A Genome-Wide Search Identifies Two Susceptibility Loci for Experimental Autoimmune Encephalomyelitis on Rat Chromosomes 4 and 10

Marie-Paule Roth, Carine Viratelle, Laurence Dolbois, Maxence Delverdier, Nicolas Borot, Lucette Pelletier, Philippe Druet, Michel Clanet and Hélène Coppin

*J Immunol* 1999; 162:1917-1922; [http://www.jimmunol.org/content/162/4/1917](http://www.jimmunol.org/content/162/4/1917)

**References**
This article cites 38 articles, 13 of which you can access for free at: [http://www.jimmunol.org/content/162/4/1917.full#ref-list-1](http://www.jimmunol.org/content/162/4/1917.full#ref-list-1)

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at: [http://jimmunol.org/subscription](http://jimmunol.org/subscription)

**Permissions**
Submit copyright permission requests at: [http://www.aai.org/About/Publications/JI/copyright.html](http://www.aai.org/About/Publications/JI/copyright.html)

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at: [http://jimmunol.org/alerts](http://jimmunol.org/alerts)
A Genome-Wide Search Identifies Two Susceptibility Loci for Experimental Autoimmune Encephalomyelitis on Rat Chromosomes 4 and 10

Marie-Paule Roth,* Carine Viratelle,* Laurence Dolbois,† Maxence Delverdier,‡ Nicolas Borot,* Lucette Pelletier,‡ Philippe Druet,‡ Michel Clanet,* and Hélène Coppin*

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune disease of the central nervous system that exhibits many pathologic similarities with multiple sclerosis. The genetic loci that contribute to mononuclear cell infiltration of the central nervous system and clinical manifestations of EAE in the rat were investigated in the F2 progeny of the highly susceptible Lewis and resistant Brown Norway strains. The data confirmed that the Lewis allele of a MHC-linked gene is necessary, but not sufficient, to confer EAE susceptibility in the F2 progeny. Subsequent analyses were thus restricted to the subset of the F2 animals with EAE-predisposing MHC genotypes. A genome-wide scan approach was performed using 103 microsatellite markers covering 85% of the genome. Two non-MHC regions were identified, one near the centromere of chromosome 4 and the other on the long arm of chromosome 10, that significantly contributed to the disease. In addition, three regions on chromosomes 9, 13, and 17 were suggestive for linkage. Congenic mapping is now needed to reduce the support intervals encoding the loci of interest to sizes amenable to physical mapping and to eventually demonstrate the involvement of some of the candidate genes of immunologic importance localized in these regions. *The Journal of Immunology, 1999, 162: 1917–1922.

Multiple sclerosis is a common T cell-mediated disease of the central nervous system (CNS) that affects at least 60 in every 100,000 people in the northern United States, Canada, and northern Europe. It is generally thought that a triggering event, such as a viral infection, combined with a genetic predisposition may underlie the disease (1). However, the identification of genetic loci that control susceptibility to multiple sclerosis has been hampered by several factors, including complex interactions of environmental factors with the predisposing genetic background, multiple genetic loci, probably each with a small contribution to the disease, and genetic heterogeneity (2–4).

Given the immunopathological similarities between experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis (5), this animal model provides a useful alternative to identify candidate loci and better understand the physiologic pathways involved in the disease process. EAE indeed is an autoimmune disease of the CNS in which the pathologic changes, i.e., encephalomyelitis and demyelination, are the consequences of T cell infiltration and recognition of CNS-associated Ags. The inflammatory CD4+ T cells that mediate EAE secrete Th1 cytokines, including IL-2, IFN-γ, and TNF-β. By contrast, CD4+ Th2-type T cells that secrete IL-4, IL-5, IL-6, IL-10, IL-13, and TGF-β are important in down-modulating inflammatory responses (6) and have the ability to suppress both the acute and relapse phases of EAE (7).

