Evidence of Viral Adaptation to HLA Class I-Restricted Immune Pressure in Chronic Hepatitis C Virus Infection

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Received 4 May 2006/Accepted 24 July 2006

Cellular immune responses are an important correlate of hepatitis C virus (HCV) infection outcome. These responses are governed by the host’s human leukocyte antigen (HLA) type, and HLA-restricted viral escape mutants are a critical aspect of this host-virus interaction. We examined the driving forces of HCV evolution by characterizing the in vivo selective pressure(s) exerted on single amino acid residues within nonstructural protein 3 (NS3) by the HLA types present in two host populations. Associations between polymorphisms within NS3 and HLA class I alleles were assessed in 118 individuals from Western Australia and Switzerland with chronic hepatitis C infection, of whom 82 (69%) were coinfected with human immunodeficiency virus. The levels and locations of amino acid polymorphisms exhibited within NS3 were remarkably similar between the two cohorts and revealed regions under functional constraint and selective pressures. We identified specific HCV mutations within and flanking published epitopes with the correct HLA restriction and predicted escaped amino acid. Additional HLA-restricted mutations were identified that mark putative epitopes targeted by cell-mediated immune responses. This analysis of host-virus interaction reveals evidence of HCV adaptation to HLA class I-restricted immune pressure and identifies in vivo targets of cellular immune responses at the population level.

Clinical and virological outcomes are variable following acute infection with hepatitis C virus (HCV); there is successful viral clearance in some individuals, while persistent infection is established in the majority of cases (35). Chronic infection is characterized by attenuated and narrowly focused CD4+ and CD8+ T-cell responses (reviewed in reference 14) in conjunction with reduced proliferative, cytokine, and cytolytic capacity of HCV-specific T cells (10, 20, 40) and loss of antigen recognition (3). Conversely, CD4+ and CD8+ T-cell responses are maintained in individuals who clear the virus (6, 17, 21, 32, 34, 42). Therefore, effective T-cell-dependent immune responses to HCV-specific viral epitopes, restricted by the presence of host human leukocyte antigens (HLA) class I (CD8+ cytotoxic T cells [CTL]) and class II (CD4+ helper T cells), appear to make an important contribution to adaptive immunity and disease outcome.

The high rate of HCV replication and mutation renders its genome susceptible to changes within and flanking HLA-restricted epitopes, thereby providing mutated HCV sequences with a means of “escaping” HLA-restricted immune responses. The relevance of HCV immune escape mutations in chronic infection (reviewed in reference 2) was first demonstrated in chimpanzees (7, 9, 41) and subsequently demonstrated in humans (4, 33). Evidence for CTL escape and reversion has been recently demonstrated in acute infection (36) and 18 to 22 years after a common-source outbreak (24). As in human immunodeficiency virus (HIV) (1), flanking epitope escape mutations affecting proteasomal epitope processing provide an effective mechanism of immune escape in HCV (26). However, direct evidence for viral escape in humans is limited, as the sequence of the transmitted virus is rarely known and most previous studies have focused on a limited number of HLA alleles (38; www.hcv.lanl.gov).

We have chosen a population-based approach to assess in
vivo associations between viral polymorphisms and HLA alleles based on our experience in chronic HIV-1 infection (22). We hypothesized that, within the functional constraints of the viral proteins, mutations that result in escape from HLA-restricted immune responses will be evident at a population level as HLA-associated mutations and mark in vivo targets of the cellular immune responses. In this study, we examined viral adaptation to HLA-restricted immune responses in individuals with chronic HCV genotype 1 infection from two host populations that show a similar HLA distribution to determine the selective pressure(s) exerted on HCV.

MATERIALS AND METHODS

Subjects. DNA and plasma samples were obtained from individuals with chronic HCV infection in the Swiss HIV Cohort Study \( (n = 59) \) and individuals from the Western Australia (WA) HIV Cohort and other chronic HCV infection cohorts in WA \( (n = 59) \). There were no differences between the cohorts in age, ethnicity, or sex \( (P > 0.1, \chi^2 \text{ and Mann-Whitney tests}) \). All Swiss individuals and 39% of WA individuals were coinfected with HIV. The CD4 \(^+\) T-cell counts in individuals with HCV/HIV coinfection were similar between the cohorts: median (interquartile range) for Swiss, 429 (305 to 560) cells/\( \mu \)l; median for WA, 378 (204 to 483) cells/\( \mu \)l \( (P = 0.13) \).

