Antibody response in Lewis rats injected with myelin oligodendrocyte glycoprotein derived peptides

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Abstract

Previous studies from our laboratory have demonstrated a predominant response to myelin oligodendrocyte glycoprotein (MOG) in patients with multiple sclerosis (MS) and showed that this molecule is able to induce in Lewis rats a chronic relapsing MS-like disease with extensive demyelination. To further study the possibility that MOG is a primary target antigen in MS, we have begun to investigate the encephalitogenicity and antibody response of different sequences of the extracellular domains of MOG in Lewis rats. We report that none of the synthetic peptides encompassing the MOG amino acid sequences 1-21, 67-87, 104-117 and 202-218 were encephalitogenic. In contrast, a single injection of MOG35-55 was able to induce severe neurological signs associated with inflammation and demyelination. All rats injected with MOG peptides 1-21, 35-55, 67-87 and 202-218 developed a high level of antibodies to their respective immunizing peptides as detected by ELISA and immunoblotting. Although all MOG peptide antisera reacted with immunoblots of native MOG separated under reducing conditions, only anti-MOG35-55 and anti-MOG202-218 antibodies reacted to native MOG, when tested under non-reducing conditions. These results indicate that the MOG35-55 peptide, which is found in the extracellular Ig V-like domain of MOG, is not only an encephalitogenic epitope but could also be an important determinant for initiating antibody-mediated demyelination. As indicated by the absence of reactivity to the other MOG peptides tested, as well as other central nervous system myelin proteins including myelin basic protein and proteolipid protein, the antibody response produced by MOG peptides is highly restricted.

Introduction

Chronic relapsing experimental autoimmune encephalomyelitis (CREAE) is a paralytic disease that is observed following immunization with central nervous system (CNS) antigens emulsified in complete Freund's adjuvant (CFA) (1,2). CREAE has long been used as an animal model for the human demyelinating disease multiple sclerosis (MS) and has been extensively studied to analyze the underlying autoimmune mechanisms leading to demyelination (1). Because myelin basic protein (MBP) and proteolipid protein (PLP) are the two most abundant CNS myelin proteins (3), they are considered putative target antigens for the pathogenic autoimmune responses occurring in CREAE (2,4). Indeed, many studies have identified the encephalitogenic peptides of MBP and PLP for various species and have characterized the immune responses to these myelin peptides (5). In particular, it is well established that the transfer of encephalitogenic CD4+ T cells specific for these MBP or PLP peptides causes EAE in naive syngeneic recipients (4). However, there is now considerable experimental evidence suggesting that other CNS components may be more relevant target antigens in immune-mediated demyelinating diseases (6-9). Among these, myelin oligodendrocyte glycoprotein (MOG) is potentially important because it is located on the outermost lamella of the myelin sheath, is expressed exclusively in CNS myelin...
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1-21
35-55
67-87
104-117
202-218

Table 1. Amino acid sequence of the synthetic MOG peptides used in this study

<table>
<thead>
<tr>
<th>Residues</th>
<th>Amino acid sequence</th>
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<tbody>
<tr>
<td>1-21</td>
<td>GQFRVIGPGHPIRALVGDEAE</td>
</tr>
<tr>
<td>35-55</td>
<td>MEVGWYRSPFSRWLYRNGK</td>
</tr>
<tr>
<td>67-87</td>
<td>GRTELKSGEGKVALRION</td>
</tr>
<tr>
<td>104-117</td>
<td>SYGEEAVELKVED</td>
</tr>
<tr>
<td>202-218</td>
<td>LHRRLAGQFLERLRNPF</td>
</tr>
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</table>

The synthetic rat MOG peptides 1-21, 67-87, 104-117 and 202-218 were gifts from Professor A. Ben Nun (The Weizmann Institute of Science, Rehovot, Israel). MOG peptide 35-55, obtained from Auspep (Melbourne, Victoria, Australia), was synthesized by standard F-moc chemistry and the purity (>95% pure) determined by reverse-phase HPLC. This panel of five MOG peptides was selected as they represent potential immunodominant sites of MOG as predicted by the 'T-site' computer program (MedImmune, Gaithersburg, MD) (20). The sequence of these peptides is shown in Table 1. MOG was purified from rat brain by affinity chromatography and the purity determined by immunoblotting with highly specific antibodies to other myelin proteins as described elsewhere (21). The non-glycosylated histidine fusion protein representing the Ig extracellular domain (amino acids 1-125) of mouse MOG (rMOG) was prepared as reported in Amor et al. (22).

