

Contrasting Effects of Low-Dose IL-2 on Vaccine-Boosted Simian Immunodeficiency Virus (SIV)-Specific CD4⁺ and CD8⁺ T Cells in Macaques Chronically Infected with SIVmac251

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IL-2, the first cytokine discovered with T cell growth factor activity, is now known to have pleiotropic effects on T cells. For example, it can promote growth, survival, and differentiation of Ag-selected cells, or facilitate Ag-induced cell death of T cells when Ag persists, and in vivo, it is thought to contribute to the regulation of the size of adaptive T cell response. IL-2 is deficient in HIV-1 infection and has been used in the management of HIV-1-infected individuals undergoing antiretroviral therapy. In this study, we investigated how continuous low-dose IL-2 affected the CD4⁺ and CD8⁺ T cell response induced by two inoculations of a canarypox recombinant SIV-based vaccine candidate in healthy macaques chronically infected with SIVmac251. These macaques had normal levels of CD4⁺ T cells at the beginning of antiretroviral therapy treatment. Vaccination in the presence of IL-2 significantly augmented Gag-specific CD8⁺ T cell responses, but actually reduced Gag-specific CD4⁺ T cell responses. Although IL-2 at low doses did not change the overall concentration of circulating CD4⁺ or CD8⁺ T cells, it expanded the frequency of CD4⁺CD25⁺ T cells. Depletion of the CD4⁺CD25⁺ T cells in vitro, however, did not result in a reconstitution of Gag-specific CD4⁺ responses or augmentation of SIV-specific CD8⁺ T cell responses. Thus, we conclude that the decrease in virus-specific CD4⁺ T cell response may be due to IL-2-promoted redistribution of cells from the circulation, or due to Ag-induced cell death, rather than suppression by a T regulatory population. *The Journal of Immunology*, 2005, 174: 1913–1921.

With the introduction of antiretroviral therapy (ART)² the replication of HIV and SIV can be suppressed to undetectable levels for prolonged intervals, provided the drugs are administered continuously. However, despite long-term suppression of viral replication, ART cannot cure the persistent viral infection, so that if the drugs are discontinued, viral replication resumes in most patients (1, 2). Because intracellular pathogens are combated by cell-mediated immunity (3–5), and both preclinical and clinical studies have demonstrated that CD8⁺ T cells are important to control viral infections (6–9), we have explored methods to attempt to boost antiviral immune reactivity, particularly the virus-specific CD4⁺ and CD8⁺ T cell responses.

The IL-2 molecule is the principal cytokine with T cell growth-promoting activity (10) responsible for the proliferative clonal expansion of Ag-selected cells (11), as well as for promoting the

differentiation and survival of effector T cells (12, 13). In addition, IL-2 has been implicated in both “positive” and “negative” selection of self-reactive cells in the thymus (14, 15), as well as the development of a subset of CD4⁺CD25⁺ cells, which are thought to suppress reactivity with self peptides, termed T regulatory cells (T-regs) (16, 17).

IL-2 as immune therapy of HIV-1 infection has been investigated in several phase I and II clinical trials and is now under investigation in large phase III clinical trials in chronic HIV infection designed to test whether high doses (9–15 million IU/day = 600 μg to 1 mg) of IL-2 given intermittently (5 days every 8 wk) together with ART can delay progression to clinical immunodeficiency by comparison with ART alone (18–30). However, this IL-2 dosing regimen is poorly tolerated, due to signs and symptoms of the systemic inflammatory response syndrome (31).

Detailed basic studies exploring the effects of IL-2 on T cell proliferation in vitro have demonstrated that resting peripheral T cells do not express the high-affinity trimeric IL-2R until activated by specific Ags (32, 33). Moreover, the concentrations of IL-2 necessary to saturate the trimeric high-affinity IL-2R are very low, ~100 pM, and most of the toxicity of high-dose IL-2 therapy is attributable to the effects of IL-2 binding to the IL-2R β- and γ_c-chain heterodimers expressed by NK cells, which have a 100-fold lower affinity for IL-2 (34).

Therefore, we have chosen a different therapeutic approach with IL-2 therapy. Our clinical studies have determined that IL-2 at low doses, ~2 million IU/day (=133 μg/day), can be given safely, without systemic side effects, for intervals as long as 6 mo to 1 year (35, 36). Moreover, the peak plasma IL-2 concentrations attained after a s.c. low-dose injection of IL-2, although low, ~25

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² Abbreviations used in this paper: ART, antiretroviral therapy; T-reg, T regulatory cell; *gpe*, *gag-pol-env*; ICS, intracellular cytokine staining.

pM, are still high enough to saturate most high-affinity IL-2Rs expressed by Ag-activated T cells, and are still too low to bind to dimeric lower-affinity IL-2Rs expressed by 90% of NK cells. IL-2 given as an immune stimulant together with ART in a randomized controlled trial resulted in an accelerated return of naive CD4⁺ T cells compared with ART alone, and had an excellent safety profile during the 6-mo administration (37).

In the past few years, we have developed a model for HIV immune-based therapies (38) in macaques either chronically or acutely infected with SIVmac251 (39, 40) and tested whether further enhancing virus-specific immune response by vaccination favors control of viral replication in the absence of ART. Because IL-2Rs are only expressed on T cells recently activated by Ags, we tested this cytokine simultaneously with a T cell vaccine in ART-treated macaques, which we expected to have low viral Ag levels. Vaccination imparted a transient virological benefit and IL-2 given with the vaccine increased the virus-specific CD8⁺ T cell response (40). However, in that study, we found that the virus-stimulated proliferative responses, as well as the frequency of CD4⁺ T cells capable of producing IL-2, were actually decreased by IL-2 administration (40).