DNA and viral RNA extraction. DNA was obtained from whole blood using the QIAamp DNA blood mini kit following the manufacturer’s guidelines. Viral RNA was extracted from plasma samples using either the QIAamp viral RNA mini kit (QIAGEN) or the COBAS AMPLICOR HCV specimen preparation kit v2.0 (Roche) according to the manufacturer’s instructions.

Bulk viral sequencing and HCV genotyping. An initial reverse transcription-PCR was performed using the primers 2412F2 and 9192R in combination with the SuperScript III one-step reverse transcription-PCR system with the Platinum Taq DNA polymerase PCR kit (Invitrogen). The first-round product was then used in nested second-round PCRs containing either the primer pairs HCV-2412F/HCV-3825R and HCV-3724F/HCV-5310R or alternatively the genotype 1-specific primers 5517M13F/6531M13R and 6430M13F/7481M13R together with the Platinum Taq DNA polymerase high-fidelity kit (Invitrogen). PCR products were purified using the Exosap method (Amersham), and BigDye Terminator (v3.1) sequencing reactions were set up with PCR or M13 primers. The samples were run on an ABI 3730 genetic analyzer, and electropherograms were analyzed using Seqscape v2.0 (ABI). Primer sequences can be obtained from the authors upon request. HCV subtypes were assigned by commercial assays (INNO-LiPA HCV II; Innogenetics, Ghent, Belgium) and confirmed by phylogenetic analysis.

HLA genotyping. HLA class I typing was performed by direct DNA sequencing using previously described methods (43). Exons 2 and 3 were sequenced separately using specific sequencing primers and performed as described above. Allele assignments were obtained using the program Assign (Conexio Genetics). In the large majority of cases, ambiguities were resolved following sequencing with allele-specific subtyping primers. However, in &lt;5% of typings, identity at exons 2 and 3 was consistent with the presence of rare alternative and null alleles, and only in these rare cases was the allele assignment consistently allocated for the most common expressed allele and haplotype combination in the relevant population.

Associations of HLA alleles with amino acid polymorphisms. Using methods previously described for the analysis of HIV (22), we created a map of the HCV amino acid residues in which polymorphism was associated with HLA alleles for the combined WA and Swiss samples. At each residue, the amino acid with the highest frequency was assigned as the consensus. The “proportion polymorphism” was determined by calculating the proportion of the population that had an amino acid different from the consensus. Residues with mixtures of the consensus and a nonconsensus residue were set as nonconsensus. Fisher’s exact test was used to assess the significance of the crude associations. In addition, the power to detect an odds ratio (OR) of &gt;2.0 or &lt;0.5 was calculated. Multiple logistic regression models were used to quantify adjusted associations between each amino acid residue (response variable) and the HLA alleles in the population (predictors). Due to colinearity introduced by HLA haplotypes, we ran three sets of models including HLA alleles from a single HLA class I locus only. Stepwise methods were used to find the most parsimonious set of HLA variables that were predictive of polymorphism. HLA variables were allowed to enter the model only if the Fisher’s \( P \) value was &lt;0.1, the minimum observed or expected cell count was &gt;5, and the power for an OR of &gt;2.0 or &lt;0.5 was &gt;20%. Models were constructed by entering variables in order from the lowest Fisher’s \( P \) value up to the point where the number of observations just exceeded 10 per variable. Then, using a standard backward elimination, variables were removed in order from the highest adjusted \( P \) value until all variables remaining in the model had a \( P \) value of &lt;0.1. All of the above criteria for model

