Cortical Demyelination Can Be Modeled in Specific Rat Models of Autoimmune Encephalomyelitis and Is Major Histocompatibility Complex (MHC) Haplotype-Related

Maria K. Storch, MD, Jan Bauer, PhD, Christopher Linington, PhD, Tomas Olsson, MD, PhD, Robert Weissert, MD, PhD, and Hans Lassmann, MD

Abstract
In recent years, a number of histopathologic studies revealed the presence of cortical demyelination in multiple sclerosis (MS). The underlying mechanisms responsible for cortical demyelination are unresolved. Recently, the presence of cortical lesions in autoimmune encephalomyelitis (EAE) induced in marmosets and Lewis rats has been demonstrated. So far, it is not known whether cortical demyelinated lesions are also present in other models of EAE. In this study, we analyzed a large spectrum of different rat strains actively immunized with myelin oligodendrocyte glycoprotein (MOG), a model strongly mimicking MS for cortical demyelination. By using sets of rat strains with the constant EAE-permissive LEW nonmajor histocompatibility complex (MHC) genome, but different MHC haplotypes, we demonstrated that considerable cortical demyelination was only found in LEW.1AR1 (RT1r2) and LEW.1W (RT1Bu) strains. These rat strains have the isotypes and alleles RT1.BBu in the MHC II region and RT1.Cu in the nonclassic MHC I region in common. Because cortical demyelination was most prominent in LEW.1AR1 rats, an additional strong influence is promoted by the RT1.Aa MHC class I allele. Demyelination was accompanied by microglia infiltration and deposition of immunoglobulins on myelin sheaths. Our study shows that extensive cortical demyelination can be reproducibly induced in certain rat strains by active immunization with MOG. Furthermore, our findings suggest that cortical demyelination in EAE depends on particular combinations of MHC I and class II isotypes and alleles. The mechanisms for this influence and any similar effects in humans will be important to define.

Key Words: Autoimmune encephalomyelitis (EAE), Cortical lesions, Myelin oligodendrocyte glycoprotein (MOG), Major histocompatibility complex (MHC), Rat.

INTRODUCTION
Although multiple sclerosis (MS) is generally considered an inflammatory demyelinating disease of the white matter, in recent years, it became evident that profound demyelination may occur in the grey matter, in particular in the cerebral cortex (1–5). Three different types of cortical lesions have been described: intracortical perivenous lesions, leukocortical plaques, and subpial demyelination (2). The vast majority of cortical demyelination is present in the form of subpial lesions, which mainly affect the outer layers of the cortex, spanning long distances along the surface of several adjacent gyri and sulci (3, 4). Cortical demyelination dominates in patients with primary and secondary progressive MS but is sparse in acute and relapsing MS (5). The pathologic mechanisms leading to cortical demyelination are so far unknown.

Experimental autoimmune encephalomyelitis (EAE) in many aspects reflects the clinical course, pathology, and immunology of multiple sclerosis (6–8). In particular, in models induced either by sensitization with central nervous system tissue homogenate or with myelin oligodendrocyte glycoprotein (MOG), demyelinated plaques within the white matter of the brain and spinal cord closely reflect the structural abnormalities found in the respective lesions in patients with MS (9, 10). Recently, the presence of cortical lesions was shown in marmoset EAE (11–13). In addition, cortical lesions could be induced in a cortical focal lesion model in LEW rats by the use of subencephalitogenic active immunization and local application of a cytokine cocktail (14). So far, however, it is unknown whether cortical demyelinated lesions are also present in other models of EAE.

The aim of this study was to determine the incidence and immunopathology of cortical lesions in a large spectrum of different rat strains actively sensitized with MOG. In this model, the combined action of T-cells, antibodies, and the...
complement cascade leads to a white matter pathology closely mimicking MS (9, 15, 16).

**MATERIAL AND METHODS**

**Animals and Sensitization Procedure**

Chronic EAE was induced in various different rat strains (Table) by active sensitization with recombinant rat MOG (rrMOG) corresponding to the N-terminal sequence of rat MOG (aa 1–125). We analyzed rat strains with different major histocompatibility complex (MHC) and non-MHC genomes (DA [RT1^av^1], BN [RT1^n], LEW [RT1^l]), rat strains with different MHC haplotypes on the LEW non-MHC genome (LEW [RT1^l]), LEW.1A [RT1^a], LEW.1W [RT1^w]), as well as 4 intra-MHC recombinant rat strains on the EAE permissive non-MHC LEW genome with recombinations between the RT1^a and RT1^w haplotypes (LEW.1AR1 [RT1^aw^1], LEW.1AR2 [RT1^aw^2], LEW.1WR1 [RT1^aw^3], LEW.1WR2 [RT1^aw^4]).

