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Increased CD4+ T Cell Levels during IL-7 Administration of Antiretroviral Therapy-Treated Simian Immunodeficiency Virus-Positive Macaques Are Not Dependent on Strong Proliferative Responses

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Brigitte Assouline,§ Michel Morre,§ Louis J. Picker,¶ and Donald L. Sodora*

CD4+ T cell depletion is a fundamental component of HIV infection and AIDS pathogenesis and is not always reversed following antiretroviral therapy (ART). In this study, the SIV-infected rhesus macaque model was used to assess recombinant simian IL-7 in its glycosylated form (rsIL-7gly) to enhance regeneration of CD4+ T cells, particularly the crucial central memory compartment, after ART. We assessed the impact of rsIL-7gly administration as single injections and as a cluster of three doses. Irrespective of the dosing strategy used, the rsIL-7gly administration transiently increased proliferation of both central memory and naive cells, in both CD4+ and CD8+ subsets, without increasing SIV levels in the blood. Administration of rsIL-7gly at intervals of 4–6 wk maximized the proliferative response to therapy but resulted in only transient increases in peripheral blood T cell counts. Although more frequent rsIL-7gly “clustered” dosing (three times weekly with 2 wk of rest and then repeat) induced only an initial proliferative burst by CD4+ T cells, this dosing strategy resulted in sustained increases in peripheral blood CD4+ T cell counts. The clustered rsIL-7gly treatment regimen was shown to increase the half-life of a BrdU label among memory T cells in the blood when compared with that of macaques treated with ART alone, which is consistent with enhanced cell survival. These results indicate that dosing intervals have a major impact on the response to rsIL-7gly in SIV-positive ART-treated rhesus macaques and that optimum dosing strategies may be ones that induce CD4+ T cell proliferation initially and provide increased CD4+ T cell survival. The Journal of Immunology, 2010, 185: 1650–1659.

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The online version of this article contains supplemental material.

Abbreviations used in this paper: ART, antiretroviral therapy; CM, central memory; EM, effector memory; FTC, emtricitabine; HAART, highly active antiretroviral therapy; PMPA, 9-β-(2-phosphonomethoxypropyl) adenine; rsIL-7gly, recombinant simian IL-7 in its glycosylated form; TrM, transitional memory.

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Antiretroviral Therapy and Evaluation of Subcutaneous Proteinkin in a Randomized International Trial were unable to demonstrate any clear clinical benefit for IL-2 therapy in HIV-infected patients. IL-15 has been less well studied, and although an effect on proliferation of CD4+ and CD8+ EM T cells has been observed (40–43), it also increases the susceptibility of CD4+ cell subsets to SIV infection (44, 45), confounding its use as an immune therapy. The potential for IL-7 as an immune therapeutic lies in its nonredundant role for maintaining T cell levels, in both increasing proliferation and preventing apoptosis in naive and CM CD4+ and CD8+ T cells (14, 22, 46–51). Studies have also contributed a great deal to documenting the expansion of therapeutically relevant cell populations, including CD4+ naive and CM subsets (14, 52–56). In nonhuman primate models, IL-7 can impact both CD4+ and CD8+ T cell compartments (14, 52–57). The effects of IL-7 were not limited to the peripheral blood and included both lymph nodes and other organs (e.g., spleen, lung, kidney, and gut) (55, 56, 58). Most of these studies however used nonglycosylated recombinant macaque IL-7 (14, 52, 54–57), and in some instances, the animals developed neutralizing Abs (52, 54, 57). Recently, IL-7 administration to both cancer and HIV-positive patients demonstrated similar increases in CD4+ T cells, indicating that IL-7 therapy could increase naive, CM, TrM, and EM CD4+ T cells (59–62). The goals of these studies were 3-fold: 1) to assess the ability of IL-7 administered to SIV-positive macaques undergoing ART to increase CD4+ T cell levels, 2) to determine the importance of CD4+ T cell proliferation for any observed IL-7-induced CD4+ T cell increases, and 3) to investigate the ability of different IL-7 regimens to achieve a CD4+ T cell recovery. These data provide evidence that IL-7 can be an effective immune therapy in SIV-positive macaques that are on effective antiretroviral therapy. In addition, these data provide insights into factors that contribute to a successful outcome following IL-7 treatment, including the identification of an optimum dosage and schedule for IL-7 to increase CD4+ T cell levels.

