Genetic influence on disease course and cytokine response in relapsing experimental allergic encephalomyelitis

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Abstract

A protracted and relapsing form of experimental allergic encephalomyelitis (EAE) develops in the DA rat after immunization with rat spinal cord homogenate (SCH) emulsified in incomplete Freund’s adjuvant (IFA). The genetic influence on this model has been analyzed by immunizing MHC congenic strains on both LEW and DA genetic backgrounds, and recombinant inbred strains between DA and E3 rats. An in situ hybridization assay was used to examine the expression of mRNA for IFN-γ, IL-4, IL-10 and transforming growth factor (TGF)-β both in sections of spinal cords and the antigen-induced expression for these cytokines by splenocytes after in vitro stimulation with encephalitogenic MBP peptides. The susceptibility of relapsing EAE after immunization with SCH in IFA in the DA strain, but not the E3 strain, was correlated with a lack of expression for TGF-β in the spinal cord. The recombinant inbred DXEB rats developed a severe EAE while surprisingly no signs of disease were observed in the DXEA strain, which shares the MHC region with the DXEB strain, after immunization with the MBP 63–87 peptide. Resistance to relapsing EAE in the DXEA strain correlated with increased non-MHC controlled expression for TGF-β and lack of IFN-γ in the spinal cord. The same pattern of cytokine expression was seen in splenocytes after stimulation in vitro with the MBP 63–87 peptide. A spreading of the immune response to the MBP 87–110 peptide was seen. Non-MHC genes controlled the quality of this response: splenocytes from MBP 63–87 immunized DXEB rats responded in vitro towards the MBP 87–110 peptide by expressing mRNA for IFN-γ, IL-10 and IL-4, whereas in the DXEA strain the corresponding response involved IL-4 and TGF-β. Taken together these data show that non-MHC controlled expression of mRNA for TGF-β is associated with resistance to EAE.

Introduction

Multiple sclerosis (MS) is a chronic relapsing inflammatory disease in which the myelin of the CNS is under attack. During recent years considerable progress has been made in the characterization of autoreactive T cells and putative autoantigenic targets in the brain. Likewise, the pathogenesis of experimental models of the disease, i.e. experimental allergic encephalomyelitis (EAE), has been further clarified. It has, however, been difficult to transform this knowledge to explain the etiology and pathogenesis of the human disease. However, the rapid advancement in genetic techniques might be useful for finding the missing clues. A number of gene regions have recently been identified in the mouse to be associated with EAE (1–3) and two of these, eae1 in MHC and eae2 on mouse chromosome 15, have been reproduced in human MS (4,5). MS is a polygenetic disease with considerable heterogeneity and for further studies we need to find relevant variants of the EAE model in different species useful for gene mapping with the goal to find gene regions which may control biological functions of crucial importance also in MS. EAE was in fact first described in humans after inoculation of rabbit spinal cord homogenate (SCH) and subsequently EAE in several species including rats have been established as models for MS (7). The induction of EAE requires SCH- or CNS-derived myelin proteins emulsified in strong adjuvants such as complete Freund’s adjuvant (CFA) containing Mycobacterium tuberculosis. In earlier studies the acute EAE in LEWIS rats...
has been shown to be controlled by both MHC and non-MHC genes (8,9), and that the induction of disease is mediated by T cells predominantly using TCR with Vβ8.2 (10). However, the introduction of foreign proteins or peptides may distort the autoimmune balance in the development of disease. It has, for example, been recently shown that only guinea pig-derived MBP 68–88 but not rat-derived MBP 68–87 are effective in inducing oral tolerance (11) and in the same vein it has been shown that the guinea pig-derived peptide induces a more severe EAE than the rat-derived peptide (12). The recent description of a relapsing EAE model in the DA rat induced with rat SCH emulsified with mineral oil by Lorentzen et al. (13) provides a new model for MS which is suitable for genetic characterization. The DA rat is highly susceptible for many autoimmune experimental models such as homologous collagen-induced arthritis (14) and diseases induced with non-immunogenic adjuvants (15) indicating that the DA strain carry genes highly susceptible to autoimmune diseases promoted by adjuvant effects. Here we present a first step genetic characterization of chronic EAE in the DA rat. To analyze the MHC influence on the protracted and relapsing form of EAE induced with rat SCH we have used MHC congenic strains on DA as well as LEW backgrounds and to analyze non-MHC genetic effects we have used recombinant inbred strains between DA and E3.

