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Evidence for Common Autoimmune Disease Genes Controlling Onset, Severity, and Chronicity Based on Experimental Models for Multiple Sclerosis and Rheumatoid Arthritis

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The pathogenicity of multiple sclerosis is still poorly understood, but identification of susceptibility genes using the animal model experimental allergic encephalomyelitis (EAE) could provide leads. Certain genes may be shared between different autoimmune diseases, and identification of such genes is of obvious importance. To locate gene regions involved in the control of EAE and to compare the findings with the susceptibility loci recently identified in a model for rheumatoid arthritis (pristane-induced arthritis), we made crosses between the encephalomyelitis- and arthritis-susceptible rat strain DA and the resistant E3 strain. Genetic analysis of animals produced in a F₂ intercross identified 11 loci associated with specific EAE-associated traits. Interestingly, five of these loci were situated at the same position as major loci controlling pristane-induced arthritis and showed similarities in inheritance pattern and subphenotype associations. Our results show that different phases of EAE are controlled by different sets of genes and that common genes are likely to be involved in different autoimmune diseases. The Journal of Immunology, 2000, 164: 1564–1568.

Materials and Methods

Animals

Rat breeding nuclei of the different strains (DA, E3) were originally generously provided by Professor Hans Hedrich (Zentralinstitut fur Versuchstierzucht, Hannover, Germany). The rats were kept in animal conditions, with specific pathogen-free status.
facilities in a pathogen-free and climate-controlled environment with 12-h light/dark cycles, housed in polystyrene cages containing wood shavings, and fed standard rodent chow and water ad libitum. The rats were 8–9 wk old when the experiment started. During the experiments, two to three rats were housed in each cage.

**Induction and evaluation of EAE**

Spinal cord homogenate was prepared from DA rats as described (1) and kept at −70°C. Rats were immunized s.c. in the base of the tail with 200 μl of an inoculum containing 100 μl IFA (Difco, Detroit, MI), 100 μl saline, and 10 mg spinal cord homogenate. All rats were weighed and examined daily for signs of EAE according to a nine point scale: 0, normal; 1, tail weakness; 2, tail paralysis; 3, tail paralysis and mild waddle; 4, tail paralysis and severe waddle; 5, tail paralysis and paralysis of one limb; 6, tail paralysis and paralysis of a pair of limbs; 7, tetraparesis; 8, premorbid paralysis and severe waddle; 9, moribund or deceased (21). A second immunization was conducted between days 72 and 76 on selected rats that included 51 rats with the highest clinical score and 51 rats with no signs of EAE. The other rats were sacrificed on day 40. Blood was obtained by cutting the tip of the tail. The rats were bled before the first and the second immunization, on day 40, and at sacrifice.

**EAE phenotypes**

The following phenotypes were determined: 1) onset of disease: first day a clinical score of 1 or higher was obtained, only diseased rats included; 2) severity: clinical score (scores 1–8) of each rat at the indicated day after immunization; 3) acute disease: rats with monophasic disease; 4) relapsing disease: rats with more than one relapse of clinical disease (a relapse was counted when the rats had an increase in clinical score of 1 or more for at least 3 days); 5) duration of disease: the number of days the rats had clinical scores 2–8; and 6) body weight: change in body weight from day 7 to 40.

**Genotyping**

Rat microsatellite markers were purchased (Research Genetics, Huntsville, AL). Tips of the tail were used for preparation of genomic DNA according to a standard protocol (23). PCR was performed in 10-μl reaction volumes containing forward and reverse primer (0.5 μM each), dNTP (200 μM), MgCl₂ (1.5 mM), Tris-HCl (20 mM, pH 8.3), Taq polymerase (0.5 U), and 20 ng genomic DNA. The forward primer was phosphorylated with 0.4 μCi [γ-32P]ATP (3000 Ci/mmol; DuPont/NEN, Boston, MA). Amplification conditions were as follows: 94°C for 3 min, followed by 25 cycles of 94°C for 15 s, 55°C for 1 min, 72°C for 90 s, and a final extension at 72°C for 7 min. The PCR was performed in a thermal cycler (MR-225; MJ Research; Watertown, MA). The PCR products were size-fractionated on 6% (AT Biochem, Malvern, PA) polyacrylamide gels in 1x TBE. Gels were exposed on autoradiographic film (Kodak XAR film, Rochester, NY or Amersham Hyperfilm MP, Arlington Heights, IL) at −70°C for 12–48 h. All markers were scored at least four times. All markers with a high logarithm of odd’s ratio (LOD) error in the haplotype analysis were rescored and retyped if necessary.

