Linkage analysis of myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis in the rat identifies a locus controlling demyelination on chromosome 18

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Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS) with a complex etiology comprising a genetically determined predisposition and a suspected autoimmune pathogenesis. Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS, which can be used to define susceptibility loci for autoimmune neuroinflammation. We have recently established a chronic relapsing EAE model characterized by inflammation and focal demyelination in the CNS by immunizing a variety of rat strains with the CNS-specific myelin oligodendrocyte glycoprotein (MOG). This model is more MS-like than any other rodent EAE model described up to now. Here we present the first systematic genome search for chromosomal regions linked to phenotypes of MOG-induced EAE in a (DA × ACI) F2 intercross. A genome-wide significant susceptibility locus linked to demyelination was identified on chromosome 18. This region has not been described in inflammatory diseases affecting other organs and the responsible gene or genes may thus be nervous system specific. Other chromosomal regions showing suggestive linkage to phenotypes of MOG-induced EAE were identified on chromosomes 10, 12 and 13. The chromosome 10 and 12 regions have previously been linked to arthritis in DA rats, suggesting that they harbour immunoregulatory genes controlling general susceptibility to autoimmune diseases. We conclude that identification of susceptibility genes for MOG-induced EAE on rat chromosomes 10, 12, 13 and 18 may disclose important disease pathways for chronic inflammatory demyelinating diseases of the CNS such as MS.

INTRODUCTION

Multiple sclerosis (MS) is a chronic inflammatory and demyelinating disease of the central nervous system (CNS). The disease is a major cause of neurological disability among adult Caucasians and no preventive or curative therapy is currently available. MS has a complex aetiology, including multiple genes and environmental factors (1,2). An autoimmune pathogenesis of MS is plausible in view of: (i) the organ selectivity of the immune attack; (ii) the increased myelin antigen immune responses in patients (3); (iii) similarities to certain EAE models (4); and (iv) its HLA association. Certain HLA haplotypes are associated with a modest increase in risk of developing MS (5,6). Besides HLA, no other chromosomal region has yet shown conclusive evidence for linkage or association with MS, although a few regions that may harbour susceptibility genes have been defined by candidate gene analysis (7) and genome-wide searches (8–12). Since there may be several susceptibility genes for MS and each one has only a modest influence on disease susceptibility (13), they will be difficult to identify (14). The use of relevant animal models, where there are no restrictions such as size of human family material, may facilitate the localization of susceptibility genes and pathogenic pathways in autoimmune neuroinflammation.

Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS (15). The most commonly studied EAE models in rats and mice are monophasic and T cell-mediated inflammatory diseases of the CNS with little or no demyelination (4). A number of non-major histocompatibility locus (MHC) chromosomal regions linked to EAE susceptibility have been identified by linkage analysis in the mouse (16–20) and rat (21–23). Interestingly, one recent study has shown that

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human syntenic regions to chromosomal regions linked to EAE susceptibility are linked to MS susceptibility in a Finnish population (24). This supports the view that there are shared susceptibility genes for EAE and MS. However, since the hitherto studied EAE models lack basic features of MS, such as focal lesions in the CNS, a pathogenic B cell response and demyelination, we believe that additional genes may be identified using a more MS-like EAE model.

It has recently been shown that a chronic relapsing EAE model with inflammation and focal demyelination in the CNS can be induced by immunizing rats with the CNS-specific myelin oligodendrocyte glycoprotein (MOG) (25–28). Synergy between T cells and an anti-MOG antibody response leads to disease manifestations in MOG-induced EAE (25). In a previous limited study we showed that MOG-induced EAE in the DA rat shares susceptibility loci with experimental arthritis (21). However, we believe that there are also organ-specific genes, which could be of major importance as therapeutic targets for MS. Therefore, we have systematically searched for chromosomal regions linked to clinical, histopathological and immunological phenotypes of MOG-induced EAE in an F2 intercross between DA and ACI rats.

