OVERT GUT IL-32 ISOFORM EXPRESSION DURING TREATED HIV INFECTION: REGULATION BY IL-17A

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BACKGROUND

The interplay between intestinal epithelial cells (IEC) and Th17 cells is key for mucosal immunity homeostasis. HIV infection provokes the impairment of intestinal barrier functions and chronic immune activation, which are not resolved by antiretroviral therapy (ART). The non-reconstitution of intestinal Th17 cells during ART causes IEC dysfunction, including an increased production of pro-inflammatory cytokines.

IL-32 is an inducer of TNF-α, a cytokine strongly associated with inflammatory diseases. Overexpression of IL-32 has been associated with the loss of virological/immunological control of viral replication in people with a history of controlled HIV infection (El-Far et al., 2016). IL-32 presents splicing variants with distinct functions, therefore the role of specific IL-32 isoforms in HIV pathogenesis remains to be investigated.

This project aims to evaluate the role of IL-32 isoforms during HIV infection, particularly in the interaction between IECs and Th17 cells in the gut-associated lymphoid tissues (GALT). Here, we monitored IL-32 isoform expression in the colon and blood of ART-treated people living with HIV (ART+PLWH), and explored the regulation of IL-32 by the Th17 hallmark cytokine IL-17A.

METHODS

1. Matched sigmoid colon biopsies and blood samples from ART-treated PLWH and uninfected individuals were used to quantify IL-32 isoforms (α, β, γ, d, ε and θ) and IL-17A mRNA by real-time RT-PCR.

2. The IEC HT-29 were treated with a panel of TLR ligands, HIV-1 NL4.3BAL or THRO strains and TNF-α, in the presence or absence of IL-17A. IL-32 isoform expression was measured by real-time RT-PCR and intracellular total IL-32 protein was measured by ELISA.

RESULTS

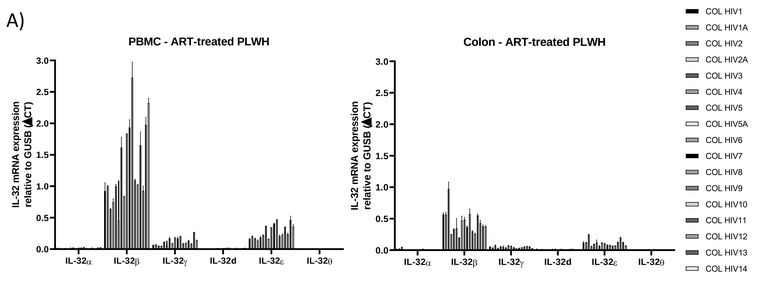


Fig. 1: A: The isoforms IL-32β, γ and ε are predominantly expressed in cells isolated from matched blood and colon biopsies of ART+PLWH, with superior IL-32 expression in the blood.

► IL-32β, γ and ε isoforms are predominant in the blood and colon of ART-treated PLWH and upregulated in comparison to uninfected controls.
► IL-32β mRNA negatively correlates with IL-17A mRNA expression in the colon.
► IL-17A downregulates IL-32 expression and production *in vitro.*

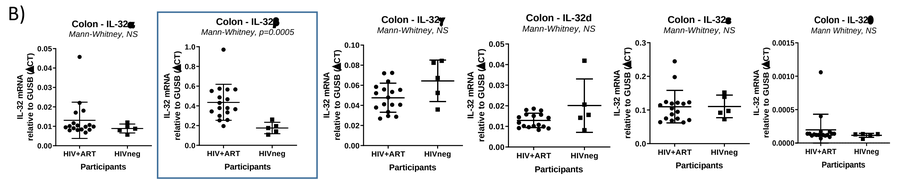


Fig. 1: B: IL-32β is significantly upregulated in the colon of ART+PLWH compared to uninfected individuals.

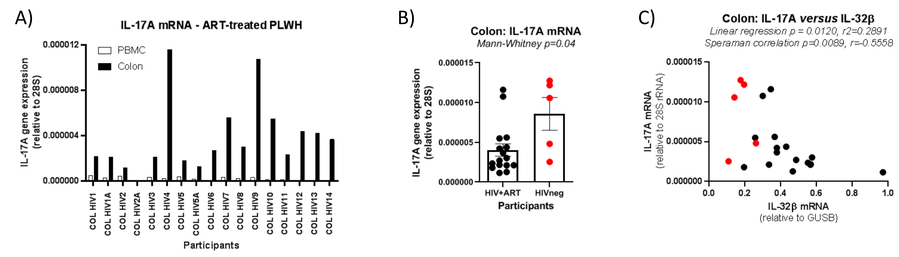


Fig. 2: A: IL-17A is absent in the blood but present in the colon of ART+PLWH. **B:** IL-17A is downregulated in the colon of ART+PLWH compared to uninfected individuals. **C:** IL-32β and IL-17A negatively correlate.

