Retrovirology

Poster presentation

P09-20 LB. Ultra-deep sequencing of full-length HIV-1 genomes identifies rapid viral evolution during acute infection

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Background

CD8+ T cell responses play a critical role in the early control of HIV. The identification of rapidly evolving sites in HIV provides a powerful means of identifying which CD8 responses are exerting strong immune pressure on the virus and may thus be driving early viral containment. Here we applied next generation sequencing technologies to accurately characterize viral evolution during acute HIV infection at high sensitivity across the complete viral genome.

Methods

Four overlapping ~3 kb amplicons spanning the HIV genome were PCR amplified from the plasma of six subjects at multiple time points during the first six months of infection. The amplicons were molecularly bar-coded, pooled, and sequenced by 454 to yield over 60 million base pairs of data, resulting in ~250-fold coverage and a sensitivity to detect minor variants down to 1%. A subset of amplicons was also sequenced by Illumina at ~8000-fold coverage.

Results

454 sequencing revealed numerous rapidly evolving sites distributed across the viral genome with a majority located in the accessory genes, and numerous mutations consistent with reversion of transmitted mutations to consensus sequence. When viral escape was observed, it was often highly complex, involving evolution at multiple positions and/or expression of multiple non-consensus residues. In some epitopes, initial CTL escape mutations reverted and were rapidly replaced by mutations at other residues within the epitope, suggesting competition between multiple escape variants for dominance over time. In several cases, the frequency of likely compensatory mutations, e.g., non-epitopic mutations, was observed to track almost perfectly over time with escape mutations, evidence of linkage of residues on viral haplotypes.

Conclusion

These data illustrate the value of next generation sequencing for the rapid, sensitive, and comprehensive identification of early HIV escape and reversion and for the characterization of epitopes under substantial early CTL pressure, a proxy for effective anti-viral CD8+ T cell activity.