HLA Class II Alleles and Chronic Hepatitis C Virus Infection


*Viral Hepatitis Division, Instituto AllA de Gastroenterologia, Hospital das Clínicas/UFMG, Belo Horizonte, Minas Gerais, Brazil; †Internal Medicine Department, School of Medicine, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; ‡Immunogenetics Division, Pediatrics Department, Escola Paulista de Medicina, Federal University of São Paulo, São Paulo, São Paulo, Brazil; †Instituto de Ciências Exatas, ICEX, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; and ‡Laboratório de Biomarcadores de Diagnóstico e Monitoração, Centro de Pesquisas René Rachou, Fundação Oswaldo Cruz, Belo Horizonte, Minas Gerais, Brazil

Abstract

The aim of this study was to investigate association of human leucocyte antigens (HLA)-DRB1 and DQB1 polymorphisms with hepatitis C virus (HCV) infection and with the occurrence of severe liver fibrosis/cirrhosis in chronically infected patients. Ninety-nine white patients, from southeast Brazil, with confirmed HCV chronic infection were included in the study. Severe fibrosis/cirrhosis (METAVIR scores F3–F4) was present in 49 patients. HLA-DRB1 specificities and DRB1*11 and DQB1*03 alleles were determined by PCR-SSP, and their frequencies were compared between patients and a control group of 108 healthy Brazilian individuals. The results confirmed previous reports of the association of DRB1*11 and DQB1*03 with protection from chronic HCV infection, but did not confirm their association with protection from severe fibrosis/cirrhosis. Furthermore, the results suggested that the polymorphic sites on HLA molecules responsible for protection from chronic HCV infection are encoded not only by the DRB1*1101 and DQB1*0301, as suggested in the literature, but also by other DRB1*11 and DQB1*03 alleles. Thus, we hypothesized that the common polymorphic residues shared by different DRB1*11 and/or DQB1*03 alleles might be responsible for selection of viral epitopes for presentation to CD4+ T cells, leading to an efficient immune response against the virus.

Introduction

Hepatitis C virus (HCV) infection is estimated to affect 170 million people, corresponding to 3% of the world population, with prevalence in different countries ranging from <1 to more than 10% [1, 2].

Outcomes of HCV infection vary widely, from asymptomatic clearance, which occurs in only about 20% of cases with acute infection, to chronic infection that may lead to complications including chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). It has been estimated that chronic HCV infection accounts for 27% of cirrhosis and 25% of HCC worldwide, and it is the major cause of liver transplantation in Europe and in the USA. Factors influencing the rate of progression of chronic hepatitis C to cirrhosis and liver cancer include alcohol abuse, duration of the infection, and, possibly, HCV viral load. In addition, viral genotype, co-infection with another type of hepatitis virus, co-infection with the human immunodeficiency virus (HIV), and male gender play also a role in the progression of disease [2, 4, 5].

The pathogenetic mechanisms for liver damage in chronic hepatitis C are not completely elucidated, but there is strong evidence that host cellular immune response is involved in the control of viral replication and contributes to hepatocellular damage. As HCV infection persists, continuous liver damage and regeneration, together with enhanced fibrogenesis, may eventually lead to cirrhosis in a proportion of patients [4–7].

The clearance of acute infection is accompanied by strong CD4+ and CD8+ T cell responses against numerous HCV-derived antigens, and there is evidence that efficacy of this immune response is influenced by the host human leucocyte antigens (HLA) molecules that present...
HLA typing. Genomic DNA was extracted from peripheral blood cells using a commercial DNA isolation kit (QiAmp DNA Mini kit; Qiagen, Hilden, Germany), according to manufacturer instructions. HLA-DRB1 (DRB1*11, DRB1*12, DRB1*14, and DRB1*16) specificities (low-resolution typing) and DRB1*11 and DQB1* alleles (high-resolution typing) were determined by PCR-SSP using commercial kits (One Lambda Inc, Canoga Park, CA, USA), according to manufacturer instructions.

Statistics. All comparisons were performed by the two-sided Fisher's exact test, except those involving HLA-DRB1*11, DRB1*12, DRB1*14, and DQB1*03, for which one-sided tests were performed, considering that these associations have been repeatedly reported in the literature. The significance level was set at \( P = 0.05 \).

