

Racial Differences in HLA Class II Associations with Hepatitis C Virus Outcomes

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A broad, vigorous CD4 T cell response, mediated by class II human leukocyte antigens (HLAs), favors hepatitis C virus (HCV) clearance. *HLA-DQB1*0301* has been associated with viral clearance in an ethnically homogeneous cohort. To validate this association and to identify other class II associations in an ethnically varied cohort, molecular class II HLA typing was performed on 200 HCV clearance and 374 matched persistently infected subjects. *HLA-DQB1*0301* was weakly associated with viral clearance in combined ethnic groups (odds ratio [OR], 0.72; 95% confidence interval [CI], 0.53–0.97) but was stronger in black subjects. In white subjects, viral clearance was associated with *DRB1*0101* (OR, 0.32; 95% CI, 0.17–0.60) and its *DQB1*0501* haplotype, whereas viral persistence was associated with *DRB1*0301* (OR, 2.36; 95% CI, 1.23–4.52) and its *DQB1*0201* haplotype. These results support a role for class II alleles in the immune response to HCV and underscore the importance of studying genetic associations in an ethnically diverse cohort.

Of persons acutely infected with hepatitis C virus (HCV), $\leq 85\%$ have viral persistence and the rest successfully clear the virus [1, 2]. Although HCV genetic diversity is associated with viral persistence [3], a number of studies indicate that host factors also are important. HCV clearance occurs less often in blacks and in human immunodeficiency virus (HIV)–infected

persons [4]. In addition, there are differences in the outcome of infection that follow a common source of the virus. For example, in an Irish cohort, 704 women were infected with HCV from contaminated anti-D immune globulin (Rhogam), 390 (55%) of whom became persistently infected [5]. In a subset of a similar cohort in Germany of women infected by contaminated Rhogam, 47.2% had viral persistence [6].

Human leukocyte antigens (HLAs) are among the most integral components of the immune system. HLAs are encoded by the most polymorphic genes known in vertebrates. The HLA molecules are divided into 2 groups, classes I and II, and present foreign antigen to CD8 cytolytic T cells and to CD4 helper T cells, respectively. Studies of early HCV infection suggest that a vigorous CD4 T cell response is associated with viral clearance [7]. Thus, a strong and broad class II–restricted T helper response is especially important in an acute HCV infection. Therefore, it is plausible that certain class II antigens present HCV epitopes more effectively to CD4 helper T cells than do others. This hypothesis has been examined, but many of the results are in conflict. In a multicohort European study, an association was detected between the class II allele *DQB1*0301* and viral clearance, which was duplicated in some, but not all, studies [8–13]. Other associations also have been reported but not reproduced [12–15]. These inconsistencies could be due to the small size of some studies, ethnic differences, or HCV genetic variability, thus emphasizing the need for large, multicenter studies in which ethnic-dependent variation is considered and other variables are controlled.

The association of class II antigens with HCV outcomes has not been investigated in North America and has never been

Received 26 January 2001; revised 16 March 2001; electronically published 30 May 2001.

Presented in part: 51st annual meeting of the American Association for the Study of Liver Diseases, Dallas, October 2000 (abstract 432).

Informed consent was obtained from all patients, and the study was approved by the Institutional Review Board at Johns Hopkins University and by individual study cohorts.

Financial support: National Institutes of Health (NIH; grants DA-00441, DA-04334, and DA-13324; HD-43200; contract CO-56000); Bureau of Maternal and Child Health and Resources Development (MCJ-060570), NIH (HD-4-3200), Centers for Disease Control and Prevention, and National Institute of Mental Health (to Hemophilia Growth and Development Study); NIH grants to New York Hospital–Cornell Medical Center Clinical Research Center (RR-06020), Mount Sinai General Clinical Research Center, New York (RR-00071), University of Iowa Clinical Research Center (RR-00059), and University of Texas Health Science Center, Houston (RR-02558); National Cancer Institute (contract CP-33002 with Research Triangle Institute to Multicenter Hemophilia Cohort Study).

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For published allele frequencies determined in ethnic groups, please refer to [21].

