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IL-13 and IFN-γ Secretion by Activated T Cells in HIV-1 Infection Associated with Viral Suppression and a Lack of Disease Progression

Robert T. Bailer,* Alvy Holloway, † Junwei Sun,* Joseph B. Margolick, ‡ Melissa Martin,* Jay Kostman,† and Luis J. Montaner*‡

The immunopathology of HIV-1 infection includes immune defects in T cell cytokine secretion, resulting in decreased Ag-specific responses. In this report, IL-13 and IFN-γ were analyzed in progressive HIV-1 disease. Both cytokines exert positive effects on Ag presentation and inhibit HIV-1 infection of macrophages. Anti-CD3/anti-CD28-activated PBMC from HIV-1-infected individuals (n = 74) compared with uninfected subjects (n = 30) secreted significantly less IL-13 (median, 0.64 ng/ml vs 2.07 ng/ml; p < 0.001) and IFN-γ (median, 40.96 ng/ml vs 129.5 ng/ml; p < 0.005). Decreased IL-13 and IFN-γ secretion in HIV infection was present in sorted CD4+ and CD8+ T cell subsets, and additional analysis determined concurrent deficiency at the protein and transcriptional level. Longitudinal analysis showed that cytokine secretion levels correlated positively with CD4 count and negatively with plasma HIV-1 viral load. Patients changing to suppressive antiretroviral therapy during the study showed increases in IL-13 and IFN-γ secretion. Overall, results show a decline in IL-13 and IFN-γ secretion in progressive HIV-1 infection and suggest a role for both cytokines as part of T cell adaptive responses associated with a lack of disease progression. The Journal of Immunology, 1999, 162: 7534–7542.

Infection with HIV-1 is characterized by the early onset of T cell dysfunction, ultimately resulting in an inability to protect the host against opportunistic pathogens. Major T cell defects following HIV-1 infection include a higher rate of apoptosis following interaction with APC (1–3) and a decreased secretion of type 1 cytokines. The differential regulation of type 1 and type 2 Th responses and the association between cytokines and immune function have led to multiple studies assessing adaptive immune function in HIV-1 infection by measuring expression and secretion of cytokines such as IFN-γ, IL-2, IL-4, IL-10, and IL-12 (4–11). Decreased secretion of type 1 cytokines such as IL-2 and IFN-γ following mitogen stimulation of T cells in progressive HIV infection is associated with a higher susceptibility to opportunistic infections (4, 9, 12–17). In contrast, the role of T cell-derived type 2 cytokines remains uncertain in spite of their clinical use in combination with antiretroviral therapy as immunotherapy targeted against chronic viral-induced activation and AIDS-associated Kaposi’s sarcoma (18–20).

IL-13, originally described as an IL-4-like type 2 cytokine (21, 22), has received limited attention in studies of HIV-1 pathogenesis in spite of its potential importance to immunotherapy as a cytokine with properties different than those of IL-4 (22, 23). 1) Receptors for IL-13 and IL-4 are differentially expressed on human T cells, B cells, and monocytes. Whereas IL-13 has IL-4-like effects on monocytes and B cells, IL-13 does not regulate T cell function due to a proposed lack of IL-13 receptor expression on T cells (22, 24). By contrast, IL-4 regulates T cell function by differentiating type 2 T cells (25). 2) IL-13 is secreted by Th0, Th1, and Th2 cells, in contrast to the secretion of IL-4 by only Th2 cells (24, 26, 27). 3) Whereas IL-4 and IL-13 are secreted by CD4+ and CD8+ T cell memory (CD45RO+) subsets, IL-13 is also secreted by the T cell-naïve subset (CD45RA+) (26, 28). 4) Calcineurin/calcmodulin pathways leading to nuclear translocation of NF-AT-1 are required for IL-4 induction, whereas IL-13 induction is independent of this pathway (28, 29). Therefore, we have focused our analysis on IL-13, as compared with IFN-γ, based on the lack of IL-13 protein secretion information in HIV-1 pathogenesis and its potential significance to adjunct immunotherapy. Specifically, IL-13 enhances Ag presentation in HIV-1-infected PBMC (21, 30, 31), inhibits macrophage HIV-1 expression (32–35), and primes macrophages for IL-12 secretion (31, 36). To our knowledge, only one cross-sectional analysis has focused on IL-13 mRNA expression as compared with IL-4 mRNA in HIV-1-infected individuals, which indicated increased IL-13 mRNA expression in unstimulated PBMC and lymph node tissue (37). It is important to expand our understanding of IL-13 from message to protein secretion by activated T cells in HIV-1 pathogenesis to indirectly assess its potential as immunotherapy relative to its secretion in disease progression.

