CO-LOCALISATION OF HEPATITIS C VIRUS DRUG-RESISTANT MUTATIONS AND IMMUNE-DRIVEN ADAPTATIONS: RELEVANCE TO THERAPY OUTCOME

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Introduction

Recent advances in molecular virology have led to the development of novel small anti-Hepatitis C virus (HCV) drugs that target specific viral proteins integral to the HCV life cycle. Preliminary studies using these agents have revealed a number of drug-resistance mutations within the target HCV proteins. Another selective force that continues to shape HCV diversity is the host's Human Leucocyte Antigen (HLA)-restricted immune response. These immune responses are stimulated by the presentation of parts of internally processed viral peptides (epitopes) in the context of the HLA molecule and hence the selection of HCV sequences targeted by the immune response is dependent on the HLA repertoire of the host. We, and others, have previously demonstrated the influence of the host's immune response on viral diversity at both the individual and population level and identified sites within the HCV genome that are under immune pressure (viral adaptation).

The issue of viral adaptation or immune 'resistance' is particularly pertinent given the development of these new anti-HCV drugs in which specific viral mutations can cause drug resistance. We hypothesize that HLA-specific viral escape in proteins targeted by the small molecules could act as drug resistance mutations and impact on treatment response. Conversely, the selection of drug resistance mutations in the same proteins that are targeted by immune responses could disrupt epitope presentation or processing and consequently impair the host's immune response. An example of this scenario is shown in Figure 1. Accordingly, an individual's HCV immune escape and drug resistance profiles are likely to be critical influences on the outcome with specific antiviral treatments.

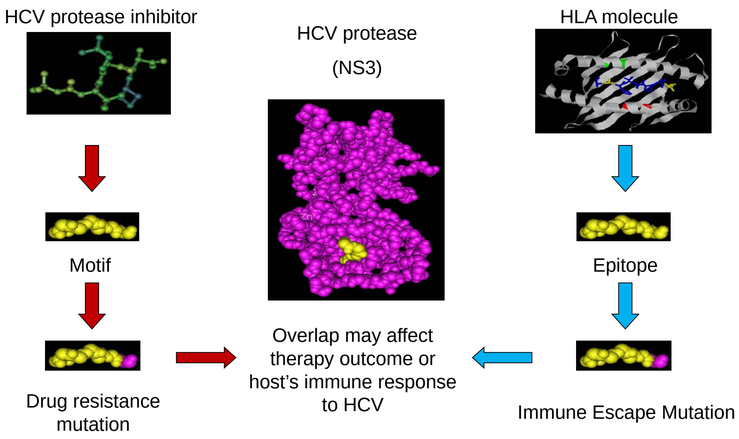

Figure 1. Overlapping selection pressures from anti-HCV drugs and the host's immune response. Convergence of drug- and immune-driven selection may influence the outcome of therapy or host's immune response to HCV. HLA repertoire refers to the expression of the HLA types within an individual.

Methods

In this study, the frequency of pre-existing drug resistance mutations within a drug-naïve population is determined and the potential for drug and immune selective pressures to intersect at sites along the HCV genome is explored.

Individuals with chronic HCV genotype 1a (n=205), 1b (n=54) or 3a (n=146) infection were recruited from Australia (1a=90, 1b=24, 3a=61), Switzerland (1a=62, 1b=21, 3a=37) and the UK (1a=53, 1b=9, 3a=48). All HCV sequences were obtained from HCV treatment-naïve individuals. Viral RNA was obtained from plasma samples using the Cobas Amplicor HCV sample prep kit (Roche Diagnostics). cDNA conversion and first round PCR products were obtained using the SuperScript III OneStep RT-PCR System (Invitrogen). Overlapping second round PCR products were amplified that covered NS3 protease and NS5B polymerase. HLA Class I and II typing was performed using sequence-based methods.

Written informed consent was obtained from participants and local Institutional Review Board approval was obtained by centers contributing to the study.

Results

Baseline profile of described anti-HCV drug resistance mutations in HCV sequences from treatment-naïve individuals with chronic infection

Known drug resistance mutations for the anti-HCV drugs were observed in NS3 protease and NS5B polymerase sequences obtained from treatment-naïve HCV mono-infected and HIV co-infected individuals (Table 1 – protease only). In the HCV genotype 1a sequences, the frequency of described anti-HCV drug resistance mutations was generally low, with the most frequent drug resistance mutation at NS3 protease T54S (4.4%). Similarly, recent studies of NS3 protease and NS5B polymerase sequences from treatment-naïve individuals with chronic HCV genotype 1 infection showed a low frequency of baseline drug resistance mutations. In summary, 21.5% of individuals with HCV genotype 1a have a variation at one or more drug resistance sites.

