IL-15 Treatment during Acute Simian Immunodeficiency Virus (SIV) Infection Increases Viral Set Point and Accelerates Disease Progression despite the Induction of Stronger SIV-Specific CD8+ T Cell Responses

Yvonne M. Mueller, Duc H. Do, Susan R. Altork, Carol M. Artlett, Edward J. Gracely, Christos D. Katsetos, Agustin Legido, Francois Villinger, John D. Altman, Charles R. Brown, Mark G. Lewis and Peter D. Katsikis

*J Immunol* 2008; 180:350-360; doi: 10.4049/jimmunol.180.1.350

http://www.jimmunol.org/content/180/1/350

---

**References**

This article cites 69 articles, 45 of which you can access for free at:

http://www.jimmunol.org/content/180/1/350.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
IL-15 Treatment during Acute Simian Immunodeficiency Virus (SIV) Infection Increases Viral Set Point and Accelerates Disease Progression despite the Induction of Stronger SIV-Specific CD8⁺ T Cell Responses

Yvonne M. Mueller,* Duc H. Do,* Susan R. Altork,* Carol M. Artlett,* Edward J. Gracely,† Christos D. Katsetos,‡ Agustin Legido,§ Francois Villinger,¶ John D. Altman,‖ Charles R. Brown,* Mark G. Lewis,** and Peter D. Katsikis2*

In this study, we examined the effect of in vivo treatment of acutely SIV-infected Mamu-A*01⁺ rhesus macaques with IL-15. IL-15 treatment during acute infection increased viral set point by 3 logs and accelerated the development of simian AIDS in two of six animals with one developing early minimal lesion SIV meningencephalitis. Although IL-15 induced a 2- to 3-fold increase in SIV-specific CD8⁺ T cell and NK cell numbers at peak viremia and reduced lymph node (LN) SIV-infected cells, this had no impact on peak viremia and did not lower viral set point. At viral set point, however, activated SIV-specific CD8⁺ cells, dendritic cells, and bone marrow stromal cells (9–12) and cytokine produced by a variety of cells including macrophages/monocytes therefore increase viral control. IL-15 is a proinflammatory cytokine; however, it has been shown to elicit an increased function and effectiveness of CD8⁺ T cells is one of the major goals in the fight against viral infections such as HIV infection. Although studies indicated the important role of virus-specific CD8⁺ T cells in HIV and SIV infection (1–5), these CTLs are unable to control viral replication and progression of HIV infection. In recent years, intrinsic defects were suggested for HIV- (6, 7) and SIV-specific CD8⁺ T cells (8) which result in reduced survival and skewed differentiation of effector memory virus-specific CD8⁺ T cells. Improving the survival and function of these virus-specific CD8⁺ T cells by cytokine therapy may therefore increase viral control. IL-15 is a proinflammatory cytokine produced by a variety of cells including macrophages/monocytes, dendritic cells, and bone marrow stromal cells (9–12) and binds to the IL-15R, composed of the specific IL-15Rα chain expressed on lymphoid, myeloid, nonhemopoietic cells, including macrophages and monocytes, NK cells, and CD8⁺ T cells (13, 14), the IL-2Rβ chain, and the common γ-chain. Transpresentation of IL-15 has been shown to be a major signaling mechanism (15–17) although this may not be true for all cell types (18). Several studies have shown the ability of IL-15 to enhance the activation, function, and proliferation of NK cells and CD8⁺ T cells (18–26), and to function as a chemoattractant for the homing of lymphocytes to peripheral lymph nodes (LN) and inflamed tissue either directly (11, 27, 28) or indirectly by inducing IL-8 and MCP-1 by monocytes (29). IL-15 overexpression suppresses progression to murine acquired immunodeficiency syndrome in mice infected with LP-BM5 murine leukemia virus (30) and can act as a plasmid adjuvant (31–33). In vitro IL-15 enhances survival, inhibits CD95-induced apoptosis, and augments effector function of HIV-specific CD8⁺ T cells (34, 35). Furthermore, IL-15 stimulates expansion of HIV-specific CD8⁺ T cells (36, 37) and restores NK cytotoxicity and deficient IL-12 production by PBMC from HIV-infected individuals (19).

The above effects of IL-15 have suggested its use as an immune augmenter in HIV infection. In previous studies, we have shown that high dose (100 µg/kg) IL-15 treatment of chronically infected cynomolgus macaques results in a 3-fold expansion of CD8⁺ T cells and NK cells. Others have shown that low-dose (10 µg/kg) IL-15 treatment of chronically infected animals may restore the


1 This work was supported by National Institutes of Health Grants R01 AI46719, AI52005, and AI62437 to P.D.K.
2 Address correspondence and reprint requests to Dr. Peter D. Katsikis, Department of Microbiology and Immunology, and Institute for Molecular Medicine and Infectious Disease, Drexel University College of Medicine, 2900 Queen Lane, Philadelphia, PA 19129. E-mail address: Peter.Katsikis@DrexelMed.edu

Received for publication July 13, 2007. Accepted for publication October 25, 2007.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Copyright © 2007 by The American Association of Immunologists, Inc. 0022-1767/07/$2.00

