Phenotypic and Functional Parameters of Cellular Immunity in a Chimpanzee with a Naturally Acquired Simian Immunodeficiency Virus Infection

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The cellular immunologic and virologic status of a chimpanzee, naturally infected with a human immunodeficiency virus type 1 (HIV-1)–like lentivirus ($SIV_{cpz\text{-}ant}$), was compared longitudinally with those of 3 HIV-1–infected and 5 uninfected chimpanzees for a period of 49 months. Evidence of immune deficiency was not observed in the HIV-1–infected chimpanzees, nor could virus be isolated from plasma. Virus could be isolated from plasma of the $SIV_{cpz\text{-}ant}$–infected chimpanzee, but clinical signs of immune deficiency were never observed. Absolute $CD4^+$ cell counts remained relatively stable, but NK cells fluctuated significantly over time and tended to correlate inversely with the virus titer in peripheral blood. Although only $CD8^+$ T cells were directly demonstrated to exert a suppressive effect on viral replication in vitro, the observed fluctuation of NK cells suggests that these cells may also be involved in the interaction with lentivirus infection in this species.

Chimpanzees can be reproducibly infected with relatively low doses of in vitro–propagated cell-free human immunodeficiency virus type 1 (HIV-1) or by injection of primary HIV-infected human plasma or peripheral blood mononuclear cells (PBMC) [1–4]. After infection, they mount HIV-specific humoral and cell-mediated immune responses that parallel those of infected humans. Virus can be isolated from PBMC but, in contrast to humans, not routinely from serum or plasma [5–7]. After >10 years of follow-up of experimentally infected chimpanzees worldwide, data to date have not revealed evidence of progression to AIDS despite occasional reports of transient lymphadenopathy and low $CD4^+$ cell counts [1, 8, 9]. One animal, inoculated with 3 different HIV-1 isolates, developed prolonged but transient $CD4^+$ lymphopenia and thrombocytopenia showing impaired proliferative responses to T cell mitogens, suggesting possible progression to AIDS [8].

In chronically infected chimpanzees, the cell-mediated immune responses to HIV-1 may differ from those in humans (e.g., the absence of autodestructive cytotoxic T cells that lyse uninfected $CD4^+$ cells) [10]. Similarities in cellular immune responses have been reported as well. Suppression of HIV-1 replication by $CD8^+$ lymphocytes from chimpanzees [8, 11] has also been observed in PBMC cultures from HIV-infected humans [12, 13].

Recently, a new primate lentivirus, designated $SIV_{cpz\text{-}ant}$, was isolated from a wild-caught, naturally infected chimpanzee [14]. To date, on the basis of DNA sequence homology, $SIV_{cpz\text{-}ant}$ has been characterized as the most divergent HIV-1–like isolate [15] and is even more divergent from the HIV-1 consensus sequence than the previously isolated $SIV_{cpz\text{-}gab}$ strain [16, 17]. The genomic relationship between $SIV_{cpz\text{-}ant}$ and HIV-1 on one hand and the occasion to study the immune responses to $SIV_{cpz\text{-}ant}$ in a naturally infected animal on the other contribute to the unique opportunity to study the natural host-virus relationship. In this study, we report on longitudinal data obtained over 4 years from a small unique cohort of HIV-1–seropositive chimpanzees, including 1 naturally $SIV_{cpz\text{-}ant}$–infected animal, and 5 seronegative controls. We compared lymphocyte subsets, proliferative and cytolytic responses, and the capacity of $CD8^+$ cells to suppress viral replication in vitro in an effort to determine the nature of the cell-mediated immune interaction with this unique chimpanzee lentivirus.

Materials and Methods

Animals. A naturally $SIV_{cpz\text{-}ant}$–infected male chimpanzee (Ch-No), ~9 years old, previously described [14], was housed at the TNO Primate Center, Netherlands, since 1991 for the duration of these studies. Serologic evidence of infection with $SIV_{cpz\text{-}ant}$ was obtained in 1989 from the first available blood sample stored at −20°C. Three experimentally HIV-1–infected chimpanzees, of which 2 (Ch-Bu and Ch-Ma) had been infected with cell-free virus from a prototype laboratory strain of HIV-1 (LAI strain) in October 1984 and the third (Ch-Co) with uncultured whole blood from an Amsterdam AIDS patient in October 1982, were housed in the same facility and were followed as HIV-infected controls [4]. Four uninfected healthy adult chimpanzees (Ch-5 through 8), colony-raised at TNO, and 1 uninfected animal at risk (Ch-Ni, caged together with Ch-No) were included as seronegative controls. Animals were observed daily for signs of illness or changes in behavior and were monitored at 4-month intervals for hematologic, immu-

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nologic, and virologic status since the start of the TNO chimpanzee cohort study in 1991.

