HIV-1 Exploits the Interplay between Epithelial Cells and Th17 Cells for Dissemination

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ABSTRACT

Background: Epithelial cells (EC) are the first to capture HIV/SIV at portal sites of entry and transmit the virus to subjacent immune cells *via* transcytosis. The interplay between EC and Th17 cells is critical for the maintenance of immune homeostasis at mucosal level as well as viral dissemination. Th17 cells produce cytokines that act on EC to promote the production of CCL20/MIP-3α, an antimicrobial peptide and chemoattractant for CCR6$^+$ Th17 cells. CCL20 was reported to limit HIV replication in T-cells by inducing APOBEC3G expression. Other studies demonstrated that CCL20 contributes to the establishment of HIV latency in CD4$^+$ T-cells and promotes dissemination from the portal sites of entry. Herein, we investigated the impact of HIV-1 on the interplay between EC and Th17 cells and studied EC-T cell *trans* infection in an environment rich in CCL20.

Methods: Intestinal EC line HT-29 was stimulated with recombinant human TNF-α and/or IL-17A in the presence or absence of the wild type R5 HIV strains NL4.3BaL and transmitted founder THRO. Memory CD4$^+$ T-cells were isolated by magnetic beads from uninfected individuals and stimulated with CD3/CD28 Abs. EC-T cell *trans* infection was studied in co-culture experiments between HIV-exposed EC and activated T-cells. CCL20, CXCL8/IL-8, and HIV-p24 levels were quantified in cell culture supernatants by ELISA. The expression of IL-17 receptor A and C (IL-17RA/RC) on EC and the intracellular expression of HIV-p24 in T-cells were quantified by FACS.

Results: IL-17A acted only in synergy with TNF-α to promote CCL20 but not CXCL8 production in EC. TNF-α treatment and HIV-1 exposure up-regulates IL-17RC expression on EC. HIV exposure further increased CCL20 production by EC in response to IL-17A and TNF-α. Although CCL20 inhibited HIV replication in T-cells cultured alone, HIV *trans* infection of T-cells co-cultured with HIV-loaded EC occurred more robustly in the presence of high levels of CCL20 levels produced by EC in response to IL-17A and TNF-α.

Conclusion: We demonstrate that IL-17A acts in synergy with TNF-α to promote CCL20 production in intestinal EC, HIV further amplifies this synergy, and HIV transmission from EC to T-cells is proportional to the magnitude of CCL20 production by EC. Together these results reveal that HIV-1 exploits the interplay between EC and Th17 cells for its own replicative advantage and that CCL20 plays an important role in HIV dissemination from the portal sites of entry.

RESULTS

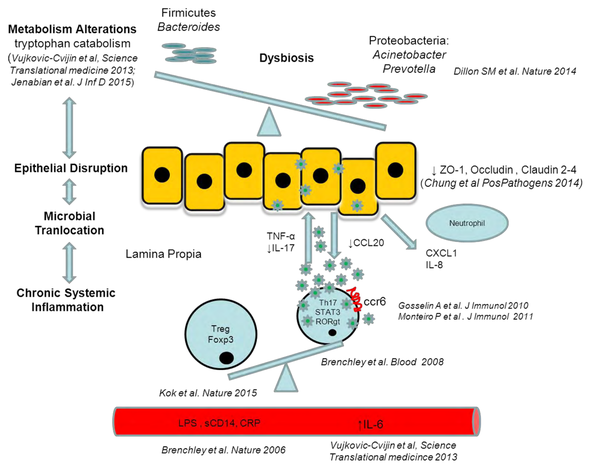


Figure 1: Mucosal immunity alterations at intestinal level during HIV/SIV infection.

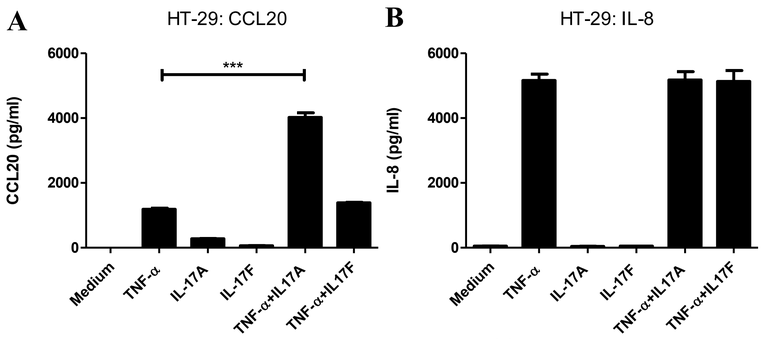


Figure 2: IL-17A acts in synergy with TNF-α to promote CCL20 but not IL-8 production in human intestinal epithelial cells. Intestinal epithelial cells (ECs; HT-29 cell line) were cultured in 48-well plates until 80% confluence. Cells were stimulated with TNF-α (10ng/ml) alone or in combination with IL-17A (10ng/ml) or IL-17F (10ng/ml) at 37°C. Levels of CCL20 **(A)** and IL-8 **(B)** were quantified in cell culture supernatants by ELISA (R&D Systems) at 24 hours post-stimulation.