Because in the above-mentioned study, most animals were immune suppressed, we wished to investigate whether IL-2 would have the same effect in immunocompetent SIVmac251-infected macaques and whether we could validate, using a larger animal number, that virus-specific CD8⁺ T cell responses would be enhanced, together with a reduction in helper responses. As well, we wanted to determine whether the decreased CD4⁺ T cell-proliferative responses corresponded with a decrease in the frequency of virus-specific CD4⁺ T cells, or whether it was attributable to intrinsic proliferative defects. Lastly, we investigated whether the CD4⁺CD25⁺ T cell population, which is augmented by high-dose intermittent IL-2 treatment (16), could have suppressive T-reg activity (41–44).

The results detailed herein confirmed our previous study in that IL-2 significantly increased the number of virus-specific functional CD8⁺ T cell responses, as monitored by tetramer staining and flow-cytometric quantification of SIV-activated cells capable of producing TNF- α and IFN- γ . However, IL-2 therapy also significantly decreased the magnitude of virus-specific CD4⁺ T cell responses as monitored by either Ag-driven lymphocyte proliferation assays or intracellular staining for cytokine production. As well, there was a significant increase in circulating CD4⁺CD25⁺ T cells in IL-2-treated animals. However, *in vitro* depletion studies did not demonstrate a greater suppressive activity mediated by this cell population in IL-2-treated macaques.

Materials and Methods

Animals and treatments

All animals were colony-bred rhesus macaques (*Macaca mulatta*). The animals were housed and handled in accordance with the standards of the American Association for the Accreditation of Laboratory Animal Care. All animals were seronegative for simian T cell lymphotropic virus type 1 and herpesvirus B before the study, and their Mamu-A*01 MHC allele status was determined by PCR with specific primers (45).

Two naive macaques were used for the IL-2 pharmacokinetics study (see Fig. 1). The remaining 11 macaques were previously inoculated with the same stock of pathogenic SIVmac251 (561) (46), and their prior treatment is summarized in Table I and described in Refs. 47 and 48. At the time of this study, all animals were aviremic, so that they were analogous to long-term nonprogressors. Macaques 3065, 3078, 3148, 3067, and 3075 were previously treated with ART, which had been suspended for >1 year. All macaques were initiated on ART that 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 *R*-9-(2-phosphonmethoxypropyl)adenine (20 mg/kg/day). Six macaques were vaccinated twice with ALVAC-SIV-*gag-pol-env* (*gpe*) (2×10^8 pfu), and five were vaccinated in a similar manner and treated with IL-2 (see Fig. 2).

Interleukin-2

Human rIL-2 was obtained from Amgen. The initial IL-2 doses were estimated from the maximum nontoxic doses defined for humans previously as 250,000 Amgen U/m² body surface area, which amounts to ~6,000 U/kg body weight, or ~0.4 μ g/kg (specific activity = 15,000 U/ μ g IL-2 protein) (35). Therefore, for a macaque of 10 kg, the initial doses used were 60,000 and 120,000 U (8 μ g). The Amgen IL-2 formulation is ~5-fold more potent than the commercially available IL-2 preparation from Chiron, so the equivalent dose of Chiron IL-2 is ~300,000–600,000 U (40–80 μ g)/macaque. Plasma IL-2 concentrations were determined using a commercially available ELISA kit from Endogen.

Quantification of plasma SIVmac RNA and CD3⁺, CD4⁺, and CD8⁺ T cell counts

SIVmac251 RNA in plasma was quantified by nucleic acid sequence-based amplification (49). Briefly, RNA extracted from plasma was subjected to isothermal amplification with primers specific for SIVmac251 and quantified by electrochemiluminescence chemistry using a coextracted internal standard. The detection limit of this assay is 2×10^3 RNA copies/input volume of 100 μ l of plasma.

CD4⁺ and CD8⁺ T cell counts were periodically determined on 100 μ l of whole blood and by FACS analysis, according to the FACS/Lyse kit (BD Biosciences) with minor modifications. Briefly, after incubating 10 μ l of a mixture containing PerCP-CD4, CD8-allophycocyanin, CD45-PE, and CD3-FITC Ab (BD Biosciences) for 30 min at room temperature, red cells were lysed by adding 2 ml of FACS/Lyse solution for 15 min. Samples were then centrifuged for 5 min at 1,200 rpm at room temperature, washed (1% FCS, 0.05% NaAzide in PBS), resuspended in 500 μ l of wash buffer, and stored at 4°C until acquired by a FACSCalibur flow cytometer (BD Biosciences). The counts were expressed as percentage of CD3⁺CD4⁺ or CD3⁺CD8⁺ lymphocytes per microliter or as absolute number of cells with these phenotypes.

Table I. Prior history and clinical features of the animals studied

Immunization Regimen	Macaque	Mamu-A*01 Status	CD4 ⁺ Count	Virus Plasma Levels	Vaccination History
ALVAC	25	–	1720	<2000	NYVAC-SIV- <i>gpe</i> + DNA
	678	+	1196	<2000	NYVAC-SIV- <i>gpe</i> + DNA
	679	+	1029	<2000	NYVAC-SIV- <i>gpe</i> + DNA
	3065	–	782	<2000	NYVAC-SIV- <i>gpe</i>
	3076	+	1572	<2000	NYVAC-SIV- <i>gpe</i>
	3148	–	1283	<2000	NYVAC-SIV- <i>gpe</i>
ALVAC + IL-2	16	+	723	<2000	NYVAC
	26	+	855	<2000	NYVAC-SIV- <i>gpe</i> + DNA
	674	+	1973	<2000	NYVAC-SIV- <i>gpe</i>
	3067	–	1537	<2000	NYVAC-SIV- <i>gpe</i>
	3075	+	852	<2000	NYVAC-SIV- <i>gpe</i>

T cell proliferation and tetramer staining

Ag-specific proliferation was measured in PBMCs from fresh blood samples. PBMCs were isolated by density gradient centrifugation on Ficoll, resuspended in RPMI 1640 medium (Invitrogen Life Technologies) containing 5% inactivated human A/B serum (Sigma-Aldrich), and cultured at 10^5 cells/well for 3 days in the absence or presence of native purified SIVmac p27 Gag, gp120, (Advanced BioScience Laboratories), or tetanus toxoid and Con A. The cells were then pulsed overnight with $1 \mu\text{Ci}$ of [^3H]thymidine before harvest. The relative rate of lymphoproliferation was calculated as fold of thymidine incorporation into cellular DNA over control (stimulation index). The stimulation index was considered positive when >3 .

