Duodenal and Rectal Mucosa Immune Reconstitution Differ after ART Initiation

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Background
Despite peripheral immunological recovery, incomplete restoration of the gut associated lymphoid tissue (GALT) during treated HIV infection is likely a major determinant of disease progression. The association between blood and GALT T-cell changes following ART initiation, and whether these changes correlate across the different gastrointestinal tract segments remain unknown.

Methodology
Peripheral blood (PB), duodenal and rectal biopsies were obtained from 11 HIV- controls and 33 ART nave HIV+ subjects participating in a randomized clinical trial comparing three different ART initiation strategies, at baseline and after 9 months of treatment. Tissue was digested into single-cell suspensions for measurements of T-cell subsets and activation phenotype by immunophenotyping.

Results
Twenty-six HIV+ subjects completed the follow-up. Median age was 37 years [25-42] vs 34 [28-43] in the control group, and median CD4 T-cell count 435 cell/mL [283-572]. Eight subjects received EFV, 10 MRV and 8 MVC+RAL, each in combination with FTC/TDF, and all achieved viral suppression. Overall, ART resulted in a significant repletion of CD4 T-cell in GALT at month 9, greater in rectum (24% to 38%) than in duodenum (12% to 17%) (all P<0.01). Also, CD8 T-cells decreased in rectum (62% to 53%) and duodenum (81% a 70%) (all P<0.01). However, the levels of both CD4 and CD8 T-cells remained very impaired relative to controls (beyond 2 SD). Changes in %CD4 in PB (24% to 40%, P<0.01) predicted %CD4 changes in colon (Beta 0.54, P=0.023) but not in duodenum (Beta -0.19, P=0.272). The %HLADR +CD38+ of CD8 T-cells was significantly higher at baseline in PB and rectal mucosa in HIV+ compared to HIV- subjects, but not in duodenum. CD8 T-cell activation significantly decreased in blood (57% to 28%, P<0.001), duodenum (65% to 52%, P=0.015) and rectum (71% to 58%, P=0.429,] and the change in PB was reflective of the smaller mucosal changes [duodenum Beta=0.39, P<0.01, and rectum Beta=0.23, P<0.01). However, the % of activated CD8 T-cells in PB and in colon did not reach normal levels. In addition, the % of PB activated CD8 T-cells at baseline predicted the extent of CD4 depletion in duodenum (Beta=-0.7, P<0.001) and colon (Beta=-0.7, P<0.001). HIV+ subjects showed similar % of naive CD8+ T-cells in colon and duodenum than controls, but not in PB. The CD8 nave/memory ratio significantly improved in blood but did not reach normal levels.

Conclusions
Following ART initiation we observed incomplete recovery of GALT CD4 T-cells. Immune reconstitution of the duodenal mucosa was impaired compared to changes in blood and rectum. The duodenal compartment may represent a portal for systemic immune activation and CD4 depletion during HIV infection.

INTRODUCTION

- Early during HIV infection gastrointestinal-associated lymphoid tissue (GALT) experiments a profound depletion of CD4 T-cells (1)
- Immunologic recovery in GALT is known to be substantially delayed compared to the peripheral compartment following successful cART (2)
- Changes in peripheral blood may non reflect changes in the gut (2)
- Disproportional increase in CD8 populations has been described in duodenal tissue (3), but it is not known whether different segments within the gastrointestinal tract experience T-cell restoration differentially
- Translocation of bacterial antigens related to this presumed persistent alteration in mucosal integrity is thought to be driving peripheral immune activation and suppressed immune restoration in peripheral CD4 T-cell counts in HIV-infected individuals (4).
- Decreased levels of mucosal repair and regeneration despite effective ART may contribute to the increased levels of inflammation and immunoactivation, described in HIV-infected patients (5) .To what extent peripheral immunoactivation reflects GALT status has not been addressed

RESULTS

- Data were available for 26 HIV+ subjects that completed the follow-up period.
- Eight subjects received EFV, 10 MRV and 8 MVC+RAL, each in combination with FTC/TDF.
- Median age was 37 years [25-42] vs 34 [28-43] in the control group
- Median CD4 T-cell count 435 cell/mL [283-572].
- All subjects in the study achieved viral suppression.

