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Spontaneous control of HCV is associated with the expression of *HLA-B*57* and preservation of targeted epitopes

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Abstract

Background and Aims—HLA Class I alleles are linked to spontaneous control of HCV and HIV-1, but for HCV the roles of particular alleles and corresponding CD8+ T-cell responses remain incompletely defined. We aimed to determine the correlations between these alleles and natural outcome of HCV and determine associated key T cell responses.

Methods—In a cohort of HCV individuals we determined HLA Class I alleles, HCV outcome, T-cell responses, and examined sequence data for mutational changes within key epitopes.

Results—Carriage of *HLA-B*57* was associated with a higher rate of viral clearance [RR=2.0, 95% C.I. 1.2–3.4] while *HLA-B*08* was associated with a lower rate [RR=0.34, 95% C.I. 0.1–0.9]. Two *HLA-B*57* restricted T-cell epitopes were targeted in spontaneous clearance; subjects with chronic viremia expressing *HLA-B*57* harbored HCV strains with a high frequency of mutations

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in key residues. *HLA-B*57*-mediated escape was supported by diminished immune recognition of these variants and acute HCV infection revealing viral evolution towards less recognized variants. Analysis of a genotype 1b strain from a single-source HCV outbreak in which *HLA-B*57* was not protective revealed sequence variations that interfere with immunogenicity, thereby preventing *HLA-B*57*-mediated immune pressure.

Conclusions—Our data indicate a role of *HLA-B*57*-restricted CD8+ T cell responses in mediating spontaneous clearance and evolution in HCV infection, and viral strains containing epitope variants that are less recognized abrogate the protective effects of *HLA-B*57*. The finding that *HLA-B*57*-mediated antiviral immunity is associated with control of both HIV-1 and HCV suggests a common shared mechanism of a successful immune response against persistent viruses.

Keywords

CD8 T cell; HLA-B57; spontaneous clearance; viral escape

Introduction

Over 170 million persons worldwide are infected with hepatitis C virus (HCV), a leading cause of liver-related morbidity and mortality.¹ Following HCV infection, chronic viremia is established in the majority of individuals while some eradicate the virus spontaneously. The ultimate outcome of HCV infection is decided by interplay between host immune responses and viral evasion mechanisms. Specifically, CD8+ T cell responses recognizing viral antigens in the context of HLA class I are primed during acute HCV infection and considered critical for clearance.² In those who proceed to high levels of viral replication, lack of control may be attributed to development of viral mutations resulting in diminished recognition by CD8+ responses.² These mutations occur in a predictable fashion based on HLA expression and significantly drive HCV evolution.³

Since CD8+ responses mediate control of HCV and other human viral infections, genetic differences in HLA class I alleles may modulate their natural outcomes. In HIV-1 infection, the *HLA-B*57*, *HLA-B*58*, and *HLA-B*27* alleles are associated with low viral loads and slower clinical progression.⁴ Immunodominant HIV-1 specific epitopes are recognized by CD8+ responses restricted by these alleles⁵ and immunological escape results in loss of viral control.⁶ On a population level, mutational change driven by these responses may result in dismantling of favorable HLA associations.⁷

In HCV, data have conflicted regarding which class I HLA alleles are associated with spontaneous clearance. In a single-source outbreak of HCV among young Irish women, carriage of either of the alleles *HLA-A*03* and *HLA-B*27* was associated with HCV clearance.⁸ In contrast, studies of cohorts infected with various HCV strains yielded different alleles associated with spontaneous clearance, including *HLA-B*57*.^{9, 10}

The aims of this study were to correlate class I HLA with HCV outcome, determine CD8+ T cell responses restricted by associated alleles, and explore mutational escape within these epitopes. We find that carriage of *HLA-B*57* and the recognition of particular *HLA-B*57*-restricted T-cell responses are important for the spontaneous control of HCV. By examining viral sequence differences and potential escape from T-cell recognition, we reconcile the disparate results from prior studies regarding this allele and expand its protective effect from HIV-1 to a second viral infection.

Materials and Methods

Ethics statement and study subjects

All subjects gave written informed consent for this study, approved by each Institutional Review Board. Subjects were enrolled from outpatient clinics from 1998 to 2007 from three hospitals (Massachusetts General Hospital, Brigham and Women's Hospital and Lemuel Shattuck Hospital). All subjects were HCV antibody positive. Race/ethnicity was self-reported and categorized as non-Hispanic Caucasian, non-Hispanic African-American, Hispanic, Asian, Native American, or Other.