Fresh PBMCs were stained with anti-human CD3 Ab (PerCP-labeled, clone SP34; BD Pharmingen), anti-human CD8 α Ab (FITC-labeled, BD Biosciences), and Mamu-A*01 tetrameric complexes refolded in the presence of a specific peptide and conjugated to PE-labeled streptavidin (Molecular Probes). Gag₁₈₁₋₁₈₉ CM9 (CTPYDINQM) (Gag_CM9)-specific tetramer was used. Samples were analyzed by FACSCalibur (BD Biosciences), and the data are presented as percentage of tetramer-positive cells of all CD3 $^+$ CD8 $^+$ lymphocytes.

Intracellular cytokine staining (ICS)

ICS was performed using the anti-TNF- α Ab with or without anti-IFN- γ Ab. A total of 10^6 fresh PBMCs in 0.2 ml of complete medium was incubated for 1 h at 37°C in the absence or presence of Gag₁₈₁₋₁₈₉ CM9 (10 $\mu\text{g}/\text{ml}$), Gag QI9 (10 $\mu\text{g}/\text{ml}$), or a pool of Gag peptides (2 $\mu\text{g}/\text{ml}$), and in the presence of CD28 and CD49d (1 $\mu\text{g}/\text{ml}$ each) on a V-bottom-well plate. After addition of 10 $\mu\text{g}/\text{ml}$ brefeldin A (Sigma-Aldrich), cells were incubated for 5 h at 37°C and processed for surface and ICS. Briefly, cells were washed with 1% FCS in PBS, surface-stained for 20 min with CD3 ϵ -FITC and CD8-PerCP (4 μl each) (BD Biosciences), washed again, and permeabilized with FACSPerm (BD Pharmingen) for 10 min at room temperature in the dark. Following two further washes, cells were intracellularly stained with PE-conjugated anti-TNF- α with or without PE-conjugated anti-IFN- γ (4 μl /well each) (BD Pharmingen), with or without allophycocyanin-labeled CD69 (1.5 μl /well each; BD Pharmingen), incubated for 20 min at 37°C , fixed with 180 μl of 1% paraformaldehyde (Sigma-Aldrich) in PBS, and analyzed by four-color flow cytometry (FACSCalibur-Multiwell Plate Manager; BD Biosciences).

CTL assay

Cryopreserved and thawed PBMCs were maintained overnight in RPMI 1640 with 20% FCS and penicillin/streptomycin in 10 ml of complete R20 medium, washed in R10, and plated in triplicate on a 96-well round-bottom plate. Mamu-A*01-positive herpes papio-transformed target cells were stimulated 2 h with 10 μg of Gag₁₈₁₋₁₈₉ CM9 or Gag QI9 peptides in 1 ml of complete medium, labeled for 2 h with 100 μCi of ^{51}Cr , and washed in Iscove's medium plus 10% FCS and RPMI 1640 plus 10% FCS with 100 U penicillin/streptomycin, and 5×10^3 cells were added to each well. Unstimulated target cells were used as a negative control. Cells were incubated for 6 h at E:T ratios of 50:1, 25:1, 12.5:1, and 6.25:1, and the percentage of released ^{51}Cr was calculated by dividing the difference between the mean cpm of experimental and spontaneous release by the difference between the mean cpm of total and spontaneous release $\times 100$.

CD25 $^+$ T cell detection and depletion

Macaque PBMCs that were depleted or undepleted of CD25 $^+$ T cells were stained with CD3 ϵ -FITC, CD4-PerCP, CD8-allophycocyanin, and CD25-PE (BD Immunocytometry Systems) for 20 min in the dark at $4-8^\circ\text{C}$ and washed with wash buffer (PBS supplemented with 1% FCS). Following incubation, the cells were washed and resuspended in 200 μl of 1% paraformaldehyde. Analysis was performed using the FACSCalibur (BD Biosciences).

Macaque PBMCs were prepared according to standard isolation techniques. The cells were passed through a prewetted 30- μm nylon mesh (catalog no. 130-041-407; Miltenyi Biotec) to remove clumps. Cells were washed, pelleted, resuspended in 100 μl of selection buffer (PBS supplemented with 0.5% FCS and 2 mM EDTA), and stained with CD25 conjugated with r-PE Ab (catalog no. 341009; clone 2A3; BD Biosciences) for 10 min at $4-8^\circ\text{C}$ in the dark. Following incubation, the cells were washed twice and resuspended in 80 μl . Twenty microliters per 10^7 cells of anti-PE microbeads (catalog no. 130-048-801; Miltenyi Biotec) was added and incubated at $4-8^\circ\text{C}$ for 15 min. The cells were washed, resuspended in 500

μl in selection buffer, and passed through magnetic MS columns for depletion of CD25 $^+$ T cells. Unlabeled effluent was collected and analyzed.

Statistical analysis

All differences were tested using repeated-measures ANOVA. Arc-sine or logarithmic transformation was applied to ICS, tetramer staining, and proliferative responses to normalize data distributions.

