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CD8+ cell depletion amplifies the acute retroviral syndrome

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The duration and severity of the symptomatology present during the early phase of human immunodeficiency virus (HIV) infection (known as the acute retroviral syndrome) is associated with alterations in the clinical profile of infection, such as a shortening of duration between infection with HIV and the onset of neurocognitive impairment and acquired immunodeficiency syndrome (AIDS). Viral-specific CD8+ cytotoxic T lymphocytes (CTLs) and CD8+ natural killer (NK) cells play a key role in antiviral immunity. Loss of CD8+ cells or their functional impairment during the early period of infection is associated with a rapid progression to AIDS in nonhuman primate studies. However, no studies have determined whether CD8+ cell loss or impairment is associated with symptoms of acute retroviral illness such as fever. In this study, the authors compared the early phase of simian immunodeficiency virus (SIV) infection in animals that were treated with the anti-CD8 monoclonal antibody cM-T807 to deplete CD8+ cells during the early period of infection (SIV+ CD8− group) to those with intact CD8+ cells (SIV+ CD8+ group). The SIV+ CD8− group had an enhanced acute retroviral syndrome when compared to the SIV+ CD8+ group. The SIV+ CD8− group also had prolonged high viral loads and distinct alterations in the proinflammatory cytokines interleukin (IL)-6 and interferon (IFN)-α, as well as in monocyte chemoattractant protein (MCP)-1. CD8+ cell depletion, therefore, appears to enhance symptoms of the acute retroviral syndrome and alters several of the immunological factors associated with the early phase of infection. *Journal of NeuroVirology* (2004) **10(suppl. 1),** 58–66.

Keywords: AIDS; fever; HIV; IL-6; MCP-1; SIV

Introduction

The duration and severity of the acute or early phase of human immunodeficiency virus (HIV) infection

appears to play a role in both the clinical profile and the rate of progression to acquired immunodeficiency syndrome (AIDS). Following HIV infection, many individuals experience an "acute retroviral syndrome," also known as "seroconversion illness," which is marked by symptoms of malaise and fever. This symptomatic period may begin preseroconversion, as early as 7 days post infection, and lasts for approximately 1 to 2 weeks, although it can be longer in some individuals (Clark and Shaw, 1993). The symptom profile during this early period of HIV infection is associated with higher viral loads (Lavreys *et al*, 2002) and a more rapid progression to AIDS (Vanhems *et al*, 2000). Individuals experiencing an acute retroviral syndrome have a higher incidence of HIV-associated minor cognitive motor disorder (MCMD) (Wallace *et al*, 2001).

Currently, the best model for studying HIV/AIDS-associated changes in a controlled environment is simian immunodeficiency virus (SIV) infection in the rhesus macaque. SIV is a primate lentivirus that is closely related to HIV (Desrosiers, 1990). SIV infects CD4+ T cells and tissue macrophages, including those in the central nervous system (CNS). The primary stage of both HIV and SIV infection is characterized by a high level of viremia and onset of specific cellular immune responses (Kuroda *et al*, 1999; Reimann *et al*, 1994). Like HIV-infected humans, SIV-infected macaques also develop cognitive and motor abnormalities, as well as altered neurophysiological signs, thus manifesting a simian form of neuroAIDS (Fox *et al*, 2000; Gold *et al*, 1998; Horn *et al*, 1998; Marcario *et al*, 1999; Prospero-Garcia *et al*, 1996).

Symptoms of an acute retroviral syndrome are also present early in infection in the SIV-infected rhesus macaque. For example, our group previously reported that the SIV-infected rhesus macaque exhibits fever occurring between 8 and 17 days post inoculation (p.i.), concurrent with a decrease in activity (Horn *et al*, 1998). We have also observed increased levels of monocyte chemoattractant protein (MCP)-1 in both the blood and cerebrospinal fluid (CSF) of SIV-infected rhesus monkeys early (14 days p.i.) in infection (Fox *et al*, 2000). Increases in MCP-1 are thought to play an important role in the recruitment of immune cells to the CNS following infection by HIV or other viruses. In addition to MCP-1, a number of other cytokines are elevated during primary SIV infection, such as interferon-alpha (IFN-α), interleukin-6 (IL-6), and interleukin-12 (IL-12) (Giavedoni *et al*, 2000; Khatissian *et al*, 1996; Zou *et al*, 1997). These cytokines likely play a role in the early immune response. Further, IL-6 and IFN act as pyrogenic cytokines (Cartmell *et al*, 2000; Kurokawa *et al*, 1996; Netea *et al*, 2000; Sundgren-Andersson *et al*, 1998) and, therefore, are likely to be involved in the fever associated with the acute retroviral syndrome.

