Primarily chronic progressive and relapsing/remitting multiple sclerosis: Two immunogenetically distinct disease entities

(HLA class II genes/HLA-DR/HLA-DQ/restriction fragment length polymorphism analysis)

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ABSTRACT HLA class II gene polymorphism was investigated in 100 patients with clinically definite multiple sclerosis (MS) by restriction fragment length polymorphism analysis of Taq I-digested DNA using DRB, DQA, and DQB cDNA probes. Twenty-six patients had primarily chronic progressive MS and 74 had relapsing/remitting MS. The latter group included patients with a secondary progressive evolution of symptoms. Both clinical forms of MS were found to be associated with the DRw15, DQw6 haplotype. In addition, primarily chronic progressive MS was positively associated with the DQBl restriction fragment pattern seen in DR4, DQw8, DR7, DQw9, and DRw8, DQw4 haplotypes, as well as negatively associated with the Taq I DQBl allelic pattern corresponding to the serological specificity DQw7. Relapsing/remitting MS was positively associated with the DQBl allelic pattern observed in the DRw17, DQw2 haplotype. These three DQBl alleles are in strong negative linkage disequilibrium with DRw15. The two susceptibility markers of each clinical form of MS act additively in determining the genetic susceptibility, as the relative risks for individuals carrying both markers roughly equal the sum of respective risks. Different alleles of the DQBl locus defined by restriction fragment length polymorphisms contribute to susceptibility and resistance to primarily chronic progressive MS as well as to susceptibility to relapsing/remitting MS. The observed immunogenetic heterogeneity between the different clinical forms of MS favors the hypothesis that primarily chronic progressive MS and relapsing/remitting MS are two distinct disease entities.

With the finding of a strong association between ankylosing spondylitis and the HLA class I antigen B27 (1, 2), a new era in the search for genetic markers in human diseases began, and various HLA antigens have been demonstrated to be associated with susceptibility to a large number of different diseases (for review, see ref. 3). Despite intense efforts the mechanisms involved in the associations between HLA antigens and diseases have not been unraveled.

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system often causing neurological deficits. The etiology as well as many aspects of the pathogenesis remain unclear. However, most current hypotheses postulate that the disease is caused by a T-cell-mediated autoimmune destruction of the myelin sheath triggered by an environmental influence, which may be viral, in the genetically predisposed individual.

Most patients with MS have a disease course characterized by relapses and remissions, termed relapsing/remitting MS (R/R MS). Many of these patients will, however, eventually enter a phase with secondary progressive evolution of symp-

toms (4). Approximately, 15–40% of the patients with MS have a primarily chronic progressive disease form (PCP MS) (4, 5); i.e., the disease has a continuous progressive evolution from onset. It has been proposed that PCP MS and R/R MS may be separate disease entities (6) since they differ with respect to epidemiology (6), age at onset (4, 5, 7), initial symptoms (4, 6), prognosis with regard to degree of disability (4, 7) and to mortality (8), and response to immunosuppression (9). Efforts to differentiate between PCP MS and R/R MS by serological HLA class II typing have yielded divergent and inconclusive results (10–12).

The extremely polymorphic HLA class II genes belong to the immunoglobulin gene superfamily and are located on the short arm of chromosome 6 with three major subregions encoding expressed molecules—DR, DQ, and DP. HLA class II antigens are transmembraneous glycoproteins composed of a heavy α chain and a light β chain. Most of the polymorphism is found within the transmembrane domain, encoded by the second exon, especially of the β chains (for review, see ref. 13). HLA antigens serve as markers for self during the ontogenetic development of the T-cell repertoire. HLA class II molecules form a complex with immunogenic determinants, which can be recognized by the T-cell receptor. Thus, the antigen-specific T-cell response is restricted by the major histocompatibility complex.