Viral load, HCV genotype, and serotype testing

Plasma HCV and HIV-1 viral RNA titers were measured using the Cobas Amplicor Monitor assay 2.0 (detection limit of 400 HIV-1 RNA copies/mL and 600 (2.8 log) HCV IU/mL, Roche Molecular Systems, Pleasanton, CA). Qualitative HCV viral RNA assays with a detection limit of 60 (1.8 log) IU/mL (Roche) were utilized for confirmation of lack of viremia. Spontaneous control of HCV was defined as viral titers less than 600 IU/mL on two consecutive plasma specimens at least three months apart in the absence of prior anti-HCV treatment, and confirmed as less than 60 IU/mL by qualitative assay if plasma was available. Chronic infection was characterized by detectable viremia. For those identified during the acute phase, HCV RNA results from later than one year after enrollment were used for classification of HCV control. Spontaneous control of HIV-1 was defined as HIV viral load less than 2000 copies/mL in the absence of antiretroviral therapy.⁴ For subjects on antiretroviral therapy (ART), the viral setpoint was defined as the mean of known HIV RNA measurements prior to ART, taken at least 1 year after HIV-1 acquisition. HCV genotyping was determined by INNO-LiPA HCV II (Bayer, Tarrytown, NY) and/or direct sequencing of the virus. Serotype was determined using the Murex HCV Serotyping 1–6 assay (Abbott, Wiesbaden, Germany).

HLA typing

The gene fragment spanning exon 2, intron 2 and exon 3 was amplified using locus-specific primers for HLA-A, -B and -C separately and the PCR products were used as the template for the sequencing reaction. For each HLA gene, two subsequent sequencing reactions (one for exon 2 and one for exon 3) were performed using exon-specific sequencing primers. The sequencing analysis was performed using the ABI Big Dye Terminator Cycle Sequencing Kit and ABI3730xl DNA analyzer (Applied Biosystems, Foster City, CA). HLA alleles were assigned based on the sequence database of known alleles with the help of the ASSIGN software developed by Conexio Genomics (Conexio Genomic, Western Australia). Ambiguous heterozygous genotypes were resolved by additional PCR and sequencing procedures using allele-specific PCR primers to selectively amplify only one of the two alleles.

Generation of CD8+ T cell lines

PBMC were stimulated with peptides and cultured with interleukin-2 to generate polyclonal cell lines, as previously described.¹¹

IFN-gamma ELISpot for comprehensive T-cell screening and comparison of peptide variant recognition

IFN-gamma ELISpot assays were performed as previously described.¹¹ Comprehensive determination of responses against overlapping peptides representing a strain of genotype 1a HCV (H77) was performed.¹¹ Peptide dilution ELISpot assays on T cell lines determined recognition at varying log₁₀ concentrations and compared the reference epitope and variants.

Decreased recognition was defined as greater than two-fold fewer SFCs produced at two or more concentrations of the variant peptide.

Tetramer staining and intracellular cytokine staining

*HLA-B*5701* tetrameric complexes containing the two peptides E2-541-NTRPPLGNW, termed NW9, and NS5-2629-KSKKTPMGF, termed KF9, were obtained commercially (Beckman Coulter, Fullerton CA). Tetramer and intracellular cytokine stainings were performed as previously described¹¹ with data collection on a FACSCalibur and analysis performed via FlowJo software (TreeStar Software, version 8.8.2).

Sequencing

Nested RT-PCR and sequencing of the predominant HCV quasispecies was performed from plasma using primers specific for genotype as previously described.^{3, 12}

Statistical analysis

Univariate associations between demographic factors, class I HLA alleles and the outcome of HCV were assessed using chi-square or Fisher's exact tests. Fisher's exact tests were used for associations between expression of *HLA-B*57* and viral mutations. Multivariable logistic regression modelling determined the independent effects of variables on HCV outcome. To reduce the possibility of type I and type II error, we restricted analyses to alleles present at sufficient frequency to detect a significant difference (greater than 8.3%, based on a calculation for 80% power) and alleles previously associated with outcome of HCV infection.^{8, 9} Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC) and graphs were generated using GraphPad Prism 3.0 (GraphPad Software, San Diego, CA).

