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IL-7 Induces Immunological Improvement in SIV-Infected Rhesus Macaques under Antiviral Therapy

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Despite efficient antiretroviral therapy (ART), CD4+ T cell counts often remain low in HIV-1-infected patients. This has led to IL-7, a crucial cytokine involved in both thymopoiesis and peripheral T cell homeostasis, being suggested as an additional therapeutic strategy. We investigated whether recombinant simian IL-7-treatment enhanced the T cell renewal initiated by ART in rhesus macaques chronically infected with SIVmac251. Six macaques in the early chronic phase of SIV infection received antiretroviral treatment. Four macaques also received a 3-wk course of IL-7 injections. Viral load was unaffected by IL-7 treatment. IL-7 treatment increased the number of circulating CD4+ and CD8+ memory T cells expressing activation (HLA-DR+, CD25+) and proliferation (Ki-67+) markers. It also increased naive (CD45RAbrightCD62L+) T cell counts by peripheral proliferation and enhanced de novo thymic production. The studied parameters returned to pretreatment values by day 29 after the initiation of treatment, concomitantly to the appearance of anti-IL-7 neutralizing Abs, supporting the need for a nonimmunogenic molecule for human treatment. Thus, IL-7, which increases T cell memory and de novo renewal of naive T cells may have additional benefits in HIV-infected patients receiving ART. The Journal of Immunology, 2006, 176: 914–922.

Infection with HIV or SIV leads to severe T lymphopenia and general immune dysfunction. Several mechanisms, including direct and indirect T cell killing, disrupted peripheral homeostasis, and impaired central de novo production, contribute to lymphopenia (1–3). Combinations of antiretroviral treatments targeting various viral proteins/functions considerably slow disease progression, allowing considerable decreases in viral load and, in most patients, significant increases in peripheral CD4+ T cell counts (4). However, although such treatments generally decrease levels of virus production to undetectable levels, they often fail to reverse lymphopenia and do not permit the restoration of specific antiviral immune responses (5–8).

IL-7, which is constitutively produced by the bone marrow, the thymus, mucosal lymphoid tissues, and lymph nodes (9), plays a crucial role in T cell homeostasis. This cytokine is implicated in thymopoiesis, in which it sustains thymocyte proliferation and survival (10, 11). It also regulates peripheral naïve T cell survival by modulating production of the antiapoptotic molecule Bcl-2 (12) and sustaining peripheral T cell expansion in response to antigenic stimulation in mice (13–15).

HIV-infected patients generally have high plasma IL-7 concentrations. The increase in IL-7 production generally occurs in the first few weeks of infection and persists throughout disease progression. The inverse correlation between plasma IL-7 concentrations and CD4+ T cell counts (16–18) suggests either a feedback mechanism for restoring peripheral T cell counts in lymphopenic patients or an increased IL-7 availability in lymphopenic hosts (15, 16, 19). Moreover, recent studies suggest that plasma IL-7 concentration might be a good predictive marker of CD4+ T cell restoration under therapy (20–22).

Previous studies in macaque models have demonstrated that exogenous IL-7 induces the expansion of naive and memory peripheral T cell populations in both healthy and SIV-infected animals (23, 24). However, the possible effects of modifying thymic production in response to IL-7 injections remain unclear.

Thymic output can be estimated by quantifying the signal joint T cell receptor excision circle (sJTREC), generated during TCRβ locus deletion, which usually precedes TCRα-chain rearrangement (25, 26). However, as recently demonstrated (26), thymic function can be estimated more accurately in HIV-infected humans by quantifying precursor T cell proliferation. Indeed, such proliferation has a direct impact on both the number of cells subjected to thymic selection and the dilution of DJβTRECs (byproducts of TCRβ-chain rearrangement). This dilution effect can be followed by measuring, in PBMC, the sJβTREC ratio (26). In contrast to sJTREC frequency, which decreases during T cell proliferation, the

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4 This work is dedicated to the memory of Nicole Israel.

5 Abbreviations used in this paper: sJTREC, signal joint T cell receptor excision circle; sIL-7, recombinant simian IL-7; ART, antiretroviral therapy; rHL-7, recombinant human IL-7; RTE, recent thymic emigrant; ISP, intermediate single positive.

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Materials and Methods

Animals and virus infection

Six young rhesus macaques (2 years old), housed and cared for in accordance with European guidelines, were included in this study. These animals were demonstrated to be seronegative for SIVmac, simian T cell leukemia virus type 1, simian retrovirus type 1 (type D retrovirus), and herpesvirus B. All of the animals were inoculated with 10 AID50 (50% animal infective doses) of the pathogenic SIVmac251 isolate (provided by A.-M. Aubertin, Institut National de la Santé et de la Recherche Médicale Unité 544, Strasbourg, France).

Antiretroviral and IL-7 treatment

All animals were injected with antiretroviral treatment at day 70 after infection, during the early asymptomatic chronic phase of infection, to mimic human thretherapy treatment. The antiretroviral treatment consisted of AZT (GluosmithKline; 4.5 mg/kg twice per day), 3TC (GlaxoSmithKline; 2.5 mg/kg twice per day), and ddi (Videx; Bristol Myers; 3 mg/kg twice per day).

rsIL-7 was originally cloned by Cytheris from Macaca mulatta and filed as Q95J83 in the “Swiss Prot” data bank; this sequence was further confirmed to be identical with the Papio papio (baboon) IL-7 sequence filed as Q8HZN1 in the same bank (27).

The rsIL-7 injections were performed after a significant decrease (2 log of viral load expressed in number of RNA copies per milliliter) in viral load due to antiretroviral treatment. Four infected animals treated with antiretroviral drugs for 34 days received injections s.c. every 2 days for 26 days with 100 µg/kg recombinant macaque IL-7 (rsIL-7) derived from Escherichia coli. IL-7 was supplied by Cytheris (CYT 01 008). These four infected macaques continued to receive ART during the period of rsIL-7 treatment. The antiretroviral treatment was stopped 17 days after the end of rsIL-7 treatment. The four infected macaques treated with both antiretroviral drugs and rsIL-7 were macaques 3, 4, 5, and 6. During the same period, the two control macaques, macaques 1 and 2, received only antiretroviral drugs. All of the animals were treated with antiretroviral drugs for 77 days.

Table I. Primers and probes for the quantification of sjTREC, DJβTRECs, and CD3γ-chain

| DNA Circles Quantification | sjTREC | Out' | CTCTCTATCTCTGCTCTGAA | In3' | GTUCCGATCTCAGAGTGTG | Out3' | ACATGGCTCCCTGGGCTGCTGTP | In5' | TAGGCCACCTCCCTTTTCAAA | DJβ1 | Out | CTCACTCTGCTGCTCTGCTTGT | In | TGGGCAAGGAGAGAAGAG | Jβ1S2 | Out | CTCTCTATCCCTCTCTGCTTG | In | TCCGCTACAGGAAAGAATG | Jβ1S3 | Out | TGGGACCTGGGAGAGCAGGA | In | AGAGGTAGGGAAGCCAGATG | Jβ1S4 | Out | TGGGCAAGGAGAGAAGAG | In | TCCGCTACAGGAAAGAATG | Jβ1S5 | Out | GAAAGTCAAGGAGAGCAGGAA | In | CTCTCTATCCCTCTCTGCTTG | Jβ1S6 | Out | ATGCTCCCTCTCTCAGGATG | In | TGGGCAAGGAGAGAAGAG | Jβ2 | Out | TGGGCAAGGAGAGAAGAG | In | TCCGCTACAGGAAAGAATG | Jβ2S1 | Out | CTCTCTATCCCTCTGCTTG | In | TGGGCAAGGAGAGAAGAG | Jβ2S2 | Out | TGGGCAAGGAGAGAAGAG | In | TCCGCTACAGGAAAGAATG | Jβ2S3 | Out | TGGGCAAGGAGAGAAGAG | In | TCCGCTACAGGAAAGAATG | Jβ2S4 | Out | TGGGCAAGGAGAGAAGAG | In | TCCGCTACAGGAAAGAATG | CD3 | Out' | AAGAAGGACTGGAGGAGAGAGA | In | GCCTCTGACGAAAGGACATGA | Jβ3 | Out | TGGGCAAGGAGAGAAGAG | In | TCCGCTACAGGAAAGAATG | CD3γ | Out' | AAGAAGGACTGGAGGAGAGAGA | In | GCCTCTGACGAAAGGACATGA

LightCycler Probes

| sj | P1 | AATAAGTGGTGACGCCCCTACAGACT fluo |
| DJβ1 | P1 | CTGGCTGCATTGGTGGGAAGGACTTTCTGCATAGAGGAGAGAG |
| DJβ2 | P1 | GATCCGATGAGGAGGCTCCCTTTTCAACCA CCCTTTTCAACCA |
| CD3γ | P1 | GGCTGTAGGATGAGGACTACCAATATTCTGTCCTTCfluoro |
| CD3 | P1 | GTGCTAGGATGAGGACTACCAATATTCTGTCCTTCfluoro |

All procedures with animals were performed after anesthesia with ketamine (Imalgène 1000; Mérial).
**TREC quantifications**

Primers specific for each of the sjTREC (6Rec-dleo), nine DJβB TREC pairs (DBJ-BJJS2 to DJ2-J3JS4), and the human CD3γ-chain of the gene (see Table I) were defined on macaque germline sequences (GenBank accession nos.: L43137, L43138, DQ805631, and DQ805632). Parallel quantification of each deletion circle and of the CD3γ-amplon was performed for each sample, using LightCycler technology (Roche Diagnostics) as previously described (28). Briefly, PBMCs were lysed by incubation in Tween 20 (0.05%), Nonidet P-40 (0.05%), and proteinase K (100 µg/ml) for 30 min at 56°C, and then 15 min at 98°C. Multiplex PCR amplification was performed for sjTREC or each of the nine DJβB TREC pairs, together with the CD3γ-chain, in a final volume of 100 µl (10 min initial denaturation at 95°C, then 22 cycles of 30 s at 95°C, 30 s at 60°C, 2 min at 72°C) using outer 3/5’ primer pairs. PCR conditions in the LightCycler experiments, performed on 1/100th of the initial PCR products, were as follows: 1 min initial denaturation at 95°C, then 40 cycles of 1 s at 95°C, 10 s at 60°C, 15 s at 72°C, Fluorescence measurements were performed at the end of elongation. TREC and CD3γ LightCycler quantifications were performed in independent experiments, using the same first-round serial dilution standard curve. This highly sensitive nested quantitative PCR assay made it possible to detect one copy in 10^3 cells for each DNA circle. The TREC and the nine DJβB TREC pairs were quantified in triplicate for all the samples studied. The sum of DJβB TREC frequencies (DJβB TREC) was estimated to be 13 times the mean of the nine measured DJβB TREC frequencies. The sj/TREC ratio corresponds to the sjTREC frequency divided by the sum of DJβB TREC frequencies (sj/TREC = sjTREC/DJβB TREC).

**Western blot analysis**

rsIL-7 was run on a 15% polyacrylamide gel containing SDS and then transferred to Immobilon-P membrane (Millipore). The membrane was incubated in blocking buffer (PBS containing 0.1% Tween 20 and 5% skimmed milk powder) for 45 min, and incubated with macaque serum at a dilution of 1/200 in this buffer for 1 h. We used 10 µg of anti-human IL-7 Ab as a positive control (R&D Systems). Membranes were washed with blocking buffer. Specifically bound Ab was detected by incubation with rabbit anti-monkey or anti-goat IgG peroxidase-conjugate (Sigma-Aldrich) as a positive control (R&D Systems). Membranes were washed with blocking buffer. Specifically bound Ab was detected by incubation with rabbit anti-monkey or anti-goat IgG peroxidase-conjugate (Sigma-Aldrich) as a positive control (R&D Systems).

**Quantification of plasma IL-7 levels**

Plasma from each sample was diluted 1:10 in PBS and then dsDNA was removed by heating at 70°C for 30 min. DNA was extracted from plasma with TRI Reagent BD Kit (Molecular Research Center). Dilutions of RNA were aliquoted and immediately frozen at -70°C. RNA from serum samples was purified with the TRI Reagent BD Kit (Molecular Research Center).

**PBMCs culture**

PBMCs from healthy donors were purified on a Ficol-Hypaque gradient and cultured in medium supplemented with 10% FCS, 1% phosphatidylserine (10,000 U/ml penicillin and 10 mg/ml streptomycin), and 2 mM l-glutamine (InVitrogen Life Technologies). Recombinant human IL-7 (rsIL-7) was added as required, at a concentration of 1 µg/ml. Plasma from one macaque was added to the culture at a final concentration of 1 µg/ml IL-7. After 24 h, the PBMCs were labeled with CD127-PE and analyzed by flow cytometry in an XL-4C machine (Beckman Coulter).

**Statistics**

Statistical analysis (Pearson’s correlation test, r and p values) was performed using the Vassar College web site. r values ≥0.3 or ≤-0.3, and p values ≤0.05 were considered significant.

**Results**

rsIL-7 transiently increases T cell counts without affecting viral load

Six juvenile rhesus macaques were infected with a primary isolate of SIVmac251. Following acute infection and the establishment of viral set point, the animals received a combination of three antiretroviral drugs: 3TC, AZT, and did. On day 34 of ART, when viral load had stabilized, two animals (animals 1 and 2) were maintained on the same regimen while the other four animals (animals 3, 4, 5, and 6) received ART plus 100 µg of rsIL-7 per kg of body weight every other day, for 26 days. Antiretroviral treatment was stopped in all the animals on day 17, after the last rsIL-7 injection. This particular IL-7 regimen was chosen on the basis of previous pharmacodynamic studies in monkeys showing full receptor occupancy 24 h after single-dose injection, and reappearance of CD127 after 48 h, reflecting receptor availability (Cytheris, unpublished observations).

Despite heterogeneous initial viral loads (10^4 to 10^6 copies/ml at set point), all of the animals displayed a drastic decrease in peripheral viral load in response to ART (Fig. 1). Viral loads reached undetectable levels for macaques 1 and 5 and were reduced by 3 to 5 log in the other four animals. Macaques 3, 4, 5, and 6 received rsIL-7 on day 104 after infection, in addition to ART. IL-7 did not affect viral load, during or after treatment or before the end of ART. In contrast, serum viral load rapidly increased in all macaques following the cessation of ART, reaching pretreatment values within a few days. Peripheral CD4+ T cell counts rapidly decreased in four infected animals (by a factor of 2 to 3 within 63 days for macaques 1, 3, 4, and 5) and decreased only slightly in the other animals (macaques 2 and 6). ART limited the decrease in peripheral CD4+ T cell counts in all treated animals. Treatment with IL-7 was immediately followed by a large increase in CD4+ T cell counts in macaques 3 (from 1000 to 3000 cells/µl) and 4 (from 2000 to 5000 cells/µl), with preinfection values reached in both cases, and by a less dramatic increase in macaque 5 (from 1200 to 2500 cells/µl). The increase in peripheral CD4+ T cell counts following rsIL-7 treatment was transient in all animals and decreased rapidly before the end of rsIL-7 treatment.

A similar pattern was observed in the CD8+ T cell compartment. ART had no effect on absolute peripheral CD8+ T cell counts, whereas rsIL-7 treatment rapidly induced a transient increase in the number of CD8+ T cells in macaques 3 and 4 (by a factor of 2 to 3). As for CD4+ T cells, macaque 5 showed a limited variation in CD8+ T cell counts. Macaque 6 showed no significant change in CD4+ or CD8+ T cell counts during rsIL-7 treatment. Thus, IL-7 therapy had a rapid effect on peripheral T cell numbers, leading to increases in the CD4+ and CD8+ T cell populations in 3 of 4 macaques.

Changes in CD4+ and CD8+ T cell counts during rsIL-7 treatment can be used to classify the animals according to their response to treatment: responder animals (macaques 3 and 4) displayed significant increases in circulating T cell levels, whereas...
macaque 5 responded less strongly (poorer responder) and macaque 6 did not respond at all (nonresponder) to IL-7 treatment.

rsIL-7 increases proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> subsets concomitantly with activation

Having demonstrated an increase in peripheral CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts in response to IL-7 treatment, we investigated whether this increase was due to peripheral T cell activation. We assessed the ongoing proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> T cells by evaluating the expression of Ki-67, a nuclear Ag expressed during and shortly after cell proliferation. Animals 3, 4, and, to a lesser extent, 5 displayed large increases in the percentages of CD4<sup>+</sup> and CD8<sup>+</sup> T cells undergoing proliferation during IL-7 treatment (Fig. 2). This effect, like the increase in absolute T cell counts, was transient, with a return to baseline either during or immediately after the end of the IL-7 treatment period. In parallel, we analyzed the expression of activation markers on T cells. In responders (macaques 3 and 4), the number of CD25 and HLA-DR-expressing CD4<sup>+</sup> T cells increased transiently during IL-7 treatment (by factors of 3.49 and 3.48, respectively, for CD25 and 4.88 and 3.32, respectively, for HLA-DR in macaques 3 and 4). In contrast, in the CD8<sup>+</sup> T cell population, CD25 expression remained stable, whereas the number of HLA-DR-expressing cells increased in macaques 3 and 4 (by factors of 3.76 and 4.29, respectively). IL-7-induced T cell activation was less pronounced in macaque 5 and was entirely absent in macaque 6. Thus, in responders, rsIL-7 transiently increased the activation of peripheral T cells, concomitant with increased proliferation.

Both naive and memory T cell populations are expanded during rsIL-7 treatment

We analyzed the effects of rsIL-7 treatment on the expansion of naive and memory T cell populations, by carrying out FACS analysis of the surface expression of CD45RA<sup>bright</sup> and CD62L<sup>+</sup>. In this analysis, naive T cells were defined as CD45RA<sup>bright</sup>. CD62L<sup>+</sup> T cell populations.
cells, with all other T cell populations defined as memory T cells. These memory T cells included CD45RA<sup>+</sup>CD62L<sup>-</sup> central (TCM) and CD45RA<sup>+</sup>CD62L<sup>-</sup> effector (TEM) memory T cells, as defined by Seder and Ahmed (29).

In responders, the number of memory T cells in the CD4<sup>+</sup> and CD8<sup>+</sup> subsets increased considerably during the IL-7 treatment period (Fig. 3). CD4<sup>+</sup> and CD8<sup>+</sup> memory T cell counts increased by a factor of 2.2 to 3.5 with respect to pretreatment values, during treatment with rsIL-7. At the peak of the IL-7 response, memory T cells accounted for 40 and 70% of circulating CD4<sup>+</sup> T cells in macaques 3 and 4, respectively, whereas memory CD8<sup>+</sup> T cells substantially outnumbered naive cells (70 and 80%).

Peripheral naive T cells also increased considerably in number during IL-7 treatment. Both CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts increased by a factor of 2.5 in macaque 3 and by factors of 2.1 and 2.5, respectively, in macaque 4. These increases were observed in the naive T cell subset 100 to 118 days after infection, clearly demonstrating that, in addition to its effects on memory T cells, IL-7 also stimulates expansion of the circulating naive T cell subset.

In macaque 5, the expansion of peripheral T cell subsets observed under rsIL-7 therapy remained more limited (Fig. 3). CD45RA<sup>+</sup>CD62L<sup>-</sup> naive T cells, which steadily declined in number following SIV infection, displayed a significant increase in number in both the CD4<sup>+</sup> and CD8<sup>+</sup> subsets under rsIL-7 therapy (by factors of 3.6 and 2.7, respectively). During the same period, similar variations were observed in the memory CD4<sup>+</sup> and CD8<sup>+</sup> subsets.

The observed expansions of the naive and memory T cell populations were only transient, with both the frequencies and absolute counts of all the subsets concerned returning to pre-IL-7 treatment values before the end of the IL-7 treatment period or by day 139.

The nonresponder (macaque 6) showed no significant change in the naive or memory CD4<sup>+</sup> and CD8<sup>+</sup> subpopulations.

Thus, under efficient ART, IL-7 therapy has, in some macaques, a rapid impact on T cell numbers leading to a transient expansion of CD4<sup>+</sup> and CD8<sup>+</sup> naive and memory T cells.

rsIL-7 induces the central renewal of naive T cells by increasing thymic function

IL-7 is the principal chemokine responsible for the proliferation of intrathymic T cell precursors (30). This mechanism is required to increase the number of double-positive cells subjected to positive and negative selection, thymic output (30). We investigated changes in thymic function during IL-7 treatment in SIV-infected macaques. We first analyzed sjTREC frequency in peripheral

FIGURE 2. rsIL-7 concomitantly increases the proliferation and activation of CD4<sup>+</sup> and CD8<sup>+</sup> subsets. T cell activation, as quantified by the expression of HLA-DR (●) and CD25 (●), was assessed in CD4<sup>+</sup> (left panel) and CD8<sup>+</sup> (right panel) T cells in ART- (macaques 1 and 2) or ART + IL-7-treated (macaques 3–6) animals. Peripheral T cell proliferation was estimated by evaluating Ki-67 expression (●). Periods of ART treatment are highlighted in light gray, with the dark gray bars indicating IL-7 treatment.
blood samples (Fig. 4a). During the first 2 mo of infection, sjTREC frequency decreased slightly in macaques 1 and 4. IL-7 injection during antiretroviral treatment did not generate readily interpretable results, in contrast to what has been reported for rhesus macaques infected with a SIVmac-H9004 nef isolate (31). However, this parameter is strongly influenced by peripheral T cell proliferation, which changes markedly during IL-7 treatment. We therefore estimated the effect of IL-7 treatment on thymic production by assessing the intrathymic proliferation of precursor T cells, through quantification of the sj;βTREC ratio, which is totally independent of peripheral T cell proliferation (26). Thymic function is initially reduced during primary SIV infection (Fig. 4b), but this effect is less marked than that in HIV-infected humans (26). During ART and IL-7 treatment, the sj;βTREC ratio rapidly increased in most of the animals (Fig. 4b). This increase was particularly large in the animals that responded well (macaques 3 and 4), suggesting that this effect was enhanced by IL-7 treatment. Moreover, the increase in sj;βTREC ratio, during the ART and IL-7 treatment period, correlated directly with variations in CD4+, CD8+, and total naive T cells counts in IL-7-treated and untreated animals (Fig. 4, c–e: $r = 0.93, p = 0.007$; $r = 0.92, p = 0.007$; and $r = 0.86, p = 0.02$). In particular, macaque 6 (●, Fig. 4), for which T cell counts continued to decline during IL-7 treatment, showed no rebound in intrathymic precursor T cell proliferation. In contrast, the sj;βTREC ratio increased for macaques 3, 4, and 5, and this increase was accompanied by the maintenance or enhancement of circulating naive T cell numbers, in both the CD4 and CD8 compartments. Thus, IL-7 treatment increases thymopoiesis.

The rsIL-7 preparation is antigenic, preventing prolonged effects of the cytokine

The lack of stability of the various effects measured following IL-7 injection prompted us to look for possible immunogenicity of the injected rsIL-7 itself, because this molecule was purified from bacterial cultures. We first analyzed levels of CD127, the IL-7Rα-chain, on the membrane of peripheral CD4+ and CD8+ T cells. Mean fluorescence intensity for CD127 decreased rapidly, suggesting that the receptor was masked or internalized during IL-7 treatment (Fig. 5a). However, the mean fluorescence intensity for CD127 returned to preinjection values before the end of IL-7 treatment, demonstrating that the cytokine was no longer able to interact with its receptor. We searched for IL-7-specific Abs in sera of the treated animals. A strong immune response to IL-7 was detected in all macaques, as early as 29 days after the start of IL-7 treatment (Fig. 5b). Ab titers reached 1/100, 1/500, 1/1000, and 1/1500 in the four animals. Finally, the neutralizing activity of the...
induced IL-7-specific Abs was tested in vitro, using human PBMCs as reporter cells. IL-7 decreased the amount of CD127 detected (Fig. 5c, left panel), but a plasma sample taken from macaque 4 on day 29 abolished the effect of the cytokine (Fig. 5c, right panel), demonstrating the presence of neutralizing Abs against IL-7. This neutralization effect may account for the transient nature of the immunological improvement observed in IL-7-treated macaques.

Discussion
We show in this study that rsIL-7 treatment, in association with ART, has a beneficial effect on the peripheral lymphopenia induced by SIV infection. We previously showed that rhIL-7 does not modify plasma, lymph node, or thymus viral load in macaques infected with the R5 SIV251 isolate and not treated with ART (24). In contrast to what has been found in in vitro studies (32–35), we show in this study that, under ART, the in vivo injection of rsIL-7 had no effect on plasma viral load in four juvenile macaques infected with the R5 primary isolate SIV251. However, we cannot exclude the possibility that IL-7 would increase viral load in the case of X4 primary isolates, because it is known to favor the in vitro replication of CXCR4-tropic viruses (35–37).

In animals that responded well, the peripheral expansion of naive and memory T cell populations coincided perfectly with the observed increase in expression of CD25 and HLA-DR activation markers (Figs. 2 and 3). This is not surprising for the memory T cell compartment, because these cells must receive activation signals triggered by a specific Ag to initiate proliferation. TCM and TEM CD8+ T cells have also been shown to proliferate in response to IL-7 (38). In the CD4 compartment, IL-7 alone did not seem to be sufficient to drive the proliferation of adult memory T cells (39). However, the situation in vivo may be different because, in SIV-infected macaques under ART, activated memory CD4+ T cells are already present in the periphery, and this population of cells can be expanded in response to IL-7 treatment.

CD4+ and CD8+ peripheral naive T cells also increase in number during IL-7 treatment, albeit to a lesser extent than memory T cells. Indeed as suggested by several in vitro studies, only a fraction of peripheral naive T cells proliferate in response to IL-7 (40).
Jaleco et al. (41) demonstrated that naive T cells from human cord blood are much more likely to proliferate in response to IL-7 than naive T cells purified from adult blood, suggesting that recent thymic emigrants (RTE) in the naive T cell subset are more likely to respond to IL-7 by increasing their rate of homeostatic proliferation. These IL-7–responding cells expressed HLA-DR. RTE are activated in the thymus. Mature precursor T cells are activated by cytokines such as TNF and IL-7 before leaving the thymic microenvironment (41). Mature thymocytes have been shown to contain activated NF-κB and the activation marker HLA-DR. These cells probably maintain a certain degree of activation during their emigration from the thymus as RTE, until their subsequent differentiation into resting naive T cells in the blood, with the loss of NF-κB activity (42).

In contrast to the quantification of sjTREC frequency, which is influenced by both central (thymopoiesis) and peripheral (homeostatic) mechanisms, quantification of the sj:JTREC ratio is a reliable marker of thymic production because it depends entirely on the proliferation of intrathymic precursor T cells (26). Using this marker, we demonstrated that IL-7 effectively promotes the in vivo proliferation of precursor T cells within the thymus (Fig. 4).

Within this organ, several subpopulations express CD127 and may therefore proliferate in response to IL-7. This is the case for early immature thymocytes, such as CD34+CD1+ and CD34+CD1+ triple-negative, CD3−CD4−CD8− intermediate single positive (ISP), and late mature CD4+ and CD8+ single-positive cells. Oka-moto et al. reported the proliferation of ISP thymocytes in IL-7–treated thymic organ cultures (30). Such proliferation during thymopoiesis is directly responsible for the increase in sj:JTREC ratio, as these ISP cells have already rearranged their TCR (ISP), and late mature CD4+ and CD8+ single-positive cells.Activated CD4+ and CD8+ memory T cells respond to IL-7 by increasing their rate of homeostatic proliferation before TCRα-chain proliferation and rearrangement occurs.

In a previous study analyzing the effect of rshL-7 injection to SIV-infected macaques (24), we demonstrated that this cytokine had no effect on the various hemopoietic populations, with the exception of T cells. This is probably also the case for the macaque cytokine. However, one major difference between the two studies is the transient effect of rshL-7. This time-limited effect may be due to differences in the injection schedule between the two studies. In the previous study, 2 × 40 μg/kg rshL-7 was injected daily, whereas we used a single injection every 2 days (100 μg/kg), on the basis of previous pharmacodynamic studies in monkeys. Because the half-life of IL-7 in vivo is ~6 h (M. Morre, personal communication), the protocol used here may not be optimal for maintaining stable serum IL-7 concentrations. In addition, despite the macaque origin of the injected IL-7, the animals rapidly developed strong IL-7–specific humoral responses (Fig. 5). It could be tempting to suggest that the rate of anti-IL-7–neutralizing Ab response may lead to the weak or nonresponsiveness in macaque 5 and 6. However, as shown in Fig. 5a, the IL-7R was undetectable, for all the treated animals, at day 118 (2 wk postinitiation of the IL-7 treatment), suggesting that the neutralizing Ab response was, at least, not sufficient at this time point to entirely neutralize IL-7. Moreover, the nonresponder animal (macaque 6) was the last one to recover CD127 expression, suggesting that the anti-IL-7 response was slower in this animal than in the others. Indeed, the end of the transient period of response to IL-7 correlated with the reappearance of surface CD127, reflecting the fact that the cytokine was no longer able to interact with its receptor, because of the presence of anti-IL-7 Abs. The production of a nonimmunogenic IL-7 molecule is being planned and should be tested to verify whether it induces sustained effects.

In conclusion, IL-7, in combination with efficient ART, can help to increase the naive T cell pool by enhancing thymopoiesis and peripheral proliferation. This may help HIV-infected patients to restore their naive T cell compartment, not only quantitatively, but also qualitatively, by increasing the diversity of naive T cells without out-firing viral load.

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Disclosures

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References


