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Myelin Oligodendrocyte Glycoprotein Induces Experimental Autoimmune Encephalomyelitis in the “Resistant” Brown Norway Rat: Disease Susceptibility Is Determined by MHC and MHC-Linked Effects on the B Cell Response

Andreas Stefferl, Uschi Brem, Maria Storch, Doris Lambracht-Washington, Carole Bourquin, Kurt Wonigeit, Hans Lassmann, and Christopher Linington

Experimental autoimmune encephalomyelitis (EAE) induced by active immunization with the myelin oligodendrocyte glycoprotein (MOG) is an Ab-mediated, T cell-dependent autoimmune disease that replicates the inflammatory demyelinating pathology of multiple sclerosis. We report that disease susceptibility and severity are determined by MHC and MHC-linked effects on the MOG-specific B cell response that mediate severe clinical EAE in the EAE-resistant Brown Norway (BN) rat. Immunization with the extracellular domain of MOG in CFA induced fulminant clinical disease associated with widespread demyelination and with an inflammatory infiltrate containing large numbers of polymorphonuclear cells and eosinophils within 10 days of immunization. To analyze the effects of the MHC (RTI I system) we compared BN (RTI ) rats with Lewis (LEW) (RTI ) and two reciprocal MHC congenic strains, LEW.1N (RTI ) and BN.1L (RTI ). This comparison revealed that disease severity and clinical course were strongly influenced by the MHC haplotype that modulated the pathogenic MOG-specific autoantibody response. The intra-MHC recombinant congenic strain LEW.1R38 demonstrated that gene loci located both within the centromeric segment of the MHC containing classical class I and class II genes and within the telomeric RTI.M region containing the MOG gene are involved in determining Ab production and disease susceptibility. This study indicates that the current T cell-centered interpretation of MHC-mediated effects on disease susceptibility must be reassessed in multiple sclerosis and other autoimmune diseases in which autoantibody is involved in disease pathogenesis. The Journal of Immunology, 1999, 163: 40 – 49.

The influence of the MHC in determining susceptibility to autoimmune diseases was first described for experimental autoimmune encephalomyelitis (EAE) (1), a prototypic T cell-mediated autoimmune disease of the CNS induced by active immunization with CNS tissue homogenates or purified myelin Ags (2). Genetic analysis of back-cross generations between EAE-susceptible Lewis (LEW) and EAE-resistant Brown Norway (BN) rats revealed that the LEW (RTI B/D) class II MHC haplotype was associated with disease susceptibility, whereas both the BN class II MHC haplotype (RTI B/D) and the BN non-MHC genetic background were associated with resistance (1, 3, 4).

The mechanism by which MHC genes modulate susceptibility to autoimmune diseases is generally discussed in terms of the effects of class I and II MHC alleles on the selection, activation, or effector function of the T cell repertoire (2, 5, 6). However, this interpretation is derived almost solely from EAE models in which disease is T cell mediated (2) and is independent of the B cell response (7). In contrast, human diseases such as multiple sclerosis (MS) are generally far more complex in that both cellular and humoral immune effector mechanisms are involved in disease pathogenesis (8–12).

This complex immunopathology is reproduced in rats with EAE induced by active immunization with the myelin oligodendrocyte glycoprotein (MOG) (13–15). MOG is a unique myelin autoantigen in that it induces both an encephalitogenic T cell response and a demyelinating autoantibody response in rodents and primates. In the LEW rat, the T cell response to MOG is only weakly encephalitogenic (16), and disease induction exhibits an absolute requirement for the MOG-specific autoantibody response (13, 16). The formation of demyelinating lesions depends on synergy between the MOG-specific T cell and autoantibody responses (13, 16). The T cell response initiates a subclinical inflammatory reaction in the CNS, disrupting the blood-brain barrier and allowing Ab to enter the CNS compartment. Activation of the complement cascade by MOG-specific Ab bound to the myelin surface then initiates demyelination while at the same time enhancing the local inflammatory response through the production of proinflammatory factors.

These observations led us to suspect that genetic susceptibility to MOG-induced EAE may be independent of MHC effects on the T cell response. We therefore investigated the susceptibility of the BN rat to MOG-induced EAE, given that this rat strain exhibits a...
generalized resistance to many purely T cell-mediated autoimmune diseases (1, 17, 18) but is susceptible to autoantibody-mediated diseases (19, 20). Surprisingly, despite its pronounced resistance to other models of EAE (1, 3), active immunization with the extracellular Ig-V-like domain of MOG (13) in CFA induced a lethal demyelinating disease of the CNS. We demonstrate that clinical disease was mediated by a high titer MOG-specific autoantibody response that overwhelmed the protective capacity of the BN genotype that suppresses disease induction by purely T cell-mediated effector mechanisms. Analysis of congenic strains either carrying the BN MHC haplotype (RT1+) on the genetic background (LEW.1N) or the MHC haplotype of LEW (RT1-) on the BN background (BN.1L) confirmed that the severity and clinical course of MOG-induced EAE were modulated by an MHC or MHC-linked effect on the anti-MOG Ab response, rather than a direct effect on the pathogenicity of the encephalitogenic T cell response. This response pattern is determined by genes located within both the centromeric part of the MHC including RT1A–RT1.C and the RT1.M region located telomeric of RT1.C, as demonstrated by the intra-MHC recombinant LEW.1R38 rat strain. MOG-induced EAE is therefore an Ab-mediated, T cell-dependent autoimmune disease of the nervous system in which the B cell rather than the T cell response can determine disease susceptibility. Moreover, both MHC and MHC-linked effects influence this B cell response and thereby modulate disease penetrance and severity. These observations suggest that the functional significance of disease-associated MHC alleles in MS should be reassessed in the context of the MOG-specific Ab response.

