IFN-α Exerts Opposing Effects on Activation-Induced and IL-7–Induced Proliferation of T Cells That May Impair Homeostatic Maintenance of CD4+ T Cell Numbers in Treated HIV Infection

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IFN-α Exerts Opposing Effects on Activation-Induced and IL-7–Induced Proliferation of T Cells That May Impair Homeostatic Maintenance of CD4+ T Cell Numbers in Treated HIV Infection

Lilian Cha,* Emma de Jong,* Martyn A. French,*† and Sonia Fernandez*

To determine whether IFN-α is a cause of the T cell hyperactivation and IL-7 signaling pathway defects that are observed in some HIV patients receiving antiretroviral therapy, we have investigated the effect of IFN-α on the proliferation of CD4+ and CD8+ T cells from healthy donors (n = 30) and treated HIV+ donors (n = 20). PBMC were cultured for 7 d with staphylococcal enterotoxin B or IL-7 in the absence or presence of 100 U/ml IFN-α8. Total and naive CD4+ and CD8+ T cells were assessed for proliferation (via Ki67 expression), CD127 expression, and phosphorylated STAT5 levels using flow cytometry. IFN-α significantly enhanced activation-induced proliferation (via staphylococcal enterotoxin B stimulation) but inhibited homeostatic proliferation (IL-7 induced) of CD4+ and CD8+ T cells. Both of these effects may adversely affect CD4+ T cell homeostasis in HIV patients. CD127 expression was increased in both healthy and HIV+ donors following culture with IFN-α8, and levels of IL-7–induced phosphorylated STAT5 were increased by IFN-α8 in healthy donors only. Hence, the inhibitory effects of IFN-α on IL-7–induced proliferation of CD4+ T cells are unlikely to be mediated by downregulation of CD127 expression or inhibition of STAT5 phosphorylation. These data suggest that increased IFN-α activity may promote the loss of T cells by accelerating cell turnover and activation-induced cell death while decreasing the renewal of T cells by inhibiting the proliferative effect of IL-7. The Journal of Immunology, 2014, 193: 2178–2186.

B etween 5 and 56% of HIV-infected individuals do not achieve a normal blood CD4+ T cell count (> 500 μL) after a mean time of 7.5 y on antiretroviral therapy (ART) (1). Persistent depletion of CD4+ T cells in lymphoid tissue, such as gut-associated lymphoid tissue, is even more pronounced than in blood (2). CD4+ T cell deficiency on ART is associated with a higher rate of mortality and morbidity (3, 4), which includes atherosclerotic vascular disease (3, 5), cardiovascular disease (4), osteoporosis and fractures (6), and non-AIDS-defining cancer (4, 7, 8).

Recovery of CD4+ T cell numbers during ART is dependent on de novo production of naive CD4+ T cells from the thymus as well as homeostatic proliferation of the naive CD4+ T cell pool. The latter process is regulated by IL-7 and its receptor via the Jak/STAT signaling pathway. The IL-7R complex is a heterodimer of an IL-7R α-chain (CD127) and the γ-chain that is common to the receptor for other γc cytokines (CD132). Defects of T cell proliferation persist in HIV patients receiving ART and are associated with T cell hyperactivation and impaired IL-7–mediated homeostasis as a result of defects of the CD127 signaling pathway. Decreased signaling through CD127 is in part a consequence of reduced CD127 expression on both CD8+ and CD4+ T cells, but postreceptor signaling pathways are also defective (9). Hyperactivation of CD4+ T cells in patients with treated HIV infection is associated with increased basal levels of the TCR signaling molecules phospho-ZAP70, -ERK, and -JNK, which appear to result in a hyporesponsiveness to signaling through the TCR (10).

Persistent CD4+ T cell depletion in HIV patients receiving ART that suppresses HIV replication is closely associated with persistent immune activation (11). However, the underlying pathogenic mechanisms are still a topic of debate. Three potential causes of immune activation have been identified, as follows: 1) increased translocation of microbial products across the gut wall (12), 2) CMV coinfection (13, 14), and 3) residual low-level HIV replication, identified in studies of ART intensification therapy with HIV integrase inhibitors (15, 16). Irrespective of whether one or more of these factors cause persistent immune activation, we and others have demonstrated that elevated expression of IFN-stimulated genes (ISG) in separated CD4+ T cells (17), separated accessory cells (18), or PBMC (19) is the strongest correlate of persistent CD4+ T cell deficiency in treated HIV patients. Increased IFN-α activity may therefore be a central component of the pathogenic mechanisms that result in poor CD4+ T cell recovery in HIV patients receiving ART.