The sequence of these peptides is shown in Table 1. The autoantibody response generated against these peptides is of restricted specificity.

Fig. 1. Proposed MOG model. MOG peptides 1–21, 35–55, 67–87, 104–117 and 202–218 are shown as bold lines.

and is highly immunogenic (10,11). Furthermore, a number of studies have demonstrated that anti-MOG antibodies are able to cause demyelination both in vivo and in vitro (12–18). The mechanisms by which such antibodies produce demyelination are varied, but include the activation of myelin proteases leading to MBP degradation (9), the activation of complement (17) and the enhanced phagocytosis of myelin by macrophages (17). Recently, we produced a chronic relapsing MS-like disease with extensive demyelination in Lewis rats by a single injection of MOG or the derived peptide, MOG35-55 (9). The clinical course and the pathology of the CNS lesions in this new animal model were very similar to those of the human disease MS. Interestingly, sera from such affected rats reacted specifically with MOG, but not with any other myelin or brain proteins (9), suggesting that as observed in the cerebral spinal fluid of MS patients (19), the autoantibody response is of restricted specificity. In order to further characterize the regions of MOG which may be encephalitogenic and able to stimulate autoreactive B cells, we immunized rats with various peptides encompassing different sequences of the extracellular domains of MOG (Fig. 1). We report here that four out of five MOG peptides tested were able to stimulate a strong humoral response. Among those, only MOG35–55 was able to induce a chronic relapsing demyelinating disease. Further, we show that the antibody response generated against these peptides is of restricted specificity.

Methods

Animals and reagents

Lewis rats were purchased from the Walter and Eliza Hall Institute of Medical Research (Melbourne, Victoria, Australia).

Histologic examination of tissues

Brains and spinal cords were dissected and immersion-fixed in 4% neutral buffered formalin. Sections of 5 μm thickness were cut and processed as described elsewhere (21).
were cut from the forebrain, cerebellum, hindbrain and spinal cord, and stained with hematoxylin & eosin or Luxol fast blue for evidence of inflammation and/or demyelination. Sections were examined without knowledge of the injection regime by two investigators (C. C. A. B. and A. Slavin) for perivascular infiltrations and demyelination.

ELISA

Synthetic MOG peptides or purified rat MOG were diluted to a concentration of 1 μg/ml in carbonate buffer, pH 9.6, and 50 μl/well coated onto 96-well microtiter plates (Disposable Products, SA, Australia) pretreated with 0.2% glutaraldehyde. Recombinant mouse MOG was used at a concentration of 10 μg/ml with 100 μl of the dilution being applied to each well. After incubation at 37°C for 3 h, the plates were washed three times with (PBS) containing 0.1% Tween 20 and blocked with 2% BSA (Boehringer, Mannheim, Germany) in PBS at 4°C overnight. The plates were then incubated with 50 μl of a 1/1,000 dilution of rat serum for 1 h at 37°C. After incubation three times, appropriately diluted biotinylated goat anti-rat IgG (Sigma, St Louis, MO) was added to the plates and incubated for 1 h at 37°C. After incubation with streptavidin–horseradish peroxidase (Amersham, Sydney, Australia) for 30 min at 37°C, the reaction products were visualized using 2,2′-azino-bis[3-ethylbenz-thiazoline-6 sulfonic acid] as a substrate and read at 405 nm with a microplate reader (Flow, McLean, VA).

