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Soluble IL-7Rα (sCD127) Inhibits IL-7 Activity and Is Increased in HIV Infection

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Soluble CD127 (sCD127) appears to play an important role in the immunopathogenesis of several chronic infections, multiple sclerosis, and various cancers. The function of sCD127 and whether it influences IL-7 bioavailability or activity is unknown. In this study, we demonstrated that recombinant and native sources of sCD127 significantly inhibited IL-7–mediated STAT5 and Akt phosphorylation in CD8+ T cells. IL-7–mediated proliferation and Bcl-2 expression were similarly reduced by sCD127. In each case, native sCD127 inhibited IL-7 activity to a greater degree than rsCD127. Anti–IL-7 activity was inherent to human plasma and could be reversed by depletion of CD127, revealing for the first time the biological activity of naturally occurring sCD127. Plasma sCD127 concentrations were increased in HIV+ individuals compared with HIV− and could be reversed by depletion of CD127, revealing for the first time the biological activity of naturally occurring sCD127. Plasma sCD127 concentrations were increased in HIV+ individuals compared with HIV− controls, correlated with IL-7 levels, and remained unchanged in HIV+ individuals following 1 y of effective antiretroviral therapy.

Determining the regulation and function of sCD127 may be critical for understanding both the pathogenesis of diseases in which IL-7 likely has a role (e.g., HIV infection, cancer) and its potential impact on IL-7 as a therapeutic approach. The Journal of Immunology, 2010, 184: 4679–4687.

I t has recently been suggested that soluble CD127 (sCD127) plays a role in the immunopathogenesis of several diseases such as multiple sclerosis (MS), acute lymphoblastic leukemia, and HIV infection (1–3). Genetic evidence has revealed an association between soluble isoforms of CD127 and chronic progressive MS, and increased levels of sCD127 transcripts have been documented in children with acute lymphoblastic leukemia. In HIV infection, individuals lacking HIV-specific cytotoxic activity have increased concentrations of sCD127 (3). Although increased levels of sCD127 have been detected in a number of disease states and have been associated with disease activity, the biological function of sCD127 and its clinical relevance remains to be established.

Soluble cytokine receptors have the ability to influence the extracellular bioavailability of their ligand and have been shown to play important roles in several aspects of the immune response including inflammation, cellular proliferation, and apoptosis. In many cases, soluble cytokine receptors act as competitive inhibitors, or, alternatively, they can enhance the activity of the cytokine. The former effect has led to the idea that the release of soluble cytokine receptors is a physiological mechanism for regulating the activity of cytokines, as production of the soluble receptor often parallels synthesis of the ligand (4). In addition to their role in modulating activity of their cytokine ligands, soluble cytokine receptors have been shown to be of prognostic value in a number of diseases and have also been developed as therapeutic agents in the treatment of a number of inflammatory diseases (5). To date, a number of soluble cytokine receptors have been identified, and their mechanisms of release, unique functions, and putative pathophysiological roles have been described (4, 5). The first identified soluble cytokine receptor was IL-2Rα (sIL-2Rα), and it has been associated with leukemia disease progression and is a marker of poor prognosis in children with Hodgkin’s disease (6). The IL-2Rα gene region, a known autoimmune susceptibility locus, possesses significant allelic heterogeneity related to either susceptibility and risk to MS and type 1 diabetes, and these allelic variants independently correlate with plasma sIL-2Rα concentrations (7). Antagonistic soluble receptors including TNFR I and II can inhibit TNF signaling and have been shown to prevent TNF-mediated tumor lysis (8). The IL-6R subunit gp130 has also been shown to inhibit the proinflammatory activity of IL-6 (9). sIL-15Rα is an example of an agonistic cytokine receptor. Pre-complexed IL-15–sIL-15Rα increases NK and CD8+ T cell proliferation and antitumor activity up to 50 times compared with IL-15 alone (10, 11).

The biological role of sCD127 is not yet known, although it is plausible that sCD127 could bind circulating IL-7, thereby decreasing IL-7 bioavailability. In HIV infection, there may be causal links between the increase in plasma IL-7 levels and higher concentrations of sCD127. Therefore, we have investigated the impact of sCD127 on IL-7 activity in human CD8+ T cells and quantified plasma sCD127 concentration in HIV infection to better understand its role in immunopathogenesis.

