MIGRATORY T CELLS ARE MORE SUSCEPTIBILE TO HIV-1 INFECTION VIA INCREASED VIRAL ENTRY

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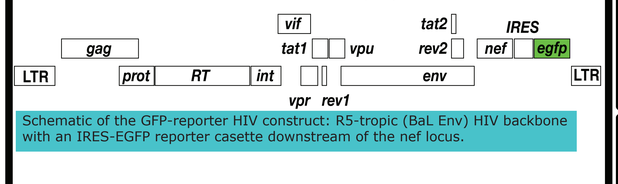
The highly organized secondary lymphoid organs are the primary site of HIV replication, transmission and CD4+ T cell depletion. This environment allows T cells to actively migrate along reticular networks in search for cognate antigen, but how these behaviors impact HIV entry and productive infection remains unclear. Previous in vitro studies often investigate HIV infection using 2D culture systems, which do not take into account the migratory behaviors of T cells in vivo. In this study, we compared the difference in behaviors, F-actin fluctuations and HIV infection of CD4+ T cells using collagen matrices that either support or restrict cell migration. The changes in cellular behaviors and phenotypes under various collagen chambers were assessed using two-photon 3D live imaging. Viral infection and cell subset analysis were performed using flow cytometry. The impact of ARV drugs and small molecule inhibitors were also explored. To measure how migration modulates viral entry and integration, HIV fusion (Vpr-Blam) assay was used. We developed a novel approach to achieve high T cell infection using collagen gels without the need for long centrifugation, cationic polymers or peptide fragments. Active migration along the collagen fibers in 3D collagen matrix results in a consistent and significant enhancement in T cell infection by cell-free R5-tropic lab-adapted (fold change (FC) increase of 5.8x) and transmitted/founder molecular HIV clones (REJO FC = 6.9x; THRO FC = 3.3x), compared to cells infected in suspension. Moreover, infection in collagen matrix results in higher HIV DNA integration (FC = 1.9x) that was not attributed to altered receptor/co-receptor expression, activation state or viability. We also observe high levels of HIV fusion (FC = 2.5x) and subsequent infection in migratory T cells, whereas non-motile T cell display low viral entry and integration. Migratory T cells can access regions where HIV density is high, indicating that the act of migration leads to more encounters with cell-free viral particles during their routine migratory surveillance. This study demonstrates that the environmental context in which initial HIV:T cell encounters occur modulate HIV entry and DNA integration efficiencies, and illustrates mechanisms by which HIV subverts T cell migratory behaviors to maximize viral dissemination to establish a chronic infection.

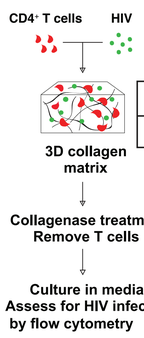
PROJECT OBJECTIVES

- To develop a novel way of improving HIV infection of primary CD4+ T cells.
- To understand how the changes in CD4+ T cell environments can impact cell migration, HIV susceptibility, and ART effectiveness.

METHODS



Schematic of the GFP-reporter HIV construct: R5-tropic (BaL Env) HIV backbone with an IRES-EGFP reporter casette downstream of the nef locus.



- 1M anti-CD3/CD28-activated CD4+ T cells
+
100,000 HIV-GFP (Incubate for 3 hours)

10X MEM	sodium bicarbonate (7.5%)	PureColl (bovine) 3.1 mg/mL	10^6 T cells in media + IL-2	Final gel volume
20µL	10µL	148µL	92µL	270µL

- Collagenase D for 10-30min at 37C

- Culture in media for 48 hours in total. Stain for CD4, p24, etc.

Optimized HIV Infection Protocol in Collagen: Day 6 activated T cells (1M) were infected with HIV-GFP (0.1M) in collagen for 3 hours at MOI = 0.1. Collagen recipe is shown on the table. Collagenase D was used to release the cells from collagen. Infection was measured using flow cytometry after 48hrs.