Increased IFN-α activity (20–22) and elevated production of IFN-α in PBMC and plasmacytid dendritic cell cultures is a characteristic of HIV infection (23) and is associated with both CD4+ T cell depletion and disease progression (21). IFN-α is an essential mediator of the innate immune response, and its ex-
pression is rapidly upregulated in response to viral pathogens, reflecting its potent antiviral activity. There are 12 functional subtypes of IFN-α, which form part of a large family of type I IFNs. All of the IFN-α subtypes are structurally similar and bind to a common receptor. Activation of the IFN-α receptor triggers a signaling cascade that initially includes the tyrosine kinases, Tyk2 and Jak1, and eventually results in activation of transcription factor complexes, such as IFN-stimulated gene factor 3 and phosphorylation and dimerization of STAT molecules, which translocate to the cell nucleus and regulate the expression of numerous ISG. Untreated HIV infection is associated with increased expression of mRNA of several ISG in monocytes (24) and T cells (20). IFN-α displays contrasting proliferation-inducing and proapoptotic properties (25). At low concentrations, IFN-α enhances cell proliferation. Conversely, at high concentrations it acts in an anti-proliferative fashion and may have detrimental effects on T cell development, maturation, and homeostasis. These actions may in part be mediated by an effect of IFN-α on IL-7 and the IL-7R as in studies assessing thymocyte differentiation and maturation, IFN-α impaired IL-7-mediated proliferation, downregulated CD127 expression, and reduced overall cell numbers in the thymus (26, 27).

IL-7 is produced by stromal cells in the bone marrow and lymphoid tissue and plays a critical role in T cell homeostasis (28). Serum levels of IL-7 are increased in HIV infection (29) and decrease after ART is commenced, which has been attributed to increased numbers of CD127⁺ T cells and receptor clearance of IL-7 (30). It is also possible that immune activation in HIV infection affects the production of IL-7 by stromal cells because cytokines potently affect the production of IL-7 from bone marrow stromal cells with IFN-γ increasing production and IL-1β reducing production (31). The effect of IFN-α on IL-7 production by stromal cells is unknown.

We hypothesized that IFN-α plays a key role in the immune activation and impaired recovery of CD4⁺ T cells that occur in a proportion of otherwise successfully treated HIV patients, by impairing homeostatic proliferation of CD4⁺ T cells and promoting accelerated T cell turnover. We have explored this by examining the effect of IFN-α on key pathways involved in CD4⁺ T cell homeostasis in both HIV patients and individuals who are not infected by HIV. We have also examined the effect of IFN-α on IL-7 production by bone marrow stromal cells.

Materials and Methods

Study groups and sample collection

Thirty healthy donors and 20 HIV-positive donors were studied. Healthy donors were categorized according to age into those <30 y (younger donors; n = 15) and >30 y (older donors; n = 15). HIV-positive donors had undergone ART for at least 12 mo, with stable suppression of viral replication (<50 copies/ml plasma HIV RNA) for >8 mo. Patients were selected on the basis of a history of extreme immunodeficiency (nadir CD4⁺ T cell counts of <40 cells/μL blood) prior to ART. A wide range of current CD4⁺ T cell counts was represented. Demographic data are displayed in Table I. This study was approved by the Human Research Ethics Committees of Royal Perth Hospital and the University of Western Australia. Informed consent was obtained from all subjects.

PBMC cultures and detection of proliferating cells and CD127 expression

Cryopreserved PBMC were thawed, washed, and resuspended at 10⁶ cells/ml in culture medium (RPMI 1640 supplemented with 10% FCS, 1% penicillin/streptomycin solution, and 2 mM l-glutamine). PBMC were seeded in 24-well plates at 10⁵ cells/well and stimulated with 1 μg/ml staphylococcal enterotoxin B (SEB; Sigma-Aldrich, Sydney, NSW, Australia) or 5 ng/ml IL-7 (R&D Systems, Minneapolis, MN) either alone or in combination with 100 U/ml IFN-α8 (PBL IFN Source, Piscataway, NJ). PBMC that were cultured without stimuli or in the presence of 100 U/ml IFN-α8 only were included as negative controls. Plates were incubated at 37°C/5% CO₂ for 7 d. PBMC were washed once in cold flow buffer (1% BSA in PBS) and then stained for surface (15 min) and intracellular (30 min) markers using the Human FoxP3 Buffer Set (BD Biosciences, San Jose, CA), according to the manufacturer’s instructions. This permeabilization buffer set was selected as it is optimal for the detection of nuclear Ags such as Ki67. The following fluorochrome-conjugated mAbs were used: CD3-V450 (clone UCHT1), CD4-APC-H7 (SK3), CD8-V500 (RPA-T8), CD45RA-allophycocyanin (H100), CCR7-PECy7 (3D12), CD127-PerCP-Cy5.5 (hIL-7R-M21), and Ki67-PE (B56) from BD Biosciences. After staining, PBMC were washed and resuspended in cold flow buffer for acquisition.