Materials and Methods

Animals and viruses

A total of 17 Indian rhesus macaques were housed at the Oregon National Primate Research Center in accordance with standards of the Center’s Animal Care and Use Committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Macaques enrolled in this study were between 5 and 7 y of age and were deemed free of Cercopithecine herpesvirus 1, D type simian retrovirus, simian T-lymphotropic virus type 1, and SIV infection. Of those 17 macaques, 8 animals remained SIV-negative and were used as healthy controls, and 9 animals were infected with SIVmac239 i.v. using 5 ng equivalents of SIV p27 (1.0

SIV viral load quantification

Plasma viral load was determined using a real-time RT-PCR assay essentially as previously described (63). The threshold sensitivity of the assay, as used for this study, was 30 SIV gag RNA copy equivalents per milliliter.

Flow cytometric analysis

Cells for flow cytometry were obtained from whole blood as previously described (64). Polychromatic flow cytometry was performed on an LSR II (BD Biosciences, San Jose, CA), and FlowJo (Tree Star, Ashland, OR) software was used for analysis. Naive and memory T cell phenotypes were defined using the markers CD28, CD95, CCR5, and CCR7 after gating CD3+ CD4+ or CD3+ CD8+ T cells. The criteria used to identify naive and memory T cell subsets were between 5 and 7 y of age and were deemed free of Cercopithecine herpesvirus 1, D type simian retrovirus, simian T-lymphotropic virus type 1, and SIV infection. Of those 17 macaques, 8 animals remained SIV-negative and were used as healthy controls, and 9 animals were infected with SIVmac239 i.v. using 5 ng equivalents of SIV p27 (1.0

mAbs

The following fluorophore-conjugated mAbs were obtained from BD Biosciences: SP34-2 (CD3; Alexa Fluor 700), L200 (CD4; AmCyten), SK-1 (CD8a; PE-Cy7, PerCP-Cy5.5, allophycocyanin-Cy7), and CCR5 (PE, PE-Cy7, PerCP-Cy5.5, allophycocyanin-Cy7). The clone 28.2 (CD28; PE-Texas Red) was obtained from Beckman Coulter (Fullerton, CA). The purified, unconjugated Ab 15053 (CCR7) was obtained from R&D Systems (Minneapolis, MN) and conjugated to biotin (Pierce Biotechnology; Thermo Scientific; Rockford, IL). CCR7-biotin was detected using Pacific blue-conjugated streptavidin from Invitrogen-Biolegend (San Diego, CA). CCR7-biotin was detected using Pacific blue-conjugated streptavidin from Invitrogen-Biolegend (San Diego, CA).

Results

rsIL-7gly administration in SIV-negative and SIV-positive ART-treated macaques induces T cell proliferation and increases T cell numbers in circulation

Previous work has demonstrated that both nonglycosylated and glycosylated recombinant macaque IL-7 exhibited induced proliferation and expansion of peripheral blood T cells (14, 52, 54, 56, 58).
rsIL-7gly dosing regimens administered

<table>
<thead>
<tr>
<th>Study Title</th>
<th>RM No.</th>
<th>Total No. of rsIL-7gly Doses</th>
<th>Interval between Doses</th>
<th>Days of rsIL-7gly Administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>rsIL-7gly treatment in SIV-negative RMs</td>
<td>Eight uninfected RMs</td>
<td>2</td>
<td>Two doses of rsIL-7gly at a 1-wk interval</td>
<td>0, 7</td>
</tr>
<tr>
<td>rsIL-7gly 2-, 4-, or 6-wk intervals</td>
<td>RM2-01</td>
<td>3</td>
<td>Two doses of rsIL-7gly at a 1-wk interval, followed by one dose at 2-, 4-, or 6-wk intervals (n = 6), 0, 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM2-02</td>
<td></td>
<td></td>
<td>21 or 35 or 49</td>
</tr>
<tr>
<td></td>
<td>RM2-03</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>RM2-04</td>
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<td></td>
<td>RM2-05</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM2-06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rsIL-7gly 6-wk intervals</td>
<td>RM2-01</td>
<td>3</td>
<td>One rsIL-7gly dose every 6 wk</td>
<td>0, 42, 84</td>
</tr>
<tr>
<td></td>
<td>RM2-03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM2-04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clusters of rsIL-7gly doses</td>
<td>RM2-05</td>
<td>9</td>
<td>Three doses of rsIL-7gly at 1-wk, intervals followed by 2 wk without, repeated three times</td>
<td>0, 7, 14, 35, 42, 49, 70, 77, 84</td>
</tr>
<tr>
<td>ART controls</td>
<td>RM2-02</td>
<td>0</td>
<td>Not applicable</td>
<td>Not applicable</td>
</tr>
<tr>
<td></td>
<td>RM2-06</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM2-08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RM2-09</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Macaques were divided into four different dosing schemes over the course of these studies. The number of animals, the number of rsIL-7gly doses that each animal received during the study, and the interval between rsIL-7gly doses are shown. Some macaques were used in multiple studies, and in each case the interval between studies was >10 wk.

