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Persistent Infection of Chimpanzees with Human Immunodeficiency Virus: Serological Responses and Properties of Reisolated Viruses

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Persistent infection by human immunodeficiency virus (HIV-1) in the chimpanzee may be valuable for immunopathologic and potential vaccine evaluation. Two HIV strains, the tissue culture-derived human T-cell lymphotropic virus type IIIB (HTLV-IIIB) and in vivo serially passaged lymphadenopathy-associated virus type 1 (LAV-1), were injected intravenously into chimpanzees. Two animals received HTLV-IIIB as either virus-infected H9 cells or cell-free virus. A third animal received chimpanzee-passaged LAV-1. Evaluation of their sera for virus-specific serologic changes, including neutralizations, was done during a 2-year period. During this period all animals had persistently high titers of antibodies to viral core and envelope antigens. All three animals developed a progressively increasing type-specific neutralizing LAV-1 versus HTLV-IIIB antibody titer during the 2-year observation period which broadened in specificity to include HTLV-IIIRF, HTLV-IIIMN, and HTLV-IIICC after 6 to 12 months. The antibody titers against both viruses were still increasing by 2 years after experimental virus inoculation. Sera from all animals were capable of neutralizing both homologously and heterologously reisolated virus from chimpanzees. A slightly more rapid type-specific neutralizing response was noted for the animal receiving HTLV-IIIB-infected cells compared with that for cell-free HTLV-IIIB. Sera from all persistently infected chimpanzees were capable of mediating group-specific antibody-mediated complement-dependent cytolysis of HIV-infected cells derived from all isolates tested. Viruses reisolated from all three animals at 20 months after inoculation revealed very similar peptide maps of their respective envelope gp120s, as determined by two-dimensional chymotrypsin oligopeptide analysis. One peptide, however, from the original HTLV-IIIB-inoculated virus was deleted in viruses from all three animals, and in addition, we noted the appearance of a new or modified peptide which was common to LAV-1 as well as to HTLV-IIIB reisolated from infected chimpanzees. It thus appears that a group-specific neutralizing antibody response as well as a group-specific cytotoxic response can develop in chimpanzees after an inoculation of a single HIV variant. This finding suggests that a common, less immunodominant determinant(s) is present on a single HIV strain which could induce group-specific antibodies during viral infection and replication. The identification of this group-specific epitope and the induction of analogous immunity may be relevant to vaccine development against human acquired immunodeficiency syndrome.

The chimpanzee is readily infected with a number of isolates of human immunodeficiency virus (HIV-1) (1, 8, 10, 14). Infection occurs rapidly, as manifested by seroconversion and reisolation of HIV within 1 to 2 months after infection (1, 8, 10, 14). Although on rare occasion transient lymphadenopathy was noted, most animals remain clinically and immunologically normal (1). As with infected humans, an active immune response persists with time and a chronic state of infection is manifested by the ability to isolate the virus from peripheral blood lymphocytes (PBLs) from both species (14). Various inocula, such as infected human plasma, tissue-culture derived virus, or virus-infected cells, have all been described as having infectious potential (1, 8, 9, 14). HIV-1 could also be serially passed from chimpanzee to chimpanzee (10).

The condition of persistent infection without clinical manifestation of acquired immunodeficiency syndrome (AIDS) in this animal model can be used to assess both the nature of the virus in the animal as well as to define immunological host responses which do not result in disease. HIVs have been described as a number of variable isolates in terms of nucleotide sequence and as highly mutable under field conditions (5). Whether significant change occurred in the virus after infection of chimpanzees with molecularly defined HIVs can now be assessed in an animal model. Members of human high-risk groups could be theoretically readily reinfected on repeated exposure by alternate virus types so that the final immune response may not represent an outcome of a single, infectious event. In experimental animal models, inoculation of a single human T-cell lymphotropic virus type IIIB (HTLV-IIIB) gp120 led to a type-specific neutralizing antibody. However, broadly specific neutralizing antibody has been described in infected humans (21, 22).

Although it can be inferred that the spectrum of group-specific neutralizing antibody resulted from one or, at most, a limited number of HIV strains, the controlled nature of the chimpanzee infection could definitely determine whether a single HIV type gives rise to a group-specific neutralizing response. Further immunological differences between HIV-infected humans and inoculated animals have recently been described in terms of complement-mediated cytotoxic antibody for HIV-infected cells which can be induced in animal models but which appears to be absent in HIV-infected humans (15). A detailed examination of humoral and cellular

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immune responses in the chimpanzee may be important in attempts to replicate a state of protection in humans by various vaccine preparations. The present studies now examine in HIV-infected chimpanzees the increase of group-specific neutralizing antibody and the appearance of antibody-mediated complement-dependent cytotoxicity (ACC) as well as define some structural and serological properties of the reisolated viruses in comparison with input HIV inocula.

