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Uncoupled Anti-HIV and Immune-Enhancing Effects when Combining IFN-α and IL-7

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Cytokine-based therapies have been examined for purging viral reservoirs and immunomodulation in HIV infection. However, single cytokines did not result in either HIV eradication or an efficient HIV-specific immune response. We hypothesize that cytokines with distinct biologic effects need to be combined for immunotherapy of HIV infection. In this study, we investigated the anti-HIV activity and immune-enhancing effects of the combination of IFN-α and IL-7. In human lymphocyte aggregate cultures infected ex vivo with the X4 HIV strain NL4-3, IFN-α/IL-7 potently inhibited HIV replication and preserved CD4+ T cells, probably by up-regulating Bcl-2. IFN-α/IL-7 also strongly inhibited R5 HIV replication. Furthermore, in allogeneic MLRs, IFN-α/IL-7 increased T cell proliferation and IFN-γ production. IFN-α alone also had strong anti-HIV activity, but neither preserved CD4+ T cells nor increased T cell responses in MLRs. IL-7 alone maintained T cells and enhanced T cell activation in MLRs, but only moderately inhibited or increased HIV replication. Thus, coadministration of IFN-α/IL-7 combines the potent anti-HIV activity of IFN-α with the beneficial effects of IL-7 on T cell survival and function. We speculate that IFN-α will block viral replication, activate APCs, and up-regulate MHC molecules, thus allowing IL-7 to display its effects for generating an efficient immune response. In this scenario, the known reactivation of latent HIV by IL-7 may be advantageous.


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3 Abbreviations used in this paper: HAART, highly active antiretroviral therapy; DC, dendritic cell; HLAC, human lymphocyte aggregate culture; OAS3, 2'-5'-oligoadenylate synthetase 3; p.i., postinfection; R5 strain, CCR5-using strain; X4 strain, CXC4-using strain; PRKR, double-stranded RNA-dependent protein kinase; Mx1, Mx protein 1.

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clinical trials of HIV therapy. In the present study, we report the anti-HIV activity and immune-enhancing effects of combinations of IFN-α and IL-7 in vitro.

Materials and Methods

Human lymphocyte aggregate cultures (HLACs)

The acquisition and processing of lymphoid tissue were approved by the local ethics committee. Human tonsils from otherwise healthy adult patients were processed within 1–5 h after tonsillectomy. HLACs were prepared by transferring minced tissue and tissue fragments into a cell strainer (70-μm pore size; Falcon) and grinding the tissue through the sieve with a syringe plunger. Erythrocytes were lysed with ACK cell-lysing buffer (BioWhittaker). Lymphoid cells were washed and transferred to 96-well, round-bottom plates at a concentration of 2 × 10^5 cells/well and cultured in 200 μl/well RPMI 1640 containing 15% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml fungizone, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1% nonessential amino acids. Cultures were performed in triplicate. One experiment comprises data from one donor; the numbers of experiments are given in the legends.

HLACs were pretreated with IFN-α (peginterferon alfa-2a; Pegasys; Roche) and/or IL-7 (Immunotools), both at 10 ng/ml unless otherwise noted, or were not treated before being infected with HIV (see below). In some experiments, HLACs were also (pre)treated with the PI3K inhibitor LY294002 (Sigma-Aldrich) at 10 μM. Cytokines and inhibitor were maintained throughout the experiment; 50 μl of culture medium was replaced every 3–4 days.

HIV infection of HLACs

HLACs were inoculated overnight with the CXCR4-using (X4) HIV strain NL-4.3 or the CCR5-using (R5) HIV strain strain 49.5 (1 ng of p24/2 × 10^5 cells in 200 μl), washed three times with PBS the next morning, and resuspended in 200 μl of fresh medium. Virus stocks were obtained, and HIV replication was determined as described previously (35).

