



HHS Public Access

Author manuscript

Genes Immun. Author manuscript; available in PMC 2014 January 01.

Published in final edited form as:

Genes Immun. 2013 ; 14(5): 330–335. doi:10.1038/gene.2013.25.

Relation of HLA Class I and II Supertypes with Spontaneous Clearance of Hepatitis C Virus

Mark H. Kuniholm^{1,#}, Kathryn Anastos^{1,2}, Andrea Kovacs³, Xiaojiang Gao^{4,5}, Darlene Marti^{4,5}, Allesandro Sette⁶, Ruth M. Greenblatt⁷, Marion Peters⁸, Mardge H. Cohen⁹, Howard Minkoff¹⁰, Stephen J. Gange¹¹, Chloe L. Thio¹², Mary A. Young¹³, Xiaonan Xue¹, Mary Carrington^{4,5}, and Howard D. Strickler¹

¹Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, NY

²Department of Medicine, Montefiore Medical Center, Bronx, NY

³Department of Pediatrics, University of Southern California, Los Angeles, CA

⁴Cancer and Inflammation Program, Laboratory of Experimental Immunology, SAIC-Frederick, Inc., Frederick National Laboratory for Cancer Research, Frederick, MD

⁵Ragon Institute of MGH, MIT and Harvard, Charlestown, MA

⁶Division of Vaccine Discovery, La Jolla Institute for Allergy and Immunology, La Jolla, CA

⁷Department of Clinical Pharmacy, University of California, San Francisco, CA

⁸Department of Medicine, University of California, San Francisco, CA

⁹CORE Center, Cook County Bureau of Health Services, Chicago, IL

¹⁰Department of Obstetrics and Gynecology, Maimonides Medical Center, Brooklyn, NY

¹¹Department of Epidemiology, Johns Hopkins Medical Institutions, Baltimore, MD

¹²Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, MD

¹³Department of Medicine, Georgetown University Medical Center, Washington, DC

Abstract

Human leukocyte antigen (HLA) genotype has been associated with probability of spontaneous clearance of hepatitis C virus (HCV). However, no prior studies have examined whether this relationship may be further characterized by grouping HLA alleles according to their supertypes, defined by their binding capacities. There is debate regarding the most appropriate method to define supertypes. Therefore, previously reported HLA supertypes (46 class I and 25 class II) were assessed for their relation with HCV clearance in a population of 758 HCV-seropositive women.

Users may view, print, copy, download and text and data- mine the content in such documents, for the purposes of academic research, subject always to the full Conditions of use: http://www.nature.com/authors/editorial_policies/license.html#terms

#Corresponding Author: Department of Epidemiology & Population Health, Albert Einstein College of Medicine, Belfer Building, Room 1308C, 1300 Morris Park Ave. Bronx, NY 10461. Phone: (718) 430-4129. Fax: (718) 430-8780. mark.kuniholm@einstein.yu.edu.

All authors declare no conflicts of interest.

Two HLA class II supertypes were significant in multivariable models that included: (i) supertypes with significant or borderline associations with HCV clearance after adjustment for multiple tests, and (ii) individual HLA alleles not part of these supertypes, but associated with HCV clearance in our prior study in this population. Specifically, supertype DRB3 (prevalence ratio (PR)=0.4; $p=0.004$) was associated with HCV persistence while DR8 (PR=1.8; $p=0.01$) was associated with HCV clearance. Two individual alleles (B*57:01 and C*01:02) associated with HCV clearance in our prior study became non-significant in analysis that included supertypes while B*57:03 (PR=1.9; $p=0.008$) and DRB1*07:01 (PR=1.7; $p=0.005$) retained significance. These data provide epidemiologic support for the significance of HLA supertypes in relation to HCV clearance.

Keywords

hepatitis C virus; HLA; human leukocyte antigen; supertype

INTRODUCTION

More than 3 million people in the United States (US) are chronically infected with hepatitis C virus (HCV),¹ and over 40% of cirrhosis and hepatocellular carcinoma in the US is attributable to HCV.² Only a minority of those initially infected with HCV are able to spontaneously clear the virus (i.e., without antiviral therapy). Those who clear HCV have been shown to have broader and more sustained CD4+ and CD8+ T cell responses against HCV antigens than those who fail to clear HCV infection (reviewed in^{3,4}), consistent with the critical role T cell-mediated immune responses are thought to play in the control of viruses, including HCV.

T cells recognize HCV peptide antigens presented on cell surfaces by human leukocyte antigen (HLA) molecules. HLA genes and molecules are highly polymorphic and this heterogeneity is thought to play an important role in differences in HLA molecule binding affinity – an important factor in determining the ability of T cells to recognize and respond to antigens.^{4,5} We and others have shown significant associations between HLA genotype and the prevalence of having cleared HCV (reviewed in⁶).