In this study, the assessment of rsIL-7gly was initially undertaken in a two-dose regimen (30 μg/kg with a 7-d interval) to assess proliferation in vivo in uninfected macaques (gray line) and compared with results obtained in SIV-positive ART-treated macaques (black line) (Fig. 1). Flow cytometric evaluation of the percentage of cells expressing the nuclear Ag Ki67 [a marker for cycling through S phase within the previous 4–7 d (65)] revealed a consistent and robust rsIL-7gly–associated increase in the percentage of Ki67+ CD4+ and CD8+ cells in the blood. This increased proliferation was observed in both uninfected and SIV-positive ART-treated macaques and within each of the T cell subsets assessed, including naive, CM, TrM, and EM (Fig. 1). However, distinctions in the rsIL-7gly response were observed, because the percentage of Ki67+ T cells in CM and TrM T cells increased rapidly and robustly, whereas the rsIL-7gly response in naive and EM cells was delayed and generally less robust (with the exception of CD8+ naive T cells, which proliferated robustly in response to rsIL-7gly) (Fig. 1). For the naive, CM, and TrM subsets, the percentages of CD4+ and CD8+ T cells expressing Ki67 returned to near baseline levels by day 21 after rsIL-7gly therapy; however, some proliferation could be observed in the EM subset after 21 d. Because EM T cells express low levels of the IL-7R (CD127) on their cell surface (56, 66–76), the proliferation of this subset likely reflects subsequent differentiation of IL-7–stimulated CM and TrM T cells rather than a direct effect on pre-existing EM T cells themselves. Importantly, irrespective of the T cell subset assessed, the differences observed in Ki67 expression were not significantly different between uninfected and SIV-positive ART-treated macaque (Fig. 1, gray line compared with black line).

The rsIL-7gly–induced proliferation of the different T cell subsets would be predicted to have an impact on the numbers of T cells in each subset. In this study, the changes in T cell levels are depicted as the fold change relative to the number of T cells that were present prior to the onset of rsIL-7gly therapy (therefore, a 2-fold change reflects doubling of the T cells present per microliter of blood). Comparing uninfected (gray line) and SIV-positive ART-treated macaques (black line), we determined that the T cells generally respond to a greater extent in the uninfected macaques, with the exception of the CM CD4+ T cells, which increased at comparable levels in both groups of macaques (Fig. 2). Overall, these data indicate that even after ART treatment the SIV-positive macaques have a reduced potential to increase T cell levels in response to rsIL-7gly when compared with uninfected macaques. However, there is also indication of the potential utility of IL-7 as a therapy for HIV-positive patients, because a 2-fold or greater change was observed in the CM, TrM, and EM CD4+ T cell subsets following rsIL-7gly treatment.

Timing of rsIL-7gly administration in SIV-positive ART-treated macaques: Dosing interval assessment and IL-7 treatment at 6-wk intervals