FIG. 1. Similar HLA-A, -B, and -C allele distributions within the Swiss and WA cohorts. Bars indicate the percentage of individuals with the respective HLA alleles, with dark gray bars indicating the HLA allele distributions within the Swiss cohort and light gray bars indicating the WA cohort. The bar corresponding to “others” groups the alleles occurring at low frequencies. The extremely polymorphic nature of HLA-B is reflected in a larger number of alleles at low frequency compared to HLA-A or -C. The HLA allele distributions for the three loci did not differ significantly between the two cohorts \( (P = 0.5, 0.1, \text{ and } 0.6 \) for HLA-A, -B, and -C alleles, respectively, by Fisher’s exact test).
building are the same as those previously published (22), except that, due to the relatively smaller size of the data set, the power cutoff used here was 20% rather than 30%. In addition, due to the inclusion of both genotype 1a and 1b HCV sequences, we reanalyzed those sites in which the consensus amino acid differed between the subtypes by reassigning the corresponding consensus amino acid for each HCV subtype.

Use of the traditional Bonferroni correction for multiple comparisons will likely be overly conservative in this application due to the large number of intercorrelated test statistics. Instead, in order to assess the proportions of associations likely to be incorrectly classified as significant due to multiple comparisons, we estimated the allele-wise false discovery rate among the associations tested (for details, see reference 8).

RESULTS AND DISCUSSION

We examined the NS3 protein of HCV because it contains several known immunogenic epitopes for the HLA-B and -A alleles and, therefore, provided an opportunity to compare our results against existing data obtained from in vitro cellular approaches. We included 59 individuals from Switzerland (Swiss) and 59 from Western Australia (WA) with chronic HCV infection and prior to the commencement of antiviral therapy for HCV infection. Of the 118 individuals in this study, 88 (75%) were infected with HCV genotype 1a and 30 (25%) were infected with genotype 1b. The profiles of the HLA-A, -B, and -C allele distributions in the two cohorts were not statistically different, with a concordance of most frequent alleles for each locus (Fig. 1). Both cohorts comprise predominantly Caucasian (Swiss, 98%; WA, 92%) and male (Swiss, 76%; WA, 73%) individuals. All Swiss individuals were recruited from the Swiss HIV Cohort Study, and 39% of the WA individuals were coinfected with HIV.

Similar viral diversity in two host populations reflects functional constraints and selective pressures within HCV. The polymorphism profiles for the two populations were remarkably similar, as shown in Fig. 2A, with conserved regions within NS3 consistent with known functionally constrained areas of
the protein such as the motifs crucial for RNA binding and ATP hydrolysis within NS3 helicase (13, 39). Within NS3 protease, active sites (16) were completely conserved and in many cases flanked by polymorphic residues (Fig. 2A). Other regions in which little variation was observed might either reflect as yet unknown structurally or functionally relevant sites or lack of immune pressure. Few sites within the NS3 protein varied in the amount of polymorphism or consensus amino acid exhibited by the two cohorts (Fig. 2B).

Phylogenetic analysis of the HCV NS3 sequences from both cohorts revealed clustering within the main genotype 1a cluster when examining changes at sites that are generally regarded as neutral or “near neutral” (synonymous; changes that do not alter amino acid). These clusters contained predominantly sequences from one cohort or the other and could reflect local transmission groups and founder effects (Fig. 3A). Interestingly, when we analyzed only those sites reflecting amino acid change (nonsynonymous), there appeared to be greater admixture, possibly reflecting common selective pressures irrespective of transmission and founder effects (Fig. 3B). We acknowl-