Results

IL-2 pharmacokinetics in rhesus macaques

The bioavailability of IL-2 in serum of macaques was assessed following IL-2 administration at 60,000 Amgen U (4 μg) or 120,000 Amgen U (8 μg) daily dose by the s.c. route to two macaques. Plasma was collected at prescribed intervals following inoculation. The same animals were reinoculated after a few months with the same dose of IL-2 to obtain duplicate results. Within 2 h from s.c. inoculation of 120,000 Amgen U of the cytokine, IL-2 reached plasma peak levels (~ 50 pM) (Fig. 1A). By 6 h after injection, the plasma level was near 10 pM, which is equivalent to the equilibrium dissociation constant (K_d) or EC_{50} of the high-affinity heterotrimeric IL-2R (Fig. 1A). At the lower dose (60,000

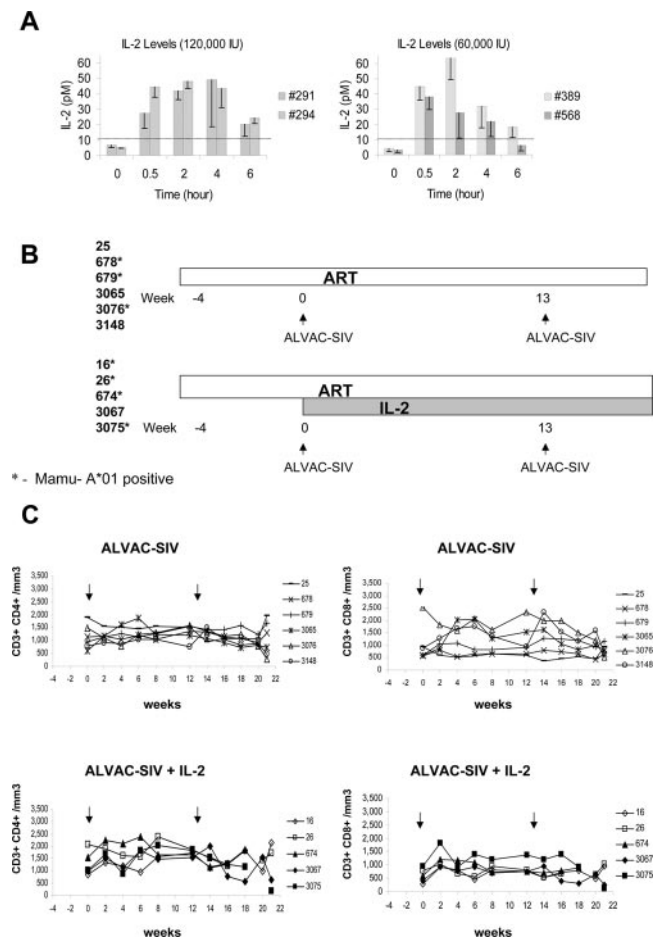


FIGURE 1. Study design and pharmacokinetics of exogenous IL-2 in rhesus macaques. *A*, Animals 291 and 294 were inoculated with a single dose of IL-2 at 120,000 IU (left panel), whereas macaques 389 and 568 received IL-2 at a dose of 60,000 IU (right panel). Plasma was collected at the time (hours) indicated. Two independent inoculations of IL-2 at the same dose in the same macaques were performed (see *Materials and Methods*). *B*, Study design and animal numbers. *, Designates Mamu-A*01-positive macaques. *C*, Absolute number of CD3 $^+$, CD4 $^+$, and CD8 $^+$ T cells in animals vaccinated with (top panels) or without (bottom panels) IL-2.

Amgen U), the IL-2 plasma level also reached 50 pM within 2 h from administration and was at or below 10 pM by 6 h (Fig. 1A). Therefore, the IL-2 dose of 120,000 Amgen U was chosen for

further studies, because, in this case, the serum IL-2 concentration for at least 6 h was equal to that necessary to half saturate the high-affinity IL-2R (50).