Restriction fragment length polymorphism (RFLP) analysis of HLA-DR and -DQ genes has been developed into a powerful tool for genomic tissue typing (14–17). The polymorphism detected by RFLP analysis correlates well with the phenotypic polymorphism recognized by serology and mixed lymphocyte culture (ref. 16; for review, see ref. 18). Additional biologically relevant polymorphism is identified by RFLP analysis compared to conventional serological tissue typing (ref. 16; for review, see ref. 18). Due to the strong linkage disequilibrium between DR and DQ subregions, DR–DQ haplotypes can be assigned by merely investigating the individual without family data (16–18).

In a preliminary study we have found (19) that PCP MS was associated with a specific heterozygous Taq 1 HLA-DQB restriction fragment pattern. To identify immunogenetic heterogeneities between the two clinical forms of MS, PCP MS and R/R MS, RFLP analysis of HLA-DR and -DQ genes was performed in a large number of clinically well characterized MS patients.

Abbreviations: RR, relative risk; MS, multiple sclerosis; PCP MS, primarily chronic progressive MS; R/R MS, relapsing/remitting MS; including patients with a secondary progressive phase; RFLP, restriction fragment length polymorphism; Pcorr58, etc., P corrected by a factor of 58, etc.; EF, etiologic fraction.

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MATERIALS AND METHODS

Patients. One hundred unrelated Swedish patients with clinically definite MS (20) were studied. Twenty-six patients had PCP MS—i.e., gradually progressive neurological symptoms from the onset of disease without remissions. Seventy-four patients had R/R MS. This latter group included patients who experienced secondary progressive evolution of symptoms after initial relapses and remissions. Clinical and cerebrospinal fluid data of the patients are given in Table 1.

Controls. One hundred healthy Swedes, HLA-DR and DQ typed by Taq I RFLP analysis, were used as controls.

Probes. The following almost full-length cDNA probes were used: DRB, a 790-base-pair (bp) HindIII-Sac I fragment of clone pII-β-3 (23); DQA, a 584-bp Rsa I-Stu I fragment of clone pI-α-5 (24); DQB, a 627-bp Ava I-Ava I fragment of clone pII-β-1 (25). Purified inserts were labeled by random hexanucleotide priming (Pharmacia) to a specific activity of 1–2 × 107 cpm/μg.

RFLP Analysis of HLA-DRB,-DQA, and -DQB Genes. DNA was extracted from peripheral blood leukocytes by phenol/chloroform extraction of proteinase K-treated nuclei in microscale. Samples of 8 μg of DNA were digested with 2 units of Taq I per μg of DNA (Boehringer Mannheim) in 20-μl reaction mixtures for 4 hr at 65°C. Total reaction mixtures were loaded on 0.7% agarose gels (20 × 20 cm). Gels were electrophoresed for 20 hr at 0.5 × TBE buffer (89 mM Tris borate/89 mM boric acid/2 mM EDTA, pH 8). Denatured DNA fragments were transferred by capillary blotting with 20× SSC (3 M NaCl/0.3 M sodium citrate, pH 7.0) to nylon membranes (Pall) and cross-linked by UV-irradiation for 3.5 min. After prehybridization for 2 hr at 42°C [50% (vol/vol) formamide/10% (wt/vol) dextran sulfate/10% Denhardt’s solution/50 mM Tris-HCl, pH 7.5/1 M NaCl/1% SDS/0.1% sodium pyrophosphate/denatured sheared salmon sperm DNA (150 μg/ml)], a 32P-labeled cDNA probe was added (15–20 × 106 cpm per membrane). (1 × Denhardt’s solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin.) Filters were hybridized for 12–18 hr at 42°C. Two 5-min washes at room temperature in 2× SSC/0.5% SDS were followed by two 30-min high-stringency washes at 60°C in 0.1× SSC/0.1% SDS. Membranes were autoradiographed (X-Omat AR5, Kodak) with intensifying screens for 2–5 days at −70°C, and RFLP patterns were analyzed (16–18).

Nomenclature. To avoid local Taq I RFLP nomenclature, the associated DR and DQ serological specificities (16, 18) are given whenever possible, using the nomenclature suggested by The Nomenclature Committee on Leucocyte Antigens 1987 (26). Allelic Taq I DRB, DQA, and DRB RFLP patterns frequently referred to in the text are described in Table 2.

