B Cells Direct R5-Tropic HIV Infection of CCR5^{neg} Naïve CD4+ T cells

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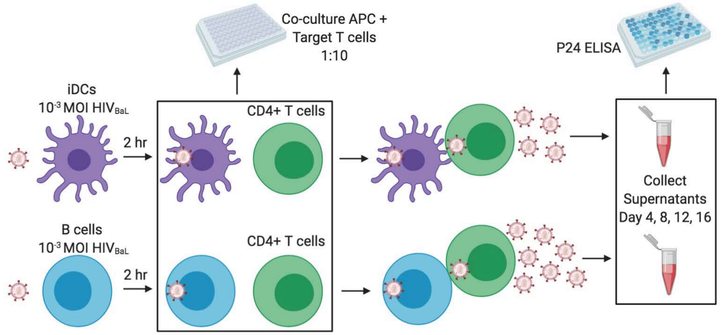
Introduction

- Latent HIV reservoirs in CD4+ T cells are a major barrier to a cure despite the success of cARTs
- Due to minimal CCR5 expression, naïve T cells (T_N) are resistant to *cis* infection in vitro, however they have been found to harbor CCR5-tropic HIV DNA[1]
- Naïve T cells also produce an equal amount or more extracellular HIV-1 RNA per infected cell as central memory (CM) T cells, a major component of the latent reservoir [1]
- HIV *trans* infection: APCs (DCs and B cells) mediate transfer of HIV-1 to CD4+ T cells via a virological synapse,[2] but this mode of transmission is inefficient in HIV+ nonprogressors (NP)[3]
- The aims were (1) to determine if T_N cells could be *trans* infected via APCs by HIV_{Bal}, a CCR5 tropic HIV strain, (2) to determine if T_N cells of HIV+ NPs harbored HIV DNA, and (3) to analyze *trans* infection ability of immature vs mature DCs (iDC, mDC)

Hypothesis

Naïve T cells are infected with HIV via *trans* infection mediated by B cells, making them an essential component of the latent HIV reservoir

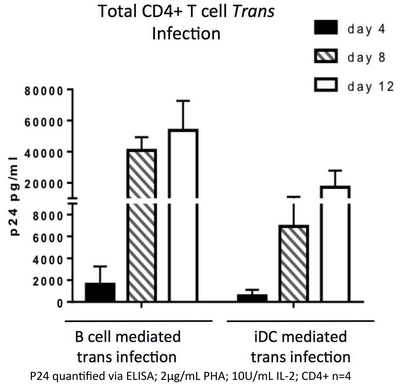
Methods



- CD4+ T cells, B cells, and monocytes were purified from PBMCs of seronegative donors by magnetic microbead separation
- B cells were activated by CD40L/IL-4 and DCs were differentiated from monocytes by GM-CSF/IL-4 and cultured for 2 and 5 days respectively
- T_N cells were purified by negative selection and cultured in PHA/IL2 or CCL-19 for 2 days
- T_N cells were purified from PBMCs of seropositive NP donors from MACS by magnetic microbead separation and analyzed via qPCR
- Cell phenotypes determined by flow cytometry

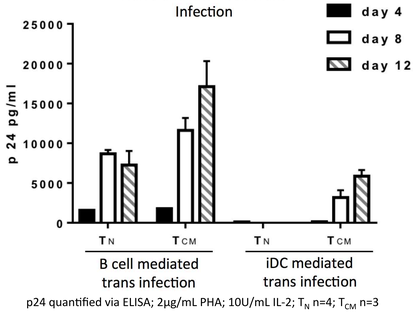
Results

PHA/IL-2 activated total CD4+ T cells are efficiently *trans* infected by B cells and iDCs



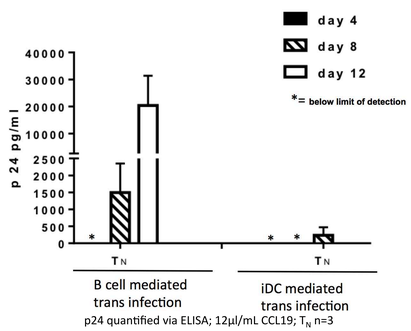
P24 quantified via ELISA; 2µg/mL PHA; 10U/mL IL-2; CD4+ n=4

PHA/IL-2 activated naïve T cells are efficiently *trans* infected by B cells, not iDCs



