Links between systemic and mucosal immunity in treated HIV infection

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INTRODUCTION

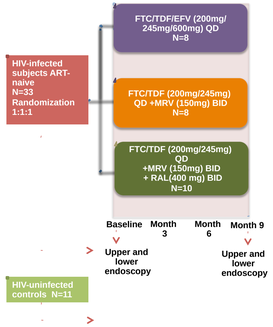
- Activation of the innate immunity (reflected by raised plasma levels of markers of gut epithelial barrier dysfunction, bacterial translocation, monocyte activation and inflammation) independently predicts mortality during treated HIV infection [1,2].
- These abnormalities have proven to be difficult to reverse, likely due to the multifaceted pathways involved in sustained immune activation and increased inflammation and the consequent array of clinical effects [3,4].
- Understanding of the underlying mechanisms might help to design therapeutic interventions to treat chronic inflammation [5].
- This study examined the correlations among markers of gut epithelial barrier dysfunction, bacterial inflammation, translocation and maturational subsets in gut mucosa.

METHODS

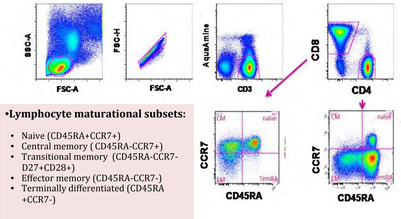
•Peripheral blood, duodenal and rectal biopsies were obtained from 11 HIV- controls and 33 ART nave HIV+ subjects participating in a randomized clinical trial comparing three different ART initiation strategies, at baseline and after 9 months of treatment.

•Tissue was digested into single-cell suspensions for immunofluorescence analysis and flow-citometry measurements.

Study Design



Flow citometry analysis on peripheral and mucosal mononuclear cells



•**Lymphocyte maturational subsets:**

- Naive (CD45RA+CCR7+)
- Central memory (CD45RA-CCR7+)
- Transitional memory (CD45RA-CCR7-D27+CD28+)
- Effector memory (CD45RA-CCR7-)
- Terminally differentiated (CD45RA +CCR7-)

•**Gating strategy** included using an FMO (fluorescence minus-one) to determine the cut-off for positive cells for CCR7for each run.

- **Immunofluorescence (IFA) analysis:** tissue was stained using polyclonal anti-CD3 rabbit and monoclonal anti-CD4 or CD8 mouse serum. Binding of CD3 and CD4 or CD8 receptors were detected simultaneously using Alexafluor 488-labeled polyclonal goat antirabbit IgG (and Alexafluor 568-labeled polyclonal goat antimouse IgG (Molecular Probes). The numbers of positive cells were counted by a single observer and presented as cells/mm^2 of lamina propria or intraepithelial regions (above the basement membrane) of duodenal mucosa.
- **Assays in cryopreserved plasma:** IL-6 (R&D Systems), sCD14 (R&D Systems), hs-CRP (CardioPhase hs-CRP assay, Siemens), lipoteichoic acid (LTA, R&D Systems) and zonulin-1 (ALPCO).
- **Statistical analysis:** Spearman's rank correlation coefficients were assessed at month 9. Longitudinal associations between changes of plasma biomarkers and variations of T cell subsets were analyzed using linear mixed models with random intercepts. Continuous variables were log-transformed when necessary to satisfy model assumptions.

RESULTS

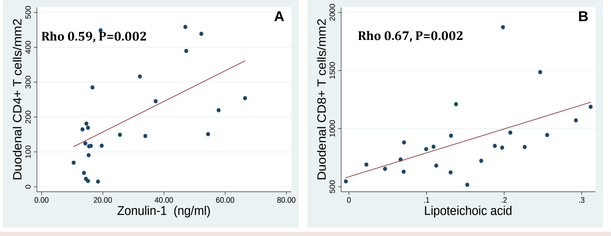
•Data were available for 26 HIV+ subjects who completed the follow-up period.
•Eight subjects received EFV, 10 MRV and 8 MVC+RAL, each in combination with FTC/TDF.
•Median age was 37 years [25-42] vs. 34 [28-43] in the control group
•Median CD4 T-cell count 435 cell/ml [283-572].
•All subjects in the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

