Class II HLA Alleles and Hepatitis B Virus Persistence in African Americans

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Persistence of hepatitis B virus (HBV) infection is likely due to the interplay of the virus and host immune response. Given its critical role in antigen presentation, allelic differences in the HLA complex may affect HBV persistence. In a prospectively followed African American cohort, molecular class I and class II HLA typing was done on 31 subjects with persistent HBV infection and 60 controls who cleared the infection. HBV persistence was significantly associated with two class II alleles, DQA1*0501 (odds ratio [OR], 2.6; \(P = .05\)) and DQB1*0301 (OR, 3.9; \(P = .01\)), the two-locus haplotype consisting of these same two alleles (OR, 3; \(P = .005\)) and the three-locus haplotype, DQA1*0501, DQB1*0301, and DRB1*1102 (OR, 10.7; \(P = .01\)). In addition, HBV persistence was associated with class II allelic homozygosity. Several class I associations with persistence were also noted but were not statistically significant after correction for multiple comparisons. These results underscore the importance of the class II-mediated immune response in recovery from HBV infection.

An estimated 300 million persons worldwide have persistent hepatitis B virus (HBV) infection and thereby are predisposed to hepatic failure and hepatocellular carcinoma [1]. After an acute infection, >90% of adults appear to recover, as indicated by clearance of hepatitis B surface antigen (HBsAg) from the serum. HBV clearance is associated with a CD8+ cytotoxic T lymphocyte (CTL) response directed against the infected hepatocyte and formation of antibodies to the HBsAg [1]. Central to this immune response is the presentation of HBV antigens in the context of HLA class I and class II molecules. Therefore, it is biologically plausible that differences in HLA alleles may affect HBV clearance. In addition, it is possible that being heterozygous for HLA alleles may be advantageous for virus clearance because of the greater number of possible HLA–viral antigen combinations [2, 3].

Certain class II alleles and a class II heterozygote advantage against persistent HBV infection have been reported from The Gambia [4, 5]. However, HBV persistence is highly dependent on the age of acquisition of infection, which differs between sub-Saharan African and economically developed countries. Class I alleles have not been associated with HBV persistence, but previous studies have not used molecular typing [5–8]. To test the hypothesis that certain HLA antigens predispose to persistent HBV infection, molecularly determined class I and class II HLA alleles were assessed in an adult African American cohort in which HBV persistence was rigorously documented.

Methods

Study subjects. The subjects are part of an ongoing study of 2921 injection drug users enrolled in Baltimore from February 1988 to March 1989, as previously described (ALIVE [AIDS link to intravenous experiences] study) [9]. For this study, a nested case-control design was used. Subjects with HBV persistence were those who had a positive test for HBsAg both at the time of enrollment and in a second sample taken at least 6 months from the first. They were also positive for HBV core antibody (anti-HBc) and negative for HBV surface antibody (anti-HBs). Controls were matched 2:1 with subjects with HBV persistence on the basis of age within 10 years, sex, race, and human immunodeficiency virus type 1 (HIV-1) status. These factors were chosen because they have been associated with HBV persistence.

Serologic testing. All serum specimens were stored at \(-70^\circ\)C, thawed, and tested according to manufacturer’s specifications for HBsAg, anti-HBs, and anti-HBc by EIAs (AUSZYME, AUSAB, and CORZYME, respectively; Abbott Laboratories, Abbott Park, IL) [10]. HIV testing was done by EIA (Genetic Systems, Seattle), and positive specimens were confirmed by Western blot (Biotech HIV-1; DuPont, Wilmington, DE) [10]. Hepatitis C virus (HCV) testing was done by second-generation Ortho HCV 2.0 EIA (Ortho Diagnostic Systems, Raritan, NJ).

HLA typing. DNA was extracted from established cell lines by phenol-chloroform extraction [11]. Class I molecular typing was...
done by use of the polymerase chain reaction sequence-specific primers (PCR-SSP), in which well-established primer pairs were used to amplify alleles [12]. Class II molecular typing was done by single-strand conformation polymorphism analysis in combination with PCR-SSP [13].