Results

HLA-DRB1 and HLA-DQB1 polymorphisms and chronic HCV infection

The frequency of DRB1*11 was lower among infected individuals than in the control group [11.1% versus 21.4%, one-sided \( P = 0.04 \), OR (odds ratio) = 0.46 CI 95%: 0.79-0.99]. Lower frequencies of DRB1*11, DRB1*12, and DRB1*14 (4.8% versus 7.8%, OR = 0.7) were observed, but the differences did not reach statistical significance (Tables 2 and 3).

The frequency of DQB1*03 was also lower among infected individuals than in the control group (36.5% versus 50.5%, one-sided \( P = 0.03 \), OR = 0.56). The frequency of DQB1*03 was lower in patients than in controls but none of these differences were statistically significant. The frequency of

| Table 3 HLA-DRB1*11 alleles frequencies (%) in 99 white Brazilian patients with chronic hepatitis C virus infection and in 103 ethnically matched healthy controls. |
|-----------------|-----------------|-----------------|
| HLA-DRB1*11 alleles | Patients | Controls |
| DRB1*1101 | 7.1 | 12.6 |
| DRB1*1102 | 1.0 | 1.0 |
| DRB1*1103 | 0 | 0 |
| DRB1*1104 | 4.0 | 7.8 |

Table 4 HLA-DQB1 alleles frequencies (%) in 99 white Brazilian patients with chronic hepatitis C virus infection and in 103 ethnically matched healthy controls.

<table>
<thead>
<tr>
<th>HLA-DQB1 alleles</th>
<th>Controls</th>
<th>All Patients</th>
<th>Patients with METAVIR scores</th>
<th>Patients with METAVIR scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 103)</td>
<td>(n = 99)</td>
<td>F0 F2 (n = 30)</td>
<td>F3 F4 (n = 99)</td>
</tr>
<tr>
<td>DQB1*02</td>
<td>2.9</td>
<td>15.9</td>
<td>32.5</td>
<td>45.8</td>
</tr>
<tr>
<td>DQB1*0201</td>
<td>1.1</td>
<td>11.2</td>
<td>20.8</td>
<td>28.8</td>
</tr>
<tr>
<td>DQB1*0202</td>
<td>5.6</td>
<td>25.0</td>
<td>25.0</td>
<td>27.1</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>DQB1*0302</td>
<td>2.0</td>
<td>16.2</td>
<td>24.7</td>
<td>35.8</td>
</tr>
<tr>
<td>DQB1*0601</td>
<td>1.2</td>
<td>3.1</td>
<td>21.2</td>
<td>31.3</td>
</tr>
<tr>
<td>DQB1*0604</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

HLA, human leukocyte antigen.

\* \( P < 0.05 \) (one-sided Fisher's exact test) in the comparison with the controls.

\* \( P < 0.05 \) (two-sided Fisher's exact test) in the comparison with the controls.

© 2011 The Authors

Scandinavian Journal of Immunology © 2011 Blackwell Publishing Ltd. Scandinavian Journal of Immunology 74, 282–287
DQ81*0501 was higher among infected individuals (34.4% versus 20.4%, two-sided P = 0.04, OR = 2.04). The frequencies of other DQB1* alleles did not differ between patients and controls (Table 4). In three patients, genotyping was not possible because of insufficient DNA.

HLA-DRB1 and HLA-DQ81 polymorphisms and the stage of liver fibrosis in patients with HCV chronic infection

The comparison of frequencies of DRB1 and DQB1 polymorphisms between 48 patients with severe fibrosis/ cirrhosis (META VIR stages F3–F4) and 48 without or with only mild fibrosis (META VIR stages F0–F2) did not reveal any significant difference.

Discussion

The purpose of this study, conducted in a white population from southeast of Brazil, was to investigate the influence of HLA class II polymorphisms on the resistance to HCV infection and also to assess the relationship between these polymorphisms and the degree of liver fibrosis in chronically infected patients. The control population was represented by white Brazilian individuals from the general population of the same geographical area and not necessarily tested for HCV infection. We do not believe that the potential occurrence of some HCV infected subjects among the controls would affect our results, because the prevalence of anti-HCV antibody positive individuals in the southeast and southern regions of Brazil has been estimated to be lower than 1.5% [46, 47].

