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/10⁶ 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.

Arch Neurol. 2011;68(4):469-472

Multiple sclerosis (MS) is the most common disease of the central nervous system to cause permanent disability in young adults. Based on strong circumstantial evidence, MS is considered to be an organ-specific autoimmune disorder, 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 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. 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 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 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. (Easy-DNA Kit; Invitrogen, Paisley, Scotland), according to the
cscribed10 on 179 asymptomatic seroconverters and 144 pa-
tients with IM. Data on an additional 31 patients with IM who
added to the analysis.

The EBV-DNA load was determined by quantitative competi-
tion in ethnicity between groups. Most control participants
were available for analysis. There was no varia-
tion across groups using the Mann-Whitney test. A P value
<.05 was considered significant. To correct for multiple test-
ing, permutation tests were performed (106 permutations).

Haplotype analyses were performed (R package haplo.stats,
Version 1.4.4; http://mayoresearch.mayo.edu/schaid_lab/
software.cfm); cohorts were treated as either case or control.

This approach, achieved using the haplo.cc function, adopts
an additive-effect linear modeling method to assess associa-
tion between the binary trait and the haplotypes. Various case-
control groupings were assessed, with 2000 control simulations
done for each. The minimum haplotype frequency for
inclusion was set at 0.005, and the most frequent haplotype was
treated as the baseline. Because sample numbers were small,
some key haplotypes may not have been modeled.

Statistical Analysis

A Fisher exact test was used to compare allele frequencies be-
tween cohorts at each of the 5 loci. All P values are presented
as uncorrected for multiple testing. Viral copy number was com-
pared across groups using the Mann-Whitney test. A P value
<.05 was considered significant. To correct for multiple test-
ing, permutation tests were performed (106 permutations).

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

<table>
<thead>
<tr>
<th>Allele</th>
<th>IM Allele Frequency</th>
<th>EBV-Positive Allele Frequency</th>
<th>OR (95% CI)</th>
<th>P Valuea</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>
<td>0.04</td>
<td>2.49 (1.29-5.01)</td>
<td>.003</td>
</tr>
<tr>
<td>HLA-DRB1*04:01</td>
<td>0.06</td>
<td>0.11</td>
<td>0.55 (0.30-0.98)</td>
<td>.03</td>
</tr>
</tbody>
</table>

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

aSignificant at P<.05.

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 significa-
cantly more frequent in the IM group (odds ratio,2.49;
95% confidence interval, 1.29-5.01; P=.003). The fre-
quency 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 hap-
lotypes were nonsignificant in all comparisons (P>.05).

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

Participants

Details on enrollment and serotyping have been published.7,8
In brief, all students enrolling at the university health center
from October 1, 1999, through September 30, 2000, were ap-
taken to partake in the study and, on recruitment, to pro-
vide 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 cen-
ter 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 re-
port 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 vari-
ation 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
was assumed to be minimal.9

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 5×106 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 de-
scribed in on 179 asymptomatic seroconverters and 144 pa-
tients 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 competi-
tive polymerase chain reaction as described by Stevens et al.11
The amplification reaction contained 30mM potassium chlo-ride, 1.5mM magnesium chloride, 10mM TRIS-hydrochloride
buffer (pH, 8.5), 200mM deoxynucleoside triphosphates, 25 pmol
of each primer (5’ primer labeled with biotin), and 1 U of Taq
polymerase. Cycling conditions were 4 minutes at 95°C; 40
cycles at 95°C, 55°C, and 72°C for 1 minute each; and 3 min-
utes at 72°C. Products were captured on a streptavidin-coated
plate and probed with digoxigenin-labeled wild-type and in-
ternal standard probes. Optical density was measured and used
to calculate the number of copies.

Downloaded From: by a Non-Human Traffic (NHT) User on 11/10/2018
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, 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. 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 shown 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, reports 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.

We observed that 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 between patients with IM carrying the HLA-DRB1*01:01 allele (median, 783 copies/10^6 peripheral blood mononuclear cells; range, 263–33 783) and those without the allele (median, 7 366 copies/10^6 peripheral blood mononuclear cells; range, 483–48 283) (Figure).
**material support:** Conacher, Giovannoni, Crawford, and McAulay. **Study supervision:** Meier, Ebers, Giovannoni, Crawford, and McAulay. **Financial Disclosure:** None reported. **Funding/Support:** Funding for this study was provided by the Medical Research Council grant G0801976. **Role of the Sponsor:** The study sponsor had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript. **Additional Contributions:** The authors thank many colleagues and collaborators at Barts and The London School of Medicine, the University of Oxford, and the Edinburgh University Health Service and its participants. The authors also acknowledge the Histocompatibility and Immunogenetics Laboratory, Royal Infirmary of Edinburgh, for typing of some IM samples.

**REFERENCES**