In a subset of 10 healthy donors, the proliferation of T cells was also assessed via CFSE labeling. Briefly, cryopreserved PBMC were thawed, washed, resuspended at 10⁶ cells/ml in warm PBS/0.1% BSA, and incubated with 10 μM CFSE (Molecular Probes) for 10 min. CFSE staining was quenched by the addition of 5 vol of ice-cold culture media, and cells were pelleted by centrifugation. Cells were washed an additional two times with ice-cold culture media before being resuspended at 10⁶ cells/ml in warm culture medium and stimulated, as described above. After 7 d of culture, cells were stained with the same fluorochrome-conjugated mAbs described above.

Assessment of STAT5 phosphorylation

Cryopreserved PBMC were thawed, washed, and resuspended in culture medium at 10⁶ cells/ml and incubated alone or with 100 U/ml IFN-α8 for 2 h at 37°C/5% CO₂. PBMC were pelleted and resuspended in 100 μL sterile flow buffer and stained with the following fluorochrome-conjugated mAbs: CD3-V450 (UCHT1), CD4-V500 (RPA-T4), CD8-PE (RPA-T8), and CD27-FITC (L128) for 15 min. PBMC were washed with flow buffer and resuspended in 1 mL culture media containing 5 ng/ml IL-7 for 15 min at 37°C/5% CO₂ before fixation and permeabilization using the Cytofix Fixation Buffer and Phosphoryl Perm Buffer III (BD Biosciences), according to the manufacturer’s instructions. After washing, PBMC were stained for CD45RA-APC-H7 (H1100) and phosphorylated STAT5 (pSTAT5)-AF647 (47) for 15 min, washed twice, and resuspended in cold flow buffer for acquisition.

Flow cytometry acquisition and analysis

Data were acquired on a FACSCanto II using Diva software (BD Biosciences). For the 7-d stimulation cultures, acquisition-stopping gates were set at 50,000 CD4⁺ T cell events defined by coexpression of CD3 and CD4 markers. The acquisition settings for the STAT5 phosphorylation protocol were fixed at 250,000 lymphocyte events defined by forward and side scatter measurements. Data files were visualized using FlowJo software (v7.6; Tree Star).

Quantification of IL-7 production by HS-27A cells

The HS-27A stromal cell line was obtained from the American Type Culture Collection and propagated in RPMI 1640 media supplemented with 10% FCS. To determine whether IFN-α modified IL-7 production by the cell line, HS-27A cells were seeded in 96-well plates at 2 × 10⁵ cells/well alone or in the presence of IFN-α8 at 1, 10, 100, or 1000 U/ml. All cultures were performed in triplicate. Supernatants were collected after 6, 24, and 48 h of incubation and stored at ~80°C. IL-7 levels in supernatants were measured using a high-sensitivity commercial ELISA (Quantikine HS Human IL-7 Immunoassay; R&D Systems), according to the manufacturer’s instructions. OD measurements were determined immediately using a Bio-Rad microplate reader set to 490 nm with wavelength correction set to 650 nm. The assay had a lower detection threshold of 0.25 pg/ml.

Statistical analysis

Statistical analyses were performed using Prism 5 (GraphPad Software, La Jolla, CA). Nonparametric tests were conducted to determine statistical significances. Wilcoxon matched pairs tests were used to compare results within healthy donor and patient groups, and Mann–Whitney tests were used to compare results between donor and patient groups. Correlation coefficients were calculated with Spearman’s tests. The p values <0.05 were considered to be statistically significant.

Results

SEB- and IL-7-dependent proliferation of T cells is negatively influenced by older age and HIV⁺ status

To determine the effects of IFN-α on T cell proliferation induced by activation via the TCR or on homeostatic proliferation induced by IL-7, we cultured PBMC with SEB or IL-7 and determined the
effect of IFN-α on proliferation by adding IFN-α8 to the cultures. IFN-α8 was chosen following a series of experiments comparing the effects of IFN-α2b, IFN-α8, and IFN-α10, which demonstrated that all three subtypes exhibited similar levels of anti-proliferative activity (Supplemental Fig. 1).

