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Challenge of Chimpanzees (*Pan troglodytes*) Immunized with Human Immunodeficiency Virus Envelope Glycoprotein gp120

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The human immunodeficiency virus type 1 (HIV-1), the causative agent of acquired immunodeficiency syndrome, infects humans and chimpanzees. To determine the efficacy of immunization for preventing infection, chimpanzees were immunized with gp120 purified from human T-cell lymphotrophic virus type IIIB (HTLV-IIIB)-infected cell membranes and challenged with the homologous virus, HTLV-IIIB. A challenge stock of HTLV-IIIB was prepared by using unconcentrated HTLV-IIIB produced in H9 cells. The titer of the virus from this stock on human and chimpanzee peripheral blood mononuclear cells and in human lymphoid cell lines was determined; a cell culture infectivity of 10^4 was assigned. All chimpanzees inoculated intravenously with 40 cell culture infectious units or more became infected, as demonstrated by virus isolation and seroconversion. One of two chimpanzees inoculated with 4 cell culture infectious units became infected. Chimpanzees immunized with gp120 formulated in alum developed antibodies which precipitated gp120 and neutralized HTLV-IIIB. Peripheral blood mononuclear cells from gp120-vaccinated and HIV-infected animals showed a significantly greater response in proliferation assays with HIV proteins than did peripheral blood mononuclear cells from nonvaccinated and non-HIV-infected chimpanzees. Two of the gp120-alum-immunized chimpanzees were challenged with virus from the HTLV-IIIB stock. One animal received 400 cell culture infectious units, and one received 40 infectious units. Both animals became infected with HIV, indicating that the immune response elicited by immunization with gp120 formulated in alum was not effective in preventing infection with HIV-1.

Acquired immunodeficiency syndrome is an immunopathological manifestation which occurs following infection by human immunodeficiency virus (HIV). Vaccines have proven to be the most effective means of controlling viral diseases, and prevention of HIV infection by vaccination is a major objective in acquired immunodeficiency syndrome research. In developing an animal viral vaccine model, the minimum requirements are an immunogen and an animal that can be infected with the virus. HIV is a retrovirus, and candidate vaccines for retroviruses include nonpathogenic or killed virus, native or recombinant subunits and synthetic peptide, infectious recombinant viruses, and anti-idiotypes (6). The advantages and dangers of each technique and whether it could be applicable only to noninfected individuals have been discussed in detail (5, 11). Experimental vaccines have been reported which protect against infection with murine, feline, and primate retroviruses (13–15, 18, 19, 25, 26).

In the work described in this report, the immunogen selected was the outer envelope glycoprotein of HIV, gp120. This glycoprotein is the most external protein on the virus. It binds to the CD4 receptor on cells (4, 16, 21), presumably as the first step in virus infection; antisera to gp120 and gp120 subunits will neutralize in vitro infection of the virus (20, 24, 28–31, 34); and glycoprotein immunogens from other retroviruses have proven successful in preventing infection (13–15). Since glycoproteins derived by genetic engineering techniques can be different from viral glycoprotein, a description of immunological responses to gp120 purified from HIV-infected cells was considered essential to defining the optimum activity of a vaccine.

Experiments designed to assess the effectiveness of HIV-1 prototype vaccines in preventing HIV-1 infection must be conducted in an animal model. Selection of the animal model is dictated by the host range of HIV. Humans, chimpanzees, and gibbon apes (Paolo Lusso, Annamari Ranki, Robert C. Gallo, Kai J. E. Krohn, Phillip D. Markham and Steven S. Kueberuwa, submitted for publication) are the only animals that have currently shown to be infectable with HIV-1. Of these, the chimpanzee is the only animal available for vaccine studies involving experimental challenge. Presently, there is no report describing clinical acquired immunodeficiency syndrome in an HIV-infected chimpanzee; therefore, the chimpanzee may not provide a suitable model for prevention of disease by vaccination. However, the chimpanzee can be used to evaluate the effectiveness of preventing primary HIV infection. Chimpanzees have been infected with plasma and HIV-1-infected cells from AIDS patients, HIV-1-infected chimpanzee blood, HIV-1-infected cells in culture, and cell-free virus (1, 8–10). It has been reported that 1,000 50% tissue culture infectious doses will infect a chimpanzee but 0.1 50% tissue culture infectious dose will not (8).