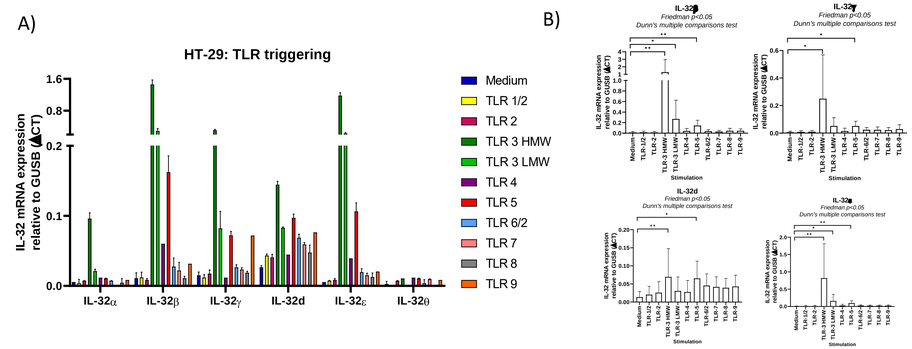


Fig. 3: A: Triggering of TLR-3 (Poly:IC HMW and LMW) and TLR-5 (Flagellin from *S. typhimurium*) pathways promotes IL-32 isoforms expression, specially IL-32β, γ and ε. **B:** Specific IL-32 isoforms expression by TLR-3 and 5 are significantly different from unstimulated cells (Medium).

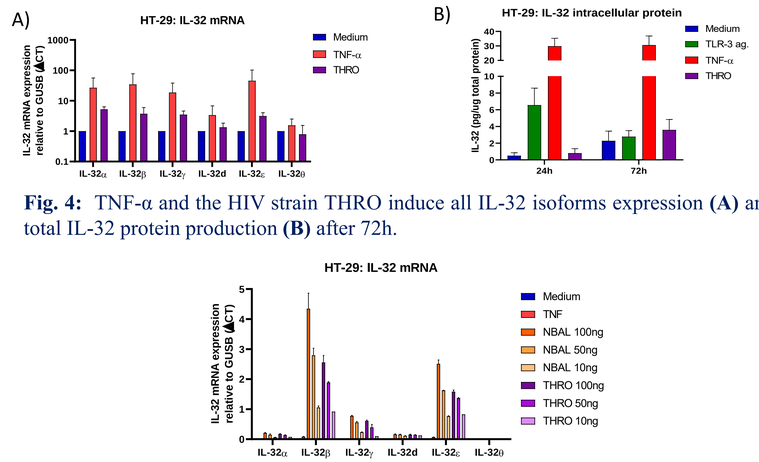


Fig. 4: TNF-α and the HIV strain THRO induce all IL-32 isoforms expression **(A)** and total IL-32 protein production **(B)** after 72h.

Fig. 5: IL-32 is strongly induced after 72h by HIV strains (N4.3Bal and THRO) with increased virulence in a dose-dependent manner.

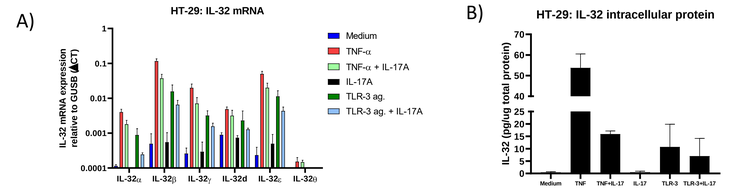


Fig. 6: IL-17A decreases TNF-α and TLR3-induced IL-32 mRNA expression **(A)** and decreases total IL-32 intracellular protein levels **(B).**

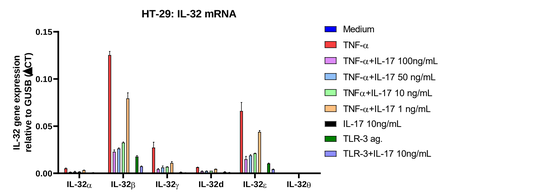


Fig. 7: IL-17A downregulates TNF-α and TLR3-induced IL-32 mRNA expression in a dose-dependent manner.

CONCLUSIONS

Our results provide a cartography of specific IL-32 isoforms overexpressed in blood and colon biopsies of ART-treated PLWH and point to the important role of Th17-specific IL-17A in the negative regulation of IL-32-driven inflammation. Our results support the importance of specific IL-32 isoforms as new therapeutic targets for preventing comorbidities in ART-treated PLWH.

References: El-Far et al. Sci Rep. 2016; Kim et al. Immunity 2005; Damen et al. Atherosclerosis. 2017; Smith et al. J Immunol. 2011; Dandekar et al. Curr Opin HIV AIDS. 2010.

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