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The Journal of Infectious Diseases 2001;184:16–21

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0022-1899/2001/18401-0003\$02.00

pursued in large cohorts with >1 ethnic group. Here, we characterized HCV clearance and persistence in 3 independent multiethnic cohorts. By using these combined cohorts, we sought to confirm the association found in Europe with *DQB1*0301* and to determine whether other class II alleles are important factors in determining HCV outcomes.

Subjects and Methods

Study subjects. Subjects in this study were participants in one of 3 studies: (1) AIDS Link to Intravenous Experience (ALIVE), an ongoing study of 2921 injection drug users (IDUs) enrolled in Baltimore from February 1988 through March 1989, as described elsewhere [16]; (2) Multicenter Hemophilia Cohort Study, a prospectively followed-up cohort of patients with hemophilia, von Willebrand's disease, or a related coagulation disorder from 16 comprehensive hemophilia treatment centers enrolled from 1982 through 1996, as described elsewhere [17]; and (3) Hemophilia Growth and Development Study, a continuing study of 333 children and adolescents with hemophilia who were enrolled from March 1989 through May 1990, as described elsewhere [18]. A nested case-control design was used. Case subjects had cleared viremia, as demonstrated by ≥ 2 instances separated by a minimum of 6 months, in which HCV RNA could not be detected in serum. Prior infection was substantiated by detection of HCV antibody (anti-HCV). Persistently infected control subjects selected from the same cohort had anti-HCV and HCV RNA in serum for ≥ 6 months. Control subjects were matched 2:1 to case subjects in the same cohort on the basis of HIV status, sex, geographic location (if applicable), and race. These factors were chosen since HIV status and race are determinants of viral clearance in the ALIVE cohort [4].

Serologic testing. Subjects who tested positive for anti-HCV by a second generation HCV EIA (HCV 2.0 EIA; Ortho Diagnostic Systems) had 2 samples, separated by ≥ 6 months, assessed for HCV RNA by a branched DNA (bDNA) assay (Quantiplex HCV RNA 2.0 assay; Chiron). Subjects with 2 samples below the limit of detection by bDNA had ≥ 1 of those samples retested with the HCV COBAS Amplicor system (Roche Diagnostics) and their antibody status confirmed by a recombinant immunoblot assay (RIBA 3.0; Chiron). Only subjects with a negative HCV RNA confirmed by COBAS were defined as clearance cases. Despite ongoing exposure in some IDUs, viral clearance by this definition appeared to be stable. Subjects with 2 positive bDNA assays were eligible to be matched to the case subjects as control subjects.

HIV-1 testing was done by EIA, and positive specimens were confirmed by means of Western blot, as reported elsewhere [16–18]. Hepatitis B surface antigen (HBsAg) status was determined by use of EIA (AUSZYME; Abbott Laboratories). All assays were done according to the manufacturer's specifications, with the exception of COBAS testing in samples that contained heparin. These samples were treated with heparinase before COBAS testing by using a Roche protocol for sample preparation of heparinized plasma. All samples used for testing had been stored at -70°C after processing and had not been used for other assays.

HLA typing. An Epstein-Barr virus-transformed cell line was established for each subject, and DNA was extracted from these cell lines by phenol-chloroform extraction. Class II molecular typ-

ing was done by polymerase chain reaction (PCR) single-stranded conformational polymorphism analysis [19], in combination with PCR sequence-specific primers [20].

Statistical analysis. All analyses were done with SAS version 6.12 software (SAS Institute). The frequencies of HLA class II alleles at *DQA1*, *DQB1*, and *DRB1* and homozygosity were compared between subjects who cleared HCV infection and those who had a persistent infection. Homozygosity was defined as having identical alleles at *DQA1*, *DQB1*, or *DRB1*. Odds ratios (ORs) were determined by conditional logistic regression and reflect the likelihood of being persistently HCV infected if carrying a specific allele. To account for the problem of significant associations arising by chance when multiple comparisons are made, we used the Bonferroni correction, which accounted for all alleles tested ($n = 40$). This correction was not applied to *DQB1*0301*, since it was tested before the multiple comparisons. Alleles that had a frequency $>5\%$ and either an uncorrected $P \leq .10$ or an OR >2 or <0.5 also were stratified by race for analysis ($n = 5$). We focused on white and

Table 1. Alleles examined in this investigation.