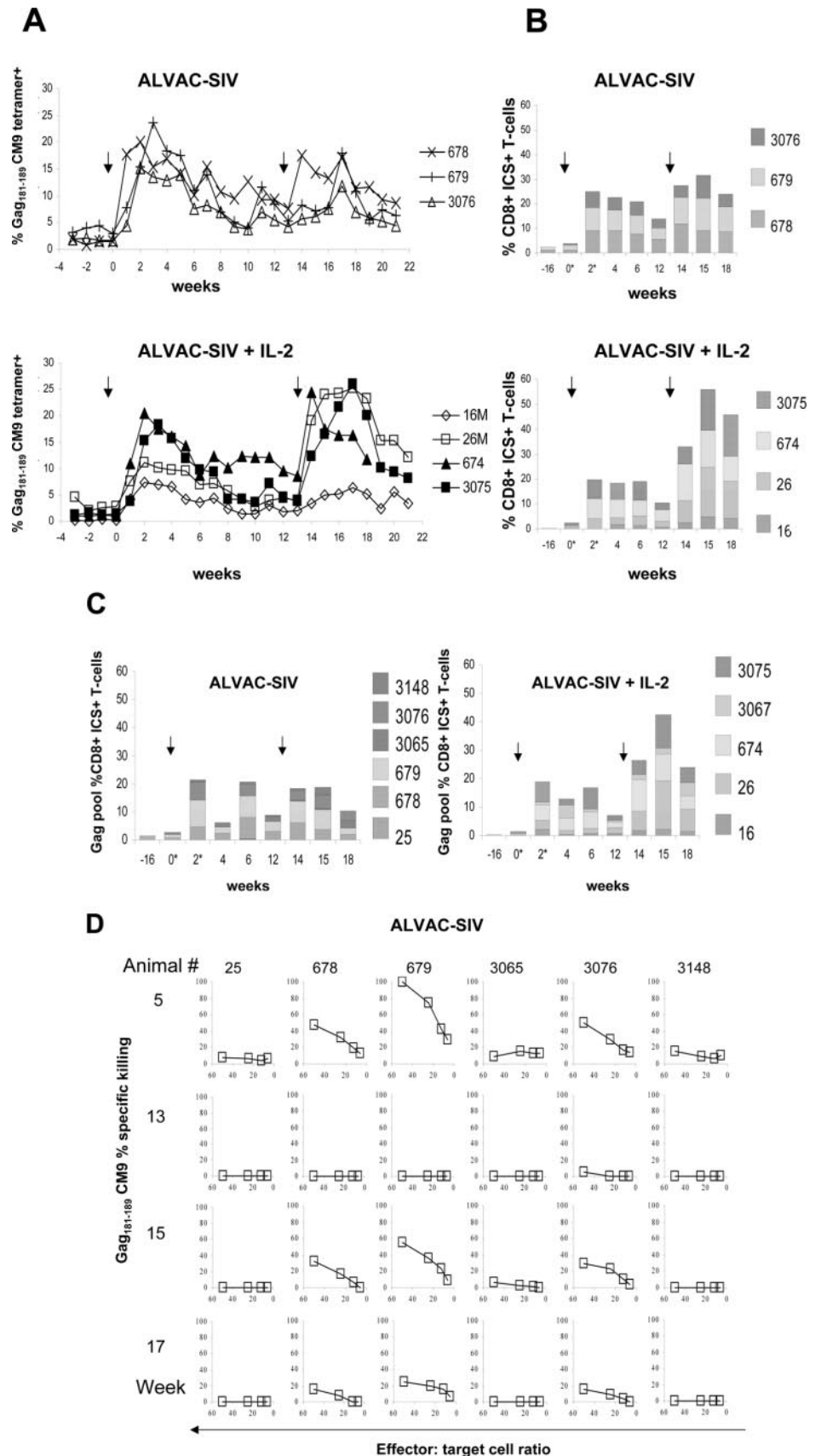


FIGURE 2. Kinetics of induction and functionality of Gag₁₈₁₋₁₈₉ CM9 CD8⁺ T cell response in vaccinated macaques with and without IL-2. *A*, Frequency of tetramer-positive cells in the blood of the Mamu-A*01-positive macaques from groups immunized with ALVAC-SIV alone (*top panel*) or with ALVAC-SIV plus IL-2 (*bottom panel*). *B*, Frequency of Gag₁₈₁₋₁₈₉ CM9-specific CD8⁺ T cells producing TNF- α following stimulation with the Gag peptide over time. *C*, Frequency of CD8⁺ T cells able to produce TNF- α following stimulation with the entire Gag peptide pool. The arrows in *A-C* indicate the time of immunization. *D-G*, Ex vivo cytolytic activity following stimulation with the dominant Gag₁₈₁₋₁₈₉ CM9 epitope (*D* and *E*) or the subdominant Gag Q19 epitope (66) (*F* and *G*). (*Figure continues*)

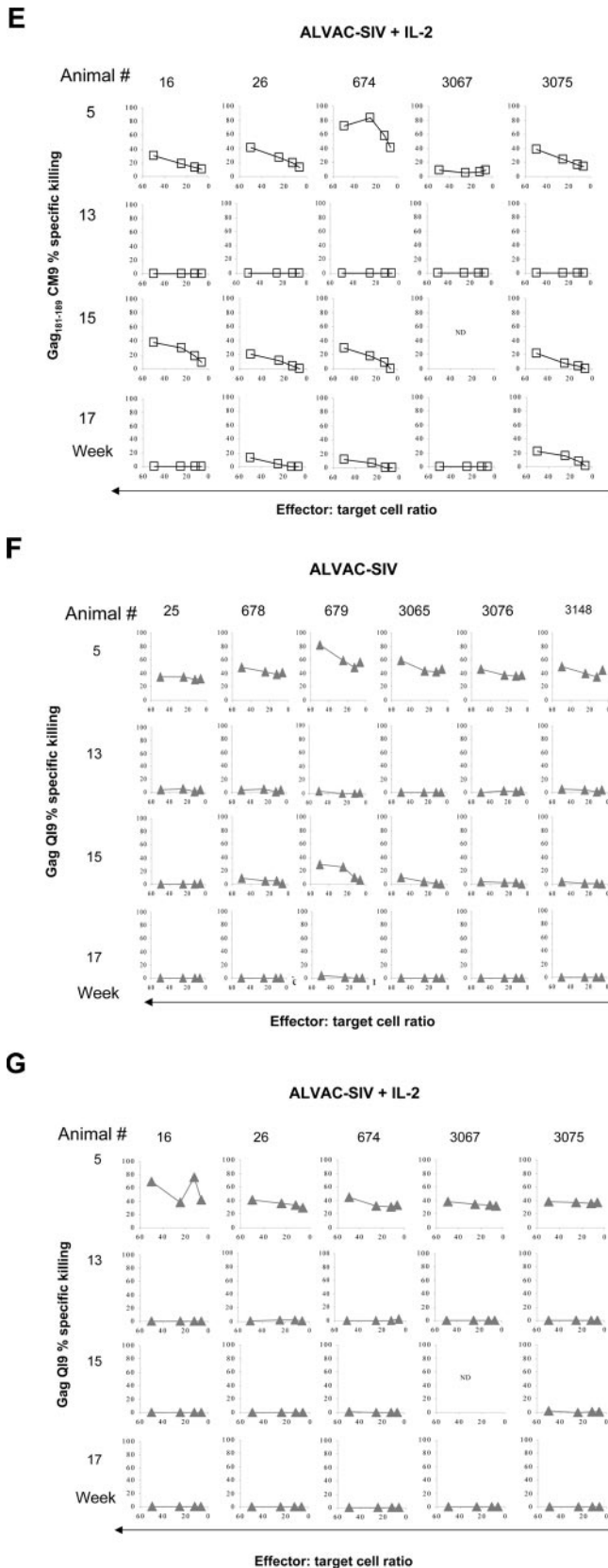


FIGURE 2. (Continued)

IL-2 induces significant increase of vaccine-induced functional CD8⁺ T cell response