Immunophenotyping of peripheral blood lymphocytes. Standard hematology was done on a Technicon H1 (Technicon Instruments, Tarrytown, NY). Lymphocyte subpopulations were analyzed in whole blood by three-color flow cytometry [18] using directly fluorochrome-conjugated monoclonal antibodies (MAbs) specific for CD3, CD4, and CD8 (Becton Dickinson, San Jose, CA). Samples were prepared according to the manufacturer's instructions and analyzed on a FACScan, using LYSIS I software (both Becton Dickinson).

Cell-mediated immune responses. PBMC were purified from freshly obtained heparinized venous blood by density gradient centrifugation. Lymphocyte proliferative responses to phytohemagglutinin (PHA; 0.5 μg/mL, purified grade; Wellcome, Dartford, UK), concanavalin A (1 μg/mL; Sigma, St. Louis), and anti-CD3 (OKT3; 200 ng/mL, purified from hybridoma culture supernatant) were measured after 4 days of mitogen stimulation. Tetanus toxoid (1/500; Wyeth Laboratories, Marietta, PA), varicella-zoster virus (1/500; Behring-Hoechst, Brussels, Belgium), and *Candida albicans* (1/500; Pasteur Mérieux, Lyon, France) were used as recall antigens. Recombinant HIV-1 p24 (10 μg/mL; Innogenetics, Ghent, Belgium) and peptides consisting of 20–25 amino acid residues covering the principal neutralizing domain of gp120 V3 loop of different HIV-1 strains (MN, SF2, HBX2, ANT-70) and 2 chimpanzee lentiviruses ($SIV_{cpz\text{-}gab}$ and $SIV_{cpz\text{-}ant}$) (Neosystems, Strasbourg, France) were used to measure cross-reactive and virus-specific proliferative responses. These responses were analyzed by [*methyl*-^{3}H]thymidine (Amersham, Kent, UK) incorporation after 6 days.

NK and anti-CD3–redirected cytotoxic T lymphocyte (CTL) activity were measured in a chromium-release assay as described [19] and are expressed in lytic units per million effector cells. One lytic unit is the number of effector cells required to lyse 30% of the 10,000 target cells. $SIV_{cpz\text{-}ant}$–infected lymphoblasts were generated by stimulating fresh PBMC of Ch-No with 0.5 μg/mL PHA and replacing the culture medium with medium containing 10 U/mL recombinant interleukin-2 (Janssen Biochimica, Beerse, Belgium) every 3 days. After 14 days, freshly isolated PBMC of Ch-No, Ch-Ni, and Ch-8 were used as effector cells and the $SIV_{cpz\text{-}ant}$–infected lymphoblasts as target cells in a chromium-release assay as described [20]. To investigate whether the lysis was major histocompatibility complex (MHC)–restricted, MAb W6/32 (anti–MHC class I, 20 μg/mL) and MAb 9.3F10 (anti–MHC class II, 20 μg/mL) were used. Both MAbs were purified from hybridoma culture supernatant.

Virus isolation from PBMC and plasma. Five million PBMC from seropositive chimpanzees were cocultured with 5×10^6 PHA-stimulated PBMC from a healthy seronegative human donor. Cultures were monitored as described [16]. Lymphocyte titers are expressed as TCID per 10^6 PBMC and plasma titers as infectious virus per milliliter of plasma.

Suppression of viral replication by $CD8^+$ lymphocytes. In a first experiment, $CD8^+$ cells from the $SIV_{cpz\text{-}ant}$–infected and 3 HIV-1–infected chimpanzees were purified with an immunomagnetic separation technique (Dynabeads M-450 CD8 and DETACHaBEAD; Dynal, Oslo) according to manufacturer's instructions. PBMC, PBMC treated with anti-CD8 [21] (MT122, provided by P. Rieber, University of Munich), CD8-depleted PBMC, and reconstituted PBMC were stimulated with 0.5 μg/mL PHA. Culture medium was replaced by medium containing 10 U/mL recombinant interleukin-2 (Janssen) after 4 days and was repeated every 3 days. Viral replication was monitored by testing the culture supernatant for the presence of viral antigens with an in-house HIV antigen capture test.