Drug resistance mutation	HCV consensus/variant(s) #					
	1a		1b		3a	
		Frequency of variant in total		Frequency of variant in total		Frequency of variant in total
V36/L/A/M/G	V/L	2/112	V	0/25	L	0/112
Q41R	Q/H	1/121	Q	0/26	Q	0/117
F43S	F	0/132	F	0/30	F	0/117
T54A/S	T/S	7/159	T	0/32	T/S	1/117
R109K	R/T	1.5/156	R	0/34	R	0/120
I153V	I/L	2.5/156	I/V	3/33	I/V	3/117
R155K/T/Q	R/T	0.5/155	R	0/33	R	0/117
A156T/V/D/S	A	0/155	A	0/33	A	0/117
D168V/A/Y	D/E	2/152	D	0/33	Q/R	1.5/117
V170A	I/V	9/154	V/I	2.5/32	I/V	6.5/117
E176G/K	E/K	0.5/161	E	0/31	S/N /G	20.5/117 1/117

Co-localisation of areas in NS3 protease and NS5B polymerase under HLA (immune) pressure and therapy selection

In Figures 2 and 3, HCV sites under HLA (immune) pressure (published HLA-restricted epitopes) are mapped onto the NS3 protease and NS5B polymerase regions that contain most of the observed drug resistance sites. In both regions, several HLA-restricted epitopes overlap with, or are flanked by, known drug resistance sites (see for example HLA-A2 restricted epitope in NS3 protease with position 54; HLA-B27-restricted epitope in NS5B polymerase with position 423). Also, a single site may be within more than one HLA-restricted epitope and be subject to selection from more than one HLA type.

Results

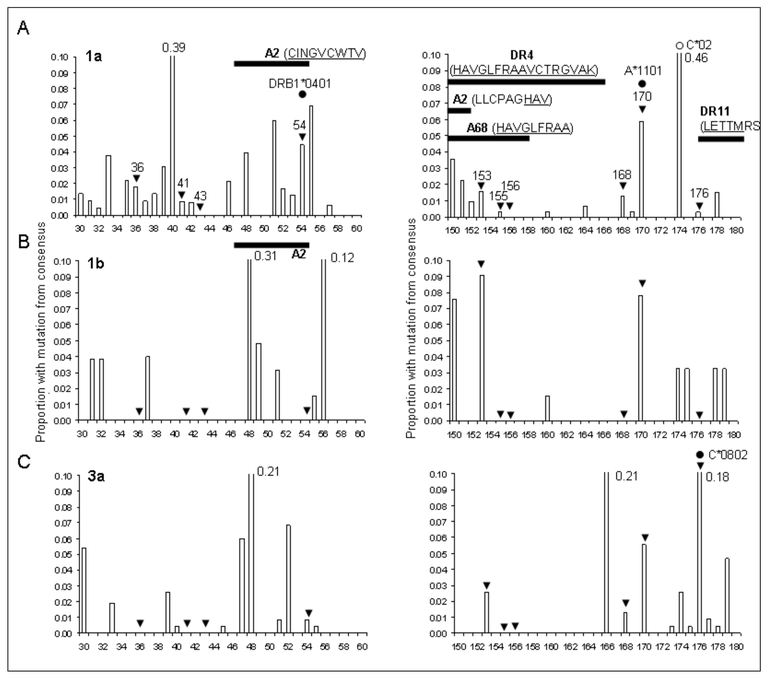

Figure 2: Overlap between drug resistance and immune escape profiles in NS3 protease. Amino acid diversity profile of the region between position 30-60 and 150-180 of NS3 protease in genotype 1a (A), 1b (B) and 3a (C) measured as the proportion of sequences with variation from consensus. Arrow-heads indicate known protease inhibitor drug resistance sites within NS3 with position indicated. Published HLA-restricted HCV epitopes are shown as black bars (epitope sequences are shown in brackets following the HLA allele; sequence falling within window is underlined).