MATERIALS AND METHODS

Animals and viral challenge. Details of the virus and methods of challenge have been previously described as part of a larger study (10). In the present study, one chimpanzee (A3E/525) received 30 ml of approximately 10^4 infectious units of HTLV-IIIB per ml. Another animal (A251/526) received 30 ml of HTLV-IIIB-infected cells (7.7×10^5 cells per ml). Last, one animal (A243A/524) received 100 ml of whole blood from another animal (A3A) that had been previously infected with lymphadenopathy-associated virus type 1 (LAV-1) (10). All animals received their inoculums intravenously.

Viruses. HTLV-IIIB and the more distantly related Haitian isolate HTLV-IIIRF (RF_{II}), United States isolate HTLV-IIIMN (MN), and French isolate HTLV-IIICC (CC) are maintained in this laboratory as persistently infected H9 cells. Titers of infectious viruses range from 3.0×10^3 to 2.5×10^4 syncytium-forming units (SFU) for HTLV-IIIB and RF_{II}, respectively, when grown in H9 cells. Comparable and somewhat higher titers were obtained by a p24 radioimmunoassay (RIA) analysis assessing antigen induction at limiting dilutions. The chronically infected H9 cells are passed biweekly and supplemented with fresh H9 cells once a week. The LAV-1 used in the inoculation of animal 524 was from a whole blood transfusion derived from a previous animal in transfer studies described earlier (10). The individual virus isolates and their cell lines are designated HTLV-IIIB/H9, RF_{II}/H9, MN/H9, CC/H9, 524/H9, 525/H9, and 526/H9, with the latter three being viruses reisolated from the chimpanzees.

Virus isolation. Reisolation of virus from persistently infected chimpanzees was accomplished at approximately 20 months after initial inoculation. Methods of isolation have been previously described (9). Briefly, Ficoll-Hypaque (lymphocyte separation medium; Bionetics Research, Inc.) gradient PBLs were stimulated in vitro with phytohemagglutinin P (1 μg/ml) for 3 days, after which T-cell growth factor (10% vol/vol) (Biotech Research Laboratories) was substituted for continued blastogenic activity. Cultures were monitored by p24 RIA analysis every week. Cocultivations were done in fresh, logarithmically grown H9 cells at a ratio of 1:1. Due to the potential of this method for isolating primate foamy viruses, supernatants from positive cultures were tested on a human glial cell line (808D) for cytopathic effects (14). PBL cultures from other normal chimpanzees were included as controls.

Radioimmune precipitation assays. As previously described, the purified proteins were radiolabeled with ^{125}I by using chloramine T (L. O. Arthur, S. W. Pyle, P. L. Nara, J. W. Bess, Jr., M. A. Gonda, J. C. Kelliher, R. V. Gilden, W. G. Robey, D. P. Bolognesi, R. C. Gallo, and P. J. Fischinger, Proc. Natl. Acad. Sci. USA, in press). Antibodies to p24 and gp120 were detected by incubating 10,000 cpm of radiolabeled antigen with 50 μl of serum diluted in RIA buffer (0.05 M Tris [pH 7.8], 0.15 M NaCl, 0.4% Triton X-100, 0.5% bovine serum albumin, 300 mg of phenylmethylsulfonyl fluoride per liter, 0.1% sodium azide) for 2 h at 37°C and overnight at 4°C. *Staphylococcus aureus* was added to each tube to facilitate precipitation of the antigen-antibody complexes. Incubation continued at room temperature, and pellets were collected at 800 × *g* for 15 min. The supernatant was discarded, and the pellets were washed by suspension in 500 μl of 0.01 M Tris hydrochloride, (pH 7.8)–0.1 M NaCl–1.0 mM EDTA–0.1% Triton X-100 and centrifuged at 1,500 × *g* for 30 min. Radioactivity remaining in the pellet was determined with a gamma counter.

Competitive RIAs. Titers were determined for the antiserum by using ^{125}I-labeled antigen, and the dilution precipitating 50% of the radiolabeled antigen was selected for the assay. Unlabeled antigen was diluted twofold in RIA buffer and incubated with the proper antiserum for 1 h at 37°C. Approximately 10,000 cpm of radiolabeled antigen was added, and incubation was continued for 1 h at 37°C and then at 4°C overnight. *S. aureus* was then added to each tube to facilitate precipitation of immune complexes, and the tubes were centrifuged at 1,500 × *g* for 30 min. The pellet was washed once with RIA buffer and recentrifuged as above. Radioactivity in the pellet was determined with a gamma counter. Unlabeled antigens included lysed retroviruses and sham virus antigen controls prepared from uninfected H9 cells. The control H9 antigen was prepared from uninfected H9 cells by being double banded in sucrose gradients and pelleted in exactly the same manner that HTLV-IIIB was purified from HTLV-IIIB-infected H9 cells. Virus was prepared by incubating sucrose-gradient purified viruses for 1 h in 0.01 M Tris hydrochloride (pH 7.8)–0.1 M NaCl–0.001 M EDTA–0.1% Triton X-100–0.05% sodium deoxycholate. Enzyme-linked immunosorbent assay determinations of the serum were evaluated for antiviral antibodies by standard techniques as described for use in a commercial test kit (Electro-Nucleonics, Inc., Columbia, Md.). Positive values were scored when absorbance (A_{492}) was greater than 0.30.

