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IL-7 Stimulates T Cell Renewal Without Increasing Viral Replication in Simian Immunodeficiency Virus-Infected Macaques

Marie-Thérèse Nugeyre,* Valérie Monceaux,† Stéphanie Beq,* Marie-Christine Cumont,† Raphaël Ho Tsong Fang,† Laurent Chène,* Michel Morre,‡ Françoise Barré-Sinoussi,* Bruno Hurtrel,† and Nicole Israël2*  

The main failure of antiretroviral therapy is the lack of restoration of HIV-specific CD4+ T cells. IL-7, which has been shown to be a crucial cytokine for thymopoiesis, has been envisaged as an additive therapeutic strategy. However, in vitro studies suggest that IL-7 might sustain HIV replication in thymocytes and T lymphocytes. Therefore, in the present study, we evaluated the effect of IL-7 on both T cell renewal and viral load in SIVmac-infected young macaques in the absence of antiretroviral therapy. This evaluation was conducted during the asymptomatic phase in view of a potential treatment of HIV patients. We show that IL-7 induces both a central renewal and a peripheral expansion of T lymphocytes associated with cell activation. No alarming modulation of the other hematopoietic cells was observed. No increase in the viral load was shown in blood or lymph nodes. These data strengthen the rationale for the use of IL-7 as an efficient immunotherapy in AIDS. The Journal of Immunology, 2003, 171: 4447–4453.

Depletion of CD4+ T lymphocytes is a characteristic feature of immune dysfunction during infection by HIV (1). Multiple mechanisms, intervening in T lymphocyte destruction and failure to reconstitute this cell population, contribute to lymphopenia (2–4).

The prevalence of abundant thymic tissue in 50% of HIV-infected adults (5) associated with an increase either of naive T cells (6, 7) or of T cell rearrangement excision circles (TREC)8 after antiretroviral therapy (ART) strongly suggests an attempt of renewal of the T cell pool by the thymus. However, the extent of thymic activity is also dependent on the stage of the disease, as shown in macaques infected by the SIV (8). Among patients with advanced disease, late thymic failure was suggested by CD4+ T cell counts remaining below normal, despite long-term suppression of viral load (9, 10).

In vivo and in vitro studies highlight the crucial role of IL-7 in thymopoiesis (11–14). Constitutively produced by the bone marrow, thymus (15), mucosal lymphoid tissues (16, 17), and lymph nodes (18), IL-7 contributes to thymocyte proliferation and survival. Moreover, recent in vitro studies in mice indicate that IL-7 also induces an increase of peripheral expansion of T lymphocytes in relation with an increase in the antigenic capacity of the APCs and in T cell survival (19). In patients with AIDS, the plasmatic level of IL-7 is higher than in healthy donors and has been shown to be inversely correlated with the level of CD4+ T cells (18). This possibly reflects an attempt to restore normal T cell levels, since these high levels of IL-7 decrease under ART when T cell count increases (18). Since IL-7 appears to be critical for both regeneration of the T cell repertoire and peripheral expansion of preexisting T cell clones, it might represent an interesting additive therapy to ART.

However, we have previously shown that IL-7 favors HIV replication in mature CD4+ thymocytes and also sustains survival of these infected cells in vitro (11). We also showed that IL-7 favors replication of X4 vs R5 variants in mature CD4+ thymocytes (20). In vitro studies by other groups suggest that IL-7 might induce HIV replication in naturally infected peripheral mononuclear cells (21) by inducing a state of virus permissiveness in quiescent T lymphocytes (22) and even in naive CD4+ T cells (23), or by inducing expression of latent virus (24). These data incited us to determine whether this cytokine modulates HIV replication in vivo. Therefore, although IL-7 would normally be associated with ART in patients, we tested the impact of IL-7 on T lymphocyte reconstitution and viral load in SIV-infected macaques in the absence of antiviral therapy.

We performed this study in young rhesus macaques to be in a situation of high thymic regenerative capacity and during the asymptomatic phase to avoid the thymus dysfunction observed in advanced disease. Furthermore, treatment of HIV patients is usually started at this stage of the disease.

Materials and Methods

Animals and virus infection

Four 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, for simian T cell leukemia virus type 1, simian retrovirus type 1 (type D retrovirus), and herpesvirus B. The animals referred to as number 1 and 2 were uninfected controls and 3 and 4 were SIV-infected animals. The macaques and 4 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).