The effect of continuous low-dose IL-2 administration in combination with ALVAC-SIV-*gpe* was assessed in 11 macaques. Ten

of the 11 macaques were vaccinated before the current study with either a combination of DNA and the NYVAC-SIV-*gpe* vaccine candidate or the NYVAC-SIV-*gpe* vaccine candidate alone, as summarized in Table I. All of these macaques had been chronically infected with SIVmac251 before treatment with ART, but at the time of this study, they had contained viral replication below a detectable level (<2000 RNA copies/100 μl input), and maintained normal levels (>700 CD4⁺ T cells/mm³) over time (Table I). Therefore, these animals were comparable to human long-term nonprogressors and were considered to have been “primed” to SIV Ags and to have protective immunity to SIV. Nevertheless, to minimize the possible contribution of subliminal viral replication to the immune response induced by vaccination, all animals were subjected to daily treatment with ART. Six of these macaques were immunized 4 wk after initiation of ART with ALVAC-SIV-*gpe* at 2 × 10⁸ pfu by the i.m. route, and vaccination was repeated in identical conditions 13 wk later (Fig. 1B). The remaining five macaques were immunized with the same regimen except that IL-2 at 120,000 Amgen U was given daily by the s.c. route up to wk 22 (Fig. 1B). During this time, CD4⁺ and CD8⁺ T cell counts were analyzed every 2 wk, and at this IL-2 dose, treatment did not result in a significant increase of CD4⁺ or CD8⁺ T cell counts in the blood, which remained within the normal ranges (Fig. 1C).

Mamu-A*01-positive macaques were included in both groups (Table I) to quantify the dominant response to Gag₁₈₁₋₁₈₉ CM9 by tetramer staining. ALVAC-SIV-*gpe* expanded the frequency of Gag₁₈₁₋₁₈₉ CM9 tetramer-positive cells in all Mamu-A*01-positive macaques. Following the first immunization, no significant difference was observed between the two groups (Fig. 2A). However, following the second immunization, the change in frequency of the tetramer response relative to the response after the first immunization was significantly higher in the animals treated with IL-2 (*p* = 0.0011), indicating that IL-2 contributed to the expansion and maintenance of this population of cells.

The ability of CD8⁺ T cells with the same specificity to produce TNF-α was assessed in parallel by ICS following in vitro stimulation with the Gag₁₈₁₋₁₈₉ CM9 peptide. In agreement with the tetramer data, significantly higher responses were observed in the IL-2-treated group after the second immunization (*p* = 0.041) (Fig. 2B). Thus, both immunological assays demonstrated that IL-2 increased the frequency of functional immune response to this dominant Gag₁₈₁₋₁₈₉ CM9 epitope.

We further analyzed the ability of CD8⁺ T cells to produce TNF-α using the entire Gag peptide array. As in the case of the Gag₁₈₁₋₁₈₉ CM9 peptide, macaques treated with IL-2 developed higher vaccine-induced Gag-specific CD8⁺ T cell response following the second immunization (*p* = 0.0009) (Fig. 2C), supporting the notion that the ability of IL-2 to increase vaccine-induced CD8⁺ T cell response is not limited to dominant epitopes.

Ex vivo CTL activity specific for the Gag₁₈₁₋₁₈₉ CM9 epitope was measured in all macaques at wk 5, 13, 15, and 17, and was found to be comparable in all of the Mamu-A*01-positive macaques from both groups. As expected, no significant CTL activity was measured in the Mamu-A*01-negative macaques 3067, 3065, and 25 (Fig. 2, D and E). This result is not unexpected because CTL activity measurement likely does not accurately reflect the frequency of the Gag₁₈₁₋₁₈₉ CM9-specific CD8⁺ T cell response. Ex vivo CTL activity was also assessed against the subdominant Gag Q19 epitope, and also in this case, no difference in the extent of this response was observed between the two groups (Fig. 2, F and G).

Vaccination-induced CD4⁺ T cell responses are significantly decreased by IL-2 treatment

In a previous study, we observed that IL-2 at the same dose used here was associated with a decrease in the proliferative response to Gag induced by vaccination with the NYVAC-SIV-*gpe* vaccine candidate (40). However, in those experiments, a parallel systematic quantitative measurement of the Gag-specific proliferative response and cytokine production by CD4⁺ T cells was not performed. Therefore, in this study, we assessed side-by-side the CD4⁺ T cell response to the entire Gag protein by ICS as well as by p27 Gag-induced [³H]thymidine incorporation at six independent time points in all 11 macaques.

Proliferative responses to Gag were significantly lower following both the first and second immunizations in macaques treated with IL-2 ($p = 0.0017$ and $p = 0.0066$, respectively, by ANOVA) (Fig. 3A), thereby confirming and extending our previous findings (40).

Interestingly, the number of CD4⁺ T cells producing TNF- α was also significantly lower in the IL-2-treated group following the entire vaccination regimen ($p = 0.015$), suggesting that IL-2 decreases the true frequency of vaccine-induced CD4⁺ T cell responses rather than altering only their ability to produce IL-2 and to proliferate (Fig. 3B).

IL-2 increases the number of CD4⁺CD25⁺ T cells

In humans, treatment with a 10-fold higher dose of IL-2 (e.g., 9–15 mU/day) is associated with an increase in the frequency of circulating CD4⁺ T cells expressing the IL-2R α -chain (CD25) (26, 51). Recently, T-reg cells with the CD4⁺CD25⁺ phenotype have been shown to be able to suppress IL-2 production and proliferation by CD4⁺CD25⁺-responding T cells, and have been described in both mice and humans (52). Therefore, we assessed whether continuous low-dose IL-2 treatment of macaques increased the frequency of this population in the blood. Flow cytometry analysis of PBMCs from the IL-2-treated and untreated vaccinated animals revealed an overall increase over time of the percentage of CD4⁺CD25⁺ T cells in IL-2-treated macaques (Fig. 4A) up to 6–7% at 6 wk of treatment, compared with <1% without IL-2 treatment (A). Surprisingly, this frequency subsequently decreased, despite continuous IL-2 treatment, and the decrease was apparently unrelated to the induction of Abs to IL-2, because the

sera of these animals did not react in Western blot with the rIL-2 protein (data not shown). It is possible that either trafficking to tissues or down-regulation of the CD25 molecule occurs after prolonged treatment with the cytokine. In either case, the level of CD4⁺CD25⁺ T cells remained higher in the IL-2-treated group throughout the study.

