Enhanced IL-32 Expression Across Immune Cells Shapes Cytokine Responses and HIV Control Persistence

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BACKGROUND

A rare subset of people living with HIV, calledHIV controllers (HIC), maintains spontaneous viral suppression in the absence of antiretroviral therapy. However, controller status is not uniformly stable: while persistent controllers (PC) maintain long-term suppression, others experience viral rebound and transition into transient controllers (TC), highlighting the need to understand mechanisms underlying durability of control[1].

Interleukin (IL)-32 is a multifunctional cytokine implicated in HIV replication and immune activation in a cell type- and context-dependent manner[2]. It is linked to antiviral interferon-γ (IFNγ) production during HIV infection[2] and suppresses HIV replication in macrophages. Opposingly, in $CD4^+$ T cells, it promotes HIV replication through induction of pro-inflammatory cytokines[3,4], while blocking HIV reactivation, possibly aiding latency[5]. Yet, its role in natural HIV control remains poorly understood.

We hypothesize that the balance between IL-32-driven inflammatory and antiviral responses support durable viral suppression, whereas disruption of these pathways contributes to the loss of spontaneous control observed in transient controllers.

METHODS

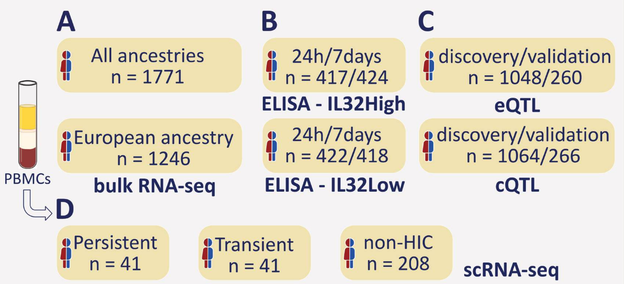


Figure 1 (A) Bulk RNA sequencing was performed in peripheral blood mononuclear cells (PBMCs) of PLWH on ART. (B) PBMCs were stimulated with viral and bacterial stimuli for 24 hours or seven days followed by quantification of cytokine production. (C) Expression and cytokine quantitative trait loci (eQTL, cQTL) mapping was performed in PLWH of European ancestry to identify single nucleotide polymorphisms associated with *IL32* expression and cytokine production. (D) Single-cell RNA sequencing data from PBMCs of persistent, transient, and non-HIV controllers was analysed.

Genetics and host-intrinsic traits regulating IL-32 mark a durable immune state that contributes to persistent HIV control.

CONCLUSIONS

- ***IL32* expression is shaped by sex and ancestry.**
- **Persistent HIV controllers have higher *IL32* expression than transient controllers.**
- **Polymorphisms associated with *IL32* expression are linked to enhanced cytokine and chemokine production upon microbial stimulation.**

RESULTS

Persistent HIV controllers express high *IL32*

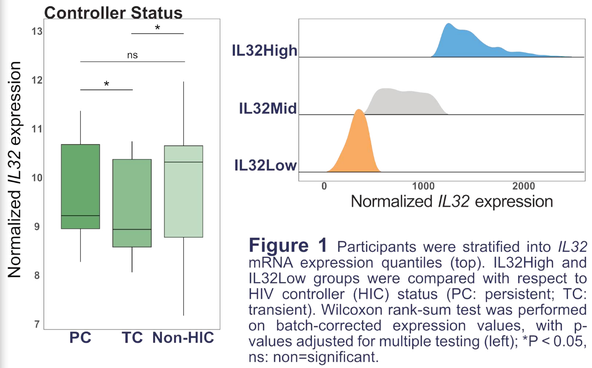


Figure 1 Participants were stratified into *IL32* mRNA expression quantiles (top). IL32High and IL32Low groups were compared with respect to HIV controller (HIC) status (PC: persistent; TC: transient). Wilcoxon rank-sum test was performed on batch-corrected expression values, with p-values adjusted for multiple testing (left); *P < 0.05, ns: non=significant.

Sex and Ancestry affect *IL32* expression

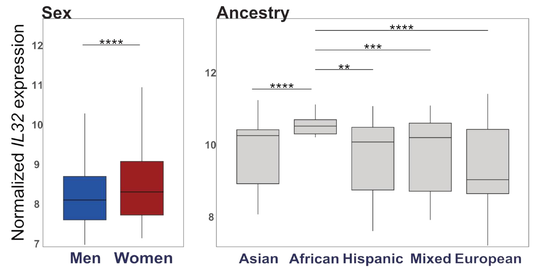


Figure 2 Participants were stratified into *IL32* mRNA expression quantiles. IL32High and IL32Low groups were compared with respect to sex at birth and self-reported ancestry. Wilcoxon rank-sum test was performed on batch-corrected expression values, with p-values adjusted for multiple testing; **P < 0.01, ***P < 0.001, ****P < 0.0001.