Immunostaining and flow cytometry

For multicolor flow cytometry, mAbs (all from BD Pharmingen unless otherwise indicated; the respective clones are given in parentheses) were used against the following extracellular Ags: CD4 (RPA-T4), CD8 (HIT8a), the α-chain of the IL-7R (IL-7Rα; R&D Systems; clone 40131), CXCR4 (12G5), CCR5 (2D7/CCR5), CD25 (M-A251), CD69 (FN50), CD38 (HIT2), CD3/CD14/CD16/CD19/CD56 (lin 1 mixture), HLA-DR (L243), CD11c (HCL-3), and CD83 (clone HB15e; PE). The percentage (given as median, (minimum-maximum)) of DCs positive for CD11c, a marker for myeloid DCs, was 99.7% (92.0–100%). The percentages (given as median (minimum-maximum)) of DCs positive for the activation marker HLA-DR and CD83, a marker for DC maturation, were 99.6% (80.6–99.9%) and 7.6% (3.5–23.6%), respectively. For MLRs, 1 × 10^7 monocyte-depleted PBls were incubated with 5 × 10^5 allogeneic or autologous DCs or cultured without DCs in 200 μl of RPMI 1640 containing 10% FCS, 100 U/ml penicillin, and 10 U/ml IL-2 (National Institutes of Health AIDS Research and Reference Reagent Program) in 96-well, round-bottom plates; cultures were treated with IFN-α, IL-7, or IFN-α/IL-7 or were not treated. Cells were harvested after 1 and 6 days of culture for additional analysis.

Real-time quantitative PCR

RNA extraction, RT, and real-time quantitative PCR were performed essentially as previously described (35). For PCR of 2′,5′-oligoadenylate synthetase 3 (OAS3), double-stranded RNA-dependent protein kinase (PKR) and Mx protein 1 (Mx1), commercially available primers and probes (Assays-on-Demand; Applied Biosystems) were used.

Results

Dose-response curves of IFN-α and IL-7 regarding their effect on HIV replication

We first determined the optimal working concentrations of IFN-α and IL-7 in dose-response curve experiments. For IFN-α, we aimed for a concentration that would incompletely inhibit HIV replication, so that any anti-HIV synergy with IL-7 could be detected. In tonsillar HLACs infected ex vivo with the X4 HIV strain NL-4.3, IFN-α inhibited HIV replication in a dose-dependent manner (Fig. 1A). At 10 ng/ml, IFN-α inhibited 69% of control HIV replication. We also considered the pharmacokinetics of IFN-α in patients. Mean trough concentrations in patients are, on the average, 8 and 16 ng/ml peginterferon alfa-2a (Pegasys; Roche) measured after 1 and 48 wk, respectively, of s.c. administration of 180 μg once weekly. Thus, we decided to use IFN-α at 10 ng/ml.

In contrast, IL-7 displayed no dose-dependent effect on HIV replication (Fig. 1B). However, in an in vitro study reported a dose-dependent effect of IL-7 on cell proliferation that was most pronounced at 10 ng/ml (37). Therefore, we used IL-7 at 10 ng/ml for the remaining experiments.

Anti-HIV activity of IFN-α combined with IL-7

We then investigated the effects of IFN-α, IL-7, and IFN-α/IL-7 on HIV replication. In X4 HIV-infected HLACs, IL-7 alone had a
variable effect on HIV replication (median (minimum-maximum) percent inhibition: 4.2% (−494.7 to 45.5%); n = 15; Fig. 2A). In contrast, IFN-α alone potently inhibited HIV replication in each experiment (58.8% (40.1–77.8%)), and IFN-α/IL-7 inhibited HIV replication slightly more than IFN-α alone (69.8% (−17.0 to 98.4%)). Notably, in five of the six cases where IL-7 alone increased HIV replication, the anti-HIV activity of IFN-α did not reduce IL-7Rα expression more than IL-7 alone. The same phenomenon was observed at different time points after infection with X4 HIV (Fig. 2C) or R5 HIV (data not shown). These data indicate that there is no cross-desensitization of IL-7Rα by IFN-α.

Effects of cytokine treatment on IL-7Rα expression

We wondered whether the dominant anti-HIV effect of IFN-α might be due to enhanced down-regulation of the IL-7R by IFN-α. To address this question, we used flow cytometry to determine cell surface expression of IL-7Rα on CD4+ T cells. Treatment of tonsillar HLACs for 4 h with IL-7 reduced the cell surface expression of IL-7Rα, whereas IFN-α had no effect compared with untreated HLACs (Fig. 3, A and B). Combining IL-7 with IFN-α did not reduce IL-7Rα expression more than IL-7 alone. The same phenomenon was observed at different time points after infection with X4 HIV (Fig. 3C) or R5 HIV (data not shown). These data indicate that there is no cross-desensitization of IL-7Rα by IFN-α.