Female rats, 8 to 14 weeks of age, were used in all experiments. All strains have been described previously (9, 15–18). MHC genotype and recombination sites have been described in the literature (15). LEW, LEW.1A, and LEW.1W have been obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany); BN from Charles River (Sulzfeld, Germany); and DA from Harlan (Winkelmann, Borchen, Germany). LEW.1AR1, LEW.1AR2, LEW.1WR1, and LEW.1WR2 have been obtained from Prof. H. Hedrich at the Medizinische Hochschule, Hannover, Germany. Homozygosity of congenic breeding pairs was routinely examined by a microsatellite marker located within the RT1 region. A total of 153 rats were analyzed in this study. The experiments were approved by the regional board.

Preparation and purification of MOG protein was performed as previously described (9, 15–17). Complete and incomplete Freund’s adjuvant was from Sigma (St. Louis, MO) and heat-killed *Mycobacterium tuberculosis* (H37Ra) was purchased from Difco (Detroit, MI). Rats were immunized under light ether anesthesia intradermally at the base of the tail with 50 to 100 μg rrMOG emulsified in complete Freund’s adjuvant containing 200 μg of heat-killed *M. tuberculosis* (DA, LEW, LEW.1A, LEW.1W, LEW.1AR1, LEW.1AR2, LEW.1WR1, and LEW.1WR2) or incomplete Freund’s adjuvant in a total volume of 100 μL (BN) as indicated in the Table.

**Neuropathology and Immunohistochemistry**

At various time points after sensitization (days 22–119 postimmunization), rats were killed and perfused through the aorta with 4% paraformaldehyde. Brains and spinal cords

---

**TABLE. Summary of rrMOG-Immunized Rats**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Induction of EAE</th>
<th>Days</th>
<th>Number</th>
<th>DM WM</th>
<th>LC</th>
<th>IC</th>
<th>SP</th>
<th>Percent DM of Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>DA (RT1^av^1)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>48–119</td>
<td>30</td>
<td>4.88 ± 0.44</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BN (RT1^n)</td>
<td>MOG/CFA</td>
<td>100 μg</td>
<td>34–70</td>
<td>35</td>
<td>4.83 ± 0.41</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LEW (RT1^l)</td>
<td>MOG/CFA</td>
<td>50–100 μg</td>
<td>22–64</td>
<td>12</td>
<td>2.09 ± 0.40</td>
<td>+ (1)</td>
<td>+ (1)</td>
<td>–</td>
</tr>
<tr>
<td>LEW.1W (RT1^w)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>40</td>
<td>9</td>
<td>1.75 ± 0.52</td>
<td>+ (1)</td>
<td>+ (2)</td>
<td>+ (3)</td>
</tr>
<tr>
<td>LEW.1A (RT1^a)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>40</td>
<td>11</td>
<td>1.64 ± 0.36</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LEW.1AR1 (RT1^aw^1)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>40</td>
<td>20</td>
<td>1.65 ± 0.16</td>
<td>+ (12)</td>
<td>+ (14)</td>
<td>+ (17)</td>
</tr>
<tr>
<td>LEW.1AR2 (RT1^aw^2)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>40</td>
<td>14</td>
<td>1.5 ± 0.31</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LEW.1WR1 (RT1^aw^3)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>40</td>
<td>10</td>
<td>1.9 ± 0.42</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>LEW.1WR2 (RT1^aw^4)</td>
<td>MOG/CFA</td>
<td>50 μg</td>
<td>40</td>
<td>12</td>
<td>2.2 ± 0.39</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Number, number of animals investigated; LC, leucocortical; IC, intracortical; SP, subpial; –, no demyelinating lesions found. The numbers in parentheses indicate the number of animals with the respective LC, IC, or SP lesions. DM WM: In rats, according to previous studies (15, 17), the degree of demyelination (DM) in the white matter (WM) was semiquantitatively described and scored as follows: 0.5, traces of perivascular or subpial demyelination; 1, marked perivascular or subpial demyelination; 2, confluent perivascular or subpial demyelination; 3, massive confluent demyelination (e.g. half of spinal cord or one optic nerve complete); 4, extensive demyelination (e.g. transverse myelitis, half cerebellar white matter or more, both optic nerves complete). The scores were evaluated in brain and spinal cord separately and then a sum score of brain and spinal cord was obtained. The percentage of demyelination in cortex (percent DM of cortex) has been assessed as described in “Methods.” Data represent mean percentage ± standard error of mean.
were dissected and routinely embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin, Luxol fast blue, and Bielschowsky silver impregnation to assess inflammation, demyelination, and axonal pathology, respectively.

