FROM GUT TO BLOOD: REDISTRIBUTION OF ZONULIN IN HIV+ PATIENTS

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Background

Damage of the gastrointestinal (GI) mucosa in HIV infection leads to microbial translocation and immune activation, which in turn contributes to non-infectious comorbidities. Combined antiretroviral therapy (cART) leads to only partial restauration of this gut damage. Several biomarkers of the epithelial barrier have been identified, however, some are not specific and others are impacted by cART. Zonulin is a modulator of epithelial tight junctions. Previous studies reported an increase of Zonulin in the blood during HIV infection while others reported a decrease. We quantified Zonulin and compared it to inflammatory markers and the viral reservoir in blood (PB), ileum (TI) and rectum (R) from HIV patients (HIV+) and controls (CTRL).

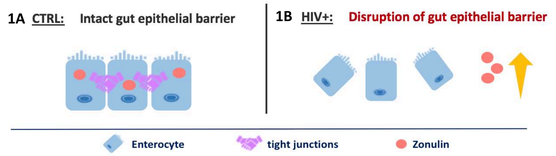


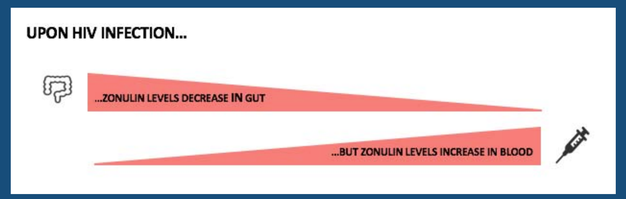
Figure 1 Intestinal Integrity: The gut epithelial barrier. (A) Intact gut epithelial barrier. **(B)** Disruption of gut epithelial barrier. Zonulin is a modulator of gut epithelial tight junctions. Loss of intestinal integrity during HIV infection is associated with elevated systemic Zonulin levels.

Methods



Biopsies and gut tissue sections from TI, R and PB were collected from 5 treatment naïve (HIV+$_{NAÏVE}$) and 10 cART-treated (HIV+$_{cART}$) HIV+ individuals and 11 controls (CTRL). Lamina propria mononuclear cells were isolated. Following flow cytometry and cell sorting of CD4+ T cells, total HIV-DNA was quantified in PB, TI and R. In serum circulating Zonulin was measured by ELISA (Immundiagnostik) and in gut sections by semi quantitative immunohistochemistry. Ultrasensitive digital ELISA (Simoa; Quanterix) was used to measure IFN-α in serum and tissue supernatants.

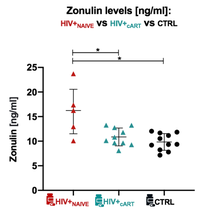
Providing insights into the role of Zonulin in the regulation of gut epithelial tight junctions in HIV infection



Results

Median CD4+T cell count [cells/µl] in HIV+$_{NAÏVE}$ was 70(30-255) versus 426(293-787) in HIV+$_{cART}$. Median time on ART [years] was 6(9-10). Circulating Zonulin levels [ng/ml] were highest in treatment-naïve HIV+ when compared to cART-treated HIV+ (p=0.04) or CTRL (p=0.0087; HIV+$_{NAÏVE}$ > HIV+$_{cART}$ > CTRL). Similarly, HIV+$_{NAÏVE}$ showed higher IFN-α and HIV-DNA levels in PB when compared to HIV+$_{cART}$ (IFN-α: p=0.04; HIV-DNA: p=0.04). In gut tissue sections however, Zonulin was higher in CTRL when compared to HIV+ patients. Circulating Zonulin in serum was negatively correlated to plasma CD4 cell count (r= -0.54, p=0.04), CD4+T cell frequencies in TI (r= -0.69 p=0.0170) and positively to IFN-α in TI (r=0.85, p=0.0061).

2A



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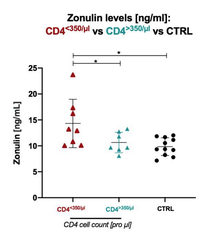

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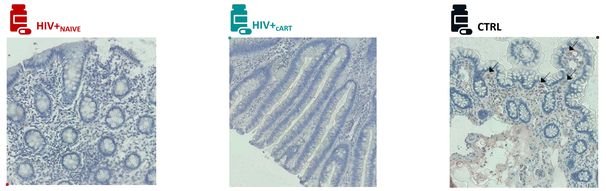


2D



Figure 2 FROM GUT TO BLOOD: Redistribution of Zonulin in HIV+ patients. (A) Systemic Zonulin was significantly higher in **(A)** HIV+$_{NAÏVE}$ when compared to HIV+$_{cART}$ and CTRL (HIV+$_{NAÏVE}$ > HIV+$_{cART}$ > CTRL) and in **(B)** HIV+ with CD4$^{<350/µl}$ when compared to CD4$^{>350/µl}$ and CTRL (CD4$^{<350/µl}$ > CD4$^{>350/µl}$ > CTRL). **(C)** Zonulin (brown/pink) semi quantitative immunohistochemistry (IHC) in paraffin embedded gut sections of HIV+$_{NAÏVE}$, HIV+$_{cART}$ and CTRL. Optical Microscropy X20. **(D)** Upon HIV infection, Zonulin levels decrease in gut but Zonulin levels increase in blood. CTRL, controls.

Methods

3A Terminal Ileum: median cell count 240.000 (IQR 63.000 – 500.000)

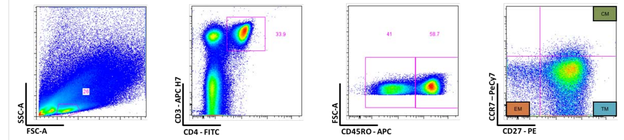
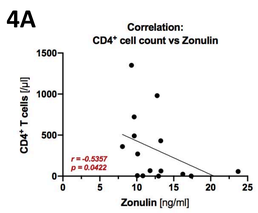


Figure 3: Exemplary flow cytometry dot plots of **(A)** terminal Ileum *(TI)* of an HIV+ patient (N°390). IQR, interquartile range; CM, T central memory cells; EM, T effector memory cells; TM, T transitional memory cells; R, rest.

Results

4A



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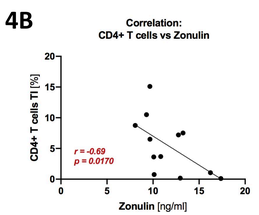

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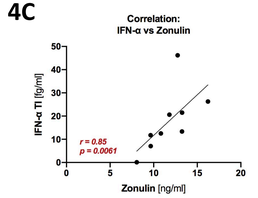

Figure 4: Systemic Zonulin: Correlation to clinical characteristics and inflammation marker in the gut.

Zonulin was correlated negatively to **(A)** CD4+ plasma cell count (r= -0.5357, p=0.0422) **(B)** CD4+ T cell frequencies (r= -0.69 p=0.0170) and positively **(C)** to IFN-α (r=0.85, p=0.0061) in the TI.

Conclusions

The data indicate that upon HIV infection, Zonulin levels decrease in gut, but increase in plasma. The latter were associated with loss of intestinal CD4+T cells and increased inflammation in the gut, suggesting that increased levels of systemic Zonulin correlate with intestinal damage. An increased understanding of the regulation of gut tight junctions during HIV infection may be crucial for the design of future therapies.

References

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