DIVERSITY OF HIV GAG SEQUENCES DURING INFECTION

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ABSTRACT:
Progression of HIV varies amongst patients, with some exhibiting a prolonged asymptomatic phase of >10yrs, whilst others are much shorter (<3yrs). Evidence indicates host HLA-type and co-receptor polymorphisms influence the onset of AIDS, but less is known about the viral contribution to this phenomenon.

A small cohort of HIV patients (n=7) were investigated for variation in Gag from both plasma and PBMCs, including where available, longitudinal samples. The viral diversity was calculated for each patient and correlated with CD4+ count and viral load (VL). During control of HIV infection, minimal diversity (1.7%) of Gag sequences in both plasma and provirus were observed. From samples studied at immune collapse (CD4+ cells <500), the average diversity was 0.8%, lower than that seen during latency. This was associated with a higher VL (average 328,097 vs. 49,246 copies/ml). For one patient (KC005) diversity during IC was slightly greater (2.4%) and may be explained by a recombination event between two or more quasispecies. Results suggest that after IC, Gag diversity decreases, possibly because peak viral fitness and escape from immunological control has been achieved.

METHODS:
Study Cohort: Patients (n=7) were selected for study based on clinical data regarding both VL and CD4+ count, defining them as either LTNP or RP. All patients were HLA-typed, and were treatment naive.

Pr55 Gag Production & Cloning: Plasma HIV-1 RNA was isolated from each patient sample using a QIAamp Viral RNA Mini Kit, whilst HIV provirus was isolated using the QIAamp DNA mini kit. RT reactions were performed using Superscript III and PCRs utilised HIV-1 clade-B specific Gag primers and Phusion™ polymerase (Fig 1). Prior to cloning, all amplicons were treated with T7-endonuclease-1 to ensure the removal of any heteroduplexes (Het§). Positive clones were then sequenced in both orientations.

Expression of Gag: To confirm an intact Gag protein, viral isolates were cloned into pcDNA4/V5-His Tag to create a fusion protein. Gag was then expressed utilising a cell free expression system, followed by western blotting with an αV5 antibody.

Data Analysis: Electropherograms were read using Chromas, sequences were confirmed as HIV Gag using BLAST and aligned using Clustal W. For each patient studied, the predominant isolate at the earliest time point was taken as the ancestral sequence and the diversity amongst patients’ quasispecies calculated from this.

RESULTS:
Pr55 Gag Amplification & Removal of Het§: Successful amplification of Pr55Gag was achieved for plasma from all patients (data for patients 10, KC65, 84 & 97 shown in Fig 1A) with (+) and without (-) RT. Provirus was only available for 2 patients and amplification of gag was achieved for both. Successful removal of Het§ was demonstrated using two HIV Clade B reference strains. Equal quantities of HXB2 and YU2 Gag were denatured and re-annealed to allow deliberate formation of Het§ (Fig 1B, lane 1). Treatment with T7-endonuclease-1 and incubation at 37°C shows removal of these on TBE-PAGE gels (Fig 1B, lane 3).

Cell Free Expression of Gag Fusion Protein: The expression of Gag fusion was confirmed by WB analysis, using a stop construct (negative control) and an HXB2 positive control (Fig 2).

Calculation of MRCA & Phylogenetic Trees: For each patient phylogenetic trees were drawn rooted to the ancestral sequence (e.g., Fig 2, Amino Acid Trees for KC134 and HIV-009). The diversity was then calculated as the % difference amongst isolates compared to the ancestral sequence (e.g., diversity of KC134 Fig 3).

CONCLUSION:
Despite the small numbers within this study, results suggest that HIV progression leads to a decrease in viral diversity, albeit slightly. This could be explained because as optimum viral fitness peaks, immune escape is achieved and the need for further viral diversity is lost as selective pressure is alleviated.

In patients who were plasma and PBMCs were available (KC005 & KC134), it was evident that Gag sequences showed a distinct evolution within each component fraction, suggesting compartmentalisation of virus. Studying these paired samples may help to understand how immune escape and viral evolution are interwoven in the two populations.

Future monitoring of patients as they progress, and the inclusion of more subjects, will further our understanding of this phenomenon and elucidate the role if any, that Gag diversity plays in HIV progression.