SEB and IL-7 induced substantial proliferation of total and naive CD4+ and CD8+ T cells in PBMC of younger, older, and HIV-positive donors (Fig. 1A–D). Overall, naive T cells were less responsive to stimulation than the total T cell population, although naive CD8+ T cells were highly responsive to IL-7. SEB was more effective at inducing proliferation than IL-7 within all T cell populations assessed, with the exception of naive CD8+ T cells, in which the reverse was observed. Proliferation was not observed in unstimulated cell cultures or those stimulated with IFN-α8 alone.

When assessing the proliferative potential of the total CD4+ T cell pool, both younger and older healthy donors were more responsive to SEB than HIV+ donors (Fig. 1A; \(p = 0.02\) and \(p = 0.0002\), respectively). Younger healthy donors also had a greater

![Figure 1](http://www.jimmunol.org/Downloadedfrom/http://www.jimmunol.org/)

**Table 1.** Demographic characteristics of HIV patients and healthy donors

<table>
<thead>
<tr>
<th></th>
<th>Younger</th>
<th>Older</th>
<th>HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>Age (years)</td>
<td>24 (20–28) (^a)</td>
<td>58 (50–73)</td>
<td>43 (33–67)</td>
</tr>
<tr>
<td>Male/Female</td>
<td>7/8</td>
<td>13/2</td>
<td>19/1</td>
</tr>
<tr>
<td>Current CD4 count (cells/μL)</td>
<td>N/A</td>
<td>N/A</td>
<td>470 (72–1334)</td>
</tr>
<tr>
<td>Nadir CD4 count (cells/μL)</td>
<td>N/A</td>
<td>N/A</td>
<td>11 (0–56)</td>
</tr>
<tr>
<td>Viral load (copies/ml)</td>
<td>N/A</td>
<td>N/A</td>
<td>&lt;50</td>
</tr>
<tr>
<td>Months on ART</td>
<td>N/A</td>
<td>N/A</td>
<td>66 (19–178)</td>
</tr>
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\(^a\)Data presented as median (range).
proliferative response to IL-7 than HIV+ donors (Fig. 1A; \( p = 0.02 \)). In the naive CD4+ T cell subset, younger healthy donors had a significantly greater proliferative response to IL-7 than older healthy donors (Fig. 1B; \( p = 0.004 \)), but no other differences between donor groups was observed.

After stimulation with SEB, a significantly greater proportion of proliferating cells was observed in the total CD8+ T cell pool in both younger and older healthy donors compared with HIV+ donors (Fig. 1C; \( p = 0.001 \) for both). IL-7–induced proliferation was also significantly higher in CD8+ T cells of younger healthy donors than HIV+ donors (\( p = 0.0007 \)). Within the naive CD8+ T cell pool, older healthy donors had a greater proliferative response to SEB than HIV+ donors (Fig. 1D; \( p = 0.02 \)), and younger healthy donors had a greater proliferative response to IL-7 than HIV+ donors (\( p = 0.03 \)).

**IFN-α8 enhanced SEB-dependent proliferation but impaired IL-7–dependent proliferation of T cells in all donor groups**

To compare the effects of IFN-α on T cell proliferation between donor groups, the proportion of proliferating cells was observed following addition of IFN-α8 to either SEB- or IL-7–stimulated cells was subtracted from the proportion of proliferating cells observed after culture with either SEB or IL-7 alone, and the difference was plotted (Fig. 1E–H). The majority of the SEB-representative plots lie in the positive region (demonstrating an overall increase), whereas the majority of the IL-7–representative plots lie in the negative region (demonstrating an overall decrease).

IFN-α8 significantly enhanced SEB-induced proliferation of total CD4+ T cells (Fig. 1E) in the younger healthy donors (\( p = 0.0001 \)), older healthy donors (\( p = 0.0007 \)), and HIV+ donors (\( p = 0.003 \)). The degree to which SEB-induced proliferation was increased by IFN-α8 was similar in all three donor groups. IFN-α8 significantly inhibited IL-7–induced proliferation of total CD4+ T cells (Fig. 1E) in each of the donor groups (\( p < 0.0001, \) \( p = 0.0003 \), and \( p = 0.0002 \), respectively). The degree of inhibition was greater in younger healthy donors than HIV+ donors (median of inhibition = 13.2% versus 4.2%, respectively, \( p = 0.004 \)).

