Poster number 0249

HIV-1 diversity in gut is associated with residual mucosal virus production on ART

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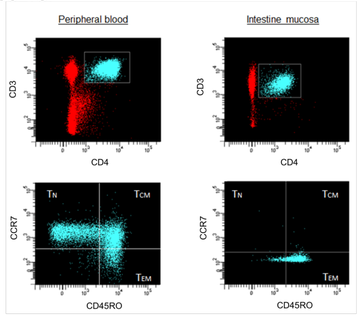
INTRODUCTION : HIV-1 persists in cellular reservoirs and some anatomical compartments despite antiretroviral therapy (ART). We compared HIV-1 in gut and blood compartments on ART, regarding differences in target cells, residual HIV-1 DNA and RNA, coreceptor usage, and virus diversity.

METHODS : Peripheral blood and duodenum samples were obtained from 17 HIV-1-infected subjects with sustained plasma VL of <50copies/ml for 5 years.

Blood and duodenal CD4⁺ T cells were phenotyped by flow cytometry (BD LSRII).

HIV-1 DNA was quantified in sorted blood and duodenal CD4⁺ T cells by qPCR, HIV-1 RNA was quantified in duodenal tissue by qRT-PCR. Virus quasispecies were characterized by next-generation sequencing of C2V3C3 *env* (454 GS Junior), with data cleaning and coreceptor usage prediction by Pyrovir software. Viral diversity in blood and duodenum compartments was assessed by haplotype numbers, adjusted-Shannon entropy, and Hill numbers. Phylogenetic analyses were preformed using CLUSTAL W. A non-parametric test for panmixia was used to assess compartmentalization.

RESULTS:



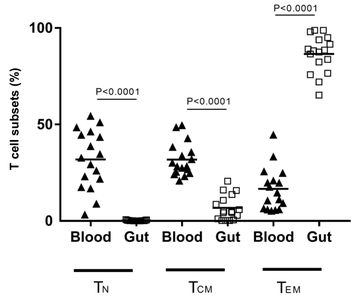


Figure 1. Characterization of T cell subsets in peripheral blood and duodenal mucosa (*lamina propria*)

Naïve T cells (**TN**, CD3⁺CD4⁺CD45RO⁻CCR7⁺), central memory T cells (**TCM**, CD3⁺CD4⁺CD45RO⁺CCR7⁺), and effector memory T cells (**TEM**, CD3⁺CD4⁺CD45RO⁺CCR7⁻) in peripheral blood and duodenal *lamina propria* were characterized by flow cytometry. **TN** and **TCM** were predominant in peripheral blood while they were scarce in the duodenum compartment (T_N 32.8% [3.3-54.45] vs. 0.1% [0-0.7], and T_{CM} 12.55% [5.15-42.35] vs. 6.2% [0.2-27], respectively). **TEM** were preponderant in the duodenal *lamina propria,* 88.5% [65.2-98.8] vs. 28.35% [20.85-49.5] in peripheral blood.

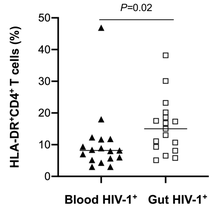


Figure 2. Frequency of HLA-DR⁺CD4⁺ T cells in blood and duodenum compartments from HIV-1-infected individuals

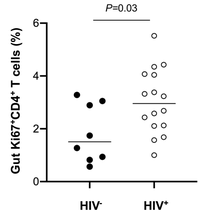


Figure 3. Frequency of Ki67⁺ in duodenal CD4⁺ T cells of HIV-1-infected individuals vs. uninfected controls

Activated CD4⁺ T cells were found at a higher frequency in duodenum than in blood compartment (HLA-DR⁺ CD4⁺ T cells, 15% vs. 8.2%, *P*<0.05). Proliferating Ki67⁺CD4⁺ T cells were found at higher frequency in the gut mucosa of HIV-1-infected subjects than in uninfected controls (2.9% vs 1.5%, *P*=0.03).

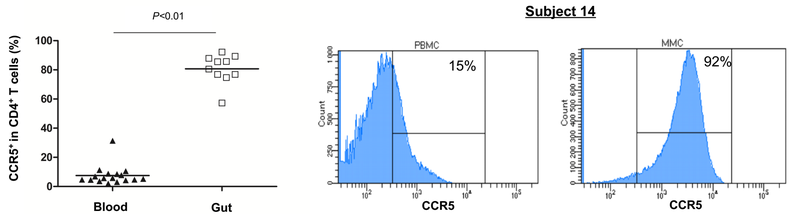


Figure 4. CCR5 expression on CD4⁺ T cells from blood and duodenum of HIV-1-infected individuals (example : subject 14)

CCR5 is highly expressed on duodenal compared to blood CD4⁺ T cells in HIV-1-infected individuals (83% vs 5.7%, *P*<0.01)

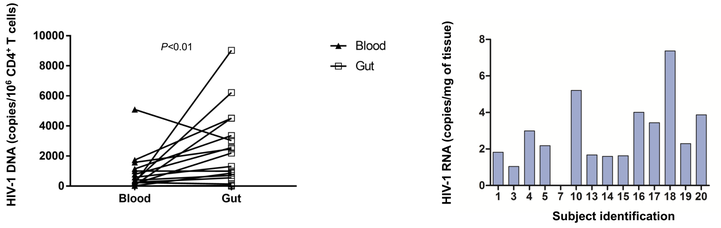


Figure 5. Quantification of HIV-1 DNA and RNA in CD4⁺ T lymphocytes and duodenal tissue of HIV-1-infected individuals

HIV-1 DNA was 6.7-fold higher in duodenum than in blood CD4⁺ T cells (328 vs 2197 copies/mL, *P*<0.01). Moreover, HIV-1 RNA was detected at low level (1-7 copies/mg) in duodenal tissue of 13/14 subjects despite being on sustained effective ART for a median duration of 5 years.