Materials and Methods

Animals and Ags

BN (RT1 +/-) rats (120–200 g) were obtained from Charles River (Sulzfeld, Germany), congenic LEW.1N (RT1 +/-) and BN.1L (RT1 +/-) rats were provided by Prof. H. J. Hedrich (Medizinische Hochschule Hannover, Germany), and LEW rats were obtained from the animal facility of the Max-Planck Institute for Biochemistry (Martinsried, Germany). For the BN.1L strain, a small deletion in the RT1.C region has previously been demonstrated; therefore its MHC haplotype is also classified as RT1.C (21). The LEW.1R38 (RT1 +/-) strain has been developed from the L x B17 recombinant inbred strain (22) by serial back-crossing on the LEW background and is maintained in the rat colony of one of the authors (K.W.).

CFA, IFA, and heat-killed Mycobacterium tuberculosis (H37Ra) were purchased from Difco (Detroit, MI). Purified protein derivative was purchased from Statens Serum Institut, Copenhagen, Denmark. Recombinant protein (MOG) corresponding to the Ig-domain (Igd) of rat MOG (aa 1–125) was expressed in Escherichia coli and purified to homogeneity (13). The purified protein dissolved in 6 M urea was dialyzed against 20 mM sodium acetate buffer (pH 3.0) to obtain a soluble preparation that was stored frozen at −20°C. The following murine mAbs were used: W3/25 (CD4); OX22 (CD45RC); R73 (rat αβ TCR); W3/25 was purified by protein G affinity chromatography from the culture supernatants of the appropriate hybridoma cell lines. OX22 and R73 were purchased from Canom, Wiesbaden, Germany.

Immunization protocols

Rats were immunized s.c. at the base of the tail with 50 μg MOG emulsified in CFA containing 225 μg of heat-killed M. tuberculosis (H37Ra) in a total volume of 100 μl. Animals were weighed and examined daily for clinical signs of EAE that were scored on the following scale: 0.5, partial loss of tail tone; 1, complete tail atony; 2, hind limb weakness; 3, hind limb paralysis; 4, moribund; 5, dead.

Generation of T cell lines and adoptive transfer experiments

MOG-specific T cell lines were generated as described previously (13). Briefly, the draining lymph nodes were removed 10 days postimmunization (dpi), and a single-cell suspension was cultured for 72 h at a concentration of 10^7 cells/ml in DMEM supplemented with glutamine, penicillin-streptomycin, sodium pyruvate, essential amino acids (Life Technologies), and 1% rat serum in the presence of the selecting Ag (20 μg/ml). T cell blasts were then isolated by density gradient centrifugation and propagated for a further 5–10 days in medium containing 15% supernatant of MLA cells (T cell growth factor (23)). Ag-specific T cell lines were subsequently maintained by cycles of Ag-specific restimulation using irradiated (5000 rad) syngeneic thymus cells as APCs, followed by expansion in T cell growth factor.

Adoptive transfer experiments were performed using freshly activated T cell blasts suspended in a volume of 1 ml DMEM. T cells were injected into the tail vein of rats anesthetized with ether. In some experiments, rats were subsequently injected i.v. with 100 mg/kg rat IgG purified by sodium sulfate precipitation from the pooled sera of rats immunized 11 days earlier with either CFA-buffer or CFA-MOG.

Enzyme-linked immunosorbent assay

Blood was collected at the times stated in the text by cardiac puncture immediately before perfusion. After clotting at 4°C, serum was collected by centrifugation and stored at −20°C. ELISA was performed with polystyrene 96-well PVC plates (Costar, Cambridge, MA) coated with 5 μg/ml Ag (3 h, 37°C) in 50 mM carbonate-bicarbonate buffer, pH 9.6. The plates were washed with PBS (0.05% Tween 20, Sigma, Deisenhofen, Germany) and blocked with 1% BSA in PBS (pH 7.4) overnight at 4°C. After washing with PBS-Tween, 100 μl of serial serum dilutions in PBS were incubated for 4 h at 37°C. Total anti-MOG levels were determined directly using 100 μl peroxidase-conjugated rat IgG and IgM-specific goat Ab (1:4000). Isotype-specific anti-MOG Ab levels were determined using 1:4000 dilutions of a panel of mouse mAbs specific for rat IgM, IgG1, Ig2a (Serotech, Oxford, U.K.) followed by a mouse-specific peroxidase conjugate (1:8000 in PBS, Dianova, Hamburg, Germany). To determine the levels of IgE, 2% dried milk powder in PBS was used as a blocking agent. Furthermore, the samples and a goat serum specific for rat IgE (1:5000) (Dunn, Asbach, Germany) as well as a HRP-conjugated donkey anti-goat serum (1:2000, Dianova) were diluted in PBS containing 0.1% milk powder. All plates were developed with o-phenylenediamine dihydrochloride (Sigma), the reaction was stopped with 3 M HCl, and optical density was determined at 490 nm.