Impact of cytokine treatment on chemokine receptor expression

One determinant of HIV entry into the cell, and hence of HIV replication, is the expression of the HIV coreceptors CCR5 and CXCR4 for R5 and X4 HIV strains, respectively. In tonsillar HLACs, the frequency of CXCR4-positive CD4+ T cells and the cell surface expression of CXCR4 on CD4+ T cells on a per cell basis were not affected by treatment with cytokines (Table 1). The same results were obtained for CCR5. Thus, the cytokines do not exert their effects on HIV replication by regulating the cell surface expression of the HIV coreceptors.

Effects of IFN-α/IL-7 on T cell numbers

A hallmark of HIV infection/AIDS is the depletion of CD4+ T cells. In vitro, this phenomenon is observed in particular in X4 HIV-infected cell or tissue cultures. Thus, we investigated whether treatment with IFN-α and/or IL-7 prevents or diminishes the loss of CD4+ T cells after infection with NL4-3. We determined the absolute numbers and fractions of CD4+ and CD8+ T cells in HIV-infected and uninfected tonsillar HLACs at the end of culture (i.e., 14 days postinfection (p.i.)). In X4 HIV-infected, untreated HLACs, CD4+ T cell levels on day 14 p.i. were only 44% of those

We wondered whether the anti-HIV effect of IFN-α would still be present at 1 and 0.1 ng/ml. As expected, we observed an inhibition of HIV replication that was 2- to 4-fold lower than with 10 ng/ml. As described below, the effects on the expression of the apoptosis marker annexin V and the antiapoptosis molecule Bcl-2 associated with IL-7 treatment were similar, as in the case of combining IFN-α and IL-7, both at 10 ng/ml (data not shown).
in uninfected, untreated cultures (Fig. 4A). Surprisingly, IFN-α did not prevent the depletion. In contrast, treatment with IL-7 alone or IFN-α/IL-7 entirely preserved CD4+ T cells. These data show that cotreatment with both cytokines is superior to treatment with IFN-α alone with regard to maintenance of CD4+ T cells in X4 HIV-infected tonsillar HLACs. As expected, R5 HIV only slightly depleted CD4+ T cells in tonsillar HLACs (Fig. 4B).

Treatment of HIV-infected HLACs with IFN-α/IL-7 or IL-7 alone resulted in even greater numbers of CD4+ T cells and CD8+ T cells than those in control cultures (Fig. 4C). In contrast, a single treatment with IFN-α had no effect on CD4+ or CD8+ T cell numbers. Thus, the increased T cell numbers are probably attributable to an antiapoptotic and/or proliferative effect of IL-7.

FIGURE 3. IFN-α’s dominant anti-HIV activity is not due to cross-desensitization of IL-7Ra by IFN-α. Tonsillar HLACs were analyzed for cell surface expression of IL-7Ra before and after 4 h of pretreatment with cytokines (A and B) and at different time points after infection with X4 HIV (C). A, The gating strategy for CD4+ T cells. Values in A–C indicate percentage of IL-7Ra-positive cells of total CD4+ T cells. B, One of three experiments is shown; the median (minimum-maximum) percentage of IL-7Ra-positive CD4+ T cells at 0 h in these three experiments was 63.6% (59.7–86.6%; data not shown). C, Data are given as the median, minimum-maximum (n = 3 for 18 h and 4 days; n = 2 for 40 h).
IFN-α, up-regulated activation markers on the surface of CD4+ and CD8+ T cells (Fig. 7). The effect was most pronounced for CD25 on CD4+ and CD8+ T cells and CD69 on CD8+ T cells, but up-regulation of CD38 or HLA-DR was minor. Thus, treatment of HIV-infected tonsillar HLACs with IL-7 alone or together with IFN-α partially activates CD4+ and CD8+ T cells.

Production of proinflammatory cytokines

The moderate to severe adverse effects of therapeutic application of cytokines may be due to induction of other proinflammatory cytokines. We investigated whether treatment of X4 HIV-infected tonsillar HLACs with IFN-α and/or IL-7 increased the production of IL-6, TNF-α, or IL-1β by measuring their concentrations in culture fluid by ELISA. Production of IL-6 and TNF-α was indeed significantly enhanced by IL-7 or IFN-α/IL-7, but IFN-α alone had no effect (Table II). In contrast, IL-1β was not significantly up-regulated by IL-7 or IFN-α. Thus, treatment of tonsillar HLACs with IFN-α/IL-7 augments the production of some proinflammatory cytokines.