However, no prior studies to our knowledge examined whether these relationships may be further characterized by grouping individual HLA genotypes according to their peptide binding affinities; often called their HLA “supertypes”^{7,8}. While several associations between HLA supertypes and the risk of HIV progression have been reported,^{9,10,11} the few studies related to HCV have focused on the laboratory identification of supertype-restricted HCV epitopes rather than clinical outcomes.^{12,13}

Therefore, we studied HLA supertypes in relation to HCV clearance in a population of 758 HCV-seropositive women in which we had previously examined associations of individual HLA alleles with HCV clearance. We were particularly interested in whether the study of HLA supertypes would provide information related to HCV clearance that could not be recognized through the study of individual HLA alleles. In this sense, our study is an evaluation of the relevance of HLA supertypes, using spontaneous HCV clearance as a

model. One challenge to studying HLA supertypes, though, has been that there is no single accepted definition of the individual alleles that constitute a supertype. Supertypes have been defined using different computational and experimental criteria for determining HLA molecule binding affinities by different scientific groups. We therefore used an agnostic approach, and assessed multiple different well characterized supertypes defined by different laboratories in order to, for the first time, study these supertypes and their relation with HCV clearance. This approach provided new information regarding which if any of these supertype classification systems had a strong relation with HCV clearance, a clinically important phenotype.

RESULTS

Study Population

We studied a multiracial population of 758 women who were HCV-seropositive at enrollment into the Women's Interagency HIV Study (WIHS), as described in our prior study of individual HLA alleles and HCV clearance.⁶ Briefly, 622 women were HCV RNA positive and 136 tested negative for HCV RNA, of whom 524 (84%) and 112 (82%) were HIV co-infected, respectively. Women who were HCV RNA positive were more likely than those who were HCV RNA negative to be Black, non-Hispanic, and (among the HIV-seropositives) to have lower CD4+ T cells levels. Age, history of injection drug use, and HIV serostatus did not differ according to HCV RNA status.

HLA Supertype Associations with HCV Clearance

Among the 71 studied HLA supertypes, seven HLA class II supertypes but no HLA class I supertypes had significant associations with HCV clearance in analyses unadjusted for multiple comparisons (shown in Supplementary Table 1). Only one of these seven supertypes, DRB3 (Greenbaum et al.),¹⁴ was significantly associated with HCV clearance following adjustment for multiple comparisons using both false discovery rate (FDR)-adjusted q values and permutation resampling-adjusted p values (Prevalence Ratio (PR): 0.35; 95% CI: 0.20–0.59; $p_{\text{unadjusted}} < 0.001$; $q = 0.008$; $p_{\text{permutation}} < 0.001$). However, three other supertypes were borderline significant using FDR-adjusted q values ($q < 0.10$), including the DR8 supertype (PR: 1.84; 95% CI: 1.19–2.85; $p = 0.007$; $q = 0.080$; $p_{\text{permutation}} = 0.917$) described by Lund et al.,¹⁵ the DR1*01 supertype (PR: 1.70; 95% CI: 1.14–2.56; $p = 0.01$; $q = 0.098$; $p_{\text{permutation}} = 0.336$) described by Nielsen et al.,¹⁶ and the A supertype (PR: 1.64; 95% CI: 1.18–2.29; $p = 0.004$; $q = 0.080$; $p_{\text{permutation}} = 0.115$) described by Ou et al.¹⁷ In an additional sensitivity analysis in which we adjusted for HIV serostatus/CD4+ T cell counts, these associations were essentially unchanged: DRB3 (PR: 0.35; 95% CI: 0.20–0.59), DR8 (PR: 1.81; 95% CI: 1.17–2.80), DR1*01 (PR: 1.73; 95% CI: 1.15–2.60), A (PR: 1.68; 95% CI: 1.20–2.35).

Table 1 shows the individual HLA alleles that comprise the four supertypes that remained significant or of borderline significance after adjustment for multiple tests. DRB3 includes two HLA alleles that were present in our study population (DRB1*03:01 and DRB1*13:02) and these two alleles had similar, significant inverse associations with HCV clearance. We then used an alternative approach – MHCcluster analysis – to further assess the clustering of

DRB3 alleles and those of the other HLA supertypes found to have significant or borderline significant associations with HCV clearance based on the sequence features defining their peptide binding grooves. MHCcluster analysis did not show that DRB1*03:01 and DRB1*13:02 clustered together according to binding groove characteristics. Instead, MHCcluster analysis found that DRB1*13:02 clustered together with the DRB1*01:03 allele that other methods included in the DR1*01 supertype. Each of the three women with a DRB1*01:03 allele was HCV RNA positive, though, and the association of the DRB1*13:02/DRB1*01:03 cluster with HCV clearance (PR: 0.33; 95% CI: 0.15–0.73; $p < 0.0001$) was similar to the association of the DRB3 supertype defined as DRB1*03:01/DRB1*13:02. We then separately incorporated the two cluster definitions, DRB1*03:01/DRB1*13:02 and DRB1*13:02/DRB1*01:03, in multivariable analyses. These models included all supertypes with significant or borderline significant associations and also the alleles not part of these supertypes that were significantly associated with HCV clearance in our prior study in this population.⁶ Both the DRB1*03:01/DRB1*13:02 (PR: 0.44; 95% CI: 0.25–0.76; $p = 0.004$ – shown in Table 2) and DRB1*13:02/DRB1*03:01 (PR: 0.45; 95% CI: 0.20–1.00; $p = 0.05$) cluster definitions retained similar inverse associations with HCV clearance in multivariable analyses, although the association of the DRB1*13:02/DRB1*03:01 cluster was no longer statistically significant.