Allele	Frequency of clearance, % (n = 400)	Frequency of persistence, % (n = 148)	Odds ratio	P
<i>DQA1*0101</i>	17.0	14.5	0.84	.31
<i>DQA1*0102</i>	19.3	22.1	1.16	.35
<i>DQA1*0103</i>	3.5	4.8	1.38	.32
<i>DQA1*0201</i>	13.8	11.8	0.82	.28
<i>DQA1*0300</i>	15.3	12.5	0.79	.19
<i>DQA1*0400</i>	7.0	7.9	1.19	.47
<i>DQA1*0501</i>	23.1	26.2	1.20	.21
<i>DQA1*0601</i>	0.5	0.1	0.25	.26
<i>DQB1*0201</i>	21.2	25.0	1.24	.16
<i>DQB1*0301</i>	24.5	18.9	0.72	.03
<i>DQB1*0302</i>	6.5	6.7	1.10	.72
<i>DQB1*0303</i>	3.4	1.8	0.49	.08
<i>DQB1*0402</i>	5.9	6.7	1.20	.50
<i>DQB1*0501</i>	17.8	12.0	0.65	.01
<i>DQB1*0502</i>	2.1	2.4	1.11	.81
<i>DQB1*0503</i>	0.8	3.4	4.19	.02
<i>DQB1*0601</i>	0.8	0.4	0.38	.29
<i>DQB1*0602</i>	12.4	15.1	1.24	.26
<i>DQB1*0603</i>	3.1	4.3	1.36	.38
<i>DQB1*0604</i>	1.3	2.3	1.70	.31
<i>DRB1*0101</i>	10.1	4.7	0.45	.001
<i>DRB1*0102</i>	3.5	3.0	0.86	.65
<i>DRB1*0301</i>	7.1	11.6	1.78	.01
<i>DRB1*0302</i>	2.5	3.8	1.55	.26
<i>DRB1*0400</i>	10.9	9.0	0.80	.30
<i>DRB1*0701</i>	13.7	11.6	0.81	.25
<i>DRB1*0800</i>	4.6	5.1	1.16	.61
<i>DRB1*0901</i>	1.8	1.5	0.83	.72
<i>DRB1*1001</i>	1.5	0.9	0.61	.38
<i>DRB1*1101</i>	7.3	6.3	0.87	.57
<i>DRB1*1102</i>	2.8	2.7	0.91	.81
<i>DRB1*1103</i>	0.5	0.5	1.0	1.0
<i>DRB1*1104</i>	2.0	1.9	0.88	.78
<i>DRB1*1200</i>	2.5	2.5	1.03	.95
<i>DRB1*1300</i>	10.6	11.3	1.05	.81
<i>DRB1*1400</i>	1.5	4.3	2.89	.02
<i>DRB1*1501</i>	7.6	8.5	1.13	.61
<i>DRB1*1502</i>	0.8	0.4	0.56	.49
<i>DRB1*1503</i>	3.3	5.6	1.74	.09
<i>DRB1*1600</i>	1.8	1.2	0.57	.28

NOTE. "n" is the no. of alleles examined in persons who either had cleared infection or had persistent infection.

Table 2. Characteristics of subjects with cleared or persistent hepatitis C virus infection.

Characteristic	Clearance (n = 200)	Persistence (n = 374)
Mean age, years	25.7	27.8
Male, %	83	83
Race, %		
White	47.5	44.7
Black	44	46.5
Other	8.5	8.8
HBsAg status, % positive ^a	16.6	4.9
HIV status, % positive	38.5	41.4

NOTE. HBsAg, hepatitis B surface antigen; HIV, human immunodeficiency virus.

^a $P < .05$.

black subjects, since other ethnic groups were not well represented in the study groups. In the racial stratification, Bonferroni correction accounted for the number of alleles stratified. Five haplotypes were examined, which were derived on the basis of known haplotypes composed of the significant alleles ($n = 5$).

