Loss of Innate Lymphoid Cells in Mesenteric Lymph Nodes occurs throughout SIV Disease Course

Joseph C. Mudd[1], Alex Ortiz[1], Claire Deleage[2], Fan Wu[1], Kenta Matsuda[1], Vanessa Hirsch[1], R. Keith Reeves[4], Jake Estes[2], and Jason Brenchley[1]

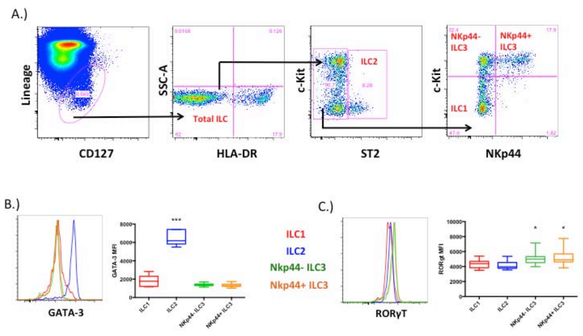
[1]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD. [2] AIDS and Cancer Virus Program, Frederick National Laboratory for Cancer Research, Leidos Biomedical Research, Inc., Frederick, Maryland, USA. [3] Harvard Medical School, Beth Israel Deaconess Medical Center, Boston, MA.

National Institute of Allergy and Infectious Diseases

Introduction

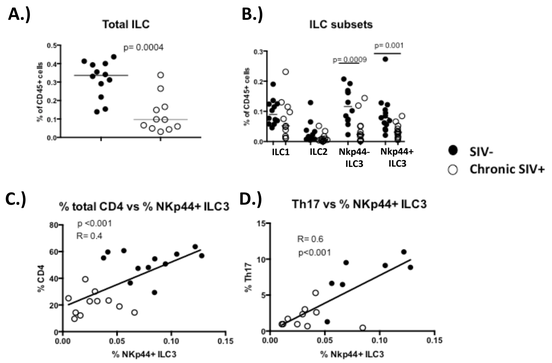
- Innate lymphoid cells (ILCs) are an arm of the innate immune system that play imporant roles in pathogen defense, barrier integrity, and tissue repair.
- ILCs lack genetically rearanged antigen receptors, but mirror many functional properties of T helper cells.
- ILCs are separated into 3 distinct lineages: ILC group 1 (ILC1s), ILC2s, and ILC3s, which parallel the transcriptional and functional characteristics of T helper 1 (Th1), Th2, and Th17 cells, respectively.
- Previous reports have shown NKp44+ type 3 ILCs (ILC3) that are important sources of IL-17 are depleted in gut tissues in HIV and SIV infection.
- It is unclear how HIV infection affect the frequencies or function of ILC2s.
- Additionally, the role of microbial tranlocation on ILC survival and function are not well understood.

Figure 1. Three subtypes of ILCs are present in Mesenteric lymph nodes (MLN) of rhesus macaques.



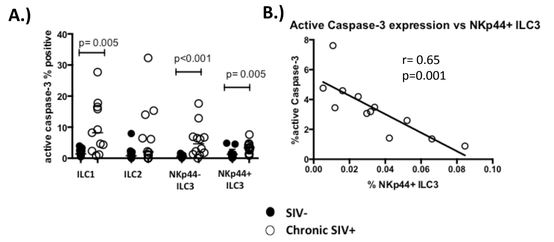
(**A**) Defining ILC subsets in the MLN of a healthy uninfected rhesus macaque. (**B**) Mean fluorescence intensity of intracellular GATA-3 expression among ILC subsets in the MLN (N= 7)(***; P<0.001; Mann-Whitney test). (**C**) Mean fluorescence intensity of intracellular ROR-gT expression among ILC subsets in the MLN (N=11; *; P= 0.03; Mann-Whitney test).

Figure 2. Frequencies of ILC3s are selectively decreased in chronic SIV+ MLNs and correlate with disease progression.



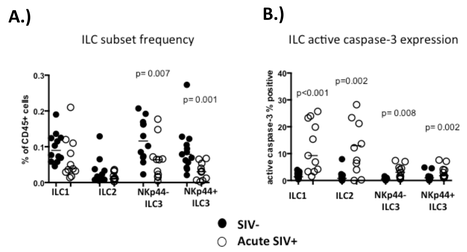
(A) Frequencies of the total innate lymphoid cell compartment, gated as Lineage-HLA-DR-CD127+, in MLNs of healthy animals (N=12) and chronic SIV+ animals (N=11). Expressed as a percentage of total viable CD45+ lymphocytes. (B) Frequencies of distinct ILC subsets in the MLNs of healthy animals and chronic SIV+ animals. Significance was determined by the Mann-Whitney U test. Relationship between NKp44+ ILC3 frequencies and (C) bulk CD4 T cells, or (D) Th17 cells in the MLN. Linear regression lines are shown (Spearman correlation).