**Methods**

**Animals**

Rat breeding nuclei of the different strains (DA, E3, DXEA, DXEB, DXEC, DA.1H, DA,1M, DA.1O, LEW.1A, LEW.1W, LEW.1N and LEW.1F) were originally generously provided by Professor Hans Hedrich (Zentralinstitut fur Versuchstierzucht, Hannover, Germany) and subsequently bred in the animal department of Medical Inflammation Research, Lund University. The rats were kept in a separate animal room under specific pathogen-free and climate-controlled conditions 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 8–12 weeks old, and were age- and sex-matched before the experiments. During the experiments, two to three rats were housed in each cage.

**Antigens**

We used the rat MBP 89–101 peptide (VHFFKNI VTPRPT), rat MBP 87–110 peptide (PVHFFKNI VTPRTPPSQGKRG), rat MBP 63–87 (TRTTHYGSLPQSGRTODENPVH) and SCH from the DA rat strain as immunogens. The peptides were synthesized on an automatic peptide synthesizer (model 430A; Applied Biosystems, Foster City, CA). After the synthesis was completed, the peptide was cleaved from the resin and purified on HPLC using reversed-phase chromatography on a C18 column eluted with an acetonitrile gradient (0–60%) in 0.1% aqueous trifluoroacetic acid. Fractions were pooled and lyophilized after analysis using plasma desorption mass spectrometry.

**Preparation of mononuclear cells from spleens**

Cell suspensions were prepared day 14 p.i. by grinding spleens through a wire mesh (1 mm²). Cells were then washed 3 times in DMEM supplemented with Glutamax-II (Life Technology), 50 IU/ml penicillin, 60 mg/ml streptomycin (Gibco, Paisley, UK) and 5% (v/v) heat inactivated FCS (Gibco). The splenocyte concentration was then adjusted to 5 × 10⁶/ml.

**Antigen-induced cellular expression of mRNA for IFN-γ, IL-4, IL-10 and transforming growth factor (TGF)-β in mononuclear cells**

To quantitate the antigen specific T cells expressing mRNA for IFN-γ, IL-4, IL-10 and TGF-β, in situ hybridization was used. In brief, 2 ml aliquots of spleen cell suspensions were applied to six-well microtiter plates (Falcon 3046; Becton Dickinson, San Jose, CA) and cultures received no exogenous antigen, MBP 63–87 (final concentration 20 µg/ml), MBP 87–110 (final concentration 20 µg/ml) or concanavalin A at final concentration of 5 µg/ml. After 24 h of culture at 37°C and 5% CO₂ and humidity, cells were then washed in sterile PBS and 1 × 10⁵ cells from each culture were dried on Probe-On slides (Fisher Scientific, Pittsburgh, PA). In situ hybridization was performed as described previously (16). Cells expressing numerous grains were counted as positive by low power dark field microscopy, and checked in higher magnification and light for accuracy. Results were expressed as numbers of labeled cells per 1 × 10⁵ plated cells. Spinal cord sections from rats sacrificed at day 14 p.i. were thaw-mounted onto Probe-On slides (Fisher Scientific). In situ hybridization was performed as for spleen cells. Results were expressed as numbers of labeled cells or infiltrates per 100 mm² tissue section.

**Induction of EAE**

Induction of EAE with MBP peptides was performed using CFA. Each rat was immunized s.c. in the dorsal root of the tail with 200 µl of an inoculum, containing 100 µl incomplete Freund’s adjuvant (IFA; Difco, Detroit, MI), 2 mg of M. tuberculosis,100 µl of saline, and 200 µg of the peptides MBP 89–101, MBP 63–87 and MBP 87–110. Induction of EAE with DA rat SCH was performed using IFA; 200 µl of an inoculum containing 100 µl IFA (Difco), 100 µl saline and 10 µg SCH was injected in the dorsal root of the tail.

**Clinical scoring**

In order to assess clinical severity, all animals were examined for disease symptoms and scored according to a nine-point scale. The clinical grading was as follows: 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 = pre-morbid or deceased.

**Statistical analysis**

Incidence of disease was evaluated by Fischer’s exact test. All other statistical analyses were evaluated by the non-parametric Mann–Whitney U-test.