Modulation of allogeneic MLRs by IFN-α/IL-7

Optimally, immune-based therapies should enhance innate and adaptive immunities. DCs are the most potent APCs in the induction of immune responses (44, 45). To determine whether IFN-α/IL-7 enhances T cell responses induced by DCs, we performed MLRs with blood monocyte-derived DCs and allogeneic PBLs in the presence or the absence of IFN-α/IL-7. T cell responses were assessed based on IFN-γ secretion and proliferation, as determined by the percentage of Ki67-positive T cells or of CFSE-labeled T cells with low expression of CFSE. Treatment with IL-7 alone or IFN-α/IL-7 increased the percentages of IFN-γ-positive CD4+ (Fig. 8A) and CD8+ (Fig. 8B) T cells and the percentage of Ki67-positive T cells (Fig. 8, C and D) relative to untreated allogeneic MLRs. IFN-α alone had no effect. Proliferation, as assessed by CFSE labeling, was lower with IFN-α/IL-7 than with IL-7 alone, but was still higher than that in untreated MLRs (data not shown). A proliferative effect of IL-7 alone or IFN-α/IL-7 was also observed with PBLs cultured without DCs, indicating that IL-7 directly activates T cells (Fig. 8, E and F). However, these effects were stronger in autologous MLRs and were most pronounced in allogeneic MLRs. We also investigated whether IFN-α/IL-7 would protect T cells from apoptosis, especially those cells that strongly proliferate. However, the percentage of apoptotic T cells, as assessed by intracellular staining for active caspase 3, was ≤1% regardless of the cytokine treatment or whether PBLs were cultured with or without DCs (data not shown). Together, these results demonstrate that the combination of IFN-α and IL-7 indeed has the potential to enhance proliferation and cytokine production of T cells after allogeneic T cell-DC interactions.

Table I. Expression of CXCRI and CCR5 on CD4+ T cells in tonsillar HLACs after a 4-h pretreatment with cytokines*

<table>
<thead>
<tr>
<th></th>
<th>IFN-α</th>
<th>IL-7</th>
<th>IFN-α + IL-7</th>
<th>No Cytokines</th>
</tr>
</thead>
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<tr>
<td>CXCRI</td>
<td>Frequencyb</td>
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<td>70.8</td>
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<td></td>
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<td>87.6</td>
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<td>10.6</td>
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<tr>
<td></td>
<td>MFI</td>
<td>9.4</td>
<td>11.2</td>
<td>10.6</td>
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a Data are given as median (min–max); p > 0.05; n = 3.

b Percentage of CXCRI-positive cells within the CD4+ T cell population.

c Mean fluorescence intensity (MFI) of CXCRI-positive CD4+ T cells.

Notes: *p < 0.05; **p < 0.01; ***p < 0.001. n = 7 for A and C; n = 5 for B.
Role of PI3K pathway in modulation of HIV replication and T cell survival by IFN-α/IL-7

To gain insight into the molecular mechanisms of the virological and immunological effects of IFN-α/IL-7, we monitored HIV replication, T cell counts, and Bcl-2 expression in tonsillar HLACs infected ex vivo with X4 HIV and cultured in the presence or the absence of the PI3K inhibitor LY294002. PI3K plays a role in both HIV replication (46, 47) and IL-7 function as a trophic factor for T cells (31, 48). With LY294002, HIV replication was very low and was completely suppressed in cultures treated with IFN-α (Fig. 9A). In contrast, treatment with IL-7 rendered cultures permissive to HIV replication, albeit to levels far below those obtained in HLACs without the PI3K inhibitor (Fig. 9, A and B). Analysis of CD4+ and CD8+ T cells counts on day 14 p.i. and Bcl-2 expression of T cells on day 7 p.i. revealed that, similar to HLACs without the inhibitor, IL-7 preserved CD4+ T cells, expanded T cells (Fig. 9, C and D), and increased Bcl-2 expression (Fig. 9, E and F), compared with cultures not treated with cytokines. IFN-α had no effect on T cell numbers and Bcl-2 expression. Thus, inhibition of the PI3K pathway abrogates HIV replication, IL-7 allows for low level HIV replication when PI3K is inhibited, and the PI3K pathway is not essential for IL-7’s survival effect on T cells in tonsillar HLACs.