Materials and Methods

Sample collection and cell isolation

All research conducted using blood from human subjects was approved by the Ottawa Hospital and Ottawa Hospital Research Institute’s Research Ethics Board (Ottawa, Ontario, Canada). Plasma was collected from Fi-cell-Paque gradients of heparinized blood from both HIV+ and HIV− individuals and stored at −80°C. The HIV+ individuals studied were recruited from the HIV clinic at the Ottawa Hospital. Blood was drawn, and plasma was isolated from individuals either naive to antiretroviral therapy or off antiretroviral therapy for >6 mo at the time of sampling.
Plasma from an additional 14 HIV+ individuals was obtained in the context of a previously reported clinical trial of highly active antiretroviral therapy (12). For in vitro tissue culture experiments, PBMCs from healthy HIV seronegative volunteers were isolated by Ficoll-Paque PLUS (Pharmacia Fine Chemicals, Piscataway, NJ) gradient separation, washed twice in PBS, and resuspended in RPMI medium 1640 supplemented with penicillin-streptomycin and 10% FCS at a concentration of 1 × 10^6 cells/ml. Isolation of CD8+ T cells from PBMCs was conducted using a MACS CD8 T Cell Isolation kit (Miltenyi Biotec, Auburn, CA) in accordance with the manufacturer’s instructions, achieving a purity of >98–99% as verified by flow cytometry. Purified CD8+ T cells were resuspended at 10^6 cells/ml in RPMI 1640 supplemented with penicillin-streptomycin and 20% FCS.

**IL-7 and IL-7R reagents**

Recombinant human IL-7 (Sigma-Aldrich, Oakville, Ontario, Canada), a lyophilized product, was resuspended in PBS at a concentration of 10 μg/ml and stored at −20°C. Two sources of sCD127 were used: recombinant human CD127-Fc chimera (CD127-Fc, R&D Systems, Minneapolis, MN) and the culture supernatants of WI-26VA4 cells (WI-sup; American Type Culture Collection, Manassas, VA), a cell line known to secrete CD127 and the culture supernatants of WI-26VA4 cells (WI-sup; American Type Culture Collection, Manassas, VA), a cell line known to secrete CD127 (clone 7417.111, R&D Systems) and anti-CD127 (clone 40131, R&D Systems). Goat anti-human CD127 polyclonal Abs (R&D Systems) generated using a five-parameter logistic curve fit equation (MasterPlex QT software, version 4, MiraiBio, South San Francisco, CA) were used to capture sCD127. The bound sCD127 was detected with human plasma samples. As a control, plasma samples were alternatively passed over an unconjugated Sepharose column.

**Cell culture**

Preincubation with CD8+ T cells, IL-7 (10–100,000 pg/ml) was preincubated with excess CD127-Fc (1000 ng/ml) or WI-sup (undiluted) or unconjugated recombinant human CD8+ T cells (1 × 10^6 cells/ml) were subsequently cultured with IL-7 alone or IL-7–CD127 complexes for various times, using a method adapted from Stoklesak et al. (10) (see below). In experiments designed to block IL-7 activity, cells were preincubated with an excess of anti-CD127 Abs (15 μg/ml) for 1 h prior to the addition of IL-7. Alternatively, IL-7 was preincubated with an excess of anti-IL-7 Abs (10 μg/ml) for 1 h before adding to cells. In addition, CD127–depleted WI supernatants (WI-sup<sub>depleted</sub>) were preincubated with IL-7 and added to cells as above.

**IL-7 signaling pathways**

The phosphorylation of STAT5 in CD8+ T cells was analyzed by flow cytometry (Alexa Fluor 488 mouse anti-human STAT5 pY694, BD Biosciences, San Jose, CA) following 15 min of culture. Specific inhibition of IL-7–induced phosphorylated STAT5 (pSTAT5) was confirmed by pre-treating cells with a Jak inhibitor (Calbiochem, Gibbstown, NJ). Preincubation of phospho-STAT5 was measured by flow cytometry. Cells were labeled with the anti-human STAT5 Ab using a fixing and permeabilization kit (Invitrogen, Carlsbad, CA) using a method modified from the manufacturer’s instructions. The cells were washed and incubated with fixing solution for 20 min at 37°C. Cells were washed again and incubated with cold methanol (100%) for 10 min at 4°C. Following another wash, cells were incubated with a fixing and permeabilization reagent and the anti-STAT5 Ab for 20 min. The proportion of phosphorylated STAT5<sub>5</sub> was calculated as follows: (mean fluorescence intensity [MFI] of cells incubated with plasma and IL-7)/MFI of cells incubated with IL-7 alone) × 100%. Phosphorylated Akt in cell lysates was assessed by Western blot after 30, 60, or 120 min of culture. As a loading control, the expression of β-actin was detected using a mouse anti-human Ab (Calbiochem). Cell pellets were washed in lysis buffer and lysates containing in lysis buffer (5 mM HEPES [pH 7.5], 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl<sub>2</sub>, and 1 mM EDTA) (40 μg/ml) for 1 h. Lysates were then centrifuged for 20 min at 14,000 rpm, 4°C, and supernatants were collected and stored at −20°C. Lysate concentrations were quantified using a BCA Protein Assay kit (Pierce, Rockford, IL). A total of 5 μg protein was resolved by 10% SDS-PAGE and blotted onto polyvinylidene fluoride membranes. Membranes were blocked overnight in 5% skim milk and incubated in a rabbit-anti-human pAkt<sub>1,4,7</sub> Ab (1:5000) followed by an anti-rabbit-IgG HRP Ab (1:10,000) (Cell Signaling Technology, Boston, MA). Proteins were detected using the Lumigen TMA-6 kit (GE Healthcare, Buckinghamshire, U.K.). Denitometry analysis of protein bands was performed using AlphaEase FC Software 6.0 (α Innotech, San Leandro, CA), and relative changes in protein expression of treated cells were calculated compared with unstimulated controls.