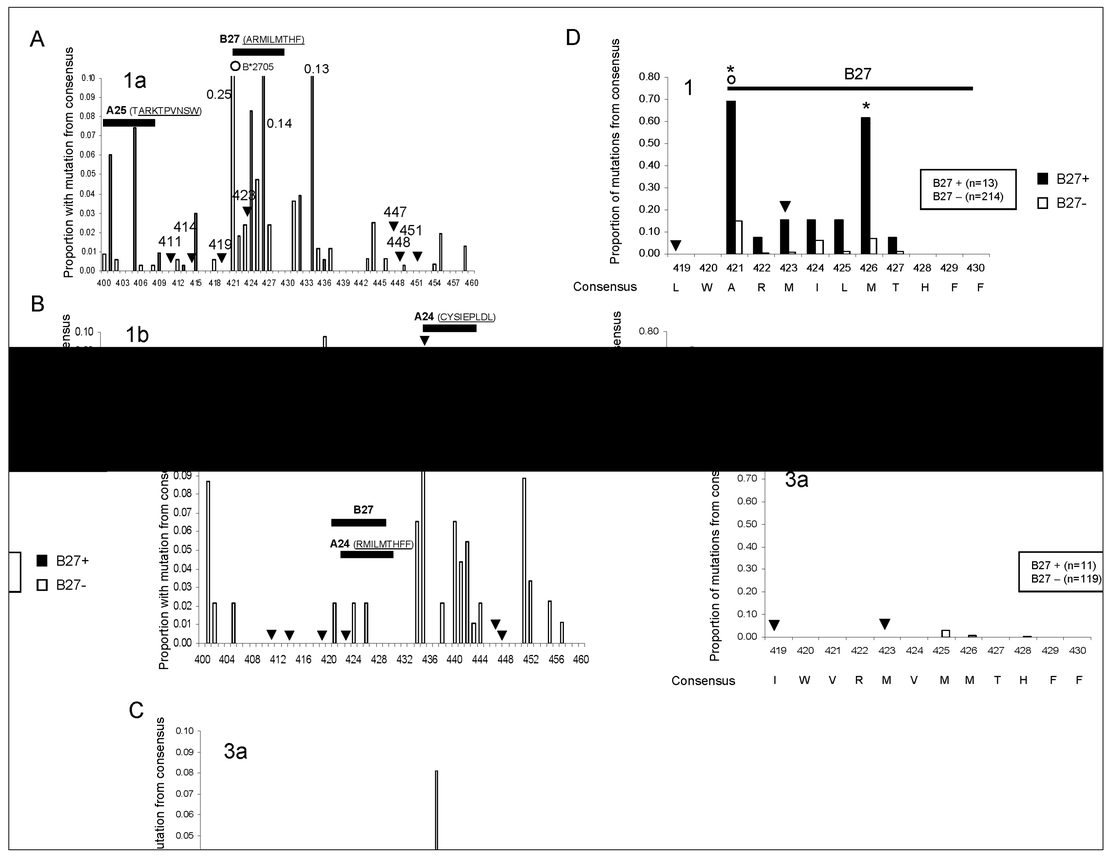

Figure 3. Overlap between drug resistance and immune escape profiles in NS5B encoding the RNA-dependent RNA polymerase. Amino acid diversity profile of the region between position 90-150 and 400-460 of NS5B in genotype 1a (A), 1b (B) and 3a (C) measured as the proportion of sequences with variation from consensus. Arrow-heads indicate known polymerase inhibitor drug resistance sites within NS5B with position indicated. Published HLA-restricted HCV epitopes are shown as in Figure 2. (D) Histograms showing the proportion of HLA-B27+ (black box) and HLA-B27- (open box) individuals with variation from consensus within and flanking the published HLA-B27 epitope shown in (A+B) for genotype 1 and 3a. P-values <0.005 (Fisher's exact test) are shown as an asterisk. Number of individuals expressing the HLA type and with sequence covering the region is shown for each HCV genotype.

Different amino acid diversity profiles for HCV genotypes/subtypes suggest differences in the position/type of immune escape and drug resistance mutations

The amino acid composition at several of the drug resistance sites can vary between the HCV genotypes/subtypes resulting in different consensus amino acids (eg NS3 protease 36, 168, 170 and 176; Table 1). In some examples, the consensus for one HCV genotype/subtype is the reported drug resistance mutation (NS3 protease 36 and NS5B polymerase 71, 482 and 499). Table 1 shows that the number and type of naturally occurring variations observed within one genotype or subtype is not necessarily present for another genotype. This suggests that genotypes differ in their ability to mutate at sites along NS3 protease and NS5B polymerase and could reflect potential differences in anti-HCV drug resistance and immune escape profiles. These findings are further supported by Figures 2 and 3. Note the marked difference in the diversity profile of the area containing the HLA-B27 epitope in NS5B (Figure 3D).

Discussion

The overlap of immune escape and drug resistance profiles for HCV suggests that knowledge of the host HLA type and HCV subtype/genotype may provide important information in defining an individual's drug regimen. In the field of HIV medicine it has already been established that synergistic effects of antiretroviral drug resistance and HLA-driven HIV adaptation results in an increased frequency of the resistant viral strain in individuals expressing the relevant HLA type and undergoing HIV treatment with the specific drug. We contend that this issue may be even more relevant to the management of HCV infection, where immune-modulating IFN-α therapy will likely remain the cornerstone of future combination treatment regimens. Accordingly, it may be useful to develop a "stratification of risk" for drug resistance based on host and viral genetics prior to the commencement of anti-HCV drugs in combination with current regimens (IFN-a).

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