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*HLA-Cw*04* and Hepatitis C Virus Persistence

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In studies of acute hepatitis C virus (HCV) infection, the early host immune response is one of the determinants of viral persistence. The class I human leukocyte antigens (HLA), which present foreign antigen to cytolytic T cells, are integral components of this response. We hypothesized that the highly polymorphic *HLA* genes affect the outcome of an HCV infection. To test this hypothesis, we molecularly typed 231 persons with well-documented clearance of an HCV infection and 444 matched persistently infected persons. *HLA-A*1101* (odds ratio [OR], 0.49; 95% confidence interval [95% CI], 0.27 to 0.89), *HLA-B*57* (OR, 0.62; 95% CI, 0.39 to 1.00), and *HLA-Cw*0102* (OR, 0.43; 95% CI, 0.21 to 0.89) were associated with viral clearance, whereas *HLA-A*2301* (OR, 1.78; 95% CI, 1.01 to 3.11) and *HLA-Cw*04* (OR, 1.78; 95% CI, 1.21 to 2.59) were associated with viral persistence. *HLA-Cw*04* is in strong linkage disequilibrium with *HLA-B*53* and *HLA-B*35*, but only *HLA-B*53* (OR, 1.70; 95% CI, 0.95 to 3.06) and the *Cw*04-B*53* haplotype (OR, 1.76; 95% CI, 0.94 to 3.26) were weakly associated with viral persistence. *HLA-B*53* has similar, but not necessarily identical, binding specificity to some *HLA-B*35* subtypes (*B*35-Px* group). The association with the *B*35-Px* group was less strong than with *HLA-B*53* alone. The association of *HLA-Cw*04* with HCV persistence was codominant (two copies of the gene were more strongly associated with persistence than one copy). However, *HLA-Cw*04* was not associated with HCV RNA levels among the persistently infected individuals. Since Cw*04 is a ligand for the killer immunoglobulin-like receptors on natural killer cells, these cells may be involved in recovery from HCV infection. Further investigation is needed to understand the relationship between class I alleles and HCV clearance.

Hepatitis C virus (HCV) is an important blood-borne pathogen that is eliminated from the host in approximately 15% of acutely infected individuals while persisting in the remaining 85% of acutely infected individuals (1, 26). The factors involved in viral clearance are not understood, but many studies suggest host differences are critical. Of 704 Irish women who were accidentally infected with the same viral inoculum (contaminated immunoglobulin D antibody), 314 (45%) cleared their infection (13). Likewise, 43% of 152 German women cleared their infection after being exposed to the same contaminated lot of immunoglobulin D antibody (16). Since the women in their respective studies received the same virus, viral diversity cannot account for the dichotomy in outcome, rather differences in host response were likely the determining factor. Another study found marked differences in the frequency of viral clearance in Caucasians and African-Americans (21). The breadth and vigor of the host cellular immune response correlated with viral clearance in a study of six chimpanzees (6). Similarly, studies in humans have shown that a stronger polyclonal cytotoxic T lymphocyte (CTL) response is associated with viral clearance (18).

The class I and class II human leukocyte antigens (HLA) are central to the host immune response and thus are ideal candidate genes to investigate for associations with HCV outcomes. Class I and class II HLA are encoded by the most polymorphic genes known and present antigen to $CD8^+$ cytotoxic T cells and $CD4^+$ helper T cells, respectively. Polymorphisms in the peptide binding regions of these molecules determine antigenic specificities and the strength of the immune response to a given pathogen. Certain HLA alleles have been shown to influence the outcome of other chronic viral infections (12, 19, 23), and a few recent studies examined class II HLA alleles in the context of HCV clearance (3, 20, 22, 25). Given the importance of the cellular immune response in HCV infection, it is reasonable to postulate that certain HLA class I molecules may present HCV epitopes to cytotoxic T cells, resulting in a protective immune response, whereas other types may participate less efficiently in clearance of the virus. To date, this hypothesis has not been investigated; thus, using three distinct cohorts of individuals, we examined whether particular HLA class I alleles are predisposing factors to either HCV clearance or persistence.