CD8+ lymphocytes play an important role in the host response to viral infections. Viral-specific cytotoxic T lymphocytes (CTLs) are CD8+ T cells that function as effector cells that specifically recognize and lyse virally infected cells. Natural killer (NK) cells also play a role in antiviral immunity, and these cells also express CD8 in rhesus monkeys. CD8+ cells, although decreasing the number of virally infected cells, can also produce deleterious effects, as they have been known themselves to be mediators of host damage following viral infection (Dixon *et al*, 1987). Loss of CD8+ cells or their functional impairment during the early period of infection results in increased viremia and a rapid progression to AIDS in animal studies, with the progression rate being correlated to timing of recovery of CD8+ cells (Schmitz *et al*, 1999a). The adaptive CTL response occurs as early as 1 week following SIV infection (Yasutomi *et al*, 1993) and within 3 to 4 weeks following primary HIV infection (Borrow *et al*, 1994; Koup *et al*, 1994; Pantaleo *et al*, 1994), and NK activity is also high during the acute phase of SIV infection (Giavedoni *et al*, 2000). Therefore, we hypothesized that the SIV-induced acute retroviral syndrome, as a model for the HIV-induced acute retroviral syndrome, would be significantly altered by early CD8+ lymphocyte depletion. We tested this hypothesis using early selective elimination of CD8+ cells in the SIV-infected rhesus macaque model to determine whether such treatment would change the presentation of the acute retroviral syndrome, and subsequently, alter the clinical profile of infection such as cytokine production or survival time.

Results

Experimental design and CD8 depletion

In order to analyze the acute retroviral syndrome, we studied 12 rhesus monkeys during the first 2 months of infection. Five monkeys were SIV infected but did not receive the CD8-depleting antibody (SIV+ CD8+ group). Seven monkeys were SIV infected and CD8 depleted (SIV+ CD8− group) by administration of cM-T807 on days 6, 9, and 13 post SIV inoculation. In this group, circulating CD8+ cells were completely depleted by day 9 p.i., and began to recover at day 31 p.i., but the number of CD8+ cells remained reduced compared to preinfection. To control for the effects of antibody treatment, two uninfected monkeys (CD8− SIV− group) were also treated cM-T807, which also produced a loss of CD8+ cells (data not shown).

Body temperature

We have previously reported that body temperature was maximally elevated at day 13 p.i. by ∼0.9°C following SIV inoculation in rhesus macaques (Horn *et al*, 1998). Consistent with our previous report, the acute retroviral syndrome was manifested by an increase in average body temperature in the SIV+ CD8+ group, although individual animals showed variability in the timing of fever. Temperature began to increase during the second week following viral inoculation, and at day 17 was elevated to 0.4°C ± 0.08°C over baseline, and peak fever was reached on day 24 p.i. (0.7°C ± 0.28°C over baseline). In the SIV+ CD8− group, temperature peaked earlier and at a higher level, to 1.5°C ± 0.08°C over baseline on day 17 p.i (Figure 1, top panel). The increased body temperature seen in the SIV+ CD8− group was significantly above that seen in the SIV+ CD8+ group on days 13 and 17 p.i. (*P* < .05; Figure 1, top panel). The SIV− CD8− group showed no alterations in temperature following mock inoculation or CD8 depletion (data not shown).