Results

To determine the effects of HLA class II on outcome of HCV infection, we examined the alleles of 200 clearance subjects and 374 persistently infected subjects (for 26 subjects only 1 control was available) for a total of 400 and 748 alleles in each group, respectively (table 1). The study group was fairly evenly distributed between blacks and whites (table 2). The group was pri-

marily men, HIV negative, and HBsAg negative. HCV clearance was associated with being HBsAg positive.

DQB1*0301 confirmation. Overall, *DQB1*0301* had an allele frequency of 24.5% ($n = 98$) among all subjects with viral clearance, compared with 18.9% ($n = 141$) among those with a persistent infection (OR, 0.72; 95% confidence interval (CI), 0.53–0.97; $P = .031$; table 3). This association was stronger in blacks (OR, 0.65; 95% CI, 0.42–1.00; $P = .054$) than in whites, in whom the association was in the same direction but not statistically significant (OR, 0.85; 95% CI, 0.54–1.33; $P = .47$). Inclusion of HBsAg status in the model did not appreciably affect this association. None of the derived *DQB1*0301* haplotypes were significantly associated with clearance (data not shown). In the complete cohort, only 6.9% of the clearance cases could be attributed to the *DQB1*0301* allele (3.6% in whites and 9.2% in blacks).

Other class II alleles. Of alleles with a frequency $\geq 5\%$, *DQB1*0501* (OR, 0.65; 95% CI, 0.46–0.92; $P = .014$; corrected $P [P_c]$, not significant) and *DRB1*0101* (OR, 0.45; 95% CI, 0.28–0.73; $P = .001$; $P_c = .040$) were more common in subjects with viral clearance. Both alleles were associated with clearance only in whites, with *DRB1*0101* (OR, 0.32; 95% CI, 0.17–0.60; $P = .0004$; $P_c = .002$) being stronger. In whites, 10.7% of clearance cases were associated with the presence of this allele. Inclusion of HBsAg status in the model did not affect these associations. The haplotype formed by *DQB1*0501* and *DRB1*0101* also was associated with HCV clearance in whites

Table 3. Class II alleles that predispose to clearance of hepatitis C virus (HCV) infection, stratified by ethnic group.

Study subjects, alleles examined	Frequency of clearance, %	Frequency of persistence, %	OR (95% CI)	P	P_c
All subjects	$n = 400$	$n = 748$			
Alleles					
<i>DQB1*0301</i>	24.5	18.9	0.72 (0.53–0.97)	.031	NA
<i>DQB1*0501</i>	17.8	12.0	0.65 (0.46–0.92)	.014	NS ^a
<i>DRB1*0101</i>	10.1	4.7	0.45 (0.28–0.73)	.001	.04
Haplotypes					
<i>DQB1*0501-DRB1*0101</i>	9.9	4.8	0.48 (0.30–0.78)	.003	.015
White subjects	$n = 190$	$n = 334$			
Alleles					
<i>DQB1*0301</i>	23.9	20.2	0.85 (0.54–1.33)	.47	NA
<i>DQB1*0501</i>	15.8	7.9	0.47 (0.27–0.84)	.01	NS ^a
<i>DRB1*0101</i>	15.7	5.4	0.32 (0.17–0.60)	.0004	.002
Haplotypes					
<i>DQB1*0501-DRB1*0101</i>	14.9	5.5	0.34 (0.18–0.65)	.001	.005
Black subjects	$n = 176$	$n = 348$			
Alleles					
<i>DQB1*0301</i>	26.5	18.6	0.65 (0.42–1.00)	.054	NA
<i>DQB1*0501</i>	19	15.8	0.81 (0.51–1.31)	.400	NS ^a
<i>DRB1*0101</i>	6.3	4.1	0.63 (0.28–1.40)	.254	NS ^a
Haplotypes					
<i>DQB1*0501-DRB1*0101</i>	6.5	4.1	0.65 (0.29–1.46)	.299	NS ^a

NOTE. For published allele frequencies determined in ethnic groups, please refer to [21]. ORs and P values were determined by conditional logistic regression. OR represents the likelihood of being persistently HCV infected if carrying a specific allele. "n" is the no. of alleles examined in persons who either had cleared infection or had persistent infection. CI, confidence interval; NA, not applicable; NS, not significant; OR, odds ratio; P_c , Bonferroni corrected P .