**Bcl-2 expression**

After 48 h of culture, intracellular Bcl-2 staining was performed using the BD Biosciences FITC-conjugated Bcl-2 Ab reagent set, following the manufacturer’s protocol (BD Biosciences). Cells were washed with PBS and resuspended in fixation buffer (100 μg/ml) (eBioscience, San Diego, CA) and incubated at room temperature for 20 min. Following incubation, cells were pelleted, washed, resuspended in permeabilization buffer (100 μg/ml) with FITC-conjugated Bcl-2 Ab (2 μg/ml) (BD Biosciences), and incubated at room temperature for 20 min. Samples were washed and then resuspended in flow staining buffer (0.5 ml) (10% FCS) (eBioscience). Analysis was performed by flow cytometry, collecting 10,000 events, with data represented as values of MFI.

**Cell proliferation**

Following isolation, cells were labeled with CFSE (CellTrace CFSE Cell Proliferation kit) using a method modified from the manufacturer’s specifications (Invitrogen Canada, Burlington, Ontario, Canada). A stock solution (5 μM of CFSE) was prepared in DMSO followed by the preparation of a working solution (8 μM) of CFSE in PBS plus 0.1% BSA. Cells were resuspended in CFSE working solution (1 × 10<sup>5</sup> cells/ml) and incubated at 37°C in the dark for 10 min. Cells were then incubated with 15 volumes of complete RPMI 1640 on ice in the dark for 5 min and then washed and resuspended in complete RPMI 1640 (1 × 10<sup>5</sup> cells/ml). Cells were cultured with medium only, PHA (2.5 μg/ml; Sigma-Aldrich), PHA plus IL-7 (10,000 pg/ml), or PHA plus IL-7 preincubated with CD127-Fc or WI-sup. The concentration of PHA used was the lowest tested concentration (i.e., submaximal dose) that induced at least two rounds of cell division among activated cells. Cells were cultured for 4 d, and then cell division was assessed by flow cytometry and analyzed using FCS Express 2 (De Novo Software, Los Angeles, CA). Individual cell divisions of the stimulated T cells were evaluated according to established methods involving CFSE analysis of T cell division (14). Data are represented as the proportion of stimulated T cells that have undergone ≥3 divisions.

**Quantification of plasma CD127 and IL-7**

Quantification of plasma CD127 was carried out using a CD127-specific Ab-bead microfluorosphere assay that has been recently developed (15). Goat anti-human CD127 polyclonal Abs (R&D Systems) were covalently coupled to carboxylated-modified fluorescent beads (Luminex, Austin, TX) were used to capture CD127. The bound CD127 was detected with biotinylated mouse anti-human CD127 Ab (clone hIL-7-R-M21; BD Biosciences) and streptavidin-PE (Invitrogen). Sample concentrations were extrapolated from standard curves of recombinant human CD127-Fc chimera (R&D Systems) generated using a five-parameter logistic curve fitting equation (MasterPlex QT software, version 4, MiraiBio, South San Francisco, CA).

In addition, an sCD127 ELISA was designed to measure sCD127 in plasma. Briefly, 96-well plates were coated overnight with mouse anti-human CD127 mAb (clone R34.34; Beckman Coulter). Plates were blocked with BSA for 1 h, washed, and then samples were incubated for 1 h. Bound sCD127 was detected following a 1-h incubation with goat anti-human CD127 polyclonal Abs (R&D Systems) and bovine anti-goat IgG-HRP. Reactions were developed using the SureBlue Reserve TMB microwell peroxidase substrate (KPL, Gaithersburg, MD) and visualized with a plate reader at 450 nm. Sample concentrations were also extrapolated from standard curves of recombinant human CD127-Fc chimera (R&D Systems).