Induction of IFN-inducible genes in HIV-infected tonsillar HLACs

IFN-α induces a number of proteins with antiviral activity (18). We wondered whether induction of IFN response genes in X4 HIV-infected tonsillar HLACs would be affected by cotreatment with IL-7. To address this question, we analyzed the expression of three well-studied IFN-target genes, OAS3, PRKR, and MX1, 1 and 4 days p.i. by quantitative real-time PCR. On day 1 p.i., all three IFN target genes were up-regulated by IFN-α alone or combined with IL-7, whereas IL-7 alone had no effect (Fig. 10). On day 4 p.i., a similar pattern was observed, albeit with less pronounced up-regulations. These data show that in tonsillar HLACs infected with HIV, induction of IFN response genes by IFN-α is maintained in combination with IL-7.

Discussion

This work is based on the evidence that single cytokines are not able to either eradicate or cure HIV. In contrast, we believe that combining cytokines, which will stimulate both the innate and adaptive immune responses, will generate an efficient HIV-specific immune response for controlling HIV. In this study we investigated the anti-HIV activity and immune-enhancing effects of the combination of IFN-α and IL-7 in vitro. Most of the experiments were performed with the X4 HIV strain NL4-3, because X4 strains are more cytopathic in vitro than R5 strains; the higher cytopathicity of X4 strains enables us to discern more clearly the potential beneficial effects of these cytokines. We demonstrated that this cytokine combination 1) has potent inhibitory activity against both X4 and R5 HIV; 2) protects CD4+ T cells from HIV-induced apoptosis, probably through up-regulation of Bcl-2, in X4 HIV-infected tonsillar HLACs; and 3) increases T cell proliferation and IFN-γ secretion in allogeneic MLRs. Although IFN-α alone also had strong anti-HIV activity, it neither preserved CD4+ T cells nor increased T cell responses in MLRs. Conversely, IL-7 alone maintained T cells and enhanced T cell activation, but only moderately inhibited or even increased HIV replication. Thus, co-administration of the two cytokines combines the potent anti-HIV activity of IFN-α with the beneficial effects of IL-7 on T cell survival and function.

In five of six experiments in which IL-7 alone enhanced X4 HIV replication, coadministration of both cytokines resulted in retention of IFN-α’s anti-HIV activity. Thus, the potent anti-HIV activity of IFN-α prevails over the potential HIV-enhancing effect of IL-7. Surprisingly, IL-7 alone had a highly variable effect on HIV replication, ranging from strong enhancement to moderate suppression of replication. Variable responses to IL-7 have also been observed for permissiveness of peripheral blood T cells to transduction with HIV-derived vectors (31). As proposed by the authors of the latter study, the variability we observed may be related to a genomic-based differential availability of a cellular transcriptional cofactor for HIV. In other in vitro studies, IL-7 almost invariably stimulated HIV replication (2, 3, 25–32), but increased viral load in only some animal studies (23, 24, 33, 34). Notably, we observed...
similar anti-HIV activity with IFN-α/IL-7 upon infection with R5 HIV.