SDS–PAGE

Tricine–SDS–PAGE was performed according to the method described by Schagger and Von Jagow (27) with minor modifications. The gel consisted of a stacking gel (4%T, 3%C), a spacer gel (10%T, 3%C) and a separating gel (16.5%T, 6%C), all prepared in 1 M Tris and 0.1% SDS, pH 8.45. Electrophoresis was performed using 0.2 M Tris, pH 8.9, as an anode buffer and 0.1 M Tris, 0.1 M Tricine and 0.1% SDS, pH 8.25, as a cathode buffer. After electrophoresis, the gels were stained with Coomassie blue R250 or transferred to a PVDF membrane (Immobilon-P; Millipore, Bedford, MA) for immunoblotting. Standard SDS–PAGE of purified rat MOG or human myelin was carried out according to the method of Laemmli using 13.5% polyacrylamide gels (28).

Immunoblotting

After transfer, PVDF membranes were blocked with 3% BSA in Tris-buffered saline (TBS). Dilutions of rat sera and the mouse monoclonal anti-MOG antibody (8-18C5) were made in TBS containing 0.05% Tween 20 and 0.25% BSA and were incubated with the membrane for 90 min. After washing three times, biotinylated goat anti-rat IgG (Sigma) or biotinylated goat anti-mouse IgG (Sigma) was added and incubated for 1 h as above. Following incubation with streptavidin–horseradish peroxidase for 20 min, reactivity was detected using ECL and autoradiography according to the manufacturer's instructions (Amersham, Sydney, Australia).

Purification of anti-MOG35–55 peptide antibody

An affinity column containing MOG35–55 coupled to immobilized dianmonodpropylamine (Pierce, Rockford, IL) was used to purify MOG35–55 antibodies. Before use, the column was washed extensively with 1 M NaCl containing 0.1% sodium azide. Serum samples from immunized rats (0.2 ml diluted 1:10 with PBS) were applied to the column, the flow through collected and bound antibodies eluted with 0.1 M glycine–HCl, pH 2.5. The columns were neutralized with 1:10 the volume of 1 M Tris, pH 9, and extensively dialysed against 0.15 M NaCl. Before testing for their antibody activity, each fraction was diluted so that the final concentration corresponded to the initial serum dilution of 1:1000.

Results

Encephalitogenic activity of MOG peptides

Consistent with our previous study (9), Lewis rats injected with whole rat MOG or with MOG35–55 developed a severe relapsing-remitting neurologic disease associated with CNS inflammation and demyelination (Table 2). The first symptoms of disease, involving weight loss and weakness of hind limbs, were observed 2 weeks after immunization. Rats recovered fully within 3 or 4 days but half of them experienced a relapse characterized by severe hind and forelimb paralysis 6–7 weeks after immunization. Histological examination at the time of severe disease revealed multifocal demyelination associated with perivascular mononuclear cell infiltrations which were most prominent in the dorsal columns of the spinal cord (data not shown). In contrast, none of the other MOG peptides tested, i.e. MOG peptides 1–21, 67–87, 104–117 and 202–218, were able to produce clinical or histological disease (Table 2).

Antibody activity to whole MOG and MOG peptides

The serum antibody reactivity of rats injected with whole rat MOG or its peptides was tested 8 weeks after immunization by ELISA and immunoblotting against the panel of immunizing peptides and whole MOG. The monoclonal anti-MOG antibody, 8-18C5, was used as a positive control for anti-MOG antibody activity.

ELISA

All rats immunized with MOG peptides 1–21, 35–55, 67–87 and 202–218 developed high levels of antibody against their immunizing peptide but did not show cross-reactivity to any of the other MOG peptides tested (Table 2). None of the sera obtained from the rats injected with MOG104–117 or from the control rats injected with adjuvant alone reacted to the MOG peptides. Likewise, none of the sera from rats injected with rat MOG had significant levels of antibody activity to any of the MOG peptides tested. Interestingly, the monoclonal 8-18C5 anti-MOG antibody did not react with any of these peptides. Each of the anti-peptide antisera was subsequently tested against the whole MOG molecule. As illustrated in Table 2, antibodies raised against MOG35-55 and MOG202-218 peptides showed high reactivity to native MOG, whilst anti-MOG1–21 antibodies showed low reactivity. Sera from animals injected with MOG67–87, guinea pig MBP or CFA alone displayed no reactivity. As expected, the 8-18C5 antibody and the sera from rats injected with whole rat MOG had high reactivity to rat MOG (Table 2). In a series of separate experiments it was found that sera from animals immunized...
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Table 2. MS-like disease and antibody response in Lewis rats immunized with MOG peptides