Genome scans performed on the progeny of experimental crosses between susceptible and resistant strains of rodents help to restrict heterogeneity of complex diseases and thus yield greater statistical power than similar approaches in humans, at the cost of representing only one segment of the genetic spectrum of a disease (8). The analysis of different strain combinations not only in the mouse (9–11) but also in other species is therefore essential to elucidate all loci that may contribute to the disease via different pathogenic pathways. Although inbred strains of rats show varying degrees of susceptibility to the induction of EAE (12), no systematic genetic analysis has been performed in this rodent species to date. Part of the difference between the highly susceptible Lewis (LEW) and resistant Brown Norway (BN) rat strains can be explained by a gene in the MHC or RT1 region (13–15). However, this locus is not sufficient to induce EAE, and there is a great deal of additional complexity that appears to be genetically determined (16, 17). Furthermore, a full-blown inflammatory reaction in the rat is not necessarily sufficient to bring about clinical symptoms, suggesting that these phenotypic traits may be controlled by separate loci (18).

In this study we investigated factors unlinked to the MHC that control both CNS inflammation and clinical manifestations of EAE by a systematic genome search performed for the first time on the F2 progeny of the LEW and BN rat strains.

Materials and Methods

Animals and disease induction

Inbred LEW and BN rats were initially obtained from the CSEAL (Centre National de la Recherche Scientifique, Orleans-La Source, France). (LEW × BN)F1 and F2 rats were bred in our facilities and maintained

---

*Centre d’Immunopathologie et de Génétique Humaine, Centre National de la Recherche Scientifique, CHU Purpan. †Ecole Nationale Vétérinaire, and ‡Institut National de la Santé et de la Recherche Médicale Unit 28, CHU Purpan, Toulouse, France.

Received for publication March 11, 1998. Accepted for publication October 29, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by grants from the Association pour la Recherche sur la Sclérose en Plaques, the Caisse d’Assurance Maladie des Professions Libérales-Provinces, the Groupement de Recherches et d’Etudes sur les Génomes, and the Région Midi-Pyrénées.

2 Address correspondence and reprint requests to Dr. Marie-Paule Roth, Cigcl, Centre National de la Recherche Scientifique, Unité Proche de Recherche 8291, Centre Hospitalier Universitaire Purpan, F-31300 Toulouse, France. E-mail address: roth@cict.fr

3 Abbreviations used in the paper: CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; BN, Brown Norwegian; LEW, Lewis; QTL, quantitative trait locus.
under conventional conditions. Myelin was extracted from guinea pig brain and spinal cord, and purified according to the procedure described by Norton and Poduslo (19). To induce EAE, rats were immunized at 10–15 wk of age by injecting in each hind footpad 0.1 ml of the antigenic solution (10 mg of purified myelin/ml) emulsified with an equal volume of CFA (Difco, Detroit, MI) supplemented with Mycobacterium tuberculosis (0.5 mg/ml; Difco) and Bordetella pertussis (2 × 10⁹ organisms/ml; Difco). This immunization procedure is known to have no effect on the resistant BN strain, but to induce strong EAE in the F₁ hybrids (20).

Clinical and histological evaluation

Rats were observed daily for clinical signs of neurological dysfunction and were scored on a scale of 0–4. They were sacrificed on day 17 after immunization for histopathological evaluation; brains and spinal cords were removed and fixed in 10% formalin. Specimens were processed through paraffin. Seven histological sections were cut at 2 µm: two transverse sections of the brain, two longitudinal sections of the cerebellum, and three longitudinal sections of the cervical, thoracic, and lumbar spinal cord, respectively. Hematoxylin and eosin staining was used to detect perivascular mononuclear infiltrates. Slides were evaluated blindly by two investigators, and histologic disease was quantitated by counting inflammatory foci with 20 or more aggregated mononuclear cells.

Genetic typing

Spleens were taken from all rats and were frozen at −70°C. DNA was prepared by digesting homogenized tissue with protein K and performing the procedure described by Norton and Poduslo (19). To induce EAE, rats were immunized at 10–15 wk of age by injecting in each hind footpad 0.1 ml of the antigenic solution (10 mg of purified myelin/ml) emulsified with an equal volume of CFA (Difco, Detroit, MI) supplemented with Mycobacterium tuberculosis (0.5 mg/ml; Difco) and Bordetella pertussis (2 × 10⁹ organisms/ml; Difco). This immunization procedure is known to have no effect on the resistant BN strain, but to induce strong EAE in the F₁ hybrids (20).