Gross motor activity

Gross motor activity was used as an additional measure of the acute retroviral syndrome, as it is
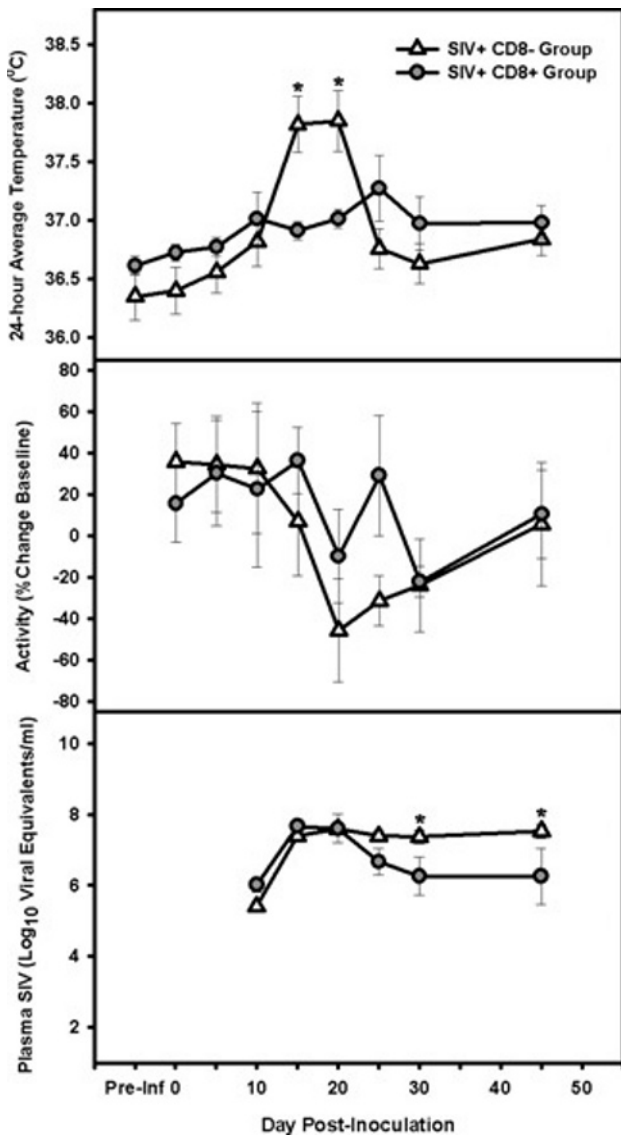
Figure 1 (**Top**) 24-hour average temperature. Temperature is significantly higher in the SIV+ CD8− group compared to the SIV+ CD8+ group on days 13 and 17 p.i. ($P < .05$). (**Middle**) Percent change from baseline activity. Although both the SIV+CD8− group and the SIV+ CD8+ group showed a decrease in activity on day 17 p.i., the decease in activity tended to be greater in the SIV+ CD8− group on day 17, as well as day 24 p.i. (**Bottom**) Plasma SIV. Plasma viral load increases to peak levels in the plasma on day 13 p.i. in the SIV+ CD8+ group and on day 17 p.i. in the SIV + CD8− group. The SIV + CD8− group maintains steady-state viral loads higher than that of the SIV + CD8+ group, with significantly higher levels occurring on days 31 and 45 p.i. ($P < .05$).

frequently reduced during malaise. In both groups, activity varied over the observation period, but was not significantly increased or decreased at any time point between groups. The changes in activity, however, did generally follow a pattern of decrease ~17 days after infection. In the SIV+ CD8+ group, activity increased over baseline ~15% to 30% through days 0 to 13 p.i., then decreasing ~10% on day 17 p.i. Activity increased once again on day 24 p.i., but

dropped by ~20% on day 31 p.i. In the SIV+ CD8− group, activity declined by 45% over baseline on day 17 p.i. and remained ~15% to 30% below baseline until day 45 p.i. (Figure 1, middle panel). Although the pattern of temperature and activity alterations in the SIV+ CD8+ group were similar to what we have previously reported (Horn *et al*, 1998), there appeared to be a trend towards the amplitude of the change being greater in the SIV+ CD8− group compared to the SIV+ CD8+ group, although not significantly different between the two groups (Figure 1, middle panel). In the SIV− CD8− group, activity remained relatively unchanged, i.e., slight increases were observed at a few time points but no decreases were observed (data not shown).

Plasma viral load
Viral load is the best correlate of disease progression in SIV/HIV infection and subsequent mortality. In the SIV+ CD8+ group, viral load reached a peak of almost 10^8 viral equivalents/per ml on day 13 p.i., by day 24 p.i., viral load had dropped almost 1 log and remained at a steady state of $10^{6.5}$ viral equivalents/per ml throughout early infection (Figure 1, bottom panel). In the SIV+ CD8− group, viral load was almost identical to the SIV+ CD8+ group until day 24 p.i. At this time point, viral load did not decline as it had in the SIV+ CD8+ group, but rather remained elevated close to or above peak levels, nearly 10^8 viral equivalents/per ml. Mean viral load in the SIV+ CD8− group was significantly higher than the SIV+ CD8+ group on days 31 and 45 p.i. (Figure 1, bottom panel).