Plasma IL-7 concentrations were measured using the IL-7 Quantikine ELISA (R&D Systems) according to manufacturer instructions. The IL-7 capture Ab was coupled to beads as described above. The conjugated beads were incubated with plasma samples (diluted 1/5 in PBS containing 0.05% Tween-20, 1% BSA, 0.05% NaN<sub>3</sub>, and 0.9 mg/ml EDTA [Sigma-Aldrich]) for 2 h at room temperature. The bound IL-7 was revealed with the biotinylated detection Ab for 1 h and streptavidin-PE. The bead analysis was done as described above. The lower detection limit of the assay was 0.5 pg/ml.

**Statistical analysis**

Data from in vitro assays were graphed and analyzed by one-way ANOVA test and the simultaneous Dunnett’s test or paired or unpaired (as appropriate) Student t tests. Plasma sCD127 concentrations were analyzed by
linear correlation analyses ($p \leq 0.05$) to determine any correlations with CD4 counts, viral loads (log transformed), or plasma IL-7 concentrations. All statistical analyses were conducted using GraphPad Prism 5.0 Software (GraphPad, San Diego, CA).

Results

sCD127 decreases IL-7–induced phosphorylation of STAT5

The principal intracellular signaling pathway of the γc cytokine receptors in T cells involves the activation of Jak-STAT signaling molecules. After 15 min of culture, increasing concentrations of IL-7 significantly induced the phosphorylation of STAT5 in CD8+ T cells, and this effect quickly plateaued (Fig. 1A), consistent with previous reports (16). This increase was evident at IL-7 concentrations as low as 10 pg/ml and plateaued at concentrations ≥100 pg/ml ($p < 0.0001$ by ANOVA) after 15 min of incubation and decreased significantly after further culture (data not shown). To determine if sCD127 had an impact on the activation of this pathway by IL-7, isolated CD8+ T cells were cultured with medium alone, IL-7, or IL-7 plus each of two sources of sCD127 (CD127-Fc chimera or WI-sup), and pSTAT5 was quantitated. Culturing CD8+ T cells with either IL-7–CD127 Fc (Fig. 1B) or IL-7–WI supernatant (Fig. 1C) complexes resulted in decreased expression of pSTAT5 compared with that seen with IL-7 alone ($p = 0.025$ and $p \leq 0.0001$, respectively). This effect was more significant with WI culture supernatant, which reduced IL-7–induced pSTAT5 expression by 52.7% (mean MFI = 17.78 ± 1.27) compared with the IL-7–rCD127-Fc chimera complexes, which reduced this by 12.3% (mean MFI = 31.46 ± 1.59; $p = 0.0001$) (Fig. 1D).

Anti-IL-7 Abs completely abrogated the effects of IL-7 on STAT5 phosphorylation (Fig. 1E). Anti-CD127 Ab (clone R34.34), also reportedly able to block the binding of IL-7 to CD127, inhibited IL-7 activity, although to a lesser extent and in the range of what was seen with sCD127 (Fig. 1F). This confirms a previous report demonstrating that this Ab has less than complete IL-7 inhibition properties (17). CD127-depleted WI-sup had no effect on IL-7–induced pSTAT5, confirming that the effect caused by WI-sup was CD127-specific (Fig. 1G).

sCD127 decreases IL-7–induced phosphorylation of Akt

The PI3K pathway is also activated upon IL-7 ligation of membrane-associated CD127 and has been associated with IL-7–induced T cell proliferation (18). Once activated, a cascade ensues, ultimately resulting in phosphorylation of Akt, a known mediator of cell cycling molecules. To determine if IL-7–induced activation of the Akt pathway is affected by sCD127, purified CD8+ T cells were treated as above and pAkt was measured by Western blot. IL-7 increased pAkt expression in a dose- and time-dependent manner. Responses peaked after 1 h of culture (Fig. 2A), consistent with published observations (16). Therefore, all further experiments involved culturing cells for 1 h with or without 1000 pg/
ml of IL-7 in the presence or absence of sCD127. The addition of IL-7 preincubated with either rCD127-Fc or WI culture supernatant significantly decreased IL-7–induced pAkt expression ($p = 0.047$ and $p = 0.028$, respectively; Fig. 2B, 2C). There appeared to be a greater inhibition of IL-7–mediated Akt phosphorylation with WI supernatant compared with CD127-Fc, although this difference did not reach statistical significance.

