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Genetic Analysis of Disease Subtypes and Sexual Dimorphisms in Mouse Experimental Allergic Encephalomyelitis (EAE): Relapsing/Remitting and Monophasic Remitting/Nonrelapsing EAE Are Immunogenetically Distinct

Russell J. Butterfield,* Elizabeth P. Blankenhorn,† Randall J. Roper,* James F. Zachary,* R. W. Doerge,‡ Jayce Sudweeks,§ John Rose,¶ and Cory Teuscher²

Experimental allergic encephalomyelitis (EAE) is the principal animal model of multiple sclerosis (MS), the major inflammatory disease of the central nervous system. Murine EAE is generally either an acute monophasic or relapsing disease. Because the clinical spectrum of MS is more diverse, the limited range of disease subtypes observed in EAE has raised concern regarding its relevance as a model for MS. During the generation of a large F₂ mapping population between the EAE-susceptible SJL/J and EAE-resistant B10.S/DvTe inbred lines, we identified four distinct subtypes of murine EAE resembling clinical subtypes seen in MS. We observed acute progressive, chronic/nonrelenting, remitting/relapsing, and monophasic remitting/nonrelapsing EAE. An additional subtype, benign EAE, was identified after histologic examination revealed that some mice had inflammatory infiltrates of the central nervous system, but did not show clinical signs of EAE. Genome exclusion mapping was performed to identify the loci controlling susceptibility to each disease subtype. We report three novel EAE-modifying loci on chromosomes 16, 7, and 13 (eae11–13, respectively). Additionally, unique loci with gender-specific effects govern susceptibility to remitting/relapsing (eae12) and monophasic remitting/nonrelapsing (eae7 and 13) EAE. The Journal of Immunology, 1999, 162: 3096–3102.

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Abbreviations used in this paper: MS, multiple sclerosis; AP, acute progressive; CNR, chronic/nonrelenting; CNS, central nervous system; EAE, experimental allergic encephalomyelitis; M-RNR, monophasic remitting/nonrelapsing; PLP, proteolipid protein; R/R, relapsing/remitting.

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with EAE have made it a useful model for MS, the difference in clinical profiles has led to concern about the suitability of EAE as an experimental counterpart of MS. This concern is addressed in the present study in which we demonstrate that five clinical subtypes are seen in mice when F2 intercross progeny of inbred strains are used.

A promising approach to understanding EAE and MS is to examine the genetic factors involved in both diseases. As with MS, both MHC-linked and non-MHC-linked factors have been associated with susceptibility to EAE (21–25). With few exceptions, the genetic dissection of murine EAE has relied on examining susceptibility and resistance among small populations of different highly inbred strains, in which varying disease-modifying genes have been fixed (15, 26). While this approach controls the genetic heterogeneity inherent in such a complex disease, it fails to provide an adequate model of the complex genetic interactions underlying MS. Failure to identify the complete spectrum of MS disease profiles in murine EAE may be due to lack of variation within inbred lines or to small sample sizes in which differences in disease subtype are not detected.

To better approximate the genetic architecture underlying susceptibility to MS, we created a large F2 population from the EAE-susceptible SJL/J and EAE-resistant B10.S/DvTe inbred lines. We report the identification of five distinct subtypes of murine EAE, namely acute progressive (AP), chronic/nonremitting (CNR), R/R, monophasic remitting/nonrelapsing (M-RNR), and benign EAE. Full genome scanning was used to identify loci important in controlling susceptibility and disease subtype in EAE. We report three novel EAE-modifying loci on chromosomes 16, 7, and 13 (eae11–13, respectively). Unique loci govern susceptibility to R/R (eae12) and M-RNR (eae7 and 13) EAE. Furthermore, we discuss the effect of gender on susceptibility to the particular subtypes of EAE. Our results strengthen the role of murine EAE as a model for human MS, and allow for the dissection of the genetic architecture underlying the different immunopathologic manifestations of disease in the CNS.