**Results**

**Development of relapsing EAE in DA, E3 and DA/E3 recombinant inbred strains**

The DA (RT1av1), but not the E3 (RT1u) strain, developed relapsing EAE after immunization with rat SCH in IFA (Table
Table 1. MHC haplotypes of DA/E3 recombinant inbred rat strains used and susceptibility to EAE induced by SCH from the DA rat strain in IFA (data refer to rats observed until day 35-41 p.i.)

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1</th>
<th>MHC region</th>
<th>Clinical incidence</th>
<th>Mean maximum severity</th>
<th>Mean onset day of disease p.i.</th>
</tr>
</thead>
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<td></td>
<td>A</td>
<td>BD</td>
<td>C</td>
<td></td>
<td></td>
</tr>
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<td>av1</td>
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<td>8/8</td>
<td>4.8 ± 0.82</td>
<td>10.4 ± 0.75</td>
</tr>
<tr>
<td>E3</td>
<td>u</td>
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<td>0/7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DXEA</td>
<td>av1</td>
<td>a a av1</td>
<td>0/8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DXEB</td>
<td>av1</td>
<td>a a av1</td>
<td>8/8</td>
<td>5.5 ± 0.82</td>
<td>&lt;10.3b</td>
</tr>
<tr>
<td>DXEC</td>
<td>u</td>
<td>u u u</td>
<td>0/8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DA.1H</td>
<td>h</td>
<td>h h n ?</td>
<td>8/8</td>
<td>4.8 ± 0.84</td>
<td>12.8 ± 0.49</td>
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<td>DA.1I</td>
<td>i</td>
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<td>5.9 ± 0.67</td>
<td>10.3 ± 0.68</td>
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<td>9.1 ± 0.14</td>
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<td>d a ?</td>
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<td>9.4 ± 0.32</td>
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<td>LEW.1A</td>
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<td>a a a a</td>
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<td>11.2 ± 1.6</td>
</tr>
<tr>
<td>LEW.1W</td>
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<td>u u u</td>
<td>4/6</td>
<td>3.8 ± 0.75</td>
<td>12.8 ± 0.75</td>
</tr>
<tr>
<td>LEW.1N</td>
<td>n</td>
<td>n n n</td>
<td>0/7</td>
<td>0</td>
<td>12.5 ± 0.87</td>
</tr>
<tr>
<td>LEW.1F</td>
<td>f</td>
<td>f f f</td>
<td>3/6</td>
<td>3.3 ± 1.2</td>
<td>12.7 ± 0.67</td>
</tr>
</tbody>
</table>

*aBased on affected animals in the group SEM.

*bUnfortunately these rats were not recorded before day 10 p.i. and when the inspections commenced all rats except one had already got disease. This rat got disease at day 12 p.i.

Fig. 1. Number of IFN-γ, IL-10, IL-4 and TGF-β mRNA-expressing cells detected in spinal cords from DA rats, compared with E3 rats, immunized with SCH. The data refer to mean number of mRNA-expressing cells per 100 mm² of spinal cord SEM and is calculated from results of seven rats in each strain. *P < 0.05, **P < 0.01.

The disease developed 10–11 days after immunization in DA rats and the subsequent disease course very reproducibly showed a protracted relapsing pattern limited to two or three relapses, similar to the earlier description of the model (13). However, in our experiments no mortality was noted. The cellular mRNA expressions for IFN-γ, IL-10, IL-4 and TGF-β were measured in sections of the lumbar part of spinal cords from DA and E3 rats sacrificed day 14 p.i. As seen in Fig. 1, no significant difference in number of mRNA-expressing cells for IFN-γ between the two strains was detected, whereas the expression of mRNA for IL-10 and TGF-β was higher in the E3 compared with the DA strain. To further analyze the genetic influence, recombinant inbred strains between DA and E3 strains were used. The DXEA (RT1<sup>av1</sup>) and DXEC (RT1<sup>u</sup>) strains were resistant (Table 1). Interestingly, the DXEB (RT1<sup>av1</sup>) strain, possessing the same MHC region as the DXEA (RT1<sup>av1</sup>) strain, developed a disease with identical clinical incidence and similar mean maximum severity compared with the DA strain (P = 0.36) (Table 1), showing that most of the susceptible genes are preserved in this strain. These data clearly show that susceptibility to EAE is under a strong influence by genes outside MHC, but do not exclude a MHC-mediated effect.