Cytokines and chemokines
Cytokines are produced during the early stages of viral infection, and have key roles in sickness behavior (Johnson, 2002). In order to examine candidate effectors in sickness behavior, serial plasma samples were taken over the course of infection and used to examine IL-6, IFN-α, and MCP-1. Analysis of plasma IL-6 levels revealed that the SIV+ CD8− group had significantly higher levels of IL-6 than did the SIV+ CD8+ group at multiple points following infection beginning at day 10 p.i. ($P < .05$; Figure 2, top panel). In both groups, IL-6 then increased. In the SIV+ CD8+ group, IL-6 levels increased slightly, peaking on day 17 p.i. The SIV+ CD8− group also peaked at day 17, but at a much higher level, and remained over baseline for the rest of the observation period (Figure 2, top panel). There were no consistent changes in plasma IL-6 in the SIV– CD8− group, although sporadic variable increases were found both before and after mock inoculation (baseline = 0.63 ± 0.46 pg/μl, range after mock infection = 0.09–3.7 pg/μl).

Plasma IFN-α showed a different pattern. Here, the SIV+ CD8+ group was significantly higher than the SIV+ CD8− group on day 10 p.i. ($P < .05$; Figure 2, middle panel). This increased level of IFN-α on day
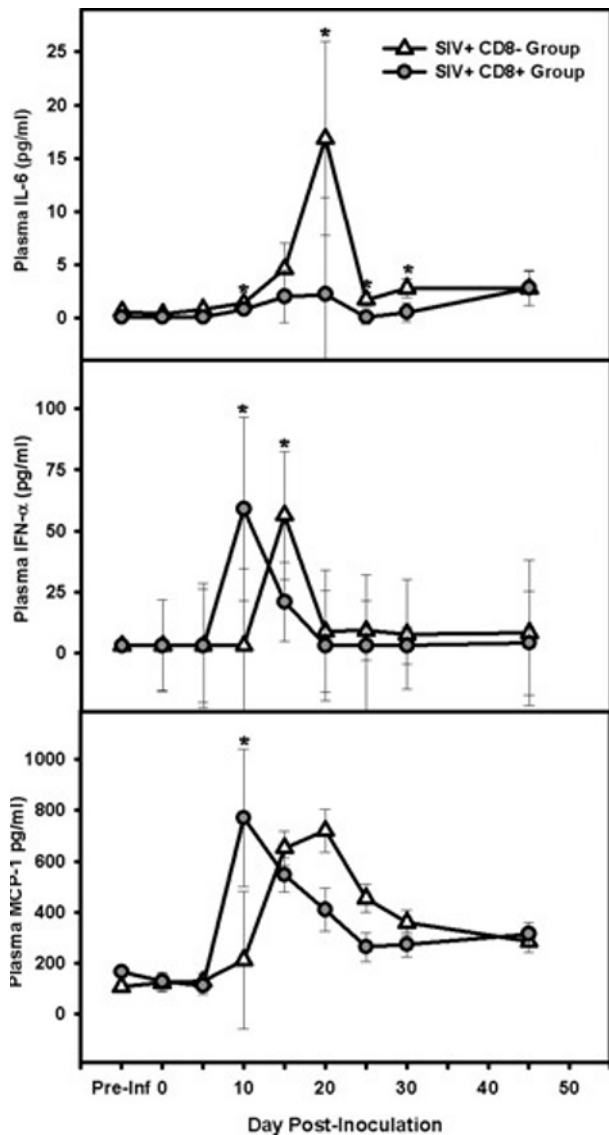
Figure 2 (**Top**) Plasma IL-6. Peak levels of plasma IL-6 occur in both groups on day 17 p.i. The SIV+ CD8− group was significantly higher than the SIV+ CD8+ group on days 10, 17, 24, and 31 p.i. ($P < .05$) (**Middle**) Plasma IFN-α. In the SIV+ CD8+ group, plasma IFN-α peaks earlier, and is significantly higher, than the SIV+ CD8− group on day 10 ($P < .05$). In the SIV+ CD8− group, the peak of IFN-α is delayed, being significantly higher on day 13 p.i. ($P < .05$). (**Bottom**) Plasma MCP-1. As with IFN-α, peak plasma MCP-1 occurs significantly earlier in SIV+ CD8+ group (day 10 p.i.) than in the SIV+ CD8− group ($P < .05$).

10 p.i. in the SIV+ CD8+ group began to decline at day 13 p.i., and returned to baseline levels by day 17 p.i., remaining there throughout the early period of infection (Figure 2, middle panel). In the SIV+ CD8− group, plasma IFN-α peaked later, at day 13 p.i,. and then remained slightly elevated over baseline and over the SIV+ CD8+ group throughout the early period of infection. Thus the CD8-depleted group showed a delayed peak of production of IFN-α relative to the CD8-intact group.