Cytokine, proliferation, and RT-PCR assays

Proliferation assays were performed in flat-bottom 96-well tissue culture plates in a total volume of 200 μl using either 5 × 10^5 lymph node cells (LNCs) or 2 × 10^4 T cells plus 5 × 10^5 syngeneic, irradiated (5000 rad) thymus cells as APCs. Ag-specific proliferation was assessed by the incorporation of [3H]thymidine (10 μCi/well) during the final 16 h of a 72-h

---

Table I. Clinical course of MOG-induced EAE in LEW, BN, and reciprocal MHC-congenic rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 Haplotype</th>
<th>n</th>
<th>Day of Onset</th>
<th>% Surviving (20 dpi)</th>
<th>Mean Score* (20 dpi)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN.1L</td>
<td>l</td>
<td>5</td>
<td>17.4 ± 1.0</td>
<td>100</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>LEW</td>
<td>l</td>
<td>8</td>
<td>15.1 ± 1.3</td>
<td>90</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>LEW.1R38</td>
<td>r38</td>
<td>6</td>
<td>13.0 ± 0.6</td>
<td>60</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>BN</td>
<td>n</td>
<td>7</td>
<td>12.1 ± 0.4</td>
<td>0</td>
<td>&gt;4*</td>
</tr>
<tr>
<td>LEW.1N</td>
<td>n</td>
<td>10</td>
<td>10.1 ± 0.9</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* After exhibiting grade ≥3.5 disease for 24 h, animals were euthanized, and their final clinical score was used for calculating the means.

In both BN and LEW.1N, the disease course was fulminant, and all animals were euthanized between 12 and 17 dpi.
culture period using a Packard Matrix 96 Direct β counter. After 72 h of production of IFN-γ, TNF-α, IL-10, and IL-4 was determined with commercial ELISA assays (Laboserv, Staufenberg, Germany) in cell culture supernatants.

IL-4 mRNA was analyzed in LNCs after 24 or 72 h of in vitro culture in the presence or absence of Ag. Total RNA was extracted from 5 x 10⁶ cells, and 1 μg was reversely transcribed into cDNA (Superscript, Life Technologies, Gaithersburg, MD). Fifty nanograms of cDNA served as a template in PCR (annealing, 60°C, 45 s; elongation, 72°C, 45 s; denaturation, 94°C, 30 s; 35 cycles). The primers were designed to amplify fragments of 700 bp for β-actin and 177 bp for IL-4. β-Actin forward, 5'-TGC TAG GAG CCA GGG CAG TAA TC-3'; β-actin reverse, 5'-TAC AAT GAG CTG CGT GTG GCC-3'. IL-4, commercially available primer-pair (Laboserv, Staufenberg, Germany).

**Histopathological analysis**

Histological evaluation was performed on paraformaldehyde-fixed, paraffin-embedded sections of brains, and spinal cords were sampled at various time points of disease. Paraffin sections were stained with hematoxylin-eosin, Luxol fast blue, and Bielschowsky silver impregnation to assess inflammation, demyelination, and axonal pathology. In adjacent serial sections, immunohistochemistry was performed with Abs against following targets: macrophages/activated microglia (ED1; Serotec), T-cells (W3/13; Sera-Lab, Crawley Down, U.K.), C9 (24), rat Ig (biotinylated anti-rat, Amersham, Little Chalfont, U.K.). Bound primary Ab was detected with a biotin-avidin technique previously described in detail (25). Control sections were incubated in the absence of primary Ab or with nonimmune rabbit serum.

The following histopathological parameters were evaluated: 1) inflammatory index: the mean number of perivascular inflammatory infiltrates
derived from an average of 15 complete cross-sections of the spinal cord of an individual animal; 2) demyelination: demyelination in the spinal cord according to the following grading system: traces of perivascular or subpial demyelination (0.5), marked perivascular or subpial demyelination (1), confluent perivascular or subpial demyelination (2), massive confluent demyelination (e.g., one-half the cross-section of spinal cord) (3), extensive demyelination (transverse myelitis) (4).

Results

The “EAE-resistant” BN rat is highly susceptible to MOG-induced EAE

Despite their resistance to EAE induced by active immunization with either myelin basic protein (MBP) or spinal cord tissue (1, 3, 4), we observed that BN rats were highly susceptible to EAE induced by sensitization with rMOG in CFA (Table I). Immunization with 50 µg MOG resulted in severe clinical disease, which progressed rapidly with virtually all animals reaching a clinical score of ≥3 within 48 h of onset. In comparison, the incidence of MOG-induced disease was lower and disease onset and progression were delayed in the “EAE-susceptible” LEW rat (Table I).

Histopathological analysis revealed the presence of widespread inflammation and demyelination throughout the CNS of BN rats with MOG-induced EAE (Fig. 1, Table II). The demyelinating lesions increased in size between days 10 and 14 postimmunization (pi) and were associated with the local deposition of C9 and

Table II. Representative pathology of MOG-induced EAE in LEW, BN, and MHC-congenic rats

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 Haplotype</th>
<th>n</th>
<th>Sample Time (dpi)</th>
<th>Inflammation (inflammatory index)</th>
<th>Spinal Cord Demyelination</th>
<th>Cellular Infiltrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEW</td>
<td>l</td>
<td>4</td>
<td>11</td>
<td>No inflammation</td>
<td>1.25 ± 0.4</td>
<td>M &gt;&gt; PMN*</td>
</tr>
<tr>
<td>BN.1L</td>
<td>l</td>
<td>3</td>
<td>21–22</td>
<td>2.1 ± 1.3</td>
<td>1.2 ± 0.5</td>
<td>M &gt;&gt; PMN</td>
</tr>
<tr>
<td>LEW.1R38</td>
<td>r38</td>
<td>3</td>
<td>11</td>
<td>No inflammation</td>
<td>2.8 ± 1.0</td>
<td>M &gt;&gt; PMN</td>
</tr>
<tr>
<td>BN</td>
<td>n</td>
<td>4</td>
<td>15</td>
<td>3.26 ± 1.17</td>
<td>1.8 ± 0.5</td>
<td>PMN &gt;&gt; M</td>
</tr>
<tr>
<td>LEW.1N</td>
<td>n</td>
<td>2</td>
<td>11</td>
<td>0.5 ± 0.4</td>
<td>0.7 ± 0.7</td>
<td>PMN (EOS) &gt;&gt; M</td>
</tr>
</tbody>
</table>

* M, macrophages; EOS, eosinophils.