The four alleles that comprise the DR8 supertype each had positive associations with HCV clearance, and though the strength of these relationships varied, three of the four alleles had PRs >2. The four DR8 alleles clustered together in MHCcluster analysis along with the DRB1*14:02 allele from the A supertype. Only two women in our population had the DRB1*14:02 allele – one HCV RNA positive and one HCV RNA negative – and addition of DRB1*14:02 to the DR8 supertype did not change the association between DR8 and HCV clearance. In multivariable analysis of all supertypes together, both the original DR8 clustering definition that included DRB1*08:01/DRB1*08:02/DRB1*08:04/DRB1*08:06 (PR: 1.76; 95% CI: 1.16–2.71; $p = 0.01$ – shown in Table 2) and the alternate clustering definition suggested by MHCcluster analysis that included DRB1*14:02 along with those four alleles (PR: 1.77; 95% CI: 1.14–2.72; $p = 0.008$) retained significant positive associations with HCV clearance.

Each of the three alleles in DR1*01 had positive associations with HCV clearance. Two of these alleles (DRB1*01:01 and DRB1*10:01) clustered together along with DRB1*01:02 from the A supertype in MHCcluster analysis, while the third allele (DRB1*01:03) did not. The association between the DR1*01 supertype and HCV clearance was essentially the same as that using the alternative clustering definition of DRB1*01:01/DRB1*10:01/DRB1*01:02. In multivariable analysis, neither the original DR1*01 clustering definition (PR: 1.50; 95% CI: 0.90–2.50; $p = 0.12$ – shown in Table 2) nor the alternative clustering definition of DRB1*01:01/DRB1*10:01/DRB1*01:02 (PR: 1.40; 95% CI: 0.80–2.44; $p = 0.25$) retained statistical significance.

While the eight A supertype alleles varied in their associations with HCV, five of the eight had positive associations that ranged from PR=1.38 to PR=2.05. The other three alleles in the A supertype had null associations. In MHCcluster analysis, four of the eight alleles in the A supertype (DRB1*04:01, DRB1*04:04, DRB1*04*05, DRB1*04:08) clustered together

while the other four alleles did not. In multivariable analysis, the association of the A supertype with HCV clearance was statistically non-significant (PR: 1.49; 95% CI: 0.97–2.28; $p = 0.07$ – shown in Table 2) as was the alternative clustering definition of DRB1*04:01/DRB1*04:04/DRB1*04*05/DRB1*04:08 (PR: 1.05; 95% CI: 0.63–1.74; $p = 0.86$).

Associations of two alleles (B*57:01 and C*01:02) that were associated with HCV clearance in our prior study in this population⁶ became statistically non-significant in multivariable analysis (Table 2). In contrast, B*57:03 (PR=1.89; 95% CI=1.18–3.02; $p=0.008$) and DRB1*07:01 (PR=1.73; 95% CI=1.18–2.53; $p=0.005$) retained significant associations with HCV clearance.

DISCUSSION

This is the first study, to our knowledge, to examine the relation of HCV clearance with HLA supertypes. Several different classification systems to define these supertypes have been proposed, based on different methods for determining which HLA molecules share similar binding affinities. Binding affinity is an important factor in determining the ability of T cells to recognize and respond to HCV antigens.^{4;5} In the current investigation, we examined multiple different well characterized supertypes defined by different laboratories, that were determined based on high-resolution HLA genotype data. Our results therefore provide important information regarding which if any of these supertype classification systems are biologically significant to HCV clearance. It is one of just a few studies that has used clinical outcomes and data to evaluate the relevance these proposed supertype definitions.

Supertypes are controversial since there is no agreed upon method for assessing the binding affinities of HLA molecules. Therefore, different research groups have reached different conclusions regarding the proper clustering of HLA alleles according to their binding affinities, into what are termed supertypes. The strongest association with HCV clearance in our study was with the HLA class II supertype DRB3, as defined by Greenbaum et al.,¹⁴ which had a significant inverse association with HCV clearance, even after correction for multiple tests using two separate methods. However, there is laboratory disagreement regarding the definition of the DRB3 supertype. The MHCcluster method, for example, did not find that DRB3 supertype alleles clustered together based on the sequence features defining their peptide binding grooves. We also note that the high-resolution HLA genotyping we conducted in this study population did not include genotyping of DRB3, DRB4 and DRB5 alleles – some of which are included in the DRB3 supertype¹⁴ – because these genes are not present in all individuals.

At least one prior study,²⁰ for example, found that the DRB3*02 allele group was positively associated with HCV clearance in a population of 41 White participants, albeit, not among 52 Black participants. WIHS women are majority non-Hispanic Black, and it is not clear from that prior study if the DRB3*02:02 allele – which is included in the DRB3 supertype – was present in that study population.²⁰ Nonetheless, the results of that prior study and of the

current investigation suggest that important insights may be obtained from examining the relationship between DRB3, DRB4 and DRB5 alleles and HCV clearance in future studies.

Three supertypes were significantly associated with HCV clearance prior to adjustment for multiple tests, and had q values of borderline significance when assessed using false discovery rate procedures. Each of these supertypes had a positive association with HCV viral clearance. The DR8 supertype reported by Lund et al.¹⁵ was especially noteworthy because the clustering of DR8 alleles was confirmed in MHCcluster analysis and its association with HCV clearance remained statistically significant in multivariable analysis. The DR8 supertype was also noteworthy because it included uncommon HLA alleles (in our population). Thus, it is only through examining these alleles within a supertype that their possible associations with HCV clearance could be recognized in the current analysis.

