Identifying Sites of "Deep Conservation" in HIV-1 for Vaccine Immunogen Design

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Background

HIV variation remains a central barrier to an effective vaccine. Immune escape contributes significantly to intra- and inter-individual HIV diversity (1). Accumulation of certain escape mutations at the population level has been suggested by i. HLA allele associations with subtype consensus residues (2) ii. Correlation between HLA allele frequency and frequency of associated viral escape mutants across diverse populations (3) and iii. Persistence of high fitness escape mutants following transmission (4). Some conserved residues may thus represent "immunologically silent" sites not subject to immune selection, rather than sites of high structural/functional constraint. We utilized known phylogenetic relationships between HIV-1 and SIVs in their natural primate hosts to identify possible sites of deeper evolutionary conservation ("high EvC") that are distinct from sites of HIV-1/human specific conservation only (Figure 1).

We hypothesized that "high EvC" residues are likely to reflect inherent constraint rather than lack of immune targeting and will demonstrate high replicative cost when mutated. In contrast, residues that are conserved among HIV-1 population isolates but not conserved among phylogenetically-related lentiviruses ("low EvC/high PopC") may represent sites of early population-level fixation of HLA- or other human-driven adaptations unique to HIV. This latter type of site may not be substantially mutationally constrained; furthermore, population-level HLA adaptation at these sites may render them poorly immunogenic in vivo. (Figure 2).

*As proof of concept, we introduced major mutations in HIV-1 at High EvC/High HIV cons sites (**Q130P,L188D,E207D,A431L**) and major mutation at a low EvC/High HIV cons sites (**E106L,Q116P,A120D**). We hypothesized that the former, but not the later, would exhibit severe functional defects. We also engineered a biochemically similar mutation at a High EvC/High site (**L188I**), hypothesizing that this would also incur substantial defects.*
No structural or functional information was used *a priori* to select these sites.

Methods

"High EvC" sites in $p24^{Gag}$ (L188, mutated to non-conservative substitution L188D and conservative substitution L188I),E207D and $p7^{Gag}$ (A431L), and a "Low EvC/High Con" site in $p17^{Gag}$ (E106L),Q116P, A120D and Q130P (Figure 1 and 2) were selected based on structure information and modeling methods and engineered into an HIV-1 NL4-3 reference strain backbone. VsVg-pseudotyped HIV-1 stocks were generated in HEK-293 cells and their $p24^{Gag}$ levels assessed by ELISA. Infectivity and replication capacity of wild-type NL4.3 and mutant viruses were assessed using an established GFP-reporter T-cell assay.

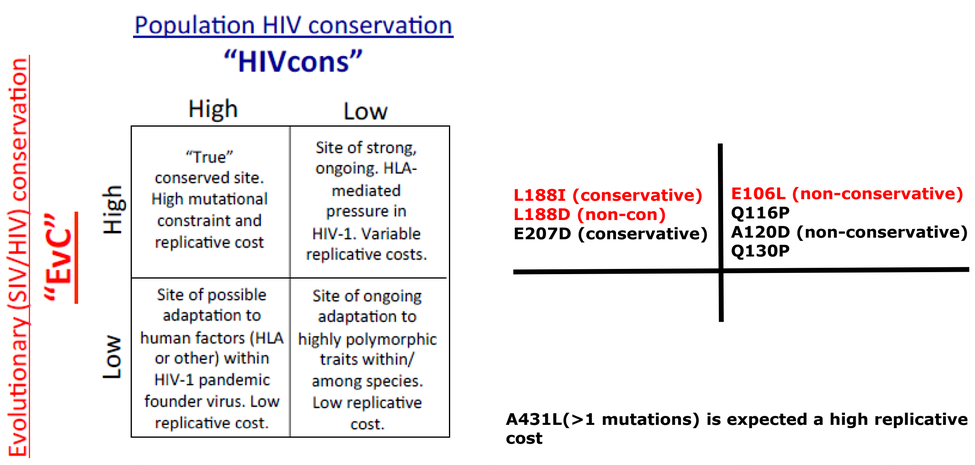

Figure 1: *Predicted characteristics of amino acid residues in HIV-1 based on EvC.*