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Recombinant human (rhIL-7) treatment

All animals (infected and noninfected) were injected s.c. with rhIL-7 (CYT 99 007, an Escherichia coli-derived rhIL-7 supplied by Cytheris, Vanves, France) twice a day at 40 μg/kg for 21 days. In the infected macaques, this treatment was performed 29 wk after infection throughout the asymptomatic phase.

Lymphocyte immunophenotyping and flow cytometry analysis

EDTA-treated blood cells were incubated for 15 min with the conjugated mAbs. Erythrocytes were lysed with the Lysing kit (Beckman Coulter, Fullerton, CA). Samples were then washed and fixed in PBS containing 1% paraformaldehyde. Analysis was performed on 5000 cells. Immunostaining was analyzed using an XL-4C cytofluorometer (Beckman Coulter France). For intracellular labeling, cells were permeabilized with the Cyto-fix/Cytoper kit (BD Biosciences, Mountain View, CA) before incubation with mAb Ki67.

Abs used for immunostaining

Cells were immunostained using the following conjugated mAbs: CD20-PE (clone L27), HLA-DR-PE (clone L243), CD8-PerCP (clone SK1), CD4-FITC, or CD4-PE (clone M-T477), CD4-PerCP (clone SK3) and CD62L-PE (clone SK1) from BD Biosciences; CD45RA-FITC (clone 2H4) from Beckman Coulter France; and Ki67-FITC, PE, PerCP (clone MOPC-21), as negative controls.

Quantification of TREC

The TREC were determined in the sorted CD4+ or CD8+ T cells of the peripheral blood according to a modified technique of Zhang et al., (25). CD4+ or CD8+ T cells were lysed and treated with protease K at 200 μg/ml for 1 h at 56°C. The quantification of TREC was performed by real-time PCR. Twenty-five microliters of reaction containing 10 μl of cell lysate (25,000 cells), 0.6 μM primers (sense, 5′-ATC ACT CTT TGT CTA GCT CCC AGC-3′; antisense, 5′-ATC TGC TGA GTT TCA TGA TTC CTC CTA-3′), 0.2 μM TaqMan probe FAM-TGC GGG CTC CAT CCT CTC GTG T-TAMRA and PCR Buffer Master Mix 1X (PerkinElmer, Wellesley, MA) containing the polymerase and its cofactors. Thermal cycling conditions consisted of 2 min at 50°C followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Amplification of a sequence within the ccr5 gene was used to normalize and quantify the number of cells. The primers used were: sense, 5′-TTC TCT GGA ATC TTC -TTC TCT GGA ATC TTC CAA AGG TGA CTG TCC TGG CTT TTC ATC ATC C-3′; and the TaqMan probe, VIC- AAC ACA GCA TGG ACG ATA GCC AGG TAC C-TAMRA)

Quantification of serum viral loads

The SIVmac serum viral loads were measured by real-time quantitative RT-PCR (26). The standard RNA was obtained from the pGEM-SZ1+ GAG plasmid (provided by A.-M. Aubertin, Strasbourg).

In situ hybridization tissue section

In situ hybridization was performed as previously described with a 35S-labeled RNA nef probe derived from the nef SIVmac142 (27).

Table 1. Impact of rhIL-7 treatment on the dynamics of hemopoietic cells

<table>
<thead>
<tr>
<th>Uninfected Animals</th>
<th>Infected Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macaque 1</td>
<td>Macaque 2</td>
</tr>
<tr>
<td>Day 0</td>
<td>Day 21b</td>
</tr>
<tr>
<td>T Lymphocytes</td>
<td>3,559</td>
</tr>
<tr>
<td>B Lymphocytes</td>
<td>1,391</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>4,700,000</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>11,000</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>5,610</td>
</tr>
<tr>
<td>Monocytes</td>
<td>220</td>
</tr>
<tr>
<td>Platelets</td>
<td>303,000</td>
</tr>
</tbody>
</table>

The number of hematopoietic cells per microliter of blood was determined in either healthy (macaques 1 and 2) or SIV-infected animals in asymptomatic stage (macaques 3 and 4) animals treated with 80 μg/kg per day of rhIL-7 at days 0, 21, and 60.

The last day of rhIL-7 treatment.

ND, Not done.