MATERIALS AND METHODS

Study participants. Subjects in this study were participants in one of three studies: (i) AIDS Link to Intravenous Experience (ALIVE) study, which is an ongoing study of 2,921 injection drug users enrolled in Baltimore, Md., from February 1988 to March 1989, as previously described (27); (ii) Multicenter Hemophilia Cohort Study (MHCS), which is a prospectively followed cohort of patients with hemophilia, von Willebrand's disease, or a related coagulation

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disorder from 16 comprehensive hemophilia treatment centers enrolled between 1982 and 1996, as previously described (10); and (iii) Hemophilia Growth and Development Study (HGDS), which is a continuing study of 333 children and adolescents with hemophilia enrolled between March 1989 and May 1990, as previously described (11). A nested case control design was used. Individual cases had cleared viremia as demonstrated by an inability to detect HCV RNA in at least two serum samples that were drawn a minimum of 6 months apart. Prior infection was substantiated by detection of HCV antibody (anti-HCV). Persistently infected control individuals were selected from the same cohort and had anti-HCV and HCV RNA in serum for a minimum of 6 months. Controls were matched 2:1 to individuals in the same cohort by human immunodeficiency virus (HIV) status, gender, geographic location (if applicable), and race. These factors were chosen because HIV status and race are determinants of viral clearance in the ALIVE cohort (21). Informed consent was obtained from all patients, and the study was approved by the Institutional Review Board at Johns Hopkins University, the National Cancer Institute, and from the individual study cohorts.

Serologic testing. Subjects who tested positive for anti-HCV by second-generation Ortho HCV 2.0 enzyme immunoassay (EIA) (Ortho Diagnostic Systems, Raritan, N.J.) had two samples, separated by a minimum of 6 months, assessed for HCV RNA by a branched DNA (bDNA) assay (Quantiplex HCV RNA 2.0 assay; Chiron Corporation, Emeryville, Calif.). Subjects with two samples below the limit of detection by the bDNA assay had at least one of the two samples retested with the HCV COBAS AMPLICOR system (COBAS AMPLICOR HCV; Roche Diagnostics, Branchburg, N.J.), and their antibody status was confirmed by a recombinant immunoblot assay (RIBA 3.0) (Chiron Corporation). Only subjects with a negative HCV RNA result confirmed by COBAS testing were considered to have cleared the viral infection. Subjects with two positive bDNA assay results were eligible to be matched to the HCV clearance subjects as controls.

HIV type 1 (HIV-1) testing was done by EIA, and positive results for specimens were confirmed by Western blotting as previously reported (10, 11, 27). Hepatitis B surface antigen (HBsAg) status was determined by EIA (AUSZYME; Abbott Laboratories, Abbott Park, Ill.). All assays were performed according to the manufacturer's specifications except for COBAS testing of samples that contained heparin. These samples were treated with heparinase prior to COBAS testing using a Roche protocol for preparation of heparinized plasma samples. All samples used for testing had been stored at −70°C after processing and had not been previously 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. High-resolution (allele level) HLA genotyping was performed using the standard sequence-specific oligonucleotide (SSO) probe typing protocols developed by the 13th International Histocompatibility Workshop (http://www.ihwg.org/protocols/protocol.htm). *HLA-A, -B*, and *-C* genes were amplified using locus-specific PCR primers flanking exons 2 and 3, the polymorphic segments of the class I genes. The 1-kb PCR products were blotted on nylon membranes and hybridized with a panel of SSO probes. *HLA* alleles were assigned to the individuals by the reaction patterns of the SSO probes based on the known *HLA* sequences. The entire exons 2 and 3 were sequenced in samples with ambiguous SSO typing results.