Comparative oligo-chymotryptic maps of viral envelopes. Envelope gene polypeptides of HTLV-III and LAV-1 were evaluated by two-dimensional chymotryptic oligopeptide mapping as previously described (17). Initial input HIVs and reisolated viruses were grown in the identical H9 cell line to compare their gp120 maps, since cell lines do have a slight effect on peptide patterns of a single isolate (W. G. Robey et al., submitted for publication).

ACC assay. Logarithmically growing target cells were labeled with ^{51}Cr and immobilized in poly(L-lysine)-coated (500 g/ml) microtiter plate wells (2×10^5 cells per well). The medium was then removed, and appropriately diluted antiserum was incubated for 1 h at 37°C. The optimal titer was determined to be 1:8 for rabbit complement (Low Tox H, Cedar Lane, Westbury, N.Y.; recommended for human lymphoid cells), and the complement was incubated with antibody-pretreated target cells for 120 min at 37°C. Serum was considered positive for ACC when the percent specific ^{51}Cr release was two times greater than the preimmune values.

Neutralization assay. Neutralizing antibody was determined by a microtiter syncytium-forming assay utilizing a sensitive clone of CEM-SS cells (P. L. Nara, W. C. Hatch, N. M. Dunlop, W. G. Robey, L. O. Arthur, M. A. Gonda, and P. J. Fischinger, AIDS Res. Hum. Retroviruses, in press). Briefly, 50,000 cells were attached to poly(L-lysine)-coated microtiter wells. Cells were then washed and inoculated with either preincubated HTLV-IIIB virus stock (200 to 300 SFU) per serum mixture (serum was diluted in

TABLE 1. Comparative serologic evaluation of persistently infected HTLV-IIIB- and LAV-1-infected chimpanzees

Animal no. (virus)	Date of sample	Antibody titer[a] to:			ELISA	Virus isolation
		p24	p121	gp120		
A243A/524 (LAV-1)	Preinfection	Neg	Neg	Neg	Neg	Neg
	1 mo	250	50	250	+	+
	2 mo	>6,250	250	>6,250	+	+
	6 mo	>6,250	250	>6,250	+	NT[b]
	1 yr	>6,250	250	>6,250	+	NT
	2 yr	>6,250	250	>6,250	+	+
A3E/525 (HTLV-IIIB)	Preinfection	Neg	Neg	Neg	Neg	Neg
	1 mo	50	Neg	Neg	Neg	+
	2 mo	>6,250	50	>6,250	+	+
	4 mo	>6,250	250	>6,250	+	NT
	1 yr	>6,250	250	>6,250	+	NT
	2 yr	>6,250	50	>6,250	+	+
A251/526 (HTLV-IIIB)	Preinfection	Neg	Neg	Neg	Neg	Neg
	1 mo	250	50	250	+	+
	2 mo	>6,250	>6,250	>6,250	+	−
	4 mo	>6,250	>6,250	>6,250	+	NT
	1 yr	>6,250	>6,250	>6,250	+	NT
	2 yr	>6,250	>6,250	>6,250	+	+

[a] Reciprocal of the highest dilution of sera that precipitated more than three times as much ^{125}I-labeled viral antigen as negative control serum.
[b] NT, Not tested.

twofold increments) or stock virus only. The cells were incubated for 1 h at 22°C, after which they were washed and replaced in complete RPMI 1640. Syncytia were counted on day 5 and compared with values for stock virus control wells. Data are represented as classical multiplicity curves, and the ratio of syncytium-forming virus neutralized (V_n) was divided by the total number of input syncytium-forming viral units (V_0). Thus V_n/V_0 was plotted on the Y axis and represents the virus-surviving fraction at twofold serum dilutions. Neutralization titers are represented as the reciprocal of the dilution at which 90% inhibition of total SFU of virus occurred.

RESULTS

Reisolation of HIVs from infected chimpanzees. Two animals were initially inoculated with HTLV-IIIB from tissue cultures, one with cell-free virus and the other with HTLV-IIIB-infected viable cells. The third animal received a transfusion of blood from a LAV-1-infected chimpanzee. At 1 month after the initial infection, virus was reisolated from all animals (Table 1) in human T-cell cultures, as determined by positive reverse transcriptase assay. Eventually the animals produced antibodies to all major structural antigens of HIV, as measured by polyacrylamide gel electrophoretic-radioimmunoprecipitation analysis (17). Reisolation both from plasma and PBLs was also attempted by cocultivation with uninfected H9 cells after 20 months of infection. After 4 weeks of cocultivation, virus was detected in the cells of all three animals as measured by reverse transcriptase and by the presence of HIV p24 and gp120 as defined by radioimmunoprecipitation. Inoculation of H9 cells with plasmas from the above animals did not result in the reisolation of HIVs.