Table 2. Tag I DRB and DQB RFLP patterns positively and negatively associated with the different clinical forms of MS are illustrated in Fig. 1.

Statistics. Data were analyzed by the χ2 test or by Fisher’s exact test, when the criteria of the χ2 test were not fulfilled. When looking for other HLA class II associations than the well-established DR2 association, gene frequencies of patients and controls were compared after subtraction of T-DRB II,T-DQB II (DRw15,DQw6)-positive haplotypes. P values of non-DR2 associations were corrected (by a factor of 29, Pcorr29) for the total number of Taq I HLA-DRB, DQA, and DQB haplotypes (n = 19) found in the healthy controls. P values were also corrected (by a factor of 2, Pcorr2) for dividing the patients into two groups. Relative risks (RRs) and etiologic fractions (EF) were calculated by methods described in refs. 27 and 28.

RESULTS

All MS Patients Versus Healthy Controls. Sixty percent of patients (n = 100) compared to 30% of controls had the T-DRB II pattern, which corresponds to the serological DR2-split DRw15 (P < 0.00005; RR, 3.5; EF, 0.43) (Table 2; Fig. 1, lane 1). Sixty-nine percent of patients compared to 50% of controls were T-DRB II-positive, which is associated with the serological DQw1-positive DQw6 (P < 0.005; RR, 2.2; EF, 0.36) (Table 2; Fig. 1, lane 2). The T-DRB II pattern (DQw6) is seen in DRw15,DQw6, Dw2 and DRw13,DQw6, Dw18 haplotypes as well as in some very rare haplotypes. The primary RFLP-defined association in all MS patients was with the T-DRB II pattern (DRw15) and not with T-DQB II (DQw6) (P < 0.001).

PCP MS Patients Versus Healthy Controls. The association of PCP MS (n = 26) with T-DRB II (DRw15) was weaker than the association found in all MS patients, 54% of patients compared to 30% of controls (Pcorr2 < 0.05; RR, 2.7; EF, 0.34) (Fig. 1, lane 1). No significant association with T-DQB II (DQw6) was observed (Fig. 1, lane 2).

Sixty-five percent of PCP MS patients compared to 38% of controls had the T-DRB IV pattern (Pcorr4 < 0.05; RR, 3.1; EF, 0.44) (Table 2; Fig. 1, lane 4). T-DRB IV in Caucasians is found in DR4,DQw8, DR7,DQw4 haplotypes (Table 2). Most of T-DRB IV-positive patients belonged to the DR4,DQw8 haplotype—42% of all PCP MS patients compared to 24% of controls (Pcorr2 < 0.05; RR, 2.3; EF, 0.24). None of the 26 patients compared to 29% of controls were T-DQB V-positive, which is associated with DQw7 (Pcorr2 < 0.05; RR, 0.06) (Table 2; Fig. 1, lane 5). Consequently, none of the DR4-positive haplotypes in PCP MS compared to 31% of DR4-positive haplotypes in controls had the T-DQB V (DQw7) pattern (P < 0.00001).

The combined occurrence of T-DRB II (DRw15) and T-DRB IV (DQw8, DQw9, and DQw4) was observed in 31% of patients with PCP MS compared to 6% of controls (P < 0.001; RR, 7.0). Eighty-eight percent of patients exhibited one or both susceptibility markers compared to 62% of controls (P < 0.05; EF, 0.70).