The association of DR1*01 with HCV clearance was not statistically significant in multivariable analysis, and only two of the three DR1*01 supertype alleles reported by Nielsen et al.¹⁶ clustered together in MHCcluster analysis. We therefore view the association of DR1*01 with HCV clearance with some skepticism, pending confirmation in other populations. Four of the eight alleles of the A supertype did not cluster together in MHCcluster analysis, and the association between the A supertype and HCV clearance was also non-significant in multivariable analysis. It is therefore unlikely that the A supertype has a biologic relationship with HCV clearance.

Surprisingly, no HLA class I supertypes were associated with HCV clearance in our study population, even in analyses that did not adjust for multiple testing. While this would seem to argue against the relevance of the HLA class I supertype classifications as they pertain to HCV, we note that HLA class I supertypes defined by Sette and Sidney,²¹ which we evaluated in the current analysis, were found to have strong associations with HIV RNA and CD4+ T cell levels in data reported by other groups.^{9;10;11}

In addition to the possible associations of supertypes DRB3 and DR8 with HCV clearance, multivariable analysis also showed a significant relation of HCV clearance with two individual alleles, namely, DRB1*07:01 and B*57:03. Two other alleles that had been found to be associated with HCV clearance in our prior investigation⁶ were no longer significant after adjustment for HLA supertypes. It is notable that B*57:03 retained a significant independent association with HCV clearance in multivariable analysis while B*57:01 did not. While B*57:01 and B*57:03 differ only by two amino acids, recent research related to the interaction of B*57 molecules with the HIV-1 Gag epitope KF11 has shown that the amino acid sequence differences between B*57:01 and B*57:03 result in the recruitment of KF11-specific CD8+ T cells with very different T cell receptor (TCR) repertoires.²² Future studies are needed to determine whether CD8+ T cell responses to B*5701-restricted HCV epitopes also differ from those to B*5703-restricted HCV epitopes. We note, though, that power to detect significant associations for B*57:01 is lower than for B*57:03 in WIHS because B*57:01 is less prevalent in this majority non-Hispanic Black cohort.

Several limitations to the interpretation of these results must be considered. First, we note that there is no single accepted method or criteria for defining HLA supertypes. Thus,

different scientific group have grouped different alleles into different supertypes. While we adopted an agnostic approach to the study of HLA supertypes, and assessed several different HLA supertype classification systems, it is possible none of these is wholly correct. The differences in methods used to define supertypes and peptide binding repertoires are reflected in both our calculation of percentage agreement between the studied supertypes and by our additional analysis using MHCcluster. Percentage agreement was high to very high (77% – 99%) between some, but not all, studied class I supertypes but was lower (37%–50%) between the class II supertypes. Further, MHCcluster provided additional alternative groups of HLA alleles that differed from the previously reported supertypes that, in the current study, had associations with HCV clearance. Second, the current study examined HCV antibody and HCV RNA data from the WIHS enrollment visit. Although most spontaneous HCV clearance occurs shortly after HCV infection, clearance of HCV RNA continues to occur over time in a small fraction of those with chronic HCV.^{23;24} Thus, it is possible that there is a small degree of misclassification in HCV viremia status that would be resolved with repeat HCV RNA testing. Lastly, we cannot exclude the possibility that the supertype associations identified in this study reflect associations of non-HLA genetic variants in linkage disequilibrium with HLA alleles.

Overall, our results suggest that the study of HLA supertypes may inform our understanding of the immunogenetic factors that influence host clearance of HCV infection. It is one of just a few studies that has used clinical outcomes and data to evaluate the relevance these proposed supertype definitions. In particular, if our results are confirmed by other groups, it would suggest that certain HLA class II binding pocket characteristics are associated with reduced (i.e., the binding pockets of the DRB3 supertype) or enhanced (i.e., DR8) host capacity to clear HCV infection, which could point to the types of peptide antigens most likely to be effective in a vaccine (one able to stimulate the immune system to clear HCV if exposed). From a research perspective, HLA supertypes may also be useful in allowing the assessment of uncommon HLA alleles. Thus, the current study suggests that studying HLA alleles according to their biologic characteristics could provide data that are relevant to larger groups of patients and provide new understanding of the role of HLA in health and disease. Conversely, the conflicting data regarding the relatedness of certain alleles suggests that methods for defining HLA binding pocket characteristics remain imperfect. Further research is needed to improve these methods, with clinical outcome studies used to adjudicate which definitions are most biologically relevant.

MATERIALS AND METHODS

Review of the HLA Supertype Literature

The PubMed database was searched using the terms “supertype” and “HLA” (or “MHC”). References cited in the articles identified through this search, as well as in review articles or book chapters, were also identified. Each report was then obtained and critically evaluated. We excluded studies that did not base their supertype definitions on high-resolution HLA genotype data,²⁵ only examined a narrow range of alleles,²⁶ or in which the supertypes reported included only a single HLA allele found in our population.^{27;28} Overall, we found 5

studies that provided class I^{15;29;30;31;32} and 5 studies that provided class II^{14;15;16;17;33} supertype definitions that met these criteria.