Because of the limited availability of chimpanzees for acquired immunodeficiency syndrome experimentation, it is

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vital that carefully evaluated lots of HIV-1 are used for experiments involving animal infection. To provide a characterized lot of HIV-1, we have prepared a large quantity of the HIV-1 variant human T-cell lymphotropic virus type IIIB (HTLV-IIIB), determined the in vitro and in vivo infectivity titers, and frozen the virus in 1-ml aliquots for use as a resource for chimpanzee infectivity studies. This stock was used in the present study as the challenge virus to test the efficacy of immunization with gp120 in alum for preventing infection with HIV.

MATERIALS AND METHODS

Preparation of virus stocks. The HIV-1 infectious stock was prepared from HTLV-IIIB-infected H9 cells (27). Infected cells from 400 ml of culture fluid were pelleted by centrifugation at 425 × *g* for 5 min, suspended in 400 ml of growth medium containing 2.5% fetal bovine serum, and incubated at 37°C in 5% CO_2. After overnight incubation, the cells were removed by centrifugation at 1,200 × *g* for 30 min. A total of 250 ml of supernatant containing HIV-1 was carefully removed from the cell pellet and diluted with 250 ml of undiluted fetal bovine serum. This mixture of cell culture fluid and fetal bovine serum was dispensed at 1 ml per vial, frozen, and stored in liquid nitrogen. The virus used in the preparation of these infectious stocks was not concentrated, because previous experiments revealed that concentration by centrifugation significantly decreased the cell culture infectivity of HIV-1 (data not shown). Virus stocks were also tested for contamination with adventitious agents including mycoplasmas; none were detected.

Animals. To conserve the scarce supply of available chimpanzees, animals previously used in experiments involving human hepatitis viruses A, B, and non-A non-B were used for this HIV vaccine trial. Many chimpanzees used in hepatitis research have received human blood products which possibly included HIV. Therefore, all animals were pretested for HIV antibodies prior to use. Housing conditions for the chimpanzees and the serological assays used to detect HIV-1 antibodies have been previously described (2). The gp120 used for immunization of the chimpanzees was purified as previously described (30). All HIV-1-inoculated chimpanzees were housed and maintained in biosafety level 3 facilities, and biosafety level 3 procedures were followed. Chimpanzees to be used in in vivo titration studies were examined and were seronegative for antibodies to envelope and core antigens of HIV-1 (2). The animals were physically examined by staff veterinarians every 2 weeks, at which time serum and blood samples were taken, analyzed for antibodies to HTLV-IIIB, and used for virus isolation.

In vitro HIV-1 titration. To determine in vitro infectivity, we recovered HIV-1 from liquid-nitrogen storage and tested it for infectivity in the continuous cell lines H9 and CEM-SS, as well as in normal human and chimpanzee peripheral blood mononuclear cells (PBMC). The titer of the virus pool on H9 cells was determined by making fourfold dilutions of the virus stock in growth medium. Virus dilutions (100 μl) were mixed with 200 μl of growth medium containing 10^5 H9 cells in six-well multiwell, cell culture plates. The cultures were incubated for 10 days, and a reverse transcriptase assay was performed on the supernatants. A reverse transcriptase value of 10,000 cpm was considered positive for virus. The assay in CEM-SS cells was conducted as previously described (24). Tenfold dilutions of the virus stock were incubated with normal human PBMC. Culture supernatants were examined weekly for HIV-1 by reverse transcriptase and HIV-1 p24 antigen capture assays. The cultures were propagated and assayed for 5 weeks, and endpoint determination depended on detection of both HIV-1 p24 and reverse transcriptase.

HIV-1 isolation from infected chimpanzees. PBMC from chimpanzees and humans were prepared by diluting heparinized blood with equal volumes of Dulbecco phosphate-buffered saline which was free of calcium and magnesium. Approximately 10 to 15 ml of Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) was used to underlay the heparinized blood, and the blood was centrifuged for 30 min at 1,500 rpm in a Sorvall RT-6000 rotor at room temperature. The PBMC were removed and washed with Dulbecco phosphate-buffered saline. The chimpanzee and human PBMC were suspended in separate flasks in growth medium (RPMI 1640 medium supplemented with 15% fetal bovine serum, 2 mM glutamine, 50 μg of penicillin-streptomycin per ml, and 100 μg of neomycin per ml) containing 2 μg of phytohemagglutinin per ml. Cells (3×10^6 to 4×10^6) were seeded into T-75 flasks and incubated at 37°C in 5% CO_2. After 48 h, chimpanzee cells were removed from the flask, pelleted by centrifugation, suspended in growth medium containing 10% interleukin-2, and adjusted to 2×10^6 to 4×10^6 cells per ml, and 20 to 30 ml was added to the flasks containing the phytohemagglutinin-stimulated adherent human PBMC. The chimpanzee and human cell mixtures were incubated at 37°C in 5% CO_2. HIV-1 expression was monitored biweekly by concentrating cell culture fluid 10-fold and analyzing it for reverse transcriptase and for HIV-1 p24 by antigen capture assays.

