OBJECTIVE: To look for HLA class II alleles and haplotypes conferring susceptibility to multiple sclerosis (MS) in the Jewish population of Israel.

DESIGN: Population-based cohort of clinically definite patients with MS tested prospectively over 7 years.

SETTING: Referral center in a neurology clinic at a university hospital in the greater Jerusalem area in Israel.

PATIENTS: A total of 162 consecutive patients with clinically definite MS from the 2 main ethnic Jewish groups in Israel: 104 Ashkenazi (80 with a relapsing remitting or secondary progressive and 24 with a primary chronic progressive course of the disease) and 58 non-Ashkenazi (36 with a relapsing remitting or secondary progressive course and 22 with a primary chronic progressive course of the disease), matched with 132 Ashkenazi and 120 non-Ashkenazi healthy controls.

MAIN OUTCOME MEASURES: The relationship between the various HLA class II alleles and haplotypes and MS, as defined by the polymerase chain reaction and sequence-specific oligonucleotide probe hybridization, among the Ashkenazi and the non-Ashkenazi Jewish sections and with respect to the different clinical courses of the disease.

RESULTS: The haplotype DRB1*1501, DQA1*0102, DQB1*0602 was found to be associated with MS among both Ashkenazi and non-Ashkenazi patients (P<.001 and P=.04, respectively). Among the non-Ashkenazi patients, a new association of haplotypes DRB1*1303, DQA1*05, and DQB1*0301 with MS was detected (P=.03). The MS susceptibility alleles, DRB1*1501, DQA1*0102, and DQB1*0602, were found in association with the Ashkenazi patients (P<.001, P=.02, and P=.01, respectively); DRB1*1501 and DRB1*1303 were more frequently observed among the non-Ashkenazi patients (P=.03, P=.04, respectively). On subdivision of the patients into clinical subgroups, associations of DRB1*0801, DQA1*0102, DQA1*0401, and DQB1*0602 with primary chronic progressive MS among the Ashkenazi patients were evident (P=.03, P=.04, P=.04 and P=.05, respectively), whereas DRB1*1501, DRB1*0301, and DQB1*0602 were associated with relapsing remitting or secondary progressive among the non-Ashkenazi patients (P=.05, P=.05, and P=.03, respectively).

CONCLUSIONS: This study, unlike previous ones, is the first to show a significant association between HLA class II alleles and MS in the Jewish population. The association with the HLA-DR2–related haplotype is similar to that among non-Jewish white patients with MS. Moreover, our data support the possibility that DRB1*1501 is the susceptibility allele responsible for the association between this haplotype and MS in the Jewish population. Our study also underscores differences in HLA profiles between Ashkenazi and non-Ashkenazi patients, and between the different clinical courses of the disease. The latter may indicate that the clinical courses of MS are influenced by the genetic background.

Arch Neurol. 1999;56:555-560

It is well documented that genetic factors are involved in the pathogenesis of multiple sclerosis (MS). As with many autoimmune diseases, the genetic susceptibility to MS is determined by genes encoded within the HLA class II region. In most population studies, the DR2-related haplotype, molecularly termed DRB1*1501, DQA1*0102, DQB1*0602, was reported to increase susceptibility to MS. This association, however, is not homogeneously distributed worldwide, as shown by the collaborative French Canadian-Sardinian study, in which a different association of MS with DR4, DQA1*0301, DQB1*0201, and DQB1*0302 alleles was found among Sardinians. Recently, complete genomic screening of affected members of families with MS indicated a linkage to the HLA region. It has also been suggested that specific amino acids in the peptide-binding groove of the HLA class II DRB1, DQA1, and DQB1 alleles may influence susceptibility to MS.
PATIENTS AND METHODS

PATIENTS AND CONTROLS

The Jewish population in Israel is divided into 2 major ethnic groups: Ashkenazi, who are of Central and Eastern European origin, and non-Ashkenazi, who are of Middle Eastern and North African origin. The prevalence of MS is more than 6-fold higher among the Ashkenazi than the non-Ashkenazi. Furthermore, these groups have distinct HLA class II profiles. All the comparisons in this study were therefore performed separately for each ethnic group.

All the patients included in the study were diagnosed as definite MS, according to Poser et al. For this study, the patients were subdivided into 2 subgroups according to the clinical course. The first subgroup included patients with a relapsing/remitting (R/R) (of at least 3 years' duration) or with a secondary progressive (SP) course. The second subgroup comprised patients with a primary chronic progressive (PCP) course who had never suffered a relapse. We combined the RR and SP patients into one subgroup as it is impossible to predict who of the patients with R/R MS will progress to SP MS.