Table 2 shows all 71 superotypes (46 class I and 25 class II) reported by these studies. The individual HLA alleles that make up each supertype are shown in Supplementary Table 2. The percentage of HLA allele agreement between class I supertype definitions has been previously reported,³⁰ with high to very high (77% – 99%) agreement found between the class I definitions of Lund et al.,¹⁵ Hertz and Yanover³⁰ and by Sette and Sidney.²¹ Percent agreement of these definitions with those of Doytchinova et al.²⁹ was, however, lower (41% - 49%).³⁰ No studies to our knowledge reported percentage of agreement between class II superotypes. We calculated percent agreement between the class II superotypes defined by Lund et al.¹⁵ and Doytchinova et al.³³ (38%), Nielsen et al.¹⁶ and Doytchinova et al.³³ (37%), and Lund et al.¹⁵ and Nielsen et al.¹⁶ (50%). Percent agreement with the HLA class II superotypes defined by two other research groups could not be determined since they used different nomenclature.^{14;17}

Study Population and Laboratory Methods

Characteristics of the HCV-seropositive women as well as the laboratory methods for HCV testing and HLA genotyping were described in our prior study of individual HLA alleles and HCV clearance in this population.⁶ Briefly, the current study focused on 1 204 women who were HCV-seropositive at enrollment into the Women's Interagency HIV Study (WIHS), an ongoing prospective, multicenter cohort study of 2 793 HIV-seropositive and 975 at-risk HIV-seronegative women enrolled through similar sources at six clinical sites. The initial WIHS enrollment was conducted between October 1994 and November 1995, and a second recruitment occurred during 2001–02.

We excluded women without known HCV RNA status (N=134), those who had not provided consent for HLA genetic testing (N=288), and the few women of race/ethnicity other than White non-Hispanic, Black non-Hispanic, or Hispanic race/ethnicity (N=24). Compared with those included in this study, the HCV-seropositive women who were excluded were younger ($p=0.03$), less likely to be non-Hispanic Black ($p<0.01$), less likely to report injection drug use (IDU) ($p<0.01$), and (among the HIV-seropositives) had lower CD4+ T cell levels ($p=0.02$).

Statistical Methods

We used multivariable log-binomial models to study associations between HLA class I and II superotypes and HCV clearance in HCV seropositive women. These models included only two variables, supertype and race/ethnicity [White non-Hispanic, Black non-Hispanic, Hispanic], consistent with recent statistical genetics studies which have shown that demographic and behavioral characteristics other than race/ethnicity are rarely associated with the genes under study and that genetic association models are generally unaffected by control for multiple covariates.³⁴ While HIV disease progression is associated with the HLA, most HCV in HIV-seropositive women is related to injection drug use and the HCV epidemic among IDU predated the HIV epidemic.³⁵ Thus, HCV is thought to typically predate HIV in these individuals, who will have cleared HCV or developed chronic infection

prior to becoming HIV infected. Consistent with this timeline, the results of our previous study of individual HLA alleles and HCV clearance in this population were largely unaffected by control for HIV and CD4+ T cell count.⁶ We conducted a sensitivity analysis in the current study, though, by adjusting those supertypes that showed at least borderline significance for HIV serostatus/CD4+ T cell counts [HIV-seronegative, HIV-seropositive with CD4+ T cell count ≥ 500 cells/mm³, HIV-seropositive with CD4+ T cell count <500 cells/mm³]. As in most prior studies of HLA genotype and disease, the analyses were conducted assuming a dominant genetic model (1 or 2 copies of a given supertype constitutes 'exposure').

A major consideration was correction for multiple hypothesis testing, since a large number of different supertypes were examined in this study. However, no single well-established method could fully address multiple testing in these data. For example, many of the supertypes that we studied are highly correlated (i.e., we studied multiple different approaches for defining supertypes based on the same HLA alleles), and this correlation violates a key assumption of false discovery rate (FDR) and similar p value correction methods which assume independent or weakly dependent hypothesis tests. Conversely, correction methods that can account for correlations, such as permutation methods, cannot take important covariates (e.g., race/ethnicity) into consideration. Therefore, as an alternative approach, we present statistical significance in several different ways: uncorrected p values, FDR-adjusted q values using the method of Storey and Tibshirani,¹⁸ and p values corrected for multiple testing by permutation resampling.¹⁹ Briefly, the q value has a somewhat different interpretation than a p value in that the q value is the rate that associations categorized as statistically significant are truly non-significant, whereas a p value is interpreted as the rate that truly non-significant associations are categorized as statistically significant. We used the $\alpha=0.05$ threshold to define statistical significance for both p and q values.

Following adjustment for multiple comparisons, we then used an alternative approach to further examine the HLA supertypes found to have significant or borderline significant associations with HCV clearance. Specifically, we used MHCcluster 1.0 (<http://www.cbs.dtu.dk/services/MHCcluster/>)³⁶ to determine the clustering of the alleles included in the HLA supertypes with significant or borderline significant associations based on sequence features defining their peptide binding grooves. MHCcluster 1.0 analysis was conducted using the default parameters of 100 bootstrap calculations and 50,000 included peptides.

As a final analytic strategy, we studied the HLA supertypes with significant or borderline significant associations with HCV clearance after adjustment for multiple comparisons together in a single multivariable model. This model included not only the HLA supertypes but also those HLA alleles not part of these supertypes that were significantly associated with HCV clearance in our prior study of individual HLA alleles in this population.⁶