3 Abbreviations used in this paper: LN, lymph node; AU, arbitrary unit.
CD4+ T cell compartment but only when viral replication is controlled by antiretrovirals (38). These studies led us to investigate the effect of IL-15 treatment during acute SIV infection in Mamu-A*01−/− rhesus macaques. Our data show that IL-15 treatment increases viral set point by 3 logs and leads to accelerated disease progression to simian AIDS in two of six animals with one developing SIV meningoencephalitis. IL-15 induced 2- to 3-fold increases in SIV-specific CD8+ T cells and NK cells during the acute infection without effect on viremia. Although increased viral set point was accompanied by reduced blood activated SIV-specific CD8+ T cells and NK cells, LN numbers of SIV-specific CD8+ T cells were increased whereas LN NK cell numbers were not affected. Expression of Ki-67 in CD4+ T cells at day 7 postinfection was positively correlated with viral load at day 84. These findings suggest that IL-15 may be altering the course of the disease by enhancing the proliferation of CD4+ T cells early during infection and promoting their migration into tissue hence increasing the target pool of SIV infection. Thus, the level of CD4+ T cell activation during acute infection may determine the viral set point months later. Finally, our findings also indicate that IL-15 treatment of acutely SIV-infected nonhuman primates may prove useful as an animal model to elucidate what controls viral set point.

Materials and Methods

Animals

The 12 Mamu-A*01−/− male Indian rhesus macaques (Macaca mulatta) included in this study were housed at the Biopac animal facility according to standards and guidelines as set forth in Animal Welfare Act and The Guide for the Care and Use of Laboratory Animals and according to animal care standards deemed acceptable by the Association for the Accreditation and Accreditation of Laboratory Animal Care International. All experiments were performed following institutional animal care and use committee approval. The animals were inoculated i.v. with 100 ID50 SIVmac251 and either treated with 100 μg/kg bodyweight rhesus macaque IL-15 or with saline (control) s.c. twice weekly for 4 wk. SIV RNA levels were measured using a quantitative TaqMan RNA RT-PCR assay (sensitivity of 200 SIV RNA copies/ml blood; Applied Biosystems) (39). Recombinant rhesus macaque IL-15 was provided through theNational Center for Research Resources (NCRR) funded Resource for Nonhuman Primate Immune Reagents (40).

Flow cytometry

Blood of animals was processed within 6 h after blood draw. Freshly density centrifugation isolated PBMC (Percoll, 1.075 g/ml; Amersham Biosciences) were stained ex vivo or used for apoptosis, cytotoxicity, and cytokine production assays. Mamu-A*01−/−/β-2-microglobulin tetratomers were loaded with SIV Gag 181–189 CM9 (CTPYDINQM) or SIV Tat SL8 28–35 (STPESANL) peptide (41). The following rhesus macaque cross-reactive anti-human Abs were used: anti-CD3 (clone SP34), -CD4 (L200), -CD8 (3B4), -CD20 (2H7), -CD25 (M-A251), -CD69 (FN50), -CD45RA (5H9), -CD62L (SK11), -HLA-DR (L243/G46-6), -Ki-67 (B56), -TNF-α (Mab11), -IL-10 (JES3-9D7), -CD3 (CD45RA, 3A9), and annexin V (BD Biosciences; eBioscience; Invitrogen Life Technologies; Southern Biotechnologies Associates). PBMC were stained with up to 10 different Abs in HBSS (Cellgro), 3% heat-inactivated fetal calf serum in 0.1 M Tris (pH 7.4), and incubated with sheep anti-digoxigenin-A [turn blue/cresyl violet and evaluated. The extent and distribution of inflammatory lesions was analyzed and rated after Boche et al. (42) as follows: (+) rare, scant/sparse; (+) frequent, scant/sparse; (+++) frequent and marked.

SIV-specific in situ hybridization

Tissues (rectum pinch biopsy samples, inguinal LN) were fixed in formalin, embedded in paraffin, and were assayed for SIV viral RNA expression by in situ hybridization as previously described (43). Briefly, the tissue sections were hybridized overnight at 50°C with either sense or antisense SIVmac239 digoxigenin-UTP labeled riboprobe, blocked with 3% normal sheep and horse serum in 0.1 M Tris (pH 7.4), and incubated with sheep anti-digoxigenin-alkaline phosphate (Roche Molecular Biochemicals) and NBT/5-bromo-4-chloro-3-indolyl phosphate (Vector Laboratories).

Statistical analysis

Statistical analysis was performed using a two-way ANOVA when data were normally distributed or could be transformed to attain that distribution. Otherwise, a series of nonparametric tests (Mann-Whitney U) between the groups at each time was used.

