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Selective Unresponsiveness to Conformational B Cell Epitopes of the Myelin Oligodendrocyte Glycoprotein in H-2b Mice

Carole Bourquin,* Anna Schubart,* Stephanie Tobollik,* Ian Mather,† Sherry Ogg, † Roland Liblau,‡ and Christopher Linington§

Autoantibodies directed against conformation-dependent epitopes of the extracellular domain of the myelin oligodendrocyte glycoprotein (MOG\textsuperscript{ex}) play a major role in the immunopathogenesis of demyelination in experimental autoimmune encephalomyelitis. We now demonstrate that one or more genes encoded within the MHC selectively censor the ability of H-2b mice to mount this conformation-dependent autoantibody response, while leaving T and B cell responses to linear MOG\textsuperscript{ex} epitopes intact. This novel form of selective B cell unresponsiveness discriminates between pathogenic and nonpathogenic Ab responses to MOG and determines whether or not Ab-dependent effector mechanisms play an important role in the pathogenesis of MOG-induced experimental autoimmune encephalomyelitis in the mouse. The Journal of Immunology, 2003, 171: 455–461.

Experimental autoimmune encephalomyelitis (EAE)\textsuperscript{1} induced by immunization with CNS Ags in CFA is an autoimmune disease that reproduces many of the clinical and pathological features of multiple sclerosis. Myelin-specific Th1 T cells initiate the inflammatory response in EAE, but the formation of large plaques of persistently demyelinated gliotic scar tissue is mediated by autoantibody responses directed against the surface of the myelin sheath (1). The major autoantigen targeted by this demyelinating autoantibody response in EAE is the myelin oligodendrocyte glycoprotein (MOG), a quantitatively minor myelin protein located at the outermost surface of the oligodendrocyte/myelin continuum (2, 3).

The MOG-specific Ab response is complex and recognizes both linear and conformation-dependent epitopes. However, the demyelinating component of this response is restricted to conformation-dependent epitopes present on the extracellular Ig-like domain of the protein (MOG\textsuperscript{ex}), whereas Abs recognizing linear MOG\textsuperscript{ex} peptides are unable to bind to the native protein and are unable to initiate demyelination in vivo (4, 5). The ability of MOG to trigger a demyelinating autoantibody response in EAE indicates that autoaggressive MOG\textsuperscript{ex}-specific B cell clones are not eliminated from the immune repertoire. This lack of tolerance is attributed to the localization of MOG within the immunologically privileged environment of the CNS, where it is sequestered from normal lymphocyte trafficking and therefore unable to trigger Ag-specific B cell tolerance (6). However, while MOG itself may not induce tolerance, a recent study identified a MOG-independent tolerogenic effect that influences the composition and pathogenicity of the MOG-specific B cell repertoire (7).

The MOG-specific hybridoma 8.18C5 secretes a conformation-dependent MOG\textsuperscript{ex}-specific mAb that can mediate demyelination in vivo in animals with EAE (4, 8). This Ab was derived from BALB/c (H-2\textsuperscript{a}) mice immunized with rat CNS glycoproteins, and its particular combination of H and L chains is clearly permissible on this parental background (9). In transgenic C57BL/6 (H-2\textsuperscript{b}) mice carrying the IgH chain of mAb 8.18C5, the transgenic H chain can pair with endogenous L chains to provide a functional MOG-specific B cell repertoire that differentiates without tolerogenic censure (6). However, attempts to replicate the fine specificity of 8.18C5 in C57BL/6 mice by simultaneously expressing both H and L chains were thwarted by the elimination of the transgenic L chain by receptor editing during development (7). This tolerogenic effect was also observed when the transgenic H and L chains were introduced into MOG-deficient mice, indicating that receptor editing was MOG independent and must therefore be mediated by some other self Ag.

We speculated that this effect might result in strain-specific differences in the pathogenicity of the MOG-specific Ab response. To test this hypothesis, we compared the B cell response to MOG\textsuperscript{ex} in different mouse strains using a combination of ELISA, FACS, and complement-dependent cytotoxicity assays to differentiate between effects on Ab responses to linear (nonpathogenic) epitopes and conformation-dependent (pathogenic) responses to the native protein. We now report that one or more genes encoded within the MHC selectively censor the ability of H-2b mice to mount a pathogenic conformation-dependent autoantibody response to MOG. This effect does not influence the ability of H-2b mice to generate T cell and Ab responses to linear MOG epitopes and is not mediated by MOG polymorphisms that might influence expression of the target autoantigen in immune tissues. This novel form of selective tolerance discriminates between conformational and linear
epitopes and determines the ability of mice to mount a pathogenic autoantibody response to MOG.