A total of 162 Jewish patients with MS were included in the study: 104 Ashkenazi (80 with the R/R-SP and 24 with the PCP course of MS) and 58 non-Ashkenazi (36 with the R/R-SP and 22 with the PCP course of MS), as well as 252 randomly selected healthy individuals who served as controls: 132 Ashkenazi and 120 non-Ashkenazi. The participants were not related to one another.

HLA CLASS II OLIGOTYPING

Genomic DNA was extracted from heparinized peripheral blood according to the salting out procedure of Miller and coworkers, with minor modifications. The DNA samples were amplified by polymerase chain reaction. Specific primer pairs of the Eleventh International Histocompatibility Workshop were used for amplification of the second exons of the DRB1*, DQA1*, and DQB1* loci. Primer sequences and amplification conditions were as described by Kimura and Sasazuki. Amplified DNA samples were blotted onto nylon membranes after electrophoresis on 2% agarose gel and visualization. Hybridization was performed using the Eleventh International Histocompatibility Workshop sequence-specific oligonucleotide probe, as well as additional probes. The probes were labeled with digoxigenin-11-2'-3'-dideoxyuridine-5'-triphosphate (DIG-11-ddUTP) and detected by chemiluminescence (Lumiphos 530 or CDP-Star; Boehringer Mannheim, Mannheim, Germany).

The nomenclature for factors of the HLA system, 1996, was used to characterize the HLA class II alleles.

AMINO ACID ANALYSIS

The presence of amino acid valine, at residue 86 of the DRB1 chain, glutamine, at residue 34 of the DQa1 chain, and leucine, at residue 26 of the DQB1 chain, was compared between patients with MS and healthy controls by HLA allelic association. Analysis was performed according to the sequence of the second exon of HLA class II alleles presented at the Eleventh Histocompatibility Workshop.

STATISTICAL ANALYSIS

Associations between HLA class II alleles and haplotypes with MS were detected using the x2 of independence with Yates correction for continuity in 2 x 2 tables. The Fisher-Irwin exact test was used when any cell value in a 2 x 2 table was less than 5. For alleles and haplotypes that were not found previously to be statistically associated with MS, the calculated P values were corrected by multiplying them by the number of comparisons. Relative risk (RR) was calculated according to Woolf’s formula.

RESULTS

DISTRIBUTION OF DRB1, DQA1, AND DQB1 ALLELES AMONG PATIENTS WITH MS

Table 1 presents the HLA allele frequencies of the DRB1*, DQA1*, and DQB1* loci.

Ashkenazi Patients

DRB1*1501 showed a significant positive association with MS (P < .001, in comparison with the control group) with an RR of 5.36. The analysis of the DQA1 and DQB1 alleles revealed a positive association of DQA1*0102 (P = .02, RR = 2.08) and DQB1*0602 (P = .01, RR = 2.62) with MS.

Non-Ashkenazi Patients

DRB1*1303, an infrequent allele among the Jewish population, showed a significant positive association with MS (corrected P value [Pc] = .04, in comparison with the healthy controls, RR = 29.84). This association was not found in the
Ashkenazi patients. The DRB1*1501 allele was also associated with MS in this ethnic group (P = .03, RR = 3.36). Among the DQA1 and DQB1 alleles, no significant associations with MS were detected in the non-Ashkenazi patients.

**HAPLOTYPES ASSOCIATED WITH JEWISH PATIENTS WITH MS**

The frequencies of haplotypes previously reported to be associated with MS were analyzed. Table 2 presents only the significant results. We found that the DRB1*1501, DQA1*0102, DQB1*0602 haplotype was more strongly associated with MS among the Ashkenazi than among the non-Ashkenazi patients (P < .001, RR = 5.00 and P = .04, RR = 3.98, respectively). As each of the alleles of this haplotype, which are in linkage disequilibrium, was found to be associated with MS in the Ashkenazi group, we tried to pinpoint this association to a particular allele. Therefore, we analyzed the frequencies of the DRB1*1501, DQA1*0102, and DQB1*0602 alleles after exclusion of individuals carrying the DRB1*1501, DQA1*0102, DQB1*0602 haplotype. Allele DRB1*1501 was observed with slightly increased frequency, both among the Ashkenazi (4 patients [4.8%]) and the non-Ashkenazi patients with MS (3 patients [3.9%]) compared with the controls (1 individual [0.8%] and 3 individuals [2.6%), respectively). DQA1*0102 was also more frequent among the Ashkenazi (14 patients [16.7%]) and non-Ashkenazi (10 patients [19.6%]) patients with MS, than the control groups (19 individuals [15.1%] and 20 individuals [17.24%, respectively). DQB1*0602 was absent in the Ashkenazi patients with MS, vs 5 (4.0%) Ashkenazi controls and it was detected in 1 non-Ashkenazi patient with MS (2.0%) vs 1 control (0.9%). These differences were not statistically significant.