Chimpanzees 524 and 525 infected with LAV-1 and HTLV-IIIB, respectively, also yielded a chimpanzee foamy virus which had both reverse transcriptase and a cytopathic syncytial effect similar to that of HIV. Unlike HIV, the foamy virus was also able to infect a non-T-4-containing continuous adherent human glial cell line. Both viruses were demonstrated to be synthesized in the same H9 cell and coexisted in culture for at least 6 months, as described previously (14).

Serological evaluation of animals after HIV infection. Sequential blood samples were regularly examined for each animal. Results of preinfection serum samples were compared with those taken years after the initial infection. Competition RIAs were performed with purified HTLV-IIIB p24 and gp120. Antibody against peptide 121 (p121; derived by genetic engineering), containing the immunodominant group-specific epitope(s) of the transmembrane gp41 protein (13), was assessed by ELISA. The standard whole virus commercially available ELISA was also performed regularly. A summation of the results is presented in Table 1. As soon as 1 month after virus injection, each animal developed antibody against at least one antigen of HIV. By 2 months after injection, maximal titers were present in all animals. No significant quantitative changes occurred during the approximately 2 years after initial injection. No substantial differences were observed between the LAV-1- and the HTLV-IIIB-injected chimpanzees receiving whole blood or cell-free virus. However, chimpanzee 526 demonstrated significantly higher virus-specific precipitating antibody titers to p121 which persisted throughout the experimental period. The maintenance of high levels of virus-specific precipitating antibodies against both the core and envelope viral antigens is compatible with persistent HIV infection.

Appearance and specificity of HIV neutralizing antibody. Although group-specific neutralizing antibody has been described in HIV-infected humans, a type-specific neutralizing antibody response has been the norm after inoculation of purified HTLV-IIIB gp120 or its subsets into a variety of animals, including primates (13, 16). Because the opportunity exists for repeated inoculation and infection with genetically divergent variants of HIV in humans, it was considered of interest to determine whether a single inoculation with one HIV variant could give rise to type-or group-specific neutralizing antibody. Neutralization was performed