In adjacent serial sections, immunohistochemistry was performed with antibodies against the following targets: MOG (Department of Biochemistry, Cardiff, U.K.), 23'-cyclophosphodiesterase (CPNase; Sternberger Monoclonals, Lutherville, MD), proteolipid protein (PLP; Department of Biochemistry, Cardiff, U.K.), macrophages/activated microglia (ED1; Serotec, Oxford, U.K.), T-cells (W3/13; Serotec), C9 (19), rat immunoglobulin (biotinylated rat; Amersham, Buckinghamshire, U.K.), and glial fibrillary acidic protein (GFAP; Lab Vision/NeoMarkers, Fremont, CA). Before staining with antibodies, antigen retrieval was performed as follows: paraffin sections were pretreated in a household food steamer device (MultiGourmet FS 20; Braun, Kronberg/Taunus, Germany) with a 60-minute incubation in a plastic Coplin jar filled with EDTA (0.05 M) in TRIS buffer (0.01 M, pH 8.5). To detect IgG and complement C9, sections were incubated with 0.03% protease from Streptomyces griseus (Sigma) for 15 minutes at 37°C. Sections were then incubated with 10% fetal calf serum (FCS) in DAKO washing buffer (FCS/DAKO; Dakopatts). Next, primary antibodies were applied in FCS/DAKO at 4°C overnight. After washing with phosphate-buffered saline (PBS), biotinylated secondary antibodies in PBS/DAKO were applied for 1 hour at room temperature (1:200; Amersham). Next, sections were washed and incubated with avidin-peroxidase (Sigma). Sections were developed with 3,3'-diaminobenzidine-tetrahydrochloride (Sigma) as a chromogen. Control sections were incubated in the absence of primary antibodies.

**Determination of Cortical Pathology**

In each animal, at least 5 coronal paraffin sections of different regions of the brain were analyzed. Cortical demyelination was analyzed by days 22 and 119 after sensitization. Sections were stained by immunohistochemistry for PLP and ED1 for determination of cortical demyelination and microglia activation. When cortical demyelination was found, the PLP-stained sections were scanned at 1500 days post injection and saved in tiff or JPEG format. Then, the images of the coronal sections were enlarged (approximately 10–15×) and printed on A4 format paper (20 × 28 cm). The exact borders of (cortical) demyelination were outlined. In case these borders could not be exactly distinguished in the printed images, sections were reinvestigated using light microscopy and the borders drawn accordingly. The graphs were overlaid by a morphometric grid (copied on photocopy film, divided in m²) and the total area of cortex as well as the area of cortical demyelination was determined manually. In addition, the type of cortical demyelination (leukocortical, intracortical, and subpial) was analyzed for each lesion. The entorhinal cortex was excluded from evaluation because demyelination could not be clearly distinguished from normal grey matter as a result of low numbers of PLP-positive myelin sheaths.

**RESULTS**

**General Neuropathology of Chronic Myelin Oligodendrocyte Glycoprotein—Autoimmune Encephalomyelitis Lesions**

Active sensitization with rrMOG-EAE resulted in widespread confluent demyelination throughout the white matter, as previously described (9, 15–18). The pathology of chronic MOG-EAE lesions in the white matter was characterized by perivenous inflammation, the formation of confluent plaques of demyelination with consecutive glial scar formation. Inflammation consisted of W3/13-positive T-cells and ED1-positive macrophages, which prevailed at later stages of disease. Active demyelination was associated with deposition of immunoglobulin and complement component 9 as has been previously shown (9).