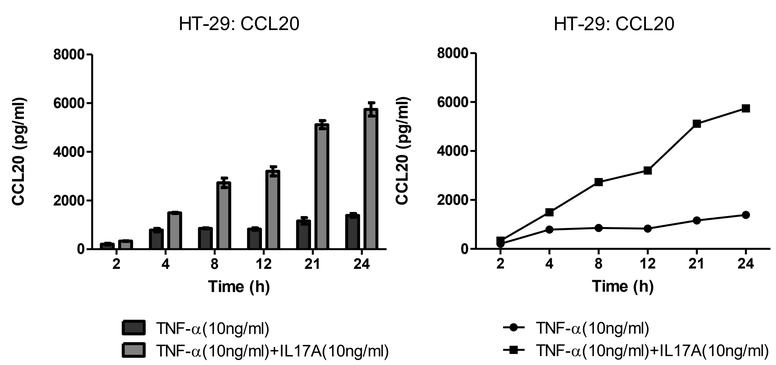


Figure 3: Time course of CCL20 production induces by IL-17A in synergy with TNF-α in human intestinal epithelial cells. The intestinal ECs HT-29 were cultured in 48-well plates until 80% confluence. Cells were stimulated with TNF-α (10ng/ml) alone or in combination with IL-17A (10ng/ml) at 37°C. Levels of CCL20 were quantified in cell culture supernatants by ELISA (R&D Systems) at different hours post-stimulation.

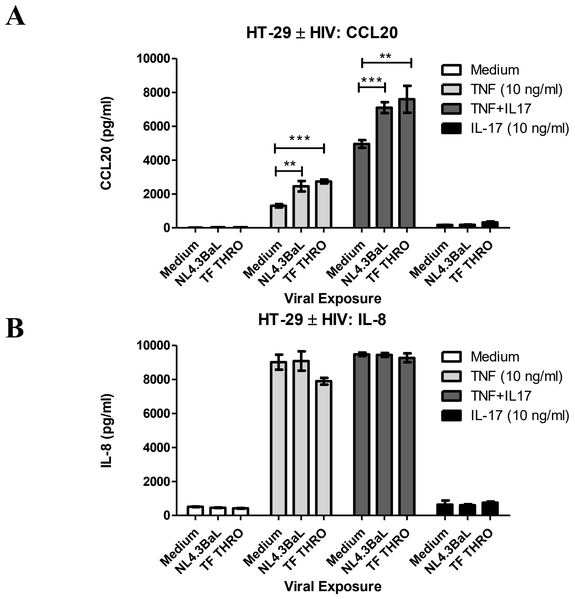


Figure 4: Effect of HIV-1 exposure on CCL20 and IL-8 production by human intestinal epithelial cells. Intestinal ECs HT-29 were cultured in 48-well plates until 80% confluence and pre-treated with TNF-α (10 ng/ml) alone or in combination with IL-17A (10ng/ml) for 3 hours and then co-treated with different HIV-1 strains (NL4.3Bal and T/F THRO) for 24 hours at 37°C. Levels of CCL20 **(A)** and IL-8 **(B)** were quantified in cell culture supernatants by ELISA (R&D Systems)

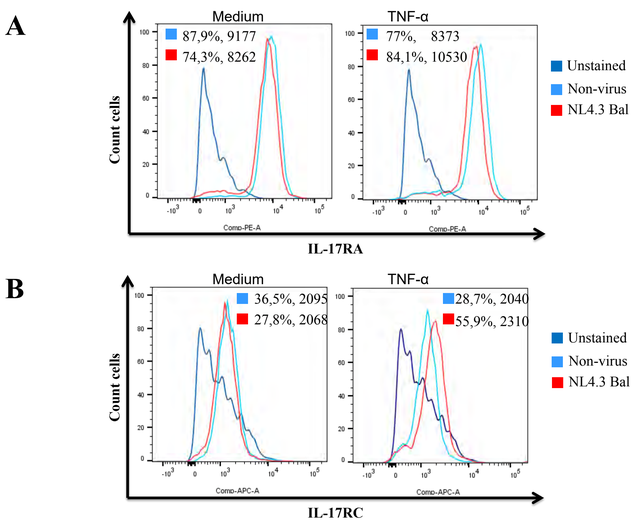


Figure 5: Human intestinal epithelial cells express higher levels of the IL-17 receptor C upon TNF-α stimulation and HIV-1 exposure. Intestinal ECs HT-29 were cultured in 48-well plates until 80% confluence. Then intestinal ECs were treated with TNF-α (10 ng/ml) and exposed to HIV-1 virus NL4.3Bal for 24 hours at 37°C. Cells were detached from the plates using Versene and stained with PE-conjugated anti-IL17 receptor A (IL-17RA) **(A)** and APC-conjugated anti-IL17 receptor C (IL-17RC) **(B)**. The phenotype of the cells was analyzed by Flow Cytometry using BD LSRII cytometer.

