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IL-7 Induces Immunological Improvement in SIV-Infected Rhesus Macaques under Antiviral Therapy[1]

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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 SIV_{mac251}. 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 (CD45RA^{bright}CD62L$^+$) 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 naive 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),[5] generated during TCRδ 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 sj:βTREC ratio (26) In contrast to sjTREC frequency, which decreases during T cell proliferation, the

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

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sj:βTREC ratio is not affected by homeostatic or Ag-induced peripheral T cell proliferation. This parameter is therefore a reliable marker for thymic function, even in HIV-infected patients and SIV-infected macaques, demonstrating variation in naive or memory T cell proliferation.

In this study, we administered recombinant simian IL-7 (rsIL-7) to SIV-infected rhesus macaques under antiretroviral therapy (ART). We first checked that IL-7 therapy did not counteract the decrease in plasma viral load obtained under ART. We then analyzed the impact of exogenous IL-7 on T cell activation and proliferation under ART. We also demonstrated that IL-7 treatment directly influenced naive T cell homeostasis by enhancing thymic function.

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 AID_{50} (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).

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

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 tritherapy treatment. The antiretroviral treatment consisted of AZT (GlaxoSmithKline; 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, DβJβTRECs, and CD3γ-chain*

DNA Circles Quantification		
sjTREC	Out5′	CTCTCCTATCTCTGCTCTGAA
	In3′	GTGCTGGCATCAGAGTGTGT
	Out3′	ACACTTGCTCCGTGGTCTGT
	In5′	TGATGCCACATCCCTTTCAA
Dβ1	Out	CTCATCTGGGCCTGTCCTTGT
	In	TGACCCAGGAGGAAAGAAG
Jβ1S2	Out	CTCTCTATGCCTTCAATGTG
	In	TCCGTCACAGGGAAAAGTGG
Jβ1S3	Out	AAGGGAACACAGAGTACTGGAA
	In	TCCCAACCTCTGCCTGAAT
Jβ1S4	Out	TGGACTTGGGGAGGCAGGA
	In	AGGAGTGGAAGGCAGCAGGT
Jβ1S5	Out	GAAACTGAGAACACAGCCAAGAA
	In	CTCATAAAATGTGGGTCAGTGGA
Jβ1S6	Out	ATCCTCCCTCTTATGTGCATGG
	In	TGAATCCAGGCAGAGAAAGG
Dβ2	Out	GGGACCAGCCCCAGAGA
	In	TCCCACCTGGTAGCTGCATT
Jβ2S1	Out	CTCCTCTGCAAATTGGTGGT
	In	CCAGCTAACTCGAGACAGGAA
Jβ2S2	Out	CACCGTGCTAGGTAAGAA
	In	GAACCCTGTTCTTAGGGGAGT
Jβ2S3	Out	TACTGGGTAAGGAGGCGGTT
	In	GAAGGACCCGAGCTGAGT
Jβ2S4	Out	GGCTGACAGTGCTCGGTAA
	In	GCGTCGCAGGGCCAGTTT
CD3	Out5′	ACTGACATGGAACAGGGGAA
	In5′	GGCTATCATTCTTCTTCAAGGTA
	Out3′	AGCTCTGAAGTAGGGAACATAT
	In3′	TTCCTGGCCTATGCCCTTTT
LightCycler Probes		
sj	P1	AATAAGTTCAGCCCTCCATGTCACACT-fluo
	P2	(Red640)-TGTTTTCCATCCTGGGGAGTGTTTCA-p
Dβ1	P1	CTGGGAGTTGGGACCGCCAGAGAGGT fluo
	P2	(Red 640)-TTTGTAAAGGTTTCCCGTAGAGTTGAATCATTGTG-p
Dβ2	P1	GATTCAGGTAGAGGAGGTGCTTTTACAA-fluo
	P2	(Red640)-AAACCCTGATGCAGTAAGCATCCCCACC-p
CD3γ	P1	GGCTGAAGGTTAGGGATACCAATATTCCTGTCTC-fluo
	P2	(Red705)-CTAGTGATGGGCTCTTCCCTTGAGCCCTTC-p