### Materials and Methods

#### Animals
Male and female SJL/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B10.S/DvTe mice were generated from breeding stock originally obtained from Dr. Chella David (Mayo Clinic, Rochester, MN). (SJL/J × B10.S/DvTe)F1 hybrids and (SJL/J × B10.S/DvTe)F2 progeny were generated in the animal colony at Brigham Young University (Provo, UT). F2 animals were generated continuously over the course of 12 mo from the same F1 hybrid breeding stock. Animals were fed Purina mouse pellets (Ralston-Purina, St. Louis, MO) and acidified water ad libitum.

#### Induction and evaluation of EAE
Induction of EAE using SJL/J spinal cord homogenate emulsified in CFA was conducted as previously described (27). Starting on day 10, mice were monitored for clinical symptoms and graded from 1–4, as follows: 0, no clinical expression of disease; 1, floppy tail without hind limb weakness; 2, hind limb weakness with or without flaccid tail; 3, hind leg paralysis and floppy tail; and 4, hind leg paralysis accompanied by floppy tail and urinary or fecal incontinence (28). Animals that progressed to a clinical score of 4 were euthanized. Mice that showed no clinical disease by day 30 were killed, and tissues were collected. Animals exhibiting clinical disease anytime from day 10 to day 30 were monitored for an additional 30 days for remission and relapse. Information on age, sex, coat color, and day of disease onset was recorded for each animal.

#### Pathologic evaluation
Animals were killed, and the brain and spinal cord were removed and fixed by immersion in 10% phosphate-buffered formalin. Transverse sections for light-microscopic analysis were cut at 5 μm from paraffin-embedded materials and stained with hematoxylin and eosin or luxol fast blue/periodic acid Schiff stain. Representative areas of the brain and spinal cord were selected for histologic evaluation based on previous reports (16, 17) and included cerebrum, cerebellum-midbrain, and spinal cord at the cervical, thoracic, and lumbar levels.

#### Genotyping and linkage analysis
Genomic DNA was isolated from liver tissue, and PCR parameters for microsatellite typing were as previously described (29). Microsatellite size variants were resolved by autoradiography on Kodak film (Eastman Kodak, Rochester, NY). A linkage map was generated with 172 informative microsatellite markers using the Kosambi mapping function in the MAPMAKER/EXP computer package (30, 31). Linkage of marker loci to disease subtype was determined using 2 × 3 χ2 tests for independence of marker genotypes between affected and unaffected groups. Permutation-derived critical values for declaration of significant linkage (32) were calculated for α = 0.10 and α = 0.05 from the distribution of the χ2 statistic from 1000 permutations of our data under the null hypothesis of no linkage at each marker. Significant linkages were reported if the χ2 statistic for a particular marker locus was greater than the critical value at α = 0.05. Suggestive linkages (α = 0.10) were reported where results replicate significant linkages identified in other studies. For identification of sex-specific loci, linkage analysis was performed on the population as a whole, and for males and females separately.

#### Results

#### Incidence and sexual dimorphism of EAE subtypes
A large cohort of (SJL/J × B10.S/DvTe)F2 mice (n = 633) was inoculated with SJL/J spinal cord homogenate and CFA for the induction of EAE. Brain and spinal cord tissues were examined for histopathology. CNS lesions were found in 454 animals (Table I), 247 of which also exhibited clinical signs of EAE. Females were more susceptible to CNS inflammation than males. While 85% of females had either brain or spinal cord lesions, only 57% of males had such lesions (χ² = 58.21, p = 2.3 × 10⁻¹⁴). Given the bias...
in susceptibility between males and females to histopathologic lesions, it is interesting that similar numbers of males and females observed in the number of males and females for both R/R and M-RNR EAE. We also observed a difference in susceptibility to particular subtypes of EAE (Table IV). AP EAE is controlled by a locus on chromosome 3 near D3Mit25 at 29 cM ($\chi^2 = 15.2$, 90% experimentwise cutoff = 14.31). Our findings replicate the identification of eae3, a locus influencing overall EAE susceptibility in both F2 (34) and backcross (35) progeny. Consistent with the findings of Encinas et al. (35), who also used the B10.S/SgMedJ and SJL/J strains, susceptibility is recessive, and inherited from the SJL parent. R/R EAE is linked to a newly identified locus on chromosome 7 at 16 cM (eae12). Maximum linkage was at D7Mit227 ($\chi^2 = 17.5$, 95% experimentwise cutoff = 15.46). Eae12 is proximal to another EAE susceptibility locus on chromosome 7, eae4 (26–50 cM). Stratification of the data by sex indicated that the effect of eae12 on susceptibility was greater in females than in males ($\chi^2$ for females = 16.5, and for males = 6.16). Susceptibility to M-RNR EAE is linked to chromosome 13 near D13Mit66 at 37 cM (eae13). In contrast to eae12, the effect of eae13 was greater in males ($\chi^2$ for males = 20.4, and for females = 15.87). Susceptibility is associated with the SJL allele. A locus on chromosome 11 at 47–52 cM (eae7), identified in a previous report affecting severity and duration of symptoms (33), is also associated with M-RNR EAE. Maximal linkage is at D11Mit36 ($\chi^2 = 20.4$, 95% experimentwise cutoff = 15.87). Susceptibility is associated with the SJL allele at eae7. No significant

