CD8+ T Cells from HLA-B*57 Elite Suppressors Effectively Suppress Replication of HIV-1 Escape Mutants

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Introduction

Elite Suppressors (or Controllers, ES) are individuals infected with HIV-1 and maintain undetectable viremia for several years. While early studies have suggested that the elite suppressor phenotype is merely a result of infection with a deficient variant, more recent studies have demonstrated that ES are infected with fully replication competent virus. Specific MHC-I molecules have been found to be overrepresented in the ES population, specifically HLA molecules B*57 (B*5801) and B*27. These MHC-I proteins are believed to confer protection by targeting conserved immunodominant epitopes.

Comparison studies of HLA-B*57+ ES and Chronic Progressors (CP) have helped to clarify the elite suppressor phenotype. CD8+ T cells of ES respond to stimulation with Gag peptides more robustly than do those of CP, showing greater proliferation as well as more granzyme A/B and perforin expression. Additionally, sequences of proviruses from ES show few to no canonical "escape mutants" from these immunodominant epitopes, whereas CP usually harbor several. Interestingly, virus sequenced from the plasma of ES frequently harbor escape mutations in these epitopes.

In this study, we addressed how ES are capable of maintaining undetectable viremia despite the presence of "escaped" variants circulating in the plasma. Specifically, we asked if CD8+ T cells from ES are capable of recognizing and inhibiting viral replication of viral variants that harbor canonical escape mutations.

Materials and Methods

NL4-3 ΔEnv::eGFP escape variant pseudotyped viruses were engineered using Agilent QuikChange Site Directed Mutagenesis kit. 293Ts were transfected with the resulting plasmid and IIIB Envelope plasmid, and virus was collected by ultracentrifugation. PBMCs were isolated from fresh whole blood by ficoll density centrifugation. CD4+ T cells and CD8+ T cells were isolated using negative and positive selection, respectively, Miltenyi beads. Cells were spinoculated at 1,200 x *g* for 2 hours at 30°C. For suppression experiments, CD8+ T cells were added to infected CD4+ T cells immediately after infection and cultured for 3 or 5 days before measuring infectivity (GFP positivity) by FACS. All studies were approved by the JHIRB. All donors provided written consent prior to participation in this study.

Results

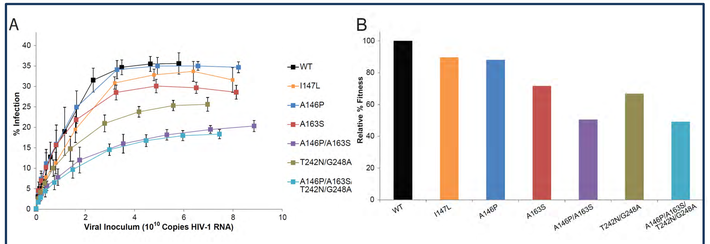


Figure 1 Fitness cost of canonical HLA-B*57 escape mutations
A. Average infection by seven NL4-3 escape variants in HIV-negative donors as measured by GFP expression. Error bars represent SEM. n=4. **B.** Relative fitness of each escape variant to wild type NL4-3 virus.

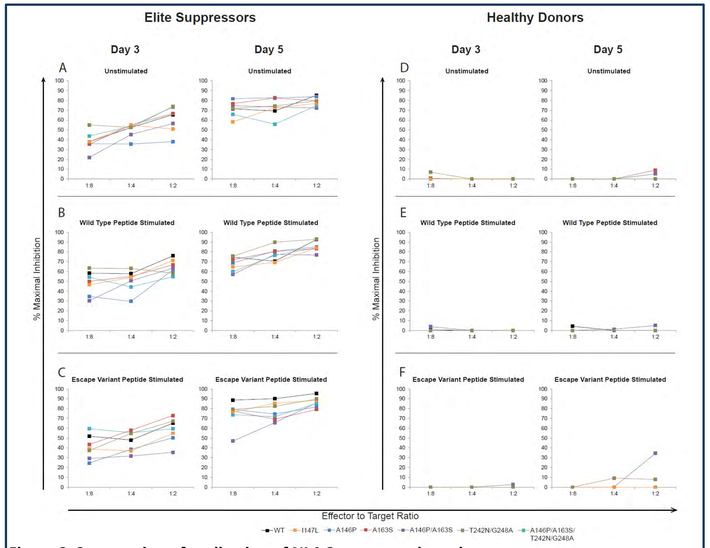


Figure 2 Suppression of replication of NL4-3 escape variant viruses
Unstimulated CD4+ T cells from HLA-B*5703+ ES (left) or HIV-negative donors (right) were infected with one of seven NL4-3 variants (wild type, black; I147L, orange; A146P, navy; A163S, red; A146P/A163S, purple; T242N/G248A, brown; A146P/A163S/T242N/G248A, teal) and cocultured with CD8+ T cells (unstimulated, top; wild type epitope peptide stimulated, middle; escape variant epitope peptide stimulated, bottom) at various effector:target ratios. Infection of CD4+ T cells was read by FACS on day 3 and day 5, and inhibition was calculated.