Within the naive CD4+ T cell subset (Fig. 1F), IFN-α8 significantly enhanced SEB-induced proliferation in both healthy donor groups (\( p = 0.01 \) and \( p = 0.001 \), respectively), but not HIV+ donors (\( p = 0.9 \)). In fact, the effect of IFN-α upon SEB-induced proliferation of naive CD4+ T cells was moderately inhibitory in HIV patients and was significantly lower than that observed in the younger and older healthy donors (\( p = 0.007 \) and \( p = 0.0005 \), respectively). Inhibition of IL-7–induced proliferation of naive CD4+ T cells by IFN-α8 was observed in all donor groups (\( p < 0.0001, \) \( p = 0.03 \), and \( p = 0.01 \), respectively) and was significantly greater in the younger healthy donors compared with both the older healthy donors and HIV+ donors, which showed a much wider range of effects (\( p = 0.002 \) and \( p = 0.02 \), respectively).

IFN-α8 enhanced SEB-induced proliferation of total CD8+ T cells in all donor groups (Fig. 1G), but the effect was only significant in the younger healthy (\( p < 0.0001 \)) and HIV+ donors (\( p = 0.0004 \)). The enhancement was greater in younger healthy donors compared with both older healthy and HIV+ donors (\( p = 0.02 \) for both). In contrast, IFN-α8 significantly inhibited IL-7–induced proliferation of total CD8+ T cells in all donor groups (\( p = 0.0002, \) \( p = 0.0001 \), and \( p = 0.003 \), respectively), and the degree of inhibition did not differ between groups.

Within the naive CD8+ T cell subset (Fig. 1H), IFN-α8 significantly enhanced SEB-induced proliferation (\( p = 0.008, \) \( p = 0.008 \), and \( p = 0.0006 \), respectively) and inhibited IL-7–induced proliferation (\( p = 0.008, \) \( p < 0.02 \), \( p = 0.008 \)). The degree of enhancement or inhibition of proliferation was equivalent in all donor groups.

In summary, the addition of IFN-α8 to PBMC cultures stimulated with SEB enhanced the observed proliferation in all T cell subsets across all donor groups. In contrast, the addition of IFN-α8 to PBMC cultures stimulated with IL-7 inhibited the observed proliferation in all T cell subsets across all donor groups. In a subset of 10 healthy donors, the effect of IFN-α8 on SEB- and IL-7–induced proliferation was assessed using both Ki67 and CFSE staining as indicators of proliferation. The results observed with CFSE were equivalent to those observed with Ki67 (Fig. 2). Representative flow cytometry scatter plots from one donor comparing the proliferation observed with Ki67 or CFSE under all conditions of stimulation are shown in Fig. 3.

**Culture of PBMC with IFN-α8 upregulated CD127 expression by T cells**

Because IFN-α8 generally reduced IL-7–induced proliferation of T cells, we examined the effect of IFN-α8 on the expression of CD127, the α-chain of the IL-7R. The mean fluorescence intensity (MFI) of CD127 expression on CD4+ T cells was significantly upregulated in the younger healthy (\( p = 0.02 \)), older healthy (\( p < 0.0001 \)), and HIV+ (\( p = 0.005 \)) donor groups after 7 d of culture with IFN-α8 (Fig. 4A). A similar upregulation of CD127 expression was observed after culture with IL-7 alone for 7 d in the younger and older healthy donors but not the HIV+ donors (\( p = 0.04 \) and \( p = 0.004 \), respectively).

**FIGURE 2.** The change in SEB- or IL-7–induced proliferation of CD4+, naive CD4+, CD8+, or naive CD8+ T cells with IFN-α8 was assessed using both Ki67 and CFSE as indicators of proliferation in 10 healthy donors. IFN-α induced an upregulation of SEB-induced proliferation (A) but inhibited IL-7–induced proliferation (B), regardless of whether Ki67 (results shown in gray) or CFSE (results shown in white) staining was used. A positive value indicates an increase in proliferation, whereas a negative value indicates a decrease in proliferation.
When naive CD4+ T cells were assessed (Fig. 4B), CD127 expression was not altered by culture with IFN-α8 in either of the healthy donor groups, but an upregulation of CD127 was observed in the HIV+ donors (p = 0.002). Culture with IL-7 alone did not influence CD127 expression in any donor group.

The MFI of CD127 expression on CD8+ T cells was significantly upregulated in the younger healthy (p = 0.01), older healthy (p = 0.05), and HIV+ (p = 0.0002) donor groups after 7 d of culture with IFN-α8 (Fig. 4C). A similar upregulation of CD127 expression was observed after culture with IL-7 alone for 7 d in the younger and older healthy donors only (p = 0.008 and p = 0.007, respectively).

The MFI of CD127 expression on naive CD8+ T cells was significantly upregulated in the younger healthy (p = 0.007), older healthy (p = 0.0002), and HIV+ (p = 0.0001) donor groups after 7 d of culture with IFN-α8 (Fig. 4D). Upregulation of CD127 expression was also observed after culture with IL-7 in the younger and older healthy donors (p = 0.02 and p = 0.01, respectively).

