Poster 00321

B Cells Augment HIV-1 Expression in Secondary Lymphoid Tissues

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BACKGROUND

- HIV-expressing (vRNA+) cells are consistently found in secondary lymphoid tissues (SLT) of people with HIV (PWH) on antiretroviral therapy (ART)
- vRNA+ cells may drive viral rebound upon ART cessation
- Little is known about what affects HIV expression in SLT

Hypothesis: B cells promote HIV expression

METHODS

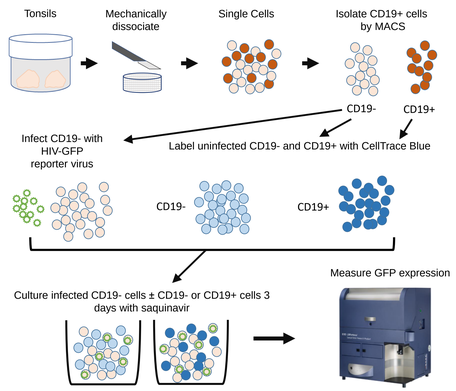
Disaggregated tonsil cells from persons without HIV were depleted of CD19+ cells by magnetic beads and CD19- to CD19+ cell ratios were determined. CD19- cells were spinoculated with X4 or R5-tropic HIV GFP reporter virus, cultured 3 days with saquinavir and dye-labeled CD19+ or CD19- cells to maintain cell ratios. Percent GFP+ of unlabeled CD3+CD8- cells and GFP median fluorescence intensity (MFI) were determined by flow cytometry.

Tonsil cells were also sorted into CD4+ T follicular helper (TFH; CD3+CD8-CXCR5hiPD-1hi), nonTFH (CD3+CD8-CXCR5+/-PD-1+/-), memory B (IgD-CD38-), pre-germinal center B (pre-GCB; IgD+CD38+), GCB (IgD-CD38+), and naïve B (IgD+CD38-). TFH and nonTFH were infected with X4 HIV, incubated with B cell subsets or control CD4+ T cells, and %GFP+ and GFP MFI assessed after 3 days.

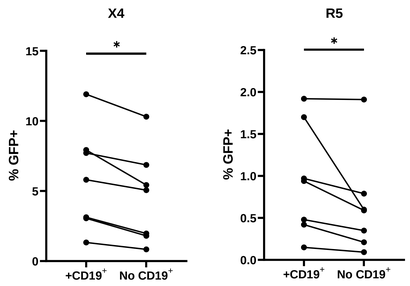
Sections of spleen from 5 male PWH on ART for >5 years and inguinal lymph node (LN) from 6 male PWH on ART for 7-29 years were analyzed by in situ hybridization for vRNA and immunofluorescent antibody staining for CD20 to determine follicular (F) regions. Frequencies of vRNA+ cells in F and extrafollicular (EF) regions, EF B cells, and percentages of EF B cells adjacent to vRNA+ cells were determined by visual inspection and quantitative image analysis. Probabilities of at least one $CD20^+$ cell adjacent to $vRNA^+$ cells (open circles) were determined using the formula: P(≥1 B cell adj) = 100% - P(no B cell adj); P(no B cell adj) = (100% - %B cells in EF)[6]. Data reported are medians. Nonparametric statistical tests were used.

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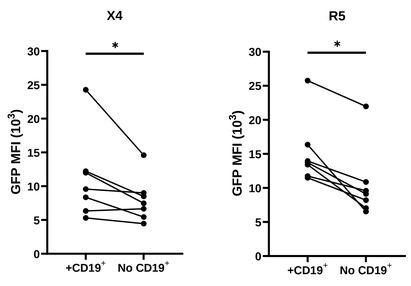
Tonsil model to study effects of B cells on HIV expression