A predominance of cytokine secretion studies in HIV-1-infected PBMC utilizing polyclonal cell stimulation via mitogen or chemical induction has limited interpretation of cytokine responses following more physiological stimulation of T cells. Ag-specific T cell activation depends on stimuli provided by APC MHC/B-7 interactions with CD3 and CD28 on T cells (38). In HIV infection, multiple steps in this interaction have been described to affect the
degree of T cell Ag-specific activation such as decreased concentrations of CD4 T cells (1), dysfunctional CD3 complex signal transduction (39), higher susceptibility for APC-mediated apoptosis (3), and predominant decreases in CD28 expression on CD8 T cells (40–43). Although up to 3-fold decreases in CD28 expression are found on CD8 cells of HIV-1-infected individuals at end stage disease, CD28 remains expressed on 68–92% of CD4 T cells, representing a large potentially responsive T cell pool to stimuli provided by APC (40, 43–45). We studied cytokine secretion by T cells from HIV-infected individuals at various stages of disease and viremia levels following stimulation via CD3 and CD28, as a model system of functional cytokine secretion by the pool of T cells able to be activated by this pathway. We compared IL-13 to IFN-γ secretion due to decreased secretion of IFN-γ in progressive HIV infection (9, 12, 16, 17, 46) and to test for differential secretion. These investigations also evaluated cytokine secretion before and after highly active antiretroviral therapy (HAART)3 to directly test the association between IL-13 and IFN-γ secretion and viral suppression.

Materials and Methods

PBMC isolation and clinical data

Venous peripheral blood samples were obtained in citrate-coated tubes following informed consent by The Wistar Institute’s phlebotomy unit (30 HIV donors) and by the Philadelphia Field Initiating Group for HIV Trials (74 HIV-1 donors), and PBMC isolated by Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient centrifugation. Ficoll-isolated cells were cultured in RPMI 1640 (Life Technologies, Grand Island, NY) supplemented with penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) and 10% heat-inactivated male AB human sera (Sigma Chemical, St. Louis, MO). A subset of 52 HIV-1 donors were repeatedly sampled for an average of 3.2 times during a period of 39 wk. Participating patients represented early and late stages of HIV-1 infection as measured by CD4 T cell/m (Fig. 1) and receiving different regimens of antiviral therapy (Table III). Antiretroviral therapy of subjects shown on Figs. 3, 4, and 5 and Table I are summarized by showing the number of reverse transcriptase inhibitors over the number of protease inhibitors (PI) (i.e., “2/1” representing two reverse transcriptase inhibitors and one PI therapy regimen). All HIV-1 patient clinical information was obtained from chart evaluation by study nurses.

Cell stimulation

PBMC (10⁶ cells/ml) or sorted cells (5 × 10⁵ cell/ml), obtained as described below, were stimulated by anti-CD3/anti-CD28 by using plates previously coated overnight at 4°C with anti-CD3 OKT3 (IgG2a) at 5 μg/ml in 0.1 M sodium carbonate buffer (pH 9.5). At the time of stimulation, wells were rinsed with PBS, and cells were resuspended in medium containing 1% (v/v) anti-CD28 ascites CK-248 (IgM) (47, 48). Preliminary experiments indicated that soluble IgM anti-CD28 was twice as effective in stimulating IL-13 and IFN-γ as plate-bound IgG2a anti-CD28 (clone 9.3) when used together with plate-bound OKT3. Supernatants were collected from unactivated and activated cultures at 4 days poststimulation based on preliminary time course analyses on infected and healthy controls demonstrating peak concentration for both cytokines at this time point.