<table>
<thead>
<tr>
<th>Immunogen</th>
<th>No. of rats</th>
<th>Serum antibody reactivity to a</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Clinical</td>
<td>Histological</td>
</tr>
<tr>
<td></td>
<td>signs</td>
<td>signs</td>
</tr>
<tr>
<td>MOG 1-21</td>
<td>0/3</td>
<td>1.33</td>
</tr>
<tr>
<td>MOG 35-55</td>
<td>5/5</td>
<td>0.04</td>
</tr>
<tr>
<td>MOG 67-87</td>
<td>0/3</td>
<td>0.06</td>
</tr>
<tr>
<td>MOG 104-117</td>
<td>0/4</td>
<td>0.08</td>
</tr>
<tr>
<td>MOG 202-218</td>
<td>0/4</td>
<td>0.10</td>
</tr>
<tr>
<td>Whole MOG</td>
<td>4/5</td>
<td>0.11</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>0/3</td>
<td>0.05</td>
</tr>
</tbody>
</table>

aAnimals were injected with 100 µg of whole rat MOG or with 200 µg of rat MOG peptides emulsified with CFA, supplemented with Mycobacterium tuberculosis. Animals were observed up to 100 days post-injection.

bSera obtained 8 weeks after immunizations were tested by ELISA at 1/1000 dilution. Results are expressed as the mean optical density (OD) units at 405 nm of duplicate determinations. Bold numbers indicate reactivities that were 3 SD or more above background. The monoclonal anti-MOG antibody (8-18C5) used as positive control reacted only with MOG (OD = 1.450) but not with any of the MOG peptides tested.

Fig. 2. Autoradiograms of immunoblots of purified rat MOG. (a) MOG was electrophoresed under reducing conditions, (b) MOG was electrophoresed under non-reducing conditions and probed with: 1, monoclonal anti-MOG antibody (8-18C5); 2, anti-MOG1-21 antibody; 3, anti-MOG35-55 antibody; 4, anti-MOG67-87 antibody; 5, anti-MOG202-218 antibody. MWS, molecular weight standard. Each antibody obtained 8 weeks after immunizations was tested at 1/400 dilution.

Immunoblotting

Anti-peptide antisera with positive antibody titers were subsequently investigated for their reactivity to whole MOG by immunoblotting. Figure 2 shows that under reducing conditions, antibodies against MOG peptides 1–21, 35–55, 67–87 and 202–218 reacted to whole MOG, with the anti-MOG35–55 antibody showing the strongest reactivity. However, under non-reducing conditions, anti-MOG35–55 and anti-MOG202–218 antibodies reacted strongly with whole MOG, while anti-MOG1–21 and MOG67–87 antibodies did not recognize whole MOG (Fig. 2) even when such antisera were tested at 1/200 dilutions (data not shown).

To further determine if the anti-MOG peptide antibodies generated in Lewis rats were only reactive to immunizing peptides or could also cross-react with the other MOG peptides, reactivity to all five peptides was tested by immunoblotting following separation by Tricine–SDS–PAGE. This electrophoretic system was used as it allows a superior resolution of small proteins (27). Figure 3 shows the Tricine–SDS–PAGE analysis of MOG peptides stained with Coomassie blue. MOG1–21 was detected as a band at 4.0–4.8 kDa, MOG35–55 at 3.7–4.4 kDa, MOG67–87 at 3.1–3.7 kDa, MOG104–117 at 1.1–1.5 kDa and MOG202–218 at 3.7–4.4 kDa. These peptides were then transferred to PVDF membranes and probed with the antisera generated against each of the five MOG peptides studied here. As illustrated by the example shown in Fig. 4, reactivity to MOG peptides 35–55, 67–87 and 202–218 was clearly demonstrated in the serum from rats injected with each of the three corresponding MOG peptides. However, none of these specific antibodies were cross-reactive with any of the other MOG peptides tested. None of the sera obtained from the control rats (CFA alone) reacted with any of the blots containing the separated peptides. No reactivity to the MOG peptides was detected with the 8-18C5 antibody and the sera from rats immunized with whole MOG (data not shown).
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Reactivity of affinity purified anti-MOG35-55 antibody

