Trifunctional T-cell Engagers Targeting PD-1 and TIGIT in Chronic HIV/SHIV Infections

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Introduction

Several studies suggest the vital role of immune checkpoint inhibitors, such as PD-1 and TIGIT, in dysregulation of CD8 T cell functional activities during chronic HIV-1/SIV infections. Importantly, these checkpoint inhibitors are also found to be highly expressed on the surface of CD4 T cells that harbor latent HIV. We have previously demonstrated that anti-HIV/anti-CD3 bispecific T-cell engager (BiTE) can be used to redirect functionally compromised follicular CD8 T cells to kill HIV infected cells *in vitro*. Here, we ***hypothesize*** that adding an extra specificity to target PD-1 and TIGIT to the BiTE will further enhance the functional activities of CD8 T cells and simultaneously lower the threshold for reactivation of HIV latently infected cells.

Methods

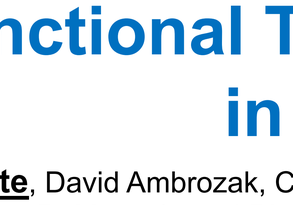
To test our hypothesis, we generated the trifunctional anti-HIV/anti-CD3/anti-PD-1 and -anti-TIGIT T-cell engagers by linking the scFvs derived from anti-TIGIT and anti-PD-1 antibodies to the BiTE molecule, respectively.

Bi-functional formats



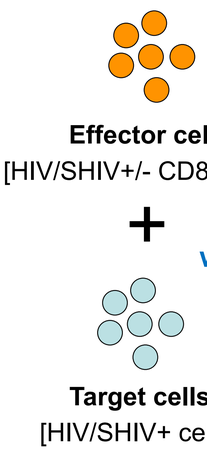
Tri-functional formats





In Vitro and *Ex Vivo* Killing Assays

Using HIV-infected cell lines and primary CD4 cells from chronically SHIV-infected LN as target cells in the *in vitro* and *ex vivo* killing assays, we tested whether trifunctional T-cell engagers (TCE) could enhance the cytolytic activities of functionally compromised CD8 T cells.

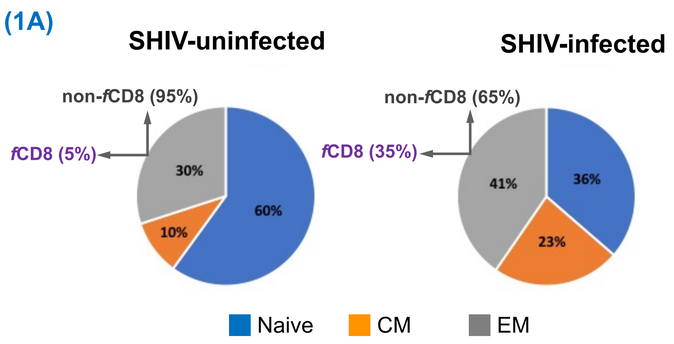


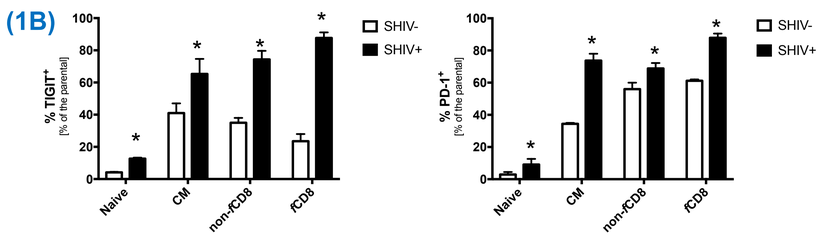
Quantify

- % target cell lysis
- *gag* DNA/RNA
- T cell activation
- CD8 polyfunctionality