Results

IL-15 treatment did not induce any severe side effects

To examine whether IL-15 treatment induced clinical side effects, animals were tested weekly for several parameters. Glucose, blood urea nitrogen, creatinine, total protein, albumin, globulin, total bilirubin, alkaline phosphatase, sodium, potassium, triglyceride lipid, and amylase concentration showed no differences when untreated and IL-15-treated animals were compared. The concentration of phosphorus was significantly reduced in the blood with IL-15 treatment at weeks 4 and 6, with no differences at later time points (data not shown), whereas the concentration of calcium was comparable between untreated and IL-15 treatment. We also observed a transient decrease in the amount of alanine aminotransferase at
weeks 3 and 4 in the IL-15-treated animals. Additionally, hematological measurements (white blood cell numbers, RBC numbers, platelets, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet numbers) were also performed weekly, with no differences observed between untreated and IL-15-treated animals. IL-15 treatment was therefore well-tolerated by the animals and did not lead to toxic side effects, which is in agreement with the results of our previous study in which we treated chronically SIV-infected cynomolgus macaques (40).

IL-15 treatment during acute infection increased viral set point

Viral load was followed in all 12 SIV-infected animals up to week 20. IL-15-treated animals had a slightly increased plasma viral load initially with $5.7 \pm 3.6 \times 10^5$ SIV RNA molecules/ml at day 7 and $20 \pm 5.9 \times 10^5$ SIV RNA molecules/ml at day 14 compared with untreated animals ($2.6 \pm 1.4 \times 10^6$ SIV RNA molecules/ml at day 7 and $10 \pm 1.5 \times 10^6$ SIV RNA molecules/ml at day 14) (Fig. 1). After week 3, viral load in the untreated animals slowly decreased whereas in IL-15-treated animals, viral load remained high. This resulted in a 1 log higher viral load in IL-15-treated animals at week 6 ($4.4 \pm 3.1 \times 10^6$ SIV RNA molecules/ml for IL-15-treated animals and $0.18 \pm 3.1 \times 10^6$ SIV RNA molecules/ml for untreated animals, $p = 0.042$) and a 3 log higher viral load at week 20 ($42 \pm 28 \times 10^6$ SIV RNA molecules/ml for IL-15-treated animals and $2.4 \pm 0.9 \times 10^4$ SIV RNA molecules/ml for untreated animals, $p = 0.017$) (Fig. 1).

Increased SIV-specific CD$^8^+$ T cells during IL-15 treatment is followed by decreased activated SIV-specific CD$^8^+$ T cells at viral set point

IL-15-treated animals at week 2 postinfection had a 2.3-fold increase in Gag- and a 3-fold increase in Tat-specific CD$^8^+$ T cells ($p = 0.044$) compared with untreated animals (Fig. 2A). However, at all later time points virus-specific CD$^8^+$ T cell numbers were comparable between IL-15-treated and untreated animals. In IL-15-treated animals, we observed 1.6-fold more CD$^8^+$ T cells at week 2 (Fig. 2B). After cessation of IL-15, the CD$^8^+$ T cell numbers were comparable between IL-15-treated and untreated animals throughout the study. No differences were found in the absolute CD$^4^+$ T cell and CD20$^+$ B cell numbers (Fig. 2B).

To determine whether IL-15 treatment altered activation of cells, we analyzed expression of the activation markers CD69, CD25, and HLA-DR. IL-15 treatment reduced the frequency of HLA-DR$^+$ Gag-specific CD$^8^+$ T cells in the peripheral blood compared with untreated animals beginning at week 6 (untreated animals: $23 \pm 3.4\%$, IL-15-treated animals: $8.2 \pm 1.3\%; p = 0.05$) (Fig. 3A). A similar increase in viral load is observed in untreated animals.
decrease was observed for total CD8+ T cells (Fig. 3A). IL-15 treatment did not alter the expression of CD69, CD25, and HLA-DR on CD4+ T cells and CD20+ B cells (data not shown).

**Reduced CD3+CD8+ NK cells during viral set point in IL-15-treated animals**

An increase in cell numbers due to IL-15 treatment was also found for CD3+CD8+ NK cells with a 2.3-fold increase at week 1 (p = 0.049; Fig. 2B). However, although IL-15 was given until week 4, the absolute NK cell numbers were not different for IL-15-treated and untreated animals from weeks 2 to 4. After week 4, IL-15-treated animals had reduced NK cell numbers compared with untreated animals with up to significantly 67% reduction at week 16 (p = 0.02; Fig. 3B). However, as mentioned below, week 30 LN from IL-15-treated animals showed no decrease in CD3+CD8+ NK cells suggesting that CD3+CD8+ NK cells are specifically reduced from circulation (Fig. 3D).

**IL-15-treated SIV-infected animals have decreased CCR5+ and CD45RA+CD62L+CD4+ T cells in peripheral blood**

We next analyzed the expression of CCR5 which is up-regulated by IL-15 (44). Although during the first 4 wk, no differences were found, at later time points the percentage of CCR5+CD4+ T cells in the IL-15-treated animals decreased to <1% whereas in the untreated animals >5% CCR5+CD4+ T cells were found between weeks 6 and 12 (p = 0.049; Fig. 3B). CCR5 expression on Gag-specific CD8+ T cells, total CD8+ T cells, CD3+CD8+ NK cells, and CD20+ B cells (untreated animals, n = 3, ▲; IL-15-treated animals, n = 3, □). Line depicts mean.
animals \( (p = 0.016); \) week 20: 10 \( \pm \) 1\% for treated and 25 \( \pm \) 6\% for untreated animals \( (p = 0.049) \). In contrast, CD45RA\(^{-}\)CD62L\(^{-}\) cells were increased in IL-15-treated animals (week 6: 63 \( \pm \) 8\%, week 20 56 \( \pm \) 4\%) compared with untreated animals (week 6: 51 \( \pm \) 9\%, week 20: 38 \( \pm \) 3\%) (Fig. 3C).

