Abstract # 242

T Follicular Regulatory Cells Are Highly Permissive to CCR5-tropic HIV-1 *ex vivo*

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BACKGROUND

The majority of HIV replication occurs in follicular helper T cells (TFH) in secondary lymphoid follicles during asymptomatic disease. Follicular regulatory T cells (TFR) are a subset of TFH that control germinal center (GC) responses. Their role in HIV-1 replication is unknown. We hypothesized that TFR are highly susceptible to HIV and evaluated potential causative mechanisms.

METHODS

Cells were disaggregated from healthy human tonsils or seronegative human lymph nodes and spinoculated with R5-tropic pNL4-3-based GFP reporter virus, NLYUV3-GFP (a gift from Dr. David Levy), or mock spinoculated, cultured for 2 days, and analyzed by flow cytometry. Due to downregulation of CD4 by HIV, cells were gated on CD3+CD8- cells and subsets within were defined as TFR (CXCR5+CD25+CD127-), GC TFR (CXCR5+PD1+CD25+CD127-), TFH (CXCR5+CD25-), and GC TFH (CXCR5+PD1+CD25-). In select experiments, tonsil TFR and TFH were sorted prior to spinoculation. CCR5 and Ki67 expression were determined at baseline prior to spinoculation in a subset of tonsil subjects. Fusion assays were performed by spinoculating BlaM-Vpr containing transmitted founder CCR5-tropic HIV-1 viruses CH40, CH58, and CH470 (obtained from Dr. Beatrice Hahn & the NIH AIDS Reagent Program) with disaggregated CD4+ isolated human tonsil cells. Statistical analyses were performed by Wilcoxon matched-pairs signed rank test using GraphPad Prism 6. Significance is denoted by asterisks where * = p< 0.05 and ** = p< 0.01.

RESULTS

Fig. 1: Representative flow gating

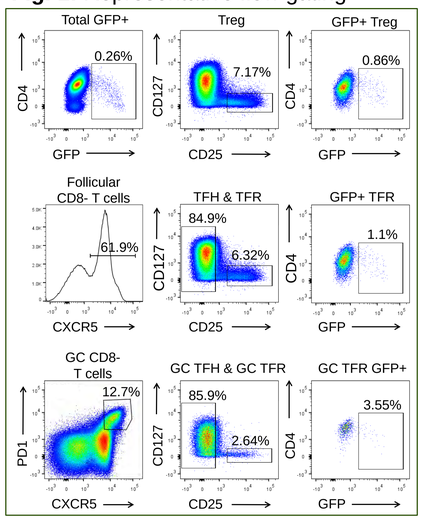


Fig. 2: Treg, TFR, and GC TFR are highly permissive to R5-tropic HIV-1

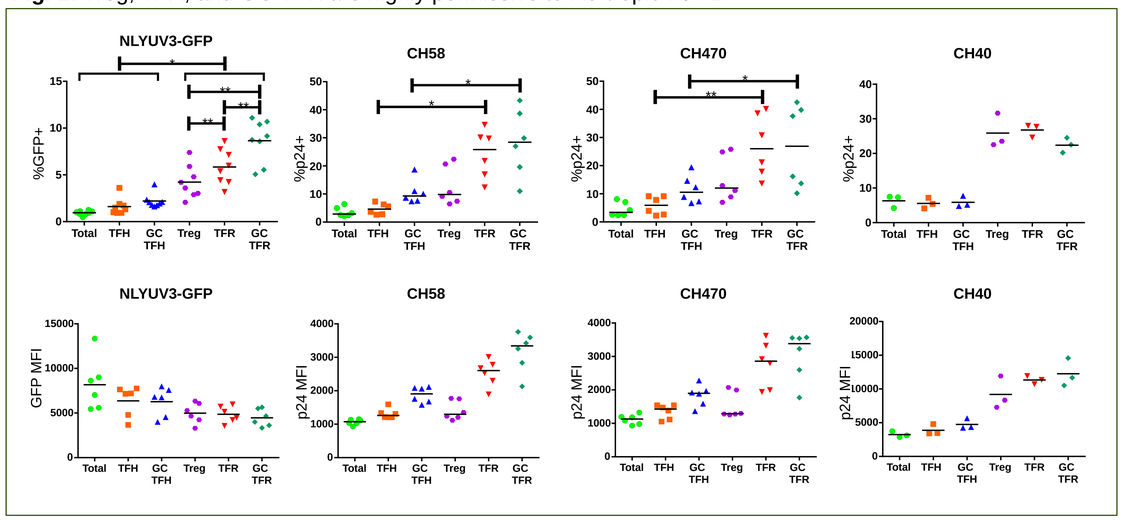


Fig. 3: Sorted TFR are highly permissive to R5-tropic HIV-1

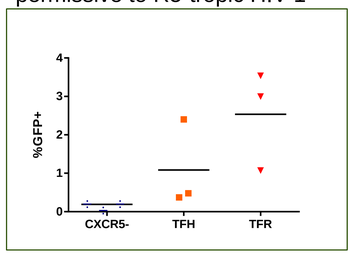


Fig. 4: Seronegative LN Treg, TFR, and GC TFR are highly permissive to R5-tropic HIV-1