**Statistical and linkage analysis**

Comparison between groups of parental strains were performed with Fischer exact test (incidence) or with the nonparametric Mann-Whitney U test (onset day, scores). Comparison between intercross F₁ female and male rats was performed with χ² test (incidence). All the statistical analyses were done using Statistics (Statsoft; Tulsa, OK) or Statview (Abacus; Berkeley, CA) software packages. Linkage analysis was performed with the Mapmaker computer package (24, 25) as described (15). An improved linkage map based on several crosses involving DA and E3 was used and can be found at http://net.inflam.lu.se/. Linkage was tested using all the phenotypes described above. Due to this multiple testing, the threshold level for significance should be higher than the formal level of 3.3 in a constrained mode of inheritance (25). The decision on designating the found linkages as EAE loci was based on the basis not only of the LOD value but also on correspondence with earlier published data on congenic strains (21) and on findings in the pristane-induced arthritis (PIA) model in the same rat cross (22).

**Results**

To map genes involved in the control of EAE, we made F₁ and F₂ crosses between the E3 and the DA strains. The disease is highly penetrable in DA rats, whereas only 25% of the F₁ rats developed disease that could be either acute or relapsing. This indicates a complex genetic control involving both recessive and dominant genes (Table I). Of the F₂ rats, 25% developed a widely variable disease course, ranging from acute EAE with one relapse followed by recovery to a more severe form of the disease with several relapses (Table I and Fig. 1). The affected rats suffered weight loss that was more pronounced in the rats with the relapsing form of EAE. Females were more prone to develop a relapsing disease (Table I).

An initial genome scan was performed using 92 F₂ rats, including 46 rats with the most severe form of the disease and 46 rats without signs of EAE. These rats were genotyped with 181 informative microsatellite markers, which covered 95% of the genome at a maximum 20-cM intermarker distance. The remaining (E3 × DA)F₂ rats were analyzed with those markers that showed suggestive linkage (LOD score >2.0). To identify loci controlling distinct features of the disease, we studied several subphenotypes: 1) the day of disease onset; 2) the maximal clinical severity; 3) the change in body weight from day 7 to 40; 4) acute EAE; 5) the duration of clinical signs (number of days during which the clinical score was between 2 and 8); and 6) the number of relapses.

Eleven gene regions associated with specific disease-connected traits (Table II and Fig. 2) were identified, and, interestingly, several of those loci coincide with loci previously identified in PIA using identical rat strains.

The onset of disease was associated with suggestive loci on chromosomes 4 and 5. Interestingly, the locus on chromosome 4 is situated at the same position as the Pia2 locus, which also controls disease onset. As is the case in the arthritis model using an identical cross (22), both onset loci contain susceptibility alleles inherited from E3: the chromosome 5 locus is inherited in a recessive and the chromosome 4 in a dominant mode.

---

**Table 1. EAE susceptibility in parental, F₁, and F₂ animals**

<table>
<thead>
<tr>
<th>Rat Strain/Cross</th>
<th>Sex</th>
<th>Incidence</th>
<th>Mean Day of Onset (± SD)a</th>
<th>Mean Maximum Score (± SD)a</th>
<th>Mean Duration (± SD)c</th>
<th>Acute Disease (%)</th>
<th>Relapsing Disease (%)</th>
<th>Mean Change in Body Weight Day 7–40°</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAa</td>
<td>M</td>
<td>7/9d</td>
<td>13 ± 6</td>
<td>6.0 ± 1.9</td>
<td>9 ± 8</td>
<td>22</td>
<td>56</td>
<td>32 ± 25</td>
</tr>
<tr>
<td>E3F</td>
<td>0/12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80 ± 3</td>
</tr>
<tr>
<td>M</td>
<td>0/3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>39 ± 9</td>
</tr>
<tr>
<td>(E3 × DA)F₁d</td>
<td>F</td>
<td>6/13</td>
<td>13 ± 1</td>
<td>5.2 ± 1.6</td>
<td>12 ± 7</td>
<td>15</td>
<td>31</td>
<td>58 ± 13</td>
</tr>
<tr>
<td>M</td>
<td>5/19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>71 ± 8</td>
</tr>
<tr>
<td>(E3 × DA)F₂e</td>
<td>F</td>
<td>27/107</td>
<td>14 ± 3</td>
<td>5.3 ± 1.7</td>
<td>23 ± 19</td>
<td>8</td>
<td>17</td>
<td>46 ± 14</td>
</tr>
<tr>
<td>M</td>
<td>30/116</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>74 ± 19</td>
</tr>
</tbody>
</table>