Statistical analysis. All statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, N.C.). The frequencies of *HLA* class I alleles and homozygosity were compared between the individuals who cleared HCV infection and those who had a persistent infection. Homozygosity was defined in two ways for the purposes of analysis: (i) having identical alleles at *HLA-A* and *-B* or *-C* or (ii) having identical alleles at two or three of these *HLA* loci. Odds ratios (OR) were determined by conditional logistic regression and reflect the likelihood of being persistently infected with HCV if carrying a specific allele.

RESULTS

We analyzed HLA class I alleles for 231 individuals with HCV clearance and 444 matched individuals who were persistently HCV infected. There were 18 persons with HCV clearance for whom only one matching persistently infected individual was identified. Although age was not a matching criterion, the mean ages in the clearance and persistently infected groups were similar at 25 and 27 years, respectively (Table 1). The two groups were not different in terms of our matching criteria, including race, sex, and HIV status. However, those with viral clearance were more likely to be HBsAg positive and thus persistently infected with hepatitis B virus ($P < 0.0001$).

TABLE 1. Characteristics of the subjects with cleared and persistent HCV infection

Characteristic	Subjects with HCV infection	
	Cleared (n = 231)	Persistent (n = 444)
Mean age (yr)	25.1	27.3
Gender (% male)	86	86
Race (%)		
Caucasian	51	49
African-American	40	41
Other	9	10
HBsAg status (% positive)	14.3[a]	4.8
HIV status (% positive)	34	35

[a] Significantly different from the value obtained for subjects with persistent HCV infection ($P < 0.0001$).

Viral clearance. Three alleles, *HLA-A*1101* (OR, 0.49; 95% confidence interval [95% CI], 0.27 to 0.89), *HLA-B*57* (OR, 0.62; 95% CI, 0.39 to 1.00), and *HLA-Cw*0102* (OR, 0.43; 95% CI, 0.21 to 0.89) were associated with viral clearance (Table 2). *HLA-B*5701* and *HLA-B*5703* accounted for over 90% of the *HLA-B*57* alleles, and of these two alleles, only *HLA-B*5701* was weakly associated with clearance (OR, 0.68; 95% CI, 0.34 to 1.36). When stratified by race, the *HLA-A*1101* and *HLA-B*57* associations were consistent in African-Americans and Caucasians. Although a strong *HLA-Cw*0102* association with viral clearance was observed in Caucasians (OR, 0.26; 95% CI, 0.11 to 0.64), this was not seen in African-Americans (OR, 1.56; 95% CI, 0.15 to 16.6). A multivariate model including HBsAg status did not modify the relationships for any of these alleles. None of these alleles formed a haplotype with class II alleles that have been associated with viral clearance in this cohort (20).

TABLE 2. HLA class I alleles that are predisposing factors to clearance of HCV infection, stratified by ethnic group

Group and *HLA* allele[a]	Frequency of clearance (%)	Frequency of persistence (%)	OR (95% CI)
All participants			
*A*1101*	5.3	2.8	0.49 (0.27–0.89)
*B*57*	7.3	4.6	0.62 (0.39–1.00)
*Cw*0102*	3.7	1.7	0.43 (0.21–0.89)
Caucasians			
*A*1101*	7.0	4.1	0.55 (0.26–1.17)
*B*57*	6.1	3.9	0.56 (0.26–1.18)
*Cw*0102*	6.5	1.8	0.26 (0.11–0.64)
African-Americans			
*A*1101*	2.1	1.1	0.50 (0.13–2.00)
*B*57*	9.1	5.2	0.58 (0.30–1.13)
*Cw*0102*	0.5	0.8	1.56 (0.15–16.6)

[a] The numbers of alleles examined (n) (double the number of persons) were as follows: for all participants, n = 462 for frequency of clearance and 888 for frequency of persistence; for Caucasians, n = 234 for clearance and 438 for persistence; for African-Americans, n = 186 for clearance and 362 for persistence.