RESULTS

Disease distribution

The incidence of clinical and/or histopathological EAE was 72% (26/36) in the DA rats, 5% (1/21) in the ACI rats and 57% (30/53) in the (DA × ACI) F1 (n = 230) rats with paresis (shaded column, female; cross-hatched column, male) and clinical and/or histopathological signs of EAE (open bars).

Figure 1. The percentage of DA (n = 36), ACI (n = 21), (DA × ACI) F1 (n = 53) and (DA × ACI) F2 (n = 230) rats with paresis (shaded column, female; cross-hatched column, male) and clinical and/or histopathological signs of EAE (open bars).

The incidence of clinical and/or histopathological EAE was 52%, whereas only 17% showed clinical signs of EAE. No difference in incidence of clinical and/or histopathological EAE between the reciprocal crosses was recorded. Fifteen of 28 (54%) F1 rats with a DA female founder, 15/25 (60%) with an ACI female founder, 31/97 (32%) F2 rats with a DA female founder and 36/133 (27%) with an ACI female founder had disease. It is therefore unlikely that mitochondrial genes have a large influence on EAE susceptibility (29).

In general, the appearance of clinical signs correlated well with histopathological disease expression. However, in a subgroup of animals throughout all strains and crosses inflammatory demyelinating lesions were present in the absence of clinical disease (Figure 1). Furthermore, on rare occasions [three (DA × ACI) F1 and five (DA × ACI) F2 rats] mild clinical signs were noted during early stages after immunization, which were not reflected in histopathological lesions at day 40. This may be due to a mild and transient inflammatory response, which was no longer detectable pathologically at later stages after sensitization.

Histopathological findings in MOG-immunized DA and ACI rats have previously been described in detail (27,28). They consisted of perivenous inflammation restricted to the CNS. This inflammatory response was in the majority of animals associated with primary demyelination, resulting in the formation of large confluent demyelinated plaques (Table 1).

The DA rats had significantly higher serum levels of anti-MOG IgG, IgG2a, IgG2b and IgG2c compared with the ACI rats, whereas there was no difference in the levels of IgG1 (Figure 2). In the F2 generation, rats with clinical and/or histopathological EAE had significantly higher serum levels of anti-MOG IgG, IgG2a and IgG2b compared with EAE-resistant rats whereas there was no difference in the levels of IgG1 or IgG2c (Figure 2).

Linkage analysis of EAE-linked loci

Forty-six rats were selected for a systematic genome search for loci linked to EAE phenotypes. They consisted of 40 rats with paresis for at least 2 days and six rats with only histopathological signs of EAE. These rats were genotyped with 175 microsatellite markers. The genotype at each marker was determined for each rat in the genome screen was 45. Genotypes were excluded when they could not be unambiguously determined.

MAPMAKER software (30) was used to construct a linkage map covering 1100 cM (Table 2). Ten additional markers could not be linked to any other marker with LOD ≥ 3. We were unable to get a higher chromosomal coverage due to lack of informative markers, possibly due to a common origin of both strains. Only ~10% of the markers tested at random in our laboratory were informative between the DA and ACI strains. To find informative markers, we therefore searched the MIT Ratmap (http://waldo.wi.mit.edu/rat/public/index.html). Only ~65% of the markers that should be informative according to the MIT Ratmap proved to be informative between our strains.

We selected the 40 rats with clinical signs of EAE for analysis at each marker as to whether there was support for linkage to a clinical EAE phenotype. Analysis in all 46 genotyped rats gave similar results. For markers showing support for linkage with a nominal P-value < 0.10 among the 40 rats with clinical
signs of EAE, all 230 (DA × ACI) F₂ rats were genotyped together with additional markers in the chromosome regions. The mean number of genotypes determined for each marker was 219. Among the 230 (DA × ACI) F₂ progeny, nine chromosomal regions were identified with a nominal P-value ≤ 0.05 in support of linkage to positive disease score, CNS inflammation or demyelination (Table 3). The coherent linkage of different phenotypes to the same genome regions shows that the phenotypes strongly depend on each other, therefore we did not correct for multiple comparisons. When evaluating support for linkage in particular regions, we considered both nominal P-values and support for linkage demon-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total no.</th>
<th>0a</th>
<th>Inflammationb</th>
<th>Demyelinationc</th>
<th>Lethald</th>
<th>Optic neuritis</th>
<th>Devic e</th>
<th>Brain</th>
<th>Spinal cord</th>
</tr>
</thead>
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<td>13</td>
<td>3</td>
<td>18</td>
<td>3</td>
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<td>1</td>
<td>0</td>
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<td>0</td>
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<td>1</td>
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<td>11</td>
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<td>17</td>
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<tr>
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<td>230</td>
<td>168</td>
<td>19</td>
<td>41</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>18</td>
<td>39</td>
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</table>