FIG. 3. Phylogenetic analyses of the NS3 region in HCV reveals clustering of neutral or “near-neutral” changes that appear to disperse when examining sites are under selective pressure. Nucleotide sequences greater than 1,000 bp in the NS3 region were aligned using the program ClustalW, and the resultant alignment was then used in the phylogenetic package Mega 3.1 (15). Phylogenetic analysis of the NS3 region was performed using the neighbor-joining method based on the modified Nei-Gojobori model for synonymous and nonsynonymous sites with pairwise deletion. Bootstrap replications ($n = 1,000$) were performed for both analyses. Sequences with less than 1,000 bp were not included; thus, not all individuals contributed to this analysis. (A) Analysis of synonymous changes reveals distinct clusters. Within the genotype 1a cluster, sequences form additional clusters which largely disappear when analyzing only nonsynonymous changes (B). Viral sequences that fall into clusters in panel A are marked with identical colors; sequences that do not fall into clusters are not colored. Triangles mark sequences from Swiss individuals, and circles mark sequences from WA individuals. For both trees, numbers at nodes are bootstrap values, indicating the percentage of 1,000 replications. Only bootstrap values above 60% are shown. The bars at the bottom of the figure indicate the number of substitutions per site.
edge that this comparison can only be made with caution given the smaller number of changes included in the nonsynonymous analysis than for the synonymous tree (reflected in the genetic distances in Fig. 3). However, the lack of clustering illustrated in the nonsynonymous tree reflects the similar HCV polymorphism profiles and consensus sequences in both cohorts, as shown in Fig. 2B.

Evidence for HLA-driven viral adaptation in HCV infection. We created a map of the HCV amino acid residues in which polymorphism was associated with HLA alleles for the combined WA and Swiss samples. Associations were considered significant if the $P$ values were $<0.05$ in multivariate analyses and after adjusting for subtype-specific differences. Positive associations were defined as HLA-associated mutations that vary from the consensus sequence, consistent with the notion of CTL escape. HLA-specific polymorphisms in which the consensus was overrepresented in the HLA-positive group were termed “negative” associations (22). We and others (18, 22) have previously hypothesized that the consensus viral sequence is “best adapted” to T-cell responses encountered in the combined HLA allele and position of the association are shown. Five of the seven positions shown had an association with an allele-wise false discovery rate of less than 10%, while two positions (NS3-1094 and NS3-1635) had false discovery rates above this cutoff. (B) The top panel shows published HLA-restricted epitopes as listed in the HCV database at http://www.hcv.lanl.gov. Specific HLA associations with viral mutations found within the NS3 region are shown as either positive associations (variation from consensus amino acid is overrepresented in the HLA-positive group) or negative associations (maintenance of consensus amino acid is overrepresented in the HLA-positive group) for HLA-A, -B, and -C alleles. Red indicates associations that fall within published epitopes, and blue indicates an association flanking a published epitope. Vertical bars indicate the amount of amino acid variation within the combined cohort for each site, and the indicated amino acid is consensus. Red vertical bars indicate nonconservative amino acid changes, and blue bars indicate conservative amino acid changes (22). Odds ratios are indicated below the HLA allele. Hashed boxes indicate likely HLA haplotype structures. The conserved helicase motifs within this region are indicated and span highly conserved residues in the combined cohort.

![Figure 4](image_url)

**FIG. 4.** Significant ($P < 0.05$) associations of HLA alleles with viral polymorphisms within NS3 mark relevant immunological sites within the virus. (A) HLA associations with viral polymorphisms ($P < 0.05$) are shown as asterisks; those within or flanking (5 amino acids) published epitopes are boxed. The HLA allele and position of the association are shown. Five of the seven positions shown had an association with an allele-wise false discovery rate of less than 10%, while two positions (NS3-1094 and NS3-1635) had false discovery rates above this cutoff. (B) The top panel shows published HLA-restricted epitopes as listed in the HCV database at http://www.hcv.lanl.gov. Specific HLA associations with viral mutations found within the NS3 region are shown as either positive associations (variation from consensus amino acid is overrepresented in the HLA-positive group) or negative associations (maintenance of consensus amino acid is overrepresented in the HLA-positive group) for HLA-A, -B, and -C alleles. Red indicates associations that fall within published epitopes, and blue indicates an association flanking a published epitope. Vertical bars indicate the amount of amino acid variation within the combined cohort for each site, and the indicated amino acid is consensus. Red vertical bars indicate nonconservative amino acid changes, and blue bars indicate conservative amino acid changes (22). Odds ratios are indicated below the HLA allele. Hashed boxes indicate likely HLA haplotype structures. The conserved helicase motifs within this region are indicated and span highly conserved residues in the combined cohort.
text of the most common or evolutionarily conserved HLA alleles in the host population.