Haplotype DRB1*1303, DQA1*05, and DQB1*0301 was positively associated with MS only in the non-Ashkenazi group (P = .03, RR = 29.84). However, in these patients the lack of association between MS and DQA1*05, DQB1*0301, suggests that the association of MS with this haplotype, was primarily due to the high frequency of DRB1*1303.

**ASSOCIATION OF HLA ALLELES WITH THE CLINICAL COURSE OF MS**

Previous studies have indicated that different courses of the disease may have distinct immunogenetic mechanisms, as different associations were found between clinical subgroups with MS and HLA alleles. Therefore, we compared the HLA profiles between patients with RR/SP MS and those with PCP MS, as well as with healthy controls (Table 3). There was a higher proportion of PCP MS among the non-Ashkenazi patients (22 patients [37.9%]) than among the Ashkenazi patients or the non-Jewish patients of European origin. This finding concurs with a previous report on the clinical course of MS in Israeli patients.

**Ashkenazi Patients With MS**

As noted in Table 3, most of the HLA susceptibility alleles were found at higher frequencies in the subgroup with PCP MS: DRB1*1501 was observed in 8 (33.3%) of the patients with PCP MS as compared with 7 (5.3%) healthy controls (P = .003), and 16 patients (20%) in the subgroup with R/R-SP MS (P = .002). However, the difference in DRB1*1501 frequency between these 2 subgroups of patients was not significant. DQA1*0102, which is in linkage disequilibrium with DRB1*1501, was detected at a significantly higher frequency in the subgroup with PCP (12 patients [50%]) than R/R-SP MS (22 patients [27.5%]) (P = .04). Similarly, DQB1*0602, which is in linkage disequilibrium with DRB1*1501 and DQA1*0102, was found at a significantly higher frequency in the subgroup with PCP (8 patients [33.3%]) than the R/R-SP MS (12 patients [15.0%]) (P = .05).

DRB1*0801 was detected only in the subgroup with PCP MS (P = .03, in comparison with the patients with R/R-SP). DQA1*0401, which is in linkage disequilibrium with DRB1*0801, also showed an association in patients with PCP as compared with R/R-SP MS (P = .03).
However, DQB1*0402 which is in linkage disequilibrium with DRB1*0801 and DQA1*0401 showed a significantly higher frequency only compared with the control group (Pc = .034) but not with the patients with R/R-SP MS. It is noteworthy that the group of patients studied (Ashkenazi with PCP MS) was too small to allow definite conclusions to be drawn.

The association of MS with the DQA1*0102 and DQB1*0602 alleles can, therefore, be mainly attributed to the high frequencies of these alleles in the patients with PCP MS among the Ashkenazi patients (Table 3).

Non-Ashkenazi Patients With MS

Most of the HLA susceptibility alleles were found more frequently in the subgroup with R/R-SP MS. DRB1*1501 was observed more often in the patients with R/R-SP (9 patients [25.0%]) than PCP MS (1 patient [4.6%]) (P = .05). Similarly, DQB1*0602, which is in linkage disequilibrium with DRB1*1501, was observed at a significantly higher frequency in the subgroup with R/R-SP (7 patients [19.4%]) as compared with PCP MS (0%), (P = .03).

The association of DRB1*1303 with MS among the non-Ashkenazi patients was confirmed in the subgroup with R/R-SP MS compared with the controls (P = .05). This allele was also more frequent in the subgroup with PCP MS (2 patients [9.1%] vs 0% in the controls) but without significant difference after the correction for number of comparisons. A larger non-Ashkenazi subgroup with PCP MS may have revealed a significant result. No significant difference in the frequency of this allele was detected between the subgroups with R/R-SP and PCP MS. In addition, DRB1*0301 showed a strong association with R/R-SP (9 patients [25.0%]), as compared with PCP MS (1 patient [4.6%]) (P = .05).

The associations of MS with the DRB1*1501 allele and haplotype can therefore be mainly attributed to the high frequencies of this allele among patients with R/R-SP MS (Table 3).

### Analysis of Particular Amino Acid Residues in the HLA Class II α and β Chains

In a search for shared amino acids between the different HLA alleles conferring MS susceptibility, valine at position 86 of the DRB1 chain, glutamine at residue 34 of the DQα1 chain, and leucine at residue 26 of the DQB1 chain were reported to be associated with the disease.14-17 In the present study, allele-specific oligotyping of DRB1*, DQA1*, and DQB1* enabled us to analyze the frequency of alleles containing these amino acids in patients with MS and in healthy controls. According to our