a No pathology.
b Inflammation alone.
c With inflammation.
d Lethal EAE before perfusion day 40 p.i.
e Devic-like, i.e. lesion in optic nerve and spinal cord.

Table 1. Summary of neuropathology

Figure 2. Anti-MOG IgG determination. Sera were sampled 12 days after immunization with MOG from the DA (n = 36), ACI (n = 21), (DA × ACI) F₁ (n = 53) and (DA × ACI) F₂ (n = 230) rats and total anti-MOG IgG and the four IgG subclasses measured. The following serum dilutions were used: IgG, 1:2000; IgG1, 1:200; IgG2a, 1:2000; IgG2b, 1:200; IgG2c, 1:200. Dots represent OD₄₅₀ nm values for individual serum samples. Differences in IgG levels between the parental strains and between F₂ rats with clinical and/or histopathological signs of EAE (F₂ aff) (n = 67) and EAE-resistant F₁ rats (F₁) (DA × ACI, n = 163), respectively, were analysed with ANOVA. *P < 1 × 10⁻²; **P < 1 × 10⁻³.
There was weak support for linkage to serum anti-MOG antibody levels \((5 \times 10^{-2} > P > 5 \times 10^{-3})\) in a number of chromosome regions (data not shown). Regions on chromosomes 4, 9, 10 and X displayed support for linkage to IgG levels and clinical and/or histopathological phenotypes of EAE. The marker D4Rat84, which is located in a region that previously showed significant linkage to anti-MOG IgG1 levels \((21)\), showed some evidence of linkage in this study \((P = 0.05)\) (data not shown).

All 230 rats were genotyped with the marker D20Mit4, which is located close to or in the MHC \((32)\). This marker showed no evidence for linkage to EAE phenotypes, suggesting that the identity in MHC serology between DA and ACI rats translates into identity of function with respect to EAE.

### DISCUSSION

We have identified regions on chromosomes 10, 12, 13 and 18 linked to clinical and/or histopathological phenotypes of MOG-induced EAE in a \((DA \times ACI) F_2\) intercross.

A locus primarily linked to demyelination, which we hereafter designate EAEDM1, was defined on rat chromosome 18. The syntenic region on human chromosome 18 has been linked to MS in a Finnish population \((33)\), but this has not been confirmed in other populations \((34,35)\). Rat chromosome 18 is syntenic to mouse chromosome 18. There is a susceptibility locus for EAE in a region on mouse chromosome 18 that does not overlap with EAEDM1 \((16)\). In a number of studies, the DA rat has been used to map susceptibility loci for arthritis and, thereby, EAEDM1 has not been included among loci linked to arthritis in this strain \((36-38)\). This suggests that EAEDM1 harbours a gene(s) specifically controlling demyelination.

There are probably a variety of mechanisms of immune-mediated demyelination in MS \((4)\). At least in a subgroup of patients, demyelinating antibodies similar to those found in MOG-induced EAE are implicated \((4,39-41)\). Other demyelinating mechanisms may include demyelination due to primary oligodendrocyte damage. Interestingly, the MBP gene, encoding a major myelin protein, is one candidate gene for demyelination on chromosome 18 \((42)\). Gene families/genes with similar functions are sometimes clustered in specific regions on the chromosomes, like the MHC. It is thus possible that this region harbours other, as yet unidentified, myelin-related genes. Hypothetically, this locus may control inflammatory mechanisms preferentially involved in demyelination as opposed to inflammatory organ destruction in other tissues such as joints. Alternatively, this locus could primarily control myelin structure or metabolism, thus affecting myelin vulnerability in MOG-induced EAE.