Analysis of the 631 amino acids within NS3 revealed 17 statistically significant associations with HLA-A, -B, and -C alleles that satisfied the conditions imposed by the statistical method. Of these, five fell within, or flanked, published HLA class I-restricted epitopes (Fig. 4). Fourteen associations fell within a region of 100 amino acids, of which 11 were within 60 amino acids in NS3 that are predominantly positioned in an external alpha-helix domain of helicase (Fig. 4 and 5). While, as outlined below, some of this clustering reflects the strong interlocus linkage disequilibrium (LD) that exists within well-defined HLA haplotypes, clustering could also be attributable to differences in the immunogenicity or functional and structural constraints of particular regions within the viral proteins.

Figures 4 and 6 illustrate the effectiveness of this approach in predicting epitope positions, correctly assigning HLA class I restriction and identifying "preferred" in vivo escaped viral mutations. For example, analysis of the amino acid sequence in HLA-B*0801-positive and -negative individuals revealed two relevant sites at P3 (K1397R) and P4 (K1398R) within the published HLA-B8 epitope at NS3-1395 (HSKKKCDEL), which have both been previously described as escape mutations (24, 36), although in our study only the K1397R association was significant (P = 0.007) (Fig. 6A). Of interest, these amino acid substitutions appear to be mutually exclusive, suggesting mutation at one of the lysine residues may be sufficient to abrogate CTL recognition and/or there is a high fitness cost for altering both residues. Furthermore, 65% of individuals carrying the HLA-B*0801 allele exhibited sequence variation within this epitope, compared with 7% of the HLA-B*0801-negative individuals (P < 0.001, Fisher’s exact test). This suggests that sequence polymorphism within this epitope is driven by escape from HLA-B*0801-restricted immune pressure and is concordant with the previously observed reversion event within this epitope following transmission to an individual that lacked the HLA-B8 allele (36). These observations and the position of this epitope within a likely structurally constrained region of the protein (Fig. 5) suggest a viral fitness hurdle that would favor consensus residues in individuals lacking the appropriate HLA allele.

Another significant HLA-B*0801 association was found in the critical P9 anchor position of the HLA-B*0801 NS3-1402 epitope (ELAAKLVAL). We also observed a negative HLA-A*0101 association with the correct escaped amino acid at P9 (F1444Y) of an immunogenic HLA-A1 epitope at NS3-1436 (ATDALMTGY) (17) (Fig. 6B). Furthermore, we found two HLA associations with residues flanking known epitopes: an HLA-A*1101 association at position P1 of the previously described HLA-A11-restricted epitope at NS3-1636 and an HLA-B*0701 association flanking the known HLA-B7-restricted epitope at NS3-1373 (www.hcv.lanl.gov; Fig. 4). Importantly, the phylogenetic analysis of the NS3 sequences does not reveal clustering of individuals that share the same HLA-restricted polymorphism (Fig. 6). This suggests that founder effects were not the major contributing factor for viral polymorphism within these epitopes.

Twelve putative CTL escape mutations were also identified in this analysis (Fig. 4), providing a basis for experimental approaches that may reveal novel HLA-restricted epitopes and associated CTL escape variants. Over 60% of the previously reported HLA class I epitopes within NS3 were HLA-A restricted (38; www.hcv.lanl.gov), while in our study, more than 75% of significant HLA-restricted associations were with HLA-B and -C alleles (Fig. 4). Although our study does not allow us to assess the relative importance of HLA-B- and -C-restricted cellular immune responses, recent reports showing the dominance of HLA-B-restricted immune responses in HCV (23) and HIV infection (11) suggest that future studies should examine the relative influence of HLA-A-, -B-, and -C-restricted responses.