FIGURE 1. HIV-1⁺ PBMC derived from patients with <500 CD4 cells/μl have decreased secretion of IL-13 and IFN-γ. IL-13 (top) and IFN-γ (bottom) secretion from anti-CD3/anti-CD28-stimulated PBMC (1 × 10⁶/ml) from 74 HIV-1-infected and 30 uninfected donors was determined. Supernatants were collected following activation and evaluated for IL-13 and IFN-γ as described in Materials and Methods, reported as cytokine quantity (picograms or nanograms) per 10⁶ PBMC (initial cell density). Results are shown by CD4 T cells/μl grouping for HIV-1 patient data. The group of all HIV⁺ donors (n = 74) includes 4 individuals with unknown CD4 concentrations. Data are presented as box plots representing the 25th–75th interquantile range, the horizontal line (with arrow) the group median, and the dashed horizontal line the group mean. *, groups with p values < 0.05 as compared with uninfected samples based on Mann-Whitney nonparametric analysis. In all cases, the nonstimulated controls contained levels of cytokines below the sensitivity levels of the assays (IL-13, 12 pg/ml; IFN-γ, 2.5 ng/ml).

Abbreviations used in this paper: HAART, highly active antiretroviral therapy; PI, protease inhibitors.
Quantification of cytokines
IL-13, IFN-γ, and IL-5 concentrations were determined by ELISA according to the manufacturer’s recommendations (Biosource International, Camarillo, TX) or by in-house RIA (IFN-γ performed as described (49) (mAbs: coating, B133.1; detection, B133.5) with commercial IFN-γ (Genzyme, Cambridge, MA) standards. All cytokine measurements were based on the average of duplicate samples. The sensitivity of IFN-γ RIA was 2.5 ng/ml, and those of commercial IL-13, IL-5, and IFN-γ ELISA were 12, 6, and 4 pg/ml, respectively.

RNase protection assays
Cellular RNA was isolated by Trizol extraction (Life Technologies), quantitated by spectrophotometer and assessed for degradation by ethidium bromide staining in denaturing gels. RNA samples (10 μg) were analyzed by RNase protection assay (RiboQuant, Pharmingen, San Diego, CA). In brief, RNA samples were hybridized with 32P-labeled riboprobes for IL-13, IL-13, and GAPDH and digested with RNases; protected regions were separated in denaturing polyacrylamide gels, dried, and quantitated on a Phosphorimager (Molecular Dynamics, Sunnyvale, CA).

Flow cytometry analysis
PBMC from uninfected and HIV-1+ donors were stained with 1) biotinylated anti-CD4 Q4120, followed by streptavidin-Red 670 (Sigma), 2) anti-CD8-FITC (Sigma), and 3) anti-CD45RO-PE (Sigma). Stained cells were separated into CD4+, CD8+, CD4+RO+, and CD8+RO+ T cell subsets by sorting on an Epics ELITE flow cytometer (Coulter Immunology, Hialeah, FL). Sorting gates were based on typical lymphocyte light scatter and isotype control fluorescence for the cell populations of interest. Sorted cell subsets were reanalyzed for purity, checked for viability by dye exclusion, and plated at 5 x 10^6 viable cells/ml in 96-well plates for stimulation and analysis as described above.

Statistical analysis
Data sets were tested for normal distributions by the Kolmogorov-Smirnov test. Nonnormally distributed groups were analyzed by the Mann-Whitney U test and Spearman’s ranked order correlation analysis. Assumptions for all statistical tests shown are fulfilled. Significant two-tailed differences were defined at an α level of 0.05 (p < 0.05). Statistical analysis was conducted with StatMost (DataMost, Salt Lake City, UT) and SigmaStat (Jandel, San Francisco, CA) software.