The increase in CD4⁺CD25⁺ T cells raised the possibility that this population might have a suppressive effect on cytokine production and proliferation, especially by CD4⁺ T cells. Because we observed a decrease in the CD4⁺ T cell response, we sought to investigate whether the depletion of the CD4⁺CD25⁺ T cells in vitro could restore the CD4⁺ T cell response. To investigate this hypothesis, total or CD25⁺ T cell-depleted PBMCs were stimulated with the entire Gag-overlapping peptide, and the ability of CD4⁺ and CD8⁺ T cells to produce cytokines was measured in the presence or absence of CD4⁺CD25⁺ T cells. Examples of the assay performed in the animals are presented for two animals in Fig. 4, B and C, and summarized in a histogram for the other four animals in D. Although depletion of the CD25⁺ T cell population was highly effective, it did not consistently result in an increase of virus-specific CD4⁺ or CD8⁺ T cell response, and overall did not differ significantly in animals vaccinated in the presence or absence of IL-2.

Discussion

In this study, we have demonstrated in macaques able to naturally contain SIVmac251 replication (long-term nonprogressors) that low-dose IL-2 given together with a T cell vaccine and maintained throughout the immunization regimen had opposite effects on vaccine-induced Ag-specific CD4⁺ and CD8⁺ T cells. IL-2 significantly increased the frequency and function of a dominant CD8⁺ T cell response, but significantly decreased the frequency of virus-specific CD4⁺ T cells. These data may be reconciled to the known biological effects of IL-2. IL-2 induces T cell proliferation but also limits T cell expansion by down-regulating the γ_c -chain and Bcl2 on cycling T cells and by causing apoptosis (53). IL-2 also causes activation-induced cell death (54) through up-regulation of Fas (CD95), Fas ligand (CD178), Fas-associating protein with death domain transcription, and down-regulation of FLICE/FLIP (55–57). The importance of the negative effect of IL-2 is supported by the finding that IL-2 and IL-2R knockout mice have lymphoid hyperplasia and autoimmune syndromes (58–60).

Thus, the effect of IL-2 likely depends on the timing and dose of administration. In fact, a recent study demonstrated that the effect of IL-2 varies greatly according to its presence at different times during the development of an adaptive immune response (61). In mice that received low doses of IL-2 during the expansion (8 days), contraction (8 to 15 days), or memory (60 days) phase of the immune response following lymphocytic choriomeningitis virus infection (61), the timing of IL-2 administration determined the extent of virus-specific CD4⁺ and CD8⁺ effector memory T cell responses. During the expansion phase, IL-2 decreased the number of CD4⁺ T cells and had no significant effect on virus-specific CD8⁺ T cell response. In contrast, IL-2 administration during the contraction phase resulted in the persistence of high virus-specific CD8⁺ and even higher virus-specific CD4⁺ T cell response up to 6 mo following infection. However, by 6 mo, the level of both CD4⁺ and CD8⁺ memory T cell responses to lymphocytic choriomeningitis virus did not differ in IL-2-treated and untreated animals, suggesting that administration of IL-2 did not increase the memory pool.

Similarly, in our study in macaques chronically infected with SIVmac251, IL-2 augmented the expansion of CD8⁺ tetramer-positive cells after vaccination but did not influence the contraction

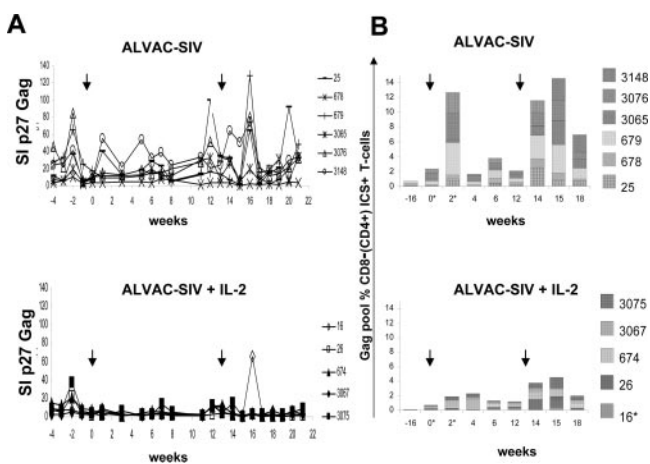
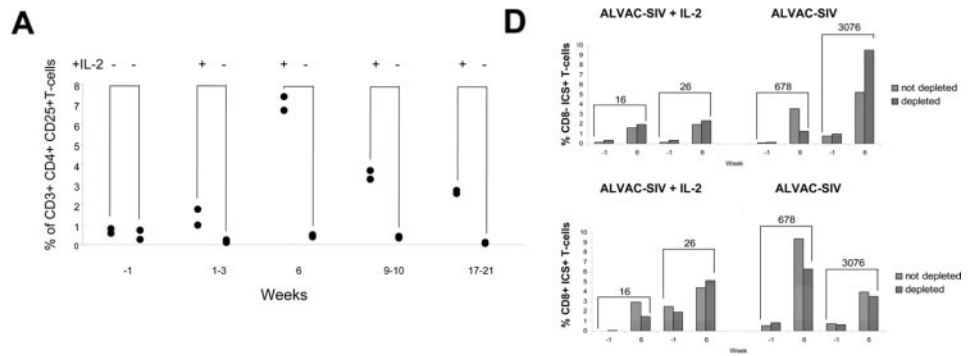
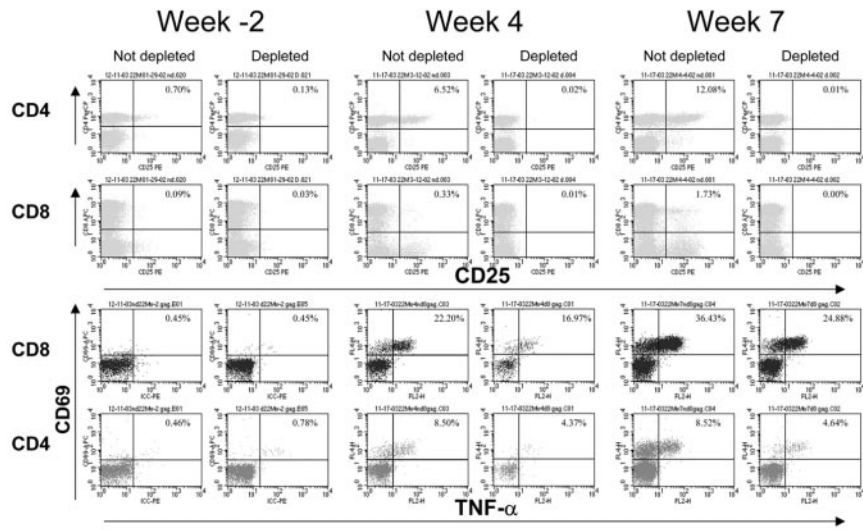