**FIGURE 1.** Patterns of lesion distribution in chronic rrMOG 1-125-induced autoimmune encephalomyelitis in different rat strains. All rats were analyzed on 40 days postinjection. Cortical demyelination is depicted in red and demyelination is shown in green in other brain areas. Widespread cortical band-like subpial plaques are exclusively found in the LEW.1AR1 (RT1^r2) and LEW.1W (RT1^r1) strains. In contrast to the profound forebrain involvement in these strains, in strains that do not have the RT1.BuDuCu isotypes in the major histocompatibility complex (MHC) II and nonclassic MHC I region (i.e. LEW.1A, LEW.1WR1, and LEW.1AR2), the lesions are mainly located in the brainstem and cerebellar white matter areas and the spinal cord. In DA (RT1^av1) rats, spinal cord, cerebellar white matter, and the optic nerves are dominantly affected. BN (RT1^u) rats exhibit major lesions in the optic system and the spinal cord.
In general, the highest incidence of lesions was found in the spinal cord, brainstem, the optic nerves, and the cerebellar white matter. Profound forebrain involvement was exclusively present in strains with the RT1.B\textsuperscript{D}C\textsuperscript{a} isotypes in the MHC class II and nonclassic MHC class I region (LEW.1W [RT1\textsuperscript{u}] and LEW.1AR1 [RT1\textsuperscript{r2}] rats) (Fig. 1). LEW.1A (RT1\textsuperscript{a}), LEW.1WR1 (RT1\textsuperscript{e}), LEW.1AR2 (RT1\textsuperscript{r3}), and LEW.1WR2 (RT1\textsuperscript{r6}) rats mainly exhibited lesions in the brainstem, the spinal cord, and the cerebellar white matter. In DA (RT1\textsuperscript{av1}) rats, spinal cord, cerebellar white matter, and the optic nerves were dominantly affected and the BN (RT1\textsuperscript{n}) strain showed major lesions in the optic system and the spinal cord as previously reported (9, 15–18) (Fig. 1).

**Major Histocompatibility Complex Genes Determine Incidence and Extent of Cortical Demyelination in Rat Myelin Oligodendrocyte Glycoprotein—Autoimmune Encephalomyelitis**

Cortical demyelination was absent in DA (RT1\textsuperscript{av1}) and BN (RT1\textsuperscript{n}) rats as well as in LEW.1A (RT1\textsuperscript{a}), LEW.1AR2 (RT1\textsuperscript{r3}), LEW1WR1 (RT1\textsuperscript{e}), and LEW.1WR2 (RT1\textsuperscript{r6}) rats (Table). Whereas LEW (RT1\textsuperscript{a}) rats occasionally exhibited leuco- and intracortical lesions, considerable subpial demyelination was present in some LEW.1W (RT1\textsuperscript{u}) and the majority of LEW.1AR1 (RT1\textsuperscript{r2}) rats (Table; Fig. 1). These rats, in addition to large demyelinating leuco- and intracortical plaques, showed extensive subpial demyelination (Table; Figs. 1 and 2).

All the MHC intrarecombinant rat strains on the EAE-permissive non-MHC LEW genome (LEW.1AR1 [RT1\textsuperscript{r2}], LEW.1AR2 [RT1\textsuperscript{r3}], LEW.1WR1 [RT1\textsuperscript{e}], LEW.1WR2 [RT1\textsuperscript{r6}]) and their parental strains LEW.1W (RT1\textsuperscript{u}) and LEW.1A (RT1\textsuperscript{a}) were completely identical with regard to EAE induction and immunization protocol (Table). A total of 33.3% (3 of 9) of the LEW.1W (RT1\textsuperscript{u}) and 85% (17 of 20) of the LEW.1AR1 (RT1\textsuperscript{r2}) rats exhibited subpial demyelination (Table).

These widespread cortical band-like subpial plaques occupied up to 39% of cerebral cortex (Table; Fig. 2C–F). We found no correlation between the occurrence of cortical plaques and the extent of white matter demyelination when all strains were analyzed together (Table). Within the LEW.1AR1 (RT1\textsuperscript{r2}) strain itself, however, we found a clear correlation between the extent of demyelination in the white matter and in the cortex (simple regression, p = 0.0003).

**Immunopathology of Cortical Lesions in Myelin Oligodendrocyte Glycoprotein-Induced Autoimmune Encephalomyelitis in the Rat**

The global incidence of cortical pathology in the different MOG-EAE rat models is shown in the Table. As described before in MS (2, 3), 3 types of cortical lesions were found in rat EAE: leuко- and intracortical lesions (Fig. 2C–F), and subpial lesions (Table; Fig. 2). Leuко- and intracortical lesions, by definition, involved not only the cortex, but also the subcortical white matter. Small intracortical lesions developed around inflamed cortical vessels (Fig. 2C–F). The largest type of cortical demyelination was the formation of band-like subpial plaques, which involved the outer cortical layers and extended in variable depth into the deeper layers (Table; Fig. 2). These lesions spanned large parts of the cortical circumference and were present in all forebrain areas (Figs. 1 and 2C). Subpial lesions frequently were confluent with periventricular intracortical lesions (Fig. 2C–F).
The early stage of subpial lesion formation was characterized by band-like subpial microglia infiltration (Fig. 2A) associated with immunoglobulin deposition on myelin sheaths (Fig. 2B). At later stages of lesion formation, widespread band-like demyelination (Fig. 2C) associated with microglia activation (Fig. 2E, F) and deposition of IgG on degenerating myelin sheaths at the border of the lesions occurred (Fig. 2G-I). In particular, the borders of subpial lesions were densely infiltrated by activated microglial cells/macrophages (Fig. 2E).