### Table II. Frequency of EAE disease phenotypes in an F2 intercross between B10.S/DvTe and SJL/J mice

<table>
<thead>
<tr>
<th>Disease Phenotypes Among Clinically Affected Progeny</th>
<th>Affected</th>
<th>APb</th>
<th>%</th>
<th>CNRb</th>
<th>%</th>
<th>R/R</th>
<th>%</th>
<th>M-RNRb</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>86</td>
<td>22</td>
<td>14</td>
<td>16</td>
<td>17</td>
<td>20</td>
<td>36</td>
<td>36</td>
<td>42</td>
</tr>
<tr>
<td>Female</td>
<td>161</td>
<td>42</td>
<td>14</td>
<td>26</td>
<td>51</td>
<td>32</td>
<td>42</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>247</td>
<td>45</td>
<td>18</td>
<td>56</td>
<td>23</td>
<td>68</td>
<td>27</td>
<td>78</td>
<td>32</td>
</tr>
</tbody>
</table>

$\chi^2$ value for independence, comparing the number of affected animals for each subtype to all other clinically affected animals.

$\chi^2 = 1.33$, 3.08, 3.99, 6.46

$\chi^2$ for independence, comparing the number of affected animals for each subtype to all other clinically affected animals.

$\chi^2 = 0.25$, 0.08, 0.046, 0.01

Unaffected and affected refer to absence or presence of clinical signs of EAE, respectively.

AP, acute progressive; CNR, chronic/nonremitting; R/R, relapsing/remitting; M-RNR, monophasic-remitting nonrelapsing.

Percentage of affected mice with given disease phenotype.

Analysis of the F2 progeny revealed multiple loci that control EAE and its subtypes (Tables III and IV). Overall disease susceptibility (Table III) is linked to two major loci seen previously by quantitative analysis (33), eae4 on chromosome 7 at 25–51 cM, and eae5 on chromosome 17 at 22–23 cM. A locus on chromosome 16 with suggestive linkage to susceptibility by quantitative analysis (33) is again suggestive of linkage by this qualitative analysis ($\chi^2 = 15.2$, 90% experimentwise cutoff = 14.82). We now give this a provisional designation eae11, based on the identification of a significant quantitative trait locus for spinal cord histopathology in this region (Blankenhorn et al., in preparation). Analysis of the data when stratified by sex show evidence of sexually dimorphic effects at eae4 and eae11 (Table III). As previously described (33), the allele from B10.S is associated with susceptibility at eae4, and the allele from SJL is associated with susceptibility at eae5 and eae11.

