Interleukin-15 but Not Interleukin-7 Abrogates Vaccine-Induced Decrease in Virus Level in Simian Immunodeficiency Virus mac251-Infected Macaques


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Interleukin-15 but Not Interleukin-7 Abrogates Vaccine-Induced Decrease in Virus Level in Simian Immunodeficiency Virus_{mac251}-Infected Macaques


The loss of CD4+ T cells and the impairment of CD8+ T cell function in HIV infection suggest that pharmacological treatment with IL-7 and IL-15, cytokines that increase the homeostatic proliferation of T cells and improve effector function, may be beneficial. However, these cytokines could also have a detrimental effect in HIV-1-infected individuals, because both cytokines increase HIV replication in vitro. We assessed the impact of IL-7 and IL-15 treatment on viral replication and the immunogenicity of live poxvirus vaccines in SIV_{mac251}-infected macaques (Macaca mulatta). Neither cytokine augmented the frequency of vaccine-expanded CD4+ or CD8+ memory T cells, clonal recruitment to the SIV-specific CD8+ T cell pool, or CD8+ T cell function. Vaccination alone transiently decreased the viral set point following antiretroviral therapy suspension. IL-15 induced massive proliferation of CD4+ effector T cells and abrogated the ability of vaccination to decrease set point viremia. In contrast, IL-7 neither augmented nor decreased the vaccine effect and was associated with a decrease in TGF-β expression. These results underscore the importance of testing immunomodulatory approaches in vivo to assess potential risks and benefits for HIV-1-infected individuals. The Journal of Immunology, 2007, 178: 3492–3504.
cells (20). In vitro, IL-15 appears to be superior to IL-7 in enhancing HIV-specific CD8\(^+\) T cell effector function (21, 22) and NK-mediated antiviral activity in autologous PBMCs (23).

In rhesus macaques (Macaca mulatta (Mamu)) chronically infected with SIVmac251, pharmacological doses of rIL-15 have been shown to increase the proliferation of effector CD4\(^+\) and CD8\(^+\) T cells and CD3\(^+\)CD8\(^-\) NK T cells (24, 25). In uninfected macaques immunized with tetanus toxoid or a live flu vaccine, rIL-15 increased long-term memory responses (26). In contrast, the administration of rIL-7 to healthy or SIV-infected macaques increased the proliferation of all T cell subsets and enhanced de novo thymic production (27–29).

IL-15 production is deficient in HIV-1 infection (30–32). In contrast, IL-7 availability is increased during primary HIV-1 infection and persists, likely as a response to or as a cause of lymphopenia. Indeed, IL-7 levels are inversely correlated with CD4\(^+\) T cell counts (33–36).

In vitro, both IL-7 and IL-15 induce HIV replication from latently infected cells of HIV-infected patients and increase the susceptibility of naive CD4\(^+\) T cells to HIV infection (37–46). Thus,

**FIGURE 1.** IL-15 abrogates vaccine-induced decrease in a viral set point. A, Schematic representation of the immunization and cytokine treatment regimens. ART was initiated at week 16 and suspended at week 41. Vaccination was performed at weeks 24, 30, and 36, as indicated by the arrowheads. The asterisks indicate the Mamu-A*01\(^+\) macaques included in the study. Cytokines were given as described in Materials and Methods. B, Pre- and post-ART plasma virus levels in each of the mock-vaccinated macaques in groups 1, 2, and 3 that were included in the evaluation of a vaccine effect on viral replication. The mean values of plasma virus level at weeks 1–16 were compared with the mean levels at weeks 45–55 in macaques from groups 1–3. D and E, Same as B and C for the macaques in groups 4, 5, and 6. Group 1 is shown for reference in E.
their use in the clinic could help to mobilize and eradicate the virus in the resting T cell population on one hand and increase virus replication on the other. In this study, we demonstrate that vaccination decreased, at least transiently, the viral set point and that neither IL-7 nor IL-15 treatment augmented the vaccine immunogenicity. Surprisingly, we also found that treatment with IL-15 abrogated the vaccine-induced decrease in the viral set point.

