Role of the HLA System in the Association Between Multiple Sclerosis and Infectious Mononucleosis

Sreeram V. Ramagopalan, DPhil; Ute C. Meier, DPhil; Margaret Conacher, PhD; George C. Ebers, MD; Gavin Giovannoni, MD; Dorothy H. Crawford, MbChB; Karen A. McAulay, PhD

Objective: To determine whether multiple sclerosis (MS) and infectious mononucleosis (IM) share common HLA associations.

Design: A prospective cohort study was conducted from October 1, 1999, through September 30, 2003.


Patients: Participants included 179 individuals who underwent asymptomatic Epstein-Barr virus seroconversion and 175 patients who developed IM.

Intervention: Genotyping for 5 classical HLA loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1).

Main Outcome Measure: Diagnosis of IM and allele frequency.

Results: Allelic analysis showed that HLA-DRB1*01:01 was significantly associated with the development of IM (odds ratio, 3.2; \( P < .001 \)). Patients with IM and HLA-DRB1*01:01 had a lower Epstein-Barr virus viral load compared with those without the allele (median, 783 vs 7366 copies/10^6 peripheral blood mononuclear cells; \( P = .03 \)).

Conclusion: HLA-DRB1*01:01 is protective against developing MS; thus, a common genetic basis between IM and MS is not supported.

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MULTIPLE SCLEROSIS (MS) is the most common disease of the central nervous system to cause permanent disability in young adults.\(^1\) Based on strong circumstantial evidence, MS is considered to be an organ-specific autoimmune disorder,\(^1,2\) but much remains to be understood about the initiation of the disease. It appears unlikely that MS results from a single causative event; rather, it is more probable that the disease develops in a genetically susceptible population as a result of environmental exposures.

There is a growing body of evidence in support of a role for Epstein-Barr virus (EBV) in determining susceptibility to MS. A key observation is the finding that individuals with a history of infectious mononucleosis (IM) have an increased risk of developing MS. A systematic review and meta-analysis\(^3\) reported a combined relative risk of MS after IM of 2.3 (95% confidence interval, 1.7-3.0). This risk has been confirmed in large population-based studies.\(^4,5\) The mechanism by which IM is associated with MS is poorly understood. A suggested mechanism is common genetic factors that predispose to both diseases. A recent prospective study\(^6\) comparing individuals who developed asymptomatic EBV infection with those who developed IM demonstrated association of IM with microsatellite markers in the HLA class I region. However, that study made no attempt to correlate risk with specific HLA genes. Given the strong association of MS with the major histocompatibility complex, we used this prospective IM cohort\(^6-8\) to investigate the effects of the HLA class I and II genes on the risk of developing IM.

METHODS

ETHICS APPROVAL

Participants were recruited through the University of Edinburgh Richard Verney Health Centre. The study was approved by the Lothian Research Ethics Committee, and all participants provided written consent. The pro-
manufacturer’s instructions, and stored at −70°C. Patients with IM. Data on an additional 31 patients with IM who previously underwent genotyping for HLA-C were performed as described by Stevens et al.11

PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral blood mononuclear cells were separated from whole blood by routine density gradient centrifugation, washed, and counted. DNA was extracted from approximately 9 x 106 cells (Easy-DNA Kit, Invitrogen, Paisley, Scotland), according to the manufacturer’s instructions, and stored at −70°C.

HLA GENOTYPING

High-resolution sequence-based typing of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 was performed as described10 on 179 asymptomatic seroconverters and 144 patients with IM. Data on an additional 31 patients with IM who previously underwent genotyping for HLA-DRB1 alone were added to the analysis.

VIRUS LOAD QUANTIFICATION

The EBV-DNA load was determined by quantitative competitive polymerase chain reaction as described by Stevens et al.11

RESULTS

Initial allele and haplotype frequencies were compared between the IM cohort (n=144) and EBV-positive group (n=179) for individuals who underwent genotyping for all 5 HLA loci.