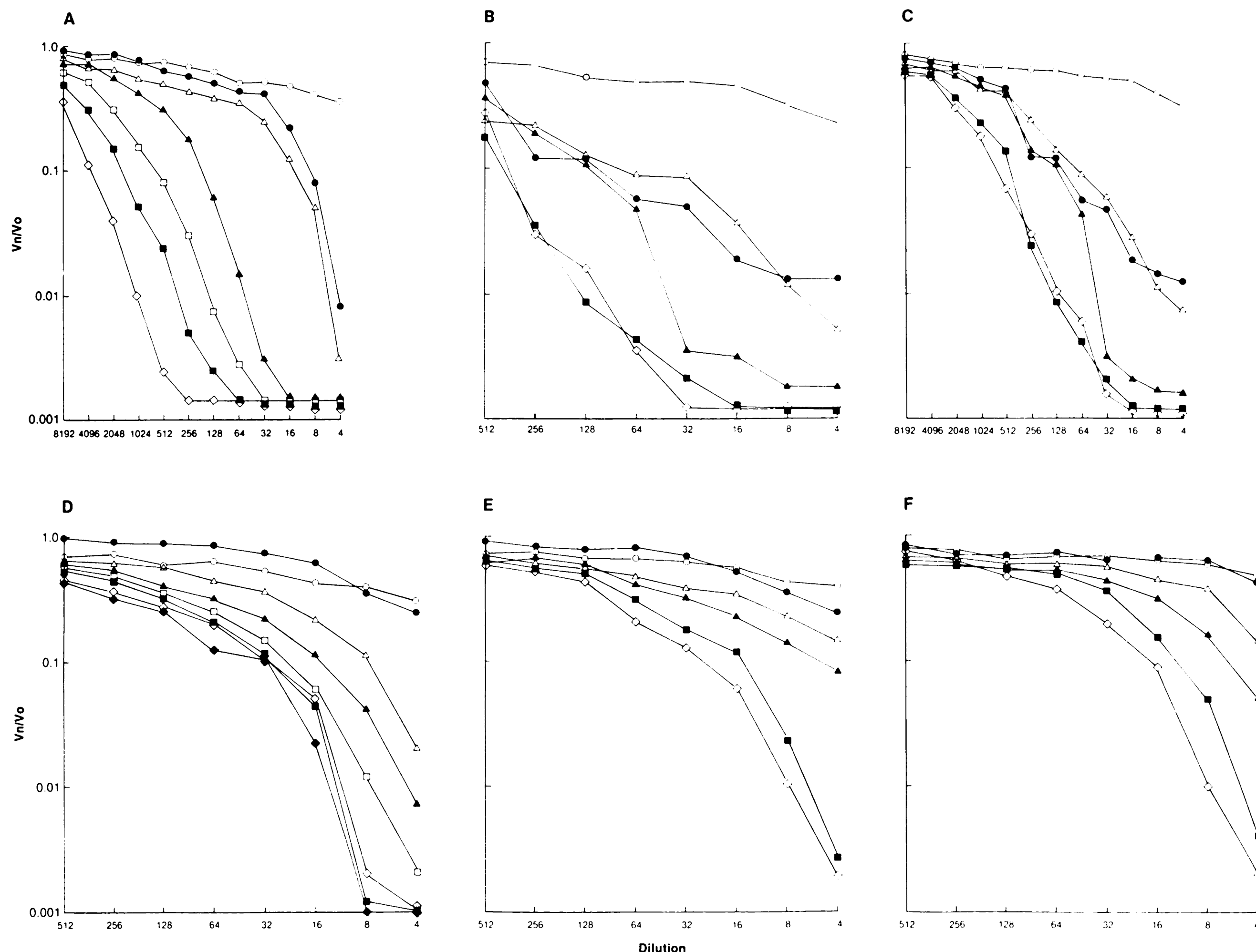

FIG. 1. Comparative sequential neutralization multiplicity curves for HIV-inoculated persistently infected chimpanzees (no. 524, panels A and D; no. 525, panels B and E; and no. 526, panels C and F). Neutralization of HTLV-IIIB (panels A through C) and RF_{II} (panels D through F) is determined by the reduction in virus-induced SFU (V_n) in the presence of twofold dilutions of pre- and postinfection serum divided by the number of total virus-induced SFU added (V_0). The symbols represent dates of different blood samples in the following order: preinfection (○), 1 month (●), 2 months (△), 3 months (▲), 6 months (□), 1 year (■), 2 years (◇), and 3 years (◆) postinfection.

by determining standard multiplicity curves against HTLV-IIIB and RF_{II}, the Haitian isolate. The latter is very distant from HTLV-IIIB and LAV-1 as defined by the actual *env* gene sequence determination, which suggests about a 20% amino acid change in the gp120 area (20). HTLV-IIIB and LAV-1 are closely related by sequence and share neutralization epitopes, as defined by goat sera raised against the gp120 of HTLV-IIIB (16).

As presented in Fig. 1, neutralizing antibody against the homologous HTLV-IIIB or closely related LAV-1 was produced rapidly and reached significant levels within 1 month after infection. By 3 months, type-specific neutralization was approximately 90% of its maximum. On the basis of virus titers used in the assay, at least 3 logs of neutralization was achievable. Antibody against HTLV-IIIB/H9 was detected more readily in the two animals given HTLV-IIIB virus or virus-infected cells and might be attributed to a larger total virus inoculum. In all three animals, group-specific neutralizing antibody appeared against the RF_{II} variant. Initially, the group-specific neutralizing titers were much lower in all three animals, and maximum achievable titers were reached by 2 years postinfection. The respective titers could be assessed as reciprocals of the dilution which gave 90% neutralization. Animal 524 had a titer of 1,024 against the homologously related HTLV-IIIB and a titer of 32 against the heterologous RF_{II} at 1 year postinfection. The two HTLV-IIIB-infected animals (no. 525 and 526) had 90% neutralizing variable homologous type-specific titers of approximately 384 and 1,024, respectively, and heterologously neutralizing group-specific titers against RF_{II} of 32 and 16, respectively (see Table 3). Group-specific neutralization was further confirmed for all three animals by neutralization of the divergent variants MN and CC (see Table 3) with comparative titers. Thus, group-specific neutralizing antibody could be induced after infection but, in this system, it was both late in appearance and significantly lower in titer than the type-specific neutralizing antibody.

Induction of ACC for HIV-infected cells. Antibody which is cytotoxic for virus-infected cells in the presence of complement appears to be important in the protective response against both murine and feline retroviruses (7, 11). Although no such antibody was observed in HIV-infected humans

TABLE 2. Analysis of ACC in HIV-infected chimpanzees

Chimpanzee no. (serum source)	Time of sample	% Cytolysis of virus-infected H9 cells	
		HTLV-IIIB	RF_{II}
A243A/524 (LAV-1)	Preinfection	3.4	NT[a]
	1 mo after	12.3	NT
	2 mo	28.6	NT
	6 mo	31.2	NT
	12 mo	32.1	NT
	24 mo	22.3	NT
A3E/525	Preinfection	4.2	5.0
	1 mo after	6.2	16.0
	2 mo	21.2	14.2
	6 mo	32.1	15.3
	12 mo	28.2	18.2
	24 mo	31.4	NT
A251/526	Preinfection	6.0	3.0
	1 mo after	21.2	16.0
	2 mo	28.2	18.0
	6 mo	21.5	20.0
	12 mo	31.2	23.0
	24 mo	26.4	NT

[a] NT, Not tested.

