Retrovirology

Poster presentation

P20-03. Identification of low frequency genetic variants during acute and early infection by parallel-allele specific sequencing

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Background

Minority viral populations present in very low abundance (<1%) are difficult to analyze by conventional sequencing analysis. Therefore, the biological implication of these rare variants has not been fully realized.

Methods

To precisely quantify frequencies of low abundance variants, we characterized a large number of viral genomes from three HIV-1 infected individuals using a newly developed parallel allele-specific sequencing (PASS) assay that can simultaneously analyze thousands of viral genomes at multiple nucleoside positions and perform linkage analysis for those sites. Several unique positions (8–15) on each genome were analyzed among hundreds or thousands of viral genomes from each sample to determine minority viral populations.

Results

Eight potential T cell escape mutations were identified by SGA sequences (7–13 per sample) after comparing the whole genome sequences obtained at screening, enrolment and various weeks (1–48) after enrolment in CH0131. To determine if such mutations were present at screening (antibody undetectable), we analyzed 1,853 partial *env* genes using the PASS assay and found two (0.11%) carrying potential T cell escape mutations. Analysis of 6,556 *nef* gene sequences showed 10 and 25 potential escape mutations (0.15% and 0.38%) at two independent Nef epitopes, respectively. Linkage analysis between independent nucleoside sites revealed numerous unique recombinant genomes at low frequencies (<1%) at the acute infection stage in two individuals (CH0047 and CH0200) infected with multiple transmitted viruses. In CH0200, PASS analysis of 821 viral genomes also identified three additional transmitted viruses present only at 2.3%–4.0% of the viral population.

Conclusion

The data indicate that some T cell escape mutations may exist at low frequencies in the early stage of the infection and are selected later. The PASS assay may serve as a useful method to detect low frequencies of T cell escape mutants, recombinant genomes, and minority transmitted viruses in HIV-1 infected individuals.