Complex impact of reversion mutations and CD8+ T cell escape mutations on HIV-1 fitness

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Abstract

Background: Mutations that revert back to the consensus sequence frequently occur in the HIV-1 genome during infection and have been considered to render the viruses more fit. However, their impact on viral fitness and interactions with the immune escape mutations have not been evaluated in their cognate transmitted/founder (T/F) viral genomes.

Methods: Our previous study showed that viruses containing both cytotoxic T lymphocyte (CTL) escape mutations and reversion mutations are as fit as the T/F viruses. To precisely determine the role of reversion mutations, we generated the T/F mutants that contained either reversion mutation alone or together with CTL escape mutations and determined their impact on the viral fitness in primary CD4+ T cells using the PASS fitness assay.

Results: The reversion mutation V247I in the TW10 CTL epitope in Gag could partially restore the fitness loss caused by the CTL escape mutation T242N in the same epitope. However, the reversion mutation V247I or I64T in Tat/Rev alone had no measurable impact on fitness of the T/F virus. The CTL escape mutations G248A in Gag and R355K in Env also did not have any detectable fitness cost. Interestingly, the CTL escape mutation G248A, like the reversion mutation V247I, could partially compensate the fitness loss caused by the T242N mutation. Both the V247I and G248A mutations together fully restored the fitness loss of the T242N mutant. Positions 242, 247 and 248 are not located at p24 pentamer or hexamer interfaces and therefore should not affect the capsid assembly. In addition, homology modeling of p24 monomers demonstrated that mutations at these positions are not expected to significantly affect stability of the helix 6 structure.

Conclusions: Our results showed that reversion mutations might not render their cognate T/F virus significantly more fit *in vitro* but could partially restore the fitness loss caused by the CTL escape mutation, and a CTL escape mutation could also partially compensate the fitness loss due to the other CTL escape mutation. These findings demonstrated that the overall viral fitness is influenced by the complex interplay of different mutations.

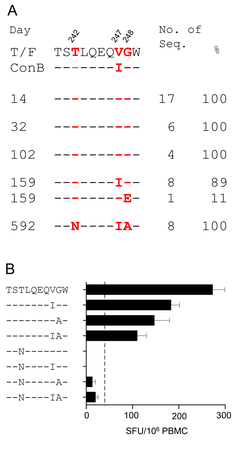

Figure 1. Frequencies of mutations in the TW10 epitope at different time point after infection (A) and the T cell responses to the wild-type and mutant TW10 peptides determined by ELISpot (B).

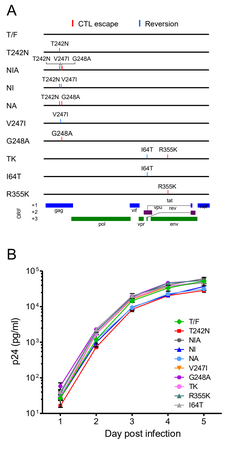

Figure 2. Schematic presentation of the mutations introduced into the CH77 T/F virus (A) and the replication kinetics of the CH77 T/F virus and its mutants (B).