Table I. Incidence and severity of clinical EAE and histologic disease in parental strains and their crosses

<table>
<thead>
<tr>
<th>Highest Clinical Scorea</th>
<th>Incidence of Clinical EAE</th>
<th>Histologic Scoreb</th>
<th>Incidence of Histologic Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>LEW</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>BN</td>
<td>9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F₁</td>
<td>3</td>
<td>4</td>
<td>11</td>
</tr>
<tr>
<td>F₂</td>
<td>135</td>
<td>12</td>
<td>28</td>
</tr>
<tr>
<td>NN</td>
<td>49</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LN</td>
<td>59</td>
<td>2</td>
<td>16</td>
</tr>
<tr>
<td>LL</td>
<td>27</td>
<td>10</td>
<td>6</td>
</tr>
</tbody>
</table>

a Rats were scored on a scale of 0 to 4, where 0 = no illness, 1 = loss of tail tone (flaccid tail), 2 = tail weakness plus hindlimb paresis, 3 = total hindlimb paralysis often accompanied by incontinence, and 4 = tetraplegia and/or death. Incidence represents the proportion of rats with clinical score ≥1.

b Histologic score was determined by counting the number of inflammatory foci with 20 or more aggregated mononuclear cells, 1 = less than 10 inflammatory foci with 20 or more aggregated mononuclear cells, 2 = 11–30 foci, 3 = more than 30 foci. Incidence represents the proportion of rats with histologic score ≥1.

The RT1.D molecule of the BN and the RT1.B molecule of the LEW rat, were determined by flow cytometry on PBL using two mAbs recognizing different grades of alcohol and embedded in paraffin. Seven histological sections were cut at 2 µm: two transverse sections of the brain, two longitudinal sections of the cerebellum, and three longitudinal sections of the cervical, thoracic, and lumbar spinal cord, respectively. Hematoxylin and eosin staining was used to detect perivascular mononuclear infiltrates. Slides were evaluated blindly by two investigators, and histologic disease was quantitated by counting inflammatory foci with 20 or more aggregated mononuclear cells.

Results

Disease phenotype

A total of 233 rats were immunized with purified myelin and observed daily for clinical signs of neurological dysfunction. The incidence of clinical disease according to strain is shown in Table I. As expected, all the LEW rats developed severe EAE, whereas none of the BN rats developed the disease. The incidence of EAE in the F₂ generation was 31.1%, a decrease of 63% from that in the F₁ generation, consistent with a polygenic mode of inheritance. Because previous studies have implicated a MHC-linked locus in the control of EAE susceptibility, we initially determined the phenotypes of the entire F₂ population for the RT1 locus. The incidences of clinical disease in rats homozygous for the LEW haplotype, heterozygous, and homozygous for the BN haplotype were 53, 34, and 0%, respectively (Table I). This is consistent with the interpretation that the LEW allele of an MHC-linked gene is necessary, but not sufficient, to confer EAE susceptibility in the (LEW × BN)F₂ progeny, and that non-MHC loci also contribute to the control of the disease. As previously reported (23), the highest clinical scores were not significantly different among F₂ rats homozygous for the EAE-predisposing MHC haplotype and those heterozygous (by Wilcoxon rank-sum test, p = 0.13), which is not in favor of a strong dose effect of Lewis MHC alleles on disease severity in this intercross.

In addition to the scoring of clinical severity, pathological lesions were evaluated by counting the number of inflammatory foci in the brain and spinal cord of each rat. Histological scores in parental strains and their crosses are given in Table I. None of the F₂ animals homozygous for the MHC BN haplotype (49 rats) had inflammatory infiltrates. In the subset of the F₂ progeny with at least one LEW haplotype (147 rats), there was no animal with clinical symptoms that did not also have histological inflammation, although 51 of 112 rats (45.5%) with an inflammatory reaction had no clinical illness. This is consistent with the previous observation that a full-blown inflammatory reaction in the rat is not necessarily sufficient to bring about clinical symptoms (18). Of note, similarly
to CFA-inoculated mice (24), the LEW rats we injected with CFA in saline did not demonstrate clinical manifestations of EAE or CNS inflammatory cell infiltration.