We next sought to determine the optimal spacing between sequential rounds of rsIL-7gly treatment so as to maximize the proliferative response, focusing particular attention on the CD4+ CM and naive T cell subsets that are likely to be key for any successful CD4+ T cell recovery. Six SIV-infected macaques were treated first with two doses of rsIL-7gly 7 d apart, and then the T cell proliferative response to a third dose of IL-7 after either 2, 4, or 6 wk was determined (Table I, rsIL-7gly 2-, 4-, or 6-wk intervals). Overall, rsIL-7gly administration in these animals was not associated with any long-term increase in plasma SIV levels, although three of the six macaques did exhibit a transient increase in plasma viral load (as high as 10-fold) at day 7 posttreatment (Table II). Administering rsIL-7gly 6 wk after the previous exposure elicited robust proliferation in CD4+ and CD8+ CM subsets following each rsIL-7gly dose in RM2-05 and RM2-06 (Fig. 3A, Supplemental Table II). Indeed, after the third dose at the 6-wk interval, macaque RM2-05 elicited the largest increase in the percentage change in Ki67 levels (31.7%) when compared with those of the other macaques in this study. For each of the 6-wk, third-dose interval macaques, the CM CD4+ T cell levels increased, transiently in macaque RM2-06 and in a more sustained manner for macaque RM2-05 (Fig. 3D, Supplemental Table II). Administration of the third rsIL-7gly dose at more closely spaced intervals, either 2 or 4 wk, resulted in proliferative responses that were more variable and generally lower than those observed in the 6-wk, third-dose macaques (Supplemental Table II). Similar results were observed in the CD8+ CM and naive T cells, with the best third-dose proliferative response being associated with the 6-wk interval and less robust responses being observed for 2- and 4-wk intervals (Fig. 3). As one might predict, the most impressive increases in levels of CM CD4+ T cells were associated with the best proliferative response following the third dose as observed in
macaques from the 2-wk (RM2-01), 4-wk (RM2-03), and 6-wk (RM2-05) dosing intervals (Fig. 3, Supplemental Table II). These data led to the hypothesis that providing a rsIL-7gly dosing strategy that optimized proliferative responsiveness in the SIV-positive ART-treated macaques would provide the optimum long-term CM CD4+ T cell increases. We went on to further investigate this hypothesis with two sets of experiments designed to assess relatively long (6 wk) and short (clustered 1 wk) rsIL-7gly dosing approaches.

To test whether the 6-wk interval administered to RM2-05 and RM2-06 would elicit long-term benefits after repeated exposures, we administered one dose of rsIL-7gly to three SIV-positive ART-treated macaques at 6-wk intervals (Table I, rsIL-7gly 6-wk intervals, three total doses). This dosing regimen was not associated with any increase in SIV plasma viremia in these SIV-positive ART-treated macaques (Table II). Peak Ki67 expression occurred 5–7 d postinjection and returned fully to baseline by day 14 (Fig. 4). As predicted from the first dosing study (Fig. 3), the second and third IL-7 administrations given at 6-wk intervals resulted in increased Ki67 expression that was generally comparable to that of the initial IL-7 response in the CD4+ CM subset (Fig. 4A, Supplemental Table II). The absolute number of CM CD4+ T cells increased as well, averaging 2- to 2.5-fold increases from baseline levels in the peripheral blood synchronous with increased Ki67 expression (Fig. 4C, Supplemental Table II). The first rsIL-7gly administration resulted in only a small increase in Ki67 expression in the CD4+ naive cell subset (Fig. 4A). The absolute number of CD4+ naive T cells did however increase in the blood following each dose (Fig. 4C). In peripheral blood, CD8+ T cell Ki67 expression was elevated in both naive and CM cells at time points just following rsIL-7gly administration (Fig. 4B). In both subsets, the increase in the absolute number of T cells was generally transient and returned to basal levels by 21–42 d after rsIL-7gly administration (Fig. 4D). These data indicate that administration of rsIL-7gly in 6-wk dosing intervals does consistently elicit robust proliferation in the CD4+ CM subset following rsIL-7gly; however, a sustained increase in CD4+ T cell levels was generally not observed.

Assessment of rsIL-7gly utilizing a three-dose cluster strategy in SIV-positive ART-treated macaques