Serological assays. The radioimmune precipitation assays were performed as previously described (2). The purified proteins were radiolabeled with ^{125}I by using chloramine-T. Antibodies to p24 and gp120 were detected by incubating 20,000 cpm of radiolabeled antigen with 50 μl of serum diluted in radioimmunoassay 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 was continued at room temperature for 15 min, and pellets were collected by centrifugation at 2,500 rpm in a Beckman J-6M centrifuge with a JS 4.2 rotor for 15 min. The supernatant was discarded, and the pellets were washed by resuspension 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.

Neutralization assays. Antibodies that neutralize HIV were detected by using an HIV-1 microdilution infectivity syncytium-forming assay (24). Virus was diluted and preincubated with cell culture medium or serial twofold dilutions of heat-inactivated serum. The virus-serum mixture was added to CEM-SS cells, and the cells were incubated at 37°C for 60 min. Following incubation, the virus-serum mixture was removed and replaced with 100 μl of RPMI 1640 medium containing 10% fetal bovine serum. The cells were examined for the presence of syncytia 5 days after infection. The neutralizing titers are expressed as the dilution of serum at which 90% of the input virus was neutralized.

T-cell proliferation assay. Cell-mediated immunity assays were performed as previously described (17). The PBMC were separated from heparinized blood by Ficoll-Paque gradient centrifugation, washed, and cultured in triplicate wells in RPMI 1640 medium and 10% (vol/vol) autologous

TABLE 1. In vitro titer determination for HTLV-IIIB infectious stock

Target cells		Titer (infectious units/ml)
Primary cultures	Transformed cultures	
Human PBMC[a]		1×10^3
Chimpanzee PBMC		1×10^4
	H9[b]	1×10^4
	CEM-SS[c]	2.5×10^4

[a] Dilutions of virus stock were incubated with normal human PBMC and monitored biweekly for 5 weeks by HIV-1 p24 antigen capture assay and reverse transcriptase assay.

[b] Dilutions of the virus stock were incubated with H9 cells and assayed after 10 days. The titer represents the highest dilution resulting in 50% infection of the cells as determined by reverse transcriptase assays.

[c] Dilutions of the virus stock were incubated with CEM-SS cells, and the titer is represented as syncytium-forming units per milliliter as determined in a quantitative virus-induction, syncytium-forming microassay (22).

plasma. Whole virions were heated for 30 min at 56°C, and 25 μg was used in the proliferation assays. Purified gp120 was also used as an antigen, and 1 μg of this purified protein was used in the assays. The cultures were pulse labeled with [^{3}H]thymidine (0.5 μCi per well) on day 5 and harvested on day 6. Incorporated [^{3}H]thymidine was measured in a scintillation counter.

RESULTS

In vitro titer of virus stocks. HTLV-IIIB stocks were recovered from liquid nitrogen and assayed on established normal human and chimpanzee PBMC and the established cell lines H9 and CEM-SS. The virus titers in human and chimpanzee PBMC were 10^3 and 10^4 infectious units/ml, respectively (Table 1). The titers in the continuous lines H9 and CEM-SS were 1×10^4 and 2.5×10^4 infectious units/ml, respectively. Variation in the assessment of the endpoint for the titration may account for the differences in titers between laboratories.