In multiple sclerosis, the extent of abnormality shown by magnetic resonance imaging is not related to the degree of clinical disability (25). Similarly, we found no correlation between the number of inflammatory foci and the clinical score in the F2 animals with histological lesions examined in this study (by Kruskal-Wallis test on ranks, \( p = 0.27 \)). The topography of the lesions may in some cases explain the silent nature of the disease. However, differences in mRNA expression of pro- and anti-inflammatory cytokines in the spinal cords of rats of different strains have been shown to correlate with their susceptibility to EAE (17). It is thus possible that the secretion by the infiltrating cells of cytokines able to down-regulate the inflammatory response may protect some rats against progression to clinical disease.

**Genetic mapping**

To map the non-MHC loci that control EAE in the rat, the subset of the F2 progeny with EAE-predisposing MHC genotypes (146 DNAs available) was typed for 103 informative markers distributed on all autosomes and the X chromosome and covering 84.4% of the genome with a spacing of 20 cM or less. As previously shown, the F2 rats require at least one MHC haplotype of the susceptible LEW strain to develop a disease phenotype. The 49 rats homozygous for the MHC BN haplotype indeed were asymptomatic and were thus excluded from the investigation to increase the power of the tests.

No locus segregating with incidence or severity of histological disease was detected in addition to the MHC by MAPMAKER/QTL (26). This may be due to the nonhomogeneous nature of the CNS-infiltrating cells in the F2 progeny. It is indeed likely that infiltration by cells secreting pro- and anti-inflammatory cytokines is independently controlled by several loci. A precise functional analysis of the inflammatory foci may thus be necessary before such genes can be identified.

To map the loci that control clinical manifestations of EAE, the genotype distribution of rats with clinical disease (\( n = 60 \)) was first compared with that of rats of the resistant phenotype (no clinical or histologic lesions; \( n = 35 \)). When a criterion of \( p < 0.05 \) was used for the purpose of completeness, a difference in these distributions was detected for markers on chromosomes 9, 10, 13, and 17 (Table II). These chromosomes were subjected to a QTL scan (26), using the penetrance scan function implemented in MAPMAKER/QTL 1.9. As shown in Fig. 1, a region on chromosome 10 fell short of significant linkage, with a LOD score peak of 4.10 (\( p = 8 \times 10^{-5} \)) at D10 Mgh10, close to the generally accepted 4.3 (\( p = 5.2 \times 10^{-5} \)) threshold corresponding to a genome-wide significance level of 0.05 (27). A LOD score of 3.45 (\( p = 3.6 \times 10^{-4} \)) above the 2.8 (\( p = 1.6 \times 10^{-3} \)) threshold for suggestive linkage (27) was obtained with marker D13 Mgh1 on chromosome 13. However, none of the markers tested within 30 cM from D13 Mgh1 (D13 Mit1, D13 Mgh3, and D13Uwm1) was informative in the present cross, and although genotyping for marker D13 Mgh1 was repeated twice to exclude typing errors, this result should be considered provisional. Of note, EAE incidence was lower in LEW/LEW homozygotes at locus D13 Mgh1 than in LEW/BN heterozygotes or BN/BN homozygotes, providing another example of an allele from a control strain contributing to increased susceptibility (11, 28, 29). Two additional regions on chromosomes 9 (peak LOD score of 2.69 at D9 Mgh4; \( p = 2 \times 10^{-3} \)) and 17 (peak LOD score of 2.87 between markers D17 Mit4 and D17 Mit5; \( p = 1.3 \times 10^{-3} \)) fell short of suggestive linkage. As shown in Table II, the genotype distribution for these markers in rats with inflammatory foci but no clinical expression was not significantly different from the expected 1:2:1 ratio. As a consequence, comparison of rats with clinical EAE (\( n = 60 \)) with those showing no clinical expression of the disease (\( n = 86 \)) leads to a

---

**Table II. Comparison of the genotype distributions of rats with clinical EAE and rats without histological lesions or rats with no clinical expression of the disease**