Memory cell distribution and spontaneous and CD95-induced apoptosis was also examined in SIV-specific CD8\(^{+}\) T cells as reduced survival and skewed memory phenotype was described as intrinsic defects for HIV-specific CD8\(^{+}\) T cells. No difference was found in the distribution of memory subpopulations of Gag- and Tat-specific CD8\(^{+}\) NK cells and total CD8\(^{+}\) T cells when IL-15-treated and untreated animals were compared (data not shown).

**Gag-specific CD8\(^{+}\) T cell number are increased in LN of IL-15-treated chronically SIV-infected rhesus macaques**

Biopsies of inguinal LN were collected 30 wk after SIV infection and IL-15 treatment. Viral loads were 25,387 \( \pm \) 17,994 SIV RNA molecules/ml blood in the IL-15-treated animals \( (n = 3) \) and 8,440 \( \pm \) 4,210 SIV RNA molecules/ml blood in the untreated animals \( (n = 3) \). The LN of IL-15-treated animals were significantly smaller (169 \( \pm \) 49 mg) than the ones from untreated animals (367 \( \pm \) 51 mg; \( p < 0.05 \)). Accordingly, total cell, lymphocyte and CD4\(^{+}\) T cell numbers per LN were significantly reduced in IL-15-treated animals. Despite this overall reduction, the percentage (data not shown) and absolute cell numbers of Gag-specific CD8\(^{+}\) T cells were increased in the IL-15-treated animals compared with untreated animals \( (p = 0.049; \) Fig. 3D). No significant differences in total CD8\(^{+}\) T cell, CD3\(^{+}\)CD8\(^{+}\) NK cell, or CD20\(^{+}\) B cell numbers were observed between LN from untreated and IL-15-treated animals (Fig. 3D).

**Percentage of day 7 Ki-67\(^{+}\) CD4\(^{+}\) T cell correlates positively with day 84 viral replication**

We next examined whether viral load at days 14 (peak) and 84 (viral set point) correlated with activation and proliferation of CD4\(^{+}\) T cells and Gag-specific CD8\(^{+}\) T cells at days 7, 14, and 84. We observed a significant increase in the percentage of Ki-67\(^{+}\) CD4\(^{+}\) T cells at day 7 postinfection in the IL-15-treated animals compared with the untreated animals (Fig. 4A). When the correlation was...
examined between the percentage of Ki-67\(^+\)CD4\(^+\) T cells on day 7 postinfection and viral load, a positive correlation was observed for day 14 viral load (\(R^2 = 0.38, p = 0.03\)) and day 84 viral load (\(R^2 = 0.78, p = 0.002\)) (Fig. 4B). However, we did not see any significant correlation when percentage of days 14 and 84 Ki-67\(^+\)CD4\(^+\) T cells were analyzed, which is in agreement with our observations that there was no difference between the percentage of Ki-67\(^+\)CD4\(^+\) T cells in untreated and IL-15-treated animals at these later time points. A positive correlation was also seen when the absolute cell numbers of Ki-67\(^+\)CD4\(^+\) T cells at day 7 and viral replication at day 14 (\(R^2: 0.72, p = 0.03\)) and day 84 (\(R^2: 0.68, p = 0.04\)) were compared. No correlation was found between percentage and absolute cell numbers of CD25\(^+\)CD4\(^+\) T cells and viral load. Whereas no correlation was found between percentage of HLA-DR\(^+\)CD4\(^+\) T cells and viral load, absolute HLA-DR\(^+\)CD4\(^+\) T cells on day 7 correlated positively with viral load at day 14 (\(R^2: 0.82, p = 0.01\)) and day 84 (\(R^2: 0.73, p = 0.03\)). When we examined the correlation between absolute SIV-specific CD8\(^+\) T cell numbers and viral replication, we found no significant correlation. The same was true for absolute cell numbers of CD25\(^+\), HLA-DR\(^+\), and Ki-67\(^+\) SIV-specific CD8\(^+\) T cells and viral load. However, the percentage of day 84 CD25\(^+\) and Ki-67\(^+\) SIV-specific CD8\(^+\) T cells correlated positively with viral load (data not
shown), indicating that increased viral replication maintains activation of virus-specific CD8+ T cells.

**IL-15 induced increased lymphocyte proliferation in SIV-infected animals**

Next, we examined whether the increased cell numbers were due to proliferation of these cells by analyzing the expression of Ki-67. No difference in Ki-67 expression of SIV-specific CD8+ T cells was observed in IL-15-treated animals (Fig. 4A). IL-15-treated animals had a significant increase in frequency of Ki-67+ total CD8+ T cells (59 ± 5.3%) compared with the untreated group (13 ± 2.1%) at week 1 (p = 0.004), but lower Ki-67+ CD8+ T cells at weeks 6 and 8 (Fig. 4A). Similarly, the IL-15-treated group had significantly more Ki-67+ NK cells at week 1 (88 ± 2%) compared with the untreated group (26 ± 6%; p = 0.004) and significantly less Ki-67+ NK cells in IL-15-treated animals at weeks 6 and 8 (Fig. 4A). Although absolute CD4+ T cell numbers did not differ between IL-15-treated and untreated animals (Fig. 2B), the IL-15-treated group had a 2-fold increase of Ki-67+ CD4+ T cells at week 1 (21 ± 2.5%) compared with the untreated animals (10 ± 1.4%; p = 0.015; Fig. 4A). At weeks 6 and 8, increased Ki-67+ CD4+ T cells were observed in the untreated but not in the IL-15-treated animals.

**In vivo IL-15 treatment did not enhance NK cytotoxicity and cytokine production by SIV-specific CD8+ T cells**

The central question of this study was whether IL-15 could enhance the innate and adaptive immune response against SIV infection. We therefore examined cytotoxicity of NK cells and peptide-mediated cytokine secretion of CD8+ T cells in untreated and IL-15-treated animals. IL-15 treatment increased the absolute numbers of TNF-α and IFN-γ-producing CD8+ T cells stimulated with the Gag peptide at week 2 (Fig. 5A). However, the absolute Gag-specific CD8+ T cell number was higher in the IL-15-treated animals, this did not result in an increase in the percentage Gag-peptide responding cells through IL-15 treatment. The same was seen when CD8+ T cells were stimulated with the Tat peptide although only an increase in IFN-γ-producing CD8+ T cells but not in TNF-α-producing CD8+ T cells was observed at week 2 (Fig. 5A and data not shown). No difference was found in IL-2 and IL-10 production by SIV-specific CD8+ T cells between untreated and IL-15-treated animals (data not shown). Finally, NK cell cytotoxicity did not differ between three IL-15-treated and three untreated animals analyzed at week 0 preinfection and weeks 1–4 and 6 postinfection (Fig. 5B).

**IL-15 treatment reduces SIV-infected cells in LN at peak viremia but increases them at viral set point**

Inguinal LN and rectal biopsies were collected from three IL-15-treated and three untreated animals and analyzed for SIV infection by in situ hybridization. No statistical difference in the number of SIV-infected cells was found in rectal biopsies when untreated and IL-15-treated animals were compared (week 1: 1.51 ± 0.39 cells/mm² and 1.44 ± 0.04 cells/mm²; week 8: 0.09 ± 0.16 cells/mm² and 0.08 ± 0.13 cells/mm² for untreated and IL-15-treated animals, respectively). In LN biopsies significantly less SIV-infected cells were found at week 2 (peak viremia) in the IL-15-treated (1.6 ± 0.8 SIV-infected cells/mm² tissue) compared with the untreated animals (7.8 ± 1.5 SIV-infected cells/mm² tissue; p = 0.021; Fig. 5C). Although no statistical differences were observed at later time points it appeared that IL-15 treatment increased the number of SIV-infected cells in LN. When infected macrophages and infected lymphocytes were estimated based on morphology, no difference in numbers of SIV-infected macrophages and lymphocytes were found between untreated and IL-15-treated groups.

**Anti-SIV Abs are decreased in IL-15-treated animals**

To examine whether IL-15 treatment altered B cell function, we analyzed anti-SIV Abs in the plasma of untreated or IL-15-treated animals at week 3 (early infection) and at week 16 (viral set point). Significant less anti-SIV Abs were found at both time points in the IL-15-treated animal group compared with the untreated one (week 3: 0.05 ± 0.03 arbitrary units (AU) vs 0.22 ± 0.06 AU, p = 0.046 and week 16: 3.3 ± 2 AU vs 16 ± 5.2 AU, p = 0.025, for IL-15-treated and untreated animals, respectively) (Fig. 5D). The decrease in Ab production was, however, not accompanied by a change in B cell numbers either in peripheral blood (Fig. 2B) or in LN (Fig. 3D). To further examine whether the reduced anti-SIV Ab concentration could result in increased viral load, correlations between Ab and viral replication were analyzed at weeks 3 and 16. No correlation was observed for week 3 Ab concentration and viral replication, however, an inverse correlation was found for week 16 Ab concentration and viral replication (R²: 0.34, p = 0.045). If IL-15-induced changes in Ab production are associated with CD4 activation/proliferation, a correlation between Ki-67+ CD4+ T cells and amount of Ab should be observed. Indeed, we found an inverse correlation between the percentage of Ki-67+ CD4+ T cells at week 1 and the amount of Abs at week 3 (R²: 0.68, p = 0.001) and 16 (R²: 0.45, p = 0.017).