SUMMARY

1) Infection using this novel collagen method, without the need for long centrifugation, exogenous cationic polymers, or peptide fragments, shows a drastic increase in the number of HIV-infected activated and resting T cells compared to cell suspension alone.

2) High infection rate is correlated to robust T cell migration, fluctuating F-actin polymerization, increased 3D velocity, and high track displacement in intact collagen.

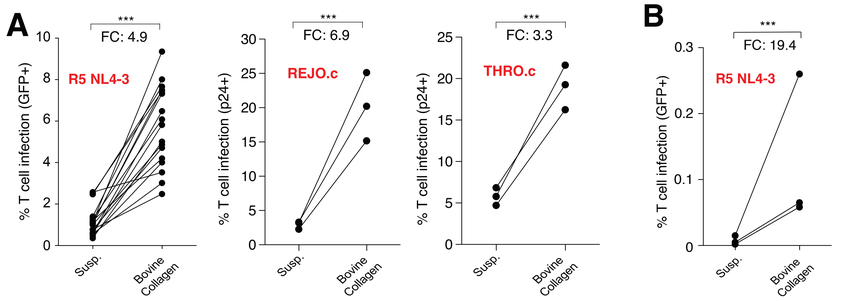
3) CD4+ T cells in lymphoid organ-like environment are very motile and are prone to higher HIV fusions and proviral integration.

FUNDING AGENCIES:

CanCURE Together, we Can CURE HIV

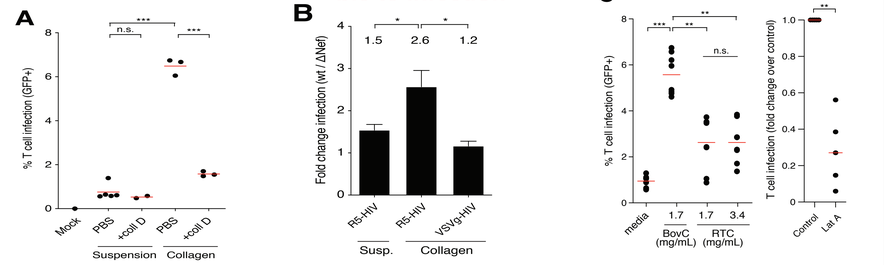
CIHR IRSC Canadian Institutes of Health Research / Instituts de recherche en santé du Canada

1) 3D collagen promotes higher infection of activated and resting CD4+ T cells at lower MOI and shorter incubation with HIV



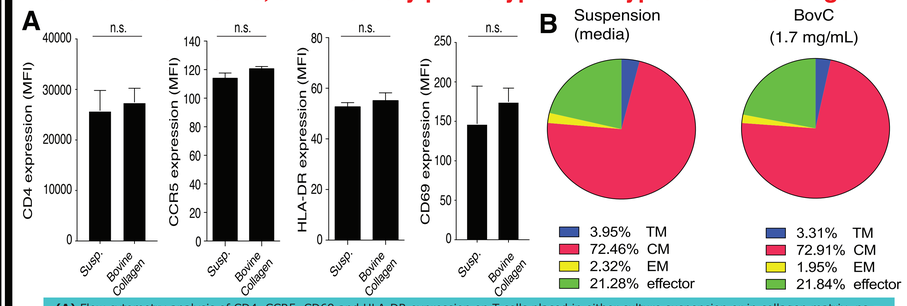
(A) Percent infection with the indicated R5-tropic HIV strains (red). Anti-CD3/CD28 activated T cells infected with R5-tropic HIV-GFP (n=17) was measured by GFP expression, and the transmitted/founder (T/F) molecular clones (n=3) by intracellular p24 staining. Each line indicates an individual donor, performed in duplicates. FC: fold change. *** p<0.001. **(B)** Percent infection of resting T cells with HIV-GFP (n=3). *** p<0.001.