Loci on chromosomes 3, 7, 11, and 13 were significant in linkage of susceptibility to particular subtypes of EAE (Table IV). AP EAE is controlled by a locus on chromosome 3 near D3Mit25 at 29 cM ($\chi^2 = 15.2$, 90% experimentwise cutoff = 14.31). Our findings replicate the identification of eae3, a locus influencing overall EAE susceptibility in both F2 (34) and backcross (35) progeny. Consistent with the findings of Encinas et al. (35), who also used the B10.S/SgMedJ and SJL/J strains, susceptibility is recessive, and inherited from the SJL parent. R/R EAE is linked to a newly identified locus on chromosome 7 at 16 cM (eae12). Maximum linkage was at D7Mit227 ($\chi^2 = 17.5$, 95% experimentwise cutoff = 15.46). Eae12 is proximal to another EAE susceptibility locus on chromosome 7, eae4 (26–50 cM). Stratification of the data by sex indicated that the effect of eae12 on susceptibility was greater in females than in males ($\chi^2$ for females = 16.5, and for males = 6.16). Susceptibility to M-RNR EAE is linked to chromosome 13 near D13Mit66 at 37 cM (eae13). In contrast to eae12, the effect of eae13 was greater in males ($\chi^2$ for males = 20.4, and for females = 15.87). Susceptibility is associated with the SJL allele. A locus on chromosome 11 at 47–52 cM (eae7), identified in a previous report affecting severity and duration of symptoms (33), is also associated with M-RNR EAE. Maximal linkage is at D11Mit36 ($\chi^2 = 20.4$, 95% experimentwise cutoff = 15.87). Susceptibility is associated with the SJL allele at eae7. No significant
loci were detected for susceptibility to the CNR or benign forms of EAE. Stratification of the data to exclude clinically affected animals revealed a locus suggestively linked (\( \chi^2 \) threshold value for the total population = 16.28, for males = 16.38, and for females = 15.88).

### Discussion

**EAE subtypes**

MS is characterized by a number of different clinical subtypes (20) that are mirrored in our intercross. Patients with R/R MS have

### Table III. Susceptibility loci linked to EAE in (B10.S/DvTe × SJL/J)\( F_2 \) mice

<table>
<thead>
<tr>
<th>Locus</th>
<th>cM</th>
<th>eae(^d)</th>
<th>Sex</th>
<th>Specificity</th>
<th>Sex</th>
<th>B</th>
<th>H</th>
<th>S</th>
<th>B</th>
<th>H</th>
<th>S</th>
<th>Total</th>
<th>( \varphi )</th>
<th>( \delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>D7Mit85</td>
<td>26.5</td>
<td>eae4</td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>33</td>
<td>62</td>
<td>14</td>
<td>40</td>
<td>114</td>
<td>46</td>
<td>19.4(^e)</td>
<td>15.1(^e)</td>
<td>NS(^f)</td>
<td></td>
</tr>
<tr>
<td>D7Mit233</td>
<td>40.0</td>
<td></td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>57</td>
<td>95</td>
<td>29</td>
<td>32</td>
<td>71</td>
<td>52</td>
<td>23.2(^e)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D7Mit39</td>
<td>50.3</td>
<td></td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>49</td>
<td>82</td>
<td>31</td>
<td>30</td>
<td>76</td>
<td>59</td>
<td>14.9(^d)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D16Mit140</td>
<td>41.0</td>
<td>eae11</td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>17</td>
<td>44</td>
<td>36</td>
<td>43</td>
<td>122</td>
<td>33</td>
<td>15.2(^d)</td>
<td>NS</td>
<td>15.3(^d)</td>
<td></td>
</tr>
<tr>
<td>D17Mit176</td>
<td>22.5</td>
<td>eae5</td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>18</td>
<td>65</td>
<td>26</td>
<td>58</td>
<td>101</td>
<td>37</td>
<td>20.7(^e)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D17Mit51</td>
<td>22.9</td>
<td></td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>18</td>
<td>64</td>
<td>27</td>
<td>58</td>
<td>116</td>
<td>36</td>
<td>16.4(^d)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>D17Mit70</td>
<td>32.7</td>
<td></td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>18</td>
<td>59</td>
<td>28</td>
<td>54</td>
<td>101</td>
<td>35</td>
<td>19.0(^e)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) EAE susceptibility locus designation according to Butterfield et al (33). eae11 is newly reported in this study.

\( ^b \) B, B10.S homozygote; S, SJL/J homozygote; H, heterozygote.

\( ^c \) \( \chi^2 \) test statistics > \( \chi^2 \) experimentwise thresholds at \( \alpha = 0.05 \) as determined by 1000 permutations of the original data (\( \chi^2 \) threshold value for the total population = 16.28, for males = 16.38, and for females = 15.88).