Results
Secretion of IL-13 and IFN-γ declines with HIV disease progression
Secretion of IL-13 and IFN-γ was measured in 30 uninfected controls and 74 HIV-1-infected donors following PBMC stimulation with anti-CD3/anti-CD28. Results demonstrated a significant decrease of IL-13 and IFN-γ secretion in HIV-1-infected patients with <500 T cells/μl as compared with uninfected donors (Fig. 1). However, cytokine secretion from enriched CD4+ T cell subsets from infected patients indicated that decreased secretion was not exclusively due to a decrease in CD4+ cell number (demonstrated below). Although median IFN-γ secretion was higher in patient samples above 500 T cells/μl, this group of responses was not significantly different from those of the uninfected control. IL-13 and IFN-γ secretion were significantly correlated (r = 0.54, p < 0.001) at all stages of disease, suggesting that both cytokine secretions are equally affected. To rule out the presence of a non-specific reduction in cytokine responses from activated HIV-1-infected PBMC, we measured IL-5 secretion in parallel with IL-13 and IFN-γ in a subset of six anti-CD3/anti-CD28 stimulated HIV-1-infected PBMC as compared with three uninfected PBMC. Results showed a lack of reduction for IL-5 in supernatants from HIV-1-infected patient samples in spite of decreased IL-13 and IFN-γ (data not shown). Taken together, these data indicate a decreased secretion of IL-13 and IFN-γ following anti-CD3/anti-CD28 stimulation with progressive HIV disease.

Both CD4 and CD8 T cell subsets from HIV-1-infected individuals secrete less IL-13 and IFN-γ
We determined the level of IL-13 and IFN-γ secretion in enriched CD4+, CD8+, CD4+RO+, and CD8+RO+ T cell subsets following flow cytometric sorting from freshly isolated uninfected (n = 3) and HIV-1-infected (n = 3) PBMC (Fig. 2, Table I). Enriched CD4+ T cell subsets following sorting from both uninfected (2 of 3) and HIV-1 (2 of 3) donors showed the greatest increase in IL-13 secretion as compared with their corresponding intact PBMC responses (Fig. 2, A and B). As expected, in uninfected donors, IL-13 secretion was shown to be a product of both CD4 and CD8 subsets, although primarily secreted from CD4+ and CD4+/CD45RO+ T cell subsets. Interestingly, in spite of an increased IL-13 secretion by HIV-1-infected CD4+ T cell subsets in 2 of 3 donors tested, total secretion levels still remained decreased by 84% in comparison with the same T cell subsets from uninfected donors (Fig. 2C). These data indicate that both total and memory subsets of CD4 and CD8 T cells from HIV-1-infected individuals with <455 CD4 T cells/μl (highest CD4 count tested) are intrinsically impaired in their ability to secrete IL-13 following cell enrichment and stimulation. In contrast to IL-13, IFN-γ secretion was not increased in enriched uninfected donor T cell subsets (data not shown), suggesting a difference between cytokines on the factors (e.g., IL-12) contributing to PBMC-stimulated cytokine secretion. In spite of the decrease in IFN-γ secretion following sorting, all HIV-1-infected subsets secreted lower concentrations of IFN-γ relative to control uninfected donor subsets. Taken together, these results suggest that decreased secretion of IL-13 and IFN-γ in progressive HIV-1-infected PBMC can be attributed in part to a decreased capacity for secretion by CD4 and CD8 T cell subsets in addition to a decrease in total CD4 T cell number.

Table I. Descriptive analysis of patient information for sorted T cell populations