In a second experiment, whole PBMC were divided into three aliquots. One aliquot was depleted of T cells with anti-CD3 (purified from UCHT1 hybridoma culture supernatant), $CD8^+$ cells were removed from the second, and a third was left untreated. By combining the CD3-depleted fraction with the CD8-depleted fraction, the suppressive effect of $CD8^+CD3^-$ NK cells could be determined separately. Untreated PBMC and CD8-depleted PBMC, reconstituted with the autologous $CD8^+$ cell fraction, served as controls.

Results

Hematologic findings and lymphocyte subsets. Results are summarized in table 1 and figures 1 and 2. White blood cell and lymphocyte counts of the 3 experimentally HIV-1–infected chimpanzees were significantly lower than in controls and generally close to the lower range of those of uninfected chimpanzees. In the $SIV_{cpz\text{-}ant}$–infected chimpanzee, the white blood cell count varied considerably, mainly because of variation in the absolute lymphocyte count, but the median values did not differ from controls. The platelet count in this animal was significantly decreased and dropped to extremely low values after 11 months of follow-up, but a hemorrhagic tendency was never observed. Other hematologic parameters always remained within the normal range.

Although the median percentage of $CD4^+$ cells and $CD8^+CD3^-$ NK cells was significantly higher in HIV-1–infected animals than in controls, the median absolute number of $CD4^+$ cells, $CD8^+CD3^+$ T cells, and $CD8^+CD3^-$ NK cells and the $CD8^+CD3^+$ T cell percentage were significantly decreased. The $SIV_{cpz\text{-}ant}$–infected animal had a normal level of $CD4^+$ cells, a decreased number of $CD8^+CD3^+$ T cells, and an increased number of $CD8^+CD3^-$ NK cells. A significant fluctuation of the $CD8^+CD3^-$ NK cell subset, parallel to but more pronounced than that of the $CD8^+CD3^+$ T cell subset, was observed during follow-up.

Analysis of proliferative responses. Results of mitogen and recall antigen stimulation are shown in table 2. After 49 months of follow-up, the median stimulation index was similar in HIV-1–infected and uninfected animals but significantly higher in the $SIV_{cpz\text{-}ant}$–infected chimpanzee. The median proliferative responses induced by the recall antigens tetanus toxoid, varicella-zoster virus, and *C. albicans* were similar or higher in HIV-1–infected chimpanzees than in controls. In contrast, soluble varicella-zoster virus and tetanus toxoid antigens induced poor proliferative responses in the $SIV_{cpz\text{-}ant}$–infected animal, but this was also the case in some uninfected controls.

Additional in vitro stimulation experiments were done with recombinant HIV-1 p24 and with four HIV-1 and two SIV_{cpz} V3 peptides at 46 months of follow-up. None of the stimuli

Table 1. Hematologic and phenotypic comparisons between $SIV_{cpz\text{-}ant}$–infected and uninfected chimpanzees and between human immunodeficiency virus type 1 (HIV-1)–infected and uninfected chimpanzees after 49 months of follow-up.

Parameter	Uninfected ($n = 5$)	HIV-1–infected ($n = 3$)	$SIV_{cpz\text{-}ant}$–infected ($n = 1$)	P (HIV)	P (SIV)
White blood cells	9.9 (4.1–20)	7.1 (3.6–11)	8.4 (3.8–14)	<.001	NS
Lymphocytes	4.5 (1.1–10)	2.1 (0.97–4.8)	3.7 (1.8–8.7)	<.001	NS
Thrombocytes	200 (139–489)	198 (133–334)	14 (9–85)	NS	<.001
$CD4^+CD3^+$ T cell %	34 (24–51)	38 (24–51)	33 (23–51)	.013	NS
$CD4^+CD3^+$ T cell count	1.3 (0.44–3.8)	0.84 (0.45–1.4)	1.4 (0.84–2.0)	<.001	NS
$CD8^+CD3^+$ T cell %	41 (20–65)	30 (16–41)	20 (12–28)	<.001	<.001
$CD8^+CD3^+$ T cell count	2.1 (0.24–4.8)	0.64 (0.24–1.9)	0.66 (0.21–2.3)	<.001	.0011
$CD8^+CD3^-$ NK cell %	17 (9–37)	20 (11–37)	29 (10–35)	.013	.0013
$CD8^+CD3^-$ NK cell count	0.74 (0.11–1.6)	0.41 (0.18–1.2)	1.1 (0.26–2.8)	<.001	.09 (NS)

NOTE. Data are median (minimum–maximum); cell counts are $\times 10^9$/L. Differences were tested by nonparametric Mann-Whitney U test. NS, not significant.

induced a significant proliferative response, neither in the $SIV_{cpz\text{-}ant}$– nor in the HIV-1–infected animals.