<table>
<thead>
<tr>
<th>Locus&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Distance Between Adjacent Markers&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Clinical EAE (1)</th>
<th>Histological Lesions Only (2)</th>
<th>Lack of Histological Lesions (3)</th>
<th>Genotype Distribution Comparisons&lt;sup&gt;c&lt;/sup&gt;</th>
<th>( \chi^2 )</th>
<th>( p ) value&lt;sup&gt;d&lt;/sup&gt;</th>
<th>( \chi^2 )</th>
<th>( p ) value&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>D9Mit3</td>
<td>1.0</td>
<td>8</td>
<td>31</td>
<td>21</td>
<td>13</td>
<td>24</td>
<td>14</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>D9Mgh4</td>
<td>0.6</td>
<td>7</td>
<td>32</td>
<td>21</td>
<td>13</td>
<td>25</td>
<td>13</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td>D9Mit2</td>
<td>—</td>
<td>8</td>
<td>31</td>
<td>21</td>
<td>13</td>
<td>24</td>
<td>14</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>D10Mit10</td>
<td>2.8</td>
<td>9</td>
<td>31</td>
<td>20</td>
<td>9</td>
<td>31</td>
<td>11</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>D10Mgh11</td>
<td>2.4</td>
<td>7</td>
<td>33</td>
<td>20</td>
<td>8</td>
<td>31</td>
<td>12</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>D10Mit9</td>
<td>2.8</td>
<td>7</td>
<td>33</td>
<td>20</td>
<td>9</td>
<td>30</td>
<td>12</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>D10Mgh10</td>
<td>1.9</td>
<td>6</td>
<td>35</td>
<td>19</td>
<td>11</td>
<td>30</td>
<td>10</td>
<td>15</td>
<td>18</td>
</tr>
<tr>
<td>D10Mgh9 (IL-4)</td>
<td>13.2</td>
<td>7</td>
<td>36</td>
<td>17</td>
<td>11</td>
<td>30</td>
<td>10</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>D10Mgh6</td>
<td>—</td>
<td>12</td>
<td>34</td>
<td>14</td>
<td>13</td>
<td>26</td>
<td>12</td>
<td>11</td>
<td>19</td>
</tr>
<tr>
<td>D13Mgh1</td>
<td>—</td>
<td>16</td>
<td>38</td>
<td>6</td>
<td>12</td>
<td>24</td>
<td>15</td>
<td>7</td>
<td>12</td>
</tr>
<tr>
<td>D17Mgh5</td>
<td>7.3</td>
<td>9</td>
<td>32</td>
<td>19</td>
<td>13</td>
<td>26</td>
<td>12</td>
<td>12</td>
<td>16</td>
</tr>
<tr>
<td>D17Mit4</td>
<td>8.6</td>
<td>8</td>
<td>34</td>
<td>18</td>
<td>14</td>
<td>27</td>
<td>10</td>
<td>16</td>
<td>14</td>
</tr>
<tr>
<td>D17Mit5</td>
<td>—</td>
<td>12</td>
<td>28</td>
<td>20</td>
<td>12</td>
<td>32</td>
<td>7</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

<sup>a</sup> Only data for chromosomal regions showing suggestive evidence of linkage to EAE are reported. The remaining data are available on request. Primers for amplification of all markers were obtained from the Whitehead Institute/MIT database (ftp-genome.wi.mit.edu/distribution/rat_sslp_releases).

<sup>b</sup> Distance in cM between two consecutive markers as calculated by MAPMAKER/EXP 3.0 on the (LEW × BN)F2 progeny using the Kosambi mapping function. The markers have been oriented according to the cytogenetic maps (30); i.e., from centromere to distal q arm for telocentric chromosomes 9 and 10, and from distal p arm to distal q arm for subtelocentric chromosome 13 and submetacentric chromosome 17.

<sup>c</sup> NN, number of F2 progeny homozygous for the Brown Norway parental allele; LN, heterozygous F2 progeny; LL, progeny homozygous for the Lewis parental allele.