Results

Demographic characteristics associated with spontaneous clearance of HCV

In a cohort of HCV antibody positive individuals (n=346), we examined factors potentially associated with HCV spontaneous clearance. Of these, age less than 45 years at baseline, not being of African-American race/ethnicity and risk other than blood transfusion were associated with viral clearance in univariate analyses (all with $p < 0.05$). The predominant strain of HCV was genotype 1a (Table 1).

Carriage of *HLA-B*57* is associated with spontaneous clearance of HCV

Next, we examined the frequency of major class I HLA alleles in our cohort and compared them to North American Caucasians (N.A. Bethesda, n=307, found at <http://www.allelefrequencies.net>¹³). *HLA-A*68* and *HLA-B*57* were overrepresented in the Boston cohort, explained by the inclusion of HIV positive and non-Caucasian populations (Supplementary Figure).

Univariate associations between relevant HLA class I alleles and HCV outcome are shown in Supplementary Table 1 and Figure 1 (as risk ratios). The allele significantly associated with clearance was *HLA-B*57* (34% vs. 17%, RR = 2.0 [95% C.I. 1.2–3.4]). Subjects carrying *HLA-A*11* or *HLA-B*35* showed a trend to clear the virus versus those without *HLA-A*11* or *HLA-B*35* (*HLA-A*11*: 32% vs. 18%, RR = 1.8 [95% C.I. 1.1–3.2]; *HLA-B*35*: 1.65 [95% C.I. 1.0–2.7]). In contrast, *HLA-B*08* was associated with a lower rate of viral clearance (7% vs. 21%, RR = 0.34 [95% C.I. 0.1–0.9]). Subjects with *HLA-B*57* had a similar distribution of gender and race when compared to the entire cohort. In a multivariable logistic regression, non-Hispanic African-American race, risk other than blood

transfusion, expression of *HLA-B*57* and non-expression of *HLA-B*08* were independent predictors of spontaneous clearance.

Dual control of HIV and HCV associated with *HLA-B*57*

Our cohort contained a significant proportion of HIV-infected individuals (45.2%), due to earlier focused recruitment.^{11, 14} When stratifying by HIV status, the *HLA-B*57* protective effect was more pronounced in those without HIV-1 (HIV negative: OR 4.8 [95% CI 1.65 – 14.3], HIV-1 positive: OR 1.5 [95% CI 0.53–4.12]).

We explored the association of *HLA-B*57* with control of HIV and/or HCV in co-infected individuals. HIV-1 viral setpoints were determined in 106 of 155 persons with HIV-1 and HCV. The prevalence of *HLA-B*57* in each subgroup is shown in Figure 2A; in a subgroup with dual control of both viruses (n=7), *HLA-B*57* was overrepresented compared to the remainder of the cohort (RR 6.0 [95% CI 1.40–26.0], p=0.03). *HLA-B*27*, another potential protective allele, was found in 1 of the individuals controlling both viruses.

To explore whether HIV-1 control was associated with HCV clearance in the subset of coinfecting subjects, baseline HIV viral loads in those with and without HCV clearance were compared. HCV clearance was associated with a significantly lower baseline HIV-1 viral setpoint (median 2513, interquartile range [IQR] 773–129,000) compared to those with HCV chronicity (median 43800, IQR 10300–121,000; Mann-Whitney, p=0.036, Figure 2B).

Two *HLA-B*57*-restricted CD8+ T cell responses dominate in *HLA-B*57* positive persons with HCV clearance

We examined HCV-specific CD8+ T cell responses in the PBMC from 16 individuals carrying *HLA-B*57*, 8 exhibiting spontaneous clearance and 8 chronically infected. In PBMC of *HLA-B*57* persons with spontaneous clearance, responses against two epitopes (E2-541-NTRPPLGNW-549, termed NW9, NS5-2629-KSKKTPMGF-2637, termed KF9) were frequently detected; 5 of 8 recognized at least one of these two epitopes and 3 recognized both simultaneously, Supplementary Table 2). HLA-restriction was confirmed using methods previously described and binding of specific T cells with tetrameric *HLA-B*5701* (data not shown).¹⁵

We next tested PBMC of individuals chronically infected with HCV (n=8) who carried *HLA-B*57*. Responses against the E2-NW9 and the NS5-KF9 were not detected in *ex vivo* ELISpot. Stimulation of PBMC with peptide and interleukin-2 revealed expansion of responses specific to either the E2-NW9 and NS5-KF9 epitopes in 4 of 8 subjects tested, both by ICS and/or tetramer staining. All 4 with detectable memory responses were infected with genotype 1a, corresponding to the reference strain (Supplementary Table 2). Overall at least one of E2-NW9 and NS5-KF9 was detected in 9 of 16 HCV+ individuals expressing *HLA-B*57*. When restricting to subjects with evidence of genotype 1 infection, 7 of 10 subjects had evidence of these responses.