An alternative hypothesis is that closely spaced, or clustered, rsIL-7gly doses as administered to RM2-01 (Fig. 3C,3F) would be able to elicit and sustain long-term increases in T cell levels when administered in multiple rounds. To evaluate the efficacy of weekly clustering rsIL-7gly administration, an experiment was designed that used a total of nine rsIL-7gly doses administered over 12 wk.
(84 d) to two macaques stably treated with a fully suppressive ART regimen (Table I, clustered rsIL-7gly doses). The dosing strategy used was to administer rsIL-7gly in three doses over 3 wk in a cluster, followed by a 2-wk washout period; this regimen was administered three times. As in the previous two dosing strategies, treatment with rsIL-7gly was not directly associated with any long-term increases in plasma viral levels (Table II). The initial three-dose cluster of rsIL-7gly administration elicited increased Ki67 expression in CM CD4+ (Fig. 5A) and CD8+ T cells (Fig. 5B, Supplemental Table II). The increase in the percentage of Ki67+ cells resulted in a corresponding increase in the absolute number of proliferating CM cells (CD4+, Fig. 5C and CD8+, Fig. 5D). Peak Ki67 expression was attained ∼7 d after rsIL-7gly administration and remained elevated until day 28, suggesting that clustered dosing elicited proliferation over a longer time than a single dose of rsIL-7gly (Fig. 5A, 5B, Supplemental Table II). Unlike the previous study (Fig. 4), rsIL-7gly–induced proliferation in both CD4+ (Fig. 5A) and CD8+ (Fig. 5B) naive T cells, though Ki67 expression was higher in the CD8+ naive T cells. Interestingly, the rsIL-7gly–induced Ki67 increase in CD4+ and CD8+ T cell subsets was attenuated in the second- and third-dose clusters (Fig. 5A–D). This attenuated rsIL-7gly response could be observed.

### Table II. Effect of rsL-7gly administration on SIV plasma viral load

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>~7 d</th>
<th>7 d</th>
<th>14 d</th>
<th>21 d</th>
<th>28 d</th>
<th>63 d</th>
<th>77 d</th>
<th>105 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>rsIL-7gly 2-, 4-, or 6-wk intervals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM2-01</td>
<td>60</td>
<td>490</td>
<td>240</td>
<td>340</td>
<td>150</td>
<td>110</td>
<td>70</td>
<td>–</td>
</tr>
<tr>
<td>RM2-02</td>
<td>30</td>
<td>340</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>40</td>
<td>–</td>
</tr>
<tr>
<td>RM2-03</td>
<td>740</td>
<td>620</td>
<td>590</td>
<td>440</td>
<td>340</td>
<td>320</td>
<td>200</td>
<td>–</td>
</tr>
<tr>
<td>RM2-04</td>
<td>50</td>
<td>40</td>
<td>30</td>
<td>150</td>
<td>30</td>
<td>40</td>
<td>50</td>
<td>–</td>
</tr>
<tr>
<td>RM2-05</td>
<td>30</td>
<td>260</td>
<td>120</td>
<td>50</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>–</td>
</tr>
<tr>
<td>RM2-06</td>
<td>8800</td>
<td>1500</td>
<td>4300</td>
<td>4400</td>
<td>3700</td>
<td>6500</td>
<td>1300</td>
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<tr>
<td>RM2-01</td>
<td>190</td>
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<td>RM2-03</td>
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<td>230</td>
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<td>120</td>
<td>110</td>
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<tr>
<td>RM2-07</td>
<td>30</td>
<td>70</td>
<td>50</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>

Plasma viral load was monitored over the course of rsIL-7gly administration in all the cohorts using a real-time PCR-based assay. Day 0 represents the first rsIL-7gly administration to the macaques. The limit of detection was 30 copies per milliliter.

**FIGURE 3.** Increased Ki67 expression patterns for determining rsL-7gly dosing intervals. rsL-7gly–induced proliferation (Ki67+) cells was assessed by flow cytometry at 2-wk intervals in groups of two macaques. The change from baseline was calculated for each subsequent point. rsL-7gly administration is indicated (↓). CM (red) and naive cells (green). A, Change in percentage of Ki67+ cells in RM2-05 and RM2-06. B, Change in percentage of Ki67+ cells in RM2-03 and RM2-04. C, Change in percentage Ki67+ cells in RM2-01 and RM2-02. A–C, The average change in Ki67 expression from both macaques is shown for naive and CM CD8+ T cells. The fold change was also determined for the absolute number of CM CD4+ T cells in each of the macaques. D, Fold change in RM2-05 and RM2-06. E, Fold change in RM2-03 and RM2-04. F, Fold change in RM2-01 and RM2-02.
in each of the T cell subsets, with the naive cells being most affected. The observed reduction in the Ki67 response appeared to be unrelated to CD127 (IL-7R) expression, because the percentage of CD127+ cells was similar at the initiation of each cluster (data not shown). Therefore, the clustered dose strategy for administering rsIL-7gly resulted in a robust increase in the number of proliferating cells during the first three dosages that was strongly reduced during the next six rsIL-7gly administrations.