<table>
<thead>
<tr>
<th>Donor</th>
<th>CD4 Cells</th>
<th>CD4 Cells</th>
<th>CD8 Cells</th>
<th>CD8 Cells</th>
<th>CD4 RO Cells</th>
<th>CD4 RO Cells</th>
<th>CD8 RO Cells</th>
<th>CD8 RO Cells</th>
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<th>Antiviral Therapy</th>
<th>CD4 T Cells/μl</th>
<th>HIV Plasma Load</th>
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<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>Uninfected 6837</td>
<td>48.2</td>
<td>99.6</td>
<td>18.5</td>
<td>95.8</td>
<td>25.2</td>
<td>96.7</td>
<td>5.5</td>
<td>87.4</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Uninfected 6840</td>
<td>44.9</td>
<td>97.1</td>
<td>35.8</td>
<td>91.7</td>
<td>16.8</td>
<td>83.2</td>
<td>7</td>
<td>82.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>Uninfected 6845</td>
<td>19.9</td>
<td>99.1</td>
<td>8</td>
<td>83.9</td>
<td>8.5</td>
<td>98.9</td>
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<td>87.2</td>
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<td>NA</td>
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<tr>
<td>HIV-1+ L-21</td>
<td>3.1</td>
<td>91.9</td>
<td>12</td>
<td>90.2</td>
<td>2</td>
<td>ND</td>
<td>5.3</td>
<td>ND</td>
<td>1997</td>
<td>2/1</td>
<td>226</td>
<td>500</td>
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<tr>
<td>HIV-1+ L-23</td>
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<td>90.5</td>
<td>16.2</td>
<td>93.9</td>
<td>3.3</td>
<td>87</td>
<td>4.3</td>
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<td>1987</td>
<td>2/1</td>
<td>25,000</td>
<td></td>
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<tr>
<td>HIV-1+ L-33</td>
<td>17.9</td>
<td>94</td>
<td>46.2</td>
<td>95.7</td>
<td>10.1</td>
<td>84.1</td>
<td>20.8</td>
<td>90.9</td>
<td>1995</td>
<td>2/1</td>
<td>455</td>
<td>25,000</td>
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</table>

* T cells were flow cytometrically sorted into CD4+, CD8+, CD4+RO+, and CD8+RO+ cell subsets. Cytokine secretion by sorted and unsorted populations is shown in Fig. 2. Cell sorting data are given as percentage of lymphocytes expressing the markers before (uns %) and after (sort %) sorting. Clinical information is presented for all patients tested. Antiretroviral therapy is reported as the number of reverse transcriptase inhibitors (RTIs) per number of PI, and viral load was determined on the day of the assay. NA, not applicable.
Reduction of IL-13 and IFN-γ at transcriptional level

To determine the relationship between decreased protein secretion and mRNA induction following T cell activation, IL-13 and IFN-γ mRNA levels were compared between healthy and HIV-1-infected donors at various stages of disease by RNase protection assay (n = 20). Protein secretion for both cytokines was also measured in parallel. Unactivated and anti-CD3/anti-CD28-activated PBMC mRNA were collected at 36 h poststimulation based on peak message levels for IL-13 and IFN-γ by time course analysis of activated uninfected samples (data not shown). RNase protection analysis was also performed on fresh unstimulated PBMC from 8 HIV-1 donors to determine general baseline levels of cytokine message present at the time of PBMC isolation and stimulation. Results showed a lack of message for either cytokine in HIV-1-infected PBMC tested at the time of isolation (Fig. 3) whereas samples following activation showed impaired IL-13 and IFN-γ message induction and protein secretion (Fig. 4). We did observe a greater sensitivity for IFN-γ message vs protein detection in 2 of 20 patients as represented in Fig. 4 by patient 97-476 who induced IFN-γ message in the absence of protein detection by RIA. Longitudinal mRNA and protein secretion from 6 patients supported an association between mRNA induction and protein secretion by showing parallel changes for both measures in sequential samples (data not shown). Overall, these results suggest that HIV-1 infection is associated with a decrease in transcriptional activation of IL-13 and IFN-γ in activated T cells which results in lower protein secretion.

Increased secretion of IL-13 and IFN-γ associated with reduction of viral load

To test the stability of T cell cytokine secretion over time and the association between viral load and cytokine secretion, we measured IL-13 and IFN-γ secretion in longitudinal samples from a subgroup of 52 from the 74 patients in the study. These patients were repeatedly sampled for an average of 3.2 times during a...
39-wk period. Results showed individual cytokine secretion responses were stable in patients showing no changes in CD4 T cell count or plasma viremia. Patient profile 25 in Fig. 5 is representative of a stable response profile showing reproducible IL-13 secretion levels during a 13-wk period. Stable deficiency profiles for both IL-13 and IFN-γ were also observed in association with elevated viral load measurements during the period of study (data not shown). A significant negative correlation between either IFN-γ or IL-13 and viral load was observed on analysis of all cytokine samples associated with viral load measurements in the study (Table II). However, the presence of a positive association between cytokine secretion and CD4 count in this same cross-sectional subgroup could not establish independent effects between viral load and CD4 count in accounting for levels of cytokine secretion.