(15), low levels of highly type-specific ACC were observed in goats inoculated with purified HTLV-IIIB gp120 (15). An analysis of the course of appearance and the specificity of ACC in HIV-infected chimpanzees is presented in Table 2. After appropriate adsorption with uninfected H9 cells, some ACC was already observed after 1 month of infection. By 2 months, maximal levels were generally observed which did not change with time during the 2-year observation period. ACC levels were significantly higher before H9 cell adsorption (60 to 70%) which were required to reduce control base line levels, using complement and antibody. No significant differences were observed between the LAV-1- or the HTLV-IIIB-infected animals. In all three chimpanzees, the response observed was group specific in that RF_{II}-infected H9 cells were also killed in the ACC assay, which is in contrast to type-specific ACC after incubation of a single purified HTLV-IIIB gp120 antigen in goats.

Physical characterization of reisolated HIVs. A rapid assessment of antigenic variabilities of HIVs can be made even without the cloning of viruses by a two-dimensional peptide analysis of the major viral envelope glycoprotein. Passage of an HIV into different human cells is also known to alter the size of the gp120 and to cause slight rearrangements of chymotryptic peptide patterns (Robey et al., submitted) Accordingly, all reisolated HIVs were grown in the H9 cell line and compared with growth of the standard HTLV-IIIB isolate from the same cell line. The HIVs reisolated from the chimpanzees were remarkably similar to each other and, in the case of the two HTLV-IIIB isolates, were very similar to the parental HTLV-IIIB. As seen in Fig. 2, two major envelope polypeptide changes from the original HTLV-IIIB were detected. The first parental peptide alteration (panel A, arrow) is absent in the three virus reisolates (panels B, C, and D). The other change (panel B, arrow) implies the formation of a common peptide among the viruses isolated from all three animals. This latter peptide is otherwise significantly reduced or absent in the original HTLV-IIIB virus stock (panel A). Only slight variations in peptide migration or relative peptide density could be observed between the viruses isolated from the LAV-1- and HTLV-IIIB-infected chimps as well as those between two laboratory LAV-1 stocks previously analyzed. It thus appears that extended passage in chimpanzees accentuated the commonality of pattern and induced only minimal changes. However, neither the original input LAV-1 nor the virus passed in a chimpanzee the first time, i.e., the input virus for animal 524, was available for comparisons.

Serologic characteristics of reisolated HIVs. Although peptide maps and basic antigenic reactivity revealed only two peptide changes in gp120, it was considered of interest to determine whether the reisolated HIVs could still be neutralized by HTLV-IIIB type-specific sera as well as whether each virus could be neutralized by its own serum or by serum from the other two animals. Although in humans a serum sample from an infected individual could generally neutralize its own virus, there was no clear-cut group-specific pattern as to neutralization efficacy and, in at least several cases, serum from an individual could not neutralize its own virus (4, 18, 21, 22). Table 3 presents a partial analysis of the HIV reisolated from IIIB-inoculated chimpanzee 526 against the three respective chimpanzee sera and

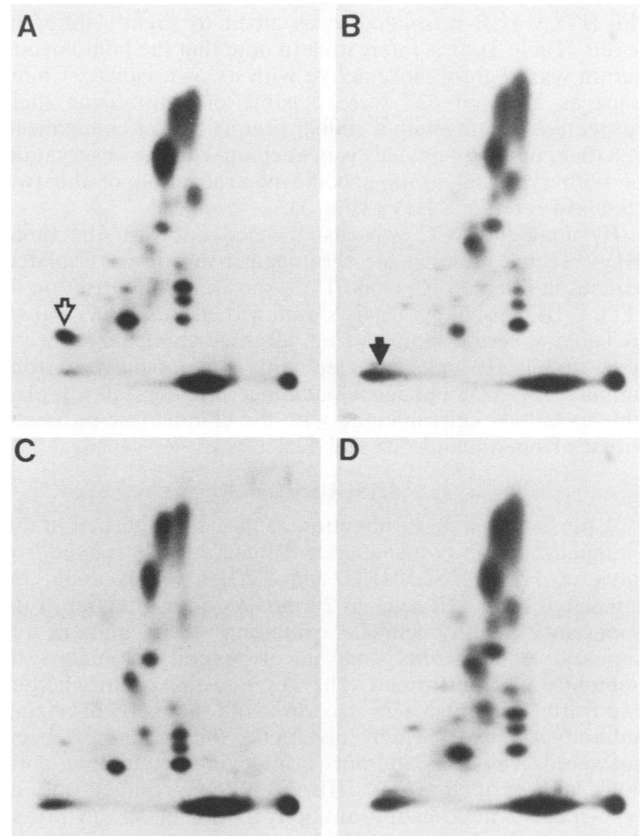

FIG. 2. Two-dimensional α-chymotryptic oligopeptide maps of reisolated HIVs and the original HTLV-IIIB external envelope glycoproteins. The ^{125}I-labeled glycoproteins were electrophoresed on preparative gels and visualized by autoradiography. The glycoprotein gel bands were excised and digested with α-chymotrypsin. The resulting peptides were subjected to electrophoresis (first dimension) and ascending chromatography (second dimension). The origin for electrophoresis is in the lower right-hand corner of each map. (A) Original HTLV-IIIB/H9 used to challenge chimpanzees 525 and 526, (B) reisolated virus from chimpanzee 525, (C) reisolated LAV-1 virus from chimpanzee 524, and (D) reisolated virus from chimpanzee 526. All virus was grown in H9 cells.

TABLE 3. Neutralization[a] of HTLV-IIIB, RF_{II}, MN, CC, and reisolated HTLV-IIIB from persistently infected chimpanzee, vaccinated goat, and human serum

Serum[b] and virus source	Titer of HIV neutralized				Reisolated HIV from chimpanzee 526 (IIIB)
	HTLV-IIIB	RF_{II}	MN	CC	
Chimpanzee A243A/524	1,024	32	10	32	256
Chimpanzee A3E/525	384	32	16	32	128
Chimpanzee A251/526	1,024	16	NT[c]	NT	1,024
Goat antiserum to HTLV-IIIB gp120	32	0	0	0	16
Human AIDS (A9)[d]	256	>512	512	>512	NT

[a] Neutralization is determined by the reciprocal of the dilution that gave a 90% reduction in total SFU of virus.
[b] Serum was obtained from animals 3 years after virus infection.
[c] NT, Not tested.
[d] Derived from an envelope-positive plasmapheresed AIDS patient.

against the standard reference goat antiserum to HTLV-IIIB type-specific gp120. The reisolated virus retained its specificity in that it was still neutralized by the type-specific goat reference serum. Each chimpanzee serum tested neutralized the HTLV-IIIB reisolated virus albeit to slightly different levels (Table 3). It is interesting to note that the homologous serum was slightly more active with its own isolate. Chimpanzees 524 and 525 were capable of neutralizing their respective isolates with a similar titer as that of chimpanzee 526 (data not shown). This is in keeping with the observation of both type-and group-specific neutralization of the two standard reference HIVs (Fig. 1).

By analogy, ACC was also carried out with the three HIV-infected chimpanzee sera against the three reisolated viruses in infected H9 cells. Type-specific goat antiserum to HTLV-IIIB gp120 cytotoxic serum was also used. As shown in Table 4, serum samples from all three chimpanzees were able to kill H9 cells infected with HIVs rederived from animals 524, 525, and 526. Goat antiserum to gp120 was also able to kill H9 cells infected with the chimpanzee-passaged viruses from animals 525 and 526.

DISCUSSION

A persistent state of infection could be established in the chimpanzee with two sequence-defined, closely related isolates of HIV, HTLV-IIIB and LAV-1. Virus could be reisolated from PBLs up to 20 months after infection from apparently healthy animals exhibiting strong immune responses. A sequential analysis of several parameters of immunity was performed. The key question as to whether exposure to a single HIV isolate could induce neutralizing antibody against a widely divergent isolate has now been answered. Thus far, attempts at inducing neutralizing antibody with purified HTLV-IIIB gp120 have resulted only in type-specific neutralizing antibody (Nara et al., in press; Arthur et al., in press). On the other hand, antibody in humans had been inferred to be group-specific, but exposure to sequential infection with less-related HIV variants could not be ruled out in most cases (12, 21, 22). Although the presence of group-specific neutralizing antibody in infected humans does not protect against the progression to clinical AIDS, preexisting group-specific neutralizing antibody may prevent primary HIV infection or participate in other mechanisms, such as ACC or antibody-dependent cellular cytotoxicity, which could limit virus spread.

In the current chimpanzee model using HTLV-IIIB or LAV-1, a type-specific neutralizing response appeared early and continued to increase in titer up to about a year after infection. The group-specific neutralizing response developed much slower and was of a lower titer, but it was still found to be increasing after 1 year, as defined by the multiplicity curves. Both the HTLV-IIIB and the RF_{II} isolates were much more difficult to neutralize with HIV antibody containing human sera than most other isolates, especially the AIDS-related virus which appeared to be highly susceptible to neutralization (22). It will be of interest to determine the relative strength of chimpanzee group-specific neutralizing antibodies against other less-related virus variants.