FIGURE 3. Kinetics of vaccine-induced virus-specific helper response to p27 Gag. *A*, The proliferative response to p27 Gag over time is presented for each animal in the two left panels. *B*, Both right panels show the virus-specific CD8⁻ (CD4⁺) response measured with the ICS assay for TNF- α following stimulation with the entire Gag peptide pool.



B 22 (IL-2 treated)



C 3076 (no IL-2)

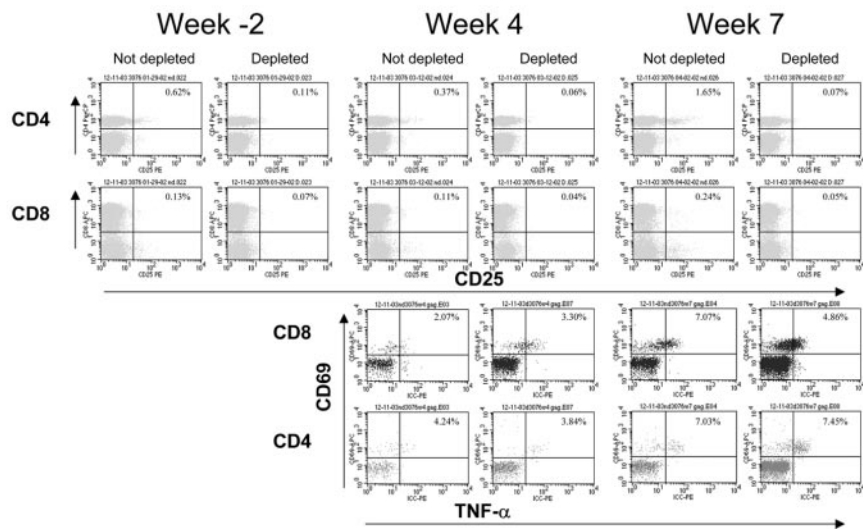


FIGURE 4. IL-2 augments the frequency of CD4⁺CD25⁺ T cells. *A*, Quantitation of CD4⁺CD25⁺ T cells in blood of four animals before treatment with IL-2 and vaccine (–) and after. *B* and *C*, Raw FACS analysis data from two animals in the study. Data on two animals are presented. Animal 22 was vaccinated and treated with IL-2 (*B*), whereas animal 3076 received vaccine only (*C*). –2 is 2 wk before treatment, and 4 and 7 wk are after vaccination with or without IL-2. In *B* and *C*, the *top two panels* demonstrate the efficiency of depletion of the CD4⁺ and CD8⁺ T cell populations, whereas the *two bottom panels* demonstrate the change in the ability of Gag-specific CD4⁺CD69⁺ or CD8⁺CD69⁺ T cells to produce TNF- α in the presence (nondepleted) or absence (depleted) of CD4⁺CD25⁺ T cells. Data for all animals tested using the same approach are summarized in *C*.

of this response. Indeed, in the long term, the frequency of tetramer-positive cells did not differ in the animals vaccinated in the presence or the absence of IL-2. The decrease in CD4⁺ T cells observed here may have resulted from trafficking of Ag-specific CD4⁺ T cells to tissues; activation-induced cell death, as IL-2 was maintained at pharmacological levels during the entire immunization regimen; or

increased frequency in the number of CD4⁺CD25⁺ T cells with suppressive activity in tissues but not in blood (62).

IL-2 administration augmented the frequency over time of circulating CD4⁺CD25⁺ T cells in the macaques studied here, as observed in HIV-1-infected individuals (63). However, this increase was transient and completely reversible once the cytokine administration was

discontinued. Initially, this finding appeared to provide a possible explanation for the decreased proliferative responses, in that both murine and human CD4⁺ T cells that also express CD25 exert suppressive activity in lymphocyte proliferative assays (42, 64). However, when tested directly, the removal of the CD4⁺CD25⁺ T cells in vitro did not result consistently in restoration of higher frequency CD4⁺ T cell responses whether the macaques were treated or not with the cytokine. Thus, the mechanism underlying the IL-2-associated decreased SIV-specific response remains unclear.

Importantly, however, our data complement findings of others in IL-2-treated HIV-1-infected individuals on ART whereby a decrease in HIV-1-specific CD4⁺ effector responses was also observed together with an increase of CD4⁺CD25⁺ T cells (63). Decreasing the frequency of virus-specific CD4⁺ T cells while maintaining functional CD8⁺ T cells may not necessarily be an undesirable result in HIV-1 infection because Ag-specific CD4⁺ T cells are preferentially infected by the virus (65). Hopefully, answers to this question may stem from ongoing human trials whereby IL-2 has been associated with vaccination.

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