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Suppression of HIV-1 plasma viral load below detection preserves IL-17 producing T cells in HIV-1 infection

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Abstract

IL-17 is proinflammatory cytokine secreted by a unique CD4+ T (Th_{17}) cell subset and proposed to play a role in host defense. We hypothesized that Th_{17} cells are lost in HIV-1 infection. HIV-1-infected children with plasma viremia below 50 copies/ml had IL-17 production, whereas those with detectable viremia had minimal secretion. These results imply viral-mediated destruction or impairment of Th_{17} cells and argue for complete suppression of viremia for reconstitution of Th_{17} cells.

IL-17 is a proinflammatory cytokine [1], unique in that it is produced by a distinct subset of human CD4+ (Th_{17}) cells [2]. Recent studies [3-9] highlight the influence of IL-17 as a mediator of tissue inflammation in several autoimmune disorders and host defense. IL-17 may have a direct role in Candida or Mycobacterial infections, and a loss of Th_{17} cells could potentially lead to vulnerability to opportunistic infections [10-13]. In this study, we assessed the impact of HIV-1 infection on Th_{17} cells in a cohort of HIV-1-infected children [14].

First, we stimulated peripheral blood mononuclear cells (PBMCs) from healthy subjects with various monoclonal antibodies (mAb) and mitogenic stimuli for detection of IL-17 production *in vitro*. We observed that stimulation with anti-CD3/anti-CD28 mAbs induced IL-17 secretion, primarily by CD4+ T cells (data not shown). In response to anti-CD3/anti-CD28 stimulation, the majority of the Th_{17} cells did not coexpress IFN-γ. In contrast, stimulation with phorbol myristate acetate/ionomycin induced IL-17 secretion from both CD4+ and CD8+ T cells, as well as dual secreting IL-17/IFN-γ T cell subsets (data not shown). These results suggest that anti-CD3/anti-CD28 stimulation is an effective method to

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Authors' contributions: L.C.N., J.M.C., and D.F.N. designed the experiments; L.C.N., J.M.C., A.R.J., J.E.S., and M.P. performed the experiments; F.E.L. and B.S.B. performed confirmatory experiments; M.P., P.J.N., and M.G.R. provided reagents; L.C.N., J.M.C., and A.R.J. analyzed data; P.J.N. and D.F.N. supervised the project and L.C.N. and D.F.N. wrote the article with input from all coauthors.

induce IL-17 secretion from CD4+ T cells *in vitro* and represents a more physiological stimulus than with mitogens.

We next tested for the frequency of Th_{17} cells in PBMCs using an anti-CD3/anti-CD28 stimulation IL-17 enzyme-linked immunosorbent spot assay in 12 HIV-1-infected children with differing levels of plasma viremia and compared this to a group of HIV-1-uninfected subjects who served as controls. The median number of IL-17-specific spot forming units (SFU) in healthy pediatric and adult subjects was 300 SFU/10^6 (range: 85–970 SFU/10^6; n = 12) and 760 SFU/10^6 (range: 110–1370 SFU/10^6; n = 7), respectively (Fig. 1a). A marked reduction in Th_{17} cells was observed in the HIV-1-infected children (median: 62 SFU/10^6; range: 10–270 SFU/10^6; n = 12). Strikingly, the infected children with HIV-1 plasma viral loads exceeding 50 copies/ml had minimal numbers of Th_{17} cells (median: 35 SFU/10^6; range: 10–95 SFU/10^6; n = 7), whereas those with suppressed viral loads had higher levels of Th_{17} cells (median: 170 SFU/10^6; range: 75–270 SFU/10^6; n = 5). Interestingly, all groups had robust IFN-γ secretion (Fig. 1b). Flow cytometry assessment confirmed that CD4+ T cells were the primary source of IL-17 following anti-CD3/anti-CD28 mAb stimulation (data not shown). We carried out a univariate logistic regression analysis to test for an association between HIV-1 plasma viral load and Th_{17} cells. We observed a statistically significant correlation between the frequency of Th_{17} cells and HIV-1 plasma viremia (Fig. 1c). It remains unclear whether the loss of IL-17 production is a cause or an effect of the increasing HIV-1 viremia. Although it is possible that this loss may reflect the depletion of CD4+ T cells, we observed no expression of CCR5 on IL-17 secreting cells (data not shown), suggesting these cells may not be targeted by the virus directly but instead be in a milieu that will not sustain Th_{17} cells differentiation *in vivo*.

In summary, we show that HIV-1-infected children with a plasma viremia below 50 copies/ml had detectable IL-17 production in contrast to those with detectable viremia. These findings are different from a previous report [15] and argue for complete suppression of HIV-1 viral replication to below 50 copies/ml as a strategy for Th_{17} cell preservation.

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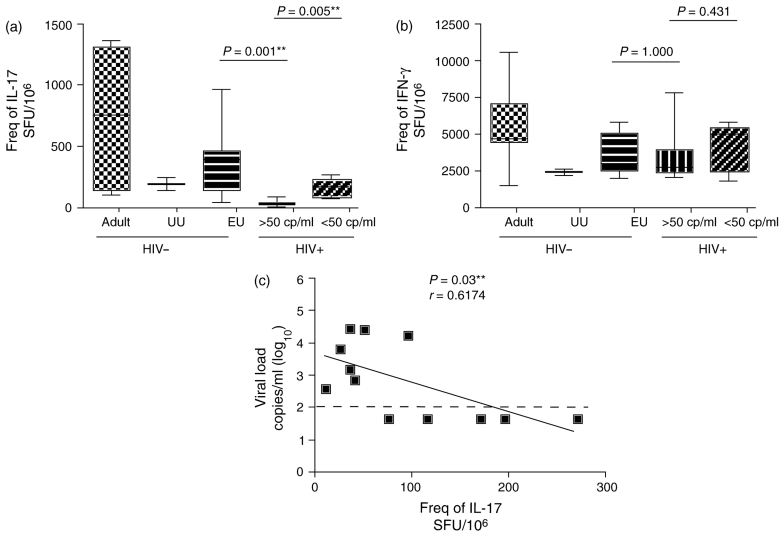

Fig. 1. Detection and comparison of the frequencies of IL-17 and IFN-γ secreting cells in peripheral blood mononuclear cells (PBMCs) derived from HIV-1-infected and uninfected subjects

Graphs depict the frequency of (a) IL-17 and (b) IFN-γ secreting PMBCs in spot forming units (SFU)/10^6 PBMCs from HIV-1-uninfected adults, unexposed uninfected children, exposed but uninfected controls and infected pediatric subjects in response to anti-CD3/anti-CD28 stimulation. HIV-1-infected pediatric subjects were segregated depending on the level of viremia to more than 50 copies/ml or less than 50 copies/ml. The ***P*-value was derived from the Mann–Whitney test and considered statistically significant if the value was less than 0.05. (c) The degree of correlation was assessed by using Spearman's rank correlation coefficient for nonparametric data between HIV-1 plasma viremia and the frequency of IL-17 SFU. EU, exposed uninfected; UU, unexposed uninfected.