Human Leukocyte Antigen Class II Alleles May Contribute to the Severity of Hepatitis C Virus–Related Liver Disease

Sophie Hué,1 Patrice Cacoub,1 Christophe Renou,6 Philippe Halfon,2 Vincent Thibault,1 Frédéric Charlotte,4 Magali Picon,7 Hervé Rifflet,8 Jean Charles Piette,1 Stanislas Pol,9 and Sophie Caillat-Zucman1

Whether the host's immune response genes influence the severity of hepatitis C virus (HCV) liver disease is controversial. Human leukocyte antigen (HLA) class II alleles were analyzed in 233 HCV RNA–positive patients with chronic active hepatitis (197 patients with Knodell index of fibrosis F0–F3 and 36 patients with index of F4). The 2 groups did not differ by sex, duration of infection, mode of contamination, alcohol consumption, or HCV genotype. Patients with cirrhosis were older than those without (56 ± 12 vs. 46 ± 14 years; P < 10−4) and had a lower DRB1*11 allele frequency (5.6% vs. 14.5%; P = .037), whereas DRB1*03 and DQB1*0201 frequencies appeared to be higher (DRB1*03, 18.1% vs. 9.6%; DQB1*0201, 37.5% vs. 23.4%; P = .04, corrected P value is not significant). Mean index of fibrosis was higher in DR3-positive than in DR11-positive patients (2.14 vs. 1.58; P = .05). By multivariate analysis, cirrhosis was associated with male sex and age >50 years. HLA class II alleles may weakly contribute to the severity of HCV liver disease. Of persons infected with HCV, only 15%–20% spontaneously clear the virus, and the rest become chronically infected.

Patients and Methods

Patients. From January 1996 to March 1998, 233 patients were prospectively enrolled in this study. All were white, living in France, and had chronic hepatitis C infection responding to the following criteria: elevated alanine aminotransferase (ALT) levels on ≥2 repeated tests, positive anti–HCV antibodies, positive HCV RNA on ≥2 repeated tests, and lack of coinfection by hepatitis B virus (hepatitis B surface antigen negative) or human immunodeficiency virus (HIV). Patients with other causes of chronic liver disease, such as alcohol-induced liver disease (>60 g/day) or autoimmune liver disease, were excluded.

Histologic status of the liver was assessed in all 233 patients by 2 independent observers who were not aware of the epidemiologic and virologic data. Results were expressed according to the Knodell histologic activity index. Patients were divided into 2 groups by stage of fibrosis. Group 1 was composed of 197 patients who were diagnosed histologically with chronic active hepatitis without cirrhosis (Knodell index of fibrosis, F0–F3). Group 2 was composed of 36 patients who were diagnosed histologically with cirrhosis (Knodell index of fibrosis, F4). No patient had hepatocellular carcinoma.

Among the 233 patients, 177 were subsequently treated with interferon (IFN)–α (ROFERON; Hoffman–La Roche) with doses of 3 × 108 U 3 times a week for ≥3 months. Responders (n = 93) were defined as having normalization of serologic activity of ALT and disappearance of serum HCV RNA within 3 months. The remaining patients (n = 84) were considered to be nonresponders (high serologic ALT activity and/or positive HCV RNA within 3 months).
**Virus testing.** The diagnosis of HCV infection was based on the detection of anti-HCV antibodies by third-generation ELISA (Ortho Diagnostic Systems). Serum HCV RNA was detected with a standardized reverse-transcription polymerase chain reaction assay by using the Amplicor HCV test (Roche Diagnostic Systems). HCV RNA quantification was determined by an amplification method that used the Amplicor HCV Monitor assay (Roche Diagnostic Systems). HCV genotype was determined in 179 patients by reverse hybridization assay (InnoLiPA HCV; Innogenetics). HCV genotype was determined in 179 patients by a reverse hybridization assay (InnoLiPA HCV; Innogenetics).

**HLA class II typing.** Genomic DNA was isolated from peripheral blood cells, and HLA class II DNA typing was done by hybridization with sequence-specific oligonucleotide probes following amplification of the second exon of the DRB1 and DQB1 genes by use of the InnoLipa HLA typing kit (Abbott). This test provides low-resolution genotyping for the major HLA-DRB1 and -DQB1 alleles.