Despite its potent anti-HIV activity, IFN-α as a monotherapy does not restore CD4+ T cell count in HIV patients (4). We asked whether the potent inhibition of HIV replication in tonsillar HLACs observed with IFN-α/IL-7 and IFN-α alone would prevent CD4+ T cell loss. In accord with previous data from other investigators (49) and our group (50, 51), CD4+ T cell numbers were not affected by R5 HIV, whereas >50% of CD4+ T cells were depleted in tonsillar HLACs infected with an X4 HIV strain over a 14-day culture. We found that IFN-α/IL-7, but not IFN-α alone, preserved CD4+ T cells. IFN-α’s antiviral activity may be outweighed by its known proapoptotic effect (52). Indeed, we observed that CD4+ T cell counts in uninfected HLACs treated with IFN-α were only 89% of those found in untreated, uninfected cultures. Furthermore, IFN-α treatment of uninfected cultures slightly increased the percentage of annexin V-positive CD4+ T cells (data not shown). However, we suggest that IFN-α alone may not provide a survival signal to uninfected CD4+ T cells that are indirectly killed by the residual replication. We showed that IFN-α alone has no effect on the expression of Bcl-2, a central factor involved in the inhibition of apoptosis (53, 54), in CD4+ and CD8+ T cells in HIV-infected HLAC. The same results were obtained with uninfected cultures (data not shown). Importantly, bystander apoptosis contributes much more to the depletion of CD4+ T cells induced by X4 HIV strains than apoptosis of productively infected cells in human lymphoid tissues ex vivo (55). Thus, the lack of CD4+ T cell preservation with IFN-α alone most likely results from a massive loss of bystander CD4+ T cells that are not protected by IFN-α. In contrast, IL-7 alone or IFN-α/IL-7 significantly up-regulated Bcl-2 in T cells, and this was associated with a reduced number of annexin V-positive T cells and a complete preservation of CD4+ T cells. Our findings are in line with previous reports demonstrating that up-regulation of Bcl-2 is a major mechanism for maintenance of T cells by IL-7 (19, 22, 38–43) and show that this mechanism may also be operative in HIV-infected cells. Thus, IFN-α must be combined with IL-7 to obtain the direct anti-HIV activity of IFN-α and maintenance of CD4+ T cells by IL-7.

Strikingly, treatment with IFN-α/IL-7 or IL-7 yielded even greater numbers of CD4+ and CD8+ T cell numbers than those in uninfected, untreated tonsillar HLACs. Primary cell cultures exhibit spontaneous cell death over time. Thus, the increased T cell numbers may be due to decreased apoptosis or enhanced proliferation. Consistent with a recent report (49), we observed proliferation of lymphoblasts, but not lymphocytes. IL-7 did not enhance proliferation in lymphocytes. Data on the proliferative effects of IL-7 on T cells are controversial; some studies report an increase in proliferation by IL-7 (23, 34, 48, 56–58), and others do not (25, 59–61). In one study, IL-7 allowed T cells to enter G1b phase, but not to complete progression through the cell cycle and proliferation (29). Importantly, depending on the type of T cells being studied, induction of proliferation by IL-7 may depend on the presence of a comitogenic stimulus (62). According to this idea, HLACs may lack or insufficiently provide such mitogenic stimulation, and even HIV appears to be an ineffective costimulus in this in vitro setting.

In contrast, such stimuli are provided in MLRs that are commonly used to evaluate the capacity of DCs to stimulate naïve T cells to proliferate and/or secrete cytokines. This stimulation typically occurs during Ag presentation of DCs to T cells (63), but also after T cell-DC interactions lacking Ag (64–66). Several adhesion molecules, including ICAM-1 and -3, participate in these DC-T cell interactions, and various DC costimulatory molecules, such as CD40, CD80, and CD86, contribute to the activation of T cells by DCs (67). We showed that IL-7, alone or with IFN-α, enhanced T cell proliferation, as assessed by Ki67 staining and CFSE labeling, and IFN-γ production in allogeneic MLRs. The effect on increased cellular proliferation is certainly not confounded by the antiapoptotic properties of IL-7 resulting in better cell survival, because virtually no apoptosis was observed in MLRs independent of the culture condition.

We were interested in the potential mechanisms of IFN-α’s dominant inhibition of IL-7’s enhancement of HIV replication. Because cytokines can induce desensitization of their own or other receptors (cross-desensitization), IFN-α might cross-desensitize IL-7R. As reported previously (58, 68), IL-7 down-regulated IL-7Rα. However, treatment of HLACs for 4 h with IFN-α/IL-7 did not reduce IL-7Rα expression more than treatment with IL-7 alone; this down-regulation was independent of HIV infection and persisted over time. Thus, IFN-α does not down-regulate IL-7Rα. Alternatively, IFN-α could down-regulate the HIV coreceptors CXCR4/CXCR5, reducing HIV entry into target cells. However, none of the cytokine treatments affected the cell surface expression of CXCR4 or CCR5. This result contrasts with other studies that describe down-regulation of CXCR4 and CCR5 by IFN-α in PBMCs or cell lines and macrophages derived from CD4+ cells from umbilical cord blood, respectively (69, 70). Conversely, IL-7 up-regulated CXCR4 expression in T cells, PBMCs, and thymocytes, and this effect was associated with increased susceptibility to infection with X4 HIV strains (28, 30, 32, 37). The disparate results from these and our experiments may be explained by the different experimental systems used.