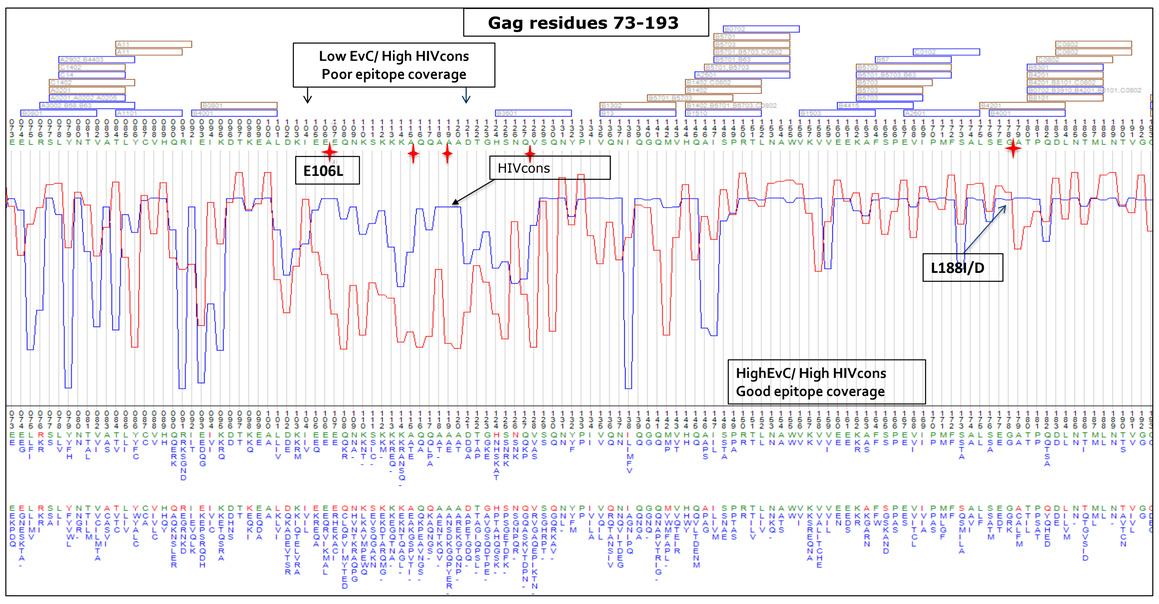

Figure 2*: Plot of EvC of residues in HIV gag **73-193** relative to HIV/SIV phylogeny. Superimposed EvC (red line) versus HIV-1 subtype B population conservation (blue line). Sites with 100% EvC and region associated with high density of known and putative CD8 T cell epitopes indicated. Superimposed EvC (red line) versus HIV-1 subtype B population conservation (blue line). Loss of EvC in the first part of the Nef region corresponds to reduced density of CD8 T cell epitopes. Epitopes mapped here include those in the LANL "A-list" and novel epitopes mapped as a result of large scale epitope mapping in Australian and US populations (5). EvC scores computed in ConSeq server (http://conseq.tau.ac.il/), which builds a phylogeny using maximum likelihood or Bayesian methods from a multiple sequence alignment of phylogenetically related primate lentiviruses. Single site scores computed in the context of the entire protein, and based on the site evolutionary rate estimated from this phylogeny, adjusted for varying numbers of protein homologues and protein length distributions. Sites **106,116,120,130** and **188** indicated*( ).

Results - EvC predicts *in-vitro* replicative capacity

"High EvC" L188 and A431 sites are 100% conserved in the SIV/HIV phylogeny. At codon 188, we engineered L188I and L188D as conservative and non-conservative substitutions respectively, and at A431 we engineered A431L. We hypothesized that any change (even conservative ones) at high EvC sites would be costly to replication. In contrast, E106L is a site conserved among all subtype B HIV-1 isolates but variable across SIVs, suggesting human host-specific conservation only. We hypothesized no or minor effects on replication capacity compared to wtNL4.3. Representative data in Figure 4.

Assessments of p24 production (Figure 4A) and infectivity (Figure 4B) were performed in triplicate with consistent results. Replication capacity experiments using VSVg pseudotyped virions (Figure 4C) in CEM-GXR cells were performed twice with consistent results. Our observations are:

1. Q130, F164Y, L188D, E207D and A431L (high EvC, non-conservative change) were significantly impaired with minimal p24 detection, negligible infectivity and no viral spread suggesting a possible viral assembly defect.
2. L188I (high EvC, conservative change) was an intermediate phenotype with high p24 generation, poor though detectable infectivity suggesting a possible entry defect, and poor/no viral spread.
3. Wildtype NL4-3 and E106L (low EvC/high PopC) were comparable with high p24 ELISA values, high infectivity, and good viral spread.