Genotypic regulation of IL-32 and cytokines

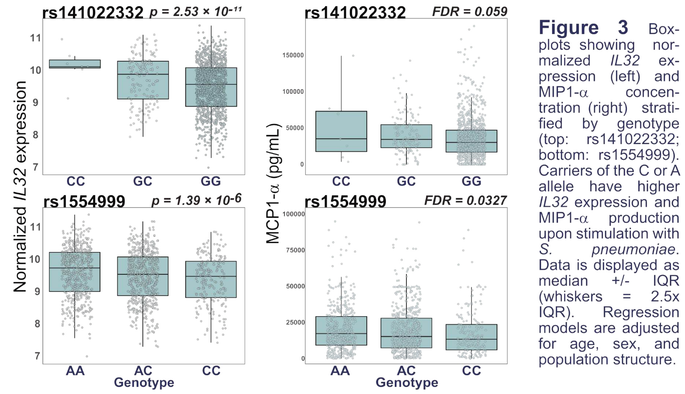


Figure 3 Boxplots showing normalized *IL32* expression (left) and MIP1-α concentration (right) stratified by genotype (top: rs141022332; bottom: rs1554999). Carriers of the C or A allele have higher *IL32* expression and MIP1-α production upon stimulation with *S. pneumoniae*. Data is displayed as median +/- IQR (whiskers = 2.5x IQR). Regression models are adjusted for age, sex, and population structure.

IL-32 regulates inflammatory responses

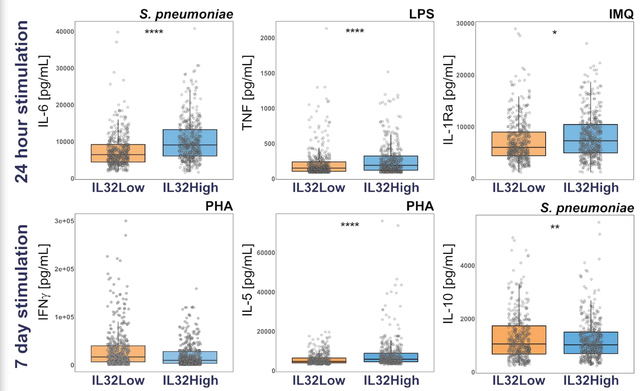


Figure 4 Cytokine responses against imiquimod (IMQ), Poly(I:C), phytohemaglutinin (PHA), *S. pneumoniae*, or lipopolysaccharide (LPS), stimulated for 24 hours or seven days, were compared between participants stratified by *IL32* expression. Data are shown in pg/mL as median +/- IQR (whiskers = 1.5x IQR). Rank-based regression analysis was performed for each cytokine-stimulus combination, adjusting for sex, age, seasonality, pandemic period, COVID vaccination status, and the first five principal components to account for population structure; *P < 0.05, **P < 0.01, ****P < 0.0001.

IL32 expression cell-specifically distinguishes persistent and transient HIV control

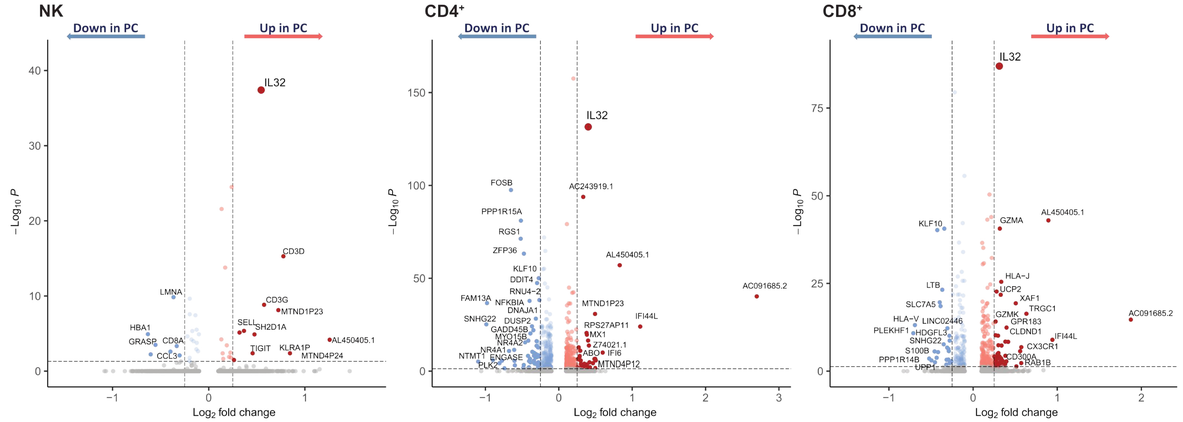


Figure 5 Volcano plots depicting differential gene expression between persistent (PC) and transient controllers within natural killer (NK) cells, $CD4^+$, and $CD8^+$ T cells. Genes are plotted by log_2 fold change and $-log_{10}$ adjusted p-value. Dashed vertical lines indicate log_2 fold change thresholds (±0.25). The horizontal dashed line denotes the adjusted p-value cutoff (adjusted p < 0.05). Genes meeting both significance and fold-change thresholds are highlighted.

ADDITIONAL KEY INFORMATION

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Sources

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