**FIGURE 3.** Representative flow cytometry scatter plots demonstrating the proliferation observed using Ki67 (top row) or CFSE (bottom row) staining in one donor. From left to right, plots for unstimulated cells, SEB-stimulated cells, SEB-stimulated cells + IFN-α8, IL-7–stimulated cells, and IL-7–stimulated cells + IFN-α8 are shown. The proportion of proliferating CD4+ T cells is indicated in each plot.

**FIGURE 4.** IFN-α8 caused an upregulation of CD127 expression in healthy and HIV+ donors. PBMC were cultured alone (unstim) or with 100 U/ml IFN-α8 or 5 ng/ml IL-7, and the MFI of CD127 expression was plotted. IFN-α8 induced an upregulation of CD127 expression in CD4+ (A), naive CD4+ (B), CD8+ (C), and naive CD8+ (D) T cells.
In summary, culture of PBMC in the presence of IFN-α8 for 7 d led to either upregulated or static CD127 expression in comparison with PBMC that were cultured alone for 7 d.

**IFN-α8 enhanced IL-7–induced pSTAT5 in all T cell populations of healthy donors**

To determine the effect of IFN-α on phosphorylation of STAT5, PBMC were pre-exposed to IFN-α8 for 2 h prior to assessment of IL-7–induced pSTAT5 expression. Overall, the frequency of pSTAT5+ CD4+ T cells, naive CD4+ T cells, and naive CD8+ T cells after stimulation with IL-7 was significantly higher in HIV+ donors (Fig. 5). This was also observed in cells pretreated with IFN-α8. In healthy donors, pretreatment of PBMC for 2 h with IFN-α8 resulted in a significantly higher frequency of CD4+ T cells ($p = 0.006$), naive CD4+ T cells ($p = 0.004$), CD8+ T cells ($p = 0.002$), and naive CD8+ T cells ($p = 0.002$) expressing pSTAT5 (Fig. 5). Pretreatment of PBMC with IFN-α8 did not significantly affect the frequency of pSTAT5+ T cells in HIV+ donors (Fig. 5). Similar findings were observed when PBMC were pretreated with IFN-α8 for 24 h (data not shown).

**IFN-α increased IL-7 production by bone marrow stromal cells**

We used HS27A cells (spontaneous producers of IL-7) to determine whether IFN-α modulates IL-7 production by bone marrow stromal cells. We first confirmed expression of the IFN-α receptor 1 and 2 subunits on the HS27A cells (data not shown). Overall, IL-7 production was low after 6 h but considerably increased after 24 and 48 h of culture. At all three time points, the highest concentrations of IFN-α8 tested (100 and 1000 U/ml) induced the HS27A cell line to produce significantly more IL-7 ($p < 0.0001$; Fig. 6). To exclude IFN-α–induced proliferation of the HS27A cells as a possible cause of the observed increase in IL-7 production, cell counts were performed on HS27A cells following culture with increasing concentrations of IFN-α8. No effect of IFN-α8 upon cell growth was observed (data not shown).

**Discussion**

In this study, we compared the proliferative potential of CD4+ and CD8+ T cells in response to activation-induced stimulation via the TCR or homeostatic proliferation via the IL-7 signaling pathway in healthy and HIV+ donor groups and evaluated the effect of IFN-α on these processes. We also examined the effect of IFN-α on production of IL-7 by bone marrow stromal cells.

We first assessed HIV- and age-associated differences in the proliferative potential of CD4+ and CD8+ T cells in the three donor groups. HIV patients were less responsive to SEB stimulation than both younger and older healthy donors. These data are in agreement with that of Downey et al. (10), who have shown that CD4+ and CD8+ T cells from HIV patients are poorly responsive to activation through the TCR. Their data were obtained using IL-2 and CD3/CD28 as a TCR stimulus rather than SEB. Interestingly, these trends were less apparent when the naive CD4+ and naive CD8+ T cell pools were assessed separately, suggesting that the memory T cell pool in treated HIV patients may be particularly unresponsive to stimulation via the TCR. This may reflect the replicative exhaustion of the memory T cell pool that is characteristic of chronic, treated HIV disease as a consequence of constant cell turnover and activation-induced senescence (32, 33).

IL-7–induced proliferation was also impaired in the HIV+ donors, but significant differences were only noted in relation to the younger healthy donors, which may reflect age rather than HIV-associated differences. The median ages of the younger and older healthy donors studied in this work were 34 and 15 y, respectively. Therefore, T cells from HIV patients on ART may have the potential to undergo “normal” (relative to age) proliferation in response to a homeostatic signal such as IL-7—in the absence of external negative factors.

