Dysregulated Gut Dendritic Cells Correlate with T Cell Activation During Untreated HIV Infection

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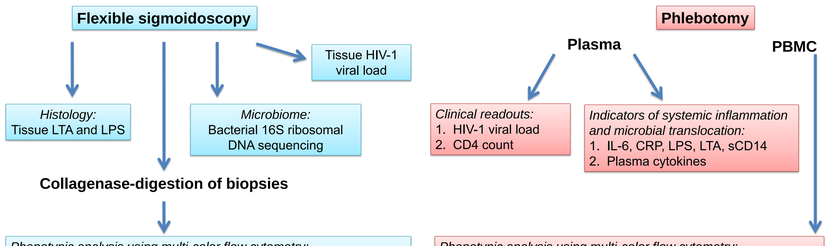
BACKGROUND

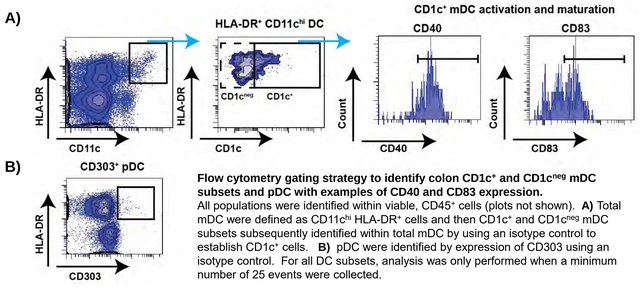
- Intestinal dendritic cells (DCs) serve as gatekeepers between the external (lumen) and internal (lamina propria) environments and are crucial in mediating the delicate balance between immunogenic and tolerogenic intestinal immune responses.
- DCs may play a role in HIV-1-associated mucosal pathogenesis:
  1. Loss of Th17/Treg balance is associated with induction of IDO by intestinal mDCs[1].
  2. Decreased frequencies of intestinal CD103$^+$ regulatory DCs during SIV infection is associated with loss of IL-17- and IL-22-producing T cells[2].
- We have previously shown lymph node DCs from untreated, HIV-1-infected subjects display a dysregulated phenotype characterized by increased CD40 and decreased CD83 expression[3]:
  1. CD40: key role in immune cell activation including T cell activation[4].
  2. CD83: indicative of DC maturation[5]. CD83 homotypic interactions may regulate DC activation and promote mucosal homeostasis[6].

OBJECTIVE

We investigated colon DC frequency and activation during chronic, untreated HIV-1 infection and evaluated associations between DC activation state and virological and immunological parameters.

STUDY DESIGN AND IMMUNOLOGICAL ASSAYS





Flow cytometry gating strategy to identify colon CD1c$^+$ and CD1c^{neg} mDC subsets and pDC with examples of CD40 and CD83 expression. All populations were identified within viable, CD45$^+$ cells (plots not shown). **A)** Total mDC were defined as CD11c^{hi} HLA-DR$^+$ cells and then CD1c$^+$ and CD1c^{neg} mDC subsets subsequently identified within total mDC by using an isotype control to establish CD1c$^+$ cells. **B)** pDC were identified by expression of CD303 using an isotype control. For all DC subsets, analysis was only performed when a minimum number of 25 events were collected.

Table 1. Subject characteristics

	Uninfected subjects	HIV-1-infected subjects
Number of subjects	14	24
Age (years)	31 (23-54)	33.5 (22-58)
Male/Female Ratio	9/5	18/6
CD4 count (cells/μl)	724 (468-1071)	445 (221-1248)*
Plasma HIV-1 viral load (HIV-1 RNA copies/ml)	-	51350 (2880-207000)

Values are shown as median (range). Statistical analysis was performed using the Mann Whitney test for comparisons between uninfected and HIV-1-infected subjects and the Fisher Exact test for comparison of categorical data. *p=0.001.

RESULTS

1 Colon DC Frequencies

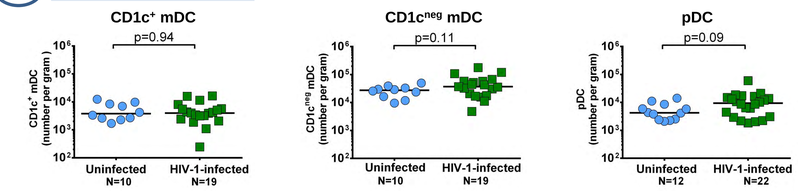


Figure 1. Frequencies of colon DC are not altered during chronic HIV-1 infection. Multi-color flow cytometry was used to enumerate frequencies of colon DC subsets from uninfected and HIV-1 infected subjects. Values are shown as the number of DC per gram of mucosal tissue. Statistical analysis was performed using the Mann Whitney test.