IFN-α induces a variety of antiviral proteins (18). In tonsillar HLACs, treatment with IFN-α or IFN-α/IL-7 resulted in an up-regulation of the three IFN-inducible genes studied, i.e., OAS3, PRKR, and Mx1; IL-7 alone had no effect. Thus, IFN-α appears to
induce an antiviral environment that is not adversely affected by cotreatment with IL-7, which might explain, at least in part, IFN-α’s dominant inhibition of IL-7’s enhancing effect on X4 HIV replication.

We also sought the reason for the great variability in IL-7’s effects on HIV replication. Permissiveness to HIV is influenced by various cellular factors, in particular the activation state and cell cycle progression (71), and activation of primary tissue cells by cytokines may be variable. Analysis of various activation markers revealed a consistent increase in CD25 on CD4+ T cells in tonsillar HLACs treated with IL-7 alone or IFN-α/IL-7. The effects of IL-7 on the other activation markers were less pronounced. Thus, IL-7’s highly variable effect on HIV replication in tonsillar HLACs cannot easily be explained by a differential modulation of the activation state of CD4+ T cells. Our data are in accord with previous studies showing a minimal effect by IL-7 on T cell activation markers (2, 31, 56, 72) and a lack of correlation between IL-7-mediated HIV permissiveness or induction of latent HIV and expression of activation markers (2, 31). In the latter studies, however, IL-7 permitted progression of quiescent T cells into the G1b phase of the cell cycle without a major effect on activation markers, and this correlated with IL-7-induced HIV permissiveness (31). Thus, depending on the genomic background, IL-7 may differentially induce a transcriptional factor that is critical for cell cycle progression and/or other cellular parameters.

To gain insight into pathways that might be activated by IL-7, we examined the effect of the PI3K inhibitor LY294002. IL-7 transduces signals via two distinct pathways, one involving PI3K and the other being the Jak/STAT5 cascade (73). PI3K is essential for IL-7’s survival effect (31, 48). It is also activated by IFN-α (74), and this activation promotes the survival of human primary B cells (75). Importantly, HIV triggers PI3K activation (46, 47). Blockade of PI3K by wortmannin, however, has no effect on the permissiveness of quiescent T cells to HIV (31). In tonsillar HLACs, inhibition of PI3K by LY294002 strongly suppressed X4 HIV replication unless the cultures were treated with IL-7. Even so, IL-7 could not completely restore HIV replication, because p24 levels were about 1/10th those measured in cultures without the
inhibitor. These findings suggest that the PI3K pathway is essential for efficient HIV replication in tonsillar HLACs, and IL-7 may bypass this requirement by inducing other effector molecules that enable HIV replication, at least partially. In contrast to previous studies, we found no role for the PI3K pathway in IL-7’s survival effect on T cells. Enhancement of CD4+ and CD8+ T cell numbers and of their Bcl-2 expression by IL-7 was observed with and without the inhibitor. We speculate that the T cell-trophic effect of IL-7 is instead mediated by the Jak/STAT pathway, the other pathway induced by IL-7. Interestingly, CD8+ T cell counts were clearly reduced by the inhibitor in HIV-infected cultures when treated with IFN-α alone or not treated with cytokines, suggesting a role for the PI3K pathway in CD8+ T cell viability.

This work provides evidence that combining IFN-α/IL-7 may be a promising approach for immunotherapy of HIV patients. We believe that only by slowing down HIV replication, activating APCs, and up-regulating MHC molecules due to IFN-α can IL-7 display its full activities, potentially enhancing the HIV-specific immune response. We believe that testing the combination of IFN-α/IL-7 would be most appropriate in HIV patients interrupting

**FIGURE 8.** IL-7 enhances IFN-γ production and proliferation of T cells in MLRs. A–D, Allogeneic MLRs, performed with DCs generated from blood-derived monocytes with IL-4/GM-CSF and monocyte-depleted PBLs, were treated or not with cytokines. Production of IFN-γ by CD4+ (A) and CD8+ (B) T cells was analyzed on day 1 of T cell-DC coculture by staining for IFN-γ. Proliferation of CD4+ (C) and CD8+ (D) T cells (gated on lymphocytes) was assessed after 6 days of MLRs by staining for Ki67 (C and D). Data are shown as the median, minimum-maximum. *p < 0.01; **p < 0.001; ***p < 0.001; n = 10. E and F, Monocyte-depleted PBLs were cocultured with allogeneic or autologous DCs or without DCs. Proliferation of CD4+ (E) and CD8+ (F) T cells (gated on lymphocytes) was assessed after 6 days of culture by staining for Ki67. One of two experiments is shown.