### Table 3. DRB1, DQA1, and DQB1 Allele Distribution According to Disease Course in Multiple Sclerosis*

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<th>Allele</th>
<th>Controls, %</th>
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<th>PCP MS, %</th>
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<th>PCP MS, %</th>
<th>P Values for R/R-SP vs PCP†</th>
<th>R/R-SP vs Controls‡</th>
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* R/R-SP MS indicates relapsing/remitting and secondary progressive multiple sclerosis; PCP MS, primary chronic progressive multiple sclerosis.
† χ² was calculated for disease courses R/R-SP and PCP.
‡ χ² was calculated for patients with R/R-SP MS and controls.
§ χ² was calculated for patients with PCP MS and controls.
|| Corrected P value.
To our knowledge, this study is the first to show a significant association between HLA and MS in the Jewish population. Although not yet conclusive, it is likely that HLA susceptibility is caused through the antigen presentation function of HLA molecules. This hypothesis also supports the involvement of an autoimmune process in MS. The fact that different HLA class II alleles are involved in susceptibility to MS in different populations, as shown in the Sardinian population, may reflect the existence of diverse pathogenic antigens in populations with different genetic backgrounds. However, the possibility of linkage disequilibrium between HLA-specific alleles and the MS susceptibility gene cannot be excluded.

In the present study, we describe a definite association between the DRB1*1501, DQA1*0102, DQB1*0602 haplotype and MS among both Ashkenazi and non-Ashkenazi Jewish patients. The differences between these 2 groups were reflected mainly in the degree and the uniformity of the association with this haplotype, which was observed at a lower frequency and lower relative risk, and only with R/R-SP MS, in the non-Ashkenazi group.

As the tri-locus haplotype DRB1*1501, DQA1*0102, DQB1*0602 displays a strong linkage disequilibrium between its alleles, it is difficult to pinpoint the primary allele responsible for susceptibility to MS. After excluding individuals who carry the DRB1*1501, DQA1*0102, DQB1*0602 haplotype from the analysis, none of these alleles appeared to show any association with MS. However, in the non-Ashkenazi group, and in the Ashkenazi subgroup with R/R-SP MS (which included most of the Ashkenazi patients), there was a positive association only with the DRB1*1501 allele and not with the other alleles of the haplotype. Furthermore, the relative risk of DRB1*1501 was higher compared with that of DQA1*0102 or DQB1*0602, both in the Ashkenazi and the non-Ashkenazi groups. Therefore, these findings may suggest that of the 3 alleles composing this haplotype, DRB1*1501 is the prime candidate for the susceptibility to MS in the Jewish population. However, there is a possibility that the susceptibility gene is not the DRB1*1501 itself, but another gene that has stronger linkage disequilibrium with DRB1 gene than with DQ genes.

Our findings reveal that an additional allele, DRB1*1303, is significantly associated with MS in non-Ashkenazi patients. This allele belongs to the serologically defined DR6 and DR13 (a DR6 subtype) family, which was found to be associated with MS in Mexican patients. However, as the latter study was based on serologic typing, it is not clear whether the reported association is attributable to the DRB1*1303 or another allele of the DR6 family. Yet, this association of MS with DRB1*1303 deserves replication in a second data sample from the same population.

Our results also show that in each of the 2 ethnic groups, there was a distinct association between a number of HLA alleles and specific subgroups with clinical MS. In the Ashkenazi group, the principal association was with the subgroup with PCP MS, and in the non-Ashkenazi group the association was mainly with the subgroup with R/R-SP MS. Specifically, the DRB1*0801, DQA1*0102, DQA1*0401, DQB1*0402, and DQB1*0602 alleles were associated only with PCP MS in Ashkenazi patients, whereas DRB1*1501, DRB1*03011, and DQB1*0602 were found mainly in the non-Ashkenazi patient subgroup with R/R-SP MS. The association of R/R-SP MS with DRB1*03011 is similar to the results reported by Olerup et al. However, in contrast to the findings of the Swedish group, no association was observed between patients with PCP MS and DR4-related alleles. It is noteworthy that the Ashkenazi subgroup with PCP MS and the non-Ashkenazi subgroup with RR/SP MS have a small number of patients, and thus the HLA class II susceptibility alleles to these subgroups could account only for a small portion of the susceptibility factors of MS in general.

In conclusion, our data demonstrate that both Jewish groups with MS (Ashkenazi and non-Ashkenazi) are similar to the non-Jewish white patients with MS, with regard to the HLA profile that influences the susceptibility to MS. These findings further demonstrate the “universality” of the MS HLA susceptibility alleles. In addition, in non-Ashkenazi patients with MS, a unique association with the DRB1*1303 allele was revealed. Finally, our data provide some indications that the clinical course of MS may be influenced by specific HLA alleles.

Accepted for publication September 10, 1998.

This work was supported in part by a research grant from Teva Pharmaceutical Industries Ltd, Netanya, Israel (Dr Brautbar).

We thank Shlomit Gen, MSc, for data analysis.

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