a Only sick animals included.  
b All rats.  
 These rats were followed until day 40.  
 Two rats died on day 12 and 13.  
° The difference between F₂ males and females in the frequency of relapsing EAE is significant (p = 0.04).
The disease severity was most strongly associated with a locus on chromosome 12 (Eae5), which contains a DA susceptibility allele of major importance not only for severity but also for the relapsing form of EAE. This locus was also identified as the major locus in PIA, controlling arthritis severity (Pia4). Other loci influencing disease severity in males were found on chromosome 1 (Eae6 and Eae7) and 20 (Eae1). Eae7 was most strongly associated with change in body weight, and Eae6 with body weight change and duration of the disease. Decrease in body weight is known to be closely correlated with clinical symptoms. A suggestive PIA locus corresponding to Eae6 was associated with arthritis severity and joint erosions (our unpublished observations). The locus on chromosome 20 (Eae1) includes the MHC region. The DA allele is associated with greater severity and incidence with a dominant inheritance pattern. An association with products of the MHC locus, in particular MHC class II, is well known in the EAE model (10) and is the only linkage observed at a significant level in most genetic studies of MS. The influence of MHC is complex and several genes other than the class II could be of importance including class I, complement factors, and TNF.

A locus on chromosome 19 (Eae8) was found to contain an allele inherited in a DA recessive mode. It controls the acute form of EAE without affecting onset or severity. This locus is most likely specific for EAE because no linkage was observed in the PIA model.

Several loci showed a suggestive association with relapses or disease duration in addition to the earlier described Eae5, Eae6, and Eae7 loci. Two loci were associated with chronicity but not with disease onset or severity, Eae9 on chromosome 6 and Eae10 on chromosome 14. Interestingly, one of these loci (Eae10) was also associated with chronic arthritis (Pia6) without affecting disease onset or severity, and, in addition, the phenotype showed a DA recessive inheritance pattern in both EAE and PIA.

Two suggestive loci on chromosome 18 showed a weak linkage to the duration of the disease. One of these is located close to the myelin basic protein gene and segregated as an E3-derived dominant allele. This is of particular interest because it has been proposed that there is linkage between the myelin basic protein gene and MS in humans (26, 27).

### Discussion

Taken together, our results suggest that the majority of the identified loci controlling various subtraits of relapsing EAE in the E3 × DA cross also are of importance for the development of arthritis (PIA). The fact that chromosomal regions that contain susceptibility genes coincide does not prove that identical genes are involved in the two diseases. The identified chromosomal regions are undoubtedly wide and contain many genes. However, there are several observations that support our hypothesis. First, the markers that gave the maximum LOD scores were identical or very closely located in the two cases. Second, the disease-promoting alleles were in all cases derived from the same rat strain in both disease models. Last, but most important, it is shown that many of the common genes in the models for MS and RA in the rat.