R/R MS Patients Versus Healthy Controls. The strength of the association with T-DRB II (DRw15) in R/R MS (n = 74) was of the same magnitude as the association found in all MS patients—62% of patients compared to 30% of controls (Pcorr2 < 0.00005; RR, 3.8; EF, 0.46) (Fig. 1, lane 1). Furthermore, 68% of R/R MS patients were T-DQB II (DQw6)-positive compared to 50% of controls (Pcorr2 < 0.05; RR, 2.2; EF, 0.37) (Fig. 1, lane 2). In R/R MS, as for all MS patients, the primary RFLP-defined association was with the T-DRB II (DRw15) allelic pattern and not with T-DQB II (DQw6) pattern (P < 0.0005).
Table 2. Allelic Taq I DRB, DQA, and DQB RFLP patterns referred to in the text and the associated serological specificities

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<thead>
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<th>DRB</th>
<th>DQA</th>
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<tbody>
<tr>
<td>II</td>
<td>II</td>
<td>II</td>
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<td>IV</td>
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<td>V</td>
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</table>

For DRB, DQA, and DQB, local Taq I nomenclature is in Roman numerals (16). kb, Kilobases; assoc. ser. spec., associated serological specificities.
*Data are from refs. 16 and 18 and O.O., unpublished data.

Thirty-two percent of R/R MS patients had the T-DQB III pattern, which is seen in the DRw17,DQw2 haplotype, compared to 15% of controls (Pcorr < 0.005; RR, 2.7; EF, 0.20)

![Fig. 1. Taq I DRB and DQB allelic restriction fragment patterns positively and negatively associated with all MS, PCP MS, and R/R MS. Lanes: 1, Taq I-digested DNA hybridized with a DRB cDNA probe; 2-5, Taq I-digested DNA hybridized with a DQB cDNA probe, as indicated. To the left of each lane, the restriction fragments characteristic for the allelic pattern are indicated in kilobases. All statistical comparisons were made with the healthy controls. Significant differences after correction for multiple comparisons are indicated: *, P < 0.05; **, P < 0.01; ***, P < 0.001.](image)

Table 2; Fig. 1, lane 3). No negative (i.e., protective) HLA class II association was found in this group of patients.

The combined occurrence of T-DRB II (DRw15) and T-DQB III (DRw17,DQw2) was found in 19% of patients with R/R MS compared to 4% of controls (P < 0.005; RR, 5.6). Seventy-seven percent of patients had one or both susceptibility markers compared to 41% of controls (P < 0.00001; EF, 0.61).

PCP MS Versus R/R MS. PCP MS as well as R/R MS were associated with T-DRB II (DRw15).

The frequency of T-DQB IV (DQw8, DQw9, and DQw4), positively associated with PCP MS, is reduced in R/R MS compared to PCP MS (P < 0.001) (Table 3). Likewise, the occurrence of T-DQB III (DRw17,DQw2) positively associated with R/R MS is reduced in PCP MS compared to R/R MS (P < 0.005) (Table 3).

DISCUSSION

The familial occurrence of MS with a tendency of increasing prevalence with increasing degree of kinship (for review, see ref. 29) suggests that genetic factors may be of importance in the etiology of MS. In a Canadian population-based study of MS in twins (30), a significantly higher concordance rate was found in monozygotic twins compared to dizygotic twins, indicating a major genetic etiologic component.

The inheritance of MS might be polygenic, with loci of the HLA class II region contributing a major part of the genetic predisposition. Linkage to the HLA region is supported by many (e.g., refs. 29, 31), but not all (32, 33), studies of haplotype sharing in affected sibling pairs. In formal segregation and linkage analysis (34), an excellent fit was obtained for a model with a dominant MS determinant tightly linked to the DR2 haplotype and a second unlinked determinant. However, as only limited data are available on the concordance rate of MS in HLA-identical siblings, the influence of non-HLA-linked genes is difficult to ascertain.

The penetration of MS in individuals carrying the associated Taq I HLA-DRB and DQB patterns is low. This does not necessarily imply that the disease is polygenic or precipitated by an environmental influence. The T-cell-receptor α/β heterodimers are formed by random genetic rearrangements. During maturation the T cells are submitted to positive and negative thymic selection controlled by the HLA antigens. Thus, the T-cell repertoire of an individual is influenced by stable genetic markers, the HLA genes, and a random genetic process. The likelihood for formation of specific T-cell clones that might contribute to disease will, therefore, be HLA-dependent. The penetrance of disease will depend on the stochastic event of forming T-cell clonal repertoires with a capacity to cause disease.