<sup>d</sup> Pearson’s \( \chi^2 \) statistics with 2 df.
F2 progeny are indicated on the MAPMAKER/EXP 3.0. The positions of all marker loci genotyped in the LOD score of 4.33 (with histological disease with markers located near the centromere trait controlling resistance to clinical symptoms in rats presenting 51). As shown in Table III, we observed significant linkage of a thus compared with that of rats with histological disease only (5).

Since no marker centromeric to D4 Mgh1 and informative in this cross was available for typing, it cannot be excluded that the locus controlling EAE in this region lies within the 5.8-cM region that separates this marker from the centromere (30).

**Locus interaction**

The fractions of F2 rats homozygous for the LEW allele, heterozygous, and homozygous for the BN allele at locus D10 Mgh10 that presented with clinical EAE were 61.3, 42.2, and 19.3%, respectively. The clinical scores were significantly different among the three groups (by Kruskal-Wallis test on ranks, p = 0.003). This suggests that the LEW allele at this locus acts in an additive fashion not only to confer EAE susceptibility, but also to determine disease severity. In contrast, two LEW alleles at locus D4 Mgh1 are necessary to significantly increase disease penetrance over that observed in rats homoygous for the BN allele. Rats with two LEW alleles at this latter locus have higher clinical scores than rats with other genotypes (by Wilcoxon rank-sum test, p = 0.0007), indicating that this predisposing genotype also influences disease severity. EAE penetrance in animals carrying different genotype combinations is shown in Table IV. The frequency of EAE in rats that do not carry a EAE-predisposing genotype at either locus is 17%, indicating that neither is absolutely required to confer EAE susceptibility. The EAE-predisposing genotype at locus D4 Mgh1 significantly increases disease penetrance associated with every D10 Mgh10 genotype, which suggests an additive effect of the gene products in the development of the disease and is consistent with the polygenic mode of inheritance of EAE susceptibility.

**Discussion**

In this study we performed the first genome-wide scan for non-MHC loci that control both the inflammatory infiltration of the CNS and the clinical manifestations of EAE, a model for multiple sclerosis, in the F2 progeny of the highly susceptible LEW and resistant BN rat strains. In addition to the MHC, we found two regions on chromosomes 4 and 10 that significantly contributed to the disease. Three other regions on chromosomes 9, 13, and 17 were suggestive of linkage, and their involvement in EAE remains to be demonstrated.

The strongest linkage was observed for a gene controlling clinical EAE in the rats presenting with histological disease with markers localized on chromosome 4, close to the IL-6 gene (31). This latter gene is an obvious candidate locus. Indeed, it encodes a cytokine that appears to be required for the establishment of the Th2 cytokine profile and to down-regulate Th1 cytokine production (32). Since Th2-type cells have been shown to suppress the acute and relapse phases of EAE without significantly reducing mononuclear cell infiltrations within the CNS (7), this cytokine might well interfere with the initiation of a Th2 response in the F2 rats with CNS infiltration but resistant to clinical EAE. The human region of conserved synteny is on chromosome 7p11.2-p21, and it is interesting to note that evidence for linkage of multiple sclerosis to microsatellite markers localized in this homologous region has been reported by the authors of two of the three genome scans recently performed in humans (33). Further investigation is now needed to determine whether IL-6 or any other gene contained in the region of interest confers susceptibility to EAE and possibly also to multiple sclerosis.

The other region significantly linked to EAE is located around D10 Mgh10 on rat chromosome 10. It is noticeable that it is homologous to a locus controlling in vitro Th1/Th2 differentiation of CD4+ T cells (34) and EAE severity (10) on mouse chromosome 11. The same region also appears to coincide with ATP7S-2, a QTL controlling elevated serum IgE production induced by gold salts in the BN rat strain (35). This IgE production is mediated by Th2
Effectors cells (36). It is thus possible that in the context of distinct genetic and environmental settings, allelic variants of a gene contained in this region contribute to several immunologically mediated pathological states, for instance by regulating the polarization of T cells to either a Th1 or a Th2 immune response. However, whether the locus controlling EAE on rat chromosome 10 and ATPS-2 are allelic or different remains to be determined. Comparison between the rat and mouse genetic maps (31, 37) reveals that this region of interest contains a large number of potentially candidate genes for which allelic variants could generate intrinsic differences between BN and LEW T cells. Such candidates include the IL-4 gene whose product directly promotes Th2 development from naive T cells; other cytokine genes, such as IL-3, IL-5, IL-9, IL-12 p40, and IL-13; and genes encoding transcription factors or signaling molecules expressed in T cells (IFN regulatory factor-1, T cell-specific transcription factor-7, and IL-2-inducible T cell kinase). The production of appropriate congenic strains will allow, through the analysis of recombination events within the region of interest, exclusion of certain candidate genes listed above and reduction of the support interval encompassing the EAE susceptibility locus on rat chromosome 10 to one that is amenable to physical mapping. Such an approach was indeed shown to allow placement of genes involved in polygenic diseases, even if they have incomplete penetrance and subtle effects, to a resolution greater than 1 cM (38).