FIGURE 2. Representative spinal cord histopathology in the late stages (20 dpi) of MOG-induced EAE in the LEW rat. Inflammation (A, B) associated by demyelination (C) was present in the parenchyma adjacent to the pia mater. Inflammation consisted of mononuclear cells (B), the majority of which were macrophages (B, D), only a few T cells and occasional PMNs were detected (E). Serial sections were stained with hematoxylin-eosin (A, B); Luxol fast blue (C); ED1 for macrophages (D); and W3/13 for T cells and granulocytes (E). A, C, ×200; B, ×850; D, E, ×330.
TNF-α accompanied by similar amounts of the proinflammatory cytokine IL-10. RT-PCR analysis of IL-4 production in LNCs. IL-4 mRNA was not detected in LNCs derived from BN rats after both 24 or 72 h of in vitro culture. However, this was not accompanied by the Ag-specific induction of mRNA for IL-4 after stimulation with MOG in vitro (Table III), and the Ag-specific induction of mRNA for IFN-γ and TNF-α was also measured in cell culture supernatants after 72 h of culture in vitro. Cytokine production in the absence of Ag was generally below the detection limit (IL-4 ≥ 12 pg/ml, TNF-α ≥ 4 pg/ml, IFN-γ ≥ 13 pg/ml, IL-10 ≥ 20 pg/ml); measurable values were subtracted from the presented data. Cytokine and restimulation data are presented as the mean of at least two representative experiments (assays performed in triplicate). In vitro proliferation and cytokine production of LNCs a

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 Haplotype</th>
<th>No Ag (cpm)</th>
<th>MOG (cpm)</th>
<th>SI</th>
<th>IL-4 (pg/ml)</th>
<th>IL-10 (pg/ml)</th>
<th>IFN-γ (pg/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IFN-IL-10 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BN.1L</td>
<td>l</td>
<td>304 ± 45</td>
<td>1112 ± 75</td>
<td>3.8 ± 0.4</td>
<td>n.d.</td>
<td>383 ± 60</td>
<td>3144 ± 984</td>
<td>133 ± 92</td>
<td>8.2</td>
</tr>
<tr>
<td>LEW</td>
<td>l</td>
<td>965 ± 115</td>
<td>5069 ± 183</td>
<td>6.0 ± 1.2</td>
<td>n.d.</td>
<td>740 ± 202</td>
<td>7085 ± 866</td>
<td>105 ± 31</td>
<td>9.5</td>
</tr>
<tr>
<td>LEW.1R38</td>
<td>r38</td>
<td>157 ± 17</td>
<td>992 ± 72</td>
<td>6.3</td>
<td>n.d.</td>
<td>1903 ± 146</td>
<td>7821 ± 653</td>
<td>164 ± 10</td>
<td>4.1</td>
</tr>
<tr>
<td>BN</td>
<td>n</td>
<td>365 ± 35</td>
<td>4017 ± 463</td>
<td>10.5 ± 1.5</td>
<td>n.d.</td>
<td>1347 ± 144</td>
<td>5294 ± 857</td>
<td>148 ± 43</td>
<td>3.9</td>
</tr>
<tr>
<td>LEW.1N</td>
<td>n</td>
<td>368 ± 67</td>
<td>2465 ± 644</td>
<td>6.2 ± 1.2</td>
<td>n.d.</td>
<td>3393 ± 183</td>
<td>9625 ± 1348</td>
<td>441 ± 158</td>
<td>2.7</td>
</tr>
</tbody>
</table>

*LNCs were prepared 10 dpi as described in Materials and Methods. The MOG-specific production of IFN-γ, IL-10, IL-4, and TNF-α was analyzed in cell culture supernatants after 72 h of culture in vitro. Cytokine production in the absence of Ag was generally below the detection limit (IL-4 ≥ 12 pg/ml, TNF-α ≥ 4 pg/ml, IFN-γ ≥ 13 pg/ml, IL-10 ≥ 20 pg/ml); measurable values were subtracted from the presented data. Cytokine and restimulation data are presented as the mean of at least two representative experiments (assays performed in triplicate) apart from LEW.1R38 (a single experiment performed in quadruplicates). SI, stimulation index, n.d., not detected.

The cellular infiltrate associated with these lesions contained large numbers of polymorphonuclear cells (PMN), together with fewer macrophages and T cells (Fig. 1, A, B, E, and F). Most striking, and atypical for rat EAE, was the presence of many eosinophils in the inflammatory infiltrate, which accounted for 15–20% of inflammatory cells invading the CNS (Fig. 1, A and B). In contrast, ED1+ macrophages were the dominant cell type in the cellular infiltrate in the LEW rat, no eosinophils were present, and only a small number of PMNs were observed in the demyelinating lesions (Fig. 2A, B, D, and E; Table II).

The neurological deficit in MOG-induced EAE is not T cell mediated

The pathogenicity of the autoimmune response to MOG in the BN rat was surprising because this rat strain is resistant to a wide range of Th1 T cell-mediated autoimmune diseases of which EAE is the prototypic example. However, the presence of large numbers of PMNs and eosinophils in the CNS was reminiscent of the histopathology induced by autoimmune Th2, rather than Th1 T cell responses in the mouse (26), raising the possibility that a Th2 T cell response to MOG was responsible for initiating clinical disease. We therefore analyzed the primary response to MOG to define the involvement of Th2 and Th1 T cell responses in disease pathogenesis.

