immunogenic regions we identified do have some overlap with those described by Souan and colleagues. Similar proteolytic processing of PrP between species may account for reduced tolerance toward certain common regions. The structured C terminal portion of PrP is underrepresented among human proteosome digests (51); thus, it may be less frequently presented during thymic T cell selection allowing some escape of T cells recognizing C terminal epitopes.

Cytokine responses to PrP peptides were heterogeneous being characterized either by striking induction of IL-6, and to a lesser extent IL-4, or a weak, mixed cytokine response. Importantly, epitopes spanning the residue 129 polymorphism were associated with distinct patterns of cytokine production. Responses to PrP 128–141 [129M] were consistently associated with high levels of IL-6 and IL-4 production that were absent in the responses to PrP 128–141 [129V]. This is the first time that the residue 129 polymorphism has been implicated in determining the quality of the immune response to PrP.

IL-6 can be produced by T cells but is also released by monocytes and B cells (52), which will also be present in a PBMC culture. Could PrP peptides drive IL-6 expression by non-T cells in culture? PrP 106–126 has previously been demonstrated to induce IL-6 production by human monocyte-derived dendritic cells (53). Furthermore, in sporadic CJD, plasma IL-6 levels may be elevated (54). However, where peptides induced very high levels of IL-6 production, this was coupled to IL-4 release, suggesting that whatever its source, IL-6 was driving a Th2-dominant T cell response. Indeed, IL-6 is required for IL-4 production by T cells undergoing Th0 to Th2 differentiation (55, 56). The overall pattern of cytokine responses suggests that PrP 128–141 [129M] and 205–225 may be able to drive a Th2-differentiated response and PrP 23–36 and 128–141 [129V] give rise to a limited Th1 response, whereas other PrP epitopes are associated with a weak Th0 profile.

It is still not clear to what extent a primed T cell response to PrP can protect against prion infection. Vaccination of A/J mice with PrP 31–50 or 211–230 in CFA led to a reduction in PrPSc level in prion-infected N2a tumor grafts (28). However, when C57BL/6 mice were inoculated with scrapie after immunization with these peptides, there was no protective response additional to the administration of CFA alone (57).

Other vaccination studies have tended to concentrate on generating anti-PrP antibody responses (15, 17–19). However, anti-PrP antibodies raised by vaccination or administered passively (58) have thus far only been effective against disease restricted to extraneural tissues, presumably as a result of poor blood–brain barrier penetration. Activation of other components of the immune system, especially those capable of safe central nervous system penetration, may therefore be a prerequisite to effective immunotherapy.

The dangers, if any, of breaking tolerance to PrP are uncertain. The only adverse side effect of anti-PrP vaccination so far reported is dermatitis with mononuclear cell invasion and destruction of hair follicles in Lewis rats several months after immunization with PrP 182–202 (50). The importance of defining T cell epitopes in self-proteins that are used as human vaccines was dramatically illustrated when 6% of patients with Alzheimer disease in a clinical trial of Aβ vaccination developed meningoencephalitis driven by T cell invasion of the central nervous system.

**TABLE 4. T Cell Responses Induced by Immunization With Native PrP in Wild-Type PrP-Expressing Animals**

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Responder Lymphocyte Species/Strain</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mo 31–50</td>
<td>C57BL/6</td>
<td>(28)</td>
</tr>
<tr>
<td>Mo 131–150</td>
<td>NOD, C57BL/6, A/J</td>
<td>(28)</td>
</tr>
<tr>
<td>Mo 151–170</td>
<td>C57BL/6</td>
<td>(28)</td>
</tr>
<tr>
<td>Mo 156–172</td>
<td>C57BL/6</td>
<td>(24)</td>
</tr>
<tr>
<td>Mo 158–172</td>
<td>C57BL/6</td>
<td>(27)</td>
</tr>
<tr>
<td>Mo 158–172</td>
<td>C57BL/6</td>
<td>(49)</td>
</tr>
<tr>
<td>Mo 182–202</td>
<td>NOD</td>
<td>(28)</td>
</tr>
<tr>
<td>Mo 211–230</td>
<td>NOD, C57BL/6, A/J</td>
<td>(28)</td>
</tr>
<tr>
<td>Ra 118–137</td>
<td>Lewis rat</td>
<td>(50)</td>
</tr>
<tr>
<td>Ra 182–202</td>
<td>Lewis rat</td>
<td>(50)</td>
</tr>
<tr>
<td>Ra 211–230</td>
<td>Lewis rat</td>
<td>(50)</td>
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</table>
The cause of this side effect is not entirely clear and may relate to a Th1-dominant response driven by the adjuvant QS-21 (59). However, an option for future vaccine development in Alzheimer disease may be to focus on the N-terminal fragment of Aβ that contains the C cell epitope but from which the immunodominant C-terminal T cell epitope has been deleted (60). These unexpected adverse effects demonstrate that mouse models with homogeneous MHC class I and II expression and/or incomplete recapitulation of human expression patterns of the target autoantigen may not be sufficient to model the effects of breaking tolerance to self-proteins in humans. Our study suggests that the immunodominant autoepitopes in human PrP reside between residue 107 and the C-terminus and that the residue 129 polymorphism quantitatively and qualitatively influences the immune response to PrP. Furthermore, PrP epitopes recognized by donor T cells ex vivo were closely matched to predicted epitopes based on known HLA-binding motifs. The optimal model for assessing a vaccine based on these sequences would be one with humanized PrP and HLA class II expression such as a double transgenic mouse. Whether a robust T cell response to these epitopes will result in harmful autoimmunity or be a prerequisite for protection from prion disease remains to be determined. However, elucidation of these immunodominant epitopes should help refine rational vaccine design for human prion diseases.

ACKNOWLEDGMENTS

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