**Statistical analysis.** HLA-DRB1 and -DQB1 allele and phenotype frequencies were determined by counting. Odds ratios (ORs) were estimated according to the Woolf formula. The level of significance was assessed in univariate analysis by using the \(\chi^2\) or Fisher’s exact test for comparisons of qualitative values or the unpaired Student’s \(t\) test for quantitative values. \(P < .05\) was considered to be significant, and the Bonferroni correction was applied for multiple tests by multiplying the \(P\) value by the number of alleles compared \((n = 25; \text{ i.e., } 13 \text{ for DRB1 and } 12 \text{ for DQB1})\) (corrected \(P\) value), except for DRB1*11, for which an association has already been reported. Differences in the means of continuous variables were assessed by the Student’s paired \(t\) test. We used the Mann-Whitney \(U\) test to compare nonparametric variables in independent samples.

Multivariate analysis was done by logistic regression. To assess the independent relationship between hepatic fibrosis and HLA frequencies, we started the multivariate analysis by including each individual HLA allele significantly different in patients with or without cirrhosis in univariate analysis adjusted for the objective criteria (age at time of study, sex, duration of infection, and genotype). Adjusted ORs and 95% confidence intervals were derived from the coefficient of the final multivariate logistic model. All analyses were performed with NCSS 6.0 statistical software (NCSS).

**Results**

**Patient characteristics.** Table 1 shows the main clinical and virologic characteristics of patients without or with cirrhosis. There was no significant difference in the sex distribution, source of HCV infection, duration of infection, and HCV genotype in the 2 patient groups. However, patients with cirrhosis were significantly older than those without cirrhosis \((56 \pm 12 \text{ vs. } 46 \pm 14 \text{ years}; \ P < 10^{-4})\).

**HLA associations with liver disease progression.** Table 2 presents the DRB1 and DQB1 allele and phenotype frequencies in patients with cirrhosis, compared with those patients with chronic active hepatitis without cirrhosis. The DRB1*11 allele frequency was lower in patients with cirrhosis than in those without \((5.6\% \text{ vs. } 14.5\%; \text{ OR, } 0.35; \ P = .037)\). Only 11.1% of patients with cirrhosis expressed the DRB1*11 phenotype, compared with 27.4% of those without cirrhosis. The frequency of the DQB1*0301 allele, which is expressed on most DR11 haplotypes in white French individuals, was not significantly different in patients with cirrhosis, compared with others \((12.5\% \text{ vs. } 21.6\%, \text{ respectively})\). Conversely, the DRB1*03 allele frequency was increased in patients with cirrhosis, compared with those without \((18.1\% \text{ vs. } 9.6\%; \text{ OR, } 2.1; \ P = .041)\). More than 30% of patients with cirrhosis expressed the DRB1*03 phenotype, compared with 16.7% of patients without. The frequency of the DQB1*02 allele, which is expressed on both DR3 and DR7 haplotypes, was also slightly higher in patients with than in those without cirrhosis \((37.5\% \text{ vs. } 23.4\%; \text{ OR, } 1.97; \ P = .012)\). However, none of these associations remained significant when the Bonferroni correction was applied. There was no heterozygote advantage for HLA class II alleles: 24 patients with cirrhosis, versus 6 patients with cirrhosis, were homo-
Table 2. HLA-DRB1 and -DQB1 allele and phenotype frequencies in patients positive for the hepatitis C virus with or without cirrhosis.

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>Allele frequency</th>
<th>Chronic active hepatitis (2n = 394)</th>
<th>Cirrhosis (2n = 72)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
<th>Chronic active hepatitis (n = 197)</th>
<th>Cirrhosis (n = 36)</th>
<th>Odds ratio (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Chronic active</td>
<td>Cirrhosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>hepatitis</td>
<td>(n = 197)</td>
<td></td>
<td></td>
<td></td>
<td>(n = 36)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*01</td>
<td></td>
<td>41 (10.4)</td>
<td>6 (8.3)</td>
<td>39 (19.8)</td>
<td>.04</td>
<td></td>
<td>4 (11.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*03</td>
<td></td>
<td>38 (9.6)</td>
<td>13 (18.1)</td>
<td>2.1 (1.1–4.1)</td>
<td>.04</td>
<td></td>
<td>33 (16.7)</td>
<td>11 (30.5)</td>
<td>.02</td>
</tr>
<tr>
<td>DRB1*04</td>
<td></td>
<td>51 (12.9)</td>
<td>9 (12.5)</td>
<td>47 (23.8)</td>
<td>.04</td>
<td></td>
<td>52 (26.4)</td>
<td>14 (38.9)</td>
<td>.02</td>
</tr>
<tr>
<td>DRB1*07</td>
<td></td>
<td>54 (13.7)</td>
<td>14 (19.4)</td>
<td>2.1 (1.1–4.1)</td>
<td>.04</td>
<td></td>
<td>2 (1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*08</td>
<td></td>
<td>11 (2.8)</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*09</td>
<td></td>
<td>2 (0.5)</td>
<td>0</td>
<td>6 (3)</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*10</td>
<td></td>
<td>6 (1.5)</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td>13 (3.3)</td>
<td>1 (2.8)</td>
<td>.02</td>
</tr>
<tr>
<td>DRB1*11</td>
<td></td>
<td>7 (1.8)</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*12</td>
<td></td>
<td>2 (0.5)</td>
<td>0</td>
<td>2 (1)</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*13</td>
<td></td>
<td>61 (15.5)</td>
<td>9 (12.5)</td>
<td>64 (34.1)</td>
<td>.02</td>
<td></td>
<td>81 (41.1)</td>
<td>21 (58.3)</td>
<td>.02</td>
</tr>
<tr>
<td>DRB1*14</td>
<td></td>
<td>14 (3.6)</td>
<td>4 (5.6)</td>
<td>16 (8.6)</td>
<td>.02</td>
<td></td>
<td>4 (11.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*15</td>
<td></td>
<td>35 (9.1)</td>
<td>7 (11.1)</td>
<td>34 (18.4)</td>
<td>.02</td>
<td></td>
<td>84 (44.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DRB1*16</td>
<td></td>
<td>4 (1.0)</td>
<td>0</td>
<td>4 (1.1)</td>
<td></td>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(DR2)</td>
<td></td>
<td>13 (3.3)</td>
<td>1 (1.4)</td>
<td>16 (8.8)</td>
<td>.02</td>
<td></td>
<td>16 (8.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DQB1*0201</td>
<td></td>
<td>92 (23.4)</td>
<td>27 (37.5)</td>
<td>1.97 (1.2–3.3)</td>
<td>.02</td>
<td></td>
<td>81 (41.1)</td>
<td>21 (58.3)</td>
<td>.02</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td></td>
<td>85 (21.6)</td>
<td>9 (12.5)</td>
<td>16 (8.8)</td>
<td>.02</td>
<td></td>
<td>84 (44.1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Only risks with a significant uncorrected P value are shown. CI, confidence interval.

* Corrected P value is not significant.

b In some patients, the DR2 subtype (DR15 or DR16) could not be determined.

mozygous for HLA-DRB1 alleles (13.9% vs. 20%; P = .43). There was no difference in HLA-DR or -DQ phenotype distribution by HCV genotype.

When patients were stratified by the Knodell index of fibrosis, the fraction of DRB1*03-positive patients increased progressively from group F0 to group F4: 9.8%, 20%, 18%, and 30.5%, respectively (χ² for trend, 4.46; P = .034) vs. F0, respectively). By contrast, the frequency of DRB1*11-positive patients was comparable in groups F0 (23.5%), F1 (30.6%), and F3 (26.2%) and only decreased in the last group, F4 (11.1%; P = .037) vs. F0, F1, and F3). Therefore, if patients were divided by mild (Knodell index of fibrosis, <3) versus severe (>3) histologic injury, the frequencies of DRB1*03 (16.2% vs. 22.7%) or DRB1*11 (27.9% vs. 20.6%) were not significantly different.