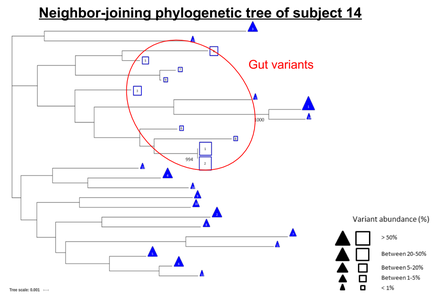
Subject ID	Blood compartment	Gut compartment
1	R5	R5
3	R5/X4	R5/X4
4	R5	R5
5	R5/X4	R5
6	R5/X4	R5
7	R5/X4	R5
8	R5/X4	R5
9	R5	R5/X4
10	X4	X4
13	R5/X4	R5
14	R5	R5
15	R5	R5
16	R5	R5
17	R5	R5
18	R5	R5
19	R5/X4	R5/X4
20	R5	R5

HIV-1 coreceptor usage was genotypicaly predicted at a clonal level from V3 sequences obtained by NGS to characterize each subject virus population. Genotypic prediction can only discriminate CCR5- vs. CXCR4-using clones. But dual-tropic R5X4 clones cannot be discriminated from pure X4 clones and both are thus classified as CXCR4-using viruses.

In the blood compartment, 9 subjects harbored only CCR5-using clones (blue) and 7 harbored both CCR5- and CXCR4-using clones (purple), while in the duodenum 13 subjects harbored only CCR5-using clones and 3 harbored both CCR5- and CXCR4-using clones. One subject harbored only CXCR4-using clones (orange) in both compartments.

The duodenum compartment was thus enriched in CCR5-using viruses.

Figure 6. Genotypic prediction of CCR5 and CXCR4 coreceptor usage of HIV-1 quasispecies characterized by NGS and PyroVir software in peripheral blood and duodenum CD4⁺ T cells.



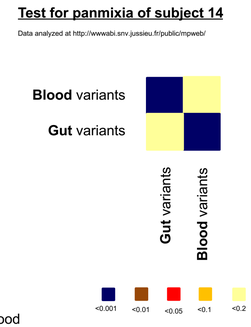


Figure 7. Compartmentalization of HIV-1 quasispecies in duodenal mucosa vs. blood

For all subjects, HIV-1 quasispecies displayed compartmentalization between the blood and duodenum (phylogenetic tree and test for panmixia, *P*<0.001).

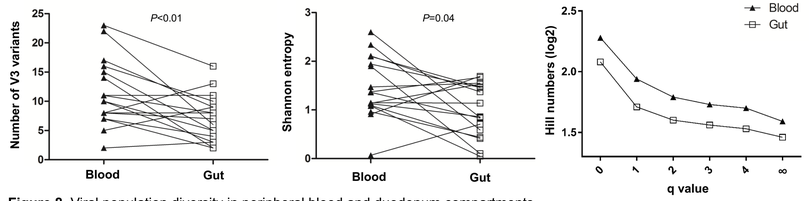


Figure 8. Viral population diversity in peripheral blood and duodenum compartments

Virus diversity in C2V3C3 *env* region was reduced in duodenum vs blood compartment. The median number of variants in the quasispecies was higher in peripheral blood than in duodenal mucosa (n=10 *vs.* 7 variants, respectively, *P*<0.01). Shannon entropy and hill numbers showed higher virus diversity in peripheral blood than in duodenal mucosa.

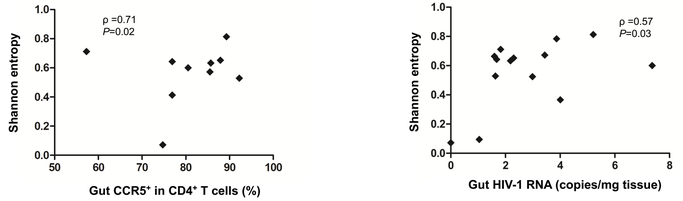


Figure 9. CCR5⁺ target cell frequency and HIV-1 residual replication are associated with higher virus diversity in the duodenum

Despite being reduced in gut vs. blood, HIV-1 quasispecies diversity in the duodenum, assessed by adjusted-Shannon entropy of C2V3C3 *env*, correlated with mucosal CCR5⁺CD4⁺ T cell frequency (ρ=0.71, *P*<0.05), and residual mucosal HIV-1 RNA level (ρ=0.57, *P*<0.05).

CONCLUSION : HIV-1 persists in the duodenum mucosa on ART with increased levels of infected cells compared to blood CD4⁺ T cells, and low-level mucosal HIV-1 RNA production. Virus diversity was reduced with enrichment in CCR5-using viruses and compartmentalization in duodenum vs. blood. However, the frequency of gut CCR5⁺ target cells and the level of HIV-1 RNA were associated with a higher virus diversity in the gut compartment, suggesting residual virus production in the gut mucosa despite sustained ART.