The effect of age was evaluated further by directly comparing proliferative responses in the younger and older healthy donor

![FIGURE 5. IL-7–induced pSTAT5 was increased by IFN-α8 in healthy donors. IL-7–induced pSTAT5 levels were examined in T cells from 10 healthy donors (circles) and 10 treated HIV+ donors (triangles). PBMC were pretreated with IFN-α8 for 2 h, and then pSTAT5 was induced by IL-7 and detected by flow cytometry. HIV+ donors had a significantly greater frequency of pSTAT5+ CD4+ (A), naive CD4+ (B), and naive CD8+ (D) T cells than healthy donors. In healthy donors only, pretreatment with IFN-α8 resulted in enhancement of IL-7–induced pSTAT5 levels in CD4+ T cells (A; $p = 0.006$), naive CD4+ T cells (B; $p = 0.004$), CD8+ T cells (C; $p = 0.002$), and naive CD8+ T cells (D; $p = 0.002$). IFN-α8 alone did not induce pSTAT5.](http://www.jimmunol.org/ Downloaded from)
Age significantly influenced proliferative responses with older healthy donors, demonstrating less proliferation of total CD8+ T cells in response to SEB and total and naive CD4+ T cells in response to IL-7. It is well established that the function of the immune system declines with age (34, 35). Diminished responses to mitotic stimuli, such as IL-2, have been observed in aging people and associated with reduced receptor signaling (36). A similar mechanism involving age-related defects in CD127 (IL-7R) signaling may affect IL-7 responsiveness.

Across all donor groups, IFN-α enhanced SEB-induced proliferation and inhibited IL-7–induced proliferation (assessed by Ki67 and CFSE staining) of both the total and naive CD4+ and CD8+ T cell populations. It would be important to confirm these effects on proliferation with live cell counting in future studies, an option that was not available to us in this study due to small cell numbers. Nevertheless, these findings suggest that IFN-α has potent, but contrasting effects on these two different activation pathways.

SEB binds to MHC class II, which stimulates the TCR to activate the MAPK pathway (37). The resulting production of various proinflammatory cytokines, such as IL-2, facilitates T cell proliferation and clonal expansion as well as promotes the production of other proinflammatory cytokines (38). Therefore, in an environment in which IFN-α levels are elevated, such as during a viral infection, IFN-α may induce CD4+ and CD8+ clonal expansion via these mechanisms. IFN-α could also enhance TCR binding and/or transduction of the signaling pathway, thereby increasing production of IL-2 and promoting further expansion of the T cell pool. Interestingly, the proproliferative effect of IFN-α on SEB-induced proliferation was least evident in the naive CD4+ T cell population from HIV+ donors—where, in fact, the effect of IFN-α was moderately inhibitory (Fig. 1F). Naive CD4+ T cell cycle entry is delayed by IFN-α exposure, perhaps as a defense mechanism to prevent cell death (25). In an environment with persistently high levels of IFN-α, as is the case in HIV disease, this delay could become permanent.

In contrast, stimulation of the IL-7 signaling pathway involves the catalytic activation of Jak1 and Jak3. This leads to the phosphorylation of STAT5, which in turn dimerizes and translocates to the nucleus, inducing gene transcription (39). Several studies have documented that IFN-α affects IL-7–mediated proliferation in fetal and postnatal thymic progenitor cells, thus impairing thymopoiesis and T cell maturation (26, 27). The effect of IFN-α on homeostatic maintenance of the naive CD4+ T cell pool (that is also governed by the IL-7 signaling pathway) has not been previously reported. Our data demonstrate that IFN-α inhibits IL-7–induced proliferation in total and naive T cells. Therefore, in contrast to its effects on activation-induced proliferation, IFN-α may exhibit antiproliferative activity during homeostatic proliferation. At physiological levels, IFN-α may play a pivotal role in regulating T cell homeostasis by establishing an equilibrium between its proliferative and antiproliferative activities. However, at high concentrations, such as in the context of HIV disease, IFN-α may also have a deleterious effect. For example, IFN-α may play an important role in inducing apoptosis of CD4+ T cells via upregulation of TRAIL (40, 41) and has been linked to increased expression of the proapoptotic molecules Bak and CD95 (Fas) on CD4+ T cells and subsequent CD95-mediated apoptosis of T cells in chronically infected HIV patients (42). Indeed, the induction of these proapoptotic signals may be sufficient to overcome IL-7–mediated cell survival signals that would promote proliferation.