As with IFN-α, the peak of MCP-1 in the plasma was different between the two groups, with MCP-1 peaking and being significantly higher on day 10 p.i. in the SIV+ CD8+ group ($P < .05$; Figure 2, bottom panel). In the SIV+ CD8− group, peak increases in plasma MCP-1 were delayed compared to the SIV+ CD8+ group, reaching peak levels on day 17 p.i. Here, MCP-1 levels remained elevated above baseline in both groups for the remainder of the observation period. There were no changes in plasma MCP-1 levels following mock inoculation in the SIV− CD8− group (data not shown).

To determine whether increases in the plasma were accompanied by similar increases in the CNS, we measured IL-6 and MCP-1 in the CSF (due to limited amounts of sample available, IFN-α was not measured in the CSF; additionally CSF was only drawn on selected dates). For IL-6, the SIV+ CD8− group levels were significantly elevated above the SIV+ CD8+ group on days 17 and 45 p.i. ($P < .05$; Figure 3, top panel). Increased CSF MCP-1 was also seen following infection, and was similarly significantly higher in the SIV+ CD8− group versus the SIV+ CD8+ group, but only on day 17 p.i. ($P < .05$; Figure 3, bottom panel).

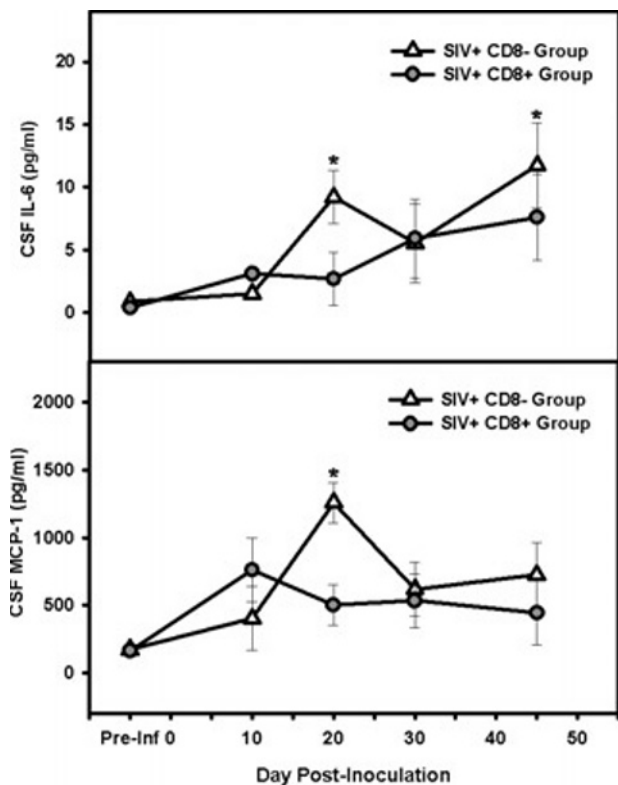**Figure 3** (**Top**) CSF IL-6. Levels in both groups peaked on day 45. IL-6 levels in the SIV+ CD8− group was significantly higher than the SIV+ CD8+ group on days 17 and 45 p.i. ($P < .05$). (**Bottom**) CSF MCP-1. In the SIV+ CD8+ group, levels peaked earlier (day 10 p.i.) than in the SIV+ CD8− group (day 17 p.i.). The SIV+ CD8− group had significantly higher levels of MCP-1 than the SIV+ CD8+ group on day 17 p.i.
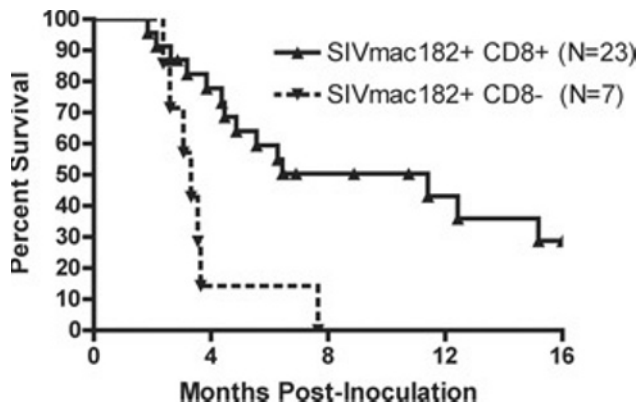
Figure 4 Kaplan-Meier survival analysis of SIVmac 182 infection. The survival of infected monkeys over time is indicated for 23 animals not receiving anti-CD8 treatment, and 7 animals receiving the CD8 depletion protocol as described. The median survival (11.4 months and 3.3 months, respectively) differs significantly ($P < .05$).