Six different alleles were identified with a frequency difference yielding a nominal P value of <.05 (Table). HLA-DRB1*01:01 was the most significant allele in the IM vs EBV-positive cohort comparison, being significantly more frequent in the IM group (odds ratio, 2.49; 95% confidence interval, 1.29-5.01; P=.03). The frequency of only HLA-DRB1*01:01 remained significant after permutation testing (corrected P = .048).

In the haplotype analysis, HLA-A*03:01–HLA-B*35:01–HLA-C*04:01–HLA-DRB1*01:01–HLA-DQB1*05:01 was significantly associated with IM (P=.03), being overrepresented in the IM cohort. All other haplotypes were nonsignificant in all comparisons (P >.05).

When we included the data from 31 patients who underwent genotyping for HLA-DRB1 alone, the association of IM with HLA-DRB1*01:01 became stronger (IM

<table>
<thead>
<tr>
<th>Allele</th>
<th>IM Allele Frequency</th>
<th>EBV-Positive Allele Frequency</th>
<th>OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*15:01</td>
<td>0.04</td>
<td>0.08</td>
<td>0.46 (0.21-0.96)</td>
<td>.04</td>
</tr>
<tr>
<td>HLA-C*02:02</td>
<td>0.01</td>
<td>0.06</td>
<td>0.23 (0.06-0.69)</td>
<td>.004</td>
</tr>
<tr>
<td>HLA-C*04:01</td>
<td>0.14</td>
<td>0.08</td>
<td>1.73 (1.03-2.94)</td>
<td>.03</td>
</tr>
<tr>
<td>HLA-DQB1*03:01</td>
<td>0.12</td>
<td>0.19</td>
<td>0.59 (0.37-0.91)</td>
<td>.01</td>
</tr>
<tr>
<td>HLA-DRB1*01:01</td>
<td>0.10</td>
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<tr>
<td>HLA-DRB1*04:01</td>
<td>0.06</td>
<td>0.11</td>
<td>0.55 (0.30-0.98)</td>
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</table>

Abbreviations: CI, confidence interval; EBV, Epstein-Barr virus; IM, infectious mononucleosis; OR, odds ratio.

a Significant at P < .05.

Details on enrollment and serotyping have been published.7,8 Procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

PARTICIPANTS

In brief, all students enrolling at the university health center from October 1, 1999, through September 30, 2000, were approached to take part in the study and, on recruitment, to provide a blood sample for EBV serologic determination. Those with EBV-seronegative results were then monitored for the development of IM while at the university (approximately 4 years). In addition, students who developed IM but were not enrolled in the original study were referred by the university health center and asked to participate. Upon diagnosis of IM (median, 10 days after onset of symptoms; range, 3-42 days), patients were examined and asked to provide a blood sample. Students with seronegative EBV test results on enrollment who did not report symptoms of IM were asked to return for further testing upon exit from the university. Students with seropositive EBV results were regarded as asymptomatic seroconverters and formed the EBV-seropositive group. Data on 179 individuals identified as asymptomatic seroconverters (controls) and 175 patients with IM were available for analysis. There was no variation in ethnicity between groups. Most control participants (78%) and all those diagnosed as having IM carried the type 1 EBV virus strain; therefore, infection with different EBV types (type 2, type 3) was assumed to be minimal.9

Of the 179 asymptomatic seroconverters, 31 were approached to take part in the study and, on recruitment, to provide a blood sample. Those who did not participate were also included in the control groupings. The null hypothesis was that there were no differences between the IM cohort (n=144) and EBV-positive group (n=179) with respect to HLA allelic frequencies. Consequently, a 2 x 2 contingency table of HLA allele frequencies was set up for each of the five loci. The chi-square test was used to compare allelic frequencies between the IM cohort and EBV-positive group. A Fisher exact test was used to compare allele frequencies between cohorts at each of the 5 loci. All P values are presented as uncorrected for multiple testing. Viral copy number was compared across groups using the Mann-Whitney test. P value <.03 was considered significant. To correct for multiple testing, permutation tests were performed (106 permutations).