TREC quantifications

Primers specific for each of the sjTREC (δRec-ψJα), nine DβJβ TRECs (Dβ1-Jβ1S2 to Dβ2-Jβ2S4), and the human CD3γ-chain of the gene (see Table I) were defined on macaque germline sequences (GenBank accession nos.: L43137, L43138, DQ005631, and DQ005632). Parallel quantification of each deletion circle and of the CD3-γ amplicon 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 DβJβ TRECs, 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 10^5 cells for each DNA circle. The sjTRECs and the nine DβJβ TRECs were quantified in triplicate for all the samples studied. The sum of DβJβ TREC frequencies (DβJβ TRECs) was estimated to be 13 times the mean of the nine measured DβJβ TREC frequencies. The sj:βTREC ratio corresponds to the sjTREC frequency divided by the sum of DβJβ TREC frequencies (sj/βTREC = sjTRECs/DβJβ TRECs).

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) at a dilution of 1/16,000 in blocking buffer. Western blots were then washed and developed using the Amersham ECL system according to the manufacturer's protocol.

Quantification of plasma IL-7 levels

IL-7 was quantified in the plasma using the IL-7 Quantikine HS kit according to the manufacturer's conditions (R&D Systems).

Abs used for immunostaining

Cells were immunostained with the following conjugated mAbs: CD20-PE (clone L27), HLADR-PE (clone L243), CD8-PerCP (clone SK1), CD4-FITC or CD4-PE (clone M-T477), CD4-PerCP (clone SK3), CD62L-PE (clone SK11), CD25-PE (clone 2A3) from BD Biosciences; CD45RA-FITC (clone 2H4), CD127-PE (clone R34.34) from Beckman Coulter; and Ki-67-FITC (clone Ki-67) from DakoCytomation; and IgG1, k-FITC, PE, and PerCP (clone MOPC-21), as negative controls.

Lymphocyte immunophenotyping and flow cytometry analysis

EDTA-treated blood cells were incubated for 15 min with the conjugated mAbs. For intracellular labeling, cells were permeabilized with the Cytofix/Cytoperm kit (BD Biosciences) before incubation with mAb Ki-67. Erythrocytes were lysed with the Beckman Coulter lysing kit according to the manufacturer's instructions. Samples were then washed and fixed in PBS supplemented with 1% paraformaldehyde, and analyzed by flow cytometry in an XL-4C machine (Beckman Coulter).

Quantification of serum viral loads

Real-time quantitative RT-PCR was performed as previously described with minor modifications, to determine serum viral load (24). Briefly, standard RNA was obtained from the pGEM-5Zf(+) GAG plasmid (provided by A.-M. Aubertin, Institut National de la Santé, Strasbourg, France). The plasmid was linearized with *Spe*I (New England Biolabs) and used for in vitro transcription, with an in vitro transcription kit and T7 RNA polymerase (Ambion). The resulting transcripts were treated with RNase-free DNase and purified on a Chroma SPIN-100 column (Clontech). Standard 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

PBMC from healthy donors were purified on a Ficoll-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 (rhIL-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 ddI. 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