Clinical outcome

All of the animals in the SIV+ CD8− group developed simian AIDS within the first 8 months following infection. Comparison of their survival with that of the five SIV+ CD8+ animals and 17 historical SIVmac182-infected animals (the SIVmac182+ CD8+ group) reveals a marked acceleration of disease. The median survival of animals in the SIVmac182+ CD8+ group was 11.4 months, whereas in the SIV+ CD8− group, median survival drops significantly to 3.3 months ($P < .05$; Figure 4).

Discussion

The presence of fever and increased IL-6 and IFN-α in the blood, and IL-6 in the CSF, during the acute phase of infection demonstrates that although the immunological milieu was disrupted in the SIV + CD8− group by selective CD8+ cell depletion, an inflammatory response still occurred and the acute retroviral syndrome was increased in severity. A comparison of temperature data from the SIV+ CD8+ group and historical data (Horn *et al*, 1998) revealed that the severity of the fever was greater in the SIV+ CD8− group. Furthermore, a shortening of the period from acute infection to the development of AIDS was observed in the SIV+ CD8− group. These results support our hypothesis that selective CD8+ cell depletion early in infection enhances symptoms of the acute retroviral syndrome and leads to an altered clinical outcome. Although a diminished CTL or NK response and increased viral load likely play a role in the shortened survival seen in these animals, it is interesting to note both the duration and severity of the acute phase of HIV infection also appear, to be associated with an altered clinical profile and rapid progression to AIDS in humans (Vanhems *et al*, 2000).

The removal of CD8 cells may also result in a greater or more persistent acute retroviral syndrome during the early period of infection, due to both persistence of high viral loads and the release of proinflammatory factors from an increased number of virally infected cells, or perhaps because of an enhanced innate immune response leading to the release of cytokines such as IL-6, IFN-α, and IL-1. Production of these factors may be involved in the altered clinical outcome seen in this study, as well as in naturally occurring rapid AIDS progression. Provocative correlations between high cytokine levels and altered clinical profiles have been observed in other studies. For example, IL-6 was significantly elevated (>100-fold) in the serum of pigtailed macaques infected with SIVsmm/PBj-14, which caused acute lethality at 8 days p.i., and high levels of IL-6 mRNA were found in lymphoid tissues of these animals (Birx *et al*, 1993). In SIVmac251-infected rhesus macaques that had rapid (<200 days) disease progression, those that developed SIV encephalitis were found to have elevated (approximately twofold) levels of IL-6 mRNA in lymph nodes (Orandle *et al*, 2001).

Both IL-6 and IFN-α key are thought to be involved in fever and sickness behavior following infection (Gruol and Nelson, 1997; Sundgren-Andersson *et al*, 1998; Kurokawa *et al*, 1996; Netea *et al*, 2000). For example, in rodents treated with an IL-6 antiserum prior the administration of the pyrogen lipopolysaccharide (LPS), there was a complete abolishment of LPS induced fever (Cartmell *et al*, 2000). Further, in HIV-infected humans with AIDS lymphoma suffering from both fever and night sweats, treatment with an anti–IL-6 monoclonal antibody alleviates both of these symptoms within a few hours (Emilie *et al*, 1994). Using knockout mice, it has also been shown that IL-6 must be present for both IL-1β– and TNF-α–induced fever (Cartmell *et al*, 2000; Sundgren-Andersson *et al*, 1998).

Sickness behavior is thought to be an adaptive response to infection; however, proinflammatory factors secreted early in infection involved in the presentation of the acute retroviral syndrome also have the potential to impact normal CNS functioning and diminish patients quality of life. For example, high levels of IL-6 have also been associated with AIDS dementia complex (Perrella *et al*, 1992; Pulliam *et al*, 1996). Further, HIV-1–infected humans experiencing acute retroviral illness with fever have a more rapid onset of clinical neurocognitive impairment when compared to infected individuals who do not exhibit such symptomology (Wallace *et al*, 2001). Fatigue is often the most prevalent symptom following HIV infection and proinflammatory factors may play a role in the onset or maintenance of this symptom.