2 Colon DC Activation and Maturation

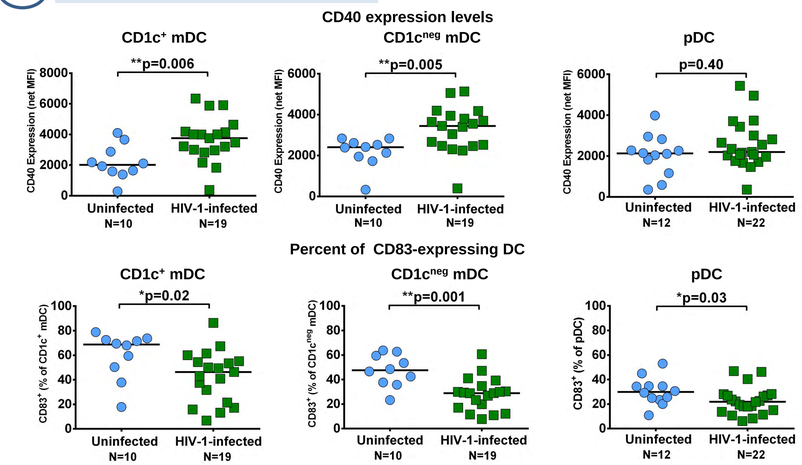


Figure 2. Colon DC from HIV-1-infected subjects have altered activation profiles. Multi-color flow cytometry was used to enumerate expression levels of CD40 (MFI) and the percent of DC expressing CD83 in uninfected and HIV-1 infected subjects. Values are shown with background (isotype) values subtracted (net). Statistical analysis was performed using the Mann Whitney test.

3 CD1c$^+$ mDC activation levels associate with constitutive mucosal cytokine production

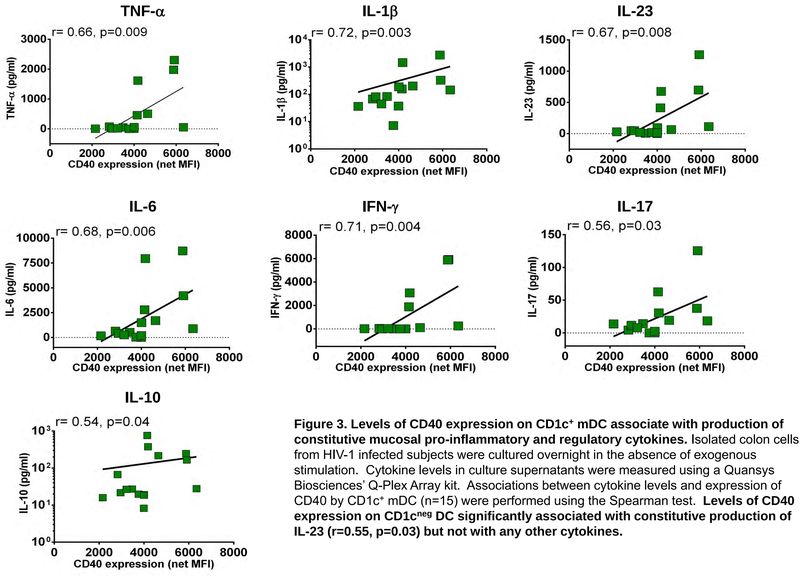


Figure 3. Levels of CD40 expression on CD1c$^+$ mDC associate with production of constitutive mucosal pro-inflammatory and regulatory cytokines. Isolated colon cells from HIV-1 infected subjects were cultured overnight in the absence of exogenous stimulation. Cytokine levels in culture supernatants were measured using a Quansys Biosciences' Q-Plex Array kit. Associations between cytokine levels and expression of CD40 by CD1c$^+$ mDC (n=15) were performed using the Spearman test. **Levels of CD40 expression on CD1c^{neg} DC significantly associated with constitutive production of IL-23 (r=0.55, p=0.03) but not with any other cytokines.**

4 CD1c$^+$ mDC activation levels associate with mucosal HIV-1 viral load

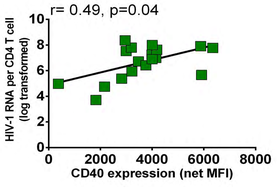


Figure 4. Levels of CD40 expression on CD1c$^+$ mDC associate with mucosal HIV-1 viral load. Total RNA was extracted from pre-weighed colon biopsies from HIV-1-infected subjects and HIV RNA quantified by real-time (RT)-PCR. HIV RNA copy numbers were normalized per CD4 T cell within each biopsy determined by flow cytometry and weight of each biopsy. Associations between mucosal HIV-1 viral load and expression of CD40 by CD1c$^+$ mDC (n=18) were performed using the Spearman test. **Levels of CD40 expression on CD1c^{neg} DC did not associate with mucosal HIV-1 viral load.**

RESULTS cont.

