Characterization of innate lymphoid cells (ILCs) in the human gastrointestinal tract demonstrates compartment-specific ILC distribution which is markedly altered in HIV infection

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Background

Innate lymphoid cells (ILC) have been shown to display critical effector and regulatory functions in innate immunity and tissue remodelling. Recent reports demonstrate ILCs to promote anatomical containment of gut commensal bacteria and to play an important role in intestinal inflammation. However, most of these studies have been performed in mouse models and, thus, the distribution of ILCs in the human gastrointestinal (GI) tract remained unclear.

In HIV infection, the translocation of microbial products from the GI tract to portal and systemic circulation is associated with chronic immune activation and disease progression, even in the setting of virus suppression with antiretroviral therapy. Little is known regarding ILCs in HIV infection and their role in microbial translocation.

Methods

In HIV(-) individuals biopsies from macroscopically normal tissue were taken in the oesophagus (n=8), the stomach (n=11), the duodenum (n=11), the terminal ileum (n=13), and the right-sided colon (n=13) during routine endoscopy. Moreover, oral biopsies (n=6) as well as peripheral blood (n=14) was studied. In addition, biopsies from the oesophagus (n=7), the stomach (n=12), the duodenum (n=12), the terminal ileum (n=8), and the left-sided colon (n=8), as well as PBMC (n=14) from HIV-infected patients under effective cART were analyzed. ILCs were phenotypically characterized by flowcytometry. Cytokine production of ILC1 subsets was studied using bead-based immunoassays (LEGENDplexTM) which allows to quantify IL-2, -13, -22, IFN-g, and TNF-a simultaneously using a flow cytometer. I-FABP serum levels were assessed by ELISA.

Conclusions

Taken together, these data demonstrate a compartment-specific dys-regulation of the intestinal ILC pool even in HIV patients under effective therapy and suggest these alterations to be involved in increased microbial translocation in HIV infected individuals.

Results

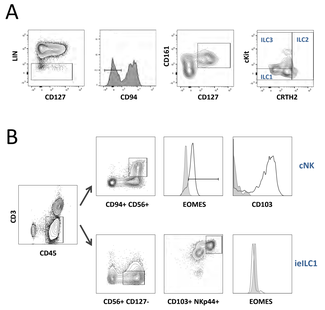


Figure 1. Gating strategy of human intestinal ILCs
Gating strategy for identification of intestinal **CD127(+) ILC1** (Lin-CD127+CD161+CRTH2-ckit-), **ILC2** (Lin-CD127+CD161+CRTH2+), and **ILC3** (Lin-CD127+CD161+CRTH2-ckit+), respectively **(A)**,
as well as for definition of **intraepithelial ILC1** (CD3-CD56+CD127-CD103+NKp44+) and **conventional NK cells** (CD3-CD94+CD56+EOMES+) **(B)**.

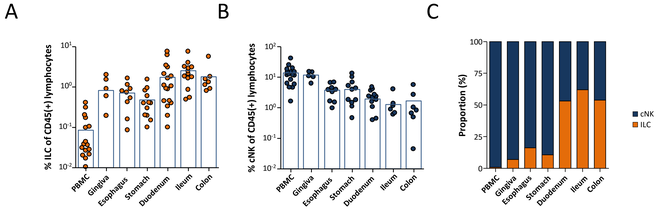


Figure 2. Compartment-specific contribution of non-conventional NK ILC and conventional NK cells
Frequency of total non-cNK ILC (referred to as ILCs) **(A)** and conventional NK cells **(B)** in the human GI tract. Figure **(C)** depicts the relative contribution of ILCs and cNK cells to the total ILC pool in the different parts of the alimentary tract. Of note, we observed an inverse correlation between frequencies of ILCs and cNK cells, with increasing ILC numbers from the oral to the distal part of the GI tract.

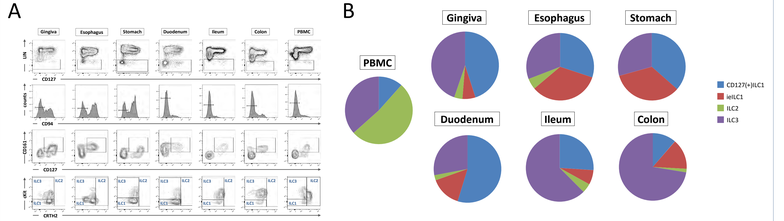


Figure 3. Compartment-specific distribution of human GI ILCs
Figure **(A)** displays exemplary flow cytometric analysis of the GI ILC pool. We found distribution of intestinal ILC subsets to be compartment-specific as we observed ILC3 to be the predominant subset in the oral cavity, the terminal ileum and the left-sided colon, whereas group 1 ILCs represented the major population in the esophagus, the stomach, and the duodenum. Frequency of group 2 ILCs was low in all intestinal compartments. **(B)**.