Results

Main experimental settings

Since there had been no previous report on IL-7 treatment in macaques when we started this study, we first had to determine the minimal dose of rhIL-7 and the duration of the treatment necessary to induce a beneficial effect on T cell reconstitution. By extrapolation from the active doses in mice, we tested 40 or 80 μg/kg per day. Eighty micrograms per kilogram per day was shown to be a more efficient dose when used during 3 wk and therefore the reported experiments were performed under these conditions.

Our study was conducted on four rhesus macaques. Animals 1 and 2 were uninfected controls, while 3 and 4 were SIV-infected animals as described in Materials and Methods. Infected animals received rhIL-7 treatment during the asymptomatic phase characterized by a stabilization of the viral load and of CD4+ T cell counts. Before rhIL-7 treatment, macaque 3 exhibited a high viral load (106 RNA copies/ml of serum) and its CD4+ T cell number dropped from 5200/μl of blood (a mean of three samplings) and stabilized around 1800/μl of blood (a mean of four samplings), whereas macaque 4 exhibited a low viral load (103 RNA copies/ml of serum) and its CD4+ T cell number was not significantly modified: from 2200 (a mean of three samplings) to 2120/μl of blood (a mean of four samplings).

Impact of IL-7 treatment on the dynamics of hemopoietic cells

Longitudinal studies were then conducted to evaluate the counts of hemopoietic cells during and after rhIL-7 treatment (as shown in Table I). Whether infected or not, the four macaques exhibited a strong increase in T lymphocyte counts during rhIL-7 treatment which persisted after interruption of the treatment (except for macaque 4 whose T cell count returned to basal level at day 60). In other respects, IL-7-transgenic mice have been previously shown to develop B cell lymphoma (28, 29). We therefore measured the B cell population: individual variability was observed in the B cell counts, but no large decrease could be seen following rhIL-7 treatment. A decrease was even observed in macaques 1, 2, and 4. We also studied the other hemopoietic cell populations and we observed a slight modification of the erythrocyte count in the four macaques. In macaque 3, a decrease of the platelets was observed, but the main decrease in the four animals concerned the neutrophils. However, this decrease did not affect the health of the animals and was reversible after the end of the rhIL-7 treatment (see at day 60).

Since our study is focused on the effect of rhIL-7 on T cell renewal, because of the particular decline of CD4+ T cells in AIDS, we studied in more detail the evolution of T cells (CD4+...
and CD8+ T cells during (from day 0 to day 21) and after (from day 22 to day 56 or 60) the treatment.

**rhIL-7 increases the absolute number of CD4+ or CD8+ T cells and this increase correlates with that of naive CD4+ or CD8+ T cells**

We first evaluated the capacity of rhIL-7 to increase the CD4+ and CD8+ T cell pools in peripheral blood. As shown in Fig. 1a, the number of CD4+ T cells increased (2- to 4-fold) and culminated around 5000 CD4+ T cells/µl in all animals (whether infected or not) and even in the presence of a high viral load (macaque 3). The response persisted at least 35 or 39 days after interruption of the treatment (except for macaque 4 whose T cell count already decreased at 39 days after treatment). However, analysis made 11 wk after the treatment (before the sacrifice of the animals) indicated a return to baseline in all animals (data not shown).

The CD8+ T cell count also increased (2- to 3-fold) under rhIL-7 treatment but this increase was not maintained throughout the treatment in the uninfected macaque 1 and in the infected macaque 4 (Fig. 1c).

The correlative increased production of naive CD4+ and CD8+ T cells (CD45RA+CD62L+), shown in Fig. 1, b and d, argues for a central renewal of these cells under rhIL-7 treatment. Of note, the percentage of naive/total CD4+ T cells (macaque 1, 85%; 2, 55%; 3, 83%; and 4, 84%) initially high (over 80%) in three of four of these young animals did not increase much with rhIL-7 treatment (macaque 1, 83%; 2, 73%; 3, 85%; and 4, 92% at day 21) except in macaque 2 which initially exhibited a lower percentage. In contrast, the percentages of naive/total CD8+ T cells were all initially lower than for the CD4+ population (macaque 1, 53%; 2, 37%; 3, 57%; and 4, 60%) and increased with the treatment (macaque 1, 70%; 2, 70%; 3, 79%; and 4, 84% at day 21).