Both BN and LEW LNCs proliferate significantly in response to MOG in vitro (Table III), and the Ag-specific induction of mRNA transcripts for IL-4 was detected by RT-PCR in BN, but not LEW LNC cultures (Fig. 3). However, this was not accompanied by the secretion of detectable amounts of IL-4 into the culture supernatants, as determined with a commercially available ELISA kit for rat IL-4 (sensitivity, ≥12 pg/ml, Table III). In contrast, both rat strains mounted a significant Th1-like response to MOG, as demonstrated by the secretion of IFN-γ in response to MOG in vitro, accompanied by similar amounts of the proinflammatory cytokine TNF-α (Table III). It therefore appears unlikely that a Th2-like response to MOG played a significant role in disease induction after immunization with MOG in CFA in the BN rat.

Paradoxically, LNCs obtained from the highly susceptible BN rat also secreted approximately twice as much of the proinflammatory cytokine IL-10 after stimulation with MOG in vitro than LEW LNCs. The resulting ratios of IFN-γ to IL-10 secretion in BN and LEW were 3.9 and 9.5, respectively (Table III). This difference in the cytokine profile was maintained in short term MOG-specific T cell lines (TCLs, Table IV) composed of a dominant population (>85–95%) of CD4+ CD45R0+ and αβ TCR+ T cells (data not presented). LEW-derived TCLs secreted large amounts of IFN-γ (>100,000 pg/ml) and substantially less IL-10 (8200 pg/ml), whereas BN MOG-specific TCLs secreted lower but similar amounts of these two cytokines (IFN-γ, 5100 pg/ml; IL-10, 4200 pg/ml) (Table IV). No mRNA transcripts for IL-4 were detected in either BN or LEW TCLs (data not presented). However, despite these marked phenotypic differences neither LEW nor BN TCLs were able to mediate severe clinical EAE by adoptive transfer. Even at cell doses of 107 T line cells per recipient, the freshly activated TCLs induced only a mild, subclinical inflammatory response in the CNS (Table IV). These observations indicate that the CD4+ T cell response to MOG, irrespective of its cytokine profile, is not directly responsible for the clinical disease induced in either BN or LEW rats by active immunization with MOG in CFA.

Clinical expression of MOG-induced EAE is Ab dependent

The pathogenicity of MOG-specific autoantibodies is well established (13, 27), yet their functional significance in the pathogenesis of EAE is normally discussed in terms of demyelination rather than a primary mechanism responsible for disease induction. However, analysis of BN and LEW rats revealed an association between the kinetics/intensity of the MOG-specific autoantibody response and the timing of disease onset and course. In BN rats, the anti-MOG Ab developed rapidly between days 5 and 10 pi to reach a titer of between 1:20,000 and 1:30,000 by disease onset. This response was maintained until day 14 pi which because of the severity of disease was the last time point from which representative samples were available (Fig. 4a). In contrast, LEW rats the MOG-specific Ab response was delayed and had reached a titer of only ≤1:1,000 by day 10 pi (Fig. 4a). At this time, LEW rats exhibit no clinical or histopathological signs of EAE. However, by the time LEW rats developed clinical signs of EAE (days 14 to 17 pi; see Table I), the Ab titer had increased to 1:5,000–1:10,000. By 10 dpi, both strains had mounted a significant T cell response to MOG in the draining lymph nodes, despite this 20- to 30-fold difference in the intensity of the MOG-specific Ab response. Moreover, in both strains, Th1 (IgG2b (Fig. 4f)) and Th2 (IgG1 (Fig. 4d)) in vivo proliferation and cytokine production of LNCs a

![Image](http://www.jimmunol.org/)

**FIGURE 3.** RT-PCR analysis of IL-4 production in LNCs. IL-4 mRNA (a) could readily be detected in LNCs derived from BN rats after both 24 and 72 h of in vitro culture in the presence of MOG. In contrast, LEW-derived LNCs failed to produce measurable IL-4 mRNA. b, β-Actin; Co, negative control; L24, L72, LEW LNC after 24 or 72 h of in vitro culture; B24, B72, BN LNC after 24 or 72 h of in vitro culture.
and IgE (Fig. 4e)-associated Ig isotypes were affected to similar extents, indicating that there was no selective enhancement of the Th2-like response to MOG in BN.

We confirmed the pathogenicity of this Ab response in vivo by the adoptive transfer of the serum IgG fraction obtained from MOG/CFA-immunized BN rats 10 dpi into recipients previously injected with a MOG-specific TCL. Although the TCLs were alone only weakly pathogenic (Table IV), combination with the MOG-reactive IgG fraction induced clinical disease and CNS demyelination in all recipients (Figs. 5 and 6) but without duplicating the eosinophilic infiltrate observed in actively immunized BN rats.

MHC-linked effects on the B cell response determine the severity of MOG-induced EAE

The above results suggest that the ability of MOG to induce clinical EAE in BN depended on the induction of a high titer MOG-specific autoantibody response, rather than the primary T cell response to this autoantigen. As the MHC can modulate Ab responses, we extended this study to investigate the susceptibility of two reciprocal MHC congenic rat strains to MOG-induced EAE.