Statistical analysis. All analysis was done with SAS version 6.12 (SAS Institute, Cary, NC). The frequencies of the HLA class I and class II types and homozygosity were compared between those with persistent HBV infection and those who had recovered from infection (controls) by χ² analysis and Fisher’s exact test when indicated. To account for the problem of significant associations arising by chance when using multiple comparisons, Bonferroni’s correction was used. Odds ratios (ORs) were determined by conditional logistic regression and reflect the likelihood of carrying a specific allele if persistently HBV-infected. Homozygosity was defined as having identical alleles at either HLA-A, -B, or -C for class I alleles or at -DQA1, -DQB1, or -DRB1 for class II alleles.

Results

Of the 2921 subjects in the ALIVE cohort, 31 tested positive both at enrollment and in a sample collected at least 6 months after, thus representing HBV persistence. No subject with HBV persistence had detectable anti-HBs, and all were anti-HBc-positive. Two matched controls were identified for all but 2 subjects with HBV persistence, for whom we were able to find only 1 control, giving a total of 60 controls among 91 study subjects. Subjects with persistence and controls were similar with respect to age, race, sex, and seropositivity to HIV and HCV (table 1).

The distribution of HLA-A, -B, -DQA1, -DQB1, and -DRB1 allele frequencies in our controls was consistent with previous reports from African American populations [14, 15]. However, two class II alleles were detected more commonly in the subjects with persistent hepatitis B: DQA1*0501 (OR, 2.6; P = .05) and DQB1*0301 (OR, 3.9; P = .01) (table 2). These two alleles and a third, DRB1*1102, are in strong linkage disequilibrium, and their two- and three-locus haplotypes were also strongly associated with persistent HBV infection (OR, 3.0; P = .005; and OR, 10.7; P = .01, respectively). DRB1*1102 and a fourth class II allele, DRB5*0200, were themselves more prevalent in subjects with persistent HBV infection (OR, 12.2 and 6.5, respectively). However, we could not ascertain with a high scientific probability whether the associations with DRB1*1102 and DRB5*0200 occurred by chance (Bonferroni-corrected P > .05). The potential fraction of HBV persistence attributable to these alleles and haplotypes was 24.6% for DQA1*0501, 23.8% for DQB1*0301, 7.3% for DRB1*1102, 7.8% for the three-locus haplotype, and 18% for the two-locus haplotype. In addition, subjects homozygous at either DQA1, DQB1, or DRB1 were two times more likely to have a persistent infection (P = .09).

We found >2-fold associations with HBV persistence for eight class I alleles. Six were more common in subjects with persistence (HLA-A*2301, HLA-A*3402, HLA-B*4901, HLA-B*4501, HLA-B*4000, HLA-Cw*1701), whereas two were detected less often (HLA-B*1400 and HLA-Cw*0800). However, as with two of the class II alleles, chance associations could not be excluded. No association with homozygosity was detected for class I alleles.

Discussion

In this report, the first investigation of the genetic basis for HBV persistence in African Americans, significant associations were found with several class II alleles. Class II molecules present antigens to CD4+ T lymphocytes, which modulate the CD8+ CTL response and are crucial to the production of neutralizing antibodies. Since HBV clearance may be mediated by both eliminating infected cells through CTL and preventing infection of additional cells through antibody, it is plausible

Table 1. Demographic characteristics of subjects with persistent HBV infection and cleared infection (controls).

<table>
<thead>
<tr>
<th></th>
<th>Persistent HBV infection (n = 31)</th>
<th>Controls (n = 60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years) ± SD</td>
<td>31.2 ± 6.7</td>
<td>33.3 ± 5.6</td>
</tr>
<tr>
<td>Race (% African American)</td>
<td>93</td>
<td>97</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>87</td>
<td>87</td>
</tr>
<tr>
<td>HIV positive (%)</td>
<td>61</td>
<td>62</td>
</tr>
<tr>
<td>Hepatitis C virus antibody (%)</td>
<td>88</td>
<td>98</td>
</tr>
</tbody>
</table>

NOTE. P > .1 for all comparisons. Antibody to hepatitis C virus was measured in 26 cases and 57 controls.

Table 2. Class II alleles that predispose to persistent HBV infection: comparison of individuals with persistent HBV infection, controls, and New York City African Americans.

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Persistent HBV infection (n = 31)</th>
<th>Controls (n = 60)</th>
<th>NYC African Americans</th>
<th>Odds ratio</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQA1*0501</td>
<td>40</td>
<td>20</td>
<td>19</td>
<td>2.6</td>
<td>.05*</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>32</td>
<td>12</td>
<td>16</td>
<td>3.9</td>
<td>.01*</td>
</tr>
<tr>
<td>DRB1*1102</td>
<td>8.5</td>
<td>0.8</td>
<td>3</td>
<td>12.2</td>
<td>.06*</td>
</tr>
<tr>
<td>DQA1<em>0301, DQB1</em>0301, DRB1*1102</td>
<td>8.5</td>
<td>0.8</td>
<td>0.03</td>
<td>10.7</td>
<td>.01</td>
</tr>
<tr>
<td>DQA1<em>0501, DQB1</em>0301</td>
<td>27</td>
<td>11</td>
<td>NA</td>
<td>3</td>
<td>.005</td>
</tr>
</tbody>
</table>

NOTE. NA, not applicable.
* Bonferroni-corrected.
that differences in the efficiency of the class II interaction with HBV antigens might be important.

In this study, HBV persistence was associated with DQA1*0501, DQB1*0301, and the haplotypes that include these two alleles and a third, DRB1*1102. Too little is known about the relationship of allelic structure and presentation of HBV antigens to explain the biologic basis for these associations. In fact, because they occur in linkage disequilibrium, the independent role of each is difficult to assess. The strongest association was with DRB1*1102 and the three-locus haplotype that includes this allele. However, DRB1*1102 occurred infrequently and thus accounted for a small fraction of HBV persistence in this cohort. The more prevalent two-locus haplotype, DQA1*0501 and DQB1*0301, could explain up to 18% of HBV persistence in this study. Nonetheless, these data suggest that, in addition to major histocompatibility complex heterogeneity, other factors influence HBV persistence.

A similar result was reported from another study examining the distribution of class II alleles in subjects with adult-onset HBV infection. Among white patients in a liver referral center in Germany, DQA1*0501 was associated with HBV persistence [6] (data regarding DQB1 were not presented). In another study in The Gambia, where most infections are acquired in early childhood, Thursz et al. [5] did not find an increased frequency of DQA1*0501 or DQB1*0301 in persistently infected persons but did find an inverse association with DRB1*1302. DRB1*1302 was not highly associated with HBV clearance in our cohort (OR, 1.2; 95% confidence interval, 0.5–2.7).

Differences between the present study and that from The Gambia may be due to the age at onset of infection, which in West Africa is chiefly perinatal or occurs in early childhood but in the United States is uncommon before adulthood. Most infections acquired in infancy and childhood persist, whereas ~95% of adult-onset infection is self-limited [1]. Given the differences in the age at onset of infection in these two studies and the importance of age in persistence, differences in the findings of these two studies are not unexpected. There could also be genetic differences between Gambians and African Americans. In addition, type 2 error cannot be excluded, because the lower end of the 95% confidence interval for the OR in this study overlaps with the data from The Gambia, and a similar effect for DRB1*1301-2 was noted in the study from Germany [6].

Class I HLA alleles have not been associated with HBV persistence in other studies [5–8]. However, previous studies used serologic testing, which is generally less precise than molecular typing. In this study, molecular typing was used, and eight potential class I correlates were found.

It has been proposed that persons with greater HLA heterozygosity are advantaged because they have a greater repertoire of molecules with which to present foreign antigens [3]. In this study, class II heterozygotes had a 2-fold-lower rate of HBV persistence. This finding was also noted in the study from The Gambia [4].

There is a >5% probability that the class II heterozygote advantage and all of the class I allele associations found in this study are due to chance. However, because these associations are biologically plausible, they merit further study. Since most adults clear HBV infection, in this study ultimately only 31 subjects met the criteria for persistence, despite the size of the initial cohort. This underscores the need for large, multicohort investigations to elucidate the genetic epidemiology of infectious diseases, as was necessary to discover the association of CCR5 and CCR2 with HIV disease progression [16].

In conclusion, this study demonstrates the importance of the class II HLA alleles in the pathogenesis of HBV infection, since persistence was associated with specific alleles, their corresponding haplotypes, and homozygosity. Additional study is needed to validate these findings and to further explore the genetic pathogenesis of HBV infection.

References