CD4 T cell depletion in immunocompetent and immunodeficient primates is associated with loss of innate lymphoid cells

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Introduction

- Immunological and structural damage to the GI tract is an important aspect of SIV and HIV-1 disease pathology.
- ILCs are important in GI homeostasis. In HIV-1+ humans, ILCs in blood are lost with similar kinetics as CD4 T cells and fail to reconstitute in chronic HIV-1+ patients on antiretroviral therapy (ART).
- Loss of ILCs is not a generalized feature of all viral infections.
- Direct viral infection is unlikely, as ILCs are not permissive to HIV/SIV infection *in vitro* or *in vivo*. The mechanisms of HIV-associated loss of ILCs are thus not understood.
- We studied ILC dynamics in SIV+ viremic, aviremic, and uninfected CD4 lymphopenic primates to understand mechanisms which underlie ILC dysfunction in HIV/SIV infection.

The importance of the gut in HIV pathology

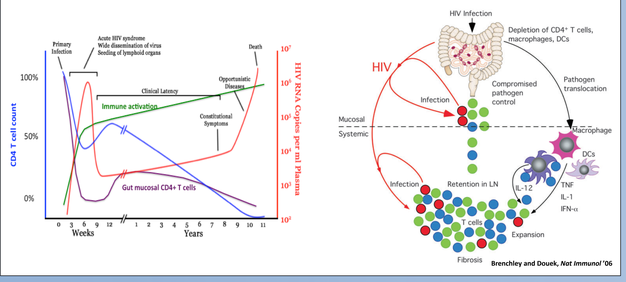
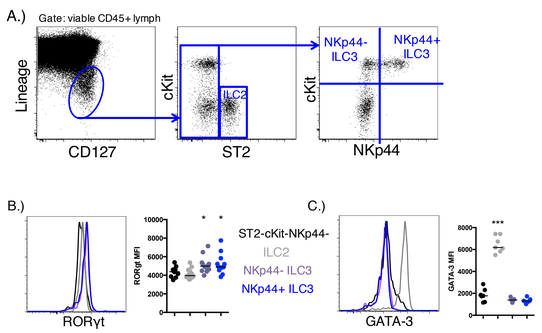
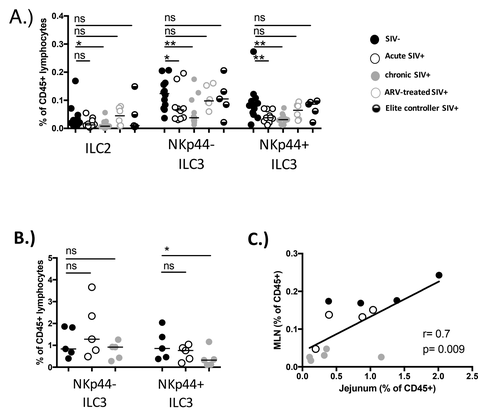


Figure 1: Distinct ILC subtypes can be defined in gut-draining LNs of Rhesus macaques



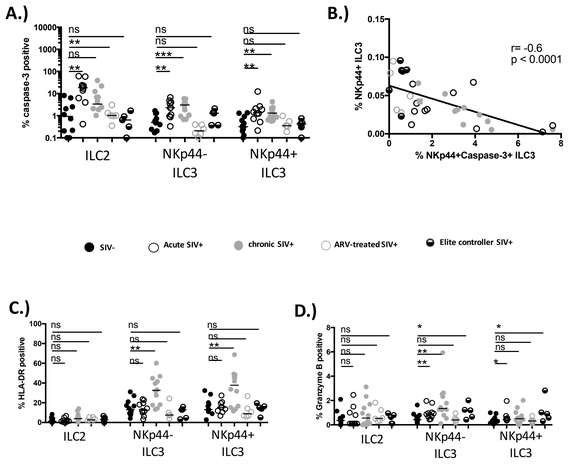
(**A**) Representative gating strategy for ILCs in MLN of a healthy animal. (**B**) RORγT expression in MLN ILC subpopulations. (**C**) GATA-3 expression in MLN ILC subpopulations.

Figure 2. Rapid loss of ILCs in the untreated SIV+ MLN is apparent in GI tissues and reversed with viremic control



(A) Frequencies of MLN ILCs in healthy uninfected RMs, untreated acute and chronic SIV-infected RMs, chronic SIV+ RMs receiving ART, and SIV-infected ECs. **(B)** frequencies of ILC3s in jejejunal cell suspensions. **(C)** correlation of NKp44+ ILC3 frequencies in animals with matching MLN and jejunal samples at time of necropsy.

Figure 3. Turnover, death and activation of ILCs in the untreated SIV+ MLN is normalized with viremic control



(A) Frequencies of MLN ILCs shown to express active caspase-3 in the uninfected and SIV+ MLN. **(B)** Relationship between NKp44+ ILC3 frequencies in the MLN and NKp44+ ILC3s expressing active caspase-3. **(C)** Percentage of MLN ILCs expressing HLA-DR. **(D)** Percentage of MLN ILCs expressing intracellular granzyme B.