Two individuals, one with spontaneous clearance and one with chronic infection, recognized another *HLA-B*57*-restricted response (NS4-1801-LTTSQTLLF-1809) but did not recognize the other two *HLA-B*57* restricted epitopes. Overall, only 2 of 16 patients recognized this latter epitope, suggesting the NS4 response is subdominant.

Evidence of escape from immunodominant *HLA-B*57*-restricted responses in chronically-infected individuals

To detect *HLA-B*57*-mediated viral evolution, we analyzed sequences derived from 134 chronically infected subjects with genotype 1a infection, comparing those with *HLA-B*57*

versus those without. Variations were highly enriched in those carrying the *HLA-B*57* allele for the two dominant *HLA-B*57*-restricted epitopes³: E2-541-NTRPPLGNW-549, odds ratio = 39, $p < 0.0001$; NS5-2629-KSKKTPMGF-2637, odds ratio = 13, $p = 0.0002$, Figure 3A). Most of the variation associated with *HLA-B*57* was contained in particular residues: for E2-546, L to X (A, Q, R, or M), OR = 16, $p < 0.0001$; NS5-2633, T to X (A, N, S, or V) OR=6.3, $p = 0.0093$ (Figure 3B). In contrast, no substantial amino acid change was found in chronically infected *HLA-B*57+* individuals against the NS4-1801-LTTSQTLLF-1809 epitope (OR 0.5, $p = 0.7$).

To determine the effect of viral polymorphisms on T cell recognition, epitope-specific cell lines were tested by peptide dilution IFN- γ ELISpot. Several variants demonstrated decreased functional recognition, consistent with escape from *HLA-B*57*-restricted responses for the E2-NW9 and the NS5-KF9 responses. All 4 major variants at position 546 exhibited partial cross-reactivity at highest concentrations but recognition decreased markedly at lower concentrations (Figure 3C). For the NS5-KF9 epitope, the infrequent M2635L variant showed equivalent recognition, but primary changes at two other positions (K2629N and T2633N) showed decreased cross-recognition (Figure 3D). The T2633A mutation resulted in complete abrogation of recognition (Figure 3D). We also examined the impact of polymorphisms within the NS5 epitope on *HLA-B*57*-restricted CD8 T cell recognition for viral sequences derived from non-B57 patients. The T2633V mutation met criteria for decreased recognition, while two other mutations (S2630A and K2632R) did not (Figure 3E). Cell lines specific for the NS5 epitope generated from two other *HLA-B*57* patients showed comparable results (data not shown). These data highlight that the preservation of these *HLA-B*57* epitopes is critical for immune recognition.

HLA-B*57-mediated protection is dependent on preservation of dominant HLA-B*57 epitopes

*HLA-B*57* was not protective in a single source outbreak that occurred in Irish women infected with HCV.⁸ Sequence data revealed mutations within the infecting strain (GenBank accession number: AF313916) in both the E2 and the NS5 *HLA-B*57*-restricted epitopes. The E2 mutation L546R in the infecting sequence is identical to a variant found in chronic HCV genotype 1a infection associated with decreased recognition (Figure 4A). The NS5 epitope included two mutations, T2633N (representing the consensus sequence for 1b viruses) and S2630K. While the single mutation T2633N shows partial cross-recognition by the prototype T-cell lines, the double S2630K-T2633N mutant from the Irish outbreak is not recognized at all even at the highest concentrations (Figures 4B and 4C). A binding prediction model using *HLA-B*5801*, a surrogate molecule sharing binding motifs with *HLA-B*57* (BIMAS, available at <http://www.bimas.cit.nih.gov>), showed a 100-fold predicted shorter dissociation time for the double mutant compared to the original 1a-derived epitope, while the 1b consensus sequence maintained binding capability compared to the 1a strain. Thus the Irish strain would avoid recognition by these two *HLA-B*57*-restricted responses.