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

Funding for the current project was provided in part by R01AI057006 (H.D.S.) in addition to National Center for Research Resources CTSA grants UL1RR025750, KL2RR025749 and TL1RR025748, and from the National Institute of Allergy and Infectious Diseases grant R01A1052065 (A.A.K). Clinical data and specimens used in this study were collected by the Women's Interagency HIV Study (WIHS) Collaborative Study Group with centers (Principal Investigators) at New York City/Bronx Consortium (Kathryn Anastos); Brooklyn, NY (Howard Minkoff); Washington DC, Metropolitan Consortium (Mary Young); The Connie Wofsy Study Consortium of Northern California (Ruth Greenblatt); Los Angeles County/Southern California Consortium (Alexandra Levine); Chicago Consortium (Mardge Cohen); Data Coordinating Center (Stephen Gange). The WIHS is funded by the National Institute of Allergy and Infectious Diseases (U01-AI-35004, U01-AI-31834, U01-AI-34994, U01-AI-34989, U01-AI-34993, and U01-AI-42590) and by the Eunice Kennedy Shriver National Institute of Child Health and Human Development (U01-HD-32632). The study is co-funded by the National Cancer Institute, the National Institute on Drug Abuse, and the National Institute on Deafness and Other Communication Disorders. Funding is also provided by the National Center for Research Resources (UCSF-CTSI Grant Number UL1RR024131). This project has also been funded in part with federal funds from the Frederick National Laboratory for Cancer Research, National Institutes of Health, under Contract No. HHSN261200800001E. This research was also supported in part by the Intramural Research Program of the NIH, Frederick National Lab, Center for Cancer Research, and by the Einstein-Montefiore Center for AIDS Research (5P30AI051519-08).

The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

References

1. Armstrong GL, Wasley A, Simard EP, McQuillan GM, Kuhnert WL, Alter MJ. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med.* 2006; 144:705–714. [PubMed: 16702586]
2. Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *J Hepatol.* 2006; 45:529–538. [PubMed: 16879891]
3. Thimme R, Neumann-Haefelin C, Boettler T, Blum HE. Adaptive immune responses to hepatitis C virus: from viral immunobiology to a vaccine. *Biol Chem.* 2008; 389:457–467. [PubMed: 18953713]
4. Burke KP, Cox AL. Hepatitis C virus evasion of adaptive immune responses: a model for viral persistence. *Immunol Res.* 2010; 47:216–227. [PubMed: 20066508]
5. Chang KM, Rehmann B, McHutchison JG, Pasquinelli C, Southwood S, Sette A, et al. Immunological significance of cytotoxic T lymphocyte epitope variants in patients chronically infected by the hepatitis C virus. *J Clin Invest.* 1997; 100:2376–2385. [PubMed: 9410918]
6. Kuniholm MH, Kovacs A, Gao X, Xue X, Marti D, Thio CL, et al. Specific human leukocyte antigen class I and II alleles associated with hepatitis C virus viremia. *Hepatology.* 2010; 51:1514–1522. [PubMed: 20169624]
7. Sidney J, del Guercio MF, Southwood S, Engelhard VH, Appella E, Rammensee HG, et al. Several HLA alleles share overlapping peptide specificities. *J Immunol.* 1995; 154:247–259. [PubMed: 7527812]
8. Sidney J, Grey HM, Kubo RT, Sette A. Practical, biochemical and evolutionary implications of the discovery of HLA class I supermotifs. *Immunol Today.* 1996; 17:261–266. [PubMed: 8962628]
9. Lazaryan A, Lobashevsky E, Mulenga J, Karita E, Allen S, Tang J, et al. Human leukocyte antigen B58 supertype and human immunodeficiency virus type 1 infection in native Africans. *J Virol.* 2006; 80:6056–6060. [PubMed: 16731944]
10. Lazaryan A, Song W, Lobashevsky E, Tang J, Shrestha S, Zhang K, et al. Human leukocyte antigen class I superotypes and HIV-1 control in African Americans. *J Virol.* 2010; 84:2610–2617. [PubMed: 20032191]
11. Trachtenberg E, Korber B, Sollars C, Kepler TB, Hraber PT, Hayes E, et al. Advantage of rare HLA supertype in HIV disease progression. *Nat Med.* 2003; 9:928–935. [PubMed: 12819779]

