Impaired IL-23 Signalling and Th17 Dysfunction in HIV Infection

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Abstract

Background

Th17 cells maintain gut homeostasis by co-ordinating a variety of innate and adaptive immune responses. HIV infection causes a profound depletion of gut Th17 cells, contributing to loss of mucosal barrier function and microbial translocation, thus driving microbial translocation and systemic immune activation. Despite normalization of circulating CD4$^+$ T cell counts with highly active antiretroviral therapy (HAART), Th17 frequency and function often remain impaired. The IL-12 family cytokine member IL-23 plays a crucial role in maintaining normal Th17 cell function. We hypothesize that HIV inhibits IL-23 signalling in Th17 cells, resulting in Th17 dysfunction.

Methodology

Th17 cells were isolated from peripheral blood of HIV-seronegative donors by magnetic selection and infected with HIV$_{CS204}$, a dual-tropic clinical isolate for 24 hours. Expression of IL-17 was examined by ELISA and flow cytometry. Levels of Th17-associated transcription factors STAT3 and retinoic acid orphan receptor C (RORC) mRNA expressed in response to IL-23 stimulation were quantified by qRT-PCR. Phosphorylation of STAT3, the primary transducer of IL-23 signalling, in response to IL-23 stimulation was assessed by flow cytometry. Th17 cells were then isolated from untreated and HAART-treated HIV-infected individuals, and STAT3 phosphorylation (pSTAT3) in response to IL-23 was examined. Expression of the IL-23 receptor protein IL23R on Th17 cells was examined by flow cytometry.

Results

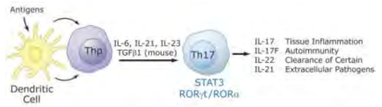
In vitro HIV infection significantly inhibited IL-17 production in response to TCR and mitogenic stimulation. *In vitro* HIV infection also significantly reduced IL-23-induced pSTAT3 and expression of STAT3 and RORC mRNA. Th17 cells isolated from untreated and HAART-treated HIV-infected individuals showed complete loss of IL-23 responsiveness. IL-6 induced pSTAT3 was unaffected by *in vitro* and *in vivo* infection, suggesting that HIV infection results in specific inhibition of IL-23 signalling. Expression of the IL-23 receptor protein IL23R was comparable between HIV-seronegative controls, untreated, and HAART-treated HIV-infected individuals.

Conclusions

These results demonstrate that *in vitro* and *in vivo* HIV infection results in impaired IL-23 signalling which is not reversed by HAART and is not a result from reduced receptor expression, demonstrating that HIV interferes with IL-23-activated signaling pathways. These findings may explain the inability of HAART to restore Th17 frequency and function and the resulting persistent chronic immune activation observed in HIV-infected individuals.

Introduction

- HIV infection causes almost complete loss of gut and blood Th17 cells
  - Th17 frequency and function remain impaired despite suppression of viral replication and CD4$^+$ T cell rebound by highly-active antiretroviral therapy (**HAART**).
- Th17 reconstitution by HAART is associated with reduced markers of microbial translocation and systemic immune activation
  - Supported by SIV model investigations of gut and blood Th17 cells
- IL-23 plays a central role in supporting and maintaining Th17 differentiation *in vivo*
  - Signals primarily through STAT3
  - STAT3 phosphorylation leads to STAT3, RORC, IL17 upregulation
- We present evidence of IL-23 signalling impairment and Th17 dysfunction in blood Th17 cells in the context of *in vitro* and *in vivo* HIV infection



Adapted from Jetten, *Nucl. Recept. Signal. (2009)*

Methods

1. Effects of *in vitro* HIV infection on IL-17 production by blood-derived and *in vitro-generated* Th17 cells were determined by ELISA and flow cytometry:

Th17 cells +/- HIV$_{CS204}$ → IL-17 expression (Flow cytometry); IL-17 secretion (ELISA)

2. The ability of T cell activators to induce STAT3 and RORC transcription in *in vitro*-infected, blood-derived Th17 cells was determined by qRT-PCR:

Th17 cells +/- HIV$_{CS204}$ → RORC/STAT3 transcription (qRT-PCR)

3. The effect of *in vitro* and *in vivo* HIV infection on IL-23-induced STAT3 phosphorylation in blood-derived Th17 cells was determined by flow cytometry

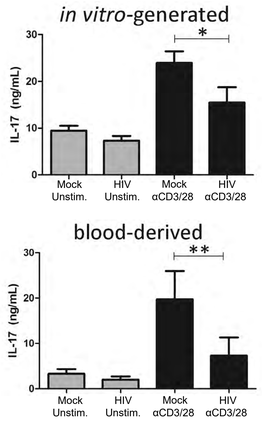
Blood Th17 cells +/- HIV$_{CS204}$ → STAT3 Phosphorylation (Flow cytometry)