\( ^d \) \( \chi^2 \) test statistics > \( \chi^2 \) experimentwise thresholds at \( \alpha = 0.10 \) as determined by 1000 permutations of the original data (\( \chi^2 \) threshold value for the total population = 14.82, for males = 14.56, and for females = 14.19).

\( ^e \) NS, not significant.

\( ^f \) cM distances between markers from our linkage map generated using the Kosambi map function within the MAPMAKER/EXP computer package (31, 32).

### Table IV. Susceptibility loci linked to AP, R/R, and M-RNR EAE in (B10.S/DvTe × SJL/J)\( F_2 \) mice

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Locus</th>
<th>cM</th>
<th>eae(^d)</th>
<th>Sex</th>
<th>Specificity</th>
<th>Sex</th>
<th>B</th>
<th>H</th>
<th>S</th>
<th>B</th>
<th>H</th>
<th>S</th>
<th>Total</th>
<th>( \varphi )</th>
<th>( \delta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP</td>
<td>D3Mit25</td>
<td>29.5</td>
<td>eae3</td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>2</td>
<td>12</td>
<td>12</td>
<td>63</td>
<td>115</td>
<td>61</td>
<td>15.2(^e)</td>
<td>NS(^d)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>R/R</td>
<td>D7Mit227</td>
<td>16.0</td>
<td>eae12</td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>13</td>
<td>37</td>
<td>1</td>
<td>68</td>
<td>132</td>
<td>73</td>
<td>17.5(^e)</td>
<td>16.5(^e)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D7Mit268</td>
<td>16.0</td>
<td></td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>1</td>
<td>15</td>
<td>1</td>
<td>65</td>
<td>160</td>
<td>47</td>
<td>17.1(^e)</td>
<td>16.4(^e)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>M-RNR</td>
<td>D11Mit36</td>
<td>47.7</td>
<td>eae7</td>
<td>( \delta )</td>
<td>( \varphi )</td>
<td>5</td>
<td>17</td>
<td>11</td>
<td>63</td>
<td>121</td>
<td>41</td>
<td>16.5(^e)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D11Mit38</td>
<td>49.0</td>
<td></td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>5</td>
<td>21</td>
<td>14</td>
<td>81</td>
<td>136</td>
<td>43</td>
<td>15.8(^e)</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D11Mit285</td>
<td>52.0</td>
<td></td>
<td>( \varphi )</td>
<td>( \delta )</td>
<td>6</td>
<td>21</td>
<td>14</td>
<td>89</td>
<td>151</td>
<td>42</td>
<td>14.6(^e)</td>
<td>20.0(^e)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) EAE susceptibility locus designation according to Butterfield et al (33). eae12 and eae13 are newly reported in this study.

\( ^b \) B, B10.S homozygote; S, SJL/J homozygote; H, heterozygote.

\( ^c \) \( \chi^2 \) test statistics > \( \chi^2 \) experimentwise thresholds at \( \alpha = 0.05 \) as determined by 1000 permutations of the original data. Threshold values for AP = 14.31 for the total population, 14.26 for males, and 14.36 for females; for R/R = 14.04 for the total population, 14.57 for males, and 14.19 for females; for M-RNR = 14.16 for the total population, 14.30 for males, and 14.34 for females.

\( ^d \) NS, not significant.

\( ^e \) \( \chi^2 \) test statistics > \( \chi^2 \) experimentwise thresholds at \( \alpha = 0.05 \) as determined by 1000 permutations of the original data. Threshold values for AP = 15.74 for the total population, 15.84 for males, and 15.53 for females; for R/R = 15.64 for the total population, 16.39 for males, and 15.86 for females; for M-RNR = 15.87 for the total population, 16.57 for males, and 15.95 for females.

\( ^f \) cM distances between markers from our linkage map generated using the Kosambi map function within the MAPMAKER/EXP computer package (31, 32).
repeated exacerbations or relapses with subsequent clinical recovery and remission of disease activity. In our F₂ mouse population, 27% of affected animals had the R/R subtype. R/R EAE is more frequent in females than in males. We observed a monophasic form of EAE (M-RNR) in 32% of affected animals in our population, consisting of an acute attack, followed by complete recovery, without further clinical signs. In contrast to R/R EAE, susceptibility to M-RNR EAE was greater in males than in females.

