The majority of reactivatable latent HIV proviruses are genetically distinct with no evidence of ongoing evolution

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Background

Two mechanisms have been proposed to contribute in to the maintenance of the HIV latent reservoir: homeostatic proliferation of latently infected cells and low level viral replication in the lymphoid tissue. Here we studied the sequences of reactivatable latent viruses obtained from a stably treated patient to assess the importance of these mechanisms.

Methods

Resting CD4+ T cells were isolated at regular intervals from the patient, underwent limiting dilution, were activated and then co-cultured with SupT1-CCR5 cells for 21 days. The supernatant was harvested for viral RNA. Regions in *gag* and *env* were analysed by Sanger sequencing. To control for artefacts from culture and sequencing, SupT1-CCR5 cells were infected with NL4-3 and underwent the same limiting dilution, culture, and sequencing processes. Pairwise comparisons were performed to obtain p-distances. Each pair of patient derived viral sequences was considered distinct if the p-distance was higher than that of the corresponding region of the sequences from NL4-3 infected SupT1-CCR5 cells. To seek evidence of viral evolution, a consensus was created from the viral sequences obtained from the first sample and sequences from samples obtained subsequently were compared against this baseline consensus.

Results

We obtained 32 sequences of reactivated latent viruses from a single patient. 18 distinct sequences could be distinguished from the *gag* region. The remaining 14 sequences segregated into five groups. However, when the *env* regions of these 14 sequences were analysed, only one ‘clonal’ group of two sequences remained. 30/32 reactivated latent viruses were distinct. If the threshold p-distance for two sequences to be considered distinct was set at the maximal (rather than average) p-distance observed in the reference set, 26/32 of reactivated latent viruses would still be considered distinct. We have not observed any increase in p-distances over 28 weeks of sampling compared to the baseline samples to suggest of accumulation of mutations.

Conclusion

Our results show that the majority of reactivatable latent viruses are genetically distinct. Persistent viral evolution was not observed in the latent reservoir harboured in peripheral blood resting CD4+ T cells in this stably treated patient.