**Materials and Methods**

**Animals**

Forty-two colony-bred rhesus macaques were obtained from Covance Research Products. The animals were housed and handled in accordance with the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care International, and the study was reviewed and approved by the animal care and use committee at Advanced BioScience Laboratories. The care and use of the animals were in compliance with all relevant institutional (National Institutes of Health) guidelines. All animals were infected by the i.v. route with SIVmac251 (561) as previously described (47–49).

**Treatment and vaccination schedule**

ART treatment consisted of i.v. administration of didanosine (10 mg/kg/day), oral administration of stavudine twice a day (1.2 mg/kg/dose), and s.c. administration of 9-(2-phosphonylmethoxypropyl)adenine (20 mg/kg/day). The macaques were vaccinated with either 10⁶ PFU of the ALVAC mock vaccine (groups 1, 2, and 3) or 10⁷ PFU of ALVAC-SIV-gag-pol-env (gpe) (groups 4, 5, and 6) at weeks 24, 30, and 36 (Fig. 1A). A cycle of s.c. rhesus macaque IL-7 treatment consisted of inoculation every 3 days starting 2 wk before immunization and maintained for one more week thereafter at a dose of 100 μg/kg for a total of eight doses. Two cycles of IL-7 treatment were given with the first two immunizations. At the time of the third immunization with ALVAC-SIV, IL-7 treatment was omitted because we had detected neutralizing Abs to IL-7 in the sera of most of the macaques (data not shown). A cycle of s.c. rhesus macaque IL-15 treatment consisted of inoculations twice a week for three consecutive weeks at a dose of 10 μg/kg for a total of six doses. IL-15 inoculation was initiated 1 wk after immunization. Rhesus macaque IL-15 did not elicit binding Abs and treatment was completed for three cycles, as planned.
Phenotypic and functional characterization of T cell subsets

Fresh PBMCs were isolated using lymphocyte separation medium (Cappel) density centrifugation. In some instances PBMCs were frozen (90% FCS and 10% DMSO) until use.

For analysis of Ki-67 levels, 1 × 10^6 cells were washed and surface-labeled with the following mAbs: CD4-PerCP (clone L200; BD Pharmingen), CD8-PE or CD8-allophycocyanin (clone 2ST8.5H7; Beckman Coulter), CD28-allophycocyanin or CD28-CyChrome (clone CD28:2; BD Pharmingen), and CD95-PE or CD95-CyChrome (clone DX2; BD Pharmagen). Surface-labeled cells were resuspended for 15 min in fixation/permeabilization solution (BD Pharmingen), washed, and labeled with anti-Ki-67 FITC (clone B56; BD Pharmingen) or isotype Abs for 25 min at room temperature.

For functional analysis of SIV-specific CD8^+ T cells, 10^6 fresh or thawed PBMCs were examined for cognate elicitation of cytokines and/or CD107a expression. To perform intracellular cytokine staining, a total of 1 × 10^6 PBMCs were incubated in RPMI 1640 medium supplemented with 10% FCS and antibiotics for 6 h in the presence of specific pooled peptides at a final concentration of 2 μg/ml each or in the presence of the superantigen staphylococcal enterotoxin B at 1 μg/ml (positive control) or medium alone (negative control). The costimulatory mAb anti-CD94d (0.5 μg/ml; BD Biosciences) was added to maximize the detection of T cells with higher activation thresholds (50); anti-CD28 mAb was not used as a co-stimulatory molecule in experiments in which it was included in the mix of Abs used for the detection of different subsets. Brefeldin A (Sigma-Aldrich) at a final concentration of 10 μg/ml was added after 1 h. The cells were then washed, stained for surface markers, permeabilized by incubation in FACS Perm/Wash solution (BD Biosciences), stained with anti-TNF-α (clone MAB11; BD Biosciences) and anti-IFN-γ (clone 4SB3; BD Biosciences) mAbs, and analyzed as described below. For combined detection of degradation and cytokine production, 10^6 fresh or thawed PBMCs were washed and incubated with 1 μg/ml each anti-CD28 and anti-CD94d mAbs and 1 μg/ml each pooled overlapping Gag peptides in a 1 ml final volume. Conjugated Abs to the granular membrane protein CD107a were supplied by M. Roederer (Laboratory of ImmunoTechnology, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD) and were added to the cells before stimulation. A negative control (anti-CD28, anti-CD49d) and a positive control (staphylococcal enterotoxin B at 1 μg/ml) were included to control for spontaneous activated production of cytokines and/or expression of CD107a. The cultures were incubated for 1 h at 37°C in 5% CO_2 followed by an additional 4 h in the presence of the secretion inhibitor monensin (0.5 μg/ml) (BD Pharmingen) and brefeldin A (10 μg/ml) (Sigma-Aldrich). After stimulation, PBMCs were washed, surface stained with anti-CD4 or anti-CD8 mAbs, washed again, and permeabilized using FACS Perm/Wash buffer (BD Biosciences). After permeabilization, the cells were washed again and stained with mAbs specific for IFN-γ and TNF-α as described above. The cells were then washed again and resuspended in 1% paraformaldehyde (Electro-Optics Microsystems) in PBS. Four-parameter flow cytometry analysis was performed using a FACSCalibur flow cytometer. List mode data files were analyzed using FlowJo software (Tree Star).