4) Robustly migrating T cells in intact fibrillar collagen are highly susceptible to infection



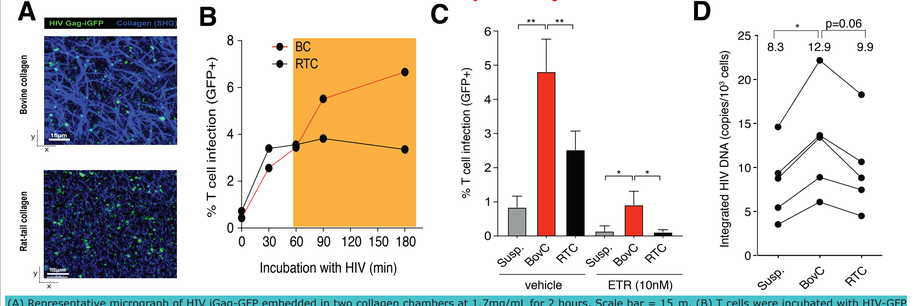
(A) T cell infection with HIV-GFP in either culture suspension or collagen matrix. Collagenase D (Coll D) was added to the indicated wells to prevent gel solidification. Mean values (n=3) performed in duplicates are shown. Red lines are median values. ns = not significant. (B) T cells were placed in culture supernatant or bovine collagen with either wildtype or DNef HIV-GFP (R5-HIV, MOI=0.1) for 3 hours, collagenase treated and assessed for productive infection after 48 hours. In some cases, virions were pseudotyped with VSVg envelope, as indicated (VSVg-HIV). Fold increase in infection by wildtype over DNef HIV are shown. n=3.* p<0.05. (C) T cell infection with HIV-GFP in either culture suspension, bovine or rat-tail collagen at the indicated concentrations. Reduction in T cell infection after LatA treatment was normalized to control infection. Red lines are median values. BovC = bovine collagen. RTC = rat-tail collagen. *** p<0.001. ** p<0.01.

2) Increased infection in collagen is not a result of changes in co-receptor, activation of T cells, and memory phenotype from Type I bovine collagen



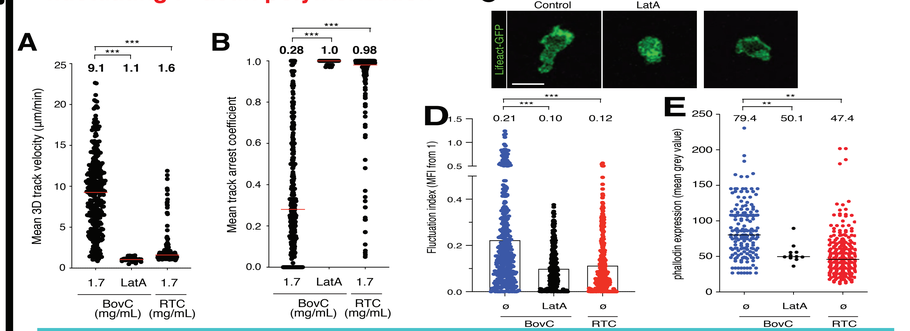
(A) Flow cytometry analysis of CD4, CCR5, CD69 and HLA-DR expression on T cells placed in either culture suspension or in collagen matrix. ns = not significant. **(B)** Phenotypic analysis of HIV-infected T cells in suspension vs collagen gels after 48 hours in either culture suspension or BovC: bovine collagen at 1.7mg/mL. Effector, EM=effector memory, CM=central memory and TM=transitional memory cell populations were defined as follows: EM = CD45RA-CD45RO+CD27-CCR7-; CM = CD45RA-CD45RO+CD27+CCR7+; TM = CD45RA-CD45RO+CD27+CCR7-

5) T cell migration in collagen augments proviral integration and decreases ART potency



