Infection with specific HIV subtypes may lead to more rapid disease progression, and in Uganda, HIV-1 subtype D infection has been associated with less favorable outcomes and differential antiretroviral (ARV) treatment responses compared to the local subtype A. Naturally occurring polymorphisms in non-B subtype viruses may also lead to differences in replicative fitness leading to distinct drug resistance patterns.

Immune activation has previously been associated with disease progression in untreated HIV-infected individuals and may also serve as an additional predictor of ARV treatment response. Subtypes A and D account for the majority of HIV infection in Uganda. We hypothesize that immunological and virological profiles between different HIV subtypes in Uganda may be predictive measures of subsequent clinical outcome.

HIV-1-positive volunteers (n = 68) were randomly recruited from a cross-sectional cohort study. All were antiretroviral treatment naive and CD4 T cell count and HIV viral load was obtained at the time of enrollment and blood draw. Institutional review board approvals were obtained from the California Department of Health Services and the Joint Clinical Research Centre Institutional Board Review. All study participants gave written informed consent. The mean age of our study population was 37 years old, and 41 (60%) were women. Median CD4 T cell counts and plasma RNA levels were 206 cells/mm$^3$ (range 4-631) and 154,313 copies/ml (range 1383-2.839 × 10$^6$), respectively. Subtyping was based on plasma sequencing for Gag and Nef as previously reported. Combined sequence results were edited using Sequencher V4.6 software (Gene Codes Corporation, Ann Arbor, Mich). The National Institutes of Health’s HIV-1 Genotyping Tool Web site (http://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.html) was used to determine the HIV-1 subtype. Polymerase gene drug resistance was analyzed using Stanford University’s HIV Drug Resistance Database (http://hivdb.stanford.edu/pages/algs/HIVdb.html). Viral nucleotide sequences were submitted to GenBank and given accession numbers AY803354 to AY803488 and EF186091 to EF186227.

Although confident assignment of viral subtype requires full-length genome sequences, practical limitations mean that it is often based on subgenomic regions. We analyzed subtype distribution based on Gag sequences. We found that subtype distribution in our study population consists primarily of subtypes A (54%) and D (37%). We next compared concordance between Gag and Nef subtyping. Fifty of the 62 Gag sequences were successfully sequenced for Nef with subtype concurrence of 74%. Recombination within genes accounted for 3% in both Gag and Nef (primarily A/D recombination, data not shown). We found no specific patterns in the recombination breakpoints (data not shown) in the inter- and intragene recombiant patterns within and between the 2 genes. No significant differences were observed in CD4 count and plasma viral load between HIV-1 subtype A and D infected volunteers (P = .186 and P = .807, respectively). There was no significant difference in subtype distribution by gender or age (P > .05).

We and others have described heightened immune activations in HIV-infected individuals from Africa compared to those from Europe and the United States. Different HIV-1 subtypes may vary in pathogenesis resulting in distinct profiles of immune activation. Activation staining was performed as previously described. CD8 and CD4 activation was defined as the percentage of CD3$^+$ CD8$^+$ CD38$^+$ HLA DR$^+$ or CD3$^+$ CD8$^-$ CD38$^+$ HLA DR$^+$ lymphocytes, respectively. Both CD8 and CD4 T cell immune activation were examined separately. CD4 T cell activation correlated

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HIV Subtypes Distribution

with CD4 count and CD8 T immune activation positively correlated with VL as we previously described (Figure 1). Because the majority of our population was infected with subtypes A and D, we focused our immune activation analysis on these 2 subtypes. We observed no significant differences in CD8 immune activation (Figure 2A, $P = .216$). In contrast, despite no significant differences in CD4 counts, CD4 immune activation was higher in subtype D compared to subtype A (Figure 2B, $P = .042$).

Although all HIV-1 subtypes are susceptible to antiviral drugs in vitro,$^{18-22}$ subtype-specific treatment responses have been observed in some cohorts.$^{19,21,23,24}$ We examined patterns of Pol polymorphism in our study population. Minor mutations in antiretroviral naïve individuals infected with non-B subtypes can exist as natural polymorphisms at positions known to be associated with drug resistance in subtype B viruses.$^{18}$ None of the study volunteers had prior exposure to ARV treatment, and not surprisingly, no primary protease inhibitor (PI) or reverse transcriptase (RT) resistance was observed. All individuals in our studies demonstrated one or more secondary PI mutations. These mutations are not uncommon in non-subtype B viral isolates and may facilitate development of resistance when present with primary resistant mutations.$^{18}$ Interestingly, the distribution of secondary PI mutations suggests subtype-specific patterns (K20 and L63, $P < .05$). Resistance in the RT region was observed in 13% of the study population, including 7% with polymorphism associated with nucleoside analog reverse transcriptase inhibitor resistance. These include L210LM, E44D, E44K, V118I, and T215D, although no subtype-specific pattern was discernable ($P > .05$,

Figure 1 T cell immune activations were measured in HIV-positive antiretroviral treatment naïve volunteers by flow cytometry using coexpression of HLA-DR and CD38. (A) CD8$^+$ T cell activation is positively associated with viral load ($P = .0028$). (B) CD4$^+$ T cell activation is inversely associated with CD4 cell count ($P = .001$).

Figure 2 T cell immune activation in HIV-1 subtype A and D. Bars represent mean values. (A) CD8$^+$ T cell activation levels were comparable between subtype A and D infected volunteers ($P = .216$). (B) CD4$^+$ T cell activation levels were significantly higher in individuals infected with subtype D ($P = .0417$).
data not shown). The observed frequency of accessory mutations in the Pol gene is consistent with prior findings. Whether differential polymorphism between subtypes in our study population confers additional selective pressure in the evolution of HIV-1 resistance remains an intriguing hypothesis.4-6

HIV-1 subtypes may confer distinct pathogenesis and impact disease progression. Our current study focuses on a study population with multiple circulating subtypes. We confirmed that subtypes A and D account for the majority of infection in this treatment-naive population. The role of T cell activation in HIV replication and pathogenesis is complex and not fully understood. Generalized immune activation is believed to be an independent mediator of HIV-mediated immunodeficiency. Discordant responses to antiretroviral therapy have also been linked to the level of cellular activation.25-27 Our observed difference in immune activation profile between subtypes suggests that antiretroviral treatment outcome may be influenced by the infecting subtype.25,26 We also report subtle differences in the distribution of Pol polymorphism between subtypes A and D. Our findings support the premise that the distinct infecting subtype may impart differential clinical outcomes in Uganda.

The small sample size of our study limits definitive conclusion on the role of subtypes. Our genotyping approach using Gag sequencing showed strong but not full concordance with Nef analyses. Thus, HIV genotypes based on a single subgenome sequence analysis may potentially dampen the observed effect of subtype-specific pathogenesis. Long-term assessment and close monitoring of larger cohorts of patients infected with non-B viruses will be necessary to determine the true clinical impact of T cell activation and polymorphism-predicting outcome in regions with multiple circulating HIV subtypes such as Uganda.

References