Materials and Methods

Mice

Female SJL (H-2b) and C57BL/6 (H-2d) mice were purchased from Charles River (Sulzfeld, Germany), and MHC congenic female mice C57BL/10 (H-2b) and B10.S (H-2a), BALB/c (H-2a), and BALB/b (H-2b) from Harlan Winkelmann (Borchen, Germany). F1 (SJL × C57BL/6) mice were bred in the animal facility at the Max-Planck-Institute for Neurobiology (Martinsried, Germany). C57BL/6 (H-2d) MOG knockout mice and sex-matched wild-type controls were bred in the Salpêtrière Hospital animal facility (Paris, France).

Antigens

The expression and purification of rat rMOG\textsuperscript{ld} were performed, as described previously (10). The exoplasmic domain of butyrophilin (BTN\textsuperscript{exo}, aa 1–216) was cloned and expressed as a soluble C-terminal hexahistidine-tagged recombinant baculovirus product (Invitrogen, San Diego, CA) and purified from the supernatant of infected High Five insect cells (BD Pharmingen, San Diego CA) by metal chelate chromatography. Peptides were purchased from Sigma/Genosys (Cambridge, U.K.).

Immunization protocols

Mice were immunized into the base of the tail with a single s.c. injection of an emulsion containing 100 µg Ag (OVA or rMOG\textsuperscript{ld} in IFA), or with 100 µg MOG\textsuperscript{ld} in CFA (Life Technologies, Rockville, MD) supplemented with 4 mg/ml inactivated Mycobacterium tuberculosis (H37 RA; Difco Laboratories, Detroit, MI). Animals were monitored regularly for clinical signs of EAE (0, no clinical disease; 1, tail weakness; 2, paraparesis; 3, paraplegia; 4, paraplegia with forelimb weakness or paralysis; 5, moribund state or death). Moribund animals were sacrificed within 24 h in accordance with federal care regulations.

Construction of expression vectors and DNA vaccination

cDNA of murine MOG was obtained, as described previously, by reverse transcription and PCR amplification of RNA isolated from mouse brain using primers that generated a fragment containing the full-length MOG coding sequence together with its signal sequence (11). A chimeric BTN-MOG cDNA construct was generated by sequential PCR amplification using the murine MOG template described above and a bovine BTN cDNA. This chimeric construct encodes an optimized Kozak sequence, followed by aa 1-216 of bovine BTN, including the signal sequence and entire exoplasmic domain, fused in-frame to the transmembrane sequence and cytoplasmic tail of mouse MOG (aa 118–218). The PCR products were cloned into the expression vector pcDNA3.1 (Invitrogen, San Diego, CA) and plasmid DNA purified from the transformed Escherichia coli strain DH5\textsubscript{x} by miniprep (Qiagen, Hilden, Germany) and sequenced to verify identity. Large-scale preparation of plasmid DNA was conducted using Qiagen EndoFree Plasmid Kits (Qiagen). Each preparation was checked by agarose gel electrophoresis, and DNA concentration was determined by agarose gel electrophoresis, and DNA concentration was determined by spectrophotometry. DNA purity was checked by agarose gel electrophoresis, and DNA concentration was determined by spectrophotometry.