In all cases at least 100,000 live events were collected for analysis.

To characterize the detection of SIV-specific CD8^+ T cells, staining was performed with pretittered, allophycocyanin-conjugated (Molecular Probes) Gag_181-189 CM9 (p11C) (CPTYDINQM)-Mamu-A^01 tetramer complexes for 30 min at room temperature (51). One hundred thousand events were collected in the lymphocyte region (R1) and analyzed with CellQuest software and PAINT-A-GATE (BD Biosciences).

Viral and cytokine RNAs

We quantified SIVRNA in plasma and tissues by nucleic acid sequence-based amplification (52) or real-time PCR. Briefly, RNA was extracted from plasma and tissues as previously described (53) and isothermally amplified using SIVmac251-specific primers. Quantification was conducted by using an electrochemiluminescence chemistry-based probe hybridization system with a coextracted internal standard. The copy number was expressed per 100 μl of plasma or per microgram of RNA, and the detection limit of the assay was 2 × 10^2 RNA copies.

Total RNA was extracted from macaque PBMCs using the guanidium thiocyanate-phenol-chloroform method modified for TRIzol (Invitrogen Life Technologies). RNA (1 μg) was reverse transcribed into first-strand cDNA in a 20-μl reaction containing 1 μM random hexanucleotide primers, 1 μM oligo(dT), and 200 U of Moloney murine leukemia virus reverse transcriptase (Promega).

cDNA quantification for TGF-β and GAPDH was performed by real time PCR as previously described (54). Results are presented as relative units of the ratio between TGF-β and GAPDH mRNA.

FIGURE 2. IL-15 increases virus levels in resting CD4^+ T cells. Box plots indicate the range of fold change in virus produced by purified resting CD4^+ T cells from the PBMCs of macaques treated with one complete cycle of either IL-7 or IL-15 or left untreated.

**End point dilution virus isolation**

Quiescent CD4^+ T cells were isolated from fresh PBMCs by negative selection. PBMCs were cultured overnight in RPMI 1640 medium. Non-adherent cells were then incubated with Abs recognizing CD8 (BD Biosciences), CD14 (BD Pharmingen), CD16 (BD Biosciences), CD19 (Beckman Coulter), CD25 (BD Pharmingen), HLA-DR (BD Biosciences), and CD69 (BD Pharmingen), followed by goat anti-mouse Ab-conjugated magnetic beads (Miltenyi Biotec). Depletion of positive cells was conducted using the autoMACS system (Miltenyi Biotec). The remaining quiescent CD4^+ T cells were cocultured in PHA/IL-2 at serial 5-fold dilutions with prestimulated primary human CD4^+ T cells and an activated human T cell line (CEM). Cocultures were maintained for 3 wk, with addition of freshly stimulated human T cells every 2–3 days. Supernatants were harvested and assayed for viral p27 by ELISA. Levels of p27 were converted into infectious units per million cells (IUPM). We took the fold change in pretreatment vs posttreatment levels of IUPM; this is defined as pretreatment IUPM levels divided by posttreatment IUPM levels. Groups were compared using two-sided Kruskal-Wallis and Wilcoxon Rank Sum tests, nonparametric tests that are powerful in small sample sizes.