Persistent outcome of acutely infected or re-infected individuals may be related to polymorphisms in HLA-B*57 epitopes at baseline or viral escape from HLA-B*57-restricted responses

We examined T cell responses in 3 *HLA-B*57* positive individuals acutely infected or re-infected with HCV. Demographic, clinical and HLA data are summarized in Table 2. Subject AC-1's clinical course was previously reported as acute infection with a genotype 1b strain with an ultimately chronic outcome.¹⁶ *Ex vivo* ELISpot and peptide-stimulated cell lines at >25 timepoints between 4 months and 2.5 years post infection during the disease course did not identify any *HLA-B*57*-restricted response, including the E2-NW9 and NS5-

KF9 epitopes (Table 2). For NS5-KF9, the initial strain contained the polymorphism T2633N as its predominating sequence and no response was detected via *ex vivo* ELISpot or peptide-stimulated lines against the autologous variant. Failure to generate these *HLA-B*57*-restricted responses was associated with chronicity, despite vigorous responses restricted by *HLA-A1* and *HLA-A2*.¹⁶

Next, we examined subject AC-2 who was acutely infected with genotype 1a virus. After initial identification with symptomatic hepatitis, this patient experienced a period of low level or undetectable viremia, but virus re-appeared at week 32 after presentation (Figure 5A). *Ex vivo* testing of PBMC by ELISpot and confirmed by tetramer staining revealed a response against the NS5 KF9 epitope, but not the E2 NW9 epitope. Viral sequences were available at week 3 before the extended period of viral control and at week 58 after establishment of persistent viremia. Phylogenetic analysis based on a 1400 kb segment of NS5 determined that week 58 virus was the identical HCV strain isolated at week 3 (data not shown). The dominant viral quasispecies at week 3 already demonstrated changes from consensus 1a sequence within each of the two *HLA-B*57*-restricted epitopes (L546Q and T2633I, Figure 5B). At week 58, the virus exhibited additional changes within the sequence targeted by the NS5 response at positions K2629E and K2632R (Figure 5B, Table 2). A cell line was generated enriched for CD8 cells targeting the NS5 epitope and when tested against the variants, dilution ELISpot revealed diminished recognition of the week 3 and the week 58 sequences, meeting criteria for escape from this response (Figure 5C). To exclude the possibility that short-term *in vitro* T-cell lines are not representative of the recognition pattern of T-cells *in vivo*, testing of the variant peptides for the NS5 epitope directly *ex vivo* found comparable results (Figure 5D). Thus, chronicity was associated with lack of priming of the *HLA-B*57*-restricted E2 response and mutational escape in the region targeted by the *HLA-B*57*-restricted NS5 response.

Finally, we examined cross-genotype immunity using PBMC from a third acutely infected subject. Subject AC-3 exhibited an initial outcome of HCV spontaneous clearance. Serotyping indicated prior infection with genotype 1 virus. HCV RNA remained undetectable for a period of >500 days and testing of PBMC revealed *ex vivo* responses against both the E2 NW9 and the NS5 KF9 epitopes. AC-3 experienced recurrent viremia with genotype 3a virus (suspected to be re-infection).¹⁴ Sequencing of regions corresponding to the genotype 1a epitopes revealed significant polymorphisms at multiple sites (mutations away from 1a consensus are underlined: E2: SLRPPSGRW and NS5: TSKKTPLGF). Testing of a cell line enriched for NS5-2629-KF9 specific cells that reacted against the 1a reference strain showed no cross-reactivity with the infecting 3a sequence (data not shown).

Thus for these three subjects we observed that viral persistence was associated with either an absence of the immunogenic epitope sequence at baseline and/or with viral escape from *HLA-B*57* restricted CD8 T cell responses.

Discussion

While CD8+ T cell responses are implicated in immune-mediated control of HCV, the precise correlates of protection remain incompletely defined. Conflicting data have been reported regarding the particular HLA class I alleles associated with spontaneous control.^{8–10} In a U.S. cohort where genotype 1a was the predominating circulating virus, we found that *HLA-B*57* was associated with spontaneous HCV clearance. Immunologic data support the role of two particular *HLA-B*57*-restricted T cell specificities in mediating immune pressure. These *HLA-B*57*-mediated responses additionally drive mutational evolution of genotype 1a virus during persistence, evidenced by cross-sectional analysis of sequences

derived from chronically infected individuals and observing immunologic escape in an acutely infected patient. Finally, by examining the sequences of 1b strains in a single-source outbreak where *HLA-B*57* was not protective, we conclude that transmission of HCV strains with pre-existing mutations in *HLA-B*57* epitopes may blunt virologic control mediated by *HLA-B*57*-restricted T-cell responses. Taken in sum, our data reconcile disparate results in prior studies examining the potential beneficial role of this allele in HCV infection.

