Poster # 00214

Germinal Center B cells Augment HIV Replication in T Follicular Helper Cells

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BACKGROUND

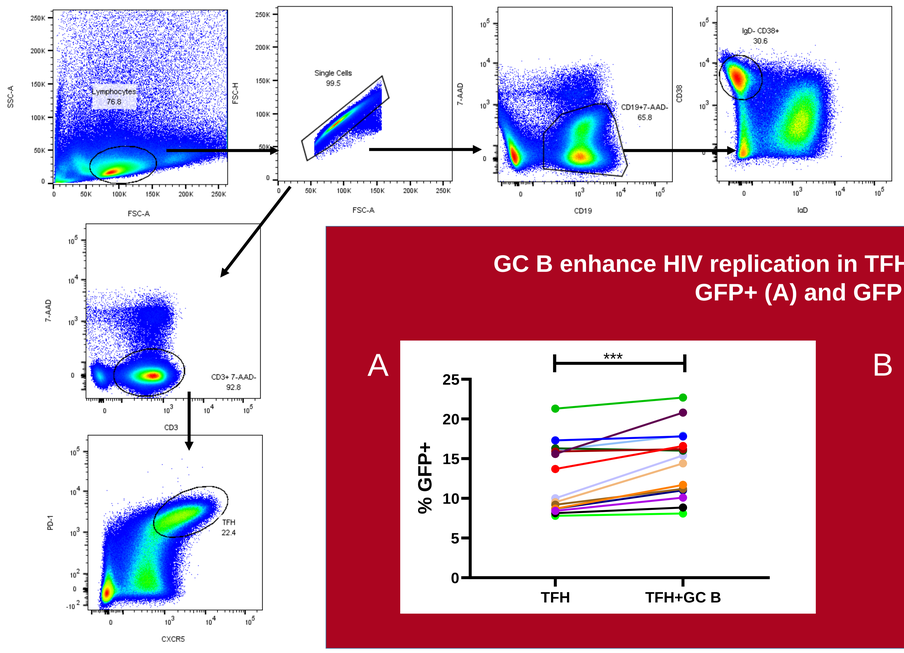
T Follicular helper cells (TFH) are highly permissive to HIV and major contributors to HIV replication in untreated and treated individuals. Much work has focused on the role of TFH in generating humoral immunity by activating germinal center B cells (GC B) through expression of the costimulatory molecules CD40L and ICOS. The impact of GC B on HIV replication in TFH, however, has not been assessed. We evaluated the impact of GC B on HIV infection, integration, and expression in TFH using a well-established ex vivo tonsil model of HIV infection.

METHODS

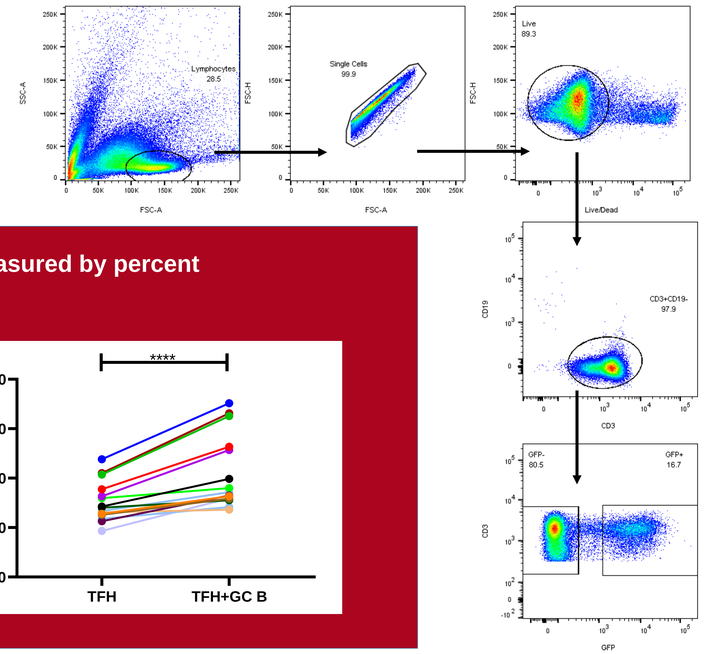
TFH ($CD3^+CD8^-CXCR5^+PD\text{-}1^{hi}$) and GC B ($CD19^+CD38^+IgD^-$) were isolated from tonsils of individuals at low risk for HIV infection. TFH were spinoculated with NLENG1-IRES, an HIV GFP reporter virus and cultured with or without GC B (1:1) in round-bottom tubes in the presence of saquinavir. Soluble ICOS (sICOS), soluble CD40L (sCD40L), and CD40 antibody were added at 5 µg/ml in a subset of experiments. Percent GFP+ TFH and GFP median fluorescence intensity (MFI) were assessed via flow cytometry after 3 days. DNA was isolated from spinoculated TFH after 18-20 hours in culture. Total and integrated HIV DNA were quantified by qPCR. TFH cell counts were quantified using counting beads. Statistical analyses were performed using nonparametric Wilcoxon and Friedman tests and post hoc Dunn's multiple comparison tests. *p<0.05, ***p<0.001, ****p<0.0001