**Analysis of SIV-specific CD8^+ T cell clonotypes**

Fluorochrome-conjugated tetrameric Mamu-A^01 complexes were used to label cognate CD8^+ T cell populations specific for the CM9 (CTPY DINQM; Gag, residues 181–189) epitope as described previously (55). Viable Ag-specific CD8^+ T cells were then isolated by flow cytometric sorting to >99% purity and extracted mRNA was subjected to a template switch-anchored RT-PCR that amplifies all expressed TCRB T cell clonotypes and an R1 sorting to a CD8^+ T cell clonotype specific for the CM9-specific CD8^+ T cell population.

**Statistical analysis**

Statistical analysis for mRNA expression was performed using the SPSS 13.0 software. Differences between groups were assessed by a nonparametric Mann-Whitney U test. Differences before and after treatment within the same group were assessed using the Wilcoxon test. Log-transformed plasma virus levels were tested using a repeated measures ANOVA model. CD4^+ and CD8^+ T cell responses were arcsine transformed and then analyzed at individual times using the ANOVA method with p values corrected by the Hochberg procedure for the multiple comparisons of ALVAC-SIV-vaccinated and cytokine-treated groups against the ALVAC control group. All p values shown in the text and figures are two-tailed.

**Results**

IL-15 abrogates the ability of vaccination to decrease plasma virus levels at set point

The ability of therapeutic vaccines to expand memory CD4^+ T cell responses has been correlated with the transient control of viral
**FIGURE 3.** IL-15 increases Ki-67 expression on effector memory CD4\(^+\) and CD8\(^+\) T cells. 

A, Examples of raw data on Ki-67 expression following one cycle of treatment with IL-15 in CD4\(^+\) and CD8\(^+\) T cells. Cell populations are defined according to the following parameters: naive T cells, CD28\(^-\) and CD95\(^-\); central memory T cells, CD28\(^-\) and CD95\(^-\); effector memory T cells, CD28\(^-\) and CD95\(^-\). The Ki-67\(^+\) T cells within each T cell subset were obtained by back-gating Ki-67\(^+\) T cells on the entire cell population in each quadrant. Representative examples of the frequency of Ki-67\(^+\) T cells in the blood of the animals treated with IL-15 are shown.

B, Mean percentage of Ki-67\(^+\) T cell subsets in mock-vaccinated and ALVAC-SIV-vaccinated macaques in the absence or presence of IL-15. Asterisks refer to significant differences \(p < 0.05\) between the ALVAC-SIV-vaccinated groups in the presence of IL-15. T\(_{\text{CM}}\), Central memory T cells; T\(_{\text{EM}}\), effector memory T cells.
replication (56). Thus, approaches able to further expand SIV-specific memory responses may synergize with vaccination. We have previously observed that, unless viral replication is suppressed by ART in SIVmac251-infected macaques, vaccination does not expand SIV-specific responses (47), and certain SIVmac251-infected macaques are able to control virus replication before ART and

**FIGURE 4.** Effect of IL-7 and IL-15 on the expansion of SIV-specific CD8+ T cells. A–C. Frequency of CM9 tetramer CD8+ T cells in the blood of Mamu-A*01+ vaccinated macaques over time in groups 4, 5, and 6 (A) and the frequency of Gag-specific CD8+ T cells producing IFN-γ or TNF-α in macaques vaccinated only or vaccinated and treated with either IL-7 (B) or IL-15 (C). Only significant p values are provided. D. Frequency of CD8+CD107+ T cells producing IFN-γ or TNF-α in blood of the immunized macaques 2 wk before immunization (week 22) and 2 wk after the last immunization (week 38).
FIGURE 4. (continued)

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maintain control of viral replication once ART is discontinued (47, 49). Therefore, we designed a study with clear inclusion criteria: 1) sustained suppression of plasma virus levels below detection (<2000 copies/ml plasma) during ART; and 2) virus levels ≥10⁵ copies/ml plasma before the initiation of ART. With these criteria in mind, 42 Indian rhesus macaques were infected i.v. with SIVmac251 and initiated on ART at week 16. The macaques were divided into six groups and, of those, group 1 (eight macaques) and groups 2 and 3 (five macaques each) were mock vaccinated in the presence or absence of IL-7 and IL-15 as controls (Fig. 1A). Groups 4, 5, and 6 (eight macaques each) were immunized with ALVAC-SIV-gpe expressing the gag, pol, and env genes of SIVmac251 (57). In addition to the vaccine, group 5 received IL-7 and group 6 received IL-15 (Fig. 1A).