5 CD1c$^+$ mDC activation levels associate with abundance of mucosa-adherent *Prevotella*

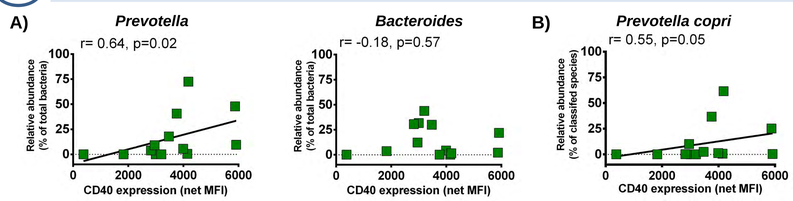


Figure 5. Levels of CD40 expression on CD1c$^+$ mDC associate with the relative abundance of mucosa-adherent *Prevotella*. We have previously reported chronic HIV-1 infection is associated with an altered intestinal microbiome including increased relative abundance of *Prevotella* in conjunction with decreased relative abundance of *Bacteroides* in colon biopsies of HIV-1-infected subjects compared to uninfected subjects[6]. Bacterial profiles of study participants were generated by broad-range amplification and sequence analysis of bacterial 16S rRNA genes. **A)** In HIV-1 infected subjects, CD40 expression levels on CD1c$^+$ mDC (n=13) associated with the relative abundance of *Prevotella*, but not with *Bacteroides*[6]. Associations between bacteria abundance and expression of CD40 by CD1c$^+$ mDC was performed using the Spearman test. **B)** At the species level, the relative abundance of *Prevotella copri* and *P. stercorea* were significantly higher in HIV-1 infected subjects compared to uninfected subjects (Mann Whitney: p=0.01 for both species; data not shown). Levels of CD40 expression on CD1c$^+$ mDC associated with the relative abundance of *P. copri* (statistical analysis performed using the Spearman test). **Levels of CD40 expression on CD1c^{neg} DC did not associate with abundance of *Prevotella*.**

6 CD1c$^+$ mDC activation levels associate with colon T cell activation

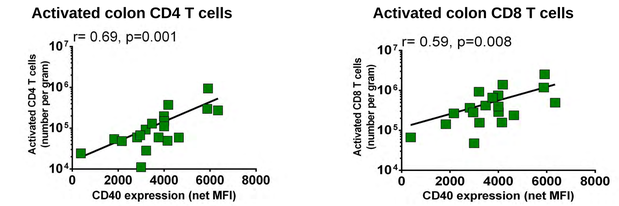


Figure 6. Levels of CD40 expression on CD1c$^+$ mDC associate with mucosal T cell activation. Multi-color flow cytometry was used to enumerate the frequency of CD38$^+$HLA-DR$^+$ colon CD4 and CD8 T cells and are reported as the number of activated CD4 and CD8 T cells per gram of mucosal tissue. Associations between the frequency of activated T cells and expression levels of CD40 by CD1c$^+$ mDC in HIV-1-infected subjects (n=19) were performed using the Spearman test. **Levels of CD40 expression on CD1c^{neg} DC significantly associated with colon CD8 T cell activation (r=0.47, p=0.04).**

7 CD1c$^+$ mDC activation levels associate with peripheral blood T cell activation

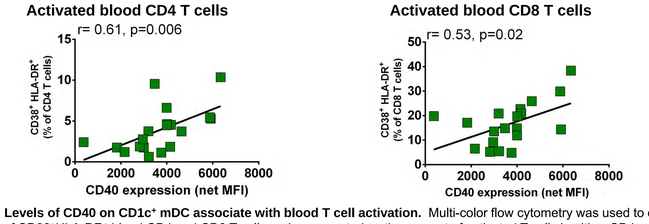


Figure 7. Levels of CD40 on CD1c$^+$ mDC associate with blood T cell activation. Multi-color flow cytometry was used to enumerate the frequency of CD38$^+$HLA-DR$^+$ blood CD4 and CD8 T cells and are reported as the percent of activated T cells in either CD4 or CD8 T cells. Associations between the frequency of activated T cells and expression levels of CD40 by CD1c$^+$ mDC in HIV-1-infected subjects (n=19) were performed using the Spearman test. **Levels of CD40 expression on CD1c^{neg} DC did not associate with blood T cell activation.**

- Minimal associations between the fraction of CD83-expressing CD1c$^+$ mDC, CD1c^{neg} mDC or pDC and virological and immunological parameters were observed.

CONCLUSIONS

- Chronic, untreated HIV-1 infection induces a dysregulated intestinal mDC phenotype characterized by increased levels of CD40 expression, but decreased expression of CD83 ***suggesting a loss of immunoregulation.***
- Levels of CD40 expression on CD1c$^+$ mDC positively associated with:
  1. Constitutive production of mucosal cytokines, mucosal HIV-1 viral load and mucosal T cell activation ***suggesting a role for intestinal mDC in HIV-associated intestinal mucosal pathology.***
  2. Increased abundance of *Prevotella* ***suggesting a link between the HIV-1-associated altered microbiome and mucosal mDC activation.***
  3. Blood CD4 and CD8 T cell activation ***thereby linking colon mDC activation levels to a marker of HIV-1 disease progression.***

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