### Table II. Production of proinflammatory cytokines in tonsillar HLACs treated with cytokines

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>IFN-α</th>
<th>IL-7</th>
<th>IFN-α + IL-7</th>
<th>No Cytokines</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α</td>
<td>38</td>
<td>62</td>
<td>60</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>(20–98)</td>
<td>(34–88)b</td>
<td>(38–110)f</td>
<td>(18–48)</td>
</tr>
<tr>
<td>IL-6</td>
<td>433.8</td>
<td>608.4</td>
<td>590.6</td>
<td>318.2</td>
</tr>
<tr>
<td></td>
<td>(145.4–950.6)</td>
<td>(237.4–1171.0)b</td>
<td>(312.0–1237.0)b</td>
<td>(164.8–738.4)</td>
</tr>
<tr>
<td>IL-1β</td>
<td>59.8</td>
<td>79.6</td>
<td>75.4</td>
<td>79.6</td>
</tr>
<tr>
<td></td>
<td>(4.0–110.0)</td>
<td>(19.8–140.0)</td>
<td>(28.8–110.0)</td>
<td>(19.8–130.8)</td>
</tr>
</tbody>
</table>

*a* Production of proinflammatory cytokines was analyzed on day 4 p.i. by ELISA; data are in picograms per milliliter and are given as median (min-max).

b *p < 0.01 vs no cytokines; n = 7.

c *p < 0.05 vs no cytokines; n = 7.
HAART after immune reconstitution, similar to a reported trial with GM-CSF (76). Administration of cytokines is frequently associated with moderate to severe side effects (77). Indeed, we observed that IL-7 increases the production of the proinflammatory cytokines TNF-α and IL-6. However, treatment of SCID-hu Thy/Liv mice with doses of IL-7 as high as 10 μg/day did not result in

FIGURE 9. Inhibition of PI3K abrogates HIV replication in tonsillar HLACs, which is partially restored by IL-7, but not by IL-7’s survival effect on T cells. HLACs were pretreated with or without cytokines in the absence or the presence of the PI3K inhibitor LY294002 at 10 μM before being infected with X4 HIV and cultured as described in Fig. 1. A and B, HIV replication in the presence (A) or the absence (B) of LY294002 was assessed by measuring the p24 Ag concentrations in the culture fluids over time. Data from three experiments are given as the mean and SEM of triplicate determinations. C and D, T cell numbers in cultures without (□) or with (■) LY294002 were determined on day 14 p.i. by staining of HLACs for CD4 and CD8 and quantification with fluorospheres (Flow-Count Fluorospheres; BD Pharmingen); absolute cell counts are shown. E and F, Expression of Bcl-2 in T cells from LY294002-treated cultures was analyzed on day 7 p.i.; Bcl-2-positive CD4+ or CD8+ T cells are shown as a percentage of the total CD4+ or CD8+ T cells, respectively. Data in C–F are given as the median, minimum-maximum. *, p < 0.05. n = 3.
cytopathicity (34). Furthermore, there is evidence that proinflammatory cytokines may be necessary components of T cell expansion driven by cytokines such as IL-2 (78–81). In conclusion, this work provides a rationale for further exploring the combination of IFN-α and IL-7 in animal models and human clinical trials of HIV infection.

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Disclosures
The authors have no financial conflict of interest.

References

FIGURE 10. Induction of IFN response genes by IFN-α. Tonsillar HLACs were pretreated, infected with X4 HIV, and cultured as described in Fig. 1. Expression of mRNAs for the IFN-inducible genes OAS3 (A and B), PRKR (C and D), and Mx1 (E and F) on day 1 p.i. (A, C, and E) and day 4 p.i. (B, D, and F) in cytokine-treated, HIV-infected tonsillar HLACs was determined by quantitative PCR and is given as x-fold relative to untreated cultures. For each sample, mRNA expression of the target gene was normalized to mRNA expression of the housekeeping gene HMBS (hydroxymethylbilene).