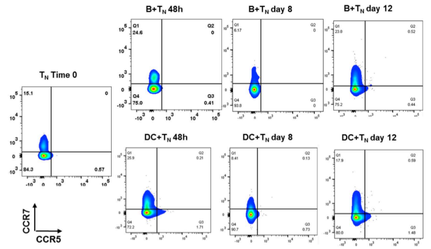
p24 quantified via ELISA; 2µg/mL PHA; 10U/mL IL-2; T_N n=4; T_{CM} n=3

CCL19 activated naïve T cells are efficiently *trans* infected by B cells, not iDCs



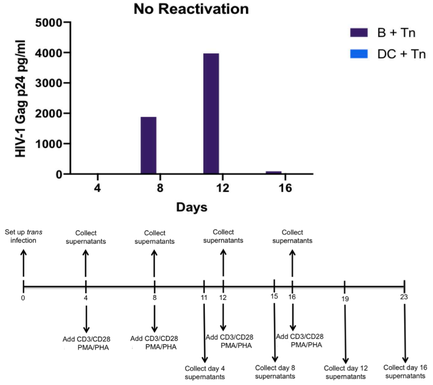
p24 quantified via ELISA; 12µl/mL CCL19; T_N n=3

Naïve T cells remain CCR5^{neg} throughout *trans* infection co-culture



T_N phenotyping via flow cytometry; data analyzed by FlowJo; BD Pharmigen PE Mouse anti-human CCR5 antibody; APC mouse IgG_{2A} CCR7 antibody; cells stained at time 0, day 2, 8, and 12 after co-culture

Naïve T cells *trans* infected by B cells harbor latent virus



On days 4, 8, 12, and 16 CD3/CD28 beads (3 beads per cell) or PMA(10nM)/PHA(10µg/mL) was added to wells; supernatants collected 7 days later

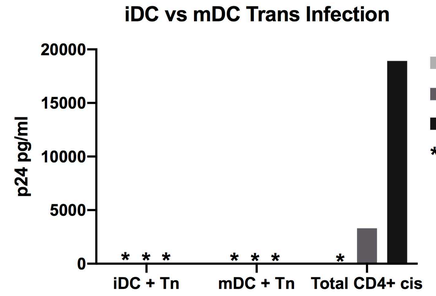
Naïve T cells from NPs do not harbor HIV DNA

Donor	Total CD4+ T cells	Naïve T cells
1	1062	0
2	1270	0
3	1110	0

qPCR; HIV DNA copies in total CD4+ T cells and naïve T cells from 3 NP donors

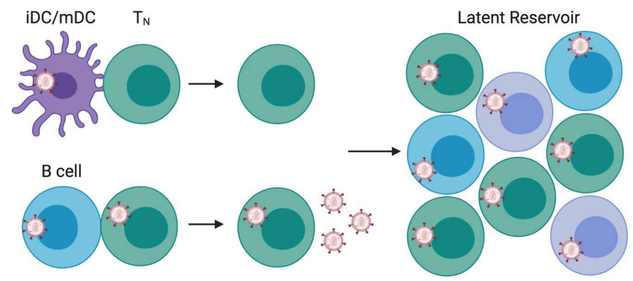
Results

Mature DCs are unable to *trans* infect CCL19 activated naïve T cells



DC maturation: monocytes activated with IL-4 and GM-CSF, cultured for 5 days with additional IL-4 and GM-CSF added on day 3; Day 5: IL-4 and CD40L added, cultured for 2 additional days; iDC and mDC phenotypes confirmed by flow cytometry; T-cell activation: 100nM CCL19

Conclusions



- B cells are able to efficiently *trans* infect naïve T cells
- iDCs and mDCs are unable to *trans* infect naïve T cells
- Naïve T cells from NPs do not harbor HIV DNA due to inability of *trans* infection
- Based on this data, we conclude that B cells are a major player in maintaining the latent viral reservoir by *trans* infecting CD4+ T cells, in particular, naïve T cells with high efficiency

Future Directions

- *Trans* infection of CCL19 activated total CD4+ T cells vs naïve T cell subset
- *Trans* infection of total CD4+ and naïve T cells with different subsets of B cells
- *Trans* infection of naïve T cells with fluorescent virus via flow cytometry
- Imaging of B cell mediated *trans* infection of naïve T cells
- Determine the cellular factors involved in differential *trans* infection of naïve T cells by B cells compared to DCs

Contact

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References

1. Nicolas Sluis-Cremer et al. Establishment and Reversal of HIV-1 Latency in Naïve and Central Memory CD4+ T cells In Vitro. 2016.
2. Giovanna Rappocciolo et al. DC-SIGN on B lymphocytes is required for transmission of HIV-1 to T lymphocytes. 2006
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