The ability of rsIL-7gly administered in clustered doses to impact peripheral blood T cell levels was assessed within the different T cell subsets. The clustered dosing regimen successfully increased peripheral blood levels of both CD4+ and CD8+ T cells within each of the virally suppressed SIV-positive macaques in the naive and CM subsets (Fig. 6). Comparing the increase in T cell number to that of macaques receiving only ART demonstrates that the effect is rsIL-7gly–mediated (Fig. 6, gray lines compared with black lines). This dosing regimen successfully increased the T cell levels in the blood, particularly the CD4+ CM cells, for the entirety of the 112-d study (Fig. 6A). In addition, despite low levels of induced Ki67 expression (Fig. 5A), naive CD4+ T cells also increased in circulation (Fig. 6A). Likewise, CD8+ CM and naive cells showed sustained long-term increases in absolute T cell numbers (Fig. 6B). In summary, these data indicate that clustering doses of rsIL-7gly in SIV-positive ART-treated macaques induced proliferation in a broad range of peripheral T cell subsets during and following the initial cluster of three doses. Furthermore, the

FIGURE 4. IL-7 administration during fully controlled SIV infection at 6-wk intervals transiently increases T cell number and proliferation. We assessed the change in Ki67+ T cells from pre-rsIL-7gly levels at each time point. Basal Ki67 levels were determined by averaging three rsIL-7gly time points prior to IL-7 administration. rsIL-7gly administration is indicated by (↓). CM (red) and naive cells (green). A, CD4+ T cells. B, CD8+ T cells. We then determined the effect of rsIL-7gly on the absolute number of T cells over time. The fold change was determined from the basal level of each T cell subset. CM (black) and naive cells (gray). C, Fold change in CD4+ T cells. D, Fold change in CD8+ T cells.

FIGURE 5. Three clustered doses of rsIL-7gly increase proliferation in peripheral T cells. Two macaques treated with stable ART therapy were administered rsIL-7gly at weekly intervals for 3wk followed by a 2-wk washout period. Proliferation in peripheral T cell subsets was monitored by flow cytometry assessing the fraction of Ki67+ cells. Each rsIL-7gly dose is indicated (↓). The change in Ki67 expression from baseline levels is shown. CM (red); naive cells (green). A, CD4+ T cells. B, CD8+ T cells. C, CD4+ T cells. D, CD8+ T cells.
ability to induce a sustained rsIL-7gly–related increase in T cell levels appears to be independent of the optimal induction of a Ki67 response in peripheral T cells.

Assessment of BrdU retention in SIV-positive ART- and rsIL-7gly–treated macaques in weekly dosing clusters

To determine whether the rsIL-7gly administrations impacted the retention of proliferating cells, we used BrdU, which is incorporated into the DNA of dividing cells and detected by flow cytometry. BrdU was administered for 4 d following the first of the nine rsIL-7gly doses in the clustered rsIL-7gly dosing study (Table 1) as a means of labeling dividing cells. We assessed the rate at which the label was lost in memory T cells in the blood following rsIL-7gly dosing. To determine the rate of loss, the amount of BrdU detected in the T cells was normalized between macaques by setting the percentage of BrdU+ cells at 1 d postlabeling at 100%. In the CD4+ memory cells, the BrdU label declined to 50% of the original amount by 14–28 d postlabeling in SIV-positive macaques treated with ART only (depicted in gray) but persisted 42–63 d postlabeling in macaques treated with rsIL-7gly (depicted in black) (Fig. 7A). rsIL-7gly administration had a greater impact on memory CD8+ T cells, which retained the label past the end of the follow-up period in macaques treated with rsIL-7gly (Fig. 7B). Therefore, in this relatively small number of SIV-positive ART-treated macaques, the addition of rsIL-7gly to these macaques increased the retention of the CD4+ and CD8+ memory cells that had been labeled during the first clustered weekly dosing.