IL-7 was administered 2 wk before vaccination to increase the pool of “naïve” cells able to respond to the vaccine. IL-7 was maintained during the first week of vaccination to favor the expansion of memory cells, as we had previously observed that IL-7 at 100 µg/kg increased the proliferation of memory T cell subsets in addition to naïve cells (28, 29). Because murine studies indicate that IL-15 is important for both the initiation and maintenance of immune responses (18), this cytokine was administered at the time of immunization and maintained for three consecutive weeks. The dose of 10 µg/kg was chosen because of the proven ability of the cytokine at this dose to increase specific responses to flu Ags in nonhuman primates (26).

Of the initial 42 macaques enrolled in the study, 20 were excluded during treatment because they failed to meet the inclusion criteria, with either virus levels below 10⁵ copies/ml plasma before ART initiation or an undetectable viral load for four consecutive weeks on ART (data not shown). One additional animal could not be evaluated because it developed diabetes and was euthanized at week 45. Thus, the virological outcome following vaccination and cytokine treatment could be evaluated in the remaining 22 animals. Before ART initiation there was no statistically significant difference in plasma virus level or CD4⁺ T cell numbers among the groups (data not shown). When mean pre-ART (weeks 6–16) plasma virus levels were compared with mean post-ART (weeks 45–55) levels, no significant differences were observed in the mock-immunized macaques that received either no cytokines or IL-15 alone (Fig. 1, B and C, groups 1 and 3). Although a significant reduction of plasma virus levels was observed when IL-7 was added to the mock vaccine (p = 0.0033), the low number of animals left in this group precluded a definitive evaluation of the effect of IL-7 treatment alone (Fig. 1, B and C, group 2). In contrast, ALVAC-SIV-vaccinated macaques with or without IL-7 had lower viral set point when ART was discontinued (p = 0.003 and 0.0007, respectively) (Fig. 1, D and E, groups 4 and 5). Surprisingly, the addition of IL-15 to the vaccine appeared to abrogate the vaccine-induced decrease in viral set point, and the pre- and post-ART plasma virus levels in this group only approached statistical significance (p = 0.053) (Fig. 1, D and E, group 6).

Following infection with SIVmac251, the number of CD3⁺CD4⁺ T cells/mm³ decreased and the CD4⁺ T cell number did not differ significantly among the groups. As expected, ART treatment resulted in a comparable increase of CD4⁺ T cells in all groups, and this increase was not affected by vaccination or cytokine treatment. Similarly, following ART suspension no significant differences were observed within the 1-year observation time (data not shown).

IL-15 but not IL-7 increases the viral reservoir

Despite the fact that both IL-7 and IL-15 increase HIV-1 replication in vitro, the data presented above suggested that the effects on SIV replication in vivo of IL-7 and IL-15 may differ. Because of the clinical implications of these findings, we wished to confirm by alternative approaches whether IL-15 increased viral replication. The infectious virus in peripheral resting T cells from the infected macaques was therefore measured before (week 21) and after one complete cycle of IL-7 (week 26) or IL-15 (week 28) treatment. PBMCs were depleted of CD8⁺ T cells and activated T cells, B cells, and monocytes by negative selection and the virus was isolated from the remaining quiescent CD4⁺ T cells (<95% pure) by end point dilution using activated human PBMCs and the CEM T cell line as targets. To increase sample sizes, all non-cytokine-treated macaques (ALVAC- and ALVAC-SIV-gpe-immunized) were analyzed together. Untreated animals had a median decrease of 6.6 (range −5.2 to −8.0) IUPM, whereas animals treated with IL-7 had a median decrease of 5.2 (range −42.8 to 0.2) IUPM and animals treated with IL-15 had a median increase of 1.1 (range −8.1 to 23.8) IUPM. We then examined the fold change in pre-treatment vs posttreatment for all three groups. Fold change is defined as the posttreatment IUPM divided by the pretreatment IUPM. Thus, fold change values of >1 indicate an increase in IUPM and fold changes of <1 indicate a decrease. The median fold change in the cytokine-untreated animals, IL-7-treated animals, and IL-15-treated animals was 0.9 (range 0 to 0.35), 0.14 (range 0.01 to 1.08), and 2.2 (range 0.5 to 3.2), respectively. The groups were significantly different by the Kruskall-Wallis test (p = 0.01). The fold change in IL-7 was statistically different from the fold change in IL-15 (p = 0.01 by the Wilcoxon rank sum test). The box plots showing the median and range of fold change by each cytokine is given in Fig. 2. A line was drawn at 1 as values of 1 indicate no change in pretreatment vs posttreatment. Values above the line show an increase in IUPM and values below 1 indicate a decrease in IUPM. These results therefore indicated that IL-7 and IL-15 have opposing effects on the viral reservoir in vivo.