The region on chromosome 10 linked to clinical and histopathological EAE phenotypes has previously been shown to be linked to experimental autoimmune arthritis in the DA rat \((36,37)\). The mouse region that is syntenic to rat chromosome 10 (Mouse Genome Informatics at http://www.jax.org) is linked to EAE susceptibility \((19)\). Furthermore, the syntenic human region on chromosome 17q22–24 (Mouse Genome Informatics) has been implicated as a region harbouring susceptibility genes for MS and other autoimmune diseases (reviewed in ref. 43). Considering that susceptibility to MOG-induced EAE did not require the disease susceptible allele in the chromosome 10 region, we believe that this region harbours a proinflammatory gene(s) that can promote, but is not required for, different autoimmune diseases. Several adhe-
sion molecule genes that hypothetically could predispose to different autoimmune diseases are located in this region (Mouse Genome Informatics).

The region on chromosome 12 linked to MOG-induced EAE susceptibility has previously been shown to be linked to pristane-induced arthritis (44) and CNS inflammation in EAE (22) in the DA rat. The syntenic human region on chromosome 7q (Mouse Genome Informatics) has shown evidence of linkage to MS and other autoimmune diseases (reviewed in ref. 43). This region may therefore also harbour a gene(s) that can promote different autoimmune diseases.

The lack of loci linked to antibody levels in this cross was a surprise, since two loci linked to antibody levels in MOG-induced EAE have previously been defined on chromosome 4 in a (DA × PVG-RT1a) F2 intercross (21). In particular, the regions on chromosomes 10, 12, 13 and 18 linked to clinical and or histopathological phenotypes of EAE in the (DA × ACI) F2 generation did not show linkage to anti-MOG antibody levels. This may indicate that serum IgG and IgG isotype levels do not reflect the presence of pathogenic demyelinating antibodies in the CNS lesions. Other reasons could be lack of power, since linkage analysis was only performed for markers for which all 230 rats had been genotyped.

In conclusion, in the very MS-like rat MOG-induced EAE we have defined a number of susceptibility loci, which control experimental disease and are of potential value in defining susceptibility loci for MS. Besides loci which appear to operate tissue specifically, a number of loci control autoimmunity in a

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**Table 3. Linkage analysis in 230 (DA × ACI) F2 rats**

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Marker</th>
<th>cM</th>
<th>Paresis*</th>
<th>Penetrance (%)</th>
<th>Demyelination*</th>
<th>Penetrance (%)</th>
<th>Inflammation*</th>
<th>Penetrance (%)</th>
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*The χ2 test was used to analyze differences in genotype frequencies between affected and non-affected rats.

Penetrance for D/D:D/A:A/A genotypes.
more general way. These loci seem to be involved in inflammatory responses per se and might harbour immunoregulatory genes. A classical locus of this type would be the MHC. On the other hand, tissue-specific loci might harbour genes which encode structure-related products or tissue-specific enzymes. The definition of these loci might be important with regard to subgroups of MS patients with linked susceptibility. Since MS is a polygenic trait and comprises a variety of disease entities, each defined locus and gene is of potential importance and worth following in more depth with regard to distribution and function.

MATERIALS AND METHODS

Animal breeding

The DA strain was originally obtained from the Zentralinstitut für Versuchstierzucht (Hannover, Germany) and the ACI strain from Harlan Sprague-Dawley (Indianapolis, IN). The DA and ACI strains have similar MHC haplotypes (45). Two reciprocal (DA × ACI) F2 intercrosses were established, each originating from one female and one male of the respective strains. The rats were routinely tested for specific pathogens according to a health monitoring program for rats at the National Veterinary Institute (Uppsala, Sweden). The local ethical committee in Stockholm approved the experiment.