Titer of the HIV-1 stocks in unimmunized chimpanzees. HTLV-IIIB was thawed and serially diluted in 1 ml of RPMI 1640 medium, without fetal bovine serum, immediately before chimpanzee inoculation. Assuming a cell culture titer of 10^4 infectious units/ml, the virus stock was diluted to yield 4,000, 400, 40, and 4 cell culture infectious units/ml. Each chimpanzee received a 1-ml intravenous injection of the appropriate virus dilution. HIV-1 infection was determined by our ability to isolate virus and detect seroconversion to the major HTLV-IIIB core protein, p24, from blood taken every 2 weeks following injection of the virus. The results of this titer determination appear in Table 2. Two weeks after the experimental inoculation, we successfully isolated virus from the chimpanzee that received the largest dose of HIV-1 (4,000 cell culture infectious units). Virus was recovered from animals that received 400 and 40 cell culture infectious units 4 weeks following inoculation. Antibody to HTLV-IIIB p24 was detected in all three of the infected chimpanzees in the first serum sample taken after virus isolation. HIV-1 has not been isolated from the chimpanzee that received 4 cell culture infectious units, nor have antibodies to HTLV-IIIB p24 been detected in the serum, even in samples taken more than 18 months after virus inoculation. We presume that inoculation of this animal with 4 cell culture infectious units did not result in infection.

HIV was readily isolated from the infected animals

TABLE 2. In vivo titer determination for HTLV-IIIB in chimpanzees

HTLV-IIIB inoculation[a] (cell culture infectious units)	Chimpanzee no.	Weeks after inoculation to virus isolation[b]	Weeks after inoculation to seroconversion[c]
First titration			
4,000	856	2	4
400	923	4	6
40	881	4	6
4	905	Negative (>1 year)	Negative (>1 year)
Second titration			
40	911	2	6
4	854	6	8

[a] The quantity of virus to be used for inoculation was determined by in vitro infection of H9 and CEM-SS cells.

[b] Blood samples were taken every 2 weeks; the PMBC were separated and cocultivated with normal human PBMC. Culture fluid was analyzed for HTLV-IIIB by a competition radioimmunoassay for HTLV-IIIB p24.

[c] Serum samples from the inoculated chimpanzees were analyzed for precipitating antibodies to HTLV-IIIB p24.

throughout the course of the experiment (Table 3). The only animal from which isolation was variable was no. 923. At weeks 12 through 16, 32, and 34, we isolated no virus from no. 923, yet we were successful in isolation attempts from all other infected chimpanzees during that time.

Immune status of vaccinated chimpanzees. As previously reported (2), we have immunized a group of eight chimpanzees with HTLV-IIIB gp120 in Alhydrogel (alum) adjuvant (Accurate Chemical and Scientific Corp., Westbury, N.Y.). Two chimpanzees were inoculated in parallel with Alhydrogel and served as unvaccinated controls. None of the 10 experimental animals showed any alterations in their absolute T4 lymphocyte numbers or shifts in their T4/T8 ratios

TABLE 3. Isolation of HIV from PBMC of chimpanzees following challenge with HTLV-IIIB

Weeks after challenge	HIV isolated[a] (animal no./challenge dose)					
	856/4,000	923/400	881/40	911/40	905/4	854/4
0	−	−	−	−	−	−
2	+	−	−	+	−	−
4	+	+	+	−	−	−
6	+[b]	+	+	+	−	+
8		+	+	+	−	+
10		+	+	+	−	+
12		−	+	+	−	+
14		−	+	+	−	+
16		−	+	+	−	+
18		+	+	+	−	+
20		+	+	+	−	+
22		+	+	+	−	+
24		+	+	+	−	+
26		+	+	+	−	+
28		+	+	+	−	+
30		+	+	+	−	+
32		−	+	+	−	+
34		−	+	+	−	+
36		+	+	+	−	+

[a] Symbols: −, virus was not isolated; +, virus was isolated. The presence of virus was determined by a reverse transcriptase assay and confirmed by an HIV-1 p24 capture assay and an HIV-1 p24 competition radioimmunoassay. The challenge dose of HTLV-IIIB is represented as cell culture infectious units.

[b] The animal died of respiratory failure following administration of anesthesia.

TABLE 4. Proliferative T-cell responses of chimpanzee PBMC toward three different HIV-1 virion preparations and toward gp120 envelope glycoprotein[a]

Chimpanzee no.	Status	Response (cpm) to following antigen[b]:				
		None	HTLV-IIIB	HTLV-IIIRF	HTLV-IIIMN	gp120
911	Unimmunized control	180	220 (1.2)	140 (0.8)	180 (1.1)	209 (1.2)
854	Unimmunized control	150	280 (1.9)	100 (0.7)	180 (1.2)	180 (1.2)
1125	gp120-alum vaccinated	220	4,800 (21.8)	2,800 (12.7)	3,700 (16.8)	2,200 (10.0)
1068	gp120-alum vaccinated	280	6,100 (21.8)	3,200 (11.4)	4,800 (17.1)	4,800 (17.1)

[a] Chimpanzee PBMC were taken at the time of challenge, which was the time of maximum precipitating antibodies.
[b] Stimulation indices are in parentheses. The stimulation index was calculated by dividing the cpm with antigen by the cpm without antigen.