B cell depletion reduces GFP+ percentages in CD3+CD8- tonsil cells

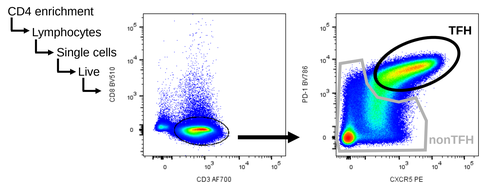


GFP MFI is reduced in CD3+CD8- tonsil cells when B cells are depleted

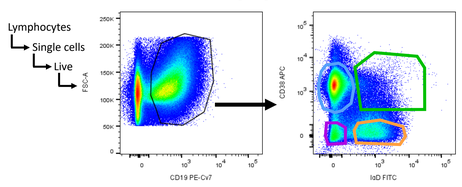


Representative gating strategies of tonsil cell populations

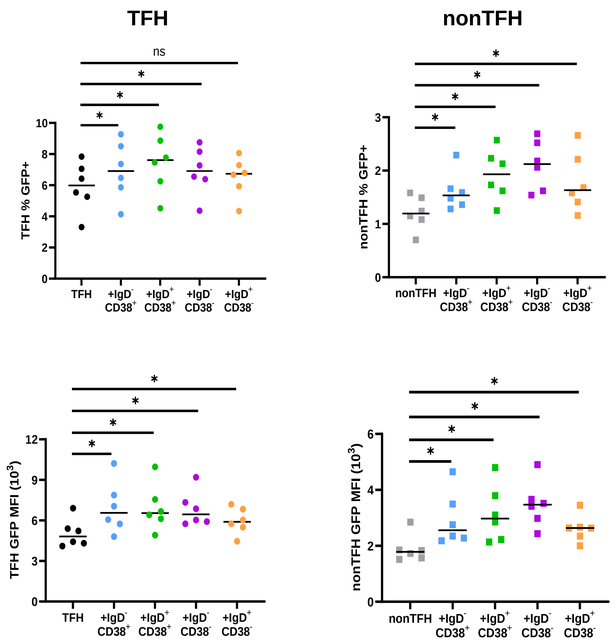
TFH and nonTFH



B cell populations



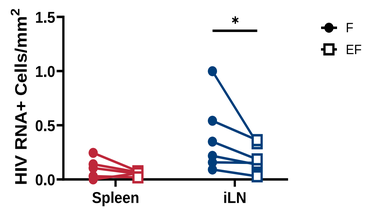
Tonsil B cell subsets induce HIV expression in TFH and nonTFH



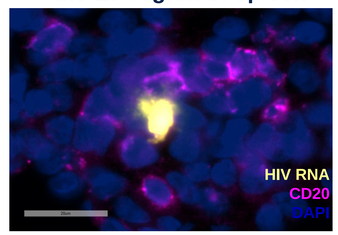
PWH on ART tissue donors

Spleens, Last Gift University of San Diego					Inguinal Lymph Nodes, University of Hawaii				
ID	Age	Years on ART	Number of vRNA+ cells evaluated	% F Area	ID	Age	Years on ART	Number of vRNA+ cells evaluated	% F Area
LG08	52	>5	40	7.7	LN-07	54	29	19	23.1
LG05	57	>5	47	6.5	LN-09	66	21	15	39.6
LG15	88	>5	52	5.1	LN-04	63	18	16	23.6
LG14	59	>5	24	0.3	LN-01	48	25	12	25.0
LG24	78	>5	13	6.1	LN-05	59	7	9	60.8
					LN-08	61	20	6	38.7

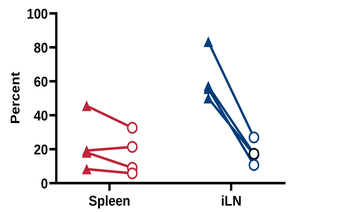
$vRNA^+$ cell frequencies are higher in B cell follicles (F) than extrafollicular regions (EF)



Representative image of a $vRNA^+$ cell and CD20 staining in the EF region of spleen



In EF regions, $vRNA^+$ cells are preferentially adjacent to B cells



▲ EF vRNA+ cells with ≥1 adjacent CD20+ cell

○ Probability of EF vRNA+ cell being adjacent to ≥1 CD20+ cell

SUMMARY

In an ex vivo tonsil model:
- HIV expression was reduced when B cells were depleted
- Purified B cell subsets induced HIV expression in TFH and nonTFH

In SLT of PWH on prolonged ART:
- vRNA+ cell frequencies were elevated in B cell-rich follicles when follicular areas were ≥5% of total tissue area
- In extrafollicular areas, vRNA+ cells were preferentially found adjacent to B cells

CONCLUSION

B cells may promote HIV expression in SLT of PWH on prolonged ART

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