**IL-15 accelerated disease progression and induced meningoencephalitis**

The increase in viral load indicated already that IL-15 treatment during acute infection may increase the normally slow progression of SIV infection in Mamu-A*01+ rhesus macaques (45, 46). We therefore followed the animals up to week 30. Two of the 6 IL-15-treated animals had to be euthanized at weeks 25 and 26, respectively, due to simian AIDS with clinical signs of CNS involvement is shown. Focal mononuclear inflammatory cell infiltrates in cerebellar (A and B) and cerebral (C) leptomeninges (lepto-meningitis). D, A microglial nodule (arrows) in the subpial stratum molecular of the cerebral cortex (early encephalitis). H&E stain shown.
molecules/ml whereas the six untreated animals had a viral load of 7.4 ± 6.6 × 10^6 SIV RNA molecules/ml blood.

One of the two animals euthanized due to simian AIDS showed clinical signs of CNS involvement such as signs of visual impairment. We therefore examined the brain to analyze whether IL-15 treatment not only accelerates progression to AIDS but also leads to early involvement of the brain. Gross examination of the brain of the two euthanized animals was essentially unremarkable. One of two animals showed a mild, focal meningoencephalitis characterized by rare, discrete, randomly distributed and scant inflammatory infiltrates—consisting predominantly of monocytes/macrophages, to a lesser extent lymphocytes—involving the cerebellar cortex. The increased viral set point of animals treated with IL-15 could be due to a direct effect of IL-15 on viral replication. In vitro IL-15 has either a marginal effect (19, 52, 53) or no effect (54, 55) on replication of HIV in naturally infected PBMC. However, in PHA-stimulated freshly HIV-infected PBMC IL-15 can induce various amounts of HIV replication that differ from study to study (44, 53–56). This suggests that the effect of IL-15 on viral replication may depend on the activation status of the cells. In agreement with this, we have observed an increase in viral load in our present study treating acutely SIV-infected monkeys when intense immune activation is occurring. In contrast, earlier studies showed that IL-15 treatment of chronically SIV-infected animals did not increase viral set point (38, 40). An important difference in acute infection may be the presence of high amounts of IL-2, as IL-2 and IL-15 synergize to increase viral replication (54). Although no IL-2 mRNA was found in peripheral blood during acute HIV infection, LN biopsies have high amounts of IL-2 mRNA (57). Thus, during acute infection endogenous IL-2 and exogenous IL-15 could synergize leading to increased viral replication. Although this could be happening in our current study of IL-15 treatment, the fact that no difference in peak viral loads was seen when animals were still being treated with IL-15 would argue against this as a mechanism of increased viral set point. Most importantly, viral set points were higher at time points long after IL-15 treatment had ceased, indicating that some other mechanism is at action.

### Discussion

Several in vitro and in vivo studies in recent years have indicated that cytokine therapy may be a promising adjunctive therapeutic treatment for HIV infection. Candidate cytokines that have been tested in animals are IL-7 (47, 48), IL-12 (49), IL-2 (50, 51), and IL-15 (38, 40, 51). The effect of such cytokine treatment on acute infection, LN biopsies have high amounts of IL-2 mRNA (57). Thus, during acute infection endogenous IL-2 and exogenous IL-15 could synergize leading to increased viral replication. Although this could be happening in our current study of IL-15 treatment, the fact that no difference in peak viral loads was seen when animals were still being treated with IL-15 would argue against this as a mechanism of increased viral set point. Most importantly, viral set points were higher at time points long after IL-15 treatment had ceased, indicating that some other mechanism is at action.

**Table 1. Histological findings in SIV-infected rhesus macaque with simian AIDS**

<table>
<thead>
<tr>
<th></th>
<th>Cerebral cortex</th>
<th>White Matter</th>
<th>Basal Ganglia/Thalamus</th>
<th>Cerebellum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptomeningial infiltrates</td>
<td>±</td>
<td>N/A</td>
<td>N/A</td>
<td>±</td>
</tr>
<tr>
<td>Perivascular infiltrates</td>
<td>±</td>
<td>±</td>
<td>‐</td>
<td>±</td>
</tr>
<tr>
<td>Multinucleated giant cells</td>
<td>‐</td>
<td>‐</td>
<td>‐</td>
<td>‐</td>
</tr>
<tr>
<td>Microglial nodules</td>
<td>‐</td>
<td>‐</td>
<td>‐</td>
<td>‐</td>
</tr>
<tr>
<td>White matter pallor</td>
<td>N/A</td>
<td>N/A</td>
<td>‐</td>
<td>‐</td>
</tr>
</tbody>
</table>

*Frequency and distribution of inflammatory lesions is rated as follows: ±, rare; scant/sparse; ‐, frequent; 0, frequent and marked; N/A, absent; N/A, not applicable.*