We hypothesized that the inhibition of IL-7–induced proliferation by IFN-α may be due to downregulation of the IL-7R α-chain, CD127. However, CD127 expression in all T cell populations, except naive CD4+ T cells from healthy donors, was upregulated by IFN-α. This result is in contrast to that of Baron et al. (26), who demonstrated a downregulation of CD127 expression following culture of thymic progenitor cells with rIFN-α. However, cells at such an early stage of primary thymic development would likely be more sensitive to IFN-α activity than mature, circulating peripheral T cells. It is also worth noting that our data report the level of CD127 expression following a period of 7 d of culture either alone or in the presence of IFN-α—which may be too long to observe IFN-α8–induced changes in CD127. To exclude this as a factor, we performed a small study using five healthy donors to assess CD127 expression after 6, 24, 48, and 72 h of culture with IFN-α8 (data not shown). Our results confirmed that IFN-α8 was not mediating its effects on IL-7–induced proliferation by downregulating expression of the IL-7R α-chain.

IFN-α also enhanced IL-7–induced STAT5 phosphorylation in all T cell populations. This is in contrast to a study by Schmidlin et al. (27) that demonstrated no significant effect of IFN-α on IL-7–mediated STAT5 phosphorylation in thymic progenitor cells. Interestingly, Erickson et al. (43) have shown that IFN-α inhibited STAT5 binding to DNA in T cells stimulated by IL-2, a cytokine very closely related to IL-7. With this in mind, a recent study by Landires et al. (44) found that the translocation of IL-7–induced pSTAT5 to the nucleus of CD4+ T cells is perturbed in patients with HIV infection. Therefore, although there was no suppressive activity of IFN-α observed upon CD127 expression and STAT5 phosphorylation, IFN-α may impair the IL-7 signaling pathway by impairing the migration of pSTAT5 to the nucleus and therefore prevent gene transcription.
It is important to note that, in addition to the STAT5 signaling pathway, the PI3K/Akt pathway is also essential in IL-7/IL-7Rα-mediated T cell homeostasis. Both pathways are primarily induced by activation of IL-7Rα, and they can act either independently or synergistically. For example, both pathways are required to stimulate T cell proliferation, whereas STAT5 signaling is more important in T cell survival (regulation of Bcl-2). In addition, STAT5 and Akt have been shown to interconnect in regulating T cell metabolism (glucose trafficking and uptake) through STAT5-dependent activation of Akt. For this study, we focused on the JAK/STAT pathway, as previous work from our group has shown a significant decrease in pSTAT5 in the total and naive CD4+ T cell compartments in HIV patients with low CD4+ T cell recovery (45). Given that the inhibition of IL-7−induced proliferation by IFN-α that we observed could not be attributed to a decrease in pSTAT5, it would be pertinent to assess the effects of IFN-α on the PI3K pathway in future studies.

The findings of our study provide supportive evidence for our hypothesis that increased IFN-α activity contributes to persistent CD4+ T cell deficiency in HIV patients receiving ART. We demonstrated that IFN-α decreased homeostatic proliferation of CD4+ T cells, which had undergone ∼5 rounds of proliferation over 7 d by 10−20%. The life span of human T cells in the absence of TCR signaling is 2–3 y (46–48); therefore, the decrease in proliferation that we have observed could have a significant cumulative effect on CD4+ T cell numbers over time. Given our findings that IFN-α also had similar effects on the proliferation of CD8+ T cells, increased IFN-α activity may contribute to the persistent deficiency of naive CD8+ T cells that is observed in some patients receiving ART (11). However, total CD8+ T cell counts are not decreased and may be increased in patients with treated HIV infection. Studies have shown that type I IFNs have an important role in inducing CD8+ T cell expansion either directly (49) or indirectly by stimulating the synthesis of IL-15, which contributes to the increased sensitivity of CD4+ T cells to apoptosis in HIV patients (23, 53).

In summary, IFN-α displays contrasting effects on TCR activation-induced proliferation and homeostatic proliferation of T cells, which together, and in association with an increased propensity of CD4+ T cells to apoptosis, would impair the recovery of CD4+ T cell numbers in some HIV patients receiving ART. Our findings provide an explanation for increased ISGs in CD4+ T cells (17) or PBMC (18, 19) of HIV patients with persistent CD4+ T cell deficiency on ART. Increased IFN-α activity may promote the loss of T cells by accelerating cell turnover and activation-induced cell death while decreasing the renewal of T cells by inhibiting the proliferative effect of IL-7. Adding therapies that inhibit IFN-α activity to ART may therefore be a strategy for increasing CD4+ T cell counts in HIV patients with persistent CD4+ T cell deficiency on ART.

Disclosures
The authors have no financial conflicts of interest.

References