TABLE 3. HLA class I alleles that are predisposing factors to persistence of HCV infection, stratified by ethnic group

Group and *HLA* allele[a]	Frequency of clearance (%)	Frequency of persistence (%)	OR (95% CI)
All participants			
*A*2301*	3.9	6.3	1.78 (1.01–3.11)
*Cw*04*	9.6	15.3	1.78 (1.23–2.59)
Caucasians			
*A*2301*	2.6	2.3	0.87 (0.32–2.41)
*Cw*04*	6.5	9.9	1.69 (0.91–3.15)
African-Americans			
*A*2301*	5.9	11.3	2.24 (1.10–4.57)
*Cw*04*	12.9	20.7	1.69 (1.02–2.79)

[a] The numbers of alleles examined (*n*) (double the number of persons) were as follows: for all participants, $n = 462$ for frequency of clearance and 888 for frequency of persistence; for Caucasians, $n = 234$ for clearance and 438 for persistence; for African-Americans, $n = 186$ for clearance and 362 for persistence.

Viral persistence. *HLA-Cw*04* (OR, 1.78; 95% CI, 1.23 to 2.59) and *HLA-A*2301* (OR, 1.78; 95% CI, 1.01 to 3.11) were associated with HCV persistence (Table 3). The association with *HLA-A*2301* was observed in African-Americans (OR, 2.24; 95% CI, 1.10 to 4.57), whereas the association with *HLA-Cw*04* was observed in both African-Americans (OR, 1.69; 95% CI, 1.02 to 2.79) and Caucasians (OR, 1.69; 95% CI, 0.91 to 3.15). A multivariate model including HBsAg status did not alter the relationship for either allele. Neither of these alleles formed a haplotype with class II alleles that have been associated with persistence in this cohort (20). *HLA-Cw*04* had a fairly strong association with viral persistence, occurred commonly, and as a haplotype with *HLA-B*35*, had been reported to be strongly associated with more rapid HIV progression (4). Therefore, further analyses of this allele were undertaken.

***HLA-Cw*04* and HCV persistence.** *HLA-Cw*04* is in strong linkage disequilibrium with the related alleles, *HLA-B*53* and *HLA-B*35*, with the former being more common in African-Americans. Of these two *HLA* alleles, only *HLA-B*5301* (OR, 1.70; 95% CI, 0.95 to 3.06) and the *B*5301-Cw*04* haplotype (OR, 1.76; 95% CI, 0.94 to 3.26) demonstrated a similar, though not significant, relationship with persistence (Table 4). *HLA-B*5301* and the *B*5301-Cw*04* haplotype were associated with persistence in Caucasians and African-Americans. When individuals with *HLA-B*5301* were excluded, the association with *HLA-Cw*04* and HCV persistence remained (OR, 1.71; 95% CI, 1.06 to 2.75). The associations with *HLA-Cw*04* could not be attributed to HIV status, since the same relationships were observed when the HIV-seronegative participants were examined separately (OR, 1.56; 95% CI, 0.98 to 2.48).

Of the 15 individuals homozygous for *HLA-Cw*04*, 14 were persistently infected. A Maentel-Haenszel test for trend indicated a codominant effect for *HLA-Cw*04* ($P = 0.009$); thus, individuals with one *HLA-Cw*04* allele were more likely to have a persistent infection, while those with both alleles almost always had a persistent infection. All four individuals homozygous for *HLA-B*5301*, three of whom were *HLA-Cw*04* homozygotes, were persistently infected; however, the Maentel-Haenszel test for trend did not reach statistical significance ($P > 0.05$).