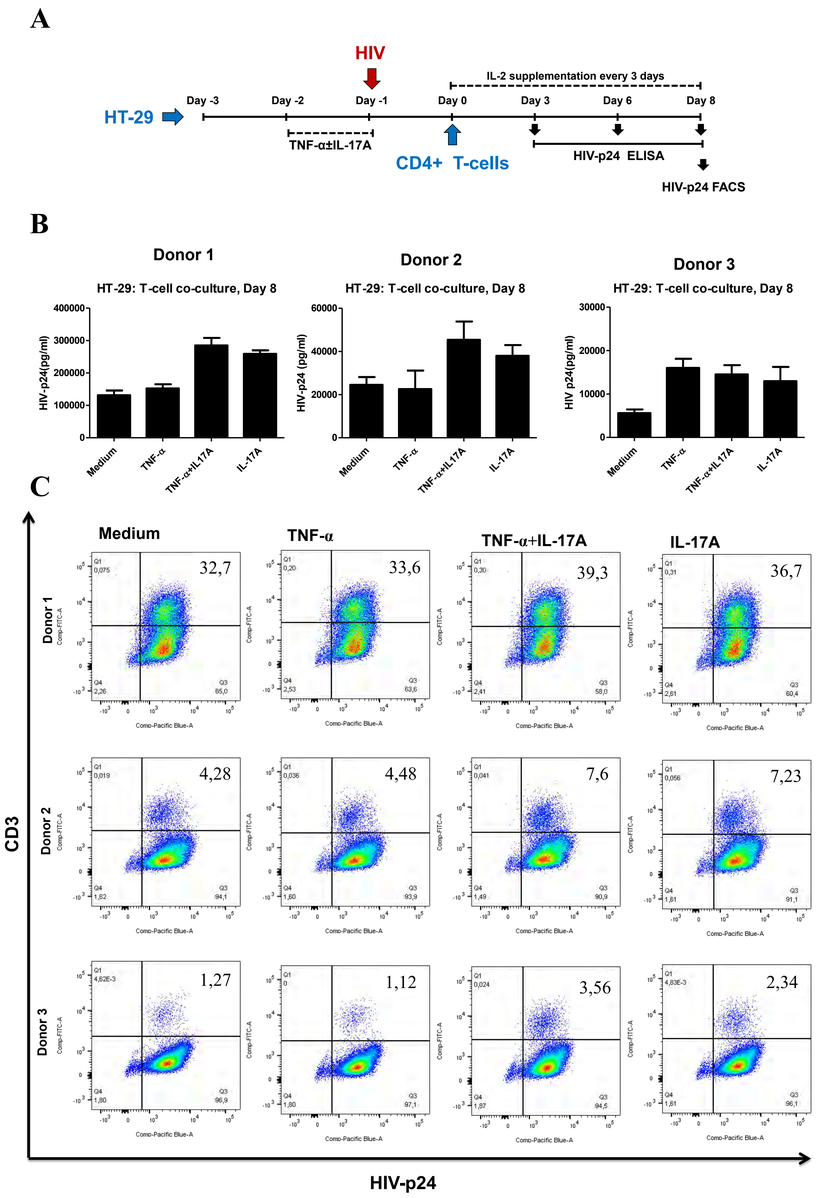


Figure 6: IL-17A in synergy with TNF-α increases the ability of human intestinal epithelial cells to efficiently transmit HIV-1 infection to CD4+ -T-cells. Intestinal ECs HT-29 were cultured in 48-well plates until 80% confluence. ECs were stimulated with TNF-α (10ng/ml) alone or in combination with IL-17A (10ng/ml) for 24 hours at 37°C and then exposed to HIV-1 T/F THRO virus for 3 hours. Unbound HIV was removed by extensive washing and cells were further cultured for 24 hours with medium alone. In parallel, memory CD4+ T-cells were isolated from HIV-uninfected individuals by negative selection using magnetic beads and stimulated with CD3/CD28 Abs for 3 days. **(A)** Cytokine-activated and HIV exposed intestinal ECs were co-cultured with CD3/CD28-activated uninfected memory CD4+ T cells (500 000 cells/well) in the presence of IL-2 (5ng/ml) for 8 days, as described in the flow chart. **(B)** Levels of HIV-p24 were quantified in cell culture supernatants at days 3, 6 and 8 after co-culture by ELISA. **(C)** Intracellular expression of HIV-p24 in T-cells was quantified at day 8 after co-culture upon staining with the following Abs: CD3 Pacific Blue and HIV-p24 FITC. Cell phenotype was analyzed by Flow Cytometry using BD LSRII cytometer.

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