Table. Alleles With a Significant Frequency Difference in Comparisons Between IM and EBV-Positive Cohorts

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<th>Allele</th>
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vs EBV-positive comparison, \( P < .001 \); odds ratio, 3.2; 95% confidence interval, 1.72-6.27); the association with all other HLA-DRB1 alleles became nonsignificant (\( P > .05 \)). There were no significant differences in HLA-DRB1 allele frequencies between the first (\( n = 144 \)) and second (\( n = 31 \)) sets of patients with IM.

Viral load data were available for a small subset of patients with IM (\( n = 32 \)). Comparison of EBV viral copy number demonstrated a significant difference (\( P = .03 \)) between patients with IM carrying the HLA-DRB1*01:01 allele (median, 783 copies/10^6 peripheral blood mononuclear cells; range, 26-33783) and those without the allele (median, 7366 copies/10^6 peripheral blood mononuclear cells; range, 483-48283) (Figure).

We observed that HLA-DRB1*01:01 is the allele most significantly associated with IM, being overrepresented in individuals who develop this syndrome compared with individuals with asymptomatic EBV infection. The HLA-DRB1*01 allele is protective in MS,12 and we found no association with the strong MS risk allele HLA-DRB1*15. Therefore, our study rules out a common genetic association between MS and IM.

The association of HLA class II with IM is functionally plausible. Epstein-Barr virus preferentially infects B lymphocytes through the binding of the major viral envelope glycoprotein Gp350 to the CD21 receptor on the surface of B cells and through the binding of a second glycoprotein, Gp42, to HLA class II molecules.13 Therefore, it may be that HLA-DRB1*01:01 is somehow more permissive for EBV entry, leading to enhanced proliferation during the initial stage of infection and effective induction of the immune response. Interestingly, HLA-DRB1*01:01 has also been shown14,15 to be a potent stimulator of CD8+ and CD4+ T-cell responses after infection with hepatitis B and mumps vaccination—both in terms of magnitude and quality of function. Furthermore, reports16 investigating the role of HLA class II molecules in the control of hepatitis C virus infection show that HLA-DRB1*01:01 possession correlates with good control and clearance of the virus, suggesting that the immune response is more effective in these individuals. Similarly, in the case of primary EBV infection, it may be that the HLA-DRB1*01:01 allele strongly binds an immunodominant EBV epitope or a selection of epitopes, leading to a more robust helper T-cell function and the subsequent heightened immune response that characterizes IM. This may in turn control initial virus replication more effectively and minimize the duration of the infection. Our viral load estimations on samples taken at similar time points (HLA-DRB1*01:01 positive: median, 11.5 days after onset of symptoms [range, 4-42 days]; HLA-DRB1*01:01 negative: median, 10 days [range, 3-28]) show a significant reduction in EBV copy number in individuals with HLA-DRB1*01:01, suggesting that immune control of virus replication is more effective in these individuals. However, the remit of this study did not allow for a detailed analysis of the immune response to confirm this hypothesis. In addition, because of the low allele frequency of HLA-DRB1*01:01, the attributable risk may be low. It may also be that HLA-DRB1*01:01 leads to more severe IM rather than susceptibility to IM.

A previous investigation6 highlighted the role of microsatellites in the HLA class I region as being associated with IM; however, the present study suggests that the HLA class II region is the principal association, as HLA-DRB1*01:01 is the most significantly associated allele in the analysis. Nevertheless, the haplotype analysis highlights the fact that HLA-A*03:01–HLA-C*04:01–HLA-DRB1*01:01 is the haplotype associated with IM, which may reflect either linkage disequilibrium or epistatic effects to increase the risk of IM.

The cohorts that we studied were perhaps underpowered to fully disentangle the effects of HLA class I and II genes as well as to detect loci exerting a small effect on IM risk, and population stratification could be an issue despite our efforts to match cohorts for ethnicity. Replication of the results obtained is therefore key to understanding in detail the genetic basis of IM. However, a common genetic association between MS and IM would mean that HLA-DRB1*15 would exert effects of similar magnitude in both diseases, which we can exclude. These data suggest that EBV plays a causal role in MS pathogenesis. Further work is warranted to understand the mechanism by which EBV influences MS risk.

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