Susceptibility to spinal cord-induced EAE in MHC congenic strains on DA and LEW backgrounds

To determine the role of MHC in relapsing EAE we used MHC congenic strains on the DA genetic background. All four strains used [DA.1H (RT1<sup>h</sup>), DA.1I (RT1<sup>i</sup>), DA.1M (RT1<sup>m</sup>) and DA.1O (RT1<sup>o</sup>)] developed relapsing diseases with high incidence (Table 1 and Fig. 2). However, the DA.1M rats developed a more protracted form of disease course in the sense that they did not recover as well during the first remission and the recovery after the first relapsing phase was slower compared with the DA strain and the other DA congenic strains (Fig. 2). The disease course in the DA.1H strain diverted from other DA congenic strains in two ways. Firstly, the onset of disease was significantly delayed compared with the DA.1M (P = 0.0007) and DA.1O (P = 0.001) strains, and, secondly, the first relapse in terms of mean maximum severity was significantly lower compared with the DA.1M (P = 0.014) and DA.1O strains (P = 0.017). The DA.1O and DA.1I strains showed a similar pattern in disease susceptibility as the DA strain. These two strains are known to carry MHC haplotypes that are intra-MHC recombinants—the av1, o and i haplotypes share the a haplotype of the class II regions, whereas the class I regions differ (17). The LEW.1A strain, carrying the highly susceptible RT1<sup>a</sup> haplotype, developed a protracted form of EAE. The disease was transient, progressing from tail paralysis to tail paralysis and uneven gait. All the LEW.1A rats developed clinical signs of disease, whereas the LEW.1N rats were resistant (Table 1). Furthermore, the LEW.1A rats showed...
earlier mean onset day of disease compared with LEW.1W ($P = 0.021$) and LEW.1F ($P = 0.014$). Thus, MHC genes exert an influence on the disease in both the LEW background and in the highly susceptible DA background. The more susceptible strains have $a$ or $c$ haplotypes in their class II regions, whereas somewhat less susceptible strains carry the $n$ haplotype of the MHC class II region.

**MBP peptide-induced EAE**

We have earlier shown that encephalitogenic responses to defined MBP-derived peptides are controlled by MHC, using MHC congenic and intra-MHC recombinant strains on the LEW background (18,19). The peptides used were derived from the region spanning from position 63 to 110 which contains the immunodominant and encephalitogenic peptides

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**Fig. 2.** A comparison with respect to mean clinical score between the DA rat strain and four different DA MHC congenic strains immunized s.c. at the tail base with SCH emulsified in IFA. The number of animals used in each group is shown in Table 1.
in the rat. To analyze the genetic control of the acute EAE induced by these peptides in comparison with the relapsing EAE we used the DA strain, E3 strain and recombinant inbred strains between these two strains. As expected from the known MHC association, DA (RT1\textsuperscript{av1}) rats immunized with MBP 87–110 (Table 2) and MBP 89–101 (Table 3) were susceptible to EAE, whereas the E3 (RT1\textsuperscript{u}) rat strain was resistant. The DXEA (RT1\textsuperscript{av1}) and DXEB (RT1\textsuperscript{av1}) rat strains developed significantly different disease courses after immunization with the MBP 87–110 peptide (Table 2). The DXEB group developed a severe EAE and showed an earlier onset day of disease (\( P < 0.0013 \)), higher clinical incidence (\( P < 0.038 \)) and severity (\( P < 0.0083 \)), compared with the DXEA strain. The DXEC rat strain showed no signs of disease (Table 2). The DXEB strain developed disease with an earlier onset day compared with the DXEA strain (\( P < 0.013 \)) after immunization with the MBP 89–101 peptide (Table 3). The difference in susceptibility to EAE between the DXEA and DXEB strains was even more pronounced when immunized with the rat MBP 63–87 peptide (Table 4). All the DXEB rats developed a severe EAE with an onset of disease as early as day 9 p.i. and surprisingly no signs of diseases were observed among the DXEA rats. The same pattern of disease course was seen in recombinant inbred rats immunized with the guinea pig MBP 63–88 peptide (data not shown). In situ hybridization was used to examine cellular mRNA expressions for IFN-\( \gamma \), IL-10, IL-4 and TGF-\( \beta \) in sections of the lumbar part of spinal cords from DXEA and DXEB strains immunized with the rat MBP 63–87 peptide. A higher number of mRNA expressing cells for IFN-\( \gamma \) were present in the spinal cord of the DXEB strain compared with the DXEA strain, whereas the TGF-\( \beta \) mRNA expression was higher in the DXEA strain compared with the DXEB (Fig. 3). Furthermore, we studied the antigen-induced cellular mRNA expression for these cytokines in cultured splenocytes from DXEA and DXEB strains immunized with the rat MBP 63–87 peptide. As seen in Fig. 4, the expression of mRNA for IFN-\( \gamma \) in response to the immunogen in splenocytes from the DXEB strain was higher compared with background, which was not seen in the DXEA strain. The number of immunogen-induced TGF-\( \beta \) mRNA-expressing cells were higher compared with background in the DXEA strain, which was not seen in the DXEB strain. Interestingly, an immune response to another rat MBP encephalitogen, the MBP 87–110 peptide, was observed. Thus, increased mRNA expressions in the splenocytes in response to the MBP 87–110 peptide compared with background were found for IFN-\( \gamma \), IL-10 and IL-4 in the DXEB strain, whereas the mRNA expressions for IL-4 and TGF-\( \beta \) were increased in response to the MBP 87–110 peptide in the DXEA strain (Fig. 4). Interestingly, the immune response to the MBP 87–110 peptide in the splenocytes showed different qualities in the two strains, the DXEA rats had an immune response lacking IFN-\( \gamma \), whereas the DXEB strain had an immune response lacking TGF-\( \beta \) (Fig. 4). Thus, in response to both the primary and secondary peptide, the non-MHC controlled expression of mRNA for TGF-\( \beta \), but not for IL-4 and IL-10, appeared to be crucial in suppressing EAE.