While we did not account for multiple comparisons, the validity of our primary HLA associations is suggested by striking parallels to two studies in cohorts with exposure to a variety of differing strains.^{9,10} In contrast, McKiernan et al. failed to detect a protective effect of *HLA-B*57* in a single-source outbreak among Irish women exposed to genotype 1b HCV via contaminated immunoglobulin.⁸ The virus in the Irish outbreak contains polymorphisms that, in the case of the NS5 epitope, are different from the general genotype 1b consensus sequence and would not allow recognition by the *HLA-B*57*-restricted responses characterized in this report. Therefore, while HLA-associations found in single-source outbreaks may be more easily detected due to the uniformity of the infecting strain, findings from such analyses may be less generalizable to other populations as the results may be influenced by the pre-existence of viral escape mutations or subtype-specific variants lacking immunogenicity in the source virus. In sum, our findings suggest that associations of certain class I HLA alleles with HCV viral outcomes are affected by the predominant circulating strains.

In a small subgroup of dually infected subjects, we find that *HLA-B*57* is overrepresented in those who spontaneously control both HIV-1 and HCV. It is possible that HIV-1 nonprogression related to *HLA-B*57* and preservation of helper T cell responses may have preserved HCV-related immunity.¹⁴ However, there may also be a common mechanism by which the allele *HLA-B*57* confers enhanced immunity against viruses. *HLA-B*57* belongs to the B58 supertype family of HLA molecules and is the allele most consistently implicated in the control of HIV-1 and lack of disease progression.¹⁷ There are several lines of evidence supporting direct roles of CD8+ T cell responses restricted by *HLA-B*57* mediating control of HIV-1 and lack of disease progression. These data include reproducibility of immunodominant responses restricted by these alleles that target proteins expressed early in HIV-1 infection,¹⁸ the association of escape from these particular responses with loss of control of virus^{19, 20} and evidence on a population level of selective pressure applied by these responses.⁷ The *HLA-B*57* association with control of HIV-1 is remarkably consistent among cohorts even accounting for differential HLA subtypes (*B*5701* in the West, *B*5703* in Africa) and infections with substantially different circulating viruses.²¹ While HIV-1-specific *HLA-B*57*-restricted epitopes across HIV-1 clades are conserved, HCV is more diverse. Chuang et al. in a smaller study found an overrepresentation of *HLA-B*57* among blood donors with evidence of spontaneous control of HCV in West Africa, where genotype 2 predominates²² but putative protective T cell responses for this genotype are unknown. Future studies should explore whether the greater viral diversity of HCV compared to HIV-1 dilutes CD8-mediated protective effects.

As in HIV-1, restriction of particular epitopes may be a key factor in determining viral control in HCV. Targeting of regions associated with viral fitness is one hypothesis that has been explored for control of HIV-1.²³ Mutations in these key epitopes may result in a loss of viral fitness for HCV, as has been described for the *HLA-B*27*-restricted epitope contained within NS5.²⁴ Particular residues in genotype 1 virus (E2-546, NS5 2629 and 2633) identified in this study may be associated with fitness costs that can be explored in entry or viral replication models.

Other hypotheses have been proposed regarding why *HLA-B*57* are associated with favorable outcomes from studies of HIV-1, including exceptional efficiency of epitope presentation to T cells²⁵ and differential T cell functionality.²⁶ Regarding the hypothesis that enhanced cross-reactivity may explain favorable HLA alleles,²⁷ enhanced cross-reactivity by responses from HLA alleles favorable for HIV-1 control (*HLA-B*57*, *HLA-B*27*, and *HLA-A*03*),²⁸ and for the immunodominant *HLA-B*27*-restricted response in HCV²⁹ has been described. Future studies parallel to those in HIV-1 should be performed using the responses identified in this study and may reveal shared mechanisms of viral control.