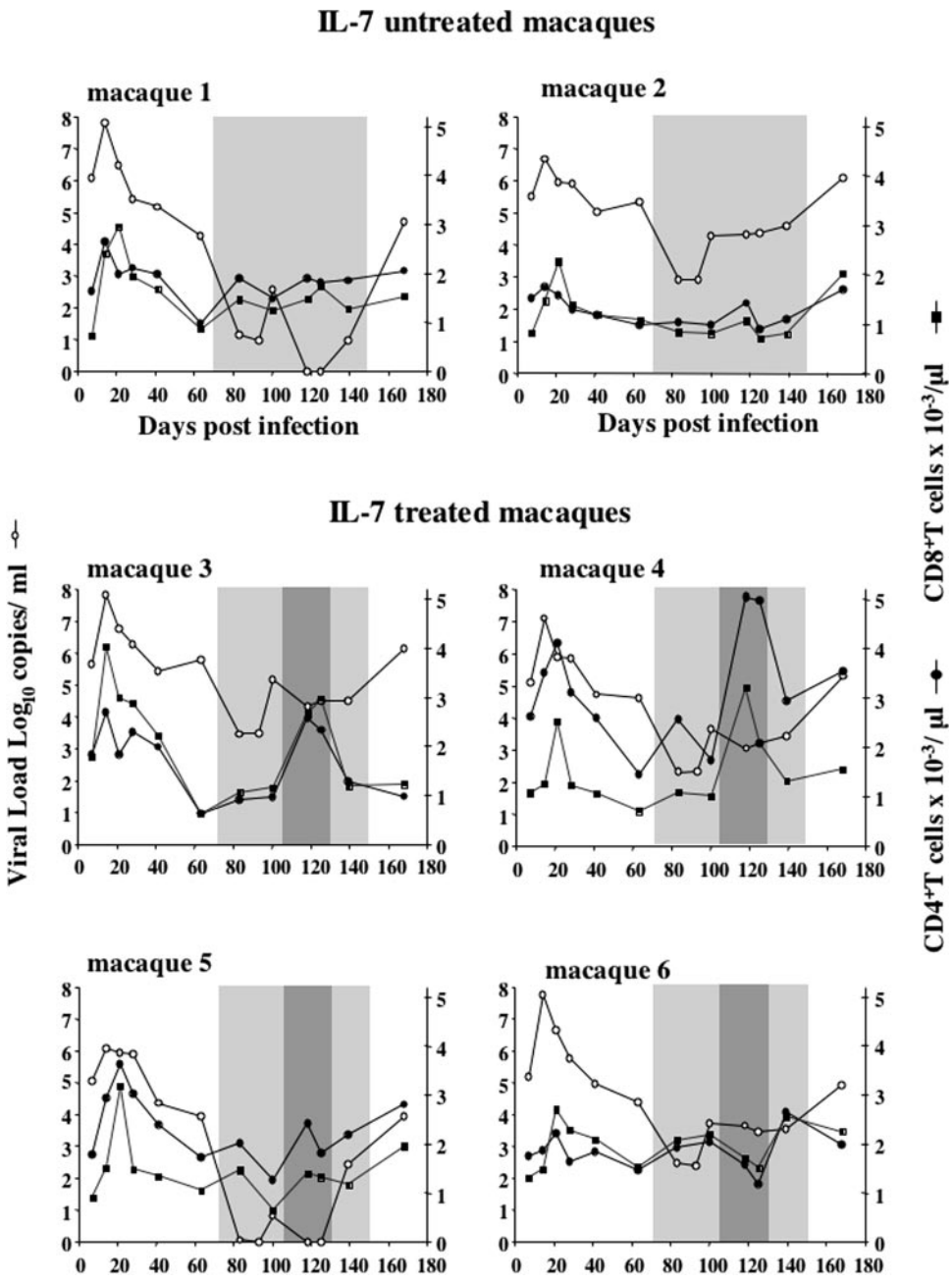

FIGURE 1. rsIL-7 transiently increases $CD4^+$ and $CD8^+$ cell counts without affecting viral load. Six young rhesus macaques were infected with the pathogenic SIVmac251 isolate. They all received ART for 70 days after infection (light gray area). Two animals (nos. 1 and 2) were maintained under this treatment, whereas the remaining four animals (nos. 3–6) were treated with ART plus 100 μg/kg rsIL-7 every other day for 26 days (dark gray area). The ○ indicate changes in viral load, and ● and ■ correspond to counts of $CD4^+$ and $CD8^+$ T cells, respectively.

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 $CD4^+$ and $CD8^+$ subsets concomitantly with activation

Having demonstrated an increase in peripheral $CD4^+$ and $CD8^+$ 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 $CD4^+$ and $CD8^+$ 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 $CD4^+$ and $CD8^+$ 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 $CD4^+$ 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 $CD8^+$ 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 and CD62L. In this analysis, naive T cells were defined as $CD45RA^{bright}$, $CD62L^+$ T

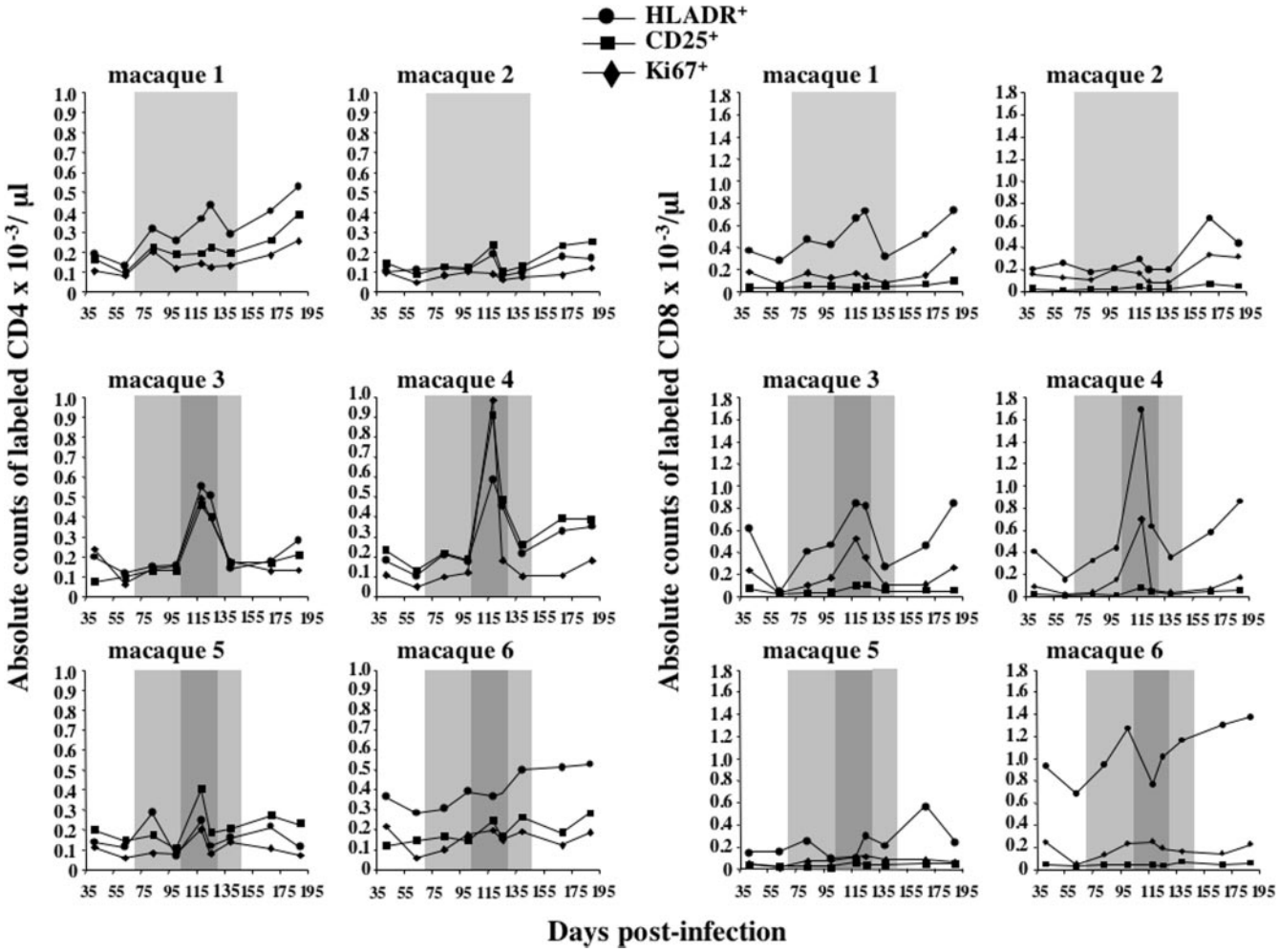

FIGURE 2. rsIL-7 concomitantly increases the proliferation and activation of $CD4^+$ and $CD8^+$ subsets. T cell activation, as quantified by the expression of HLA-DR (●) and CD25 (■), was assessed in $CD4^+$ (*left panel*) and $CD8^+$ (*right panel*) T cells in ART- (macaques 1 and 2) or ART + IL-7-treated (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.

cells, with all other T cell populations defined as memory T cells. These memory T cells included $CD45RA^-CD62L^-$ central (T_{CM}) and $CD45RA^{low}CD62L^+$ effector (T_{EM}) memory T cells, as defined by Seder and Ahmed (29).