Increased viral set points in IL-15-treated animals could be due to increased CD4^+^ T cell targets for infection. IL-15 can directly affect CD4^+^ T cells and macrophages, therefore increasing the target cell pool for SIV infection. The first studies describing the effect of IL-15 and IL-15Rα knockout mice concluded that CD4^+^ T cells are not affected in these mice (27, 58). This is in accordance with our study of IL-15-treated chronically SIV-infected cynomolgus macaques where we have not observed any changes in activation, cell numbers, and memory phenotype of blood CD4^+^ T cells (40). However, we and others have observed that under certain circumstances, IL-15 can indeed act as costimulator albeit weak for memory CD4^+^ T cells (38, 59–61). Furthermore, mitogen-activated human CD4^+^ T cells express the IL-15Rα chain (62) and IL-15-stimulated effector memory CD4^+^ T cells can migrate into tissue (38), suggesting that IL-15-responsive CD4^+^ T cells may migrate to sites of viral infection and replication. In the present study, we did observe increased Ki-67 expression in CD4^+^ T cells at week 1 in IL-15-treated animals, however, this was not accompanied by increased peripheral blood CD4^+^ T cell numbers. This may indicate CD4^+^ T cells migration into tissue. We have not observed increased infected lymphocytes in inguinal LNs or rectal biopsies in the acute infection. It is possible that CD4^+^ T cells migrated into GALT, which was shown before to be one of the major site of viral replication (63). In week 30 LN, the absolute CD4^+^ T cell number is decreased, suggesting that CD4^+^ T cells in our animal model may be migrating preferentially into GALT and not LN. We found that CD45RA^-^CD62L^-^ CD4^+^ T cells are diminished in the blood of IL-15-treated animals which suggests that these cells are either migrating into GALT or they are being consumed at sites of viral replication, because memory CD4^+^ T cells are the prime target for SIV infection. IL-15 treatment also reduced blood CCR5^-^ CD4^+^ T cells, something previously reported in chronic infection (63). Because IL-15 up-regulates CCR5 on human CD4^+^ T cells (44, 64), the loss of blood CCR5^-^ CD4^+^ T cells suggests migration of these cells from peripheral blood into tissue and increased likelihood of infection with the CCR5-tropic SIVmac251 virus. This, however, remains to be determined. It is unlikely that monocytes, another target cell population for SIV infection, is the source of increased viral replication as the numbers and CCR5 expression of monocytes were not affected in IL-15-treated animals. That indeed activation and proliferation of CD4^+^ T cells early during acute SIV infection can alter the viral replication months later was further supported by the positive correlation we have found between percentage and absolute cell numbers of Ki-67^-^CD4^+^ T cells at day 7 and viral load at days 14 and...
84. Absolute numbers of HLA-DR⁺CD4⁺ T cells was also positively correlated with early (day 14) and late (day 84) viral replication. These findings suggest that viral replication during viral set point is already at least partially determined at day 7 by the magnitude of CD4⁺ T cell activation and proliferation.

Besides altering replication and target cell populations of SIV, changing the immune response against SIV could be another mechanism contributing to the disease acceleration and high viral loads induced by IL-15 treatment. The failure of NK cells and SIV-specific CD8⁺ T cells to control virus could result in the increased viral set point found in IL-15-treated animals. Although we only observed increases in blood SIV-specific CD8⁺ T cell numbers at week 2 in IL-15-treated animals, increased numbers of Gag-specific CD8⁺ T cells were found in LNs even at week 30. Because numbers of blood HLA-DR⁺ Gag-specific CD8⁺ T cells were reduced in IL-15-treated monkeys, this may suggest migration of activated cells into tissues. The fact that viral replication is higher in IL-15-treated animals despite having more SIV-specific CD8⁺ T cells in the LNs suggests that these cells may be either functionally defective or die there. If activated virus-specific CD8⁺ T cells accumulate at sites of viral replication, bystander killing or functional defects could be enhanced especially if viral replication is high. Further studies are necessary to determine whether SIV-specific CD8⁺ T cells in LNs and possibly other sites are fully functional or exhausted effector CD8⁺ T cells.

IL-15 treatment did reduce significantly the numbers of blood NK cells without increasing them in LNs. This may suggest an impairment of NK cells to control viral replication. In contrast, it could suggest that the homeostasis of NK cells has been affected with possibly higher turnover of these cells as a result of higher viral replication in the IL-15-treated animals. It should be stressed that for both reduced activated SIV-specific CD8⁺ T cells and NK cells and the accompanied increased viral loads, two alternative explanations can be given. Either loss of activated SIV-specific CD8⁺ T cells and NK cells is resulting in reduced control of viral replication and hence increased viral set points in IL-15-treated animals, or alternatively, increased viral replication due to immunological mechanisms such as increased seeding of target cells results in exhaustion of activated SIV-specific CD8⁺ T cells and NK cells and thus the reduced numbers.

We also observed a decrease in anti-SIV Abs in IL-15-treated compared with untreated animals. However, no concurrent decrease in B cell numbers in peripheral blood and LN was observed. More studies are necessary to examine more carefully the effect of IL-15 on B cell function. The decreased anti-SIV Abs could indeed influence the outcome of SIV infection, as it was shown before that during progression to HIV and SIV, Abs specific for HIV and SIV decrease or are absent (65, 66). We observed an inverse correlation between the amount of anti-SIV Abs and viral replication at week 16. However, no correlation of anti-SIV Abs at week 3 and viral replication at week 3 was observed, indicating that although anti-SIV Abs are already significantly decreased early in SIV infection, this does not associate with viral set point.