12. Chang KM, Gruener NH, Southwood S, Sidney J, Pape GR, Chisari FV, et al. Identification of HLA-A3 and -B7-restricted CTL response to hepatitis C virus in patients with acute and chronic hepatitis C. *J Immunol.* 1999; 162:1156–1164. [PubMed: 9916747]
13. Wentworth PA, Sette A, Celis E, Sidney J, Southwood S, Crimi C, et al. Identification of A2-restricted hepatitis C virus-specific cytotoxic T lymphocyte epitopes from conserved regions of the viral genome. *Int Immunol.* 1996; 8:651–659. [PubMed: 8671652]
14. Greenbaum J, Sidney J, Chung J, Brander C, Peters B, Sette A. Functional classification of class II human leukocyte antigen (HLA) molecules reveals seven different supertypes and a surprising degree of repertoire sharing across supertypes. *Immunogenetics.* 2011; 63:325–335. [PubMed: 21305276]
15. Lund O, Nielsen M, Kesmir C, Petersen AG, Lundegaard C, Worning P, et al. Definition of supertypes for HLA molecules using clustering of specificity matrices. *Immunogenetics.* 2004; 55:797–810. [PubMed: 14963618]
16. Nielsen M, Lundegaard C, Blicher T, Peters B, Sette A, Justesen S, et al. Quantitative predictions of peptide binding to any HLA-DR molecule of known sequence: NetMHCIIpan. *PLoS Comput Biol.* 2008; 4:e1000107. [PubMed: 18604266]
17. Ou D, Mitchell LA, Tingle AJ. A new categorization of HLA DR alleles on a functional basis. *Hum Immunol.* 1998; 59:665–676. [PubMed: 9757949]
18. Storey JD, Tibshirani R. Statistical significance for genomewide studies. *Proc Natl Acad Sci U S A.* 2003; 100:9440–9445. [PubMed: 12883005]
19. Westfall, PH.; Young, SS. *Resampling-Based Multiple Testing: Examples and Methods for p-Value Adjustment.* Wiley-Interscience; 1993.
20. Harris RA, Sugimoto K, Kaplan DE, Ikeda F, Kamoun M, Chang KM. Human leukocyte antigen class II associations with hepatitis C virus clearance and virus-specific CD4 T cell response among Caucasians and African Americans. *Hepatology.* 2008; 48:70–79. [PubMed: 18537178]
21. Sette A, Sidney J. Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism. *Immunogenetics.* 1999; 50:201–212. [PubMed: 10602880]
22. Yu XG, Lichterfeld M, Chetty S, Williams KL, Mui SK, Miura T, et al. Mutually exclusive T-cell receptor induction and differential susceptibility to human immunodeficiency virus type 1 mutational escape associated with a two-amino-acid difference between HLA class I subtypes. *J Virol.* 2007; 81:1619–1631. [PubMed: 17121793]
23. Eyster ME, Sanders J, Goedert JJ. Viral clearance occurs very early during the natural resolution of hepatitis C virus infection in persons with haemophilia. *Haemophilia.* 2004; 10:75–80. [PubMed: 14962224]
24. Raghuraman S, Park H, Osburn WO, Winkelstein E, Edlin BR, Rehmann B. Spontaneous clearance of chronic hepatitis C virus infection is associated with appearance of neutralizing antibodies and reversal of T-cell exhaustion. *J Infect Dis.* 2012; 205:763–771. [PubMed: 22293431]
25. Cano P, Fan B, Stass S. A geometric study of the amino acid sequence of class I HLA molecules. *Immunogenetics.* 1998; 48:324–334. [PubMed: 9745009]
26. Tong JC, Tan TW, Ranganathan S. In silico grouping of peptide/HLA class I complexes using structural interaction characteristics. *Bioinformatics.* 2007; 23:177–183. [PubMed: 17090577]
27. Mohanapriya A, Nandagond S, Shapshak P, Kanguane U, Kanguane P. A HLA-DRB supertype chart with potential overlapping peptide binding function. *Bioinformation.* 2010; 4:300–309. [PubMed: 20978603]
28. Kanguane P, Sakharkar MK, Rajaseger G, Bolisetty S, Sivasekari B, Zhao B, et al. A framework to sub-type HLA supertypes. *Front Biosci.* 2005; 10:879–886. [PubMed: 15569626]
29. Doytchinova IA, Guan P, Flower DR. Identifying human MHC supertypes using bioinformatic methods. *J Immunol.* 2004; 172:4314–4323. [PubMed: 15034046]
30. Hertz T, Yanover C. Identifying HLA supertypes by learning distance functions. *Bioinformatics.* 2007; 23:e148–e155. [PubMed: 17237084]
31. Reche PA, Reinherz EL. Definition of MHC supertypes through clustering of MHC peptide-binding repertoires. *Methods Mol Biol.* 2007; 409:163–173. [PubMed: 18449999]

32. Sidney J, Peters B, Frahm N, Brander C, Sette A. HLA class I supertypes: a revised and updated classification. *BMC Immunol.* 2008; 9:1. [PubMed: 18211710]
33. Doytchinova IA, Flower DR. In silico identification of supertypes for class II MHCs. *J Immunol.* 2005; 174:7085–7095. [PubMed: 15905552]
34. Kuo CL, Feingold E. What's the best statistic for a simple test of genetic association in a case-control study? *Genet Epidemiol.* 2010; 34:246–253. [PubMed: 20025064]
35. Pybus OG, Cochrane A, Holmes EC, Simmonds P. The hepatitis C virus epidemic among injecting drug users. *Infect Genet Evol.* 2005; 5:131–139. [PubMed: 15639745]
36. Nielsen M, Justesen S, Lund O, Lundegaard C, Buus S. NetMHCIIpan-2.0 - Improved pan-specific HLA-DR predictions using a novel concurrent alignment and weight optimization training procedure. *Immunome Res.* 2010; 6:9. [PubMed: 21073747]

Associations of HLA supertypes and their component alleles with HCV viremia among HCV seropositive women - shown are those HLA supertypes that retained significant or marginally significant ($\alpha < 0.10$) false discovery rate (FDR)-adjusted p values after adjustment for multiple comparisons