Induction and clinical assessment of EAE

Recombinant rat MOG corresponding to the N-terminus of the protein (amino acids 1–125) was expressed in Escherichia coli and purified to homogeneity by chelate chromatography (46). The purified protein in 6 M urea was dialyzed against phosphate-buffered saline (PBS) to obtain a preparation that was stored at −20°C. Rats, 9–15 weeks old, were anesthetized with ether and injected once intradermally at the base of the tail with a 200 µl inoculum, containing 50 µg recombinant rat MOG in saline, and emulsified (1:1) with incomplete Freund’s adjuvant (Difco, Detroit, MI). At each immunization session rats from the parental strains were included as controls.

Rats were examined daily for signs of EAE and weight from day 7 post-immunization (p.i.) until being killed at day 40 p.i. The clinical scoring was as follows: 0, no illness; 1, tail weakness or paralysis; 2, hind leg paraparesis or hemiparesis; 3, hind leg paralysis or hemiparalysis; 4, tetraparesis or moribund. Ataxia was routinely assessed. An index of weight loss was calculated as (weight at immunization − lowest weight after immunization)/weight at immunization.

Anti-MOG IgG subclass determination

Sera for antibody measurements were sampled on day 12 p.i. ELISA plates (Nunc, Roskilde, Denmark) were coated with 2.5 µg/ml (100 µl/well) recombinant rat MOG (amino acids 1–125) overnight at 4°C. Plates were washed with PBS, 0.05% Tween 20 and free binding sites were blocked with 5% skimmed milk in PBS, 0.05% Tween 20 for 1 h at room temperature (RT). After washing, diluted serum samples were added and the plates were incubated for 1 h at RT. Plates were then washed and diluted rabbit anti-rat IgG and γ-chain-specific IgG (Nordic, Tilburg, The Netherlands) were added and incubated for 1 h at RT. Subsequently, unbound antibodies were removed by washing prior to the addition of a peroxidase-conjugated goat anti-rabbit antiserum (Nordic) diluted in PBS, 0.05% Tween 20 (1:10 000). After 30 min incubation plates were washed thoroughly and bound antibodies were visualized by addition of 3,3′,5,5′-tetramethylbenzidine (Sigma, Stockholm, Sweden). The enzymatic reaction was terminated with 1 M HCl after 15 min incubation in darkness and the optical density was read at 450 nm. Rats immunized with the same MOG preparation were included as a positive control and sera from non-immunized rats as a negative control on all ELISA plates.

Histopathological examination

At day 40 p.i. rats were deeply anesthetized and perfused via the left ventricle of the heart with 4% paraformaldehyde.
Genomic DNA from the rats in the F2 generation was prepared from tail tips according to a standard protocol (47). Microsatellite markers were used for genotyping, using PCR essentially as previously described (48), except that the primers were end-labeled with [γ-33P]ATP. Primers were obtained from Research Genetics (Huntsville, AL) or from GENSET (Paris, France). Some primers were a kind gift from Dr Howard J. Jacobs (Medical College of Wisconsin, Milwaukee, WI). The PCR products were size fractionated on 6% polyacrylamide gels and visualized by autoradiography. All genotypes were scored manually and double-checked.

Statistical analysis

For linkage analysis of EAE susceptibility loci the Pearson χ2 test was used to determine whether the observed genotype distribution among rats with a positive clinical score significantly deviated from the expected D/D:A/A frequencies assuming that the alleles segregate randomly. The same test was used to analyze whether there was a significant difference in observed genotype distribution between non-affected and affected rats, i.e. with a positive clinical score, CNS inflammation or demyelination, respectively. For linkage analysis of EAE severity the Kruskal–Wallis test was used to determine whether, among rats with a positive clinical score, different genotypes were associated with differences in maximum score. The Kaplan–Meier method was used to analyse differences in day of onset between genotypes among rats with a positive clinical score. Anti-MOG antibody levels were analysed by ANOVA.

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REFERENCES