Discussion

Immunotherapeutics have the potential to complement existing therapies that are designed to suppress HIV replication, particularly in those patients in whom CD4+ T cell reconstitution is not optimum following HAART (13–16). Cytokines involved in T cell homeostasis (e.g., IL-2, IL-7, and IL-15) have been proposed as immunotherapeutics during HIV infection (13, 15, 16, 27). The recent failure of IL-2 in clinical trials (31) highlights the challenges identifying and implementing an immune therapeutic treatment in HIV-infected patients. The strength of IL-7 for use as an HIV therapeutic lies in its ability to potentially increase the levels of two important CD4+ T cell subsets, naive and CM (14, 22, 46–51), through both cycling (58, 77–80) and cell survival (58, 81–84). This activity has already been addressed in nonhuman primates infected with SIV, although the IL-7 used in these studies was not optimum because it was not produced in mammalian cells (tended to elicit IL-7–specific neutralizing Abs) and the dosing strategy was generally for a short duration (14, 52–57). More recently, clinical studies in humans have also been undertaken, one in cancer patients by Sportès et al. (62) and two in HIV-positive patients conducted by Levy et al. (59) and Sereti et al. (61). Both Sportès et al. and Levy et al. document significant expansion of CD4+ and CD8+ T cells lasting from 28 d to 48 wk (59). Notably, in agreement with the nonhuman primate model (14, 52–57), the human studies indicate that IL-7 administration effectively expands both naive and memory T cells (59, 62). Although both human studies reported increased cell cycling, the study by Sportès et al. (62) also indicated a role for IL-7–mediated T cell survival effects, as opposed to proliferative effects, as the principle contributor to CD4+ T cell expansion.

Our approach in this study was to use the SIV/macaque model to further elucidate the efficacy of immune therapy using rsIL-7gly treatment in a context where ongoing virally mediated killing was minimized. Previous work in the nonhuman primate model was conducted with 80–100 µg/kg/day (14, 54, 55) or regimens of 100 µg/kg/day every other day (52, 57, 72). On the basis of work published in 2007 by Dereudre-Bosquet et al. (57) that provided evidence that the Ki67 response to IL-7 administration peaked at 7-d intervals, regardless of IL-7 administration in the interim, we chose a 7-d dosing interval as the minimum and then further assessed the 2-, 4-, and 6-wk dosing intervals. This study used a relatively low-dose (30 µg/kg/wk) administration of rsIL-7gly as well as washout periods between doses to reduce the frequency of administration while retaining what we hypothesized would be an optimal CD4+ T cell response to rsIL-7gly. In addition, we sought to characterize further in the macaque model rsIL-7gly’s impact on different T cell subsets following these different dosing intervals. The dosing interval study (Fig. 3) assessed the impact of varying the third dose to a 2-, 4-, and 6-wk spacing in SIV-positive ART-treated macaques that had previously been administered two weekly injections of rsIL-7gly. Overall, the third dose resulted in a muted Ki67 proliferative response compared with those of the earlier doses. We observed that spacing the third dose to a 6-wk interval resulted in the best peak Ki67

![FIGURE 6. rsIL-7gly administration results in long-term increases in absolute T cell number during fully controlled SIV infection. Where applicable, rsIL-7gly administration is indicated (↓). The mean absolute T cell count of four macaques treated with ART alone (gray square), RM2-05 (closed circle), and RM2-07 (open circle) is shown in each figure (mean ± SEM). A, Fold change in naive and CM CD4+ T cell subsets. B, Fold change in naive and CM CD8+ subsets.](http://www.jimmunol.org/content/vol189/issue6)
response within macaque RM2-05 and a 1.6-fold increase in CD4+ CM cells. In addition, although the 2-wk interval third dose was associated with a smaller percentage of Ki67+ CM CD4+ T cells, the numbers of peripheral CM CD4+ T cells still increased to >2-fold the pre–rsIL-7gly levels. These data enabled us to generate the hypothesis that spacing the rsIL-7gly dosages 6 wk apart would result in robust Ki67 responses, whereas closely spacing the dosages could result in superior fold increases in CM CD4+ T cells.