The LEW.1N strain carries the RT1 n haplotype of BN on the LEW background, the BN.1L strain the RT1 l haplotype of LEW on the BN background. The RT1 n haplotype clearly segregated with early disease onset, an acute fulminant disease course (Table I), and a high titer Ab response to MOG (Fig. 4). In contrast, the RT1 l haplotype was associated with later onset of disease, decreased susceptibility, and a delayed rise in anti-MOG Ab titers. This MHC-associated effect on the kinetics of anti-MOG Ab responses in LEW and BN affected both Th1- and Th2-associated Ig isotypes equally (Fig. 4, g and h). The dominant role of the autoantibody response in determining differences in disease onset/severity in LEW and BN

Table IV. Cytokine profile and pathogenicity of MOG-specific T cell lines

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 Haplotype</th>
<th>No Ag</th>
<th>MOG</th>
<th>SI</th>
<th>IFN-γ</th>
<th>IL-10</th>
<th>IFN-γ:IL-10</th>
<th>n</th>
<th>Clinical score</th>
<th>Inflammatory index</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEW n</td>
<td>l</td>
<td>253.0 ± 22.3</td>
<td>6,520.0 ± 93.6</td>
<td>26</td>
<td>110,103 ± 33,893</td>
<td>8,210 ± 5,332</td>
<td>13.41</td>
<td>10</td>
<td>0.12 ± 0.7</td>
<td>1.6 ± 0.3</td>
</tr>
<tr>
<td>BN.1L</td>
<td>l</td>
<td>287.5 ± 81.2</td>
<td>5,615.0 ± 98.6</td>
<td>20</td>
<td>60,474 ± 27,797</td>
<td>17,536 ± 5,144</td>
<td>3.45</td>
<td>4</td>
<td>0.5 ± 0.2</td>
<td>2.7 ± 0.6</td>
</tr>
<tr>
<td>BN n</td>
<td>l</td>
<td>136.7 ± 7.9</td>
<td>3,883.0 ± 311.7</td>
<td>28</td>
<td>5,144 ± 1,259</td>
<td>4,201 ± 2,371</td>
<td>1.22</td>
<td>4</td>
<td>0 ± 0</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>LEW.1N</td>
<td>l</td>
<td>849.0 ± 45.2</td>
<td>9,285.5 ± 137.8</td>
<td>11</td>
<td>46,801 ± 4,583</td>
<td>16,221 ± 3,301</td>
<td>2.89</td>
<td>4</td>
<td>0.50 ± 0.4</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

T cell lines were selected in vitro by one to two rounds of restimulation as described in Materials and Methods. Cytokine and restimulation data are presented as the mean of at least two representative experiments, all assays performed in triplicate. Pathogenicity was determined by the adoptive transfer of 10^7 freshly activated T cell blasts into syngeneic recipients; clinical scores are given for day 7 posttransfer. SI, stimulation index.

T cell transfer data for LEW taken from Ref. 13.

specific autoantibody response, rather than the primary T cell response to this autoantigen. As the MHC can modulate Ab responses, we extended this study to investigate the susceptibility of two reciprocal MHC congenic rat strains to MOG-induced EAE. The LEW.1N strain carries the RT1 n haplotype of BN on the LEW background, the BN.1L strain the RT1 l haplotype of LEW on the BN background.

The RT1 n haplotype clearly segregated with early disease onset, an acute fulminant disease course (Table I), and a high titer Ab response to MOG (Fig. 4). In contrast, the RT1 l haplotype was associated with later onset of disease, decreased susceptibility, and a delayed rise in anti-MOG Ab titers. This MHC-associated effect on the kinetics of anti-MOG Ab responses in LEW and BN affected both Th1- and Th2-associated Ig isotypes equally (Fig. 4, g and h). The dominant role of the autoantibody response in determining differences in disease onset/severity in LEW and BN

FIGURE 4. The anti-MOG Ab response in LEW, BN, and MHC-congenic rats. The Ab response to MOG was determined by ELISA in all four rat strains. a. Total IgG + IgM was analyzed in serial serum dilutions of BN (□) and LEW (○) rats, and titers were ~30-fold higher in BN rats by day 10 pi. b. This reflected the kinetics of the Ab response, which had already reached near maximal level in BN by day 10 pi. This was a general effect on both Th1 (IgG2b (f)) and Th2 (IgG1 (d) and IgE (h))-associated isotypes. Rapid isotype switching in the BN rat was also indicated by the decline of IgM (c) by day 14 pi. Comparison of the parental and congenic strains (day 10 pi) indicated that the MHC had a major influence on the Ab response; BN and MHC congenic LEW.1N rats developed similar, high titer Ab responses, irrespective of the isotype studied (IgG2b (g); IgE (h)). In contrast, LEW rats produced very low titers of all isotypes, and the congenic BN.1L rats produced intermediate titers. Data points represent the mean ± SD of five to eight animals in all experiments.

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strains was supported by analysis of the T cell response to MOG in the MHC congenic rat strains. Similar to the situation in the parental strains, the MOG-specific T cell responses exhibited a Th0/Th1 cytokine phenotype synthesizing large amounts of IL-10 and IFN-γ, but no detectable IL-4 (Tables III and IV) and were unable to mediate severe clinical EAE by adoptive transfer (Table IV). These observations confirm the importance of the MOG-specific Ab response in determining the clinical course and time of onset of MOG-induced EAE in the rat, but using only the MHC congenic strains LEW.1N and BN.1L we were unable to further define the MHC genes involved in this effect.

Intriguingly, the MOG gene is itself located within the telomeric region of the MHC (28, 29), raising the possibility that MOG polymorphisms (22) may themselves influence the autoantibody response. To exclude this possibility, we took advantage of the rat strain LEW.1R38. The RT1 r38 haplotype represents an intra-MHC recombination between LEW and BN and was identified in

FIGURE 5. Co-transfer of MOG-specific T cells and Abs in BN rats. MOG-specific T cells (10^7) were transferred into BN rats, followed 4 days (arrow) later by the serum Ig fraction derived from either MOG-immunized rats (■) or CFA-immunized controls (○). Rats receiving BN-derived MOG-specific Abs developed paralysis and weight loss, whereas animals receiving control Abs remained clinically healthy.

FIGURE 6. Representative neuropathology of adoptive transfer of MOG-specific BN T cells either alone or in the presence of BN anti-MOG Ig. The transfer of T cells alone induced a subclinical inflammatory response in the CNS composed of mononuclear cells (C) without demyelination (A). In contrast, in the presence of MOG-reactive Ig, the T line cells induced a predominant PMN infiltrate (D) that was associated with demyelination (B). A, B, Luxol fast blue myelin stain (×150); C, D, hematoxylin-eosin stain (×700).

FIGURE 7. Polymorphism for the MOG gene in the RT1 haplotypes l and n. Genomic DNA of rat strains LEW, LEW.1N, LEW.1R38, BN, and BN.1L was digested with the restriction enzyme HindII, separated in a horizontal 0.8% TAE-agarose gel, and blotted onto a nylon membrane. Hybridization was performed in 6× SSC/5× Denhardt’s solution, 10% dextran sulfate, 0.5% SDS at 68°C with the ^3P-labeled MOG exon 2 probe derived by PCR from the mouse MOG gene.

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FIGURE 8. Ab response to MOG in LEW, LEW.1N, and LEW.1R38 rats. Serial dilutions of sera from four to six animals obtained 10 dpi (a) or 14–15 dpi (b) were analyzed for the presence of anti-MOG Abs (total IgG + IgM) by ELISA. LEW.1N rats (○) had the highest titers of anti-MOG Abs on day 10 pi, followed by intermediate levels in the LEW.1R38 (●) and low levels in the LEW (◆) rat. By day 14–15 pi, Ab titers in the LEW.1N and LEW.1R38 rat were indistinguishable, whereas LEW still had lower levels of total MOG-specific IgG and IgM at this time point. Both Th1 (IgG2b (■)-) and Th2 (IgE (▲)-)associated isotypes followed a similar pattern, and by day 17 pi Ab levels in LEW were similar as the endpoint titers in the other strains.

mapping studies of RT1.M class I genes representing homologues of the H-2 M genes of the mouse (22). The recombination breakpoint in r38 defines the RT1.M region representing the most telomeric part of the rat MHC. Demonstration of a RFLP of the MOG gene in LEW and BN allowed mapping of this gene to the RT1 complex with the LEW.1N strain and, more specifically, to the RT1.M region with the r38 haplotype (Fig. 7) (22). Surprisingly, we observed that active immunization of LEW.1R38 rats resulted in a fulminant disease course that was similar to that seen in LEW.1N rather than LEW (Table I) although disease onset was delayed by ~2 days (Table I). Also, histopathological changes in LEW.1R38 were similar to those in LEW.1N in that inflammatory infiltrates were characterized by large numbers of PMNs. The r38 haplotype therefore enhances disease susceptibility even in the presence of virtually the entire RT1 complex (regions RT1.A–RT1.C) of the RT1 haplotype associated with low susceptibility to MOG-EAE. Once again, this effect does not seem to be directly related to the T cell response, because we could not detect any differences to either the parental LEW strain or LEW.1N in the primary T cell response to MOG (Table III). However, the r38 haplotype did influence the MOG-specific B cell response. The intra-MHC recombinant rats mounted a B cell response with intermediary kinetics to MOG as compared with LEW and LEW.1N (Fig. 8). This indicates that the anti-MOG Ab response is influenced by genes located within regions both centromeric and telomeric of the recombination breakpoint in r38. Again, in all strains disease onset was concomitant with the development of a significant anti-MOG response that reached comparable titers at the endpoint of analysis (Fig. 8).

Discussion

In this study, we demonstrate that disease susceptibility, severity, and clinical course in MOG-induced EAE are determined by the autoantibody response to the target Ag, rather than the encephalitogenic potential of the associated T cell response. This effect is not trivial in that it renders the BN rat highly susceptible to MOG-induced EAE, the pathogenic MOG-specific Ab response overwhelming those mechanisms that protect this rat strain against purely T cell-mediated autoimmune diseases of the nervous system (3, 17, 18).

Previous studies have associated the resistance of BN to MBP-induced EAE with TNF-α polymorphisms (30), a robust HPA axis response (31), defects in Ag processing (32), and differential expression of class II molecules (33). More recently, it was shown that BN but not LEW rats are susceptible to Th2-dependent autoimmune diseases induced by treatment with either gold salts or HgCl2. The adoptive transfer of autoreactive Th2 T cell lines into CD8-depleted BN but not LEW recipients induces an autoimmune syndrome characterized by polyclonal B cell activation, increased titers of autoAbs, proteinuria, and glomerular nephritis (20). Conversely, LEW rats are resistant to HgCl2-induced autoimmune disease, and treatment with HgCl2 will suppress EAE in this strain (34). In the context of EAE, Th1 and Th2 T cell responses are seen as being mutually antagonistic by virtue of the reciprocal regulatory effects of Th1 (IFN-γ, IL-12)- and Th2 (IL-4, IL-5, IL-10)-associated cytokines (35). The adoptive transfer of neuroantigen-specific Th2 T cell lines or clones (26, 36) induces a subclinical eosinophilic infiltrate in the CNS of normal recipients, whereas in immunodeficient hosts these T cells mediate clinical disease associated with a PMN infiltrate (37).