Several progressive subtypes of MS have been described, including primary progressive, secondary progressive, and progressive relapsing (20). Each of these subtypes is characterized by a steady accumulation of neurologic deficits over time, with some variation between the subtypes in the amount of recovery between relapses. The CNR EAE subtype in our population is analogous to these progressive forms of MS, accounting for 23% of affected animals. AP EAE, seen in 18% affected mice, resembles the malignant MS subtype. Both are characterized by swift progression of disease resulting in severe disability or death a short time after onset.

Benign MS accounts for up to 20% of MS cases (12). This mild disease subtype is characterized by a lack of neurologic deficit for 15 yr after onset (20). The finding of incidental cases of MS in autopsy series unsuspected in life suggests that the disorder can in fact be entirely subclinical (36). It is known that mice can have histologic signs of EAE, such as lymphocytic and monocytic CNS infiltrates, without exhibiting clinical signs (16, 17). This benign subtype of EAE was seen in 54% of animals with histologic lesions.

As with MS (13), females in our population were more susceptible to EAE than males. Differences between males and females in susceptibility to EAE have been known for some time. Recent reports have begun to characterize and explain these differences (37–41). In a model using myelin basic protein as the inducing Ag, young SJL/J males were entirely resistant to disease induction, while females were susceptible (37). Another model using PLP peptide 139–151 as the inducing Ag found that SJL/J males undergo a monophasic disease, while females exhibit a chronic relapsing disease (38). Consistent with these observations, M-RNR EAE in our population was more frequent in males than in females, and R/R EAE was more frequent in females than males. Interestingly, given the extreme gender bias in susceptibility to histopathologic signs of EAE, males and females progressed in similar proportions to clinical signs of EAE. This observation suggests that the sexual dimorphism in susceptibility to EAE may be regulated by genes controlling the ability of inflammatory mediators to infiltrate the CNS. In this respect, a recent study has implicated sex hormones in the homing of mononuclear cells to the CNS and the production of IFN-γ in the induction phase of EAE (41).

Genetic analysis

Loci controlling susceptibility to EAE were found on chromosomes 7 (eae4), 17 (eae5), and 16 (eae11), and have been identified in a previous report by quantitative analysis (33). Significance of these loci is confirmed in this report by qualitative methods. Analysis of the data when stratified by sex suggests a sex-specific effect at both eae4 and eae11. In addition to these loci, we have found that loci on chromosomes 3 (eae3), 7 (eae12), 11 (eae7), and 13 (eae13) affect the clinical subtype of EAE.

Two previous reports have identified an EAE susceptibility locus on chromosome 3 (eae3) on an interval between 30 and 53 cM (34, 35). In our population, eae3 is linked with susceptibility to AP EAE. Susceptibility is associated with the SJL allele. A locus controlling susceptibility to Thiefer’s murine encephalomyelitis virus-induced demyelination (Tmevd2) is also found on this region of chromosome 3 (42). Eae3 and Tmevd2 may in fact represent a single gene or gene cluster involved with demyelinating disease or CNS pathology (42). Susceptibility loci to autoimmune ovarian dysgenesis and insulin-dependent diabetes mellitus (Aod2 and Idld3, respectively) also map to this region of chromosome 3, although proximal of eae3 (43, 44).

A newly identified locus on chromosome 7 (eae12) is linked to R/R EAE. A sex-specific effect at this locus may explain the increased susceptibility of females to this disease subtype. Interesting candidate genes in the region of eae12 include myelin-associated glycoprotein (Mag), an EAE-inducing Ag at 11 cM, and TGF-β1 at 6.5 cM (www.informatics.jax.org). Two groups have linked susceptibility to systemic lupus erythematosus to this region of chromosome 7 (45, 46). This colocalization may represent a single gene important for autoimmunity in general. A recent study has implicated a region on chromosome 19q syntenic with eae12 in susceptibility to MS in North American/Northern European Caucasian and Chinese populations (47).