To confirm that the reactivity of the anti-MOG35-55 antibodies to native MOG was not due to diverse antibody populations, serum samples taken 6 and 10 weeks post-injection of MOG35-55 were purified using a MOG35-55 affinity column. As exemplified by the results shown in Fig. 5, flow-through fractions tested by ELISA had little reactivity against native MOG or MOG35-55. By contrast, bound antibodies reacted strongly to both preparations. Interestingly, affinity-purified anti-MOG35-55 antibodies from the 10 weeks sample reacted more strongly to native MOG than did antibodies from the 6 weeks sample (Fig. 5).

Reactivity of anti-MOG peptide antisera to human myelin

In order to ascertain if the anti-MOG-specific peptide antisera could recognize native MOG in myelin, and perhaps also react with other myelin components, human myelin proteins were separated under non-reducing conditions, transferred to membrane and probed with the anti-MOG peptide antisera. Among the four anti-MOG peptide antibodies tested, only those directed to MOG35-55 and to MOG202-218 reacted strongly with the 28 kDa form of MOG and, to a lesser extent, with the 55-58 kDa form of MOG (Fig. 6). No reactivity to other myelin components could be observed with these sera. (Fig. 6). Both MOG bands were also detected with the monoclonal 8-18C5 antibody.

Discussion

Although considerable attention has been given to the possible pathogenic role played by autoreactive CD4+ T cells in MS, it should be emphasized that primary demyelination could also be mediated by autoantibodies (8,11,29). Indeed, one of the important immunopathological features of this disease is the intrathecal synthesis of excessive amounts of Ig. These Ig are restricted in their heterogeneity (19) with some showing autoreactivity to CNS antigens (30,31). Whilst the role of such autoantibodies in the pathogenesis of MS is still undefined, there is increasing evidence suggesting that the extensive immune-mediated demyelination seen in the CNS of such patients is probably caused by a synergy between autoreactive T and B cells (14,15,29).

As part of our ongoing efforts aimed at identifying the putative CNS components responsible for triggering the autoimmune responses leading to demyelination, we recently reported that MOG, an exclusive and quantitatively minor component of CNS myelin, could induce in Lewis rats a relapsing-remitting neurologic disease with extensive plaque-like demyelination (9). The experiments described in this paper verify the encephalitogenic potential of rat MOG in Lewis rats and demonstrate its ability to stimulate a vigorous B cell response. Moreover, by using synthetic peptides encompassing five different sequences of the extracellular domains of rat MOG (amino acid sequences 1-21, 35-55, 67-87, 104-117 and 202-218) we confirm that one of the predominant encephalitogenic epitopes of MOG for the Lewis rat is located in the Ig-like domain, between amino acid residues 35 and 55 (9). Indeed, none of the other four peptides representing other extracellular regions of MOG were able to induce neurological signs or histological lesions within the CNS. These results are consistent with those of Linington et al. (29) who showed that none of the T cell lines specific

Fig. 3. Tricine-SDS-PAGE analysis of MOG peptides after Coomassie blue staining. MWS, molecular weight standard; lane 1, MOG202-218; lane 2, MOG104-117; lane 3, MOG67-87; lane 4, MOG35-55; lane 5, MOG1-21.