### Table II. Chromosome regions showing evidence of linkage to EAE-associated traits

<table>
<thead>
<tr>
<th>OTL</th>
<th>Chromosome</th>
<th>Marker</th>
<th>S</th>
<th>O</th>
<th>D</th>
<th>A</th>
<th>R</th>
<th>BW</th>
<th>Inheritance Pattern</th>
<th>Variance Explained (%)</th>
<th>Corresponding PIA Loci (LOD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eae1</td>
<td>20</td>
<td>D20Rat41/Mgh4</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>DA dominant</td>
<td>5</td>
<td>Pia1: severity/chronicity, DA dominant (3.0)</td>
</tr>
<tr>
<td>Eae5</td>
<td>12</td>
<td>D12Mit2/Rat9</td>
<td>13.0</td>
<td>3.6</td>
<td>5.0</td>
<td>14.0</td>
<td>2.3</td>
<td></td>
<td>DA recessive</td>
<td>28</td>
<td>Pia4: severity, DA additive (8.4)</td>
</tr>
<tr>
<td>Eae6</td>
<td>1</td>
<td>D1Mit7/Mgh12</td>
<td>3.3</td>
<td>3.2</td>
<td>2.2</td>
<td>5.6</td>
<td></td>
<td>DA recessive</td>
<td>25</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Eae7</td>
<td>1</td>
<td>D1Mit7/Mgh12</td>
<td>4.5</td>
<td>4.0</td>
<td>5.3</td>
<td></td>
<td>DA recessive</td>
<td>20</td>
<td>Pia5 (suggestive): severity, DA recessive (3.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eae8</td>
<td>19</td>
<td>D1Mit13/Mgh12</td>
<td>3.3</td>
<td>3.2</td>
<td>2.2</td>
<td>5.6</td>
<td></td>
<td>DA recessive</td>
<td>25</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Eae9</td>
<td>6</td>
<td>D6Mgh3/Rat10</td>
<td>2.7</td>
<td>2.4</td>
<td>3.7</td>
<td></td>
<td>DA recessive</td>
<td>35</td>
<td>Pia6: chronicity, DA recessive (4.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eae10</td>
<td>14</td>
<td>D14Mit5/Wox11</td>
<td>2.5</td>
<td>3.5</td>
<td></td>
<td></td>
<td>DA recessive</td>
<td>35</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eae11</td>
<td>4</td>
<td>D4Wox30:Mgh14</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E3 dominant</td>
<td>25</td>
<td>Pia2: onset, E3 dominant (3.9)</td>
<td></td>
</tr>
<tr>
<td>Eaux</td>
<td>5</td>
<td>D5Mit10/Wox21</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E3 recessive</td>
<td>23</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Eaev</td>
<td>18</td>
<td>D18Mgh1/Wox13</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>E3 dominant</td>
<td>15</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Eaez</td>
<td>18</td>
<td>D18Mit7/Wox1</td>
<td>2.4</td>
<td>2.3</td>
<td></td>
<td></td>
<td>E3 dominant</td>
<td>5.0</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* QTL, Quantitative trait loci. The loci names Eae2, Eae3, and Eae4 are reserved for earlier reported loci on chromosomes 4, 10, and 9 associated with various forms of EAE in other rat strain crosses (28, 29). The suggestive loci on chromosomes 14 and 4 were designated (as Eae10 and Eae11) because they correspond to earlier defined PIA loci. Eaux, Eave, and Eaez are nondesignated suggestive loci.

* LOD score values are given for the indicated inheritance pattern for the following traits: S, severity; O, onset; D, duration of disease; A, acute disease; R, relapsing disease; BW, change in body weight. Phenotypes are described in Materials and Methods.

* LOD score values given for the corresponding PIA loci using the same markers as indicated for the EAE loci. The data have been published (22) or obtained from a yet unpublished (DA × E3F2) cross (the Pia7 locus and suggestive loci on chromosome 1).

* Only found in males.

* Only found in females.
the identified susceptibility loci had similar inheritance patterns and subphenotype associations in the two models. The latter observation strengthened the observation that different phases of the disease are controlled by distinct sets of genes and in particular that such subtraits could be identically controlled in two different inflammatory diseases such as arthritis and encephalomyelitis. However, a comparison of some of the PIA and the EAE loci did show differences in the effects of gender. Thus, the Pia2 was found only in females whereas the corresponding Eae11 was found in all animals. The Pia6 was found in all animals whereas the corresponding Eae10 was found only in females. These differences could possibly be dependent on different sex influences on the two diseases, which may modulate the phenotypic expression associated with the identified loci. For example, females are more prone to develop relapsing EAE than males, whereas in PIA the sex effect is mainly affecting day of onset and incidence. Furthermore, preliminary evidence suggest that the Y chromosome influences the disease in opposite ways in the two diseases (our unpublished observations).

Undoubtedly, the PIA and EAE are differently induced and have different pathogenesis, but they also share certain phenomena such as a tissue-specific destruction and a relapsing pattern of disease development. In both diseases, regardless whether it starts early or late, or whether it is mild or severe, a relapsing pattern of disease may evolve that in part might be controlled by the same genes. Further subphenotyping of the disease and correlation with loci...
that are shared or are distinguished, will shed more light on the critical pathogenic events. This would suggest that similar patho-
genetic mechanisms are responsible for several subtraits of rheuma-
toid arthritis and MS.

In contrast, we would also expect involvement of a large number of different genetic settings for a single trait. Earlier reported genome scans using another rat strain combination (DA × BN), and with different induction protocols that tend to give more acute disease, identified three loci (on chromosomes 4, 9, and 10) asso-
ciated with maximal disease severity that were not found in the
presently analyzed strain combination (28, 29). Interestingly, how-
ever, in the latter study a suggestive linkage for disease severity
was observed on chromosome 12, corresponding to the presently
identified Eae5 locus, and on chromosome 4, corresponding to the
severity-associated Eae3 locus in the mouse. In addition, the Eae5
locus on chromosome 12 corresponds closely to a major locus
recently shown to control a model for uveitis, experimental allergic
uveitis, using another set or rat strains (30). However, it is more
likely that analyses of crosses with different parental strains will
reveal various sets of genes controlling EAE, i.e., we expect a
similar degree of genetic heterogeneity that is observed in the con-

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