**DISCUSSION**

We demonstrated that severe cortical demyelination occurs in LEW.1W (RT1u) and LEW.1AR1 (RT1r2) rat strains actively immunized with MOG. In comparison to published work in marmosets and a focal lesion EAE model (11–14), we demonstrated, in addition, that the presence of such lesions is genetically controlled by MHC genes. Like in MS, the dominant cortical pathology in LEW.1W and LEW.1AR1 rats consisted of band-like subpial demyelination, which affected the outer layers of the cortex and spanned long distances of the cortical surface. Thus, MOG-EAE in these rats qualifies for an excellent model to study cortical pathology. Magnetic resonance imaging of cortical plaques is still not satisfactory. Our relatively cheap and highly reproducible model may help to develop magnetic resonance imaging techniques for unequivocal identification of cortical lesions. Furthermore, experiments can be designed to clarify the functional consequences of cortical demyelination, a question that is so far unresolved in patients with MS.

Although all analyzed rat strains revealed extensive white matter demyelination, considerable subpial demyelination was only found in the LEW.1AR1 (RT1r2) and LEW.1W (RT1u) rats. These strains have the MHC isotypes and alleles RT1.BDw and RT1.Cw in the nonclassic MHC I region in common (17). Additionally, a very strong influence was promoted by the classic RT1.Aa MHC I allele of the LEW.1AR1 (RT1r2) rat. RT1.A is the classic MHC I gene of the rat in contrast to nonclassic MHC I genes encoded within the RT1.C region (15). Our data thus suggests that the incidence and extent of cortical demyelination in EAE is regulated by genetic influences from the MHC I and II isotypes and alleles. To elucidate the exact mechanisms of preferential cortical demyelination in LEW.1AR1 rats, mechanistic studies will be performed.

The MHC effects observed in our study are of interest for several reasons. There is a strong association of MS with the human leukocyte antigens, and especially the DR2b genes (20, 21). In addition, complex interactions with influences of both disease predisposing and protective influences are now apparent (22) just as have been observed in EAE (15, 23, 24). Influences from the MHC I region may promote cortical pathology through different scenarios. Classic MHC I molecules are restriction elements for CD8+ T-cells. Therefore, the high incidence of cortical lesions present in the LEW.1AR1 (RT1r2) rats might be dependent on CD8+ T-cells. In addition, the LEW.1AR1 rats are peculiar in regard to peptide loading of RT1.Aa alleles as a result of a polymorphism in the TAP2 genes (15, 25). These major and complex effects of genes within the MHC (now also shown to be valid for a phenomenon such as cortical demyelination) should be important to decipher mechanistically. This is not easily done in human outbred populations with variations in environmental influences but can be approached in the current experimental paradigm by genetic means and protein chemistry. Such studies are ongoing in our laboratories. Our observations also suggest that an MS subphenotype such as cortical demyelination could be studied on the basis of a genetic regulation from the human leukocyte antigens.

LEW.1AR1 (RT1r2) and LEW.1W (RT1u) are different from the other investigated rat strains in several aspects (17). They show only minimal macrophage recruitment in active lesions, the dominant effector cells being activated microglia. This microglia infiltration is associated with less axonal bystander damage. In contrast, lesions in the other rat strains are characterized by extensive and dominant macrophage infiltration in the demyelinating plaques and pronounced axonal damage. The disease course in LEW.1AR1 (RT1r2) and LEW.1W (RT1u) rats is milder and more chronic as compared with the other rat strains. Furthermore, LEW.1AR1 (RT1r2) and LEW.1W (RT1u) exhibit profound involvement of the forebrain, which contrasts the dominant affection of the spinal cord, brainstem, cerebellar white matter, and optic nerves in chronic EAE of the other investigated rat strains (Fig. 1). Whether these differences are related to the formation of cortical plaques remains to be determined.

In summary, we show that extensive cortical demyelination can be modeled in certain rat strains by active sensitization with MOG. In the future, this will allow studying of pathogenetic mechanisms involved in cortical demyelination in more detail. Furthermore, our data suggest that the incidence and extent of cortical demyelination in EAE depends on particular combinations of MHC I and II isotypes and alleles. The mechanisms for this influence and any similar effects in humans will be important to define and such studies are ongoing.

**ACKNOWLEDGMENTS**

The authors thank Marianne Leisser, Angela Kury, and Ulrike Köck for expert technical assistance.

**REFERENCES**