IL-15 significantly increases Ki-67 expression on both effector CD4⁺ and CD8⁺ T cells

At first we investigated which T cell subset was affected by the cytokine treatment by measuring the expression of the Ki-67 cell cycle progression marker on total naïve and memory CD4⁺ and CD8⁺ T cell subsets defined by the CD28 and CD95 markers (58). In SIV-infected macaques, IL-7 induced the proliferation of naïve T cell subsets within the CD4⁺ and CD8⁺ T cell populations that acquire a “memory-like” phenotype as previously described both...
in mice and macaques (28, 59) (data not shown), impairing our ability to properly quantitate T cell subsets following IL-7 treatment. In contrast, IL-15 mainly increased the Ki-67 marker on CD4^+ and CD8^+ effector memory T cells (Fig. 3A). The increase in the percentage of Ki-67^+ effector memory CD4^+ and CD8^+ T cells was observed in both groups receiving IL-15, whether immunized with the ALVAC-SIV-gpe or with the ALVAC vector only (Fig. 3B). However, this increase was significant only in the group of animals immunized with ALVAC-SIV-gpe. The small size of group 3 (two animals) likely accounts for this discrepancy (Fig. 3B).
Neither IL-7 nor IL-15 affects frequency, function, or clonotype recruitment of SIV-specific CD8\(^+\) T cells

To assess whether IL-7 or IL-15 augmented vaccine-induced immune responses, we measured the frequency of the dominant Gag\(_{181-189}\) CM9 response by tetramer staining in the Mamu-A\(^*\)01\(^-\) animals and the immune response to the entire Gag peptide by intracellular cytokine staining in Mamu-A\(^*\)01\(^-\) animals. At weeks 25 and 26 following the first immunization, the Gag\(_{181-189}\) CM9 tetramer\(^+\) T cells were expanded in all immunized Mamu-A\(^*\)01\(^-\) macaques; however, neither IL-7 nor IL-15 significantly augmented this vaccine-induced immune response and did not affect the extent nor kinetics of the tetramer response when viral replication resumed after ART suspension (Fig. 4A). One potential caveat to this finding is the demonstration that IL-15-expanded effector memory T cells tend to exit the blood circulation, potentially lowering the number of effectors detected among PBMCs (25).

Next, we measured the ability of memory T cells to produce TNF-\(\alpha\) and IFN-\(\gamma\) after stimulation with the entire Gag peptide pool before, at time 0, and 3 and 6 wk after immunization. Vaccination significantly increased the total Gag-specific CD8\(^+\) T cell response (\(p < 0.0001\)), and this increase was reflected mainly in the central memory T cell compartment (Fig. 4B). Although IL-7 had no discernible effect on Gag-specific CD8\(^+\) T cell responses, IL-15 significantly increased Gag-specific CD8\(^+\) central memory T cells in mock-vaccinated macaques, and there was a trend toward an increase in cognate effector T cells as well (Fig. 4C). However, as with the tetramer data presented in Fig. 4A, there was no significant increase in excess of that induced by ALVAC-SIV alone.