^a $P > .05$.

Table 4. Class II alleles that predispose to persistence of hepatitis C virus (HCV) infection, stratified by ethnic group.

Study subjects, alleles examined	Frequency of clearance, %	Frequency of persistence, %	OR (95% CI)	<i>P</i>	<i>P_c</i>
All subjects	<i>n</i> = 400	<i>n</i> = 748			
Alleles					
<i>DRB1*0301</i>	7.1	11.6	1.78 (1.13–2.79)	.013	NS ^a
Haplotypes					
<i>DQB1*0201-DRB1*0301</i> ^b	7.0	11.8	1.80 (1.14–2.85)	.012	.060
<i>DQAI*0501-DQB1*0201-DRB1*0301</i> ^b	7.0	11.7	1.79 (1.13–2.83)	.013	.065
White subjects	<i>n</i> = 190	<i>n</i> = 334			
Alleles					
<i>DRB1*0301</i>	7.0	14.4	2.36 (1.23–4.52)	.010	.050
Haplotypes					
<i>DQB1*0201-DRB1*0301</i>	6.6	14.7	2.51 (1.28–4.91)	.007	.035
<i>DQAI*0501-DQB1*0201-DRB1*0301</i>	6.6	14.5	2.48 (1.27–4.86)	.008	.040
Black subjects	<i>n</i> = 176	<i>n</i> = 348			
Alleles					
<i>DRB1*0301</i>	7.4	9.6	1.24 (0.63–2.45)	.53	NS ^a
Haplotypes					
<i>DQB1*0201-DRB1*0301</i>	7.6	9.7	1.21 (0.61–2.38)	.59	NS ^a
<i>DQAI*0501-DQB1*0201-DRB1*0301</i>	7.6	9.7	1.21 (0.61–2.38)	.59	NS ^a

NOTE. For published allele frequencies determined in ethnic groups, please refer to [21]. ORs and *P* values were determined by conditional logistic regression. OR represents the likelihood of being persistently HCV infected if carrying a specific allele. “*n*” is the no. of alleles examined in persons who either had cleared infection or had persistent infection. CI, confidence interval; NS, not significant; OR, odds ratio; *P_c*, Bonferroni corrected *P*.

^a *P_c* > .05.

^b *DQAI*0501* and *DQB1*0201* were not significant when analyzed as separate alleles (data not shown).

(OR, 0.34; 95% CI, 0.18–0.65; *P* = .001; *P_c* = .005) but not in blacks (OR, 0.65; 95% CI, 0.29–1.46; *P* = .299).

*DRB1*0301* was weakly associated with viral persistence in whites (OR, 2.36; 95% CI, 1.23–4.52; *P* = .010; *P_c* = .050; table 4). Inclusion of HBsAg status with *DRB1*0301* in the model modestly strengthened this association (OR, 2.85; *P* = .006; *P_c* = .030). Both the *DQB1*0201-DRB1*0301* (OR, 2.51; 95% CI, 1.28–4.91; *P* = .007; *P_c* = .035) and the *DQAI*0501-DQB1*0201-DRB1*0301* (OR, 2.48; 95% CI, 1.27–4.86; *P* = .008; *P_c* = .040) haplotypes were associated more strongly with persistence in whites. In this cohort, *DQAI*0501-DQB1*0201* was in strong linkage disequilibrium with *DRB1*0301*. Homozygosity at *DQAI*, *DQB1*, or *DRB1* was not associated with HCV persistence.

Discussion

We studied 200 HCV clearance subjects and confirmed the association of *DQB1*0301* with HCV clearance and also demonstrated differences in the strength of association on the basis of ethnicity. Of interest, the association was stronger in black subjects, although 2 European studies, in which the association was initially discovered, consisted primarily of European white subjects [8, 10]. Since none of the *DQB1*0301* haplotypes in this study demonstrated the same association, it is likely that this allele, rather than its haplotypes, is the more important determinant of outcome.