Fig. 4. Autoradiograms of immunoblots of MOG peptides. MOG peptides (2.5 μg) were electrophoresed using Tricine-SDS-PAGE and probed with (a) anti-MOG35-55 antibody, (b) anti-MOG67-87 antibody and (c) anti-MOG202-218 antibody. MWS, molecular weight standard; lane 1, MOG1-21; lane 2, MOG35-55; lane 3, MOG67-87; lane 4, MOG104-117; lane 5, MOG202-218. Each antibody obtained 8 weeks after immunizations was tested at 1/400 dilution.
for MOG peptides 1-20, 61-80, 98-117 and 198-218 were encephalitogenic in Lewis rats, but a T cell line directed to MOG peptide 35-55 was able to transfer histological EAE at 0.1 µg/ml.

The strong encephalitogenic potential of the 35-55 sequence of MOG is further emphasized by its ability to induce a relapsing-remitting neurological disease in some strains of mice (32,33). The finding that MOG35–55 can induce immunopathogenic responses in different species as well as in animals of the same species having a different MHC is at variance with what is observed with MBP and PLP where animals with different MHC haplotypes respond to distinct epitopes of MBP and PLP (2,4,5). Investigations are therefore currently underway in our laboratory to ascertain if the susceptibility of various animals species to develop the MOG-induced MS-like disease is controlled by gene(s) associated with or outside the MHC.

In a recent study, Potter et al. (34) reported that when mice were immunized with the encephalitogenic PLP peptide 178–191, the anti-PLP peptide antibodies in the serum of these mice were cross-reactive with three other PLP peptides (97–110, 209–217 and 215–228) and that none of the anti-PLP peptide antibodies reacted with native PLP. Our findings are at variance with those obtained with PLP peptides, in that none of the antibodies raised against a specific peptide, including MOG peptide 35–55, were cross-reactive with any of the other MOG peptides. Some of these anti-MOG peptide antisera were also able to react with the native MOG. As removal of MOG35–55 antibodies by immunosorbition eliminated the reactivity to native MOG, it is likely that the reactivity was restricted to this peptide, although we cannot completely rule out the possibility of other B cell epitopes within the MOG molecule. Given the strong antibody response directed to some of the MOG peptides, it is somewhat surprising that no antibodies to other myelin antigens could be detected. This is particularly so in animals injected with MOG35–55 as these rats had extensive plaque-like demyelination. Whether or not antibodies to other myelin antigens are produced, but remain localized in the brain, needs further investigation. The reason for the difference between the findings obtained with PLP peptides and the current results with MOG peptides is unclear but may be related to their immunogenic properties and/or their respective amount in myelin. While PLP is the major structural protein of myelin, representing some 50% of total protein, MOG represents only ~0.05% of total protein (3,21,35). However, despite these quantitative differences, MOG must have a high immunogenic potential since immunization with CNS tissue homogenate generates high levels of anti-MOG antibodies (11). On the other hand, one would expect that following demyelination the level of MOG available for antigen presentation would be relatively low. Accordingly, the number and types of antigen-presenting cells effectively presenting MOG may be highly restricted, a situation which is unlikely to occur in the case of the quantitatively more abundant PLP molecule.

MOG is a protein of 218 amino acids which is predicted to have two transmembrane domains and an Ig-like extracellular domain (36–38). It is also thought that residues 200–218 form an extracellular ‘tail’ (36). In the present investigation, antibodies were produced against three peptides derived from the Ig-like domain, i.e. MOG1–21, MOG35–55 and MOG67–87, and to the MOG peptide 202–218, corresponding to the extracellular tail. All antisera reacted to whole MOG under reducing conditions in Western blots; however, under non-reducing conditions, only antisera to MOG35–55 and MOG202–218 reacted with whole MOG. The reason why some of our anti-MOG peptide antibodies are able to recognize the
native MOG whilst others are not is uncertain, but the failure of some anti-peptide antibodies to recognize their counterparts in the native protein is a well known observation. While the three-dimensional structure for MOG has not been reported, a recent analysis performed by Linsley et al. (39) reveals a similar overall structural homology between the V-like Ig domain of MOG and that of the co-stimulatory molecule B7. Using the B7 model, we found no obvious structural reasons which could explain the difference in reactivity of the anti-MOG peptides to the native MOG. Peptides 1-21, 35-55 and 67-87 have similar charge and hydrophilicity, are found in similar locations within the Ig domain, containing sequences that form part of both the β-sheet and loop regions, and all should have significant portions exposed to the surface of MOG. Therefore, and as suggested by the absorption study described in Fig. 5, the stronger binding of the anti-MOG35-55 antibody to the native MOG may simply be due to a maturing of the antibody response resulting in higher affinity antibodies. In view of the fact that the MOG peptide 35-55 represents an immunodominant epitope of MOG and is encephalitogenic, this is perhaps not unexpected since an effective threshold of high-affinity antibodies bound to oligodendrocytes would be required to initiate the cascade of events leading to demyelination. However, as described elsewhere (40), it is unlikely that the ability of a MOG peptide to produce antibodies capable of recognizing native MOG is the only prerequisite for development of neurological signs.