When patients were compared by DR phenotype, the mean Knodell index of fibrosis was significantly higher in DR3-positive (2.1 ± 1.5) than in DR3-negative patients (1.7 ± 1.4; P = .05) or in DR11-positive patients (1.58 ± 1.3; P = .05). The mean total Knodell index was similar in the different groups (7.5 ± 3.5 vs. 6.8 ± 3.5). Age, sex, duration of infection, and HCV genotype did not differ between DR3-positive and DR11-positive patients. In multivariate analysis, the presence of cirrhosis (but not absence of cirrhosis) was associated with male sex and age > 50 years (P < .001) but not with any DRB1 or DQB1 allele.

Response to treatment with IFN. There was no significant difference between responders and nonresponders to IFN by age, sex, duration and source of infection, HCV genotype distribution, and Knodell activity index. There was also no association between IFN response and HLA class II alleles. DRB1*03 frequency was 11.9% in nonresponders and 11.1% in responders, and DRB1*11 frequency was 13.7% in nonresponders and 13.9% in responders.

Discussion

Primary HCV infection can run either a limited persistent course or a severe chronic outcome. The DRB1*11 and/or DQB1*0301 alleles have been reproducibly involved in spontaneous HCV clearance in different European populations [2–6], although, in Irish women exposed to HCV genotype 1b from a single inoculum, viral clearance was associated with the DRB1*0101 and DQB1*0501 alleles [7–9]. Differences in the strength of association depending on ethnicity have been described, and the DQB1*0301 effect is stronger in black than in white subjects [14]. In addition, HLA class II alleles influence the variance in virus load over time in persons infected with HCV1b [15].

Association of HLA alleles with HCV liver disease progression in chronically infected patients is more controversial. Either no allele has been associated with accelerated progression to cirrhosis [3, 4, 7] or results have been controversial, and, thus, a clear pattern of how HLA influences disease progression has not emerged [2, 5, 8–13]. Our study confirms and extends, in a large series of well-characterized HCV chronically infected patients, the protective role of DRB1*11 on progression of liver disease. DRB1*11 was a protective factor against a severe outcome of chronic HCV infection in different groups [8, 10, 13]. By contrast, Thursz et al. [4] did not show any HLA difference...
between patients with mild (Knodell index of fibrosis, <3) and severe (≥3) histologic injury in very large study groups recruited from 8 European countries. Of note, we only observed HLA differences when we compared patients with extreme manifestations of the disease (patients with or without cirrhosis) but not when we stratified the patients by presence of mild versus severe fibrosis. Indeed, analysis of persons who represent the extremes of severe progression and nonprogression increases the strength of the signal studied as we observed in HIV-infected patients [16]. Thus, reanalysis of patients from the series of Thursz et al. [4] might provide complementary information.

In addition to the protective effect of DRB1*11 on progression of liver disease, we suggest a potential role of the DRB1*03 and DQB1*0201 alleles on progression to HCV cirrhosis. This is in accordance with the previously reported association of the DRB1*03-DQB1*0201 haplotype with viral persistence in white patients [9, 13]. However, this effect is weak and needs to be validated in future studies.

What are the mechanisms by which HLA class II molecules may modulate the course of HCV liver disease? Because HCV is not cytopathic for the infected cells, the immune response may play an important role in the hepatocellular damage during HCV infection. Binding of HCV peptides to HLA molecules is the first step for the initiation of this immune response. Because of the extensive polymorphism of HLA, the strength of the response will vary among individuals. The DRB1*11 and DQB1*0301 alleles may present immunogenic HCV antigens in a configuration that could be effectively recognized by helper T cells. This would explain how DRB1*11, together with other factors, favors self-limiting infection. In subjects who do not clear the virus, DRB1*11 might also be critical to limiting the spread of the virus within the infected host and for keeping HCV liver injury under control. Of interest, CD4-positive T cell epitopes have been characterized in patients with self-limited hepatitis, and most immunodominant epitopes were presented in the context of DRB1*11 or DQB1*0301 molecules [17]. Identification of protective HLA alleles might thus provide clues to the development of vaccine. One must keep in mind, however, that even if HLA plays a role in HCV-related liver fibrosis, the role is much weaker than the 2 major factors—advanced age and male sex.

Acknowledgment

We thank Sylvie Dantin (Roche) for helpful contributions.

References