The congenic lines produced will also serve to test the hypothesis that the locus controlling EAE on rat chromosome 10 and ATPS-2 may be the same gene. This is of particular interest because the prevalence of IgE-mediated allergic diseases is significantly decreased in patients with multiple sclerosis (39). This latter observation suggests that the genetic factors that promote susceptibility to Th1-mediated inflammatory diseases in humans may protect against the development of Th2-mediated diseases (39). In that respect, it is noteworthy that susceptibility to high IgE levels and asthma in humans is linked to the chromosomal region 5q31.1 that contains the IL-4/IL-5 gene cluster (40) and is syntenic to the region of rat chromosome 10 that contains both ATPS-2 and the locus shown in this study to confer susceptibility to EAE.

Acknowledgments

The expert technical assistance of M. Amardeilh, C. Demangel, M. Perey, and M. T. Ribouchon is gratefully acknowledged. We thank H. Villaroya for providing us with purified myelin, M. T. Bihoreau and D. Gauguier for the D4Wox32 primers, and M. Daly for prerelease of MAPMAKER/QTL 1.9.

References


The Journal of Immunology 1921

Table III. Comparison of the genotype distributions of rats with clinical EAE and rats with histological lesions only

<table>
<thead>
<tr>
<th>Locus</th>
<th>Distance Between Adjacent Markers</th>
<th>Clinical EAE</th>
<th>Histological Lesions Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NN</td>
<td>LN</td>
</tr>
<tr>
<td>D4Mgh1</td>
<td>1.5</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>D4Wox32</td>
<td>13.1</td>
<td>9</td>
<td>25</td>
</tr>
<tr>
<td>D4Mgh2</td>
<td>0.2</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>D4Mgh14</td>
<td>11.5</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>D4Mgh3</td>
<td>—</td>
<td>15</td>
<td>27</td>
</tr>
</tbody>
</table>

* Only data for chromosomal regions showing suggestive evidence of linkage to EAE are reported. The remaining data are available on request. Primers for amplification of all markers except D4Wox32 were obtained from the Whitehead Institute/MIT database (ftp-genome.wi.mit.edu/distribution/rat_sslp_releases).

* Distance in cM between two consecutive markers as calculated by MAPMAKER/EXP 3.0 on the (LEW × BN)F1 progeny using the Kosambi mapping function. The markers have been oriented according to the cytogenetic maps (30); i.e., from centromere to distal q arm for this telocentric chromosome.

* NN, number of F2 progeny homozygous for the Brown Norway parental allele; LN, heterozygous F2 progeny; LL, progeny homozygous for the Lewis parental allele.

* Pearson’s x² statistics with 2 df.

* p values.

Table IV. Penetrance values for different genotype combinations at loci D4Mgh1 and D10Mgh10

<table>
<thead>
<tr>
<th>Locus</th>
<th>D10Mgh10</th>
<th>D10Mgh10</th>
<th>D10Mgh10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LL</td>
<td>LN</td>
<td>NN</td>
</tr>
<tr>
<td>D4Mgh1 LL</td>
<td>90</td>
<td>66.7</td>
<td>28.6</td>
</tr>
<tr>
<td>D4Mgh1 LN or NN</td>
<td>47.6</td>
<td>33.9</td>
<td>16.7</td>
</tr>
</tbody>
</table>

* Percentage of all animals with a specific genotype combination that presented with clinical EAE.