TABLE 4. Association of *HLA-Cw*04*, *Cw*04-B*35*, *Cw*04-B*53*, *B*35-Px*, and *B*35-PY*, stratified by ethnic group

Group and *HLA* allele or haplotype[a]	Frequency of clearance (%)	Frequency of persistence (%)	OR (95% CI)
All participants			
Alleles			
*Cw*04*	9.6	15.3	1.78 (1.23–2.59)
*B*5301*	3.5	5.8	1.70 (0.95–3.06)
*B*35*	7.0	7.6	1.07 (0.68–1.69)
*B*35-Px*	5.7	7.9	1.43 (0.89–2.29)
*B*35-PY*	4.8	5.4	1.11 (0.66–1.88)
Haplotypes			
*Cw*04-B*5301*	3.1	5.2	1.76 (0.94–3.26)
*Cw*04-B*35*	4.8	6.5	1.36 (0.80–2.31)
*Cw*04-B*35-Px*	4.4	6.9	1.63 (0.97–2.76)
*Cw*04-B*35-PY*	3.5	4.8	1.33 (0.73–2.43)
Caucasians			
Alleles			
*Cw*04*	6.5	9.9	1.69 (0.91–3.15)
*B*5301*	0.4	0.7	1.82 (0.19–17.89)
*B*35*	6.1	7.3	1.17 (0.60–2.28)
*B*35-Px*	3.0	3.4	1.08 (0.42–2.73)
*B*35-PY*	3.5	4.6	1.31 (0.56–3.07)
Haplotypes			
*Cw*04-B*5301*	0.4	0.7	1.82 (0.18–17.9)
*Cw*04-B*35*	4.4	6.4	1.41 (0.65–3.05)
*Cw*04-B*35-Px*	1.8	2.8	1.53 (0.48–4.82)
*Cw*04-B*35-PY*	3.1	4.4	1.35 (0.55–3.34)
African-Americans			
Alleles			
*Cw*04*	12.9	20.2	1.69 (1.02–2.79)
*B*5301*	7.5	12.7	1.77 (0.95–3.30)
*B*35*	8.6	5.5	0.61 (0.31–1.20)
*B*35-Px*	8.6	13.0	1.57 (0.87–2.85)
*B*35-PY*	7.5	5.2	0.67 (0.33–1.35)
Haplotypes			
*Cw*04-B*5301*	6.5	11.3	1.84 (0.95–3.59)
*Cw*04-B*35*	5.4	3.9	0.69 (0.30–1.58)
*Cw*04-B*35-Px*	7.0	11.3	1.69 (0.88–3.24)
*Cw*04-B*35-PY*	4.8	3.9	0.77 (0.33–1.81)

[a] The numbers of alleles examined (*n*) (double the number of persons) were as follows: for all participants, $n = 462$ for frequency of clearance and 888 for frequency of persistence; for Caucasians, $n = 234$ for clearance and 438 for persistence; for African-Americans, $n = 186$ for clearance and 362 for persistence.

The peptide binding cleft for the class I alleles consists of nine pockets (2). The B and F pockets, which bind the second amino acid (P2) and the carboxyl-terminal amino acids of the bound peptide, respectively, are the most important determinants of binding specificity. Thus, we examined the alleles which had either identical B or F binding pockets to Cw*04. *HLA-Cw*14* is the only allele identical at the B pocket, and *HLA-Cw*1801* is the only allele which shares an identical F pocket to *HLA-Cw*04.* Neither of these was associated with viral persistence (OR, 0.62 and 0.68, respectively).

We also investigated whether *HLA-Cw*04* was associated with a higher level of HCV RNA among those who were persistently infected. The HCV RNA levels from the first specimen in this study were log transformed, and the median values in those with and without *HLA-Cw*04* were determined. No difference between the groups was observed (median of 6.6 for both groups).

