Published in final edited form as:

J Acquir Immune Defic Syndr. 2011 May 1; 57(1): e17–e19. doi:10.1097/QAI.0b013e3182169050.

The Association between HIV-1 Subtype C Antiretroviral Resistance and HLA Prevalence in Southern India

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To the Editors

Antiretroviral drug resistance and escape from cytolytic T lymphocytes (CTL) are major obstacles in the control of HIV replication. Therefore, it is important to understand the complex interactions of viral escape and the host immune response, particularly in association with specific drug-resistant mutations (DRM) to 1 or more of the 3 extensively used antiretroviral treatment (ART) classes [nucleoside reverse transcriptase (RT) inhibitors (NRTI), non-nucleoside RT inhibitors (NNRTI), and protease (PR) inhibitors (PI)]. Unlike the mutations allowing escape from CTL, DRM localize to particular genomic sites and lead to specific amino acid changes. DRM conferring NRTI and NNRTI resistance occur in RT codons 1–335, whereas DRM conferring PI resistance occur within PR codons 1–99, based on standard HXB2 numbering.¹ These regions of HIV-1 *pol* also contain a number of putative CTL epitopes. If these CTL epitopes remain immunogenic after incorporating drug resistance mutations, the well-documented constraints on drug resistance mutations could potentially be exploited to prime the immune system in anticipation of emerging mutants.^{2,3} Similarly, if epitopes incorporating drug resistance mutations exhibit enhanced CTL immunogenicity, then possibly the selection of mutations by ART could modulate the CTL response against HIV. To explore these connections, we investigated how DRM and CTL pressure overlap to select for specific mutations in HIV-1 subtype C from south India.

We studied 45 patients PR and RT sequences, who were receiving second-line ART therapy to investigate how a number of common antiretroviral drug resistance mutations in HIV *pol* affect CTL recognition with relation to a predefined specific HLA allele derived peptide. HIV-1 genotyping was done using Viro-Seq v.2.0 genotyping system with analysis software v2.8 (Celera, CA), the nucleic acid sequences were aligned pair wise using clustal W in Molecular Evolutionary Genetic Analysis software for windows version 4.0. The reference sequences were obtained from Los Alamos National Laboratory database. Selection pressure was estimated as dN/dS using SLAC model (www.datamonkey.org).

The study cohort was predominately male (33) (80%), and the average age was 35 ± 9 years. Median CD4 count was 146 cells per milliliter (IQR: 8–472), and median PI-exposure was for 16.3 months (range: 6–38 months). The vast majority of the sequences were HIV-1 subtype C (98%) and contained high rates of DRM; of 45 sequences, 33 (73%) had resistance to PI, 41 (91%) to NRTI, 33 (73%) to NNRTI, and 24 (53%) had triple-class resistance. Analyses were used to identify codons across the cohort, which were under selection pressure and found 20 (4.6%) sites under positive selection, 161 (37%) under negative selection, and the rest to be neutral (58%). Of the positively selected sites, PR codons 10, 16, 24, 74, 82, and 90 and RT codons 69, 184, 188, and 190 were associated with putative epitopes and DRM (Fig. 1). For the amino acid sites in PR, we found: (1) HLA A*6802 and A*7401 targeted an epitope containing PR position 10 (L10F); (2) HLA B*1503 and B*57 targeted positions 16, 24, 73, 74, and 76; (3) HLA A*0201 targeted position 76; (4) HLA B*81 targeted positions 82, 84, 85, and 90. Additionally, the PR positions 82 and 90 including positively targeted selected DRM epitopes. Among the positively selected amino acid sites in RT, (1) HLA B51 targeted positions 184; HLA A*0201 targeted positions 184, 188, and 190; and HLA A2 targeted positions 184 and 188. Considering DRM positions, the HLA haplotypes A*0201 and B*3501 along with B*51 and B*18 were related to DRM sites M184V in RT and with HLA alleles A*0201, A*6802, A*7401, B*1503, B*57, and B*81 in PR. The HLA allele frequencies of the above alleles among the Indian populations, particularly in south India with different indigenous caste groups, are genetically heterogeneous as documented in published literature.^{4–9} Therefore, it is necessary to understand south Indian HIV-1 patient HLA profiles to understand the influence of the CTL epitopes generated against the HIV-1 subtype C drug-resistant mutants.

Acknowledgments

We are most grateful to the clinical and laboratory staff at YRG Centre for AIDS Research and Education, VHS, Chennai, India, for their facilitation of the study.

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FIGURE 1.

A and B, Schematic representation of regions of the HIV-1 *pol* gene encoding known CTL epitopes that overlap with common drug resistance mutation sites. The HLA restriction of each epitope is indicated in the wild-type subtype C (top) sequence. Underlined amino acids indicate mutations positively selected ($dN/dS > 1$) and associated with antiretroviral drugs in the bottom sequence.