In the present study an EF of 0.70 is found for the presence of the RFLP-defined alleles T-DRB II (DRw15), T-DQB IV...
Table 3. Frequencies of Taq I HLA-DQB allelic restriction fragment patterns contributing to susceptibility and resistance to PCP MS compared to R/R MS

<table>
<thead>
<tr>
<th>Pattern</th>
<th>Frequency, %</th>
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<th>P</th>
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<tbody>
<tr>
<td></td>
<td>PCP MS</td>
<td>R/R MS</td>
<td>Controls*</td>
</tr>
<tr>
<td>Taq I DQB IV (DQw8,DQw9,DQw4)</td>
<td>65</td>
<td>27</td>
<td>38</td>
</tr>
<tr>
<td>Taq I DQB V (DQw7)</td>
<td>0</td>
<td>19</td>
<td>29</td>
</tr>
<tr>
<td>Taq I DQB III (DRw17,DQw2)</td>
<td>4</td>
<td>32</td>
<td>15</td>
</tr>
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</table>

NS, not significant. *values are as follows: for PCP MS, 26; for R/R MS, 74; for controls, 100.
†P corrected by a factor of 8.
‡Positively associated with PCP MS.
§Negatively associated with PCP MS.
* Positively associated with R/R MS.

(DQw8, DQw9, DQw4), or both in PCP MS, and an EF of 0.61 is found in R/R MS for the occurrence of T-DRB II (DRw15), T-DQB III (DRw17,DQw2), or both. These figures are in good agreement with the concept that most of the genetic susceptibility to MS is conferred by genes in the HLA class II region.

In population studies of MS, associations were first observed with the HLA class I antigens A3 and B7. However, subsequently stronger associations were found with the class II specificities Dw2 and DR2. In most population studies of Caucasian MS patients consistently strong associations with the Dw2 and DR2 specificities have been obtained (ref. 35; for review, see ref. 36). A Dw2 association has also been observed in American black MS patients. A weak DR3 association has been observed in a few studies (37). In Arabian (38) as well as in Sardinian (39) MS patients an association with the DR4 antigen has been found. A weak association with the Dw1 antigen (DR2 associated) was found in Scottish MS patients (11), but this finding has not been confirmed (12, 40). An association with the Dw4 specificity has been reported in Swedish MS patients (41). However, in a Norwegian study (42), where a Dw4 association was also observed, this was interpreted to be secondary to a primary DR2 association. It is worth noting that in population studies of DP antigen frequencies in Norwegians (43), the Dutch (44), and Danes (45), weak linkage disequilibrium between the DR2 and Dw4 specificities has been observed.

In our MS patients, the well-known DR2 association is confirmed (for review, see ref. 36). In addition, the association is found to be with the RFLP pattern T-DRB II that corresponds to the serological DR2-split DRw15. The three cellurally defined specificities associated with DR2 (Dw2, Dw12, and Dw21) can readily be distinguished by Taq I RFLP analysis and DR, DQA, and DQB linkage analysis (18, 46). All T-DRB II (DRw15) positive patients in our study have the Taq I RFLP patterns corresponding to Dw2, which is in accordance with previous findings (35).

An association with T-DQB II, corresponding to the Dw1-split DQw6, is also observed. Based on RFLP data this association is statistically secondary to the T-DRB II (DRw15) association (P < 0.001). However, as the protein sequences of the polymorphic amino-terminal domain of the DRB1 (for review, see ref. 13) and the DQB1 (47) gene products most likely are unique in the DRw15,DQw6,Dw2 haplotype, it is not possible by Taq I RFLP analysis or by serological DR and DQ typing to determine the biologically primary association.

As it has been suggested that PCP MS and R/R MS may be distinct diseases (6), it is of great interest to note the finding of positive and negative associations with different RFLP-defined DQB1 alleles in the two clinical forms of MS.