The stronger association of *DQB1*0301* with persistence in blacks may be related to inherent differences between blacks and whites or to stronger linkage of this allele to the actual

disease gene in this group. The strength of association for *DQB1*0301* in this study (OR, 0.72; *P* = .031) was much weaker than in the previous European studies (OR, 0.45; *P* = .004 [11]), perhaps because of a lower allele frequency in our cohort, compared with that in the European cohorts. Another possibility is the higher prevalence of HCV genotypes 2 and 3 in Europe than in the United States, where HCV genotype 1 is prevalent. If *DQB1*0301* were more efficient at presenting epitopes from these genotypes than from genotype 1, a greater effect would be seen in Europe. However, this is difficult to substantiate, because the genotype is unknown in most clearance cases. Third, the association may be with a gene that is tightly linked to *DQB1*0301*, and this linkage may be stronger in European cohorts than in the cohorts in this study. Fourth, the clearance subjects in our study may be inherently different, since our study subjects probably had repeated exposure and reinfection through multiple transfusions or injection drug use, whereas the majority of subjects in the European cohorts probably had a single infection with HCV from a transfusion. Thus, our subjects may have higher levels of virus or may have been exposed to a greater diversity of virus quasi species. *DQB1*0301* may not be as effective in presenting antigen in persons with repeated exposure to the virus.

As in our study, *DRB1*0101* also was identified in the cohort of Irish women as being more common in those able to clear the virus [13]. Since the Irish cohort looked only at *DRB1* alleles, the potential association with *DQB1*0301* or the *DQB1*0501-DRB1*0101* haplotype remained unknown. Of interest, Lamonaca et al. [22] found that *DRB1*0101* had a high binding affinity to 3 of the 4 highly immunogenic epitopes that

they identified in the HCV genome, supporting the finding of its association with viral clearance. Since the Irish cohort was limited to white women of European descent and since our study showed the association only in the white subjects, this allele may be less important for clearance in blacks or it may be more tightly linked to the true disease gene in whites. Alternatively, the lower frequency of this allele in blacks may decrease the power to find an association.

The association of *DRB1*0301* with viral persistence was significant only in whites after use of the Bonferroni correction, and the same relationship was identified in a cohort from Thailand [12]. Unfortunately, the significance of this allele in the Thai study was difficult to determine, because it was limited to 43 clearance subjects and 57 unmatched persistently infected subjects. Our study, in a different ethnic population, also suggests that the *DRB1*0301* haplotypes with *DQB1*0201* and *DQA1*0501* influence viral persistence in whites. Of interest, this allele also has been associated with autoimmune hepatitis [23] and with chronic HCV, compared with that in uninfected control subjects [24]. It also is possible that this allele is linked to another gene associated with viral persistence.

It was reasonable to postulate that homozygous persons have fewer alleles with which to present immunogenic HCV epitopes, resulting in a narrower protective response. Although our study was much larger than that of Thursz et al. [11], we also did not find a class II heterozygote advantage with HCV clearance. The failure to confirm this hypothesis may be due to insufficient power to detect the association, although a study larger than this one may not be undertaken. It also suggests that perhaps other unidentified determinants of viral clearance, working in conjunction with class II alleles, are also important. For example, the immune cells involved in the very early response in the liver also may have an important role in establishing the outcome of an acute HCV infection.

In this multiethnic study, we showed differential associations of *DQB1*0301*, *DRB1*0101*, and *DRB1*0301* with associated haplotypes in ethnic groups. Our lack of associations with other class II alleles is also an equally important finding, since we studied a large number of people in ethnic groups that differ from those previously studied. Weak HLA effects could still potentially be overlooked. Nevertheless, targeting the epitopes presented by *DQB1*0301* and *DRB1*0101* for vaccines that will boost the T helper response may be effective in a wide range of ethnic groups.

Although the effects of class II alleles identified in this study are important in determining viral outcomes, this accounts for only a small percentage of the persons in the persistence and clearance groups. Thus, other genes, acting either alone or in concert with other genetic determinants and environmental factors, need to be identified to elucidate the differences in the host response to this rapidly mutating virus.

Acknowledgments

We thank Karen Nolt for technical assistance and the study subjects whose participation made this investigation possible.

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