Because IL-15 and, to some extent, IL-7 increase effector function in vitro, we measured CD8\(^+\) effector T cells more accurately using Abs to CD107, a phenotypic marker of degranulation. Following the stimulation of PBMCs with the entire Gag peptide pool, the ability of CD8\(^+\) T cells to degranulate and produce TNF-\(\alpha\) and IFN-\(\gamma\) was measured before immunization (week 22) and, following all immunizations and cytokine treatment, at week 38. Although the differences found in Gag-specific effector memory CD8\(^+\) T cells among the groups studied did not reach statistical significance, there was a trend toward an increase in the number of Gag-specific effector CD8\(^+\) T cells in the IL-15-treated group, likely reflecting the ability of IL-15 to increase the proliferation/survival of effector T cells (Fig. 4D).

Although IL-7 and IL-15 had no significant effects on the magnitude or functional attributes of the virus-specific CD8\(^+\) T cell response above and beyond those endowed by ALVAC-SIV vaccination alone, clonotypic fluctuations induced by these cytokines could potentially mediate differential effects on viral replication. To assess the effects of IL-7 and IL-15 on the clonotypic composition of SIV epitope-specific CD8\(^+\) T cell responses, we sorted viable CM9-Mamu-A\(^*\)01 tetramer "CD8\(^+\)" T cells from MHC class I allele-matched macaques in groups 4, 5, and 6 and to >99% purity and conducted a longitudinal molecular analysis of constituent clonotypes using an unbiased template switch-anchored RT-PCR as described previously (55). There was a significant decrease in overall clonality across the cohort after 4 wk of ART before vaccination (week 20) compared with a postprimary infection pretreatment time point (week 12) (\(p = 0.0312\); upon the subsequent cessation of ART after immune manipulation (week 41), no significant changes in clonality were observed (Fig. 5). Further, no significant differences in clonality between groups were detected at week 41 (Fig. 5).

IL-7 and IL-15 treatment and SIV-specific CD4\(^+\) T cell responses

Although the effect of IL-7 and IL-15 on CD8\(^+\) T cell responses is well documented, less is known about how these cytokines affect Ag-specific CD4\(^+\) T cell responses. As observed for the Gag-specific CD8\(^+\) T cell responses, vaccination significantly increased SIV-specific CD4\(^+\) T cell responses (\(p = 0.0014\)). Surprisingly, IL-7 treatment and vaccination resulted in a significant expansion of Gag-specific CD4\(^+\) effector T cells (\(p = 0.026\)) (Fig. 6, A and B). These findings are unexpected, because IL-15 induced the cell cycle progression of effector CD4\(^+\) T cells (Fig. 3, A and B). Thus, although IL-7 and IL-15 did not significantly augment the CD8\(^+\) T cell response in conjunction with vaccination, they affected different subsets of memory CD4\(^+\) T cells.

IL-7 decreases TGF-\(\beta\) production

IL-7 is known to decrease production and signaling mediated by TGF-\(\beta\), a cytokine that increases macrophage susceptibility to HIV-1 infection (60), increases HIV replication (61), and negatively affects immune function in HIV-1-infected patients (62). Because these data suggested a decrease in virus levels in IL-7-treated animals, we speculated that IL-7 may have affected TGF-\(\beta\) production. We therefore investigated TGF-\(\beta\) expression in RNA obtained from macaque PBMCs before (week 16) and after (weeks 26, 34, 46) treatment with cytokines by real-time PCR. A significant decrease in TGF-\(\beta\) expression over time was observed only in the IL-7-treated group, and this effect was durable because, at 5 wk after ART suspension (week 46) the levels of TGF-\(\beta\) in blood remained significantly lower in the group treated with IL-7 (Fig. 7). Importantly, the level of mRNA expression of other cytokines such as IL-2 and IL-10 and that of the negative immune regulator IDO were not affected by IL-7 treatment (data not shown).

Discussion

The immune response to HIV/SIV fails to abate virus replication, suggesting that methods to potentiate the adaptive host response may be of clinical benefit. Both IL-7 and IL-15 are cytokines produced by stromal cells that directly affect the homeostatic proliferation of memory T cells and regulate effector function (63, 64). Prior studies on naive or SIV-infected macaques treated with rIL-7
protein have demonstrated that this cytokine alters T cell homeostasis (27, 28, 65) without altering SIV replication (66). The use of rIL-15 has been pioneered in naïve macaques alone or together with Ags (26) and has been demonstrated to increase the frequency of long-lived CD4+ and CD8+ T cells. IL-15 treatment of a small number of SIV-infected macaques was reported not to affect viral replication (24, 25). However, none of the previous studies have investigated whether IL-7 or IL-15 could augment either a preexisting or an SIV vaccine-induced T cell response. In this study we have modeled the clinical use of both IL-7 and IL-15 together with a live poxvirus vector-based SIV vaccine in SIVmac251-infected rhesus macaques.