In several animal retrovirus systems such as murine or feline leukemia, ACC for infected cells also appeared to be important for long-term protection from disease (7, 11). Neutralization of murine and feline retroviruses as well as that of HIV does not require added complement (7, 11). In the murine AKR system, virus-infected cells readily persist in the presence of only hyperimmune neutralizing antibody and resume viral replication as soon as the antibody is withdrawn (6, 19). To eliminate the cells that contained infectious provirus, ACC was necessary. In the case of human HIV infection, neutralizing antibody appears to exist without a complement-mediated cytotoxic potential (15). This represents a salient difference from the capacity of inducing type-specific ACC with HTLV-IIIB gp120 in lower species and the current finding of group-specific ACC in LAV-1- or HTLV-IIIB-infected chimpanzees. It may be that ACC plays a significant role in the prevention of clinically observable immune impairment in chimpanzees which are clinically infected with HIV. Additional parameters of antibody-dependent cellular cytotoxicity and cytotoxic lymphocyte responses are also being assessed in these animals.

Because many HIV variants which may be further mutable are known, the possibility existed that group-specific neutralizing and ACC responses occurred with the gradual appearance of less-related mutants of the input viruses (5, 12). Accordingly, the reisolated viruses of all three animals were subjected to partial structural and immunological analyses. Two-dimensional peptide maps of gp120, the most variable region of the HIV genome, have been examined and readily revealed small differences in peptides among closely related isolates. Passage of HIV through a different human cell line resulted in small changes which are reversed to the original pattern after passage back to the original cell line, and the size of gp120 was variable in different human cells presumably because of variable host cell glycosylation. Accordingly, peptide maps of the reisolated HIVs from

TABLE 4. ACC of HIV-infected cells by sera from LAV-1- or HTLV-IIIB-infected chimpanzees

Serum source	% Cytolysis of H9 cells infected with				
	Original HTLV-IIIB	RF_{II}	HIV from no. 524	HIV from no. 525	HIV from no. 526
Goat antiserum to gp120	15	3.2	NT[b]	21	16
Chimpanzee A243A/524 (1 yr PI[a])	NT	NT	23	17	21
Chimpanzee A3E/525 (1 yr PI)	29	18.2	16	19	22
Chimpanzee A251/526 (1 yr PI)	21	23	24	21	24

[a] PI, postinfection.
[b] NT, Not tested.

chimpanzees were prepared for viruses produced in the H9 cell line and compared with HTLV-IIIB also grown in H9 cells. Passage of HTLV-IIIB-infected cells or virus in chimpanzees resulted in two envelope peptide alterations after several years of infection in the animals. The LAV-1 virus that was used to infect the chimpanzee was not available and, as described elsewhere (W. G. Robey, submitted for publication), several somewhat divergent passages of LAV-1 exhibited significant peptide map differences even after a shift to the H9 cell line.

The significant observation appears to be a lack of extensive change of the HTLV-IIIB virus after 2 years of infection in the chimpanzee when compared with its parental HTLV-IIIB. A common peptide seemed to have been acquired by all three chimpanzee isolates. Only very minor differences in peptide position between virus from animal 524 and those of animals 525 and 526 were observed. The subtlety of change would appear to require definitive analysis of cloning and sequencing to determine the point at which mutations might have occurred. The above minor changes are compatible with results of studies of viruses sequentially isolated and cloned from infected humans in which only very small alterations occurred with time (12). In each case, the caveat exists that the predominant species of HIV has not changed very much and that more major structural variants could have occurred but are not currently detected as significant entities. Immunological parameters of the isolated viruses confirmed that significant changes had not occurred. Each virus contained the same neutralizing epitopes as defined by the type-specific neutralizing goat antiserum to HTLV-IIIB gp120 which previously was capable of neutralizing both LAV-1 and HTLV-IIIB. Each chimpanzee serum sample also effectively neutralized not only its own virus but also neutralized reisolated HIV from one other animal. Although this might have been expected, at least several cases are known in which human anti-HIV antibody could neutralize heterologous HIVs but could not neutralize the virus isolated from the very same individual (4, 18, 21, 22). ACC data on reisolated HIVs were in keeping with the observation that no immunologically significant changes occurred in the HIV after 2 years of chronic infection in the chimpanzee.

The studies of vaccine development against AIDS in humans would seem to depend on reproducing a state of protective immunity analogous to that found in humans previously exposed to HIV but who have eliminated or so controlled the infection that no clinically detectable impairment of immune function was detected. Unfortunately, such protected status in humans has not yet been defined, and no definitive aspects of humoral or cellular immunity have been associated with a truly immune status (21, 22). Studies as to how the chimpanzee controls the virus may be the only alternative. The induction of analogous group-specific antibody and cell-mediated immune profiles may be required to induce a broad protection from primary infection in both chimpanzees and humans and further set the standards for an effective vaccine.

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