Although IL-15 treatment induced a 2- to 3-fold increase in SIV-specific CD8⁺ T cells during acute infection, it was rather surprising that this had no effect on peak viremia. IL-15 could be enhancing the proliferation or survival of these cells. IL-15 inhibits CD95-induced apoptosis of HIV-specific CD8⁺ T cells (34, 60) and in vivo IL-15 induces massive expansion of CD8⁺ T cells (40). Given that SIV-specific CD8⁺ T cells are already >90% Ki-67⁺ as we show, it is unlikely that IL-15 is augmenting their proliferation; most likely, we are inhibiting the death of these cells. The fact that IL-15 increased SIV-specific CD8⁺ T cells and NK cells by 2- to 3-fold without any impact on peak viremia indicates how potent an immune response induced by an effective vaccine may need to be to impact on viral replication during acute infection.

IL-15 not only increased the viral set point but also led to accelerated disease progression to simian AIDS in two of the six treated animals. In one of the two animals, histology revealed that the brain was affected in this animal. Neuropathologically, SIV-infected macaques are known to reveal four distinct lesion profiles: minimal changes, early encephalitis, leukoencephalopathy, and encephalitis (42). Detailed morphological evaluation of one animal that died of AIDS in our study revealed evidence of minimal changes and incipient early encephalitis in the form of rare mononuclear inflammatory cell infiltrates in the cerebral and cerebellar, in conjunction with rare microglial nodules in the subpial portions of the cerebral cortex. The overall paucity of meningeal and parenchymal infiltrates coupled by the lack of multinucleated giant cells, substantial cortical and subcortical presence of microglial nodules and/or myelin pallor in the cerebral hemispheric white matter argue against the diagnosis of a full-blown SIV encephalitis or leukoencephalopathy. Our findings suggest that this animal suffered from early minimal CNS SIV-associated disease and incipient early encephalitis. It should be noted that previous studies to date have failed to establish a relationship between neuropathological findings, numbers of SIV-replicating cells, T cell infiltration, and repertoires of proinflammatory cytokines (42). However, a recent study suggests that IL-15 mRNA levels in the brain are increased in SIV infection and that this may indeed lead to maintenance of SIV-specific CD8⁺ T cells in the CNS (67). Our results could therefore be interpreted in different ways. Either IL-15 treatment increased the numbers of activated CD8⁺ T cells that could enter the brain and may therefore induce cytotoxic damage and inflammatory pathology, or more SIV-infected cells could enter the brain in the IL-15-treated animals and promote brain pathology. Furthermore, as mentioned above, we found much lower anti-SIV Abs in IL-15-treated animals compared with untreated animals. In a study using SIV/Δ-induced immunodeficiency disease CNS involvement was observed in animals which did not mount a good Ab response to the virus (68). This could indicate that the significant lower amount of anti-SIV Abs found in the IL-15-treated animals at weeks 3 and 16 may be involved in the CNS damage we have observed. A strong anti-SIV response was also found in the plasma and cerebrospinal fluid of slow progressors but not of fast progressors when rhesus macaques were infected with SIVmac251 (69). Detailed studies will be necessary to reveal the mechanism responsible for this early involvement of the CNS in IL-15-treated animals and whether Ab levels play a part in this.

In summary, we have shown here that IL-15 treatment for 4 wk during acute SIV infection of rhesus macaques increased the viral set point in all animals and accelerated disease progression to simian AIDS in two of six animals with one of the animals developing SIV meningoencephalitis. Although 2- to 3-fold more SIV-specific CD8⁺ T cells and NK cells were observed in IL-15-treated animals this had no impact on peak viremia although numbers of infected cell in LN biopsies were reduced. Whereas activated SIV-specific CD8⁺ T cells and NK cells were reduced in the blood of IL-15-treated animals at viral set point, SIV-specific CD8⁺ T cells were increased in LN while NK cell numbers did not differ with IL-15 treatment. Early activation of CD4⁺ T cells followed by reduction of blood CCR5⁺ and CD45RA⁺ CD62L⁺ CD4⁺ T cells with IL-15 treatment suggest an increased use of these cells as targets of infection that leads to increased viral set point and infected cells in LN. The positive correlation between day 7 Ki-67⁺ CD4⁺ and HLA-DR⁺ CD4⁺ T cells and viral replication at day 84 further supports a direct association between early CD4⁺ T cell activation...
and viral set point. Finally, this study suggests that IL-15 treatment of acutely SIV-infected nonhuman primates may serve as a useful model to understand what controls viral set point in HIV infection.

Acknowledgments
We thank Louise Bertrand (Department of Neurobiology and Anatomy, Drexel University College of Medicine) for assisting with figure preparations. We thank the NCRR-funded Resource for Nonhuman Primate Immune Reagents for the recombinant rhesus maque IL-15.

Disclosures
The authors have no financial conflict of interest.

References


IL-15 TREATMENT DURING ACUTE SIV INFECTION