In responders, the number of memory T cells in the $CD4^+$ and $CD8^+$ subsets increased considerably during the IL-7 treatment period (Fig. 3). $CD4^+$ and $CD8^+$ 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 $CD4^+$ T cells in macaques 3 and 4, respectively, whereas memory $CD8^+$ T cells substantially outnumbered naive cells (70 and 80%).

Peripheral naive T cells also increased considerably in number during IL-7 treatment. Both $CD4^+$ and $CD8^+$ 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). $CD45RA^{bright}CD62L^+$ naive T cells, which steadily declined in number following SIV infection, displayed a significant increase in number in both the $CD4^+$ and $CD8^+$ 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 $CD4^+$ and $CD8^+$ 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 $CD4^+$ and $CD8^+$ 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 $CD4^+$ and $CD8^+$ $CD45RA^{bright}CD62L^+$ 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

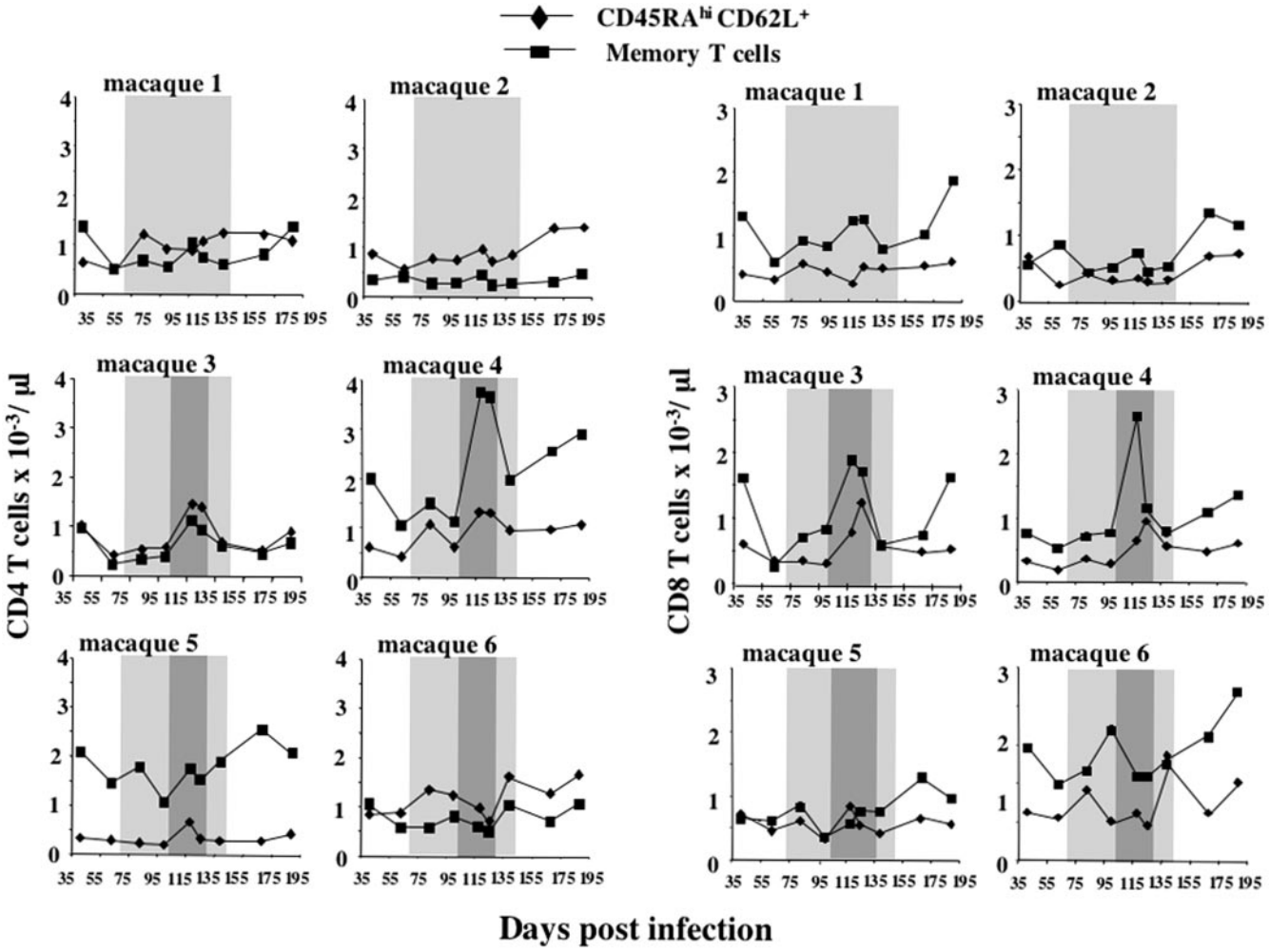

FIGURE 3. Naive ($CD45^{bright}CD62L^+$) and memory $CD4^+$ and $CD8^+$ T cell counts increased in rsIL-7-treated animals. The frequency of naive (♦) and memory (■) T cells, as defined by the expression of CD45RA and CD62L surface Ags, was followed in $CD4^+$ (*left panel*) and $CD8^+$ (*right panel*) peripheral T cell subsets during treatment with either ART alone (macaques 1 and 2) or ART^+ IL-7 (macaques 3–6). Periods of ART treatment are highlighted in light gray, with the dark gray bars corresponding to IL-7 treatment.