Figure 4. IL-17 production, inflammatory IL-1β and IFNγ gene expression transcripts are elevated in ILC3s of the untreated SIV+ MLN

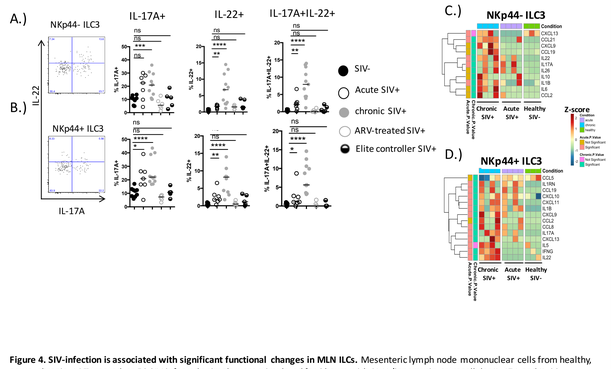
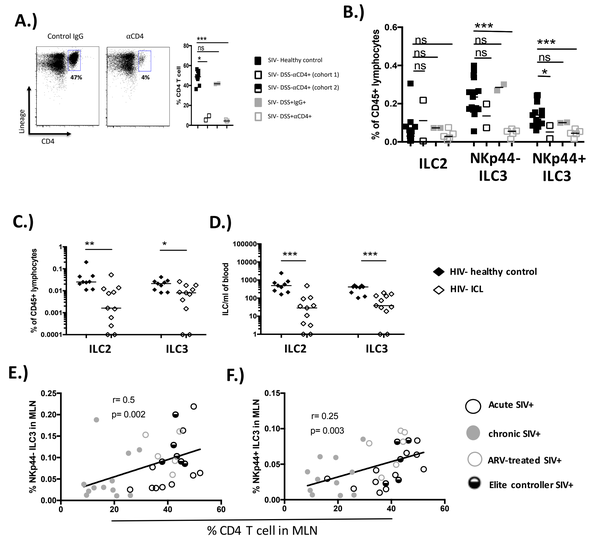


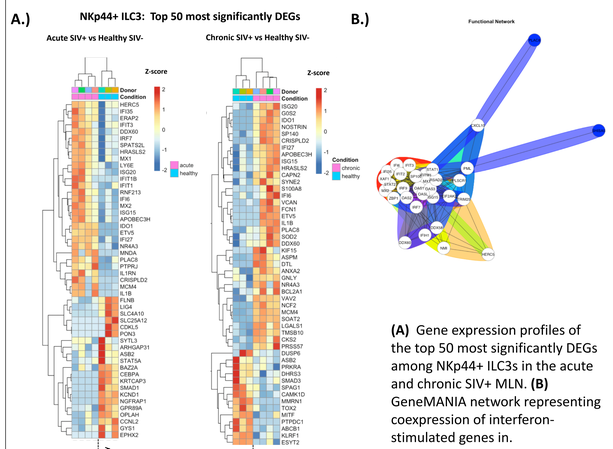
Figure 4. SIV-infection is associated with significant functional changes in MLN ILCs. Mesenteric lymph node mononuclear cells from healthy, acute, chronic, ART-treated, or EC SIV-infected animals were stimulated for 6 hours with PMA/ionomycin. Intracellular IL-17A and IL-22 expression was measured in NKp44- (**A**) or NKp44+ (**B**) ILC3. Gene expression profiles of selected cytokines and chemokines produced by NKp44- (**C**) and NKp44+ (**D**) ILC3s in the SIV- and the untreated SIV+ MLN. Color scheme in both heatmaps represents number of standard deviations above (red) or below (blue) the mean. Statistical significance was determined by the Mann-Whitney test for (A) and (B). Statistical significance of (C) and (D) was determined using the Wald test with Bonferroni correction for multiple comparisons.

Figure 5. ILCs are deficient in CD4-lymphopenic HIV/SIV-uninfected human and nonhuman primates.



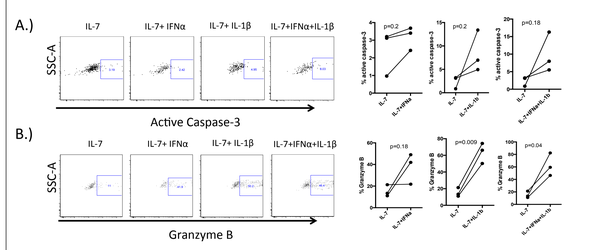
(**A**) effect of anti-CD4 treatment in MLNs of SIV- animals. (**B**) frequencies of ILCs in the MLN of healthy control, SIV-uninfected DSS treated, αCD4-treated, or animals receving both treatments. **(D)** Frequencies and **(E)** absolute numbers of blood ILCs in healthy and ICL human subjects. Correlation between **(F)** NKp44- ILC3 and **(G)** NKp44+ ILC3 percentages and CD4 T cell percentage in the MLN of SIV+ RMs.

Figure 6. Gut-resident NKp44+ ILC3s are highly responsive to Interferon *in vivo*



(A) Gene expression profiles of the top 50 most significantly DEGs among NKp44+ ILC3s in the acute and chronic SIV+ MLN. **(B)** GeneMANIA network representing coexpression of interferon-stimulated genes in.

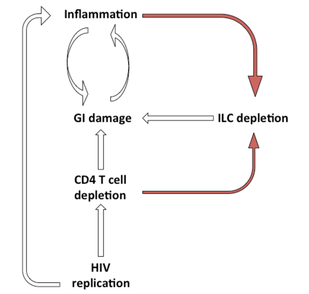
Figure 7. *In vitro* exposure to IFNα and/or IL-1β regulates NKp44+ ILC3 survival and cytotoxicity



ILC subpopulations from MLNs of healthy animals were sorted and stimulated in the presences or absence of IL-1β or IFNα. Active caspase-3 expression **(A)**, and granzyme B **(B)** were measured after 6 days of culture in NKp44+ ILC3.

Conclusions

- Our data support a model whereby viral replication contributes indirectly to ILC loss both by direct depletion of CD4 T cells and by induction of host inflammatory mediators.
- Strategies that enhance CD4+T-cell reconstitution, or target inflammatory mediators may hold promise in reconstituting ILCs in treated HIV-1+ subjects



Model depicting proposed determinant of ILC loss with concordant CD4 loss leading to GI damage