**Downregulation of IL-7–induced Bcl-2 expression by sCD127**

Complementing its role in T cell development and homeostasis, IL-7 increases the expression of cell survival and antiapoptotic molecules including Bcl-2. After 48 h of culture with IL-7 (1000 pg/ml), intracellular Bcl-2 expression in isolated CD8$^+$ T cells increased significantly ($p = 0.01$; Fig. 3C). Preincubation of IL-7 with rCD127-Fc did not significantly decrease Bcl-2 expression (Fig. 3A, 3C) compared with IL-7 alone. In contrast, WI-sup's did decrease IL-7–induced Bcl-2 expression ($p = 0.03$; Fig. 3B, 3C). Inhibition of IL-7 activity was more evident in the presence of anti–IL-7 or anti-CD127 Abs (Fig. 3D, 3E). This effect was further confirmed to be CD127-specific because CD127-depleted WI-culture supernatant had no effect on IL-7–induced Bcl-2 expression (Fig. 3F).

**IL-7–induced proliferation is reduced by sCD127**

It has been well described that IL-7 induces T cell proliferation and hence is a potential IL-7 activity that could be inhibited by sCD127. To investigate this possibility, isolated CD8$^+$ T cells were CFSE-labeled and cultured for 5 d with submaximal concentrations of the mitogen PHA. Such stimulation enables CD8$^+$ T cells to respond to the proliferative effects of IL-7 (Fig. 4A, 4B), as reported previously (19, 20). The proliferation of mitogen-stimulated CD8$^+$ T cells increased significantly in the presence of IL-7 ($p = 0.02$). When IL-7 was preincubated with rCD127-Fc or WI-supernatant, the proportion of cells dividing three or more times reduced significantly compared with cells stimulated in the absence of these sources of sCD127 ($p = 0.05$ and $p = 0.02$, respectively; Fig. 4C–E). Analysis of the proportion of cells dividing $>1$ division yielded similar results (data not shown). Unlike the inhibition of STAT5 phosphorylation and Bcl-2 expression, there was no significant difference in the proliferative effects of CD127-Fc compared with WI-supernatant.

**Activity of sCD127 in human plasma**

Plasma samples were preincubated with IL-7 for 1 h, cultured with CD8$^+$ T cells for 15 min, and then STAT5 phosphorylation was measured by flow cytometry. Thirteen independent plasma samples from eight different donors possessed anti–IL-7 activity, as its addition to isolated CD8$^+$ T cells inhibited IL-7–induced phosphorylated STAT5 levels. This provided the opportunity to determine if sCD127 was responsible for this activity and therefore may be able to block IL-7 activity in vivo. sCD127 was depleted from the plasma by CD127-Ab affinity chromatography, whereas duplicate samples of plasma were passed through an un conjugated
The depletion of sCD127 was confirmed using an sCD127-specific ELISA (data not shown). Depletion of sCD127 from 10 of these 13 samples reversed the anti–IL-7 activity, restoring pSTAT5 levels to near those seen in the absence of plasma (Fig. 5A, 5B). In plasma samples that did not possess anti–IL-7 activity, depletion of sCD127 had no effect on IL-7–induced STAT5 phosphorylation (data not shown, n = 8).

These results indicate that sCD127 present in human plasma is capable of inhibiting IL-7 activity, revealing for the first time its biological activity.

**Plasma CD127 concentrations are increased in HIV infection**

The expression of plasma sCD127 can be detected in both HIV− and HIV+ individuals, as shown previously by Western blot analysis.

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**FIGURE 3.** Increased expression of intracellular Bcl-2 by IL-7 is reduced by a native source of sCD127. A and B, Representative histograms depict the intracellular Bcl-2 expression of isolated CD8+ T cells cultured with medium (black line), IL-7 (black fill), and IL-7 plus rCD127-Fc chimera (dark gray fill) or IL-7 plus WI-26VA4 supernatants (light gray fill) for 48 h of culture. The dotted lines represent unstained cells. C, The expression of Bcl-2, shown as MFI, is summarized in the bar graphs (n = 6). *p = 0.01; **p = 0.03 by Student t test. This downregulation was CD127-specific as anti–IL-7 (D) and anti-CD127 (E) decreased IL-7–induced Bcl-2 expression, whereas CD127-depleted WI-26VA4 supernatants had no effect (F).