Given the strong antibody response seen following immunization with MOG202-218 peptide, the observation that these antibodies are able to recognize native MOG, plus the previous report that this overall sequence is able to generate a peptide-specific T cell response in Lewis rats (29), it is unclear why this peptide failed to induce clinical or histological disease. However, several possibilities can be contemplated. In a study aimed at assessing the in vivo pathogenic potential of a panel of anti-MOG mAb, Piddlesden et al. (17) reported a striking variation in the ability of these antibodies to initiate demyelination in Lewis rats with MBP-induced EAE. This difference in demyelinating activity was not related to specificity for a given antibody, since all were shown to be CNS myelin specific, all recognized MOG epitopes on oligodendrocytes and all stained oligodendrocytes in a similar manner (17). Thus, like some of these mAb, the anti-MOG202-218 antibodies may not be pathogenic. Alternatively, this peptide may not be accessible to the pathogenic humoral immune response. Indeed, it has been presumed that the C-terminal region of native MOG forms an extracellular 'tail' but, as suggested by Gardinier et al. (36), it is possible that this region is not exposed at the outer surface of the myelin sheath but is in fact intracellular. If this is the case, it is unlikely that this sequence of MOG would act as a target for primary antibody-mediated demyelination and thus explains the inability of MOG202-218 to induce disease.

Several papers have reported that the 8-18C5 anti-MOG mouse mAb, raised against rat cerebellar glycoproteins, possesses demyelinating activity both in vivo and in vitro (12-17). We clearly demonstrate here that 8-18C5 does not react to any of the extracellular peptides of MOG tested in this study but did react with the native MOG. Thus, although 8-18C5 mediates demyelination, the site of antibody binding of 8-18C5 is either different from that of the anti-MOG35-55 antibody or directed against a conformational determinant localized within this region. The possibility that antibodies to MOG35-55 are able to initiate demyelination in Lewis rats is currently under investigation in our laboratory, using anti-MOG35-55 antibodies and T cells sensitized to MBP (14).

In summary, the results of this study corroborate and extend our (9) and other reports (29) showing that MOG, a minor CNS myelin component, is capable of inducing immune-mediated demyelination and a highly restricted B cell response in Lewis rats. These findings implicate MOG as an important primary target autoantigen for immune-mediated demyelination in MS, a contention supported by our recent demonstration of a predominant T cell response to MOG in the peripheral blood of MS patients, the suggested presence of anti-MOG antibody in the CSF of such patients (41) and the well documented demyelinating activity of MOG reactive antibody both in vivo and in vitro (12-17). Taken together, these results emphasize that a synergism between a T cell response and anti-MOG antibodies recognizing native MOG or some of its determinants, is critical for the development of severe demyelinating disease. Further investigations into the autoimmune-mediated demyelination pathways generated by MOG peptides may not only provide important insights into the pathogenetic mechanisms of MS but also have distinct implications for the development of future specific therapeutic approaches to MS.

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Abbreviations

CFA complete Freund's adjuvant
CNS central nervous system
CREAE chronic relapsing experimental autoimmune encephalomyelitis
EAE experimental autoimmune encephalomyelitis
MBP myelin basic protein
MOG myelin oligodendrocyte glycoprotein
MS multiple sclerosis
PLP proteolipid protein

References

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