Viral load in addition to CD4 count as a determinant of IL-13 and IFN-γ secretion was suggested by analysis of patient data according to the amount of therapy they were taking and by the analysis of patients starting de novo suppressive HAART regimens. First, classification of patients by amount of therapy they were taking and by the number of reverse transcriptase inhibitors per number of PI. Not shown are results from unstimulated controls for each donor showing no detectable cytokine mRNA or protein. Supernatants evaluated for IL-13 and IFN-γ as described in Materials and Methods (assay sensitivity levels: IL-13, 12 pg/ml; IFN-γ, 2.5 ng/ml), reported as cytokine quantity (picograms or nanograms) per 10^6 PBMC (initial cell density). nd, not determined.

Increased cytokine secretion was not restricted to de novo suppressive HAART because patients changing HAART regimens due to noncompliance also showed increases in cytokine secretion. Patient 17 in Fig. 5D illustrates these latter patients by showing a change of HAART regimen (wk 3) resulting in better suppression of viremia and an increase in IL-13 and IFN-γ secretion. Taken together, results showed that T cell cytokine secretion of IL-13 and IFN-γ is a stable functional parameter of T cell function in the absence of clinical progression.

Discussion

We report the first study of decreased IL-13 mRNA expression and protein secretion by activated T cells in progressive HIV-1 infection. Although a decrease in multiple cytokines such as IL-2, IFN-γ, and IL-12 are proposed to contribute to disease progression, our data directly addressed secretion of IL-13 based on the lack of any data on its secretion during HIV-1 pathogenesis in spite of its clear potential as an immunotherapeutic (e.g., enhanced Ag presentation (21, 31), inhibition of HIV-1 in vitro infection (32–35, 50, 51), antiinflammatory effects (22), and priming for IL-12 secretion (31, 36)).

In general, a significant decrease of IL-13 and IFN-γ secretion was observed in stimulated PBMC from HIV-1-infected individuals whose CD4 count was <500 CD4 T cells/μl (Fig. 1). However, individual examples of decreased cytokine secretion with higher than 500 CD4 T cells/μl were observed as shown in Fig. 4. In support of a decrease in cytokine secretion due to T cell-specific factors rather than a decrease in total CD4 number alone, IL-13 and IFN-γ remained deficient following activation of enriched CD4, CD8, or respective CD45RO memory subsets as compared with uninfected controls (Table I, Fig. 2). We interpret these results to indicate that in addition to decreasing CD4 T cell number, an inherent deficiency in secretion of IL-13 and IFN-γ from CD4 and CD8 T cell subsets is present in progressive HIV-1 infection.

Potential mechanisms acting to contribute to decrease cytokine expression may include HIV-1 gp120/CD4-mediated alteration of the protein tyrosine kinase fyn and lck pathways involved in CD3-mediated signal transduction (39, 52), alteration of the CD3/TCR...
complex (39), and total CD28 expression (39, 53, 54). In contrast to defects associated with the T cell CD3 complex, CD28 signaling has been suggested to remain functional in HIV infection (55). However, CD28 expression by CD8 T cells subsets in progressive HIV infection has been shown to be preferentially decreased, which may provide a potential mechanism to account for the increased deficiency of IL-13 secretion between activated CD8 vs CD4 T cells subsets from the same HIV-1-infected patient (Fig. 2) (40–43). The presence of multiple viral and host factors determining total levels of T cell activation and cytokine expression is consistent with the variability of cytokine secretion profiles observed between otherwise comparably HIV disease-staged donors. Therefore, as a T cell product from activated CD4 or CD8 type 1 and type 2 T cells (24, 26, 27), the decrease of IL-13 secretion would suggest this cytokine is not expressed at normal levels during immune activation of T cells following interaction with MHC-II and B-7 on APC.

The presence of viral-induced mechanisms affecting the activation of cytokine genes at a step before transcription is consistent with the observation of decreased IL-13 and IFN-γ mRNA in association with decreased protein secretion in HIV-1-infected donors.