Cytolytic activity. Median NK cell and redirected CTL activity measured during follow-up are shown in table 2. Median NK activity was similar in uninfected and HIV-1–infected animals and significantly higher in the $SIV_{cpz\text{-}ant}$–infected chimpanzee. However, at month 11, NK activity was very low in this animal, but this was also the case for the relative and absolute number of NK cells (figure 2). Redirected CTL activity was present in all animals but at a significantly lower level in HIV-1–infected than in the uninfected animals and the $SIV_{cpz\text{-}ant}$–infected chimpanzee.

At 49 months of follow-up, we investigated whether fresh PBMC were able to lyse $SIV_{cpz\text{-}ant}$–infected lymphoblasts. These lymphoblasts had been cultured for 14 days and were actively producing virus, since viral antigens were detected in the culture supernatant. Results of this experiment are shown in table 3. Autologous effector cells lysed 15% of the target cells at an effector-to-target ratio of 40:1. The addition of anti–MHC class I and class II MAbs reduced but did not prevent lysis. Allogeneic effector cells from 1 of 2 seronegative chimpanzees lysed the infected target cells as efficiently as did the autologous effector cells.

Virus isolation from plasma and PBMC. Virus titers were measured in PBMC and in plasma of peripheral blood. Virus could not be routinely isolated from plasma or from PBMC from HIV-1–infected chimpanzees with standard virus isolation methods. In contrast, virus was always isolated from plasma or PBMC (or both) of the $SIV_{cpz\text{-}ant}$–infected chimpanzee at each time point, although titers were extremely low at month 8. After this time interval, the absolute number of NK cells and NK activity dropped to their lowest value measured during this study. Virus could be isolated from PBMC with relatively stable titers. A remarkably high plasma virus titer (>500 TCID/mL of plasma) was observed after 11 months of follow-up when NK activity and the absolute number of NK cells and $CD8^+$ T cells had reached their lowest values. During the months that followed, a marked drop in the number of platelets in peripheral blood was observed together with a 2- to 3-fold increase in number of lymphocytes, a 3-fold increase in number of $CD8^+$ T cells, and a 6-fold increase in number of NK cells.

Suppression of viral replication. Suppression of viral replication by $CD8^+$ lymphocytes was measured after 30, 33, and 38 months of follow-up (figure 3). Viral antigen could not be detected in any of the culture supernatants of PBMC or CD8-depleted PBMC of the HIV-1–infected animals during 24 days of culture. In contrast, viral antigen was clearly detectable in the culture supernatant of unfractionated stimulated PBMC of the $SIV_{cpz\text{-}ant}$–infected animal at 30 and 38 months but not at 33 months. Cultures of CD8-depleted PBMC and CD8-reconstituted PBMC tended to become positive more rapidly than unfractionated cells, in contrast to PBMC to which the anti-CD8 MAb MT122 was added.

At 33 months of follow-up, viral antigen could be detected only when PBMC were first depleted of $CD8^+$ cells. Furthermore, as shown in figure 3B, virus production could be demonstrated only after depletion of $CD8^+CD3^+$ T cells but not after depletion of $CD8^+CD3^-$ NK cells.

Discussion

To date, chimpanzees experimentally infected with HIV-1 have not developed clinical evidence of progression to AIDS. Our findings add new data and further substantiate previous observations that the immunologic abnormalities detected in HIV-infected humans are not observed in experimentally HIV-infected chimpanzees [4, 22–25]. An opportunity to gain further insight into the unique biology of HIV-1 infection in chimpanzees arose with the identification of a wild-caught chimpanzee with a naturally occurring lentivirus infection, the isolate of which is a divergent member of the HIV-1 subfamily of primate lentiviruses.

Comparison of lymphocyte subsets and various cell-mediated immune parameters revealed several unique differences between HIV-1 infection in chimpanzees and humans. In HIV-1–infected humans, in vitro T cell proliferative responses to

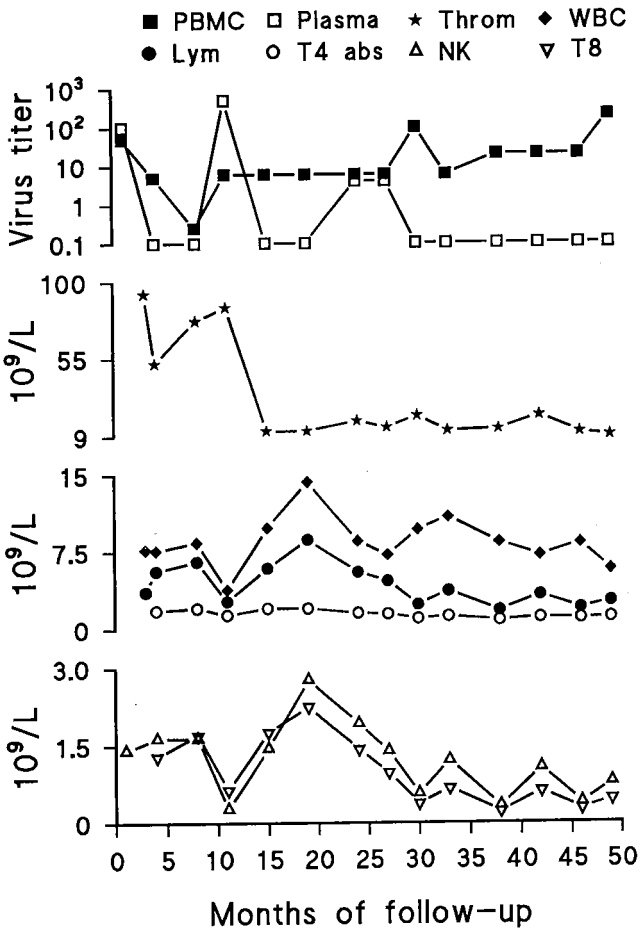

Figure 1. Forty-nine months of follow-up of hematology results, lymphocyte subset measurements, and virus titers in peripheral blood of naturally $SIV_{cpz\text{-}ant}$–infected chimpanzee. PBMC, virus titer in peripheral blood mononuclear cells; Plasma, virus titer in plasma; Throm, thrombocytes; WBC, white blood cell count; Lym, lymphocyte count; T4 abs, absolute $CD4^+$ T cell count; NK, absolute count of $CD8^+CD3^-$ NK cells; T8, absolute $CD8^+CD3^+$ T cell count. Corresponding results for 5 human immunodeficiency virus (HIV)–seronegative and 3 HIV-1–seropositive chimpanzees are given in table 1.

recall antigens and anti-CD3 are often impaired and generally precede the loss of proliferative responses to mitogens [26, 27]. During 49 months of follow-up, these animals had normal levels of $CD4^+$ cells and normal in vitro responses to mitogenic and antigenic stimuli. The lack of a proliferative response to recombinant HIV-1 p24 in the 3 HIV-1–infected chimpanzees was not unexpected, since these animals have been shown to have a dormant HIV-1 infection resulting in a decline or absence of the humoral (data not shown) and cellular immune responses. The lack of proliferative responses to recombinant HIV-1 p24 and V3 peptides in the $SIV_{cpz\text{-}ant}$–infected animal is more puzzling and probably indicates a lack of cross-reactive T cell epitopes rather than a loss of reactivity. However, natural infection with $SIV_{cpz\text{-}ant}$ was characterized by extracellular viremia, fluctuation of NK cell levels, and persistent thrombocytopenia. In this animal (Ch-No), absolute $CD4^+$ T cell counts consistently fluctuated within the normal range over time. In contrast, significant changes in the percentage and absolute number of $CD8^+CD3^-$ NK cells were seen. A similar but less pronounced trend was obtained for the absolute number of $CD8^+CD3^+$ T cells.

In humans, $CD8^+$ T cells play an important role in the immune response to HIV [28] either by their direct cytolytic activity [29] or by their capacity to suppress HIV replication by a noncytotoxic mechanism [12]. Similar to findings in humans, suppression of HIV replication by $CD8^+$ cells has been demonstrated in experimentally HIV-1–infected chimpanzees [8, 11–13]. Studies by Castro et al. [11] demonstrated that $CD8^+$ lymphocytes, even when obtained from uninfected chimpanzees, were able to suppress HIV replication in vitro by a noncy-

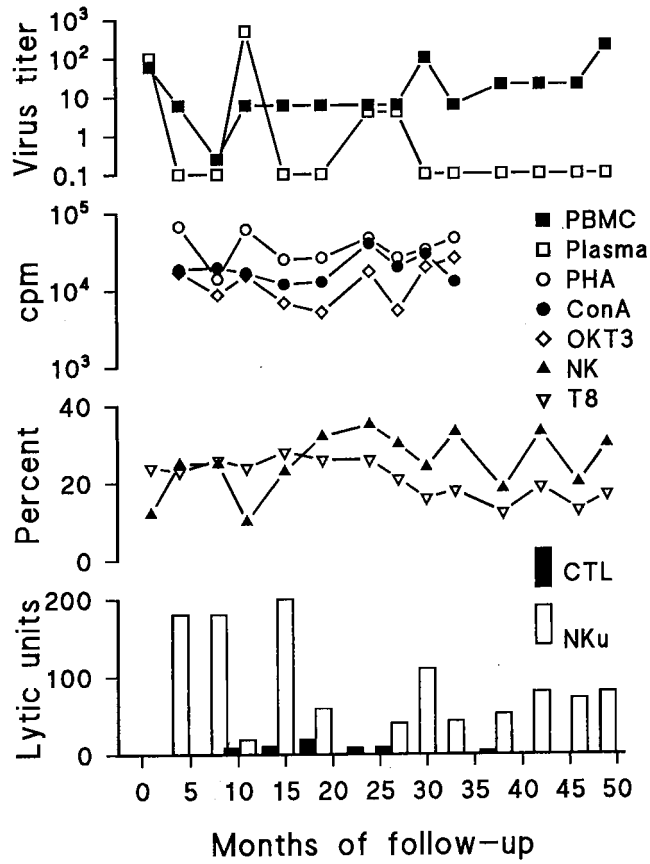

Figure 2. Forty-nine months of follow-up of virus titers, lymphocyte subsets, and parameters of cellular immunity in naturally $SIV_{cpz\text{-}ant}$–infected chimpanzee. PBMC, virus titer in peripheral blood mononuclear cells; Plasma, virus titer in plasma; PHA, proliferative response to phytohemagglutinin; ConA, proliferative response to concanavalin A; OKT3, proliferative response to anti-CD3 MAb; NK, percentage of $CD8^+CD3^-$ natural killer cells; T8, percentage of $CD8^+CD3^+$ T cells; CTL, redirected cytolytic T cell activity (not available before 7 months, at 30 or 33, and after 36 months); NKu, NK activity (not available at 1 and 24 months). Corresponding results for 5 human immunodeficiency virus (HIV)–seronegative and 3 HIV-1–seropositive chimpanzees are given in tables 1 and 2.

Table 2. Comparison of lymphocyte proliferation and cytolytic activity between $SIV_{cpz\text{-}ant}$–infected and uninfected chimpanzees and between human immunodeficiency virus type 1 (HIV-1)–infected and uninfected chimpanzees after 49 months of follow-up.

Parameter	Uninfected (n = 5)	HIV-1–infected (n = 3)	$SIV_{cpz\text{-}ant}$–infected (n = 1)	P (HIV)	P (SIV)
Lymphocyte blast transformation (cpm × 10^{-3})					
Mitogens					
Phytohemagglutinin	11 (3.5–111)	8.3 (4.4–48)	33 (14–67)	NS	<.001
Concanavalin A	6.7 (2.1–33)	5.4 (2.0–14)	19 (12–40)	NS	<.001
Anti-CD3	5.4 (0.9–62)	4.8 (1.9–16)	16 (5.1–25)	NS	.02
Recall antigens					
Candida albicans	10 (1.3–41)	19 (2.5–32)	8.2 (7.3–17)	NS	NS
Varicella-zoster virus	5.6 (0.9–54)	18 (8–34)	1.9 (0.5–4.3)	NS	.019
Tetanus toxoid	1.8 (0.1–19)	9.8 (4.5–23)	1.1 (0.2–3.7)	.0048	NS
Cytolytic activity (lytic units/10^6 cells)					
NK activity	48 (6–105)	32 (9–78)	76 (19–200)	.03	.011
Redirected cytotoxic T lymphocyte activity	11 (0–40)	7 (0–33)	10 (5–20)	.03	NS

NOTE. Data are median (minimum–maximum). Differences were tested by nonparametric Mann-Whitney U test. NS, not significant.