Significant correlations between soluble markers of inflammation and maturational T cell subsets in the gut mucosa at month 9 of ART

Soluble Marker	Marker	Rho.	P value
Zonulin-1	Duodenal CD4+ T cell density	0.59	0.002*
	Rectal CD8+ naïve T cells	-0.51	0.009
	Duodenal CD8+ naïve T cells	-0.53	0.004*
	Rectal CD8+ CM	-0.58	0.001
	Duodenal CD8+ EM	-0.44	0.002
	Duodenal CD8+ Naïve/Mem	0.52	0.004
	Rectal CD8+ Naïve/Mem	0.54	0.008*
Lipoteichoic acid	Duodenal CD8+ T cell density	0.67	0.025*
Soluble CD14	-	-	-
Interleukin-6	Duodenal CD8+ CM	-0.47	0.016
	Duodenal CD8+ TEMRA	0.58	0.018
	Rectal CD4+ TM	-0.51	0.007*

** Denotes a P value <0.005 for the association between longitudinal changes of plasma biomarkers and changes of T cell subsets (as fixed effects) in linear mixed effects models. All significan P values in these models reflect significant associations between longitudinal variations levels of soluble markers maturational T cell subsets.*

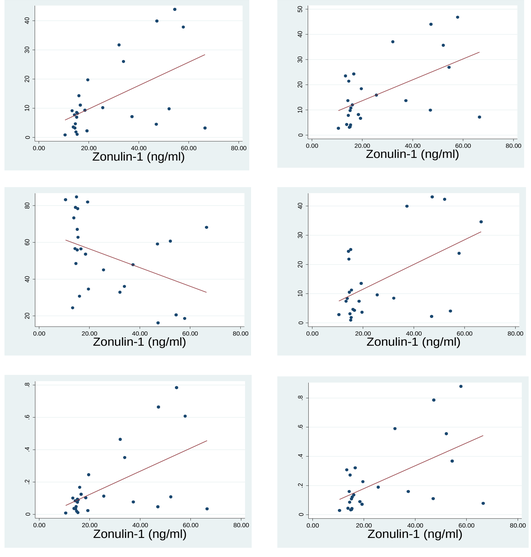
Duodenal T cell density correlates with plasma levels of zonulin-1 and lipoteichoic acid at month 9 of ART



A. Zonulin-1 levels positively correlated with duodenal CD4+ T cell density. In a linear mixed effects model, changes of duodenal CD4+ T cell density positively correlated with changes of zonulin-1 (Coef. 0.13, P=0.004)

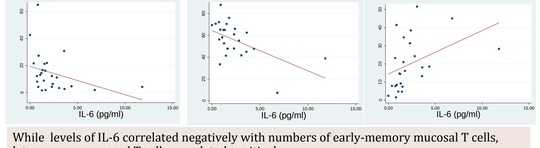
B. Lipoteichoic acid levels positively correlated with duodenal CD8+ T cell density. In a linear mixed effects model, changes of duodenal CD8+ T cell density positively correlated with changes of LTA (Coef. 0.46, P=0.023).

A number of T cell subsets in colon and duodenum correlated with plasma zonulin-1 levels at month 9 of ART



Improvement of T cell maturation (naïve and early-memory subsets) and higher naïve/memory ratio) correlated with higher zonulin-1 levels.

Mucosal subsets correlated with levels of IL-6 at month 9 of ART



While levels of IL-6 correlated negatively with numbers of early-memory mucosal T cells, late-memory mucosal T cells correlated positively.

CONCLUSIONS

- We observed a number of significant correlations between maturational T cell subsets in the gut and systemic markers of gut barrier integrity (zonulin-1), bacterial translocation (LTA) and inflammation (IL-6).
- In contrast, these intestinal T cell subsets did not correlate with monocyte activation (sCD14), which may be related to different pathogenic pathways.
- Strategies aimed at modulating chronic inflammation may require targeting the gut immunity .

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