Other host genes may also be responsible for this shared protective effect. Genetic association studies have suggested that various killer cell Ig-like receptor (KIR) alleles modulate the protective effect of HLA in HIV-1;³⁰ however, these alleles are different than the KIR allele that correlates with HCV protection.³¹ It is also possible that *HLA-B*57* is in linkage disequilibrium (LD) with other genes that would mediate HCV control. While we did not perform HLA-class II analysis, LD of *HLA-B*57* is closest with *HLA-DRB1*07*, an allele so far not appreciably linked to control of HCV.³²

We did not explore why in the present study and others no association between viral clearance and the allele *HLA-B*27* was detected^{9, 10} in distinction to the association observed in Ireland.⁸ An immunodominant *HLA-B*27*-restricted response has been identified and viral divergence towards mutations associated with immunologic escape was seen in *HLA-B*27* positive individuals.³³ Since the *HLA-B*27*-restricted epitope within NS5 is preserved as the consensus sequence in both subtypes 1a and 1b, viral differences do not account for the lack of association in this study compared to others.³⁴

In summary, we report an association between the class I allele *HLA-B*57* and HCV outcome and provide data that implicate two particular CD8+ T cell responses as relevant to control of HCV. These findings suggest a protective mechanism of *HLA-B*57* that is common to both HIV-1 and HCV; however, for HIV-1 the protective effect extends across clades while for HCV this protective effect may be diluted even by minor strain-specific polymorphisms. If a small number of changes in key epitopes can abrogate the protective effect of a class I allele, evolution towards escape within key epitopes may result in dismantling of HLA-associations, as observed for *HLA-B*51* and HIV-1 in the Japanese population.⁷ Thus, diversity and escape from class I responses represent major challenges for immunity against these viruses based on CD8 T cell responses.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

(ALT)	alanine aminotransferase
(ART)	antiretroviral therapy
(ELISPOT)	enzyme-linked immunospot
(IDU)	intravenous drug user
(LP)	lymphoproliferative
(SFC)	spot-forming cell

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Figure 1. Association of HLA Class I types with HCV outcome

Risk ratios with 95% confidence intervals representing the effect size of the association of various HLA types and HCV chronicity or clearance. An asterisk (*) represents p values less than 0.05.

Figure 2. Association of outcomes of both HIV-1 and HCV in mono- and co-infected individuals
 (A) Prevalence of *HLA-B*57* in various subgroups present in this study. Spontaneous control of HCV was defined as viral titers less than 600 IU/mL on two consecutive plasma specimens at least three months apart in the absence of any history of anti-HCV treatment. If subjects were identified during the acute phase, HCV RNA values greater than 1 year from the acute phase of infection were used for classification. All with HCV control includes HIV positive subjects. Spontaneous control of HIV-1 was defined as HIV-1 viral load less than 2000 copies/mL in the absence of antiretroviral therapy. (B) HIV-1 viral loads were measured at time of enrollment (if off therapy) or were determined from measured values prior to starting antiretroviral therapy (see methods). Co-infected persons with HCV clearance exhibited lower HIV-1 viral titers compared to co-infected persons with HCV persistence.

Figure 3. Polymorphisms in HCV genotype 1a virus associated with *HLA-B*57* and relative recognition by CD8+ T cell responses

(A) Association of polymorphisms with *HLA-B*57* carriage for 2 of the 3 epitopes tested, revealing enrichment of changes in the E2 NW9 and NS5 KF9 epitopes targeted by *HLA-B*57*-restricted responses. The analysis counts mutations within the epitope at any position, as described in reference (3). (B) Specific variants noted in the two epitopes listed, stratified by *HLA-B*57* status. X represents multiple variants at that site; for E2-NW9 at position 546, L to X (A, Q, R, G, or M); for NS5-KF9, changes include position 2629 K to X (Q, N, S), position 2630 S to X (A, H, R, C, T), position 2633, T to X (A, N, S, or V). For the NS5 epitope, this analysis reveals a change in position NS5-2632 (K to R) that was found more frequently in non-B57 individuals. (C–E) Peptide-titration curves showing the number of spot-forming cells (SFC) in an ELISpot containing enriched cell lines specific for (C) E2-NW9 and (D) NS5-KF9 (square) versus peptides containing variants found in *HLA-B*57* individuals, while (E) shows a cell line enriched for NS5-KF9 specific cells tested against variants found in non-*HLA-B*57* individuals.