Table II. IL-13 and IFN-γ secretion are negatively associated with HIV-1 viral load

<table>
<thead>
<tr>
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<th>IFN-γ</th>
<th>CD4 T Cells/µl</th>
<th>HIV-1 Viral Load</th>
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<tr>
<td><strong>IL-13</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>33</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>r</td>
<td>0.5675</td>
<td>0.4512</td>
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<tr>
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<td>n</td>
<td>33</td>
<td>33</td>
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</tr>
<tr>
<td>r</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
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</tr>
<tr>
<td>r</td>
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<td>0.0161</td>
</tr>
<tr>
<td>p</td>
<td></td>
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<td>0.0161</td>
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*Spearman’s ranked order correlation analysis for independent cytokine secretion samples associated with measurements of HIV-1 mRNA during the study from 33 independent individuals.*
PBMC (Fig. 4). Of interest was our inability to reproduce a previous report of increased message for IL-13 in unstimulated PBMC from eight HIV-1-infected donors (Fig. 3) (37). Although the reasons for difference in results remain uncertain (i.e., sensitivity between gene expression assays, the variability of IL-13 expression by CD4 count or antiretroviral therapy as shown in this study, and sample processing), our study conclusively shows a decrease of IL-13 gene expression and protein secretion following CD3/CD28 T cell activation in progressive HIV infection.

T cell cytokine secretion of IL-13 and IFN-γ appear to be a stable functional parameter in the absence of clinical changes in disease progression (Fig. 5A). By contrast, patients starting or modifying HAART regimens showed changes in cytokine secretion consistent with a negative association between viral load and either IL-13 or IFN-γ secretion (exemplified in Fig. 4, B–D). Increases in IL-13 and IFN-γ secretion in patients who responded to therapy by decreasing their viral load are in concert with previous demonstrations of increased redistribution of circulating CD4 memory cells (CD45RO), recovery of PHA proliferative responses at 1–2 wk (56, 57), and an increase in CD28+ expression as early as 1 wk after initiation of therapy (56) (42). Therefore, increased IL-13 and IFN-γ secretion in these patients may reflect the summation of: 1) functional capacity of redistributed memory (CD45RO+) CD4+ T cells able to secrete both cytokines (26); 2) an increase in expression of CD28; and 3) the absence of viral-induced disturbances to the CD3 signal transduction complex (39, 53, 54). Although we interpret increases in cytokine secretion to be associated with immune reconstitution as a result of viral suppression, we cannot rule out independent effects by HAART on T cell function and cytokine secretion. Interestingly, no significant decrease or increase in IL-13 or IFN-γ secretion from HIV-1-infected PBMC over uninfected sample levels was observed at the onset of suppressive antiviral therapy, indicating a reconstitution of cytokine levels comparable with those measured in uninfected controls.

Contrary to showing IL-13 as a T cell product that is selectively overexpressed in association with HIV-1 disease progression as would be expected according to recent hypotheses on HIV-1 pathogenesis (37, 58), our data would indicate an impairment in the development of T cell-mediated type 2 responses based on the recently described dependent contribution of IL-13 to these responses (59). On the other hand, the role of IL-13 in early immune responses or type 1 immunity remains largely undefined, although it is a secreted product from CD45RA, Th0, and Th1 T cells (24, 26, 28). Overall, we interpret a decrease of IL-13 and IFN-γ secretion from activated T cells in HIV infection to be consistent with the presence of a general state of T cell immunodeficiency following APC-mediated activation affecting both type 1 and type 2 responses. Although our cross-sectional and longitudinal analyses show that T cell secretion of IL-13 and IFN-γ is not associated with immune responses from patients who are viremic or at late stages of HIV-1 disease, additional longitudinal studies would be needed to determine whether changes in cytokine secretion signal a change in disease progression.

The demonstrated effects of both IL-13 and IFN-γ to have direct antiviral effects on HIV-1 macrophage infections (32–35, 50, 51), to enhance Ag presenting function (21, 31), and to be associated with improved disease status (increased CD4 or decreased HIV viral load) suggest a contribution by these cytokines in maintaining immune function following HIV-1 infection. It remains to be tested whether IL-13 or IFN-γ may be beneficial as adjunct immunotherapy with antiviral chemotheraphy due to their association with a lack of disease progression and their proposed effects on viral and immune regulation.

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