Table 1

HLA supertype	^{1,2,3,4} HCV RNA positive (N=622)	HCV RNA negative (N=136)	PR (95% CI)	Unadjusted p value	False discovery rate (FDR)-adjusted q value	Permutation resampling-adjusted p value
DRB3 supertype	176 (32%)	14 (12%)	0.35 (0.20, 0.59)	0.000	0.008	0.000
DRB1*03:01	99 (18%)	8 (7%)	0.38 (0.19, 0.75)	0.005		
DRB1*13:02	87 (16%)	6 (5%)	0.35 (0.16, 0.77)	0.009		
DR8 supertype	47 (8%)	17 (15%)	1.84 (1.19, 2.85)	0.007	0.080	0.917
DRB1*08:01	3 (1%)	3 (3%)	2.89 (1.17, 7.13)	0.021		
DRB1*08:02	3 (1%)	3 (3%)	2.56 (1.20, 5.47)	0.015		
DRB1*08:04	36 (6%)	9 (8%)	1.36 (0.74, 2.50)	0.324		
DRB1*08:06	5 (1%)	2 (2%)	2.14 (0.65, 7.07)	0.214		
DR1*01 supertype	52 (9%)	21 (18%)	1.70 (1.14, 2.56)	0.010	0.098	0.336
DRB1*01:01	41 (7%)	17 (15%)	1.74 (1.12, 2.68)	0.013		
DRB1*01:03	3 (1%)	0	5	5		
DRB1*10:01	9 (2%)	5 (4%)	1.96 (0.98, 3.95)	0.058		
A supertype	132 (24%)	44 (38%)	1.64 (1.18, 2.29)	0.004	0.080	0.115
DRB1*01:01	41 (7%)	17 (15%)	1.74 (1.12, 2.68)	0.013		
DRB1*01:02	33 (6%)	9 (8%)	1.38 (0.76, 2.50)	0.296		
DRB1*04:01	29 (5%)	6 (5%)	0.93 (0.44, 1.95)	0.844		
DRB1*04:04	20 (4%)	5 (4%)	0.92 (0.41, 2.07)	0.850		
DRB1*04:05	10 (2%)	5 (4%)	2.05 (1.01, 4.17)	0.048		
DRB1*04:08	1 (0%)	0	e	e		
DRB1*10:01	9 (2%)	5 (4%)	1.96 (0.98, 3.95)	0.058		
DRB1*14:02	1 (0%)	1 (1%)	1.91 (0.46, 7.83)	0.370		

PR: Prevalence Ratio; 95% CI: 95% Confidence Interval

¹ Supertypes were defined as in prior studies by Greenbaum et al.¹⁴ (DRB3 supertype), Lund et al.¹⁵ (DR8 supertype), Neilson et al.¹⁶ (DRB1*01 supertype), and Ou et al.¹⁷ (A supertype)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

- 2 Percentages reflect the number of women who were homozygous or heterozygous for the indicated supertype or genotype amongst those with complete DRB1 genotype information (N=675)
- 3 Analyses were conducted using log-binomial models with adjustment for race/ethnicity (non-Hispanic Black, non-Hispanic White, Hispanic)
- 4 Associations of HLA supertypes that did not show significant or marginally significant associations after adjustment for multiple comparisons are shown in Supplementary Table 1
- 5 No estimate due to model non-convergence

Table 2

Independent associations of HLA supertypes and HLA alleles with HCV viremia among HCV seropositive women - shown are those HLA supertypes that retained significant or marginally significant ($\alpha < 0.10$) false discovery rate (FDR)-adjusted q values after adjustment for multiple comparisons and those alleles not part of these supertypes that were significantly associated with HCV viremia in our prior study in this population¹

HLA supertype	PR (95% CI)	p value
DRB3	0.44 (0.25, 0.76)	0.004
DR8	1.76 (1.14, 2.72)	0.01
DR1*01	1.50 (0.90, 2.50)	0.12
A	1.49 (0.97, 2.28)	0.07
Individual alleles		
DRB1*07:01	1.73 (1.18, 2.53)	0.005
B*57:01	1.44 (0.70, 3.00)	0.32
B*57:03	1.89 (1.18, 3.02)	0.008
C*01:02	1.32 (0.72, 2.43)	0.37

PR: Prevalence Ratio; 95% CI: 95% Confidence Interval

¹ Results from a single multivariable log-binomial model with adjustment for race/ethnicity (non-Hispanic Black, non-Hispanic White, Hispanic)

Table 3

Previously described HLA supertypes by laboratory (see Methods for inclusion criteria, and Supplementary Table 2 for a list of individual HLA alleles included in each supertype).

Class I	Supertype (number of HLA genotypes included in the supertype)¹	Ref
Doytchinova et al.	A2 (15), A3 (22), A24 (3), B7 (33), B27 (23), B44 (25), C1 (14), C4 (12)	29
Hertz & Yanover	A1 (10), A2 (8), A3 (6), A24 (3), B7 (7), B27 (3), B39 (4), B44 (6)	30
Lund et al.	A1 (10), A2 (8), A3 (8), A24 (3), B7 (12), B27 (8), B39 (4), B44 (11), B58 (6), B62 (3)	15
Reche et al.	A2 (6), A3 (6), A24 (2), AB (2), B7 (6), B15 (2), B27 (2), B44 (2), B57 (3), Bx (3)	31
Sidney et al. ²	A1 (10), A2 (9), A3 (14), A24 (3), A01/A24 (2), B7 (24), B27 (19), B44 (16), B58 (8), B62 (6)	32
Class II		
Doytchinova & Flower	DR1 (10), DR3 (2), DR4 (14), DR5 (18), DQ1 (8), DQ3 (4)	33
Greenbaum et al.	Main DR (6), DR4 (3), DRB3 (2), Main DQ (4), DQ7 (2)	14
Lund et al.	DR1 (2), DR4 (5), DR8 (4), DR11 (3), DR13 (4), DR15 (2)	15
Nielsen et al.	DR1*01 (3), DR1*03 (2), DR1*04 (5), DR1*08/11 (2)	16
Ou et al.	A (8), De (5), E (6), Dr (8)	17

¹Restricted to supertypes that included at least two HLA genotypes present in our study population

²The supertypes defined by Sette and Sidney²¹ are very similar to those defined by Sidney et al.³², this latter study was included in the current investigation