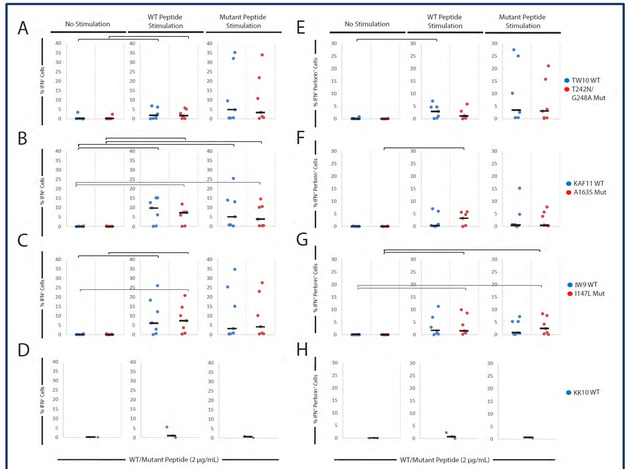


Figure 4 Intracellular cytokine staining of ES CD8+ T cells
PBMCs from HLA-B*5703+ ESs that were stimulated with either wild type or escape variant epitope peptides for 7 days or PBMCs that were unstimulated were (re)stimulated overnight with individual peptides. CD8+ T cells were analyzed for IFN-γ (**A-D**) or coexpression with perforin (**E-H**).

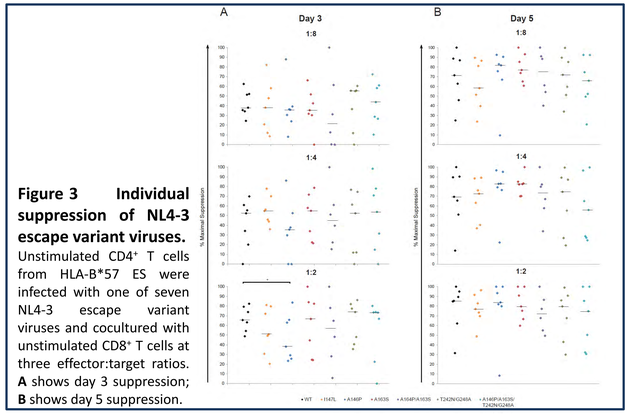


Figure 3 Individual suppression of NL4-3 escape variant viruses.
Unstimulated CD4+ T cells from HLA-B*57 ES were infected with one of seven NL4-3 escape variant viruses and cocultured with unstimulated CD8+ T cells at three effector:target ratios. **A** shows day 3 suppression; **B** shows day 5 suppression.

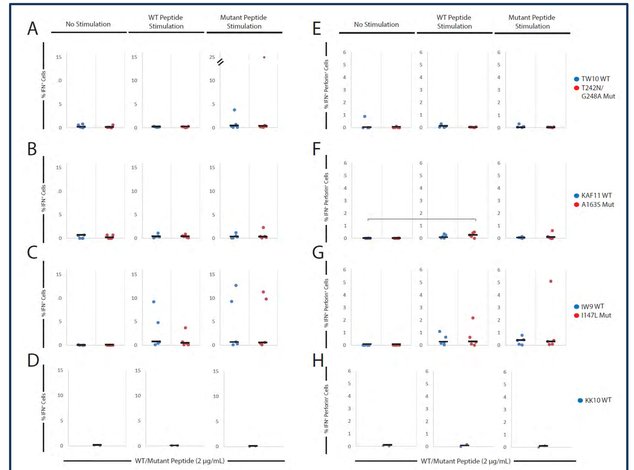


Figure 5 Intracellular cytokine staining of ES CD8+ T cells
PBMCs from HLA-B*5703+ CPs that were stimulated with either wild type or escape variant epitope peptides for 7 days or PBMCs that were unstimulated were (re)stimulated overnight with individual peptides. CD8+ T cells were analyzed for IFN-γ (**A-D**) or coexpression with perforin (**E-H**).

Conclusion

CD8+ T cells from ES are capable of suppressing replication of viruses despite the presence of "escape mutations" in HLA-B*57-restricted Gag epitopes; while reduced fitness of mutated variants may also play a role in the elite suppressor phenotype *in vivo*, viremic HLA-B*57+ patients often harbor these mutations, suggesting an ability for robust growth despite potential fitness costs. Additionally, our data suggest a protective *de novo* responses in ESs and some CPs that are capable of responding to both wild type and "escape" variant peptides. Induction of CD8+ T cells that could recognize and kill cells infected with either wild type or escape variant virus would be advantageous for a CTL-based vaccine.

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