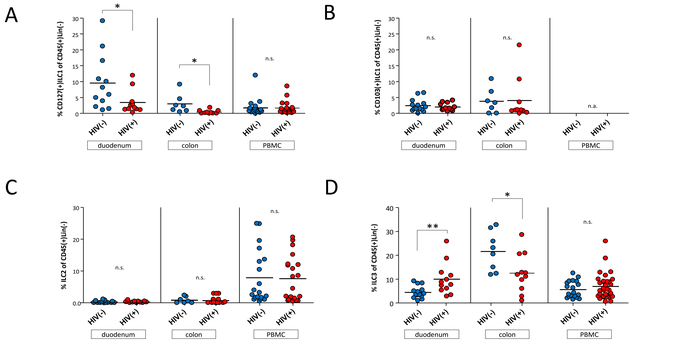


Figure 4. Alterations of the intestinal ILC pool in HIV patients under effective cART
Lymphocytes isolated from the duodenum, the colon, and the circulating blood obtained from controls (blue) or HIV patients (red) were analysed with respect to frequency of CD127(+)ILC1**(A)**, CD103(+)ILC1 **(B)**, ILC2 **(C)**, and ILC3 **(D)**, respectively. We found frequency of ILC3 to be significantly reduced in the colon of HIV patients **(D)**. In the duodenal samples, however, frequency of ILC3 was significantly higher in HIV(+) patients compared to HIV(-) controls. Regarding group 1 ILCs we found HIV infection to be associated with significantly decreased frequency of CD127(+)ILC1 in both compartments of the GI tract **(A)**, whereas frequency of CD103(+)ILC1 did not differ between HIV patients and healthy controls **(B)**. In addition, we found frequency of ILC2 to be similar between both groups **(C)**. Of note, frequency of circulating ILC subsets did not differ between HIV patients and controls.

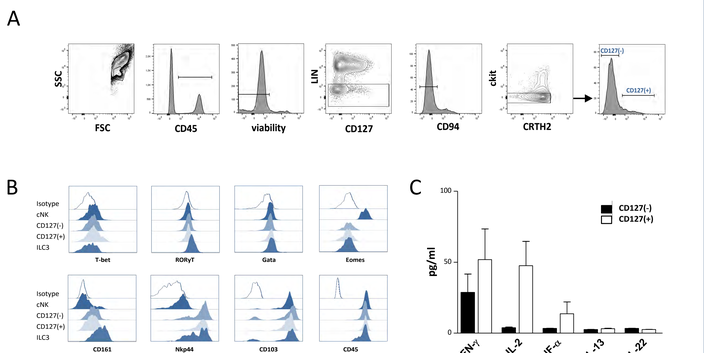


Figure 4. Functional and phenotypically characteristics of CD127(-)ILC1-like cells in comparison to CD127(+)ILC1
(A) Gating strategy for definition of Lin(-)CD45(+)CD94(-)CD161(+)c-kit(-)CRTH2(-)CD127(-)ILC1-like cells. We found CD127(-)ILC1 to display expression levels of T-bet similar to that found in CD127(+)ILCs but to be negative for Eomes, GATA, and RORgT, which further suggested Lin(-)CD45(+)CD94(-)CD161(+)c-kit(-)CRTH2(-)CD127(-) cells to be closely related to CD127(+)ILC1 **(B)**. Moreover, these cells were found to be capable of producing IFN-g, whereas only negligible secretion of IL-13 or IL-22 was observed, resembling the cytokine pattern characteristic for group 1 ILCs **(C)**.

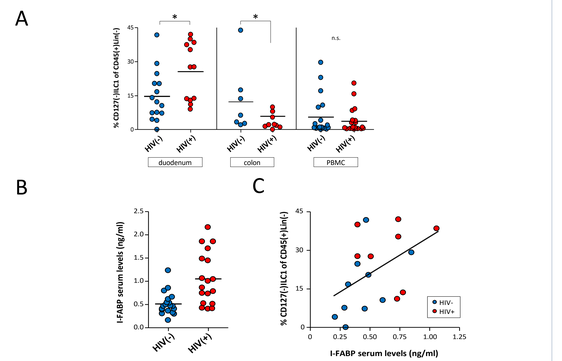


Figure 5. CD127(-)ILC1-like cells in HIV infection
CD127(-)ILC1-like cells were significantly enriched in the duodenum but reduced in the colon of HIV patients **(A)**. Serum levels of I-FABP have been used to correlate intestinal impairment and microbial translocation. In our cohort, I-FABP serum levels were significantly higher in HIV(+) than HIV(-) subjects **(B)**. Of note, frequency of duodenal CD127(-)ILC1-like cells was positively correlated with serum levels of I-FABP, suggesting that HIV-mediated alteration of this cell population might be involved in microbial translocation **(C)**.