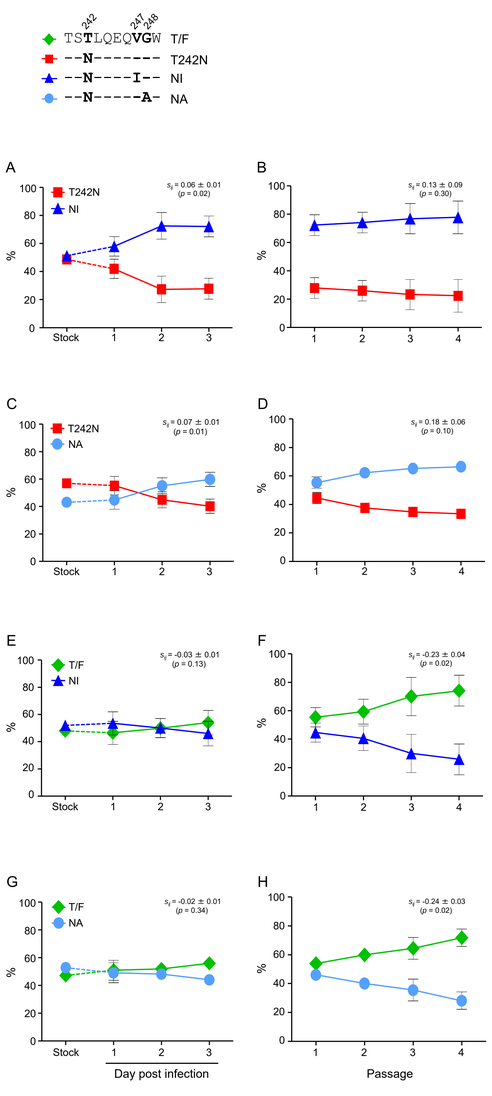

Figure 3. Partial restoration of the fitness loss of T242N mutant by compensatory mutations V247I and G248A in single- and multiple-passage fitness assay.

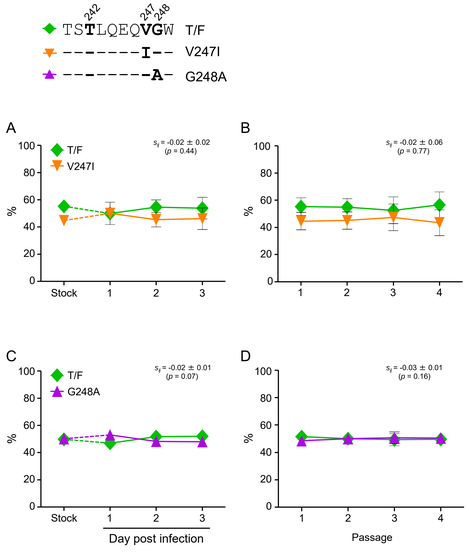

Figure 4. The V247I or G248A mutation alone did not cause detectable fitness changes in their cognate T/F virus in single- and multiple-passage fitness assay

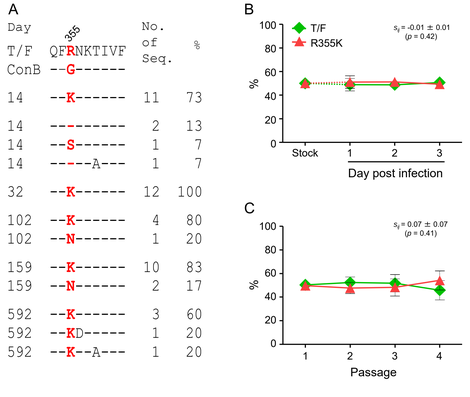

Figure 5. No detectable fitness cost of the early CTL escape mutation R355K in Env in single- and multiple-passage fitness assay.

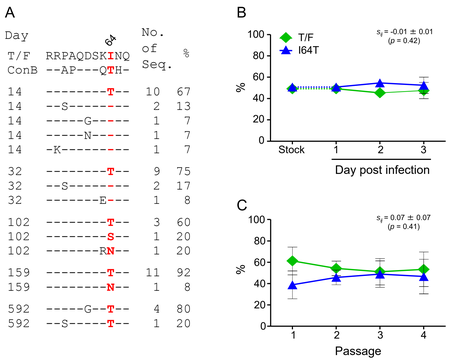

Figure 6. No detectable fitness cost of the early reversion mutation I64T in Tat/Rev overlapping region

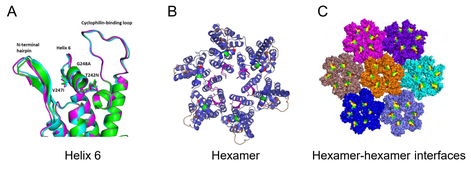

Figure 7. Structural modeling of mutations in the TW10 epitope in the p24 protein of Gag.

Summary

- The reversion mutation V247I or the partial CTL escape mutation G248A alone partially compensates the fitness loss caused by the T242N mutation, and both together can fully restore the fitness loss to the T/F level.
- Two reversion mutations (V247I and I64T), one early CTL escape mutation (R355K) and one partial CTL escape mutation (G248A) each alone did not cause detectable fitness impact on their cognate T/F virus *in vitro*.
- The CTL escape mutation can also compensate the fitness loss caused by another CTL escape mutation.
- The overall HIV-1 fitness is influenced by a complex interplay of different mutations.