blood samples (Fig. 4*a*). 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-Δnef 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. 4*b*), 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. 4*b*). 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-7R α-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. 5*a*). 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. 5*b*). Ab titers reached 1/100, 1/500, 1/1000, and 1/1500 in the four animals. Finally, the neutralizing activity of the

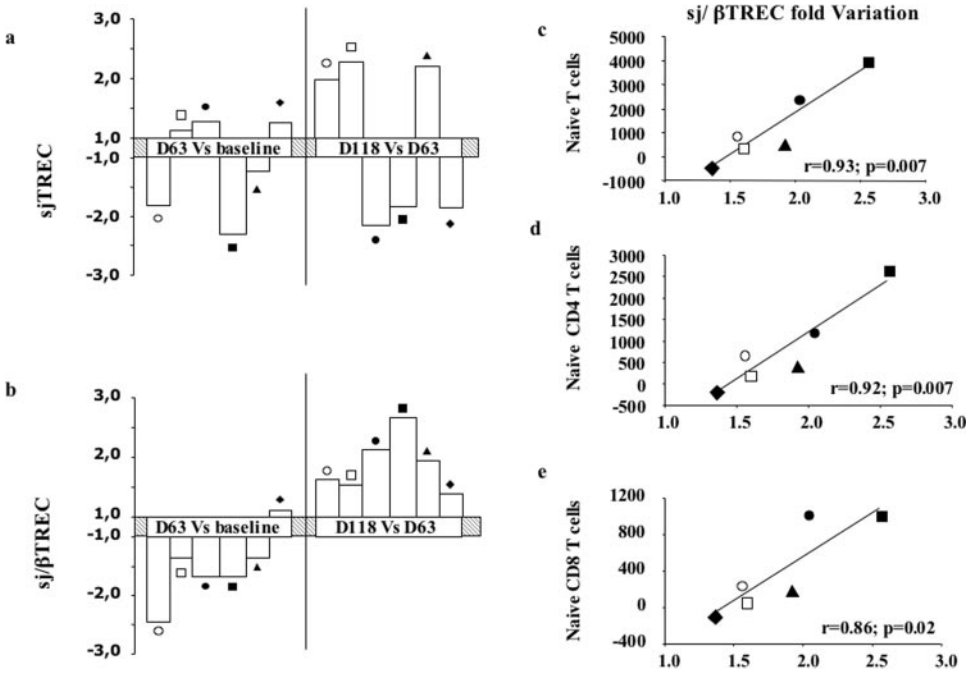

FIGURE 4. Analysis of thymic function in rsIL-7-treated animals. Changes in sjTREC (*a*) and sj:βTREC ratio (*b*) between baseline and day 63 (pre-ART) are presented on the *left part* of *a* and *b*. Changes in these parameters between days 63 and 118 (at peak rsIL-7 activity) are presented on the *right part* of these panels. Each animal is represented by a different symbol: macaque 1, ○; macaque 2, □; macaque 3, ●; macaque 4, ■; macaque 5, ▲; and macaque 6, ◆. The correlations between variations of sj:βTREC ratio and fluctuations in number of naive T cells, naive $CD4^+$ T cells, or naive $CD8^+$ T cells between days 63 and 118 are shown in *c*, *d*, and *e*, respectively. Pearson's correlation coefficients and their associated probabilities are shown for each graph.