totoxic mechanism. We report that suppression of viral replication by $CD8^+$ lymphocytes, though not always effective, was also observed in the $SIV_{cpz\text{-}ant}$–infected chimpanzee. The successful suppressive activity exerted by $CD8^+$ cells and observed at month 33 coincided with an expansion of the absolute number of $CD8^+CD3^-$ NK cells and, to a lesser extent, of $CD8^+CD3^+$ T cells and with a drop in PBMC viremia, suggesting a possible role of $CD8^+CD3^+$ T cells and $CD8^+CD3^-$ NK cells in controlling $SIV_{cpz\text{-}ant}$ replication. However, the observation that the suppressive activity demonstrated in vitro was exerted by $CD8^+CD3^+$ T cells and $CD8^+CD3^-$ NK cells argues against this assumption. Unfortunately, data on suppressive activity were not obtainable at earlier time points at months 11 and 19, which may have further substantiated this observation. The reason the CD8-reconstituted PBMC tended to become positive more rapidly than untreated PBMC is unclear, but the successive manipulations might have affected the $CD8^+$ cells, resulting in a partial loss of their suppressive activity. Indeed, the addition of anti-CD8 MAb (MT122) to unfractionated PBMC of HIV-1–infected humans has been shown to abolish the suppressive activity of human $CD8^+$ cells in vitro [21]. However, we were not able to demonstrate a similar effect of this antibody on PBMC of the HIV-1– or $SIV_{cpz\text{-}ant}$–infected chimpanzees. Since MT122 was shown to bind to chimpanzee $CD8^+$ cells (data not shown), the impact of the binding of this MAb to $CD8^+$ cells on viral replication in vitro seems to differ in chimpanzees and humans.

Table 3. Anti-$SIV_{cpz\text{-}ant}$ cytolytic activity in fresh autologous and allogeneic peripheral blood mononuclear cells to cultured $SIV_{cpz\text{-}ant}$–infected lymphoblasts.

Donor	$SIV_{cpz\text{-}ant}$ status	% ^{51}Cr release at effector-to-target ratio 40:1	20:1	10:1
Ch-No	Positive	15	5	4
+ anti–MHC-I		7	6	0
+ anti–MHC-II		10	0	0
Ch-Ni	Negative	15	9	9
Ch-Ha	Negative	6	4	2

NOTE. Anti–MHC-I and –MHC-II, monoclonal anti–major histocompatibility complex class I (W6/32) and class II (9.3F10).

The cytolytic potential of T cells in chimpanzees was assessed in an anti-CD3–redirected CTL assay and found to be similar in infected and uninfected chimpanzees. This activity is very low in seronegative humans and increases significantly in a disease-related fashion after infection with HIV-1 [20]. Interestingly, fresh unstimulated T cells from uninfected chimpanzees, unlike normal human T cells, displayed relatively high levels of redirected CTL activity. The significance of this observation regarding the different pathogenicity of HIV-1 in chimpanzees and humans remains to be further elucidated. $SIV_{cpz\text{-}ant}$–specific CTL activity was assessed in a single experiment using $SIV_{cpz\text{-}ant}$–infected lymphoblasts as target cells. A low-degree lysis of infected target cells was observed, but there is no indication that this lysis was MHC-restricted. On the contrary, the addition of anti–MHC class I and class II antibodies could not inhibit the lysis. In addition, lymphocytes from 2 uninfected chimpanzees lysed the infected target cells as well and point to nonspecific lysis.

In HIV-1–infected humans, NK activity diminishes as the infection progresses to AIDS [30]. In the $SIV_{cpz\text{-}ant}$– and HIV-1–infected animals, NK activity was at similar levels to unin-