RESULTS

Representative TFH and GC B sort

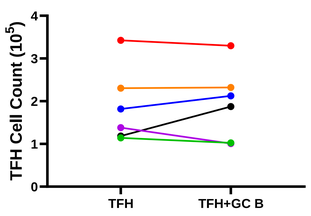


Representative flow analysis post culture

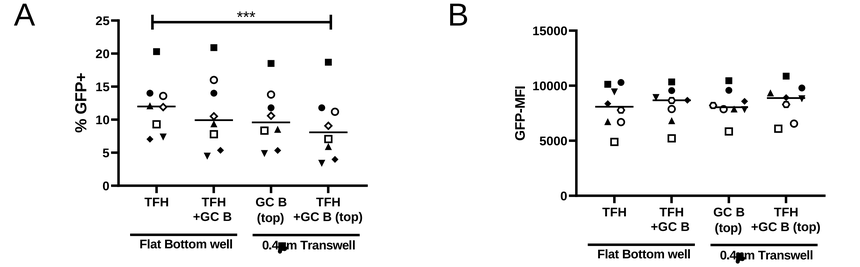


GC B enhance HIV replication in TFH as measured by percent GFP+ (A) and GFP MFI (B)

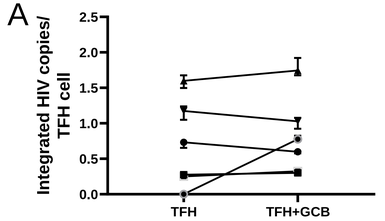
GC B do not impact TFH viability



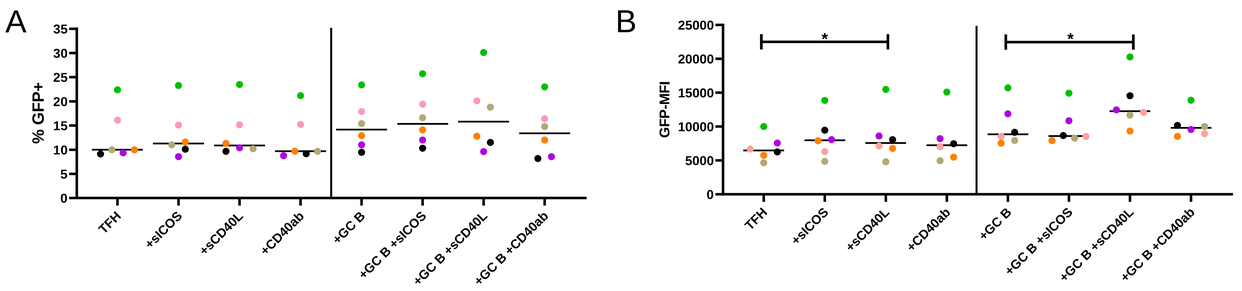
GC B distributed in flat bottom wells or transwell inserts do not augment percent GFP+ (A) and GFP MFI (B) in TFH



GC B do not affect integrated (A) or total (B) HIV DNA in TFH



Costimulatory molecules do not influence percent GFP+ (A) or GFP MFI (B) in TFH:GC B cultures



CONCLUSIONS

- GC B augment HIV replication in TFH through direct contact.
- Addition of soluble ICOS or CD40L does not further amplify GC B effects on HIV replication in TFH.
- A better understanding of the mechanisms that underlie GC B-mediated effects on HIV replication could present new targets for suppression of HIV replication in B cell follicles.

ACKNOWLDEGEMENTS

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