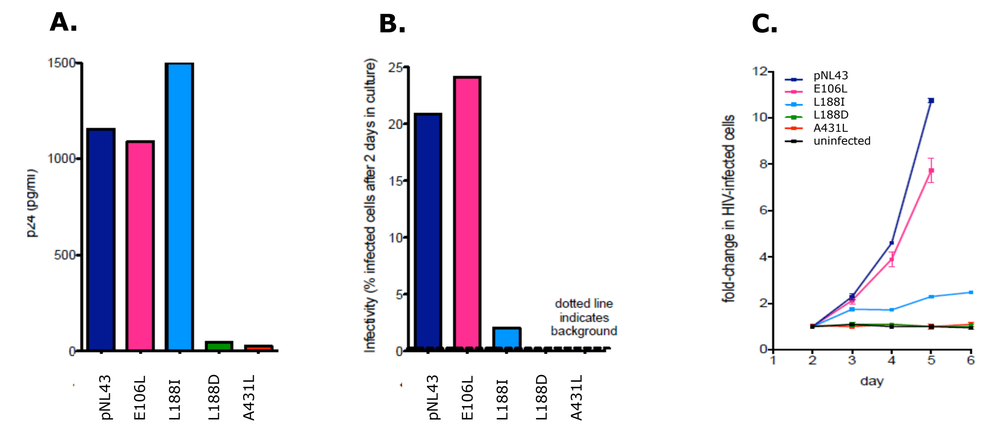

Figure 3: *A: P24 ELISA, B: Infectivity, C Replicative Capacity using VSVg pseudotyped viral stocks, duplicate cultures initiated at 2% MOI on day 2.*

Comparison of EvC values at sites tested using mutagenesis studies (6) in many instances reflect characteristics predicted in the schema

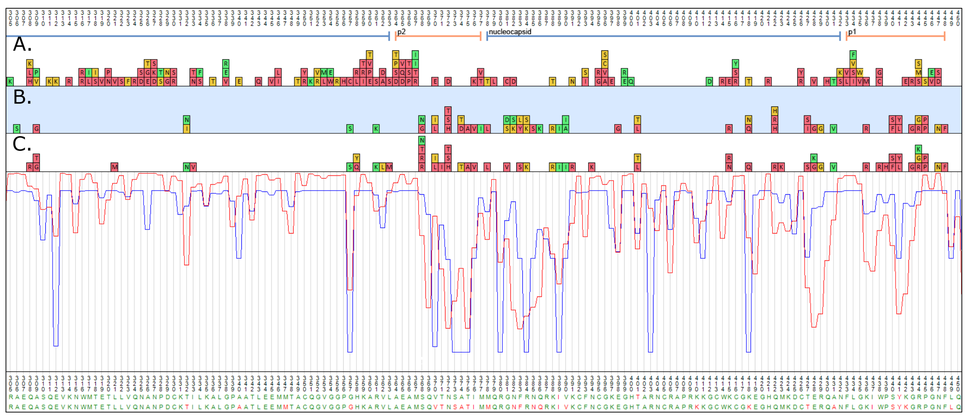

Figure 4: *EvC mapped against replicative capacity effects at specific sites in published HIV-Gag mutagenesis experiments (6). Red denotes severe lethal change, yellow intermediate and green comparable to wildtype. Note many sites not previously examined and frequently the specific mutations tested (A) are not those observed either in population HIV (B) or SIV sequences (C).*

Discussion

In all tested cases, high EvC correctly predicted a lethal or near lethal effect of mutation whereas absolute population-level conservation alone did not appear to confer any significant constraint. No structural or functional information was used *a priori* to select these sites or specific substitutions .

The initial sites tested support the hypothesis that EvC can be used as a probe to identify virological constraint and/or potential host adaptation early in the pandemic. In this case, E106L falls in an area that is poorly covered by known T-cell epitopes and may represent a site of HIV adaptation to human-specific immunity at the population-level.

Using EvC in a systematic way to understand functional aspects of HIV proteomes and host-specific selection pressures may be applied in the development of HIV immunogens as well as in rational drug design.

References

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Acknowledgments

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