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HLA Class II Alleles and Chronic Hepatitis C Virus Infection

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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** alleles were determined by PCR-SSP, and their frequencies were compared between patients and a control group of 103 healthy white 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*1–16*) 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*1101*, *DRB1*1104*, *DQB1*03* and *DQB1*0301*, 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% 0.00–0.95]. Lower frequencies of *DRB1*1101* (7.15 versus 12.6%, OR = 0.52) and *DRB1*1104* (4.0% versus 7.8%, OR = 0.47) alleles 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 frequencies of the alleles *DQB1*0301*, **0302* and **0303* were lower in patients than in controls but none of these differences were statistically significant. The frequency of

Table 2 HLA-*DRB1* specificities frequencies (%) in 99 white Brazilian patients with chronic hepatitis C virus infection and in 103 ethnically matched healthy controls.

HLA *DRB1*	Controls (*n* 103)	All Patients (*n* 99)	Patients with METAVIR scores F0 F2 (*n* 50)	Patients with METAVIR scores F3 F4 (*n* 49)
*DRB1*01*	27.1	29.3	34.0	24.5
*DRB1*03*	21.3	20.2	20.0	20.4
*DRB1*04*	19.3	17.2	22.0	12.2
*DRB1*07*	26.2	27.3	26.0	28.6
*DRB1*08*	9.7	13.1	12.0	14.3
*DRB1*09*	1.9	4.0	6.0	2.0
*DRB1*10*	0	4.0	8.0	0.0
*DRB1*11*	21.4	11.1*	12.0	10.2
*DRB1*12*	1.9	3.0	2.0	4.1
*DRB1*13*	23.3	30.3	26.0	34.7
*DRB1*14*	9.7	5.0	0.0	10.2
*DRB1*15*	22.3	20.2	16.0	24.5
*DRB1*16*	6.8	4.0	4.0	4.1

HLA, human leucocyte antigens.
*$P < 0.05$ (one-sided Fisher's exact test) in the comparison with the controls.

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

HLA, human leucocyte antigens.
No difference reached statistical significance.

Table 4 HLA-*DQB1* alleles frequencies (%) in 96 white Brazilian patients with chronic hepatitis C virus infection and in 103 ethnically matched healthy controls.

HLA-*DQB1*	Controls (*n* 103)	All Patients (*n* 96)	Patients with METAVIR scores F0 F2 (*n* 48)	Patients with METAVIR scores F3 F4 (*n* 48)
*DQB1*02*	36.9	44.8	45.8	43.8
*DQB1*0201*	11.6	18.8	20.8	16.7
*DQB1*0202*	25.2	26.0	25.0	27.1
*DQB1*0203*	0.0	0.0	0.0	0.0
*DQB1*03*	50.5	36.5*	43.7	29.2
*DQB1*0301*	23.3	17.7	20.8	14.6
*DQB1*0302*	17.5	12.5	14.6	10.4
*DQB1*0303*	9.7	5.2	6.2	4.2
*DQB1*0304*	0.0	1.0	2.1	0.0
*DQB1*0305* *0312*	0.0	0.0	0.0	0.0
*DQB1*04*	7.8	15.6	16.7	14.6
*DQB1*0401*	1.0	0.0	0.0	0.0
*DQB1*0402*	6.8	15.6	16.7	14.6
*DQB1*05*	41.7	42.7	45.8%	39.6
*DQB1*0501*	20.4	34.4**	43.8	25.0
*DQB1*0502*	9.7	3.1	2.1	4.2
*DQB1*0503*	9.7	5.2	0.0	10.4
*DQB1*0504*	1.9	0.0	0.0	0.0
*DQB1*06*	40.8	53.1	47.9	58.3
*DQB1*0601*	2.9	1.0	2.1	0.0
*DQB1*0602*	13.6	19.8	16.7	22.9
*DQB1*0603*	14.6	21.9	25.0	18.7
*DQB1*0604*	13.6	7.3	0.0	14.6
*DQB1*0605*	1.9	0.0	0.0	0.0
*DQB1*0606* **0608*	0.0	0.0	0.0	0.0
*DQB1*0609*	3.9	3.1	4.2	2.1
*DQB1*0610* *0620*	0.0	0.0	0.0	0.0

HLA, human leucocyte antigens.
*$P < 0.05$ (one-sided Fisher's exact test).
**$P < 0.05$ (two-sided Fisher's exact test) in the comparison with the controls.

*DQB1*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-*DQB1* 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 (METAVIR stages F3–F4) and 48 without or with only mild fibrosis (METAVIR 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*0501* 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*0501* 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 (METAVIR scores F0–F2) and with severe fibrosis/cirrhosis (METAVIR 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*3* (*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.

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