**rhIL-7 increases T cell activation and proliferation**

It has been recently shown that the human CD4+ T lymphocytes expressing the HLA-DR activation marker specifically proliferate in the presence of rhIL-7 in vitro (30). We thus wondered whether rhIL-7 might up-regulate in vivo this activation marker and whether this activation correlates with the capacity to proliferate, as characterized by the expression of the Ki67 marker. As shown in Fig. 2a, an increase in the absolute number of CD4+HLA-DR+ T cells.
as shown in Fig. 3, despite the high rate of proliferation due to rhIL-7 treatment, the TREC values were maintained in healthy macaques, arguing therefore for an increase in thymic output. In contrast, we observed a marked decline (day 21) in the TREC values in infected animals. Since the magnitude of the production of naive T cells was presumably the same in the four animals, we can argue that this decline was the result of a higher rate of T cell proliferation. This high level of proliferation was not obvious from the counts of Ki67+ cells (Fig. 2, b and d) but the overall rate of proliferation might have been impaired by the rate of apoptosis due to infection. Therefore, we suggest that this particular decline might be due to an increased rate of transition between naive and memory T cells to compensate for the destruction of activated T cells by infection. Of note, interruption of the treatment at day 21 limits the decrease in TREC numbers.

**rhIL-7 does not modify the viral load in the serum nor in the lymph nodes**

In vitro studies indicate a possible induction of virus replication in IL7-treated thymocytes (11) or lymphocytes (21, 23). Furthermore, we noticed that rhIL-7 significantly increased lymphadenopathy. This increase in the size of lymph nodes might be linked to the increased level of cell activation and might lead to an increase in virus replication. Therefore, it was of importance, in view of a possible use of rhIL-7 in humans, to determine the effect of rhIL-7 treatment on the viral load in the serum, lymph nodes, and thymus. As shown in Fig. 4, no significant increase in serum viral load was observed in macaques 3 and 4. Similarly, no increase in viral load was observed in the serum of two older macaques (5 years old) treated with rhIL-7 also during the asymptomatic phase (data not shown). Lymph nodes of macaques 3 and 4 were collected before (at the beginning of the asymptomatic phase, 7 wk after infection) and 11 wk after rhIL-7 treatment. Viral load in the lymph nodes was determined by in situ hybridization for SIVmac nucleic acid. We evaluated the median of the number of infected cells per square millimeter in the paracortical area in three distinct sections of lymph nodes. Viral load in lymph nodes correlated with that determined in the serum: macaque 3 characterized by a high serum viral load exhibited a high number of infected cells (a median of 15 productively infected cells/mm² which did not increase (9/mm²) after rhIL-7 treatment), whereas very few positive cells (below

**FIGURE 3.** rhIL-7 treatment decreases the TREC in infected macaques. Each line represents an individual animal, either healthy (macaques 1 and 2) or SIV-infected in asymptomatic stage (macaques 3 and 4), treated with 80 μg/kg per day of rhIL-7 treatment between days 0 and 21. TREC counts are per 10⁶ CD4+ or CD8+ cells.
cells expressing the viral genome. The discussion (first step toward a possible use of IL-7 as an adjuvant immunotherapy in AIDS, we focused our study on two major questions. The virus was searched for in the serum, lymph nodes, and thymus of the two infected macaques (animals 3 and 4). The viral load was determined by real-time quantitative RT-PCR in the serum and by in situ hybridization for SIV RNA in the lymph nodes and thymus. The photomicrographs were taken at the same original magnification (×40). The dark spots indicate the cells expressing the viral genome.

1/mm² irrespective of the treatment) was observed in macaque 4. In conclusion, rhIL-7 treatment did not modify the viral load either in the serum or in the lymph nodes.

Regarding the thymus, in situ hybridization, performed 11 wk after rhIL-7 treatment on macaques 3 and 4, showed no positive cells (Fig. 4). Furthermore, the thymus architecture was perfectly conserved, suggesting no insult caused by the virus at that time of infection (43 wk after infection in total).

Discussion
As a first step toward a possible use of IL-7 as an adjuvant immunotherapy in AIDS, we focused our study on two major questions. The first one was to determine whether IL-7 permits a restoration of T cell number even in the presence of an ongoing infection and the second was whether this cytokine modifies the level of virus replication in vivo. For this reason, we analyzed in SIV-infected macaques the effect of a 3-wk rhIL-7 treatment during the asymptomatic phase of infection in the absence of ART. The implications of this study are therefore different from those derived from the study published recently by Fry et al. (31), which reports the effect of IL-7 in infected animals treated with ART and immunization (therefore precluding the observation of the effect of IL-7 on viral load).