To more clearly assess the advantages and disadvantages of the different dosing studies, we undertook two studies, the first administering rsIL-7gly every 6 wk and a second administering rsIL-7gly in clustered weekly intervals. We repeated each dosing regimen for three cycles, assessing the effect of rsIL-7gly on CD4+ and CD8+ naive and CM cells after each dose. When we administered rsIL-7gly at 6-wk intervals, we elicited robust proliferation but little long-term increase in T cell number, whereas clustering the dosages in 1-wk intervals resulted in a sustained CM CD4+ T cell increase in the two macaques studied. These observations suggest that the cumulative effect of clustering rsIL-7gly doses may be important to maintaining the self-renewing capacity of CM cells. Two further observations from these studies support the hypothesis that mechanisms other than inducing detectable increases in proliferation, for example, increased cell survival in the periphery, may contribute to increasing absolute cell number in macaques. First, CD4+ naive cells increased absolute count in the periphery with little detectable change in Ki67 expression. Second, when IL-7 was administered in a three-dose cluster, despite observing a muted Ki67 response to the second and third rounds of IL-7 treatment, in the same macaques, the absolute T cell count continued to increase with each sequential cluster of IL-7 administration (Figs. 5, 6). These results suggest that IL-7 has therapeutic benefits contributing to expanding the peripheral T cell pool that are not always resulting from increased proliferation but rather due to antiapoptotic survival effects of IL-7. We conclude that the optimal dosing regimen would likely be one with closely spaced (weekly) IL-7 doses that maximizes the benefit from both proliferative and survival effects of the cytokine. Further, we would hypothesize that a cluster of IL-7 doses (three at 1-wk intervals) could be used as a stopgap measure in HIV-positive HAART-treated patients that do not recover CD4+ T cell levels above a certain target, for example, 500 CD4 cells per microliter of blood. Frequent monitoring of these patients would be required to determine whether additional IL-7 clustered dosing were warranted and could be readministered should the patients peripheral blood CD4 count drop below a target value at subsequent visits.

The importance of maintaining the self-renewing capacity of the CM CD4+ T cell population with regard to disease progression has been described in previous studies (24–26). These data suggest that IL-7 administration can effectively regenerate the CD4+ CM subset, directly countering a major effect of viral pathogenesis. Further, the results from our Brdu labeling studies indicate that the cells dividing during the first round of rsIL-7gly have a longer retention time in the peripheral blood compared with those of the controls. The most likely hypothesis to explain these findings is that rsIL-7gly has a direct antiapoptotic role and is preventing apoptosis, thereby prolonging the life span of memory CD4+ T cells. Indeed, a recent study by Beq et al. (58) demonstrated that rsIL-7gly administration to macaques resulted in suppressed levels of apoptosis (measured by assessment for active forms of caspases 3, 8, or 9 as well as DNA fragmentation). Beq et al. (58) further demonstrated that by inducing T cell trafficking to mucosal sites, such as the intestine, rsIL-7gly may actually be able to assist in repair of SIV-induced damage at these sites as well. IL-7 administration impacts naive T cell population dynamics in addition to memory cell populations. Increasing the absolute number of naive T cells could potentially provide ancillary immunological benefits, such as increased memory precursor populations, that may be beneficial for patients.

The studies in HIV/AIDS patients, in particular those conducted by Levy et al. (59), are promising and provide strong support for further developing IL-7 as an immunotherapeutic (61). The macaque models, which have thus far been highly consistent with the studies in humans, enable the undertaking of highly controlled SIV infections and more extensive sampling, providing higher resolution of the events following rsIL-7gly administration and better determination of the systemic effects of rsIL-7gly administration. Of some concern from the macaque studies is the monkey-to-monkey variation that we are observing in the different rsIL-7gly–treated groups, indicating that every patient might not benefit equally from the use of IL-7 as an immune therapeutic. However, it is possible that the variability could in part be due to the macaques in this study having relatively high levels of CD4+ T cells following ART treatment (ranging from 519 to 1054 CD4+ T cells per microliter of blood) (Supplemental Table 1). One important positive finding in these studies is that SIV-positive macaques with an ART-controlled infection respond to rsIL-7gly in a manner similar to SIV-negative macaques, with proliferative bursts and a potential for a sustained increase in multiple CD4+ T cell subsets. Overall, these studies provide further evidence that IL-7 can be used effectively as an immunotherapeutic to increase the absolute number of CD4+ T cells to assist in the recovery of the immune system dysfunction induced by the SIV or HIV infections. It will be important to focus future studies on assessing the functionality of the T cells that are responding to IL-7 therapy to determine that they can perform necessary functions when exposed to Ags or pathogens.

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Disclosures
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References
IL-7 THERAPY OF SIV-POSITIVE ART-TREATED MACAQUES