(2). Two of the gp120-alum-immunized chimpanzees (no. 1068 and 1125) were selected as candidates for challenge with HTLV-IIIB. These animals were boosted with 50 μg of gp120 formulated in alum. This was the fifth HTLV-IIIB gp120 immunization; it took place 244 days after the initial inoculation and 98 days after the fourth immunization. Precipitating antibody responses were 1:1,600 and 1:3,200 for no. 1068 and 1125, respectively. The maximum neutralizing antibody titers detected in the gp120-alum-immunized chimpanzees was 1:16 (2). Sera from the gp120-alum-immunized chimpanzees neutralized only the HIV isolate HTLV-IIIB, indicating that the neutralization antibody response continued to be type specific.

Proliferative T-cell response in immunized animals. The HIV-specific T-cell response was assessed by using PBMC taken 2 weeks after the fifth immunization. The PBMC from the gp120-alum-immunized chimpanzees were responsive to all three HIV antigens, as well as the purified gp120 (Table 4). In both vaccinated animals, the maximum response was seen when HTLV-IIIB was used as the antigen. The PBMC from the unimmunized controls were negative for all HIVs tested, as well as the purified gp120.

Challenge with HTLV-IIIB. Two immunized and two unimmunized chimpanzees were inoculated with HTLV-IIIB whose titer had been determined in vivo. The vaccinated animals were challenged 2 weeks following the fifth immunization. Chimpanzee no. 1125 was challenged intravenously with 400 cell culture infectious units of HTLV-IIIB, and chimpanzee no. 1068 was challenged with 40 cell culture infectious units. The unimmunized chimpanzees were inoculated with 4 and 40 cell culture infectious units. Blood was collected from the animals at biweekly intervals, and the sera were examined for antibody-related parameters (precipitating antibody to HTLV-IIIB gp120 and p24, and neutralizing antibody). The PBMC were separated from heparinized blood and used for virus isolation and T-cell stimulation assays.

When the serological responses of gp120-vaccinated and challenged chimpanzees were compared with the responses of unvaccinated, challenged animals (Fig. 1), it is evident that a strong anamnestic immune response against HTLV-IIIB gp120 was present in the vaccinated animals. This anamnestic response peaked at approximately 42 days after the challenge. It was first observed 28 days after challenge in chimpanzee no. 1068. Precipitation of gp120 by sera from the control animals, no. 854 and 911, was found at 84 and 56 days after challenge, respectively. The immune response peaked 4 to 5 months postchallenge, and the maximum gp120 precipitation titer was significantly lower in the controls than in the vaccinated animals. Precipitating antibody titers to HTLV-IIIB p24, indicative of infection, were observed in all animals 48 to 56 days after challenge. In a separate study conducted 4 months later, two additional chimpanzees were inoculated with 40 and 4 cell culture infectious units to assess the stability of the frozen virus stocks and determine the minimum dose needed to infect a chimpanzee. When cell culture assays were performed, HIV-1 titers did not change on storage from the initial estimates (data not shown). Protocols for virus preparation, inoculation, chimpanzee housing, and virus isolation were identical to those used in the in vivo titer determination experiment described above.

In this second experiment, both chimpanzees became infected with HIV-1, as determined by our success in isolating virus from PBMC and by the development of antibodies to HTLV-IIIB p24 (Table 2). Infection was detected in the animal receiving 40 cell culture infectious units at 4 weeks after inoculation; by 6 weeks, the animal had detectable levels of antibodies to HTLV-IIIB p24. The chimpanzee receiving 4 cell culture infectious units also became infected; virus was isolated from PBMC taken at 6 weeks, and the animal seroconverted 8 weeks after virus inoculation. The interval from inoculation to virus isolation and seroconversion was longer in this animal than in any of the other inoculated chimpanzees. The fact that one of two chimpanzees inoculