T-Cell Responses to Human Immunodeficiency Virus (HIV) and Its Recombinant Antigens in HIV-Infected Chimpanzees

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Peripheral blood lymphocytes from chimpanzees infected for 3 months to more than 3 years with human immunodeficiency virus (HIV) had normal T-cell proliferative responses after stimulation with a variety of recall antigens and mitogens, indicating that HIV infection does not cause detectable immunological impairment in chimpanzees. This finding contrasts with that obtained in HIV-infected humans, who often have impaired T-cell reactivity. Peripheral blood lymphocytes from most HIV-infected chimpanzees that were studied also had strong proliferative responses to purified HIV as well as to HIV envelope glycoproteins isolated from the virus, to recombinant HIV envelope glycoproteins gp120 and gp41, and to HIV *gag* protein p24. The HIV-specific T-cell responses in HIV-infected chimpanzees may contribute to prevention of the development of acquired immunodeficiency syndrome in this species.

The human immunodeficiency virus (HIV), isolates of which include human T-cell lymphotropic virus type III, lymphadenopathy-associated virus, and acquired immunodeficiency syndrome (AIDS)-associated retrovirus, is the etiologic agent of AIDS (2, 10, 16). AIDS is a disease in humans that is associated with the loss of T helper cells, impaired immunological functions, lymphadenopathy, wasting syndrome, opportunistic infections, and malignancies (11-13, 17). Since our first report (1) describing the chimpanzee as a model in AIDS research, several other investigators (7-9) have also demonstrated the exquisite susceptibility of this animal species to infection with HIV. However, to date, no chimpanzees have developed AIDS, and only an occasional animal has been found to develop lymphadenopathy within 32 weeks after HIV infection and concomitant significant decrease in T helper cells and mitogen responsiveness (1, 5, 6). The immune mechanisms that may play a role in preventing AIDS are not clear. Virus-specific T-cell-mediated immunity, which has been found to be absent or weak in asymptomatic HIV-infected persons and in patients with AIDS (19; Zarling et al., manuscript in preparation), is important in resistance to disease caused by enveloped viruses (14, 22) and may also play a role in preventing the progression from HIV infection to AIDS in chimpanzees.

We previously reported that immunization of macaques and chimpanzees with a recombinant vaccinia virus expressing HIV envelope glycoproteins gp120 and gp41 elicits HIV-specific T-cell responses (20, 21). However, it had not been ascertained whether infection with HIV induces an HIV-specific T-cell response in chimpanzees and whether T-cell responses to other antigens are normal or impaired in these HIV-infected animals. We report here that HIV-infected chimpanzees have normal lymphocyte proliferative responses to xenogeneic lymphocytes in mixed leukocyte cultures; to recall antigens, including herpes simplex virus and cytomegalovirus; to the T-cell mitogen phytohemag-

glutinin and concanavalin A; and to the B-cell mitogen pokeweed mitogen. Most important, we demonstrated that peripheral blood lymphocytes (PBL) from HIV-infected chimpanzees proliferate in vitro in response to stimulation with purified live and inactivated HIV, lentil-lectin purified envelope glycoprotein (env) extracted from HIV, and recombinant HIV antigens rgp120, rgp41, and rp24.

MATERIALS AND METHODS

Chimpanzees. A total of 18 chimpanzees (*Pan troglodytes*) was included in this study. The sex distribution was 13 males and 5 females ranging from 6 to 14 years of age. Most of the animals had antibodies to hepatitis B virus and/or were hepatitis B or non-A, non-B carriers. When this study was done, the time post-HIV infection ranged from 3 months (animal X110) to 6 months (animals X64 and X134) to 3.5 years (animal X114). None of the animals after HIV inoculation had displayed any signs of disease, abnormal lymphocyte subsets, or mitogen responsiveness except chimpanzee X114, which had transient lymphadenopathy approximately 3 years before this study (1, 5, 6). The experimental chimpanzees (X110, X115, X123, X64, and X134) had been inoculated one time or repeatedly with the HIV-infected human plasma or with the HIV strains human lymphotropic virus type III, AIDS-associated retrovirus type 2, or lymphadenopathy-associated virus type 1. That the animals had been infected with HIV was confirmed by demonstrating persistent HIV seropositivity by enzyme immunoassays, indirect immunofluorescence, Western blots (immunoblots), and, in most chimpanzees, isolation of HIV from their lymphocytes.

Proliferative responses of chimpanzee PBL. PBL from chimpanzees were isolated by Ficoll-Hypaque gradient centrifugation and washed three times with phosphate-buffered saline. PBL (1×10^5 or 2×10^5) were cultured in 96-well plates containing RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with 10% heat-inactivated autologous serum or pooled serum from HIV-

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seronegative humans. The cells were cultured alone or with the following mitogens or antigens: pokeweed mitogen (10 µg/ml), UV-inactivated herpes simplex virus type 1 (KOS strain; 10⁶ PFU/ml before UV inactivation), UV-inactivated cytomegalovirus (AD169 strain; a 1:128 dilution which was found optimal in preliminary proliferation assays), sucrose gradient-purified intact live HIV (5 µg of protein per ml) grown in human lymphocyte antigen class II antigen-negative CEM cells or HIV (5 µg/ml) inactivated by treatment with psoralen and UV light, lentil-lectin column-purified envelope glycoproteins isolated from purified HIV (env, 1 µg/ml) (21), recombinant gp120 (5 µg/ml) expressed in Chinese hamster ovary cells (15), recombinant gp41 (1 µg/ml) (4), and p24 (1 µg/ml) (3) expressed in *Escherichia coli* (3, 4). As a negative control antigen we used a fusion protein (1 µg/ml) consisting of Trp LE *E. coli* protein fused to a peptide from the VP3 region of foot-and-mouth disease virus. The lentil-lectin purified env was found by silver-stained gels to be more than 90% pure (21), and the recombinant proteins gp120, gp41, and p24 were 99, 85, and 85% pure, respectively, as determined by sodium dodecyl sulfate gel electrophoresis, Coomassie blue staining, and Western blotting. To measure T-cell proliferative responses to xenogeneic lymphocytes in mixed leukocyte cultures, 1 × 10⁵ PBL were stimulated with 2 × 10⁵ X-irradiated (2,500 R) pooled human lymphocytes. Four to 6 days later, 1 µCi of [³H]thymidine ([³H]TdR; New England Nuclear Corp., Boston, Mass.) was added to each of three or four replicate wells. After 6 to 18 h of incubation, cells were harvested with a multiple-well harvesting apparatus, and [³H]TdR incorporation was determined by liquid scintillation counting. The stimulation index (SI) was calculated by dividing the mean counts per minute of [³H]TdR incorporated into cells containing antigen by the mean counts per minute of [³H]TdR incorporated into cells in wells without antigen.

Western blot analysis. Sera from HIV-infected and control chimpanzees were analyzed with a commercial kit (Bio-Rad Laboratories, Richmond, Calif.). HIV was disrupted and inactivated before undergoing sodium dodecyl sulfate-polyacrylamide electrophoresis and was transferred to nitrocellulose strips. The serum samples were diluted 1:100 with the wash-diluent solution and were incubated with the strip for 30 min. Unbound material was aspirated, and the strip was washed. An enzyme-linked antibody to human im-

munoglobulin G was incubated with the strip for 30 min. The unbound conjugate was removed, the strip was washed, and a substrate solution was allowed to react for 30 min. The reaction was terminated by aspirating the substrate and washing the strip. The appearance of purple bands indicated a positive reaction.

RESULTS AND DISCUSSION

Results of the first experiment (Table 1) indicate that PBL from chimpanzees infected with HIV for 6 months to more than 3 years proliferated within the same range as did PBL from uninfected chimpanzees in response to the recall antigens herpes simplex virus and particularly cytomegalovirus, to which most chimpanzees are seropositive (Eichberg, unpublished data). The PBL of the infected chimpanzees also had normal proliferative responses to X-irradiated human PBL in mixed leukocyte cultures and to the B-cell mitogen pokeweed mitogen. Also, the PBL had normal T-cell proliferative responses to the T-cell mitogens concanavalin A and phytohemagglutinin (data not shown). In contrast to the situation with HIV-infected humans, who often have impaired lymphocyte proliferative responses to the T-cell mitogens and a variety of antigens or mitogens (12, 17, 18), no indication was obtained from this study for impaired responses to several antigens or mitogens in HIV-infected chimpanzees.

To determine whether HIV infection of chimpanzees induces HIV-specific T-cell responses, we exposed PBL from the HIV-infected chimpanzees to HIV and HIV antigens and monitored the in vitro stimulation. PBL from all HIV-infected chimpanzees demonstrated positive proliferative responses (SI, ≥3) after exposure to live or inactivated HIV (lymphadenopathy-associated virus) with an SI ranging from 8.3 to 14.3 and 4.1 to 18.1, respectively (Table 1). PBL from the infected chimpanzees had SIs ranging from 4.6 to 19.3 after stimulation with lentil-lectin purified envelope glycoprotein (env) containing gp120 and gp41. By fluorescence-activated cell sorter analysis with monoclonal antibodies, it was found that 40 to 60% of HIV- or env-stimulated cells expressed the T-cell antigen CD4, and 40 to 60% expressed the T-cell antigen CD8. All the control animals had proliferative SIs of less than 3.0 after stimulation by both live and inactivated HIV and by the envelope

TABLE 1. Proliferative responses of PBL from HIV-infected and control chimpanzees to HIV and recall antigens^a

Animal no.	SI for:						
	Pokeweed mitogen	Human PBL	Herpes simplex virus	Cytomegalovirus	Live HIV	Inactivated HIV	HIV env
Infected chimpanzees	>8 in 7/7	>35 in 7/7	>3 in 3/7	>3 in 7/7	>3 in 6/6	>3 in 7/7	>3 in 7/7
X110	59.3	149.0	10.2	10.7	14.3	14.9	10.0
X114	37.2	74.6	2.4	5.0	8.3	10.5	7.9
X118	8.2	38.6	2.0	4.5	NT	4.1	10.1
X133	44.8	150.0	3.2	28.4	9.2	18.1	19.3
X139	15.3	40.9	1.9	20.4	10.0	17.7	7.5
X134	11.6	35.0	20.9	5.6	14.3	10.9	5.3
X64	15.9	65.0	2.7	10.6	8.7	6.4	4.6
Control chimpanzees	>8 in 4/4	>35 in 4/4	>3 in 2/4	>3 in 3/4	>3 in 0/4	>3 in 0/4	>3 in 0/4
X140	14.6	259.0	1.0	1.8	1.4	1.6	1.6
X174	53.5	72.3	8.2	10.8	1.8	2.7	NT
X175	49.5	52.6	8.1	6.6	1.7	2.9	1.9
X207	14.0	53.1	2.5	3.0	2.0	1.0	1.1

^a PBL were stimulated as described in Materials and Methods. Cells were labeled for 6 h with [³H]TdR before being harvested. SI was calculated as described in Materials and Methods. NT, Not tested.

glycoprotein env. Chimpanzee X110, when tested in this assay, had been infected for only approximately 3 months, and chimpanzees X134 and X64 had been infected for approximately 6 months before being tested, whereas the other animals had been infected for from 1 to 3.5 years previously. From these data, it is apparent that even after several years of infection with HIV, T-cell responses to HIV and to HIV envelope glycoproteins persist in HIV-infected chimpanzees.

We next examined the proliferative response to the recombinant envelope glycoprotein gp120 that was cloned and expressed in mammalian cells (15). Several concentrations of gp120 were tested, and the responses were measured on days 5, 6, and 7. With all chimpanzees tested, peak proliferative responses occurred with a final concentration of gp120 of 5 μ g/ml, but the peak proliferative response in the animals varied between days 5 and 7. The results shown in Table 2 are those from the day of peak proliferative response, with gp120 at 5 μ g/ml. The results from chimpanzees infected repeatedly with HIV-infected plasma or human lymphotropic virus type III derived from infected H9 cells indicate that PBL from these chimpanzees have SIs ranging from 2.5 to 13.3 (Table 2). In contrast to these results, the SIs of PBL from uninfected chimpanzees were 0.9 to 1.0. The results shown for chimpanzee X110 were obtained before infection of this animal with HIV. With six chimpanzees that were inoculated one to three times with the HIV isolate AIDS-associated retrovirus type 2, five had SIs of 3.8 or higher after exposure to gp120, whereas the SI of the control animal was 1.1 (Table 2).

Besides the T-cell response to gp120, we determined whether HIV-infected chimpanzees would also respond to recombinant gp41 and p24 expressed by transfected *E. coli* (3, 4). Also, we examined whether there would be a correlation between proliferative responses to various HIV antigens and the presence of antibodies to recombinant gp120, gp41, and p24 in chimpanzees, as determined by Western blots. All infected chimpanzees demonstrated strong antibody responses against the HIV *gag* (p24) antigen and both envelope glycoproteins (gp120 and gp160) and responded, although more weakly, to gp41 (Fig. 1). However, noticeably absent were the anti-gp120 and anti-gp160 responses in chimpanzee X110, who was infected 16 weeks before the results shown in Fig. 1 and Table 3 were obtained. When we assayed the PBL of these same animals for lymphoproliferation after stimulation with various HIV antigens, we found that chimpanzee X110 likewise lacked a proliferative re-

FIG. 1. Western blot analysis of sera from HIV-infected and control chimpanzees. Lanes 1 to 6 are for infected chimpanzees X110, X114, X118, X119, X133, and X139, respectively. Lane 7 is for control chimpanzee X140. KD, Kilodaltons.

sponse to gp120 but did respond to gp41 and p24 (Table 3). Four of the six infected chimpanzees demonstrated positive proliferative responses to gp120, five of the six responded to gp41, and five of the six responded to p24. PBL from infected and control chimpanzees did not respond proliferatively to foot-and-mouth disease virus, the negative control antigen (data not shown). Thus, the majority of the HIV-infected chimpanzees, whether inoculated once or multiply with HIV and whether infected for a few months or for more than 3 years, exhibited T-cell proliferative responses to HIV, to HIV envelope glycoproteins, and to the *gag* protein p24.

To our knowledge, this is the first demonstration of normal T-cell responses to a variety of antigens and mitogens and, most important, to HIV and HIV recombinant antigens by chimpanzees infected with human AIDS virus. Although HIV infects CD4⁺ lymphocytes in chimpanzees, as it does in humans, the PBL of HIV-infected chimpanzees, including

TABLE 2. Proliferative responses of PBL from HIV-infected and control chimpanzees to recombinant gp120^a

Kind of chimpanzee	HTLV-III ^b -infected			ARV ^c -infected		
	Animal no.	Response (cpm of [³ H]TdR [SI] to:		Animal no.	Response (cpm of [³ H]TdR [SI] to:	
		No antigen	rgp120		No antigen	rgp120
Infected	X114	675	8,961 (13.3)	X88	2,351	8,863 (3.8)
	X118	13,075	43,275 (3.3)	X97	592	7,457 (12.6)
	X119	2,392	5,875 (2.5)	X115	7,125	28,203 (4.0)
	X133	378	2,564 (6.8)	X117	7,720	11,892 (1.5)
	X139	8,893	48,121 (5.4)	X120	1,016	5,216 (5.1)
				X123	968	5,549 (5.7)
Control	X110	1,597	1,519 (1.0)			
	X140	2,167	2,017 (0.9)	X193	651	741 (1.1)

^a PBL were stimulated with 5 μ g of recombinant gp120 expressed in mammalian cells per ml as described in Materials and Methods. Cells were labeled for 18 h with [³H]TdR before being harvested.

^b HTLV-III, Human lymphotropic virus type III.

^c ARV, AIDS-associated retrovirus.

TABLE 3. Proliferative responses of PBL from HIV-infected and control chimpanzees to recombinant envelope (rgp120 and rgp41) and *gag* (rp24) proteins^a

Kind of chimpanzee	Animal no.	Response (cpm of [³ H]TdR [SI] to:			
		No antigen	rgp120	rgp41	rp24
Infected	X110	712	720 (1.0)	5,364 (7.5)	9,826 (13.8)
	X114	1,287	34,995 (27.2)	21,725 (16.9)	21,700 (16.9)
	X118	1,184	1,949 (1.6)	4,003 (3.4)	5,921 (5.0)
	X119	14,143	56,867 (4.0)	33,220 (2.3)	41,465 (2.9)
	X133	1,021	5,648 (5.5)	3,854 (3.8)	6,382 (6.2)
	X139	1,027	18,050 (17.6)	10,018 (9.8)	9,469 (9.2)
Control	X140	949	984 (1.0)	3,156 (3.3)	1,904 (2.0)
	X178	546	277 (0.5)	841 (1.5)	964 (1.8)

^a PBL were stimulated with 5 µg of rgp120 per ml or with 1 µg of rgp41 or rp24 per ml, as detailed in Materials and Methods. Cells were labeled for 18 h with [³H]TdR before being harvested.

some infected for over 3 years, appear to function normally in response to a variety of antigens and mitogens and to respond to intact HIV, to envelope glycoproteins gp120 and gp41, and to the *gag* protein p24. In contrast, in previous studies, humans infected with HIV for unknown periods were found to have impaired T-cell responses to various antigens and mitogens (12, 17, 18), and in our preliminary experiments and in those of others (19; Zarling et al., in preparation), only a minority of HIV-infected humans demonstrated a T-cell proliferative response to HIV. It is thus tempting to speculate that the T-cell responses to HIV and its antigens that we have detected in HIV-infected chimpanzees may contribute to the observed resistance to development of AIDS in this primate species.

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