Figure 1

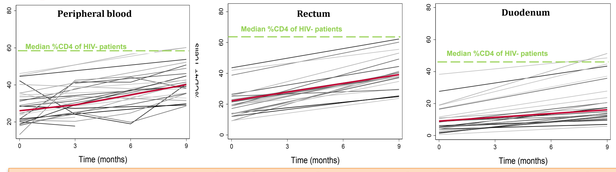


Figure 1: Overall, ART resulted in a significant repletion of CD4 T-cell at month 9 in PB (24%-40%) and GALT, being greater in rectum (24% to 38%) than in duodenum (12% to 17%) (all P<0.01)

Changes in %CD4 in PB predicted %CD4 changes in colon (Beta 0.54, P=0.023) but not in duodenum (Beta -0.19, P=0.272).

Figure 2

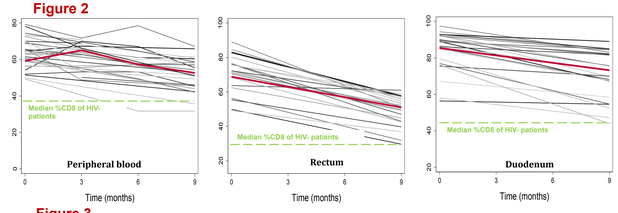


Figure 2: Along with this changes, CD8 T-cells decreased in PB in rectum (62% to 53%) and duodenum (81% a 70%) (all P<0.01)

Figure 3

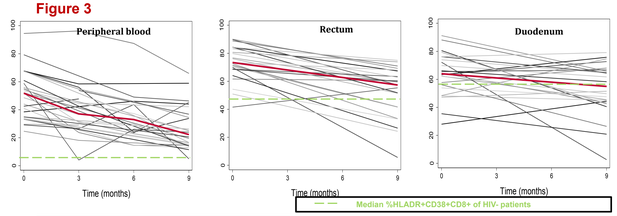


Figure 3: The % of activated CD8 T-cells was significantly higher at baseline in PB and rectal mucosa in HIV+ compared to HIV- subjects, but not in duodenum.

CD8 T-cell activation significantly decreased in blood (57% to 28%, P<0.001), rectum (71% to 58%, P=0.429] and duodenum (65% to 52%, P=0.015). Change in PB was reflective of the smaller mucosal changes [duodenum Beta=0.39, P<0.01, and rectum Beta=0.23, P<0.01).

METHODS

- Peripheral blood (PB), duodenal and rectal biopsies were obtained from 11 HIV- controls and 33 ART nave HIV+ subjects participating in a randomized clinical trial comparing three different ART initiation strategies, at baseline and after 9 months of treatment.
- Tissue was digested into single-cell suspensions for measurements of T-cell subsets and activation phenotype by immunophenotyping
- Lymphocyte maturational subsets were defined as follows: naive T cell (T_N, CD45RA+CCR7+), central memory (T_{CM}, CD45RA-CCR7+), transitional memory (T_{TM}, CD45RA–CCR7-), effector memory (T_{EM}, CD45RA-CCR7-), terminally differentiated (T_{EMRA}, CD45RA+CCR7-) for CD4+ and CD8+ cells.
- T-cell activation was defined as co-expression of HLA-DR and CD38 on respective lymphocyte population.
- Gating strategy included using an FMO (fluorescenceminus-one) to determine the cut-off for positive cells for CCR7, CD38, and HLA-DR for each run.

Correlations between the % HLADR+CD38+CD8+ T Cells on PB at baseline and the extent of CD4 depletion in GALT

Figure 4

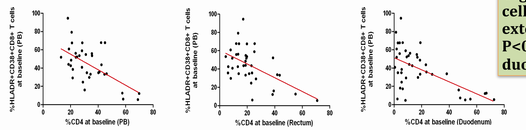
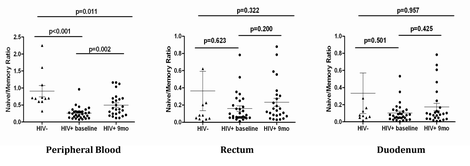


Figure 4: Percentage of activated CD8+ T cells in PB at baseline correlated with the extent of CD4 depletion in PB (Beta=-0.9, P<0.001), rectum (Beta=-0.7, P<0.001) and duodenum (Beta=-0.7, P<0.001).

Figure 5



HIV+ subjects showed similar percentage of naive CD8+ T-cells in rectum and duodenum than controls, but not in PB. The CD8 naive/memory ratio significantly improved in blood but did not reach normal levels (Figure 5).

CONCLUSIONS

- **Following ART initiation we observed incomplete recovery of GALT CD4 T-cells.**
- **Immune reconstitution of the duodenal mucosa was impaired compared to changes in blood and rectum.**
- **Collectively, our results suggest that the duodenal compartment may represent a portal for systemic immune activation and CD4 depletion during HIV infection**

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