When comparing the results of our study with previous cellular based studies, we found concordant results for HLA-B8 epitopes (significantly more associations within than outside epitopes); however, we found no significant associations within published HLA-A2 epitopes (Fig. 7). The premise of this approach is that it identifies in vivo targets of the immune response, and, therefore, the lack of significant associations for some HLA-A alleles would suggest that HLA-A-restricted mutations within these epitopes...
may not be critical for viral immune escape in chronic HCV infection. Interestingly, our analysis did not detect significant HLA-restricted mutations within two HLA-A2 immunodominant epitopes at NS3-1073 and NS3-1406 (Fig. 8). This is in concordance with recent reports which describe a limited degree of sequence variability within the A2 NS3-1073 epitope (30, 31), possibly due to a high viral fitness cost of mutations within this epitope (30). Hence, high viral fitness cost may also limit the variability of HCV within some immunologically relevant epitopes. Using a population-based approach, the lack of HLA-restricted associations in immunodominant epitopes could be used to direct the search for motifs within the viral sequence in which mutations may impart a high viral fitness cost or are not

FIG. 6. HLA-restricted mutations in individuals with chronic HCV infection within published HLA class I-restricted epitopes. The first line indicates the published epitopes, and the second line indicates the consensus amino acid from both cohorts, with significant associations found in this study shown by white letters on a black background and with an arrow. Identity with consensus is indicated by dots, and deletions are indicated by white dashes on a black background; the sequences are sorted by the presence of the respective HLA alleles. Mutations within epitopes are shown as white letters on a black background, outside epitopes are shown in dark gray, and positions that appear to contain HCV genotype 1b-specific polymorphisms are boxed. A lowercase letter indicates the presence of two major viral quasispecies including consensus. Sequences from individuals infected with HCV genotype 1b are marked by a closed circle. Individuals with incomplete sequence for the respective epitopes were excluded. HLA-B*0801- and HLA-A*0101-positive individuals are marked with circles in the respective phylogenetic trees. Trees include all sites and were generated as described in the legend to Fig. 3. Individual sequences with mutations at positions with significant associations are marked with closed circles; those with consensus amino acids are marked with open circles. (A) Demonstration of a positive HLA association with viral polymorphism in two overlapping epitopes (B8-1395 and B8-1402). The K1397R and L1410X substitutions were significantly \( P = 0.007 \) and \( P = 0.006 \) in multivariate analyses, respectively, see Materials and Methods) more frequent in HLA-B*0801-positive than in HLA-B*0801-negative individuals. One individual has a large deletion spanning two neighboring HLA-B*0801 epitopes (marked with a closed triangle) and could not be included in the phylogenetic analyses. (B) Demonstration of a negative HLA association with viral polymorphism (F1444Y) within the known HLA-A1-1436 epitope. The consensus amino acid F appears to be maintained within the HLA-A*0101-positive individuals compared to the increased incidence of mutation from consensus (Y) in the HLA-A*0101-negative individuals. The phylogenetic analyses do not reveal clustering of individual sequences that share the same HLA-restricted polymorphism.
subject to ongoing immune pressure in vivo. This approach is unlikely to identify new epitopes within highly conserved regions, as the premise for the identification of HLA-restricted mutations marking putative epitopes is based on viral polymorphism. Comprehensive analysis of the relevant epitopes needs to be complemented by cellular assays using overlapping peptides which span these highly conserved regions.

Another critical biological observation was the association of particular HLA-B and -C alleles (and to a lesser extent HLA-A) at identical positions within NS3. These are likely to reflect the strong interlocus LD that exists within well-defined HLA haplotypes (5). These include the common Caucasian haplotypes HLA-A*0101, -B*0801, and -C*0701 and HLA-A*0301, -B*0701, and -C*0702. Figure 4 clearly shows the haplotype structure at positions 1384, 1397, and 1410 within NS3. As the LD between HLA-B and -A is less than that observed for HLA-B and -C, one would expect these observations to occur predominantly for HLA-B and -C as shown in Fig. 4. Hence studies describing epitopes restricted to a particular HLA-B or -C allele using cellular assays on a small number of individuals may be subject to the confounding issue of haplotype structure. These characteristics within the major histocompatibility complex have made it difficult to assign a primary causative gene to the large number of diseases associated with the region (5).