**FIGURE 4.** IL-7–induced proliferation of CD8+ T cells is reduced by sCD127. Representative scatter plots illustrate the cell division of CFSE-labeled CD8+ T cells stimulated for 5 d with medium only (gray dots) or PHA (black dots) (A) and PHA plus IL-7 (10,000 pg/ml) (B), PHA plus IL-7 preincubated with rCD127-Fc (C), or PHA plus IL-7 preincubated with WI-sup (D). Cells in divisions ≥3 divisions are shown in hatched boxes, and the proportion (%) of cells are indicated. E, The proportions of CD8+ T cells undergoing ≥3 divisions are summarized in the bar graph. *p = 0.02; **p = 0.05; ***p = 0.02 by Student t test; n = 5.
(21). In this study, the concentration of sCD127 was quantified in the plasma of 55 HIV+ individuals not receiving antiretroviral therapy and compared with 58 HIV− controls, using a recently described CD127-specific Ab-microfluorosphere assay (15).

The mean concentration of plasma sCD127 was significantly higher in HIV+ individuals (228.3 ± 110.7 ng/ml) compared with healthy HIV− controls (175.7 ± 161.7 ng/ml) (p = 0.024) (Fig. 6A). Statistical significance remained following analysis of the data with exclusion of a single very high value in the HIV+ group (sCD127 = 859 ng/ml) (p = 0.05). To determine if the concentration of sCD127 was associated with measures of HIV disease progression known to correlate with CD127 expression on CD8+ T cells (e.g., CD4 count, viral load, plasma IL-7), potential correlates of sCD127 concentration in HIV+ individuals were examined. The mean ± SD CD4 count of the HIV+ individuals was 369.35 ± 195.20 cells/μl. The mean ± SD viral load (plasma HIV

FIGURE 5. Plasma sCD127 reduces IL-7 activity. Undepleted or CD127-depleted plasma samples were preincubated with IL-7 (1000 pg/ml) for 1 h and then added to isolated CD8+ T cells and cultured for 15 min. The expression of pSTAT5 was then analyzed by flow cytometry. A, Two representative histograms of pSTAT5 expression by CD8+ T cells are shown. Cells were cultured with IL-7 (1000 pg/ml) preincubated with medium only (black line), undepleted plasma (dark gray), or CD127-depleted plasma (light gray). B, Depletion of sCD127 reversed the anti–IL-7 activity of plasma in 10 of 13 samples. The data are summarized in C. *p = 0.006 by paired Student t test.

FIGURE 6. Plasma sCD127 concentrations are increased in HIV infection. A, Plasma sCD127 was measured in HIV− (n = 58) and HIV+ (n = 55) individuals using a CD127-specific Luminex assay. Plasma from HIV+ individuals had significantly more sCD127 than HIV− individuals. *p = 0.024 by Student t test. The lower detection limit of this assay was 50 pg/ml (dotted line). There were no correlations between plasma CD127 concentrations and CD4 counts (B) or viral loads (C) (log transformed). D, Plasma sCD127 correlated positively with plasma IL-7 concentrations in HIV+ individuals (p = 0.024, r = 0.305). The lower detection limit of the IL-7 assay was 0.5 pg/ml.
analyses were not conducted in the HIV returns to near normal levels in HIV + individuals following highly active antiretroviral therapy (Fig. 7). Very low or undetectable (≤8 pg/ml) concentrations may decrease during antiretroviral therapy-induced immune reconstitution. Therefore, plasma sCD127 was quantified in samples from antiretroviral naive HIV + individuals receiving highly active antiretroviral therapy (12). Plasma sCD127 concentrations did not change significantly over 48 wk of effective therapy (Fig. 7).

Discussion
Despite the discovery of a soluble form of CD127 capable of binding IL-7 almost 20 y ago (13), a functional role for the soluble receptor has not been described. Because there is an indication that sCD127 plays a role in the immunopathogenesis of a number of diseases (24, 26–29), and there are ongoing clinical investigations of IL-7 as a potential HIV or cancer therapeutic, we sought to investigate whether sCD127 was capable of influencing IL-7 activity. Both signaling and functional outcomes of IL-7 in CD8 + T cells were inhibited by sCD127. In addition, depletion of CD127 from plasma revealed for the first time the biological activity of naturally occurring human CD127.