Data

CD8 T cell dynamics and functionality during SHIV-infection





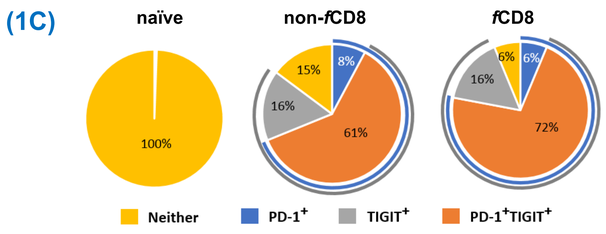
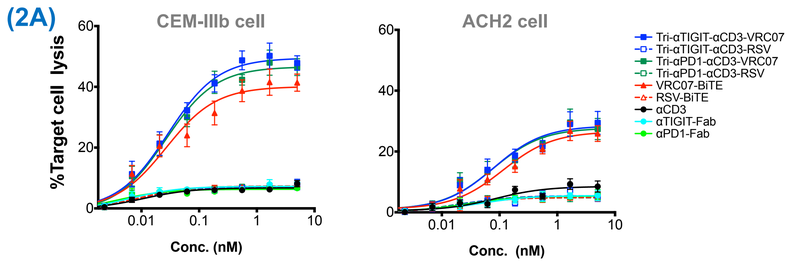
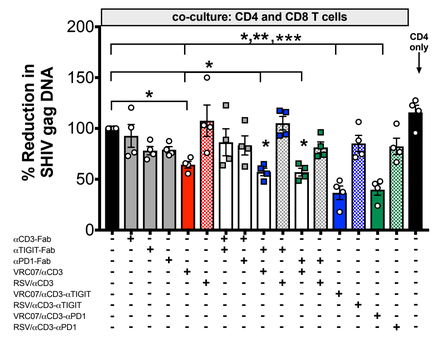
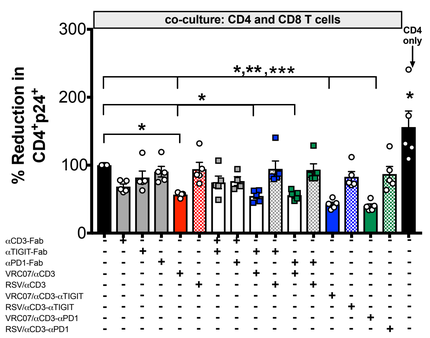


Figure 1. (A) frequency of CD8 subsets in uninfected VS chronically SHIV-infected LN; (B) comparison of PD-1 and TIGIT expressions in CD8 subsets of uninfected VS chronically SHIV-infected LNs; and (C) frequency of PD-1 and/or TIGIT expressions in CD8 subsets of chronically SHIV-infected LNs use in the study. (n > 5)

Trifunctional anti-HIV/anti-CD3/anti-PD-1 and anti-TIGIT increases the cytolytic activities of CD8 T cells







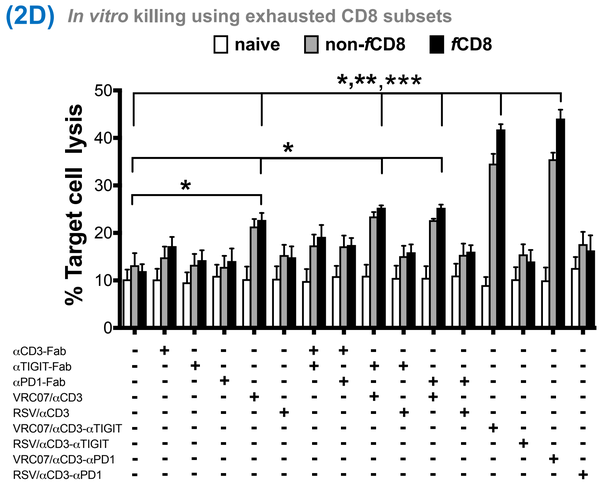
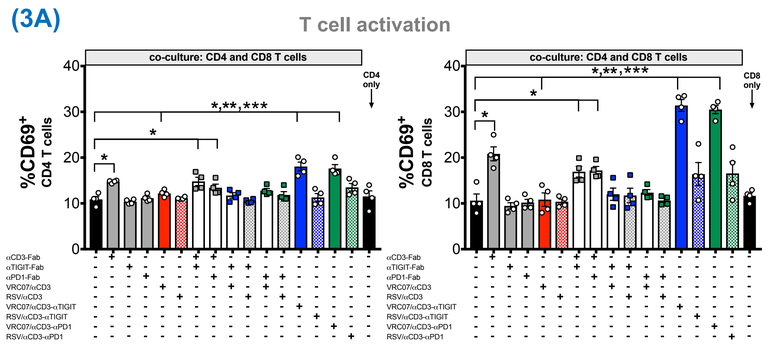