In contrast to eae12, a locus on chromosome 13 (eae13) is linked to susceptibility to M-RNR EAE in males, but not females. The SJL allele at this locus is responsible for increased susceptibility to the M-RNR disease subtype. A sex-specific effect at eae13 may give males a greater ability to control disease once it is initiated. It is tempting to speculate that eae13 might be directly responsive to testosterone levels. In this regard, it has been reported that castrated males immunized with PLP peptide 139–151 display a relapsing form of EAE in contrast to intact males that display a monophasic disease (41). In light of the sex-specific effect at eae13, it is interesting to note the presence of rsl, a locus controlling the regulation of sex-limited protein in this region of chromosome 13 (48). Steroid 5α-reductase 1 (Srds1a) at 39 cM, which is involved in the synthesis of dihydrotestosterone, is an interesting candidate gene for both rsl and eae13. Other candidate genes in this region include Ihh at 35 cM, Cita2a at 36 cM, and Cita2 at 38 cM (www.informatics.jax.org).

Mechanistically, both eae12 and eae13 may be involved in epitope spreading, a phenomenon associated in some reports with relapses (49). Eae12 may be involved with presentation of secondary CNS Ags, or access to the CNS of T cells reactive to secondary Ags. Conversely, eae13 could inhibit epitope spreading associated with relapses by causing a shift in the response to secondary CNS Ags from a pathogenic Th1 response to a protective Th2 response.

An additional locus significant in M-RNR EAE is eae7 on chromosome 11. Eae7 was identified in a previous report for its effect on severity and duration of symptoms (33). Shorter duration of symptoms and decreased severity of disease were associated with the SJL allele at this locus. Consistent with these observations, in the present study, the SJL allele predisposes for a shorter, less severe (M-RNR) disease subtype. An interesting candidate gene in this region is the inducible nitric oxide synthase gene (Nos2) at 46 cM. Nitric oxide and other intermediates in the nitric oxide synthesis pathway have been implicated in the CNS pathology in EAE (50). Consistent with the effect of eae7 in M-RNR EAE, increasing evidence suggests that the Nos2 gene can have a protective role in EAE (51, 52). Other candidate genes in the region include the family of small cytokines (Sclva–12) at 46–48 cM (www.informatics.jax.org). Susceptibility loci for two other autoimmune disorders colocalize to this region of chromosome 11, Orch3 in autoimmune orchitis at 44.5 cM, and Idld4 in insulin-dependent diabetes mellitus at 44 cM (53, 54).

The appearance of different EAE subtypes in our population and the unique genetic components underlying disease subtype emphasize the usefulness of large F₂ populations in the genetic dissection.
of complex traits. F₂ populations allow for more complex genetic interaction between loci, resulting in increased variation in phenotypes and emergence of new phenotypes unobserved in less complex models. A large population allows for the observation and genetic analysis of subtle differences in phenotype that may be attributed to random variation in smaller populations. Of particular interest in our population are the genetic differences governing the susceptibility to particular disease subtypes and the influence of sex on these genetic differences. It is worth noting that appropriate genetic modeling using other EAE-S strains such as PL/J, B10.PL/SnJ, and BALB/cByJ may reveal additional clinical, histopathologic, and sex-specific effects not observed in our population. Investigation of the genetics underlying different disease subtypes in EAE may lead to a greater understanding of the genetic control of immune system regulation in general, e.g., underlying control of tolerance, Th1/Th2 dichotomy, tissue-specific homing of lymphocytes, and mechanisms of suppression of ongoing immune responses. Further analysis of loci with specific effects may explain the general immunologic differences between males and females, and lead to a better understanding of the interplay between the endocrine and immune systems.

Recent genome scans have not resulted in the expected identification of MS susceptibility loci in the human genome (4–8). Difficulty in controlling for gene interaction (epistasis) in an outbred population, and the strong sexual dimorphism in the expression of disease may explain these disappointing results. Our observations suggest that different disease subtypes have different underlying genetic control, and that this control may differ between the sexes. Thus, underlying genetic differences may explain the heterogeneity in disease course and prognosis characteristic of MS. Differences in disease between the sexes, and between patients with different disease courses may be responsible for confounding the interpretation of human MS genetic data or masking the presence of susceptibility loci. In light of our findings, MS genetic studies should be stratified more stringently with respect to sex and disease type.

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References


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