Figure 3. ILCs in the MLN of chronic SIV+ rhesus macaques are apoptotic



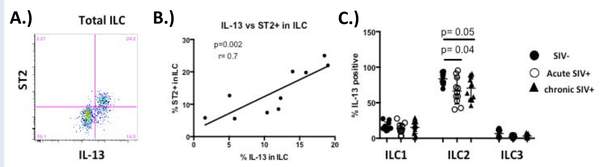
(A) Intracellular active Caspase-3 expression in healthy uninfected animals (N=12) and chronic SIV+ animals (N=12) was determined with an antibody that recognizes the active form. Statistical comparisons were performed with the Mann-Whitney U test. (B) Relationship between Caspase-3 expression of NKp44+ ILC3 and frequency of NKp44+ ILC3 in MLNs of chronic SIV+ rhesus macaques. Linear regression lines are shown (Spearman correlation).

Figure 4. ILCs in the SIV+ MLN are lost early in disease course.



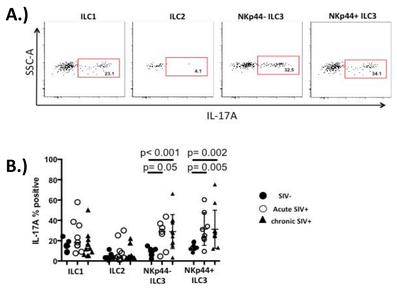
(A) Frequencies of distinct ILC subsets in the MLNs of healthy animals (N=12) and acute SIV+ animals (N=10) at 14 days post-infection. (B) Intracellular Caspase-3 expression in healthy uninfected animals and chronic SIV+ animals was determined with an antibody that recognizes the active form. Statistical comparisons were performed with the Mann-Whitney U test.

Figure 5. IL-13 production in ILC2s are decreased in the SIV+ MLN



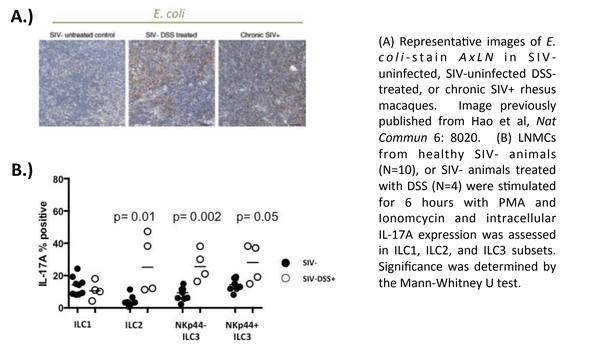
(A) LNMCs from a healthy animal were stimulated for 6 hours with PMA and Ionomcyin and intracellular IL-13 production was plotted against ST2 surface expression in the innate lymphoid compartment. (B) Relationship between ST2 surface expression and IL-13 production in the innate lymphoid cell compartment (N=10). (C) LNMCs from healthy SIV- animals (N=10), acute SIV+ animals (N=11), or chronic SIV+ animals (N=11) were stimulated for 6 hours with PMA and Ionomcyin and intracellular IL-13 expression was assessed in ILC1, ILC2, and ILC3 subsets. Significance was determined by the Mann-Whitney U test.

Figure 6. Acute and chronic SIV infection is marked by elevated IL-17A production in ILC3s in the MLN.



(A) LNMCs from a healthy animal were stimulated for 6 hours with PMA and Ionomcyin and intracellular IL-17A production was assessed in major ILC subsets. (B) LNMCs from healthy SIV- animals (N=10), acute SIV+ animals (N=11), or chronic SIV+ animals (N=11) were stimulated for 6 hours with PMA and Ionomcyin and intracellular IL-17A expression was assessed in ILC1, ILC2, and ILC3 subsets. Significance was determined by the Mann-Whitney U test.

Figure 7. GI damage caused by DSS treatment of healthy rhesus macaques increases IL-17A production in ILCs.



(A) Representative images of *E. coli*-stain *AxLN* in SIV-uninfected, SIV-uninfected DSS-treated, or chronic SIV+ rhesus macaques. Image previously published from Hao et al, *Nat Commun* 6: 8020. (B) LNMCs from healthy SIV- animals (N=10), or SIV- animals treated with DSS (N=4) were stimulated for 6 hours with PMA and Ionomcyin and intracellular IL-17A expression was assessed in ILC1, ILC2, and ILC3 subsets. Significance was determined by the Mann-Whitney U test.

Conclusions

- Distinct ILC subsets can be defined in MLNs of nonhuman primates that express known ILC lineage transcription factors.
- ILC3s in the MLN undergo apoptosis early in SIV infection and this is sustained throughout disease course.
- ILC2s in the MLN secrete less IL-13 in SIV-infected animals, while IL-17A production is enhanced in ILC3 in MLNs of SIV-infected animals.
- Microbial translocation may contribute to heightened IL-17A production in MLN ILC3s.

Acknowledgements

We thank all members of the Brenchley lab for helpful discussions and the NIAID veterinary staff.

Funding for this study was provided in part by the Division of Intramural Research/NIAID/NIH. The content of this poster does not necessarily reflect the views or policies of DHHS, nor does the mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government