CD40L/IL-4 Stimulated B Cells as Efficient Mediators of HIV-1 Binding and Trans Infection

343

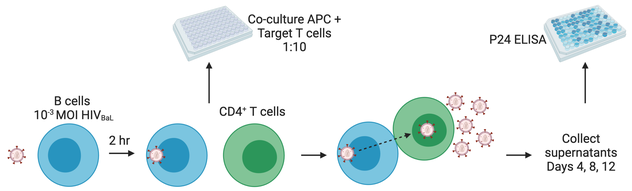
Abigail Gerberick[1], Allison DePuyt [1], Peter Shoucair[2], Robbie Mailliard[2], Nicolas Sluis-Cremer[2], and Charles Rinaldo[2]

[1]Department of Infectious Diseases and Microbiology, School of Public Health and [2]Division of Infectious Diseases, Department of Medicine, School of Medicine, University of Pittsburgh, Pittsburgh, PA, United States

BACKGROUND

- Cell-to-cell HIV-1 transmission is a potent mechanism of HIV-1 pathogenesis
- B and CD4+ T cells frequently interact in secondary lymphoid organs (SLO), thus potentially promoting B cell-mediated HIV-1 trans infection
- In SLO, B cells are stimulated though both stromal (BAFF) and CD4+ T cell (IL-4, IFN-g, CD40L) signals, however the impact of these signals on the efficiency of HIV-1 binding and trans infection remains unknown

METHODS



- Human B and CD4+ T cells were isolated from blood of people without HIV
- B cells were left unstimulated or stimulated with CD40L/IL-4, CD40L/IFN-g, BAFF/IL-4, or BAFF/IFN-g for 48h
- For trans infection, see above figure
- For HIV-1 binding, B cells were incubated with HIV-1 Gag-iGFP (MOI 10^{-1}) and analyzed by flow cytometry
- Single cell RNA sequencing was performed via the BD Rhapsody and Illumina platforms post 10h stimulation and data were analyzed using the Seurat packaged in R Studio

RESULTS

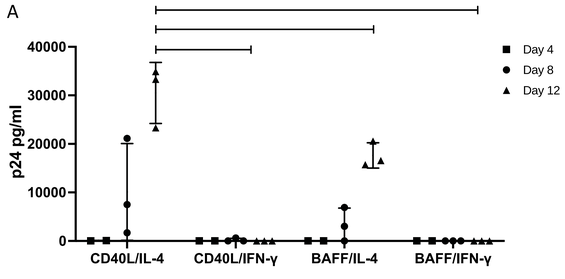
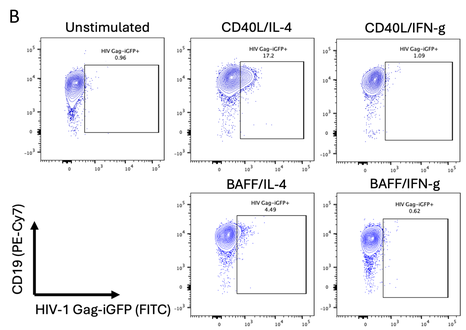


Figure 1. CD40L/IL-4 Stimulated B cells efficiently trans infect CD4+ T cells **(A)** One way ANOVA (p<0.0001) followed by Dunnett's multiple comparisons on day 12. n=3. **p=0.0043 ****p<0.0001

RESULTS



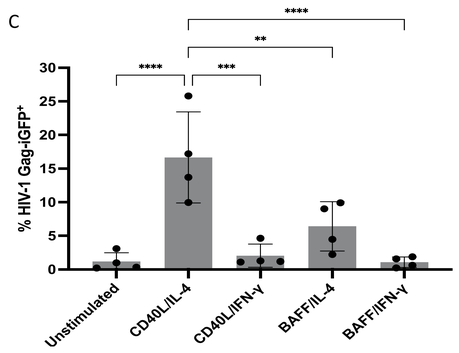
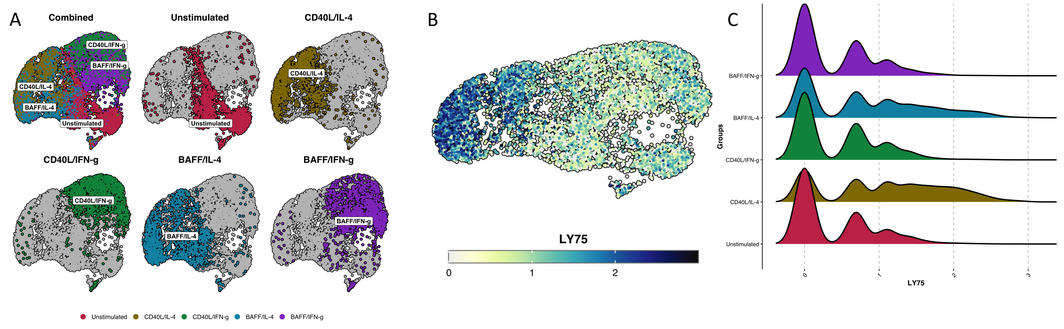
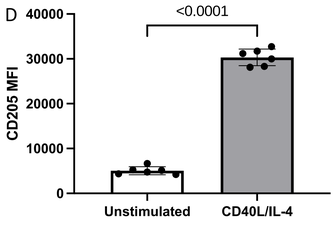
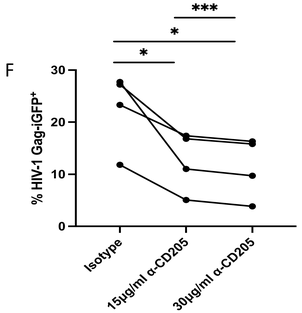