sCD127 reduced the activation of IL-7 signaling pathways, as indicated by the decreased phosphorylation of STAT5 and Akt (Figs. 1, 2). Activation of STAT5 by IL-7 has been associated with increased glucose uptake and activation of Akt (16), and murine models suggest that STAT5, although not independently, may promote long-term survival of T cells by inducing Bcl-2 production (30). In the case of the phosphorylation of STAT5, and to a lesser degree Akt phosphorylation, native CD127 (WI supernatant) inhibited IL-7 signaling to a greater degree than its recombinant counterpart. This suggests that possible conformational differences between native and recombinant sources of sCD127 affect the nature of their interactions with IL-7, such as receptor-ligand binding affinities. In a separate issue, anti–IL-7 and anti–CD127 Abs decreased IL-7 activity more than either source of sCD127, demonstrating that natural inhibition of IL-7 activity can be enhanced by artificial means. Phosphorylation of Akt, a PI3K pathway molecule common to γc cytokines, is important for promoting T cell proliferation (18). Indeed, we demonstrated that preincubation of IL-7 with sCD127 resulted in decreased proliferation of CD8 + T cells in vitro (Fig. 4). The limited reduction in IL-7–induced Bcl-2 expression by sCD127 suggests that there is a low threshold of IL-7 signaling required to produce Bcl-2. This would not be surprising given that in homeostasis, limited physiological concentrations of IL-7 in the circulation (22, 31) are able to maintain the survival of a vast T cell population. Collectively, inhibition of early IL-7 signaling pathways by sCD127 can result in measurable decreases of IL-7–associated activities in vitro, suggesting the sCD127 is an IL-7 antagonist in this setting and does not exhibit pleiotropic effects in vitro. Opposing effects of soluble cytokine receptors have not been demonstrated in vitro; however, there have been discrepancies such as with sIL-15Rα, which inhibited IL-15 activity in vitro (32) yet enhanced this activity in vivo (10, 11, 33). The depletion of sCD127 from plasma suggests that the inhibitory properties of sCD127 observed in vitro may be of biological relevance to the activity of IL-7 in vivo. It is not clear if this would occur in all individuals, as decreased activity was observed in 13 of 21 experiments (62%), and in two cases where plasma decreased IL-7 signaling (Fig. 5B), depletion of sCD127 further decreased IL-7 activity, suggesting that other factors in the plasma may be involved.

The concentration of sCD127 (1000 ng/ml) required in this study to reduce IL-7–induced activity of CD8 + T cells in vitro was near the range of plasma IL-7 concentrations in healthy and HIV + individuals (175.7 ng/ml and 228.3 ng/ml, respectively; Fig. 5), and tissue concentrations may be significantly higher (34). As such, the physiological consequences of this may be more pronounced than those observed in vitro. Other soluble cytokine receptors known to be produced at similar concentrations in vivo include sTNFR with concentrations approaching 2000 ng/ml (35), and up to 75 ng/ml of IL-6R has been detected in the sera of HIV + individuals (36). However, comparably lower concentrations of sIL-2Rα (6 ng/ml) were measured in the serum of patients with MS, yet these concentrations were able to inhibit IL-2 activity in vitro (37). The primary source(s) of sCD127 in plasma has not been determined, but may be the result of alternative splicing of mRNA transcripts encoding the membrane-bound form of CD127 (13), receptor shedding (21), receptor cleavage (38), or a combination thereof.

Using a quantitative CD127 assay, it was determined that plasma sCD127 concentrations were higher in HIV + individuals compared with HIV – individuals, consistent with our previous report and confirming the initial report by Carini et al. (3, 21). Recent reports describe the detection of sCD127 in human plasma, but either decreased levels (39) or similar levels (40) in HIV-infected individuals compared with healthy individuals. In these reports, two different ELISA methods with different Abs were used. In the first report, a polyclonal anti-CD127 Ab for plate coating and a monoclonal Ab (41031 clone, R&D Systems) for detection of plate-bound sCD127 was used, whereas the second report describes what appears to be a similar assay, but uses mAbs for coating (M21 clone, BD Biosciences, or 40131, R&D Systems) and a polyclonal Ab for detection. It is unclear if IL-7 in plasma interferes with CD127 detection by either of these assays, and it is possible that elevated levels of IL-7 in HIV + individuals lead to decreased detection of sCD127 in this setting. Furthermore, the concentrations of sCD127 detected in these reports are consistently <50 ng/ml, which is the lower threshold of the LumineX assay and considerably lower than the levels measured by this

![FIGURE 7](http://www.jimmunol.org/DownloadedFrom/FIG7.jpg)
sensitive assay. It is also possible that the narrow detection range of the ELISA prevented the detection of a correlation between sCD127 concentration and IL-7 concentration in HIV+ individuals as was observed in this study (Fig. 6D).