Figure 2. Effects of T cell-engagers on (A) percentage of CEM-IIIb and ACH2 target cell lysis, when SHIV+ LN CD8 T cells were used as effector cells; (B) the level of SHIV *gag* DNA of CD4 cells from SHIV+ LN, when autologous CD8 T cells were used as effector cells; (C) level of CD4+p24+ infected cell in the latency model system; and (D) percentage of CEM-IIIb target cell lysis, when sorted CD8 subsets from chronically SHIV-infected LNs were used as effector cells. 2nM of TCEs were used in (B,C,D). * significantly different from "No TCE"; ** significantly different from "VRC07/αCD3"; *** significantly different from indicated group [p < 0.05]. (n > 3)

Trifunctional anti-HIV/anti-CD3/ anti-PD-1 and anti-TIGIT activates and enhances polyfunctionality of T cells



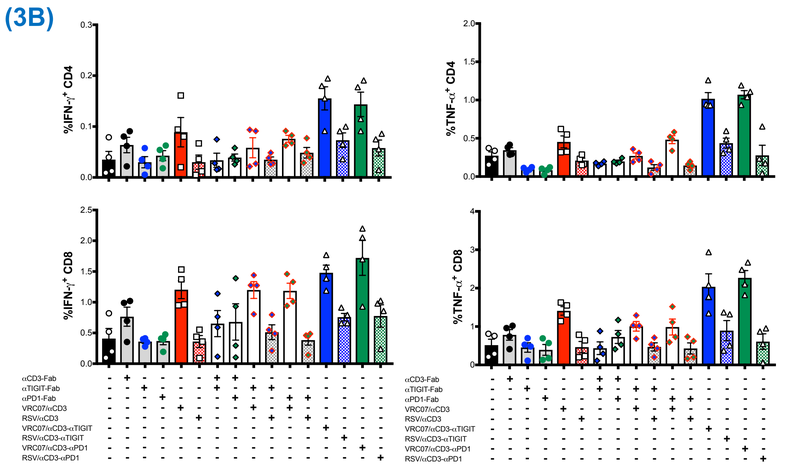


Figure 3. Effects of T cell-engagers on (A) frequency of CD69+CD4 and CD69+CD8 T cells; (B) gag-specific polyfunctionality of SHIV+ LN CD8 and CD4 T cells (normalized to no gag-pooled peptide stimulation). 2nM of TCEs were used. * significantly different from "No TCE"; ** significantly different from "VRC07/αCD3"; *** significantly different from indicated group [p < 0.05] (n > 3)

Results

We found that trifunctional anti-HIV/anti-CD3/anti-TIGIT and –anti-PD-1 increased the cytolytic activities of CD8 T cells than anti-HIV/anti-CD3 BiTE in the *in vitro* and *ex vivo* killing assays. We also demonstrated that trifunctional anti-HIV-1/anti-CD3/anti-TIGIT and –anti-PD-1 could enhance the CD8 T cell lysis of the latently infected cells using a primary HIV latency model. Furthermore, trifunctional anti-HIV-1/anti-CD3/anti-TIGIT and –anti-PD-1 were shown to increase antigen-specific CD107a degranulation, levels of granzyme B, cytokine release by CD8 T cells, which could potentially underlie the observed increase in the killing capability of CD8 T cells.

Conclusion

Our results indicate that the use of trifunctional T cell engagers targeting immune checkpoints, TIGIT and PD-1, may serve as novel immunotherapeutic strategies to eliminate infected cells in HIV infected individuals.

Acknowledgement

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