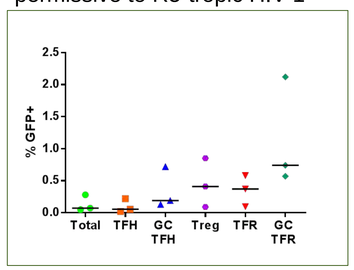


Fig. 5: Treg, TFR, and GC TFR express high levels of activation markers and CCR5

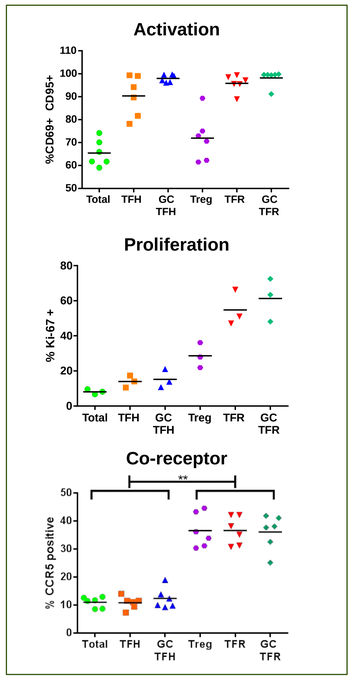
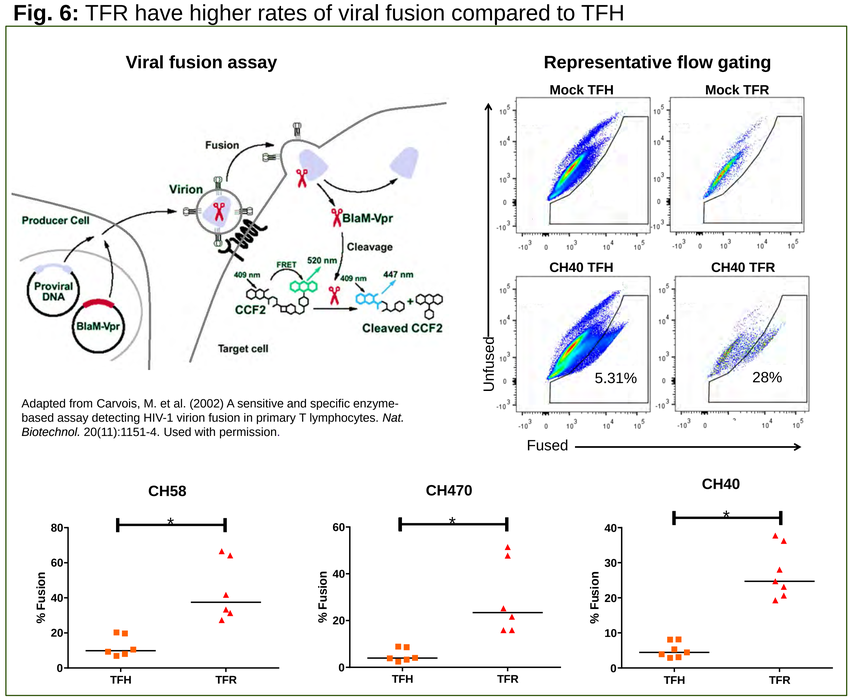


Fig. 6: TFR have higher rates of viral fusion compared to TFH



Adapted from Carvois, M. et al. (2002) A sensitive and specific enzyme-based assay detecting HIV-1 virion fusion in primary T lymphocytes. *Nat. Biotechnol.* 20(11):1151-4. Used with permission.

SUMMARY

- Treg, TFR, and GC TFR are highly permissive to R5-tropic HIV-1 *ex vivo.*
- TFR and GC TFR are highly proliferative based on Ki-67 staining.
- A significantly greater proportion of Treg, TFR, and GC TFR express the co-receptor CCR5 compared to total T cells, TFH, and GC TFH.
- Viral fusion takes place in a greater proportion of TFR compared to TFH during R5-tropic HIV-1 infection.

CONCLUSIONS

TFR are highly permissive to R5-tropic HIV *ex vivo*, which may promote HIV infection and replication within secondary lymphoid follicles *in vivo*. Much of this increased permissivity is due to fusion taking place in a greater proportion of TFR compared to TFH. This, in turn, is likely due to the fact that a greater percentage of TFR express the CCR5 co-receptor compared to TFH. Additionally, TFR are highly proliferative, based on Ki-67 staining. As proliferating T cells are a major target cell of HIV, this likely contributes to the increased permissivity of TFR compared to TFH.

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