In PCP MS an association with T-DQB IV is found. This RFLP pattern is seen in DR4,DQw8, DR7,DQw9, and DRw8,DQw4 haplotypes. The amino acid sequences of the DQB chain probably differ between these three haplotypes (47). However, a significant proportion of T-DQB IV-positive PCP MS patients exhibit Taq I DRB, DQA, and DQB RFLP patterns corresponding to the DR4,DQw8 haplotype (Pcorr < 0.05). The five Dw types (Dw4, Dw10, Dw13, Dw14, and Dw15) associated with the serological specificity DR4 cannot be separated by RFLP analysis. The distribution of these cellulary defined specificities is not known in PCP MS. If the distribution does not markedly differ between DR4-positive PCP MS patients and controls, it would be tempting to assume that the observed DR4,DQw8 association might primarily be with the DQBl locus. However, this would have to be confirmed by specific sequencing.

A negative (i.e., protective) association is observed with T-DQB V (DQw7) in PCP MS. This Taq I DQBl allelic pattern is mainly seen in the DR4,DQw7, DRw11,DQw7, DRw12,DQw7, and DRw8,DQw7 haplotypes. The amino acid sequences of the first domain of the DQB chains are identical in all these haplotypes (47). However, the amino acid sequences of the DQA (47) and DRB (for review, see ref. 13) chains differ. Thus, one might speculate whether the protective effect may be conferred by the DQBl gene product.

In R/R MS a positive association is found with T-DQB III (DRw17,DQw2). It is of interest to note that no association is found with the Taq I DQBl pattern seen in the DR7,DQw2 haplotype. Although the RFLP patterns of these two haplotypes are different, the sequences of the membrane-distal domain of the DQB chains are identical (47). However, the amino acid sequences of the first domain of the DQA chains differ (47) between the two DQw2 haplotypes, which may explain why an association is only found with the DQB pattern of the DRw17,DQw2 haplotype and not with the DR7,DQw2 haplotype. No association is observed with the Taq I DQA RFLP pattern of the DRw17,DQw2 haplotype. This DQA pattern is also found in DRw11,DQw7 and DRw12,DQw7 haplotypes. The sequences of the polymorphic domain of the DQA chains in the haplotypes exhibiting this Taq I DQA pattern are identical (47). Furthermore, the two RFLP patterns (T-DRB IV and T-DRB VIII; Table 2) associated with DRw17 have identical sequences of the second exon of the DRBl locus (48). They differ only in the DRB3 locus, which encodes the DRw52 specificities (49). Thus, RFLP data obtained in the present study supported by available sequence information confirms that R/R MS is associated with the specific DQA-DQB heterodimer of the DRw17,DQw2 haplotype, where neither the sequence of the DQA or the DQB chain is unique. However, it is not possible to exclude that the biologically primary association might be with the DRBl locus of DRw17,DQw2 haplotype.

In the present study the well-established DRw15,Dw2 association in MS is confirmed. Furthermore, different RFLP-defined alleles of the DQB1 locus are found to con-
tribute to susceptibility and resistance to PCP MS as well as to susceptibility to R/R MS. Due to the strong linkage disequilibrium between DR and DQ subregions, it is, as previously mentioned, not possible by RFLP analysis to exactly determine which DR loci, DQ loci, or both that confer susceptibility to the different clinical forms of MS. This issue has to be addressed by sequencing of the polymorphic second exon of DRB1 and DQB1 genes in haplotypes positively associated with MS. The protective association observed in PCP MS might primarily be with the DQB1 locus.

It is of great interest to note that the differences in frequencies of the DQB RFLP patterns positively associated with PCP MS and R/R MS are more pronounced when comparing the two forms of MS with each other than when comparing PCP and R/R MS patients with controls (Table 3). The observed immunogenetic heterogeneity gives strong support to the hypothesis that PCP MS and R/R MS are two distinct disease entities. This may well prove to be of great importance when studying the etiology and pathogenesis of MS and also when the effect of different therapeutic regimes are evaluated.

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