Association of *HLA-B*35* and *HLA-B*53* subtypes with viral

persistence. *HLA-B*53* and *HLA-B*35* can be grouped according to their binding preferences at the B and F pockets (28). We tested the hypothesis that the *HLA-B*5301* association may be stronger if it is combined with functionally similar *B*35* subtypes. In this panel of subjects, eight *HLA-B*35* subtypes and one *HLA-B*53* subtype (*B*5301)* were identified. Based on peptide binding preferences, the *HLA-B*35* and *HLA-B*53* subtypes can be divided into two groups: (i) those binding proline at P2 and tyrosine at P9, *B*35-PY* (*B*3501, B*3508,* and *B*3517*) and (ii) those binding proline at P2 but not recognizing tyrosine at P9, *B*35-Px* (*B*3502, B*3503, B*3504, B*3512, B*3522,* and *B*5301*). Neither the *B*35-PY* group nor the *B*35-Px* group were associated with HCV clearance or persistence (OR of 1.11 and 1.43, respectively). The association with the *Cw*04-Px* haplotype and viral persistence (OR, 1.63; 95% CI, 0.97 to 2.76) was similar to that of the *Cw*04-B*5301* haplotype.

Effect of heterozygosity on outcome. We also hypothesized that individuals with greater class I allelic diversity, i.e., those who are fully heterozygous, may be able to present a wider array of antigens and thus be more likely to clear HCV. The individuals who were homozygous at one locus were not more likely to have a persistent HCV infection (OR, 1.27; 95% CI, 0.81 to 1.98) than those who were fully heterozygous. Likewise, the individuals who were homozygous at two or three loci were not more likely to be persistently infected (OR, 1.11; 95% CI, 0.50 to 2.47) than those who were fully heterozygous or homozygous at only one locus; however, only 28 people were homozygous at two or three loci.

DISCUSSION

In this investigation, which is the first to examine HLA class I alleles in the context of HCV clearance, we used the largest known clearance panel to date with carefully matched controls. We found that *HLA-A*1101*, *-B*57,* and *-Cw*0102* were associated with viral clearance and that *HLA-A*2301* and *-Cw*04* were associated with viral persistence. Detailed analysis of *HLA-Cw*04* and the alleles with which it forms haplotypes, namely, *HLA-B*35* and *HLA-B*53*, did not uncover an association stronger than with *HLA-Cw*04* alone. While the biologic basis for these *HLA* associations remains to be proven, they cumulatively could account for outcomes in up to 18% of the subjects in these cohorts. Thus, although class I *HLA* genes are potentially important, other genes that regulate or affect the immune response should also be examined.

The *HLA-B*57* association with viral clearance was present in both Caucasians and African-Americans. Interestingly, *HLA-B*57* has also been associated with slow progression of HIV-1 disease in different ethnic populations (8, 15); thus, this allele may have some protective activity in a variety of chronic viral illnesses. Alternatively, *HLA-B*57* may be in linkage disequilibrium with a gene that promotes elimination of viral infections. Finding the association of *HLA-Cw*0102* with clearance only in Caucasians is not necessarily surprising and points to the importance of examining more than one ethnic group. This observation suggests that *HLA-Cw*0102* is either linked to a true susceptibility gene or interacts with another gene that is present only in Caucasians.

In this study, individuals with either *HLA-Cw*04* or *HLA-B*5301*, which are in strong linkage disequilibrium, had an almost twofold-increased risk of being persistently HCV infected. Despite the relatively large size of this clearance cohort, there were too few subjects to precisely distinguish which allele was most important. However, several pieces of evidence suggest that the *HLA-Cw*04* allele is the critical factor and that the *HLA-B*5301* association was present due to its linkage disequilibrium with *HLA-Cw*04*. First, the relationship with *HLA-Cw*04* was present even in those without the *HLA-B*5301* allele. If *HLA-B*5301* were driving the association, a decrease in the magnitude would be expected when *HLA-B*5301*-positive individuals were excluded from the analysis. An analysis of *HLA-B*5301* excluding those with *HLA-Cw*04* could not be done because of the limited number of subjects with this profile. Second, a strong codominant effect of *HLA-Cw*04* was noted, demonstrating that those with two copies of the allele rarely eliminate the virus while those with one copy may overcome the predisposition toward viral persistence. Only 3 of the 14 *HLA-Cw*04* homozygous individuals were also homozygous for *HLA-B*5301*, which also supports an association with *HLA-Cw*04*. Third, when functionally similar subtypes of *HLA-B*5301* were grouped (*Px* group), the association with persistence was not strengthened.