The observation that the cellular infiltrate in MOG-induced EAE in BN contained large numbers of polymorphonuclear leukocytes (PMNs) and eosinophils was initially taken to indicate that a Th2 T cell response was involved in disease pathogenesis. However, we were unable to demonstrate the secretion of the classical Th2 cytokine, IL-4 by either primary LNC cultures or short term MOG-specific T cell lines following antigenic stimulation in vitro. Moreover despite the presence of IL-4 mRNA transcripts in BN LNCs we were unable to demonstrate a selective enhancement of IgE, relative to other Ig isotype responses to MOG in BN as compared with LEW, LEW.1N, and BN.1L. These observations suggest that disease in BN is independent of the induction of a substantial Th2 T cell response to MOG, and at present the molecular basis for the eosinophilic infiltrate seen in BN is unclear. This pathology was only observed in the context of the BN genotype, and preliminary studies have failed to demonstrate the local expression of either IL-5 or eotaxin expression in the CNS, although these molecules play a critical role in recruitment of eosinophils into other organs (38, 39). However, the large numbers of PMNs found in the lesions may reflect the acuteness of the fulminant Ab-driven disease in BN, LEW.1N and LEW.1R38 rats, given that similar infiltrates have previously been demonstrated in acute cotransfer paradigms of EAE (40, 41).

Although the primary encephalitogenic Th1 CD4+ T cell response is necessary for disease induction, we were able to demonstrate that clinical expression of disease is mediated by Abs rather than T cells. Indeed despite large differences in their ability to synthesize IFN-γ none of the MOG-specific TCLs derived from any of the four strains studied were able to induce severe clinical EAE by adoptive transfer. This was noted previously for LEW rats (13, 16) and now appears to be an Ag, rather than strain related phenomenon. Interestingly, not only MOG-specific but also MBP and P2 protein-specific TCLs can be derived from Ag primed BN.
donors that are pathogenic in vivo [18, 42] indicating that protection from actively induced disease is not maintained by deletional mechanisms. This finding is concordant with the observation that the resistance of BN to MBP-induced EAE can be overcome by using carbonyl iron as the adjuvant [43].

The subclinical T cell response to MOG is essential for the induction of clinical EAE as it is responsible for transiently disrupting the blood brain barrier [44], which in BN occurs in the presence of a very high titer autoantibody response. Ab activating the CNS binds to the myelin sheath and activates the complement cascade [45] which may directly lead to myelin destruction [24, 46]. The local production of C3a and C5a then initiates an increasing spiral of inflammation, further B cell dysfunction, and demyelination [24, 44]. In the presence of an established high titer Ab response to MOG this results in a catastrophic cycle of increasing CNS inflammation and demyelination that cannot be controlled by counterinflammatory mechanisms, such as the up-regulation of complement inhibitory proteins within the CNS compartment [47]. These observations are clinically relevant as there is an increasing body of evidence implicating Ab and complement mediated mechanisms in the immunopathogenesis of demyelination in MS [9, 10]. Moreover, in a subgroup of patients with acute fulminate disease, demyelination is associated with a PMN eosinophilic infiltrate together with complement and Ig deposition similar to that seen in MOG-induced EAE in the BN rat (H. Lassmann, personal communication).

Comparison of the kinetics of the Ab response in LEW and BN and the corresponding MHC congenics support this concept in that disease onset/severity correlated with the serum anti-MOG Ab titer, and these effects were associated with the MHC; the RT1^d haplotype was associated with severe early onset disease and a rapid high titer Ab response to MOG, whereas in the context of the RT1^a haplotype both the Ab response and disease onset were delayed. Adoptive transfer experiments also demonstrated that neither the background genotype nor MHC haplotype grossly influenced the pathogenicity of MOG-specific TCLs in this strain combination. Although MHC genes are well known to influence the B cell response, we did not anticipate this effect on the kinetics and magnitude of the anti-MOG Ab response would be sufficient to induce clinical EAE in the BN rat. The MHC genes responsible for this effect remain to be identified but it should be noted that there is a partial Ag specificity as the RT1^d haplotype supports a high Ab response to MOG (this study) and OVA [48, 49], but not MBP [50] or PPD (A. Steffler, unpublished observation). This may implicate a role for class II MHC genes as they support the Ag/peptide specific expansion of CD4^+ T cells that can provide B cell help.

However, the results obtained with the intraMHC recombinant LEW.1R38 rat indicate that two distinct regions of the MHC influence the Ab response and thereby disease severity in MOG induced EAE. In line with previous studies demonstrating a major effect of the class II region [RT1.B/D] in a set of recombinants between parental haplotypes RT1^a and RT1^d there is clear evidence for an effect of the segment spanning the RT1.A–RT1.C region that includes RT1.B/D [51]. A new and unexpected finding was the additional strong effect of the telomeric RT1.M region. Interestingly, the gene encoding the target Ag MOG itself is located within this region (28, 29) and is known to be polymorphic between LEW and BN [22], making it a prime candidate for this effect. However, a sequence analysis of MOG-1gd cDNA has not revealed any differences between the strains used in this study (data not shown). This does not exclude genetic differences outside of the coding region that may influence the expression or turnover of MOG, and hence its antigenic properties. We are currently investigating this possibility further. It should also be noted that apart from MOG, other genes contained in this gene segment of the r58 recombinant haplotype may also influence disease susceptibility to EAE and/or the Ab response to MOG.

This study demonstrates that both MHC and MHC-linked effects can influence disease penetrance and severity via the B cell response in MOG-EAE, a model that closely resembles MS [52]. In view of recent reports implicating anti-MOG Abs in the pathogenesis of MS [53] the functional significance of MHC-associated susceptibility genes should be re-assessed in the context of the MOG-specific B cell response in MS.

References


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