4. Expression of the IL-23 receptor chains on blood-derived Th17 cells from HIV-seropositive and HIV-seronegative donors was determined by flow cytometry (IL23R) and Western blot (IL-12Rβ1)

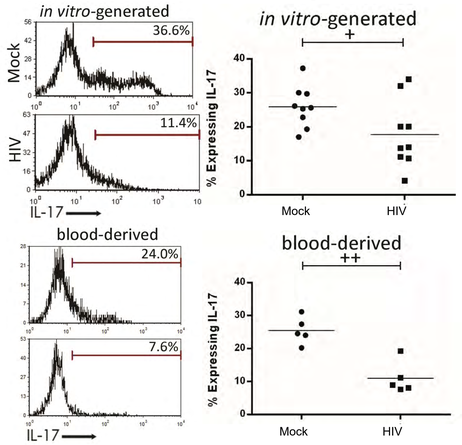
Blood Th17 cells
A. HIV Seronegative
B. HIV Seropositive
→ IL23R Expression (Flow Cytometry); IL-12Rβ1 expression (Western Blot)

Results

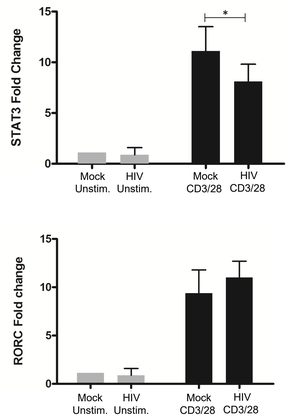
1. HIV Infection reduces IL-17 secretion



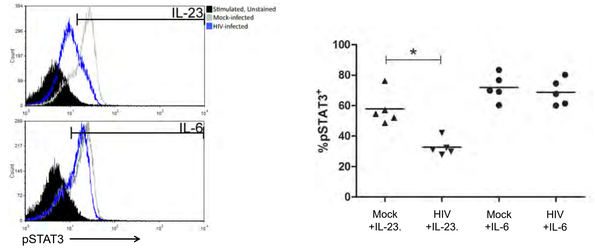
2. HIV Infection reduces IL-17 expression



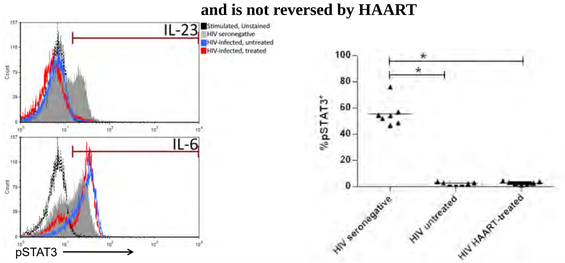
3. HIV Infection reduces STAT3 transcription



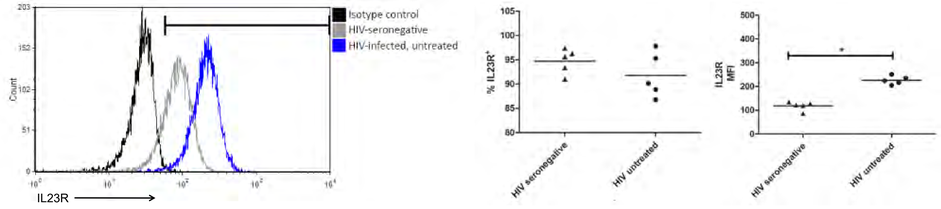
4. *In vitro* HIV Infection inhibits IL-23 signalling by blocking STAT3 phosphorylation



5. *In vivo* HIV Infection inhibits IL-23 signalling by blocking STAT3 phosphorylation and is not reversed by HAART



6. *IL-23 receptor expression is unaffected by in vivo* HIV Infection



Conclusions

1. **Secretion and expression of IL-17 by blood and *in vitro*-derived Th17 cells is significantly inhibited following *in vitro* HIV infection.**
2. **Transcription of STAT3 by Th17 cells is significantly reduced in response to T cell activators following *in vitro* HIV Infection.**
   - Transcription of RORC is unaffected
3. **IL-23-induced STAT3 phosphorylation in blood-derived Th17 cells is significantly inhibited by *in vitro* HIV infection**
4. **IL-23-induced STAT3 phosphorylation in blood-derived Th17 cells is completely inhibited in blood-derived Th17 cells from HIV-seropositive individuals**
5. **Inhibition of IL-23-induced STAT3 phosphorylation by *in vivo* HIV infection is not reversed by HAART**
6. **IL-23 receptor expression is comparable between HIV-seropositive and HIV-seronegative donors**
   - Suggests interference with downstream mediators is involved in mechanisms of IL-23 signalling inhibition

References

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