The mechanisms leading to CNS dysfunction in HIV infection are not yet completely understood. Neurons are not infected by the virus and, therefore, the cause(s) of CNS dysfunction are indirect. As cells

of the monocyte lineage are the targets for HIV infection in the brain, products of infected and/or activated macrophages are prime candidates for inducing CNS damage (Kaul *et al*, 2001). Interestingly, the number of macrophages present in the brain of infected individuals can be correlated with the severity of HIV dementia (Glass *et al*, 1995).Macrophage entry to the CNS is assisted by an increase in the level of chemoattractant molecules such as MCP-1. We have previously shown that MCP-1 is elevated in the CSF relative to the plasma 2 weeks after SIV infection in non–CD8-depleted SIV-infected monkeys (Fox *et al*, 2000); similarly here. Although different time points were examined, we find MCP-1 elevated early in the SIV+ CD8− monkeys, as well as the SIV+ CD8+ controls. The increase of MCP-1 in the CSF likely contributes to the influx of macrophages into the brain, which in turn may produce factors that lead to CNS damage. Indeed, MCP-1 is elevated in the CSF of those with HIV encephalitis, HIV dementia (Cinque *et al*, 1998; Conant *et al*, 1998; Kelder *et al*, 1998), as well as in monkeys with SIV encephalitis (Zink *et al*, 2001). Further, absence or loss of function of CD8+ cells during the early period of infection may favor a higher penetration of infected and/or inflammatory cells into the CNS, leading to increased incidence of encephalitis or other CNS alterations.

In this study, we altered the immunological milieu through selective CD8+ cell depletion in SIV-infected rhesus macaques, and by doing so, we demonstrated that these animals exhibit increased signs of illness early in infection. Our data show that these animals experience greater fever. Further, these SIV+ CD8− animals also experience greater or more prolonged alterations in IL-6 compared to SIV+ CD8+ animals, thus suggesting a role for CD8+ cells in the regulation of the inflammatory response. Although it is difficult to compare the course of AIDS in CD8-depleted animals to human AIDS, it is interesting to note that these animals had greater symptomatology and prolonged high viral loads early in infection, and this correlated with severely diminished survivability. We therefore propose that increased symptomatology, viral load, and proinflammatory factors during the early phase of infection may be predictors of disease course, both in terms of CNS disease and survivability. Diminished CD8 function, either effector or regulatory, can thus alter both time course and outcome of SIV infection.

Materials and methods

Animals
Using a within-subjects design, male rhesus macaques, 3 to 5 years old, weighing 4 to 7 kg, free from SIV, type D simian retrovirus, and herpes B virus were obtained from Covance (Alice,

TX) and Charles River (Key Lois, FL) for use in three of the groups: SIV-infected, CD8-depleted (SIV+ CD8− group; $N = 7$), SIV-infected, non–CD8-depleted (SIV+ CD8+ group; $N = 5$), and uninfected, CD8-depleted (SIV− CD8− group; $N = 2$). A fourth SIV-infected, non–CD8-depleted group (SIVmac182+ CD8+ group; $N = 23$), comprised of animals from current and previous studies, was utilized for historical physiological and survival data, some of which have previously been published (Fox *et al*, 2000; Horn *et al*, 1998). Animals were kept in a biocontainment facility, housed individually in stainless-steel cages, which permit olfactory, visual, and auditory contact with other monkeys in the room. The room was maintained on a 12/12-h light/dark cycle (lights on 6:00 AM; lights off 6:00 PM) and at a temperature of 25.0°C. Each animal had *ad libitum* access to food and water. All procedures were performed with approval of The Scripps Research Institute's Institutional Animal Care and Use Committee consistent with National Academy of Sciences *Guide for Animal Care and Use* (1996).

Physiological monitoring
Animals were implanted with telemetry transmitters (10TA-D70) capable of detecting body temperature and gross motor activity (Data Sciences International, St. Paul, MN) using aseptic surgical techniques under anesthesia. Transmitters were implanted into a subcutaneous pocket in the left flank by making a transverse incision 2 cm caudal to the left scapula. After device implantation, the incision was closed using 3.0 polyglactin sutures. Data acquisition was achieved through Data Sciences hardware (Receiver plate RLA 2000, St. Paul, MN) and software (Dataquest A.R.T. Version 2, St. Paul, MN). Sampling was taken every 10 s but averaged into 10-min bins. The average of these 10-min bins over the 24-h period prior to each blood and CSF sampling point was used for analysis. Animals were given 4 weeks to recover from surgery before beginning the experimental protocol.

Viral infection
A cell-free inoculum stock of SIV (SIVmac182) was obtained after serial passage of SIV-infected microglia (Watry *et al*, 1995). At 10:00 AM each animal ($N = 12$, 7 of which were subsequently CD8-depleted, see below) received SIVmac182 stock (0.9 ng of p27 gag antigen) diluted into RPMI-1640 by intravenous (i.v.) injection.