The results observed in this study confirmed previous findings of other authors regarding the resistance to chronic HCV infection conferred by DRB1*11 [11, 13, 15–17, 20, 21, 27, 28, 30, 31, 36–38], and DQB1*03 specificities [12–15, 17, 20, 21, 23, 26, 27, 36–38]. These genes are in tight linkage disequilibrium, and a strong association between the haplotype DRB1*1101, DQB1*0301 and maintenance of a multispecific CD4+ T helper response that conferred protection against HCV infection has been observed [27].

Concerning which DRB1*11 allele would be associated with protection, most of the studies have found DRB1*1101, which is the most common DRB1*11 allele in Caucasian populations and is in linkage disequilibrium with DQB1*0301. In the present study, we observed lower frequencies of not only DRB1*1101, but also of DRB1*1104, an allele that has also been reported to be associated with protection and that is also in linkage disequilibrium with DQB1*0301 [15, 18, 21]. The association with both DRB1*1101 and DRB1*1104 could suggest that the resistance to HCV infection conferred by HLA-DRB1*11 is because of polymorphic sites common to molecules encoded by DRB1*1101 and DRB1*1104 or simply could be reflecting the fact that both these alleles are in linkage disequilibrium with DQB1*0301. One argument in favour of the primary association with DQB1*03 is that one study found DQB1*0301 [14] and another one reported association with DQB1*0302 [12], without identifying DRB1*11 as a relevant factor.

Concerning the association of DQB1*03 alleles with protection from chronic HCV infection, we observed that not only DQB1*0301, but also DQB1*0302 and DQB1*0303 presented lower frequencies in patients than in controls. None of these differences reached statistical significance, probably because of the low number of subjects. Of note is that, as mentioned earlier, the association with DQB1*0302 has already been described in Northern European Caucasians [12]. Taking into consideration these findings, we suggest that the residues in HLA-DQ beta molecules responsible for protection are present not only in molecules encoded by DQB1*0301 but also by DQB1*0302 and DQB1*0303, which could be relevant in the field of vaccine development.

The increased frequency of DQB1*0301 in patients was of borderline statistical significance (P = 0.04, without correction for multiple comparisons) and need to be validated in future studies. This finding is in contrast with reports of association of DQB1*0301 with protection from HCV infection reported in two studies [24, 26] and with the lack of any kind of association observed in most of the published studies.

Association of HLA alleles with liver disease progression in patients with chronic hepatitis C is controversial, mainly regarding the protection of severe fibrosis/cirrhosis associated with DRB1*11 and/or DQB1*03, found by some [25, 28, 29, 31, 42] but not by other [16, 18, 20, 21] authors. In the present study, the frequency of DRB1*11 in patients without or with only mild fibrosis (META VIR scores F0–F2) and with severe fibrosis/cirrhosis (META VIR scores F3–F4) were essentially the same (12.2% versus 10%). No significant differences between these two groups were observed for DQB1*03 or DQB1*0301 frequencies, although higher frequencies were observed in patients with F0–F2 scores. In conclusion, our data do not corroborate findings of other authors regarding protection from evolution to more severe liver fibrosis conferred by DRB1*11 but do not rule out a possible protective role of DQB1*03 (DQB1*0301).

We believe that the main contribution of our study to the complex issue of HLA associations with HCV infection outcomes is that the results suggested that the polymorphic sites on HLA molecules responsible for protection from chronic infection are encoded not only by the DRB1*1101 and DQB1*0301 alleles but also by other DRB1*11 and DQB1*03 alleles. Our hypothesis is
that these common polymorphic residues, particularly those shared by DQB1*03 alleles, are responsible for the selection of particular viral epitopes to be presented to CD4+ T cells, leading to an efficient immune response against the virus. This idea should be further tested, as it has implications in the context of vaccine development.

Acknowledgment

This work was supported by grants from the Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Associação Fundo de Incentivo à Psicofarmacologia (AFIP). We thank the patients and controls who volunteered to participate in the study.

References


© 2011 The Authors
Scandinavian Journal of Immunology © 2011 Blackwell Publishing Ltd. Scandinavian Journal of Immunology 74, 282–287
47 Yu RB, Heng X, Ding WL et al. The association between the genetic polymorphism of HLA-DQA1, DQB1, and DRB1 and serum alanine aminotransferase levels in chronic hepatitis C in the Chinese populations. *J Gastroenterol Hepatol* 2008;23:1391-402.