**Discussion**

The relapsing EAE model in rats induced with autologous SCH in mineral oil is an improved model for MS and useful
Table 4. MHC haplotypes of DA/E3 recombinant inbred rat strains used and susceptibility to EAE induced by the rat MBP 63–87 peptide (data refer to rats observed until day 39–40 p.i.)

<table>
<thead>
<tr>
<th>Strain</th>
<th>RT1 MHC region</th>
<th>Clinical incidence</th>
<th>Mean maximum severitya</th>
<th>Mean onset day of disease p.i.a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DXEA</td>
<td>av1</td>
<td>a</td>
<td>av1</td>
<td>0/8</td>
</tr>
<tr>
<td>DXEB</td>
<td>av1</td>
<td>a</td>
<td>av1</td>
<td>7/7</td>
</tr>
<tr>
<td>DXEC</td>
<td>u</td>
<td>u</td>
<td>u</td>
<td>0/8</td>
</tr>
</tbody>
</table>

aBased on affected animals in the group ± SEM.

For genetic analyses. By using different rat strains, some of them sharing MHC, we found a strong influence not only by the MHC region but also by genes outside MHC. The E3 strain was resistant to induction and developed mRNA-expressing cells for TGF-β and IL-10 in the spinal cord in contrast to the susceptible DA strain. The study also shows that the immune process leading to EAE after induction with peptides might not be the same as that triggered by immunization with the entire spinal chord. For example, the DXEA strain was resistant to EAE induced by SCH emulsified in IFA (Table 1) and MBP 63–87 emulsified in CFA (Table 4), whereas the same strain developed disease using different MBP peptides emulsified in CFA (Tables 2 and 3). The present genetic characterization of rat spinal cord-induced EAE differs from earlier studies by several aspects of importance for further genetic analysis (8,9,20). In the present study no foreign antigens have been used for induction of the disease, whereas in earlier studies the SCH was derived from guinea pig and mixed with mycobacteria-containing adjuvants. In earlier studies only acute, self-limited, disease courses were obtained, whereas in the present model a relapsing prolonged disease course is seen. In the light of earlier demonstration of an MHC-mediated control of the guinea pig spinal cord/CFA-induced disease (9) it might not be surprising to find that MHC plays an important role also in the autologous model. It is, however, not obviously so, which has been clearly demonstrated in the collagen-induced arthritis model in the rat. In this model certain MHC alleles are associated with the induction using heterologous type II collagen but not with the autologous type II collagen most likely dependent on the predominant presentation of the immunogenic non-self pep-
tides (14). Nevertheless, the finding that also the autologous spinal cord-induced relapsing EAE is under MHC control imply that certain myelin protein peptides play a crucial role mediating the disease. Indeed, the most immunogenic autologous MBP peptides (63–87, 89–101 and 87–110) all showed a MHC-associated pattern similar to the spinal cord-induced disease as has also been partly shown in previous studies (18,19). The fact that both DA and LEW rats with the n haplotype are relatively more resistant than their corresponding congenic strains suggests an important role for the MHC class II region. However, the influence by MHC may be complex and several genes could be of importance. Our earlier findings of a regulatory role of class I regions in MHC (19) underscores the importance of a further careful analysis of the role of the different MHC genes. A comparison between rat strains sharing MHC showed dramatic differences in disease susceptibility indicating a strong influence by genes outside MHC. Among our studied rat strains, the E3 strain was the most resistant. Interestingly, in situ hybridization analysis of the lumbar spinal cord obtained from E3 rats showed mRNA-expressing cells for TGF-β and IL-10, whereas the susceptible DA rats lacked such expression. This is in accordance with our earlier findings that recovery from disease is associated with such a cytokine expression (16). Thus, genes controlling the cytokine