It is not necessarily surprising that neither of the alleles with an identical B or F binding pocket to Cw*04 were associated with viral persistence, since peptide binding is determined by more than one pocket. However, if one of these alleles had also been associated with viral persistence, then there would have been evidence for the importance of a particular pocket in HCV infection.

One could speculate that *HLA-Cw*04* presents HCV peptides that lead to an ineffective immune response or that it interacts with the virus or another host protein that favors viral persistence; however, data supporting such mechanisms do not exist. Alternatively, data do support the hypothesis that the association with *HLA-Cw*04* could be linked to its putative interaction with natural killer (NK) cells, which are important in clearing many viral infections and may also be important in HCV clearance (14, 17). Cw*04 behaves as a ligand for one of the NK cell receptors among the killer immunoglobulin-like receptor group. Unlike CTLs, NK cells are programmed to kill the target cell unless its inhibitory receptor is engaged by a target cell ligand such as Cw*04. It is possible that NK cells have an important role in determining HCV clearance early in an acute infection but are less important once a persistent infection has been established. The association of *HLA-Cw*04* with viral persistence but not with HCV RNA levels would support this hypothesis. It is logical that NK cells may be important for viral clearance initially, since 65% of all lymphocytes in the healthy, uninfected liver are NK cells or T cells that express NK receptors (NKT cells) (9). Other viruses, such as HIV (5), can specifically downregulate *HLA-A* and *-B* alleles, but it is not known if HCV is capable of such immune evasion. If it is, then the CTL response, which relies on these antigens, may be less important for viral clearance than are NK cells. A single study of NK cells in HCV-infected persons demonstrated decreased NK cell activity in individuals persistently infected with HCV than in population controls (7).

The association of *HLA-Cw*04* with HCV persistence is modest, but as recently discussed elsewhere, OR of less than

two for individual genes are not unexpected in disease outcomes which are multifactorial, such as HCV infection, and may be dependent upon the presence of other genes (24). For example, if the association of *HLA-Cw*04* and HCV persistence is determined by Cw*04 binding with a receptor, then the right combination of Cw*04 and receptor would need to be present for the effect to be seen. Such potential interactions could be with killer immunoglobulin-like receptors or with receptors that are yet to be described.

It seems plausible that individuals who are fully heterozygous at class I alleles can present a more diverse array of epitopes to cytotoxic T cells and thus may be more apt to clear the virus. Although we demonstrated an association between *HLA-Cw*04* homozygosity and HCV persistence, it was surprising that an overall association between class I homozygosity and viral persistence was not detected. More subjects may need to be studied to detect such an association if homozygosity at two or three loci is indeed needed to see an effect.

The strengths of this study include the large clearance panel with controls matched for factors important for viral clearance and the rigorous class I typing methodology used. Despite these strengths, there are a few important limitations. First, we did not have enough power to detect associations with very low frequency alleles. Second, due to the large number of and fairly even distribution of class I alleles, the frequency of any individual allele is low; thus, none of the alleles identified in this study would be significant if we accounted for multiple comparisons. Thus, it is important for these associations to be verified in other large, well-constructed cohorts and to consider the biologic plausibility of these associations such as we have done for *HLA-Cw*04*. Finally, causality cannot be determined by such an association study.

In summary, several HLA class I molecules are associated with either HCV clearance or persistence supporting the importance of the cytolytic T-cell response to the outcome of an HCV infection. The association of *HLA-Cw*04* with HCV persistence and its potential relationship to innate immunity also merit further investigation. If all the alleles found to be associated with an HCV outcome were verified, they would still account for only a small percentage of HCV outcomes; thus, the search for polymorphisms in other immune regulatory genes should continue.

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