Figure 4. HLA-B*57-restricted responses against genotype 1a virus do not recognize peptides corresponding to the infecting virus from a genotype 1b outbreak
 Peptide titration curves of highly enriched cell lines specific against the (A) E2-NW9 and (B) NS5-KF9 epitope against the reference sequence, genotype 1b consensus, and the corresponding sequence from the Irish strain. Panel (C) is an intracellular cytokine staining for interferon-gamma, testing the cell-line enriched for CD8 T-cells recognizing NS5-KF9 against the reference sequence, the genotype 1b consensus, and the corresponding sequence from the Irish strain.

A AC-2 HLA A1,2 B51,57 Cw6,15

B

Weeks after presentation	E2 NW9 NTRPPLGNW	NS5 KF9 KSKKTPMGF
Week 3	Q.....	I.....
Week 58	R.....	E.....RI.....

Figure 5. Longitudinal analysis of an acutely infected individual (AC-2) with *HLA-B*57*-restricted response against NS5

Panel A shows the clinical course: after a detectable viral load, viral control was seen for several months. Virus became detectable and sequencing revealed this was the same initial strain, mutated in the regions that may be targeted by E2-NW9 and NS5-KF9 (bulk sequence indicating the majority sequence is shown in Panel B). Panel C shows the effect of the mutations found within NS5-KF9 on T cell recognition; the T to I mutation at position 2633 suggested partial recognition, the additional mutations at positions 2629 and 2632 that emerged at week 58 resulted in abrogation of recognition from the NS5-KF9 response. Panel D shows *ex vivo* data at a timepoint with a relatively large response, showing similar patterns of recognition of the autologous mutations and indicating that stimulation did not select only the most avid and/or least cross-reactive T-cells in cultured cell lines.

Table 1
Characteristics of MGH HCV cohort, classified by virologic outcome

Characteristic	HCV Antibody Positive	
	Persistent infection (n=280)	Spontaneous clearance (n=66)*
Gender (% male)	67%	64%
Age (% >45 years) ^a	52%	35%
Race (%)		
Caucasian	75%	82%
African-American ^b	13%	4%
Hispanic	7%	14%
Other	5%	0%
Risk factor (%)		
Injection drug use	60%	71%
Blood products ^c	13%	3%
Sexual	8%	9%
Other / Unknown	19%	17%
HIV status (% positive)	43%	51%
Genotype		Unknown
1	66%	
2	10%	
3	9%	
4	4%	

* Plasma was available to confirm HCV RNA less than 60 in 57 of 66 subjects.

^a Age <45 correlated with spontaneous clearance OR 2.04 [1.12–3.56]

^b African-American or other race negatively correlated with spontaneous clearance compared to Caucasian/Hispanics, OR 0.29 [0.09–0.97]

^c Risk of blood product exposure negatively correlated with spontaneous clearance, OR 0.20 [0.05–0.86]

Table 2
Summary of three HLA-B*57 individuals with acute HCV infection

AC-1 experienced a genotype 1b initial infection treated with IFN- α but then relapsed with the same virus, detailed in reference #16. AC-2 presented with symptomatic acute hepatitis C infection with genotype 1a; after a period of control for several months, virus recurred. AC-3 was found to have spontaneous clearance with serotyping indicating prior genotype 1 infection, then was reinfected with a genotype 3a virus. Abbreviations: IDU = injection drug use, "unk" for unknown clinical data at the time of previous infection for patient AC2, ETR = end of treatment response, SVR = sustained virologic response, E2 = envelope protein 2, NS5 = nonstructural protein 5.

	Age	Sex	HIV	Class I HLA	Peak ALT	Genotype	B*57-restricted Responses	Sequences (changes from cognate epitope are underlined)	Outcome
AC-1 (initial infection) (relapse post therapy)	15	F	-	A1,2 B40,57 Cw 7,15	579 1721	1b	None	NS5-2629: ...N... NS5-2629: ...N...	ETR then relapse SVR after re-treatment
AC-2 (initial infection) (relapse after initial control)	31	M	-	A1,2 B51,57 Cw6,15	2101 577	1a	E2-541 negative NS5-2629 positive	E2-541: ...Q... NS5-2629: ...I... E2-541: ...R... NS5-2629: E..RI...	Initial control Chronic
AC-3 (1 st infection) (2 nd infection)	unk 44	M M	unk +	A2 B44,57 Cw 5,6	unk 109	1 (serotype) 3a	E2-541 positive NS5-2629 positive	Unk E2-541: SL...S.R. NS5-2629 T....L...	Cleared Chronic