Figure 1. CD40L/IL-4 Stimulated B cells efficiently bind HIV-1 **(B-D)** HIV-1 Gag-iGFP binding by stimulation **(B)** Representative donor **(C)** One way ANOVA followed by Dunnett's multiple comparisons. N=4. **p=0.0038, ***p=0.0001, ****p<0.0001







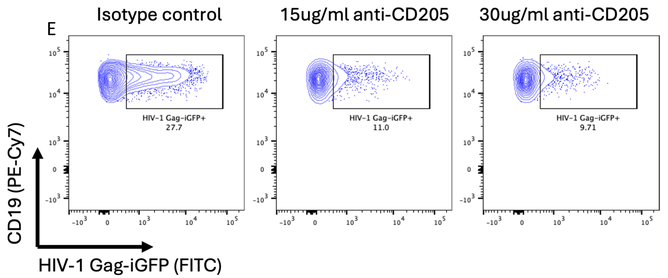


Figure 2. CD40L/IL-4 Stimulated B cells bind HIV-1 via CD205 **(A)** B cell gene expression by stimulation **(B,C)** CD205 mRNA expression by stimulation **(D)** CD205 protein expression. N=6. paired t test **(E,F)** HIV-1 Gag-iGFP binding post treatment with anti-CD205 polyclonal antibody **(F)** Representative donor **(G)** n=4. Paired t test. *p<0.03 ***p=0.0004

RESULTS

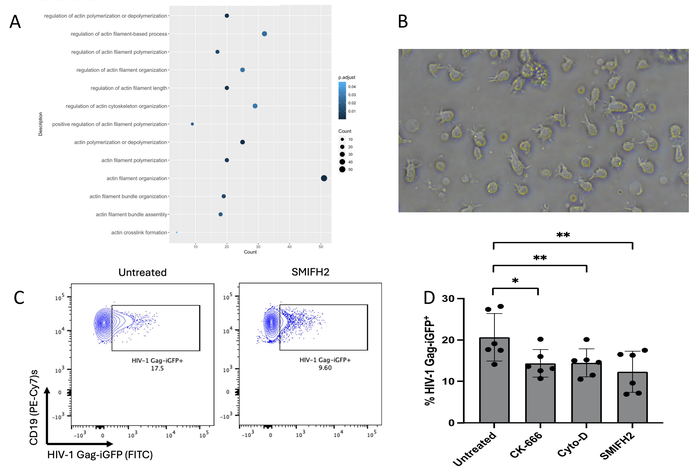


Figure 3. CD40L/IL-4 Stimulation promotes actin polymerization and HIV-1 binding **(A)** Gene ontology pathways. CD40L/IL-4 vs all but BAFF/IL-4 **(B)** CD40L/IL-4 stimulated B cells. Light microscopy 40x magnification **(C,D)** HIV-1 binding post actin polymerization inhibitors (C) Representative donor **(D)** n=6. Paired t tests. *p<0.03 **p<0.01

CONCLUSIONS

- CD40L/IL-4, an immunologically relevant stimulation, primed B cells to efficiently bind HIV-1 and mediate trans infection of CD4+ T cells *in vitro* as compared to the other stimulation conditions
- B:T cell interactions in SLO likely play a key role in HIV-1 pathogenesis by facilitating HIV-1 trans infection
- This study identified the signals (CD40L/IL-4) and receptor/gene expression (CD205/actin) that enable B cells to bind and transfer virus to CD4+ T cells
- This information could lead to the development of treatments to reduce persistence HIV-1 infection and maintenance of the viral reservoir

ACKNOWLEDGEMENTS

- Special thanks to Patrick Mehta, Kathy Hartle, Lori Caruso, Mounia Alaoui-El-Azher, Rinaldo Flow Lab, the Mailliard lab, and the MACS/WIHS Combined Cohort Study (MWCCS)
- This work was supported by: T32-AI065380, R0-1AI118403, U01-HL146208, and U01-AI35041