The biological role that sCD127 has in vivo remains to be established, although these data suggest that the inhibition of IL-7 activity by its soluble receptor may be of clinical relevance (Fig. 5). Furthermore, these effects may be more pronounced in primary and secondary lymphoid organs, where IL-7 concentrations would be expected to be much higher than in the plasma (34). The concentration of sCD127 in HIV+ individuals did not correlate with other prognostic markers such as viral load or CD4+ T cell counts; however, there was a positive correlation with plasma IL-7, which is known to be increased in HIV disease (41) (Fig. 6).

Despite the immunoreostorative effects of highly effective antiretroviral therapy, which include increased CD4 counts and the re-expression of membrane-bound CD127 to near normal levels (24, 25), sCD127 concentrations remained stable over time (Fig. 7). Similarly, plasma IL-7 concentrations have been shown to remain elevated with effective antiretroviral therapy (26, 27, 42, 43). Sustained concentrations of sCD127 despite the favorable response to antiretroviral treatment is analogous to what has been observed with sIL-2Rα, which is increased in severe MS yet is not influenced by effective treatment (37). Stable concentrations of sCD127 in HIV infection may prevent or diminish any potential immunoreostorative effects of elevated concentrations of IL-7.

The present data, derived from in vitro experiments, suggest that sCD127 inhibits the activity of IL-7. The fact that IL-7 activity was not completely abrogated in the presence of excess amounts of sCD127 suggests low affinity between sCD127 and IL-7. It has been shown that IL-7 activities are mediated through high-affinity interactions between IL-7 and membrane-bound CD127 (44). The soluble form of a cytokine receptor may be expected to have lower affinity for its ligand compared with its membrane-bound form, as is the case with soluble IL-2Rα (5). It is however possible that sCD127 could act as a carrier of IL-7 in vivo over time, thereby increasing its half-life as has been seen with other cytokine receptors such as sIL-15Rα, which enhanced the activity of IL-15 in mice in vivo (10, 11). In fact, a recent report demonstrated that binding IL-7 with a unique, nonblocking, anti–IL-7 mAb was actually able to promote greater T cell expansion in vivo compared with IL-7 alone (45). Lastly, whether this inhibition of IL-7 activity occurs in other cell types expressing CD127 in health and disease is not known. Macrophages increase surface CD127 expression in response to HIV Tat protein, whereas CD4+ T cells decrease expression in HIV infection, similar to CD8+ T cells (24, 27). Expression of CD127 is limited to immature B cells through early pre-B stages and human B cells, unlike T cells, are not dependent on IL-7 (46). Therefore, cell-type specific factors may influence the activity of sCD127.

There is evidence suggesting that sCD127 may be a marker of immunopathogenesis in several diseases. A trend of decreased mRNA encoding both membrane-associated CD127 and sCD127 was detected in individuals with MS expressing a GCA haplotype of CD127, which is associated with chronic progressive MS (1). Similarly, a recent study of an HIV+ cohort associated CD127 gene haplotypes derived from two exonic single nucleotide polymorphisms (Thr244Ile and Val356Ile), both implicated with the progression of MS (47), with rapid HIV disease progression (48). The expression of mRNA encoding sCD127 was detected in leukemic cell samples of children with acute lymphoblastic leukemia, although no comparison was made to healthy controls (2). Another possible functional role of sCD127 in vivo may involve decreasing CTL activity. This has previously been suggested by Carini et al. (3), who detected more sCD127 in HIV+ individuals with undetectable HIV-specific CTL activity compared with those with detectable CTL activity. It has been suggested that, in health, the concentration of IL-7 is limited (49). Our results suggest that the presence of increased amounts of sCD127 in disease would counter this otherwise altruistic model of IL-7 bioavailability given its ability to inhibit the activity of IL-7.

Exogenous IL-7 has been shown to preferentially promote the homeostatic proliferation of human naïve T cells in vivo (50), a desirable clinical outcome for lymphopenic patients. Initial clinical trial results indicate that IL-7 therapy improves T cell survival and proliferation of nonhuman primates and in humans in the context of HIV infection and in cancer (51–53). The influence of sCD127 on IL-7 activity may be relevant to IL-7 therapy in which high concentrations of IL-7 are achieved (up to 10 μg/kg) (51), and this may become quite pronounced if the exogenous IL-7 induces additional release of sCD127. This resulting increase in the concentration of plasma sCD127 may potentially reduce the efficacy of the exogenous IL-7. Elucidation of the exact role of sCD127 on IL-7 physiology and function in vivo, both under normal conditions and in disease, is needed to more fully understand its role in the regulation of the immune system, its potential role in disease pathogenesis, and the impact it may have on IL-7 therapies in HIV+ individuals or in cancer patients.

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References


