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 sites 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.

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, 10/19, 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-naive 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 position 155. Similarly, recent studies of NS3 protease and NS5B polymerase sequences from treatment-naive 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.

**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).