We report here that IL-7 exhibits the remarkable capacity to increase T cell number in monkeys, in particular by increasing the pool of CD4⁺ and CD8⁺ naive T cells. This increase reaches the same level whether the animals have been infected or not. Of note, the CD4⁺ response persisted for at least 5 wk after interruption of the rhIL-7 treatment. Although the increase in T cells was similar in magnitude to that observed by Fry et al. (31), in contrast to these authors, we observed an increase in activated HLA-DR⁺ cells. The percentage of these cells among the total CD4⁺ varied for each animal and was independent of infection. It reached around 30%, which represents an 8-fold increase above normal level. The fact that the kinetics of HLA-DR⁺ cells correlates with that of Ki67⁺ cells strongly suggests that these activated cells, mostly memory cells, are those that are able to proliferate in the presence of IL-7.

These data are in agreement with the study of Jaleco et al. (30) who showed that under conditions where the HLA-DR⁺ cells are removed, purified human CD4⁺ T cells fail to proliferate in response to IL-7. Several ex vivo studies in humans also indicate that IL-7 does not promote the proliferation of naive T cells (32–34) except in the case of the immature naive cells of the human cord blood which have been identified as recent thymic emigrants (33, 35). Our conclusions differ from those of Fry et al. (31) who observed an expansion of naive T cells. This difference is probably due to the very high IL-7 concentration (500 µg/kg per day) used by these authors, which presumably leads to the proliferation of some recent thymic emigrants still existing in these young animals. Since we observed that the number of Ki67⁺ cells slightly exceeded those of HLA-DR⁺ (except for macaque 3), we cannot exclude the possibility that a few non-fully activated cells might be induced to proliferate. Nevertheless, most of the naive cells did not seem to proliferate and their increased number was probably attributable to de novo generation. The TREC values in the CD4⁺ and CD8⁺ T cells, measured in healthy animals, argued for an increase in thymic output since they remained stable despite the high level of peripheral expansion. In contrast, the marked decline in TREC values observed in infected animals might result from an increased rate of transition between naive and memory T cells to compensate for the destruction of the activated cells by infection.

TREC levels in CD8⁺ cells also decreased, probably because cell death also affects CD8⁺ cells. For instance, it has been shown that human CD8⁺ cells are driven to apoptosis by indirect mechanisms following infection (36).

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IL-7 INFLUENCES T CELL RENEWAL But Not SIV REPLICATION

load, as might be predicted from in vitro studies (11, 20, 21–24). Regarding the serum viral load, no increase was observed in the four infected macaques (two young and two old animals) in response to IL-7, irrespective of the absolute value of the viral load at the start of the treatment. Although difficult to compare with our study since it deals with infected monkeys undergoing ART and immunization, the study by Fry et al. (31) concludes of the same lack of effect of IL-7 on the serum viral load. We did not determine the viral load in peripheral CD4+ T cells to limit the volume of blood samples. However, it seems likely that modification in T cells would be reflected in the serum during the 39 days of the experiment and particularly during IL-7 treatment. Besides viral load was also determined in T cells in lymph nodes, and no difference was observed irrespective of rhIL-7 treatment. However, this lack of increase in blood and lymph nodes was observed in a situation where the thymus remained uninfected. We have previously shown that rhIL-7 is able to sustain a viral reservoir in mature thymocytes (39). Therefore, it is not excluded that, in more advanced disease conditions, in the case of viral insult to the thymus, rhIL-7 might indirectly increase the peripheral viral load. Of note, a study by Napolitano et al. (40) concludes that, under conditions of thymus infection in the model of HIV-infected SCIDhu mice, IL-7 does not increase viral replication in thymocytes.

In conclusion, our findings represent an encouraging step toward the use of IL-7 in the treatment of immunodeficiency associated with AIDS in human patients. In particular, an important feature of IL-7 treatment is that it can increase the number of CD4+ T cells even in the presence of the virus (particularly illustrated in the case of macaque 3). This is important not only for an immune reconstitution of infected individuals (who are currently under ART), but even more so in patients who ill-respond to the ART and whose levels of CD4+ T cells continue to decrease.

However, additional experiments are needed to confirm the long-term effects of rhIL-7 and to determine whether it will be necessary to proceed by cycles of treatment, for instance to allow neutrophil regeneration. Given the immunogenicity of the cytokine and the appearance of Abs against rhIL-7 at late time points of the experiments (data not shown), it would be wise to use the simian counterpart of rhIL-7 in studies exploring this effect.

Acknowledgments

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