The vaccination of HIV-1-infected individuals (67) or SIV-infected macaques (47, 49, 56, 68) with these vaccine modalities results in an expansion of virus-specific adaptive CD4+ and CD8+ T cells that is nevertheless insufficient to control viral replication in the long term. In SIVmac251-infected macaques we have previously demonstrated a correlation between vaccine-induced CD4+ and CD8+ T cell responses and set point viremia following ART suspension (49), and the phenotypic characterization of SIV-specific CD8+ T cells demonstrated an inverse correlation with Ag-specific central memory CD8+ T cells and plasma virus level (68). Thus, we hypothesized that approaches able to increase the frequency of memory T cells may augment the benefits of vaccination.

The rationale for the use of IL-15 and IL-7 in the treatment of HIV/SIV infection stems from the fact that production of IL-15 is decreased in HIV-1 infection. IL-7 expression, in contrast, is high in both HIV-1 and SIV infection, but its levels are nevertheless insufficient to maintain the homeostatic proliferation of T cells (69). Importantly, both cytokines increase HIV replication in vitro (37–46), posing a dilemma for their therapeutic use in HIV-1-infected individuals.

We found that, although neither cytokine was able to further augment vaccine-expanded CD8+ T cell responses, both cytokines significantly augmented vaccine-expanded CD4+ T cell responses in SIVmac251-infected macaques treated with ART. This finding is consistent with the notion that the vaccination of infected animals may have already induced the maximal expansion of SIV-specific CD8+ T cell responses but that the effect of cytokines may become more evident when CD4+ T cell responses are low, as in HIV/SIV infection.

IL-7 treatment alone was not associated with increased plasma virus level and, if anything, a lower level of virus was found in resting T cells, while IL-15 had the opposite effect. The contrasting effects of these cytokines on in vivo viral replication is surprising, because both cytokines reactivate HIV-1 replication in vitro (46). This difference could be explained by the differential effect of these cytokines on T cell subsets in vivo. IL-7 increases the proliferation of all T cell subsets, but mainly of naive cells that are not a cell target for HIV/SIV. In contrast, IL-15, by massively expanding CD4+ effector T cells and altering their migration pattern (25), may augment SIV replication and dissemination. Because of this, the effect of vaccination would be curtailed as was observed in this study. Indirect mechanisms, however, could also explain the differential effects of IL-7 and IL-15 on viral replication in vivo. In our study, IL-7 significantly decreased the expression of TGF-β. IL-7 also decreases TGF-β expression in murine models of pulmonary fibrosis (70, 71). TGF-β potentiates HIV infection (72) and is directly immunosuppressive. In addition, TGF-β plays a role in the generation and maintenance of CD4+CD25+ Treg cells that, in turn, may dampen the adaptive immune response to HIV/SIV. Indeed, an accumulation of Treg cells is observed in tissues of HIV-1-infected individuals (73, 74) and SIV-infected macaques (74). CD25+ T cell depletion of human or macaque PBMCs in vitro results in the restoration of virus-specific immune response (54). Accordingly, CTLA-4 blockade in chronically infected macaques results in a decrease of TGF-β expression and virus levels in tissues (54).

Interestingly, a recent report demonstrated that pharmacological treatment of human volunteers with rIL-7 results in a decrease of the frequency of Treg cells (75, 76). Although it is possible that the TGF-β down-regulation by IL-7 may decrease Treg numbers, the fact that IDO was not affected suggests a more direct effect of IL-7 on TGF-β expression.

In sum, these data demonstrate that the risks and benefits of potential novel therapies for HIV-1 infection should be evaluated carefully in suitable animal models whereby regimens of administration can be evaluated thoroughly. This is especially pertinent for cytokine-based interventions in which the pleiotropic effects of these soluble mediators render the overall outcome unpredictable.

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Disclosures

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References


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