expression in the CNS may therefore be of importance for disease susceptibility. Analysis of the genetic influence on the immune response was made after immunization of the rats with the immunodominating MBP peptides derived from the 63–110 region. An immune response towards the MBP 87–110 peptide was seen 14 days after immunization with the MBP 63–87 peptide. This could be due to a response towards endogenously derived peptides, as we have previously shown to occur using the same peptides in the corresponding mouse model (21), although we have not in the present experiment formally excluded the possibility of a cross-reactive process. Interestingly, the cytokine phenotype of the redirected immune response was controlled by non-MHC genes as shown by a comparison between the DXEA and the DXEB strains. An interesting difference was the ability to express TGF-β which has earlier been shown to be of importance in the down-regulation of an inflammatory response in EAE. Expression of TGF-β in CNS has been provided as the explanation for the bystander effect in oral tolerance and neutralization of TGF-β leads to enhanced disease (22). We have earlier shown that the antigen-specific induction of TGF-β is controlled by the MHC class I region, peptide specific and that depletion of CD8+ T cells enhances the susceptibility. The present findings suggest that also genes outside MHC control this important phenotype. Another dramatic difference was the ability to express mRNA for IFN-γ that also seems to be of non-MHC gene control. Previous studies have shown that the ability to express IFN-γ is controlled by MHC, and that strains which do not secret IFN-γ in response to the antigenic peptide are immunological non-responders and resistant to disease (23). Our present findings show that the rats respond with other cytokines and that the ability to respond with IFN-γ is controlled not only by MHC genes but also by genes outside MHC. In contrast, we could not find any significant difference in the ability to respond with IL-4 or IL-10 indicating that genes controlling Th2 responsiveness may not differ in the analyzed strains. The importance of the cytokine pattern for disease susceptibility and development is, however, still unclear but the findings that the same differences in mRNA expression are found in the lumbar spinal cord as in the antigen-specific immune responses in vitro indicate a relation. The involvement of cytokines in the disease process, whether secondary or primary, and the interaction with other regulatory mechanism may be possible to sort out after the clarification of which non-MHC gene loci and genes control disease susceptibility and cytokine secretion patterns respectively. However, it is also likely that the MHC region of the DA strain contributes to the relapsing character of the disease, although this topic is not directly addressed here. The DA rat has a remarkable susceptibility to develop EAE in response to various myelin proteins such as MBP, various MBP peptides or proteolipid protein (24), suggesting a promiscuity in binding of peptides derived from myelin proteins by the DA MHC class II molecules. It is also interesting to note that the DA and Dxeb, but not the DXEA, strains develop chronic arthritis (25) indicating that a detailed comparative genetic analysis of the DA versus the E3 strains may reveal genes controlling chronicity in tissue-specific autoimmune disease.

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Abbreviations

<table>
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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>CFA</td>
<td>complete Freund's adjuvant</td>
</tr>
<tr>
<td>EAE</td>
<td>experimental allergic encephalomyelitis</td>
</tr>
<tr>
<td>IFA</td>
<td>incomplete Freund's adjuvant</td>
</tr>
<tr>
<td>MBP</td>
<td>myelin basic protein</td>
</tr>
<tr>
<td>MS</td>
<td>multiple sclerosis</td>
</tr>
<tr>
<td>SCH</td>
<td>spinal cord homogenate</td>
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<td>TGF</td>
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