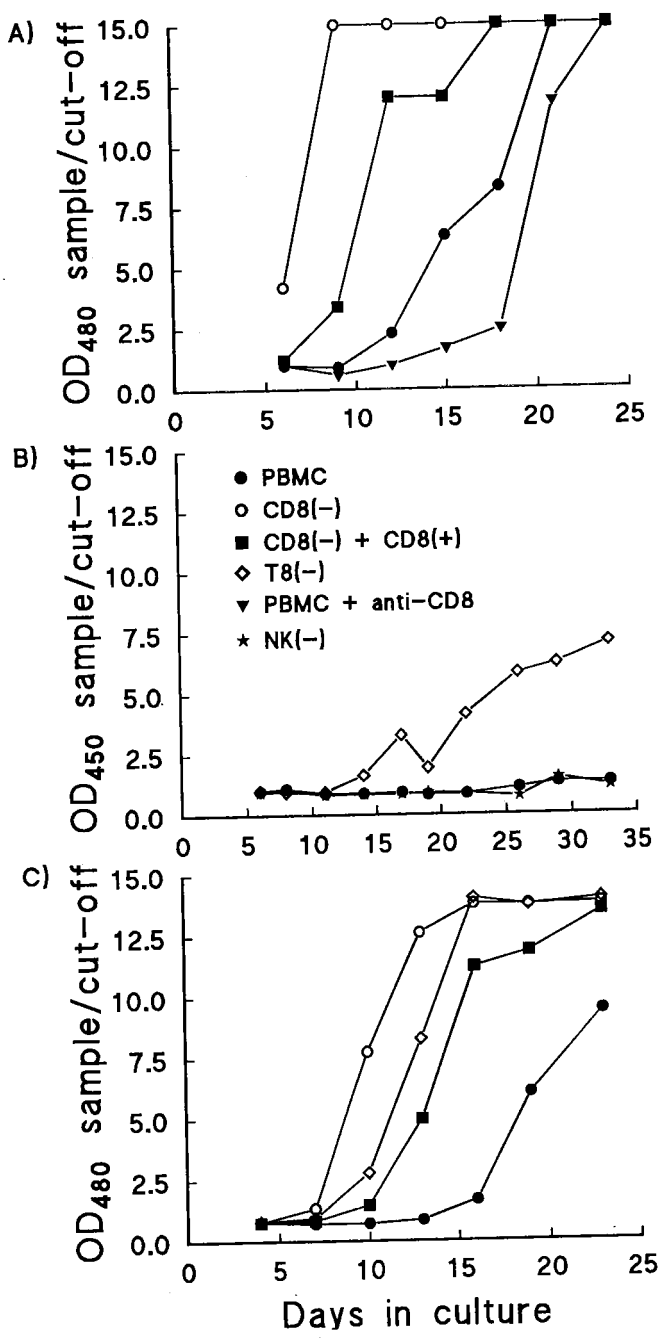

Figure 3. In vitro suppressive activity on virus production in stimulated lymphocyte cultures of $SIV_{cpz\text{-}ant}$–infected chimpanzee at 30 (**A**), 33 (**B**), and 38 (**C**) months of follow-up. PBMC, unfractionated peripheral blood mononuclear cells; $CD8(^-)$, PBMC depleted of $CD8^+$ cells; $CD8(^-)$ + $CD8(^+)$, CD8-reconstituted PBMC; $T8(^-)$, PBMC depleted of $CD8^+CD3^+$ T cells; PBMC + anti-CD8, PBMC cocultured with anti-CD8 MAb (MT122); $NK(^-)$, PBMC depleted of $CD8^+CD3^-$ cells. OD_{480}, optical density at 480 nm.

fected controls, confirming observations by others [22]. Although elimination of $CD8^+CD3^-$ NK cells in two experiments did not result in increased viral growth, it is intriguing that the lowest number of NK cells and the lowest NK activity were also found at the time of a pronounced plasma viremia in this animal. The number of NK cells increased dramatically thereafter, whereas the viremia decreased. Therefore, it is still not excluded that NK cells could have a role in regulating viral replication in naturally lentivirus-infected chimpanzees. The NK activity measured in chimpanzees was always much higher than the NK activity we have measured in humans (data not shown). Even at the lowest effector-to-target ratios, significant target lysis was observed in normal and infected chimpanzees. However, we share the concern of others that the K562 cell line may not represent the most appropriate target for chimpanzee NK effector cells [22] and, hence, it is too premature to relate the different pathogenicity of HIV-1 in infected humans and chimpanzees to differences in NK activity in both species.

In conclusion, assessment and comparison of the cellular immune status of the naturally $SIV_{cpz\text{-}ant}$–infected chimpanzee with that of experimentally HIV-1–infected chimpanzees did not reveal evidence of immune deficiency suggestive of overt pathogenic effect in this species. The only clear response to the fluctuation in viral replication seems to be the marked expansion of the absolute number of $CD8^+CD3^-$ NK and $CD8^+CD3^+$ T cells. Whether or not NK cells play an important role in preventing HIV-1 and $SIV_{cpz\text{-}ant}$ from becoming pathogenic in chimpanzees remains to be elucidated. The lack of pathogenicity of HIV-1 and $SIV_{cpz\text{-}ant}$ in chimpanzees is probably multifactorial but may include the persistence of potent NK activity.

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