CD8 depletion
The SIV+ CD8− group ($N = 7$) was treated with the anti-CD8 antibody cM-T807 in order to deplete CD8+ lymphocytes, using a slight modification of the original protocol (Schmitz *et al*, 1999b). On day 6 post SIV inoculation, each animal was injected

subcutaneously with 10 mg/kg of cM-T807 and on days 9 and 13 p.i., each animal was infused through the saphenous vein with 5 mg/kg of cM-T807. As a control, a third group of animals (SIV− CD8− group; $N = 2$) were mock inoculated with RPMI-1640 alone (vehicle for SIV inoculation) and then treated with the same doses cM-T807 also on days 6, 9, and 13 post mock inoculation. This produced a significant loss of CD8+ lymphocytes in both groups (approximately >90% days 9 to 17).

Blood, CSF, and tissue sampling
Serial blood and CSF samples were taken at the same time (10:00 AM) on days both prior to and following SIV inoculation and mock inoculation. Because *in vivo* CSF volumes are less than blood volumes, CSF samples were taken at fewer time points than plasma during the early period of infection. All procedures were performed after intramuscular administration of 10 to 20 mg/kg ketamine as anesthesia. Blood samples were drawn from the femoral vein into EDTA-treated tubes, and plasma was separated from cells by centrifugation. CSF was obtained from the cisterna magna by percutaneous puncture with a 25-gauge needle. CSF samples containing >0.1% blood contamination (determined by red blood cell [RBC] counts) were discarded.

Peripheral mononuclear cells
Buffy coats obtained from centrifugation of EDTA-anticoagulated blood were placed in Ficoll-Histopaque (Pharmacia Biotech, Uppsala, Sweden) gradient centrifugation for isolation of the mononuclear fraction. After washing, cells were enumerated in a Coulter Z2 (Coulter, Miami, FL) and resuspended in complete RPMI-1640, containing 10% fetal calf serum (FCS) at a concentration of 10^7/ml.

Flow cytometry
Cells isolated as described above and were stained with antibodies diluted according to a previous titration in staining buffer (Hank's balanced salt saline [HBSS] with 2% FCS and 0.01% NaN$_3$). The antibodies used for the staining were anti-monkey CD3-biotin (clone FN-18, Biosource) followed by Streptavidin-PerCP or Streptavidin-APC (Pharmingen, San Diego, CA), anti-human CD8-(phycoerythrin) (PE) (clone DK25, Dako), and anti-CD4-PE (clone OKT4, hybridoma obtained from ATCC (Rockville, MD),

with the secreted antibody purified and PE-conjugated in the laboratory). The proper isotype controls (Pharmingen) were also used. The cells were then processed through a FACSort cytometer before analysis of data with CellQuest software (Becton-Dickinson Immunocytometry Systems, San Jose, CA).

Viral quantitation
Viral load was determined by Bayer Reference Testing Laboratory (Emeryville, CA), by measuring SIV RNA in plasma with the quantitative branched DNA (bDNA) signal amplification assay.

Cytokine and chemokine measurement
Plasma and CSF samples were stored at −80°C until the time of assay. Samples were then thawed and tested for IL-6 (Human Quantikine and Quantikine HS; R&D Systems, Minneapolis, MN), IFN-α (human; Endogen, Woburn, MA) and MCP-1 (Human Quantikine; R&D Systems), by enzyme-linked immunosorbent assay (ELISA) using the standard procedures described in the product information sheet for each kit. For accuracy, when the level of a particular cytokine was above or below the sensitivity range of the assay, samples were retested using either a high (as available) or standard sensitivity kit to get accurate levels. Due to a limited amount of CSF collected, some cytokines were measured only in the plasma.

Statistical analysis
After determining the normality and homogeneity of variances of the SIV+ CD8+ and SIV+ CD8− groups (the SIV− CD8− group was not included in the analysis due to its small sample size, and therefore was not represented in any of the graphs), statistical analyses were performed on either raw or log 10 transformed data using repeated-measure two-way anlysis of variance (ANOVA) with significance set at the $P < .05$ level. Significant main effects were analyzed using the SNK *post hoc* test. All of the above statistical tests were run using the software programs SigmaStat (SPSS Science, IL). Data are presented in the text as mean ± SEM. Statistical comparisons of the SIVmac182+ CD8+ group ($N = 23$) and the SIV+ CD8− group ($N = 7$) were made using the log-rank and chi-square tests using Prism software (version 4.0, GraphPad, San Diego) with significance set at the $P < .05$ level.

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