We may expect that CTL immune pressure in coinfected individuals is compromised compared to that in HCV-monoinfected individuals and in this case would likely reflect a reduced number of HLA-restricted associations. However, previous studies showed an overall similar breadth and magnitude of CTL responses in HIV-negative and HIV-positive patients (12), although declining CD4+ T-cell counts were associated with lower levels of HCV-
FIG. 8. Lack of significant HLA associations within known immunodominant HLA-A2 epitopes. (A) A2-1073. (B) A2-1406. Examination of the two HLA-A2 epitopes finds no significant accumulation by multivariate analysis (see Materials and Methods) of mutations at single residues within the HLA-A*0201-carrying individuals. All three patients with the Y1082F mutation affecting proteasomal processing were infected with HCV genotype 1b, as were the individuals in the experimental setting described by Seifert and colleagues (26). The first line indicates the published epitopes, and the second line indicates the consensus amino acid from both cohorts. Identity with consensus is indicated by dots, and deletions are indicated by white dashes on a black background; the sequences are sorted by the presence of the respective HLA alleles. Mutations within epitopes are shown by white letters on a black background, outside epitopes are shown in dark gray, and mutations that appear specific to HCV genotype 1b are boxed. A lowercase letter indicates the presence of two major viral quasispecies, including the consensus. Sequences from individuals infected with HCV genotype 1b are marked by a closed circle; one individual with a large deletion is marked by a closed triangle. Individuals with incomplete sequence for the respective epitopes were excluded.
specific CD8+ T cells. Similarly, diminished immunological pressure in advanced HIV disease (CD4+ T-cell count below 200 cells/µl) has been associated with a decrease in HCV quasispecies (25, 37). The large majority (90%) of the coinfected individuals in this study had a CD4+ T-cell count above 200 cells/µl, and most (67%) individuals were treated with antiretroviral therapy and had undetectable HIV RNA. Given these characteristics, we would expect that the HLA class I-driven immune pressure in the coinfected individuals would be similar to that for the monoinfected individuals. However, although the majority of the patients coinfected with HIV were under successful antiretroviral therapy, we cannot exclude the possibility that coinfection alters the shape of the T-cell repertoire and the hierarchy of recognized T-cell epitopes, as has recently been described by Selin and Welsh (27).

In conclusion, we have utilized a population-based approach to identify (i) polymorphic and conserved sites that are likely to reflect structural or functional constraints in the HCV genome, (ii) significant associations of specific HLA alleles with HCV mutations within and flanking published T-cell epitopes (including likely critical anchor residues), and (iii) putative HLA-restricted CTL escape mutations within NS3. Furthermore, the HLA associations are relevant to two geographically distinct chronic HCV infection cohorts with similar HLA distributions. Given the paucity of published HLA-restricted epitopes within HCV, this method will provide a resource that could both complement and validate cellular approaches aimed at characterizing the host’s immune response in chronic infection. However, given the large amount of diversity between the HCV genotypes (28, 29), we would envisage that this analysis will need to be performed on large cohorts of specific viral genotypes.

In HIV, this approach has been substantiated by the confirmation of predicted viral escape mutations, including the first description of viral escape mediated by disruption to intracellular epitope processing caused by a mutation outside an epitope (1). In several instances to date, the use of this approach was able to predict the location, amino acid substitution, HLA subtype association, and biological mechanism of escape and reversion kinetics (19). The analysis here should identify in vivo targets of cellular immune responses in chronic HCV infection and provide critical information for the design of future vaccines.

ACKNOWLEDGMENTS

We thank David Nolan, Andrew Lucas, Marion McInerney, Saroj Nazareth, Joanne Young, Victoria O’Brien, Charlotte Ekstrom, and Suzanne English. We acknowledge the help and support from our colleagues at the Centre for Clinical Immunology and Biomedical Statistics, Royal Perth Hospital and Murdoch University, and the Department of Clinical Immunology and Biomedical Genetics, Royal Perth Hospital. We thank Paul Klenerman for helpful comments on the manuscript.

This work was supported by the National Medical and Research Council of Australia. S.G. was supported by a Healy fellowship from the Raine Medical Research Foundation. A.R. was supported by a Fellowship for Prospective Researchers from the Swiss National Science Foundation.

The authors do not have any competing financial interests.

REFERENCES