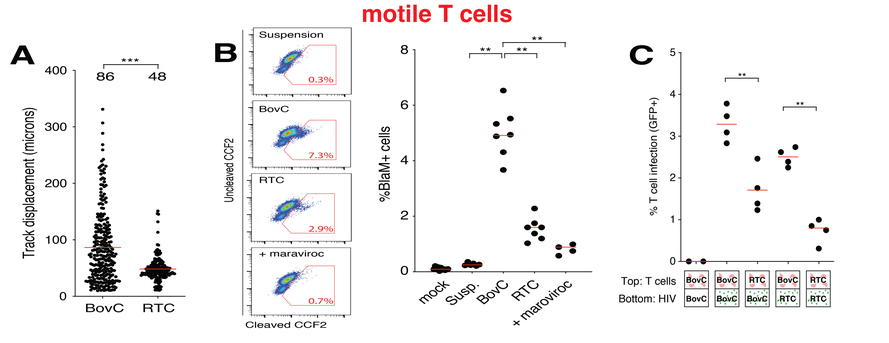
(A) Representative micrograph of HIV iGag-GFP embedded in two collagen chambers at 1.7mg/mL for 2 hours. Scale bar = 15 m. (B) T cells were incubated with HIV-GFP in collagen for the indicated times, collagenase treated, and assessed for productive infection after 48 hours by flow cytometry. Yellow shaded box indicates collagen gel matrix solidification. n=2. (C) T cells were pre- and incubated with HIV-GFP (MOI=0.1) in the presence of 10nM each tenofovir, emtricitabine and raltegravir (ETR) for 3 hours in culture suspension or collagen and productive infection analyzed by flow cytometry after 48 hours. BovC = bovine collagen. RTC = rat-tail collagen. Representative data from three independent experiments are shown. (D) Integrated HIV DNA copies per 103 T cells at 6-hour post infection (MOI=0.01) in either culture suspension or the indicated collagen gels. Each line indicates an individual donor (n=5) performed in duplicates. * p<0.05, ** p<0.01. *** p<0.001. ns = not significant.

3) Collagen fiber promotes robust migration, stabilized cell polarity, and fluctuating F–actin polymerization



(A) Mean 3D track velocity and (B) Mean track arrest coefficient of T cells embedded in bovine or rat-tail collagen. LatA = Latrunculin A. Red lines and numbers indicate median values. Data from two experiments and analysis from 7 movies are shown. (C) Representative micrograph of T cells transduced with Lifeact-GFP embedded in the indicated collagen preparation. Scale bar = 10um. (D) Rectangular ROIs were used to longitudinally measure MFIs at randomly chosen sites in non-polarized cells and at the leading edge of polarized cells. Fluctuation index (standard deviation from the baseline "1" value) measurements at all timepoints for each condition. Black bars and numbers indicate median values. *** p <0.001 (E) T cells in each experimental condition were fixed in collagen gels in situ and stained with Texas Red-conjugated phalloidin. Total cell phalloidin expression (mean grey values) is shown for all timepoints. ** p<0.01.

5) High 3D velocity and displacement leads to enhanced levels of viral fusion in motile T cells



(A) Track displacement of T cells embedded in bovine or rat-tail collagen. (B) T cells were incubated with HIV virions containing Blam-Vpr in either culture suspension, bovine or rat-tail collagen for 3 hours, and then loaded with CCF2. Cells were fixed and assayed by flow cytometry (% cleaved CCF2 in red box). As a control, CCR5 antagonist, maraviroc (1µM) was used to pre-treat cells. n=3. Red lines are median values. ** p<0.01 (C) Dual-collagen overlay assay. HIV-GFP was embedded into the lower collagen matrix (bottom), then overlaid with collagen containing T cells (top) for 6 hours. T cells were cultured for another 48 hours before flow cytometry analysis. N=2. Red lines indicate median values. Red lines are median values. BovC = bovine collagen. RTC = rat-tail collagen. ** p<0.01.