induced IL-7-specific Abs was tested in vitro, using human PBMCs as reporter cells. IL-7 decreased the amount of CD127 detected (Fig. 5*c*, *left panel*), but a plasma sample taken from macaque 4 on day 29 abolished the effect of the cytokine (Fig. 5*c*, *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 SIV_{251} 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 SIV_{251}. 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. T_{CM} and T_{EM} $CD8^+$ cells have also been shown to proliferate in vitro 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).

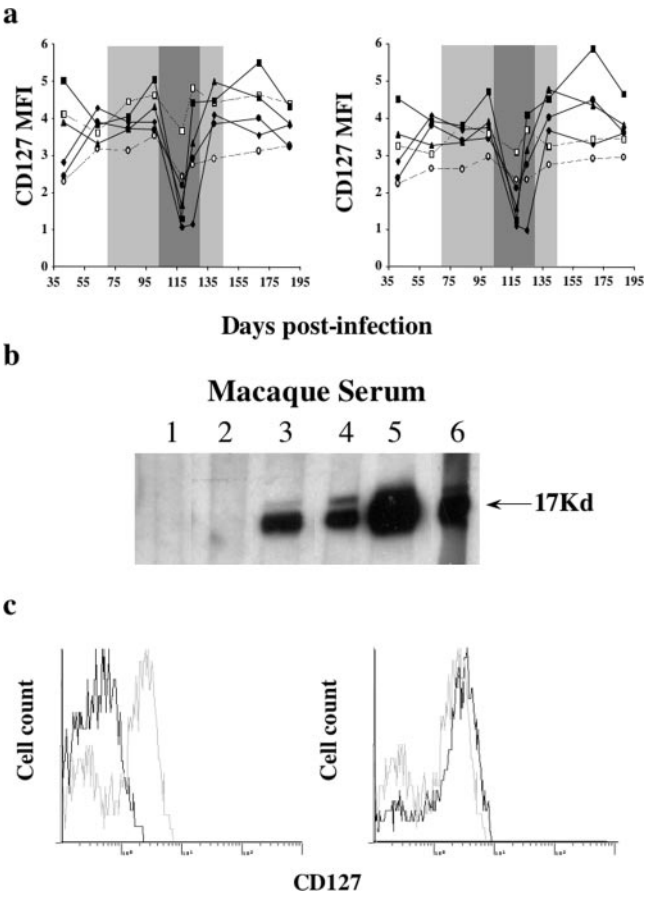

FIGURE 5. rsIL-7-neutralizing Abs. *a*, Mean CD127 fluorescence intensity (MFI) of $CD4^+$ (*left panel*) and $CD8^+$ (*right panel*) T cells. Symbols are as in Fig. 4. *b*, Detection of anti-IL-7 Abs in the plasma of four IL-7-treated animals (*lanes 3–6*). *Lanes 1–2* represent untreated animals 1 and 2. *c*, Neutralization of exogenous IL-7 by plasma from IL-7-treated animals. CD127 expression was analyzed on PBMCs alone (gray lines; *left* and *right panels*) or cultured with rhIL-7 in the absence (dark line; *left panel*) or presence (dark line; *right panel*) of plasma from an immune macaque (macaque 4).

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:βTREC 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. Okamoto 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:βTREC ratio, as these ISP cells have already rearranged their TCRβ-chain and proliferate before TCRα-chain rearrangement occurs.

In a previous study analyzing the effect of rhIL-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 rsIL-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 rhIL-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. 5*a*, 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 influencing viral load.

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Disclosures

The authors have no financial conflict of interest.

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