T cell proliferation and ELISA

A single cell suspension was prepared from the draining lymph nodes 10 days post infection (p.i.) and cultured in DMEM supplemented with glutamine, sodium pyruvate, penicillin, streptomycin, nonessential amino acids, and 2-ME (complete DMEM) containing 1% mouse serum in the presence of rMOG\textsuperscript{ld} or 2.5 µg/ml Con A. Proliferation assays were performed using 2 × 10\textsuperscript{5} cells/well in 96-well plates in a total volume of 200 µl. Ag-specific proliferation was assessed by [\textsuperscript{3}H]thymidine incorporation for the last 16–18 h of a 3-day culture period using a Packard Matrix 96 Direct beta counter (Meriden, CT). For cytokine assays, 10\textsuperscript{5} cells/well were cultured in 1 ml complete DMEM in 24-well plates, and the supernatant was collected after 48 h and stored at −80°C. Cytokine ELISA kits were purchased from Endogen (Biorad, Eching, Germany), and assay was performed according to the manufacturer’s instructions. All assays were performed in triplicate. Ab levels were determined by ELISA using serum obtained from peripheral blood and stored at −20°C. Ninety-six-well vinyl assay plates were coated with 10 µg/ml of rMOG\textsuperscript{ld}, BTN\textsuperscript{exo}, or peptide in PBS. After blocking with 1% BSA, the assay plates were incubated with test serum. Alkaline phosphatase-conjugated goat anti-mouse IgG (Southern Biotechnology Associates, Birmingham, AL) was used to detect specific binding. Color was developed by the addition of p-nitrophenyl phosphate, and the OD was read at 405 nm.

FACS analysis

The expression vector pcDNA3.1 (Invitrogen) containing the complete mouse MOG cDNA was transfected into a mouse myeloma cell line (Ag8) to generate a MOG-expressing target cell line (Ag8/MOG), as described (11). Cells were maintained in complete DMEM with 10% FCS and 1 mg/ml G418. To detect Ab responses to native MOG\textsuperscript{ld}, transfected and untransfected cells were washed with PBS/1% FCS and incubated with test serum (1/30) for 1 h on ice. After washing, cells were stained with Cy2-labeled anti-mouse IgG (Dianova, Hamburg, Germany) for 1 h and washed again; propidium iodide was added to exclude dead cells; and cells were analyzed immediately by FACS (BD Biosciences).

Complement-dependent Ab-mediated cytotoxicity assay

Anti-MOG Ab-mediated cytotoxicity was determined by incubating in triplicate 2 × 10\textsuperscript{6} Ag8 or Ag8/MOG cells/well in complete DMEM/1% FCS containing test serum at a dilution of 1/30 in the presence or absence of freshly reconstituted rabbit complement (Behring, Marburg, Germany) at a final dilution of 1/30. After incubation for 45 min at 37°C with 10% CO\textsubscript{2}, cell viability was determined using the tetrazolium salt WST-1 (Roche, Germany), and absorbance was read at 450 nm.

Results

The MOG\textsuperscript{ld}-specific Ab response in H-2\textsuperscript{b} mice does not recognize the native protein expressed at the cell surface

To compare B cell responses to MOG\textsuperscript{ld} in H-2\textsuperscript{b} (C57BL/6 and C57BL/10) and H-2\textsuperscript{a} (SJL and B10.S) mouse strains, mice were immunized with rat rMOG\textsuperscript{ld} in IFA. Blood was collected 4 wk later, and the serum Ab response was analyzed by FACS to identify responses to the native murine protein expressed at the surface of a MOG-transfected mouse myeloma cell line. In addition, sera were analyzed by ELISA to determine the response to the recombinant Ag and to map its specificity using a panel of overlapping synthetic MOG\textsuperscript{ld} peptides.

FACS analysis revealed that H-2\textsuperscript{b} mice mounted an Ab response to native, glycosylated MOG\textsuperscript{ld} determinants expressed on the surface of MOG-transfected cells in vitro, whereas this response was absent in MOG\textsuperscript{ld}-immunized H-2\textsuperscript{a} mice (Fig. 1). In contrast, all four mouse strains were found to have mounted an Ab response to bacterially derived rat MOG\textsuperscript{ld} when assayed by ELISA, irrespective of their genetic background or MHC haplotype (Fig. 2A). Furthermore, epitope mapping using synthetic MOG peptides demonstrated that the Ab response to the recombinant protein recognized specific linear MOG\textsuperscript{ld} epitopes in all four strains (Fig. 2, B and C).

![FIGURE 1](http://www.jimmunol.org/DownloadedFrom/10.4049.jimmunol.96-0946/sf_1)
C57BL/6 and C57BL/10 mice both mounted a MOG IgG specific Ab response to aa sequences 1–26 and 50–74, and in addition C57BL/6 mice also recognized aa 27–50. In B10.S mice, the specificity of the Ab response was as previously described for SJL mice and recognized epitopes present in peptides 1–26 and 50–87 (Fig. 2, B and C) (11). These results demonstrate that the absence of a response to the native protein in H-2 b mice is not associated with a generalized lack of responsiveness to MOG IgG epitopes, but that H-2 b mice are selectively unresponsive to those MOG IgG conformation-dependent epitopes implicated in the pathogenesis of Ab-dependent demyelination (4, 5).

MOG-specific Ab responses are not cytotoxic in H-2 b mice

The inability of MOG IgG-specific Abs to bind to the native extracellular domain of MOG in H-2 b mice indicates that these Abs are unlikely to be cytotoxic. This was confirmed using sera from MOG IgG-immunized C57BL/10 and B10.S mice in an in vitro complement-dependent cytotoxicity assay. As previously described for SJL mice, sera from MOG IgG-immunized B10.S mice mediate the Ag-specific, complement-dependent lysis of MOG-transfected Ag8 cells in vitro (Fig. 3A) (11). In contrast, sera obtained from MOG IgG-immunized C57BL/10 mice did not exhibit Ag-specific cytotoxicity (Fig. 3A). No significant difference in cell survival was observed with any sera when assay was performed with untransfected target cells or in the absence of complement (data not shown). Absence of complement-dependent cytotoxicity in H-2 b mice was not due to the absence of a complement-fixing IgG2a Ab response, as immunization with MOG IgG in IFA induced MOG IgG-specific IgG1 and IgG2a isotypes in both mouse strains. High levels of both isotypes were detected in B10.S mice, whereas in C57BL/10 mice we observed a bias toward Abs of the IgG1 isotype (Fig. 3B).

The different capability of B10.S and C57BL/10 mice to mount a conformation-dependent and cytolytic Ab response to MOG IgG was found to correlate with their susceptibility to develop EAE following immunization with MOG IgG in IFA. This protocol is generally assumed not to induce disease, as it skews the immune response in favor of counterinflammatory Th2 T cell responses (12, 13). Nonetheless, five of six B10.S mice developed clinical signs of EAE between days 15 and 29 p.i. after immunization with MOG IgG in IFA (mean maximum score: 2, 1), whereas none of the six C57BL/10 mice immunized showed any clinical signs of disease in this time period (Fig. 3C).

**FIGURE 2.** The Ab response to MOG in H-2 b mice recognizes rMOG IgG and linear peptide determinants. A, IgG specific for rMOG IgG was measured by ELISA in naive and MOG IgG-immunized H-2 s and H-2 b mice. IgG specific for a panel of overlapping MOG IgG peptides was determined by ELISA in C57BL/6 mice (B) and in B10.S and C57BL/10 mice (C). All sera were pooled from three or more mice and diluted 1:100 4–5 wk after immunization with MOG IgG in IFA. Data are representative of at least two independent experiments.

**FIGURE 3.** The Ab response to MOG IgG in H-2 b mice is not cytotoxic. A, Survival of MOG-transfected target cells was measured after incubation with serum from naive and MOG IgG-immunized congenic mice in the presence of complement. Cell survival was determined by metabolization of the tetrazolium salt WST-1. Differences in cell survival between naive and immune sera were highly significant in B10.S mice (p < 0.01), but not in C57BL/10 mice (NS). B, MOG-specific IgG1 and IgG2a isotypes were determined by ELISA in the serum of naive and MOG IgG-immunized congenic mice 4–5 wk p.i. A and B, All sera were pooled from three or more mice and diluted 1/30 4–5 wk after immunization with MOG IgG in IFA. Data are representative of two independent experiments. C, Clinical course of EAE in MHC-congenic mice after immunization with MOG IgG in IFA.
Selective loss of responsiveness to conformation-dependent B cell epitopes is not associated with a defect in the Th1 T cell response

Previous studies demonstrated that both H-2<sup>a</sup> and H-2<sup>b</sup> mice can mount an encephalitogenic response to MOG T cell epitopes, indicating that the inability of H-2<sup>b</sup> mice to mount a conformation-dependent and pathogenic Ab response was not associated with T cell tolerance (14). This was confirmed following analysis of the T cell response in the draining lymph nodes of C57BL/10 and B10.S mice 10 days after immunization with MOG<sup>led</sup> in CFA. In accordance with other studies, both strains supported a strong and quantitatively similar Th1 MOG<sup>led</sup>-specific T cell response as determined by both T cell proliferation and production of IFN-γ (Fig. 4, A and B), but not of the Th2 cytokine IL-4 (data not shown). The effect of the H-2<sup>b</sup> haplotype on the conformational B cell response is therefore not associated with a haplotype-specific effect that influences the Th1 response to MOG<sup>led</sup>.

H-2<sup>b</sup> mice are unresponsive to MOG-DNA vaccination

To rule out that immunization with bacterially derived nonglycosylated rat MOG rather than the native murine protein was not responsible for the failure of H-2<sup>b</sup> mice to mount a conformation-dependent response to MOG<sup>led</sup>, we investigated the Ab response to murine MOG<sup>led</sup> following vaccination with a DNA construct encoding the full-length mouse autoantigen. DNA vaccination leads to expression of Ag by host cells in its native conformation and with appropriate posttranslational modifications, and elicits Abs directed to native epitopes (15). As described previously, MOG-DNA vaccination induced a strong autoantibody response to MOG<sup>led</sup> in SJL mice, which was shown to be purely conformation dependent and cytolytic (Fig. 5A) (11). In contrast, MOG-DNA vaccination did not induce any detectable MOG<sup>led</sup>-specific Ab response in C57BL/6 mice (Fig. 5A), although vaccination with DNA encoding a control protein, bovine BTN, induced a strong Ab response in both C57BL/6 and SJL/J mice (Fig. 5B). Bovine BTN exhibits both structural and sequence homologies to MOG<sup>led</sup> (Fig. 6), indicating that the failure of C57BL/6 to mount a B cell response to MOG<sup>led</sup> is highly discriminating and Ag specific.

We extended this study to investigate the Ab response to MOG following MOG-DNA vaccination in the MHC congenic mouse strains B10.S and C57BL/10, and BALB/b and BALB/c. Once again, the H-2<sup>b</sup> MHC haplotype was associated with an inability to mount a MOG<sup>led</sup>-specific Ab response following MOG-DNA vaccination (Table I). To investigate whether the effect of the H-2<sup>b</sup> allele was dominant, we examined the Ab response to MOG-DNA vaccination in 13 F<sub>1</sub> (SJL × C57BL/6) mice. Intriguingly, in F<sub>1</sub> mice, the MOG<sup>led</sup>-specific IgG levels were approximately one-half those obtained in SJL/J mice (n = 6), indicating that a gene dosage effect may be operating (average OD<sub>405 nm</sub> assessed for individual mice: SJL, 0.78; F<sub>1</sub> (SJL × C57BL/6), 0.45; p < 0.01).

Unresponsiveness to MOG-DNA vaccination is independent of the MOG gene

MOG is itself located within the telomeric region of the MHC, raising the possibility that unresponsiveness to MOG-DNA vaccination in H-2<sup>b</sup> mice was due to an H-2<sup>b</sup>-specific MOG polymorphism (16). We therefore investigated the Ab response induced by MOG-DNA vaccination in MOG-deficient MOG<sup>-/-</sup> H-2<sup>b</sup> mice.

**FIGURE 4.** The MOG-specific T cell response in the MHC-congenic strains B10.S and C57BL/10 is similar. T cell cultures were prepared from lymph node cells 10 days after immunization with MOG<sup>led</sup> in CFA. A, T cell proliferation after 72-h incubation without Ag, with 2–50 μg/ml MOG<sup>led</sup>, or with Con A. B, Production of IFN-γ in cell culture supernatant after 48 h. Differences between the strains are not significant at both 10 and 50 μg/ml MOG<sup>led</sup> (A and B).

**FIGURE 5.** H-2<sup>b</sup> mice are unresponsive to MOG-DNA vaccination. A, ELISA of the MOG<sup>led</sup>-specific IgG Ab response in serial dilutions of serum from MOG-DNA-vaccinated mice 28 days p.i. Sera were pooled from three or more mice. Data are representative of three separate experiments. B, BTN-specific serum IgG Ab response in control DNA- and BTN-DNA-vaccinated mice 28 days p.i. (Sera were assayed individually. BTN-DNA: C57BL/6, n = 5; SJL, n = 2. Control DNA: C57BL/6, n = 4; SJL, n = 1.)
MOG-DNA vaccination exacerabates EAE in SJL mice, but not in C57BL/6 mice

We previously demonstrated that the autoantibody response induced by MOG-DNA vaccination in SJL mice enhances the severity of EAE irrespective of the identity of the encephalitogen (11). We predicted that because C57BL/6 mice are nonresponders to MOG-DNA vaccination, we would not see a similar potentiation of disease in this strain. In SJL mice vaccinated with MOG-DNA, the onset of EAE induced by immunization with the proteolipid protein peptide 139–154 was 3–4 days earlier than in mice vaccinated with control DNA, and disease severity was increased (Fig. 7A). In contrast, prior vaccination with MOG-DNA had no significant effect on either onset or severity of EAE in C57BL/6 mice immunized with the encephalitogenic peptide MOG 55–55 (Fig. 7B).

Discussion

Our study identifies a highly discriminating mechanism in H-2b mice that differentiates between conformation-dependent and linear MOG 55–55 B cell epitopes, and selectively censors their ability to generate a cytolytic, conformation-dependent autoantibody response to this CNS autoantigen. This effect was seen not only after active immunization with rat MOG 55–55 in adjuvant, but also following vaccination with a full-length mouse MOG cDNA construct. The importance of the conformation-dependent component of the MOG-specific B cell repertoire in the pathogenesis of Aβ-mediated demyelination was first shown using a panel of MOG-specific mAbs derived from BALB/c (H-2b) mice sensitized with myelin glycoproteins isolated under non-denaturing conditions (9, 17). These mAbs induce demyelination in vivo when passively transferred into animals with EAE and are specific for conformation-dependent MOG 55–55 epitopes (4, 17). Recently, studies in the marmoset further established that recognition of conformational features is a prerequisite for the pathogenicity of the MOG 55–55-specific Ab response (5).

Our analysis of H-2b mice actively immunized with MOG 55–55 supports this interpretation. The MOG-specific Ab response in the H-2b strains C57BL/6 and C57BL/10 only recognizes linear MOG 55–55 epitopes that are not accessible to Ab when the protein is in its native conformation. Consequently, H-2b-derived MOG 55–55-specific Abs are incapable of activating complement at the membrane surface and lysing MOG-expressing target cells in vitro. This is in stark contrast to the MOG 55–55-specific Ab response in H-2d (SJL/J, B10.S) and H-2b (BALB/c) mice that has a significant conformation-dependent and potentially cytopathic component.

The inability of C57BL/10 mice to mount a cytolytic Ab response to MOG may be one factor that contributes to their resistance to EAE induced by immunization with MOG 55–55 in IFA. This protocol is generally considered only weakly encephalitogenic, as it induces a bias toward a counterinflammatory Th2 T cell response, but nonetheless, this protocol will induce severe EAE in the context of an appropriate genotype (12, 13). In the current study, immunization with MOG 55–55 in IFA skewed Ig isotype usage in favor of a dominant Th2-associated MOG 55–55-specific IgG1 response in C57BL/10 mice, suggesting a marked difference in the ability of B10.S and C57BL/10 mice to develop Th2 T cell responses to this autoantigen. Although complement fixation by MOG-specific Abs is isotype dependent, it has been demonstrated using a panel of mAbs that IgG1 responses to MOG can fix complement and mediate demyelination in vivo (8, 17). This suggests that the shift in Ig isotype usage in H-2b mice is unlikely to be responsible for the inability of this strain to mount a cytolytic Ab response to MOG. This MHC-dependent effect on the balance of Th1 to Th2 T cell responses to MOG is currently under investigation in our laboratories, and may provide a murine parallel of

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a Determination of the MOG-specific IgG response by ELISA 4–6 wk after MOG-DNA vaccination in different strains of mice. All sera were assayed individually at a dilution of 1/100, and the average OD 405 is presented.
MHC-dependent effects on susceptibility to MOG-induced EAE in the rat (18, 19).

The mechanism that censors the conformation-dependent Ab response to MOG in H-2b mice is still a matter of speculation. The responsible genes are encoded within the MHC, the sequence, organization, and function of which are now understood in great detail. MOG was itself considered a candidate gene, as it is located within the MHC, and low levels of MOG transcription are reported to occur within primary and secondary lymphoid organs (3, 16, 20). These observations raised the possibility that strain-specific differences in MOG expression outside the CNS could influence the composition of the B cell repertoire. However, ablation of MOG expression in H-2b mice fails to restore the conformation-dependent Ab response, indicating that loss of responsiveness is not mediated by MOG itself. The MHC also encodes multiple gene products involved in Ag degradation, processing, and presentation. Polymorphic variation affecting these pathways could either result in the degradation of the target structure on the protein or generate a MOG-specific T cell repertoire that fails to provide efficient T cell help for the conformation-dependent Ab response in H-2b mice. Although these possibilities cannot be excluded at present, lack of T cell help appears unlikely, as the MHC congenic strains C57BL/10 and B10.5s mount quantitatively and qualitatively similar T cell responses to MOG.

An alternative mechanism is that the presence of a conformational mimic of MOG encoded within the MHC mediates this tolerogenic event. B cell tolerance relies heavily upon the elimination of self-reactive B cells with defined specificities during development in the bone marrow and subsequently in the periphery during B cell differentiation. Negative selection is normally discussed in terms of the induction of tolerance by either clonal deletion, anergy, or receptor editing mediated through contact with the nominal autoantigen (21). However, in analogy to molecular mimicry in which structural and sequence homologies between foreign and self Ags can trigger autoimmunity and in some cases tolerance, it is possible that tolerance to one self Ag may be induced by cross-reactivity of the B cell receptor with another component of self (22–24). The demonstration that the transgenic L chain of a demyelinating MOG-specific mAb is eliminated by receptor editing in H and L chain double-transgenic B cells in MOG-deficient C57BL/6 mice suggests that cross-reactivity does occur (7).

Although the identity of this putative cross-reactive autoantigen is unknown, candidate Ags may be encoded within a cluster of BTN family genes located between the classical MHC class II and III regions of the murine MHC (25). The N-terminal Ig-like domain of these BTN-like proteins has a very high level of sequence and structural homology to MOG (16). Moreover, the genes encoding these proteins are polymorphic, and preliminary data indicate that there are strain-specific differences in their expression patterns between H-2b and H-2d mice (M. Pagany, unpublished observations). Active immunization with bovine BTN itself induces an encephalomyelitis in the Dark Agouti rat due to molecular mimicry with MOG (26). This immunological cross-reactivity is, however, restricted to the T cell response to these Ags. BTN itself is not encoded within the murine MHC, indicating that this milk protein is not responsible for the effects described in this study. Whatever the mechanism responsible for censoring the pathogenic Ab response to murine MOG in H-2d mice, a recent study suggests that it is highly sensitive to amino acid substitutions that may subtly alter the conformation of the extracellular domain, as C57BL/6 mice can mount a pathogenic Ab response to the human protein (27). Murine and human MOG (MOG) differs at only 11 aa positions, including a S > P substitution at position 42 (Fig. 6). Which of these amino acid substitutions is responsible for the ability of the human protein to induce this pathogenic response has still to be determined. Although negative selection is an essential step in selecting the B cell repertoire, there is also evidence that immature B cells undergo positive selection dictated by the specificity of their Ig receptor (28). Using IgH chain transgenic mice, these authors find evidence of positive selection within a small fraction of the mature B cell receptor repertoire (28), a situation analogous to the selection of the T cell repertoire that also depends on a combination of positive and negative selection steps. Intriguingly, F1 (SJL × C57BL/6) mice develop MOG-specific Ab titers following MOG-DNA vaccination that are intermediate to those obtained in the parental responder (H-2d) and nonresponder (H-2b) strains, suggesting a gene dosage effect that could be compatible with either partial negative or enhanced positive selection.

In summary, using MOG as a model for B cell-mediated autoimmunity in the CNS, we demonstrate the existence of a genetically determined mechanism that renders H-2d mice selectively unresponsive to pathogenic conformation-dependent B cell epitopes of MOG. This MHC-dependent effect is not associated with an effect on either the response to nonpathogenic linear MOG B cell epitopes or the MOG (MOG)-specific T cell response. This mechanism may account in part for the observation that conformation-dependent Ab responses to MOG (MOG) are only detectable in a small percentage of multiple sclerosis patients (29).

Note: While this paper was in preparation, we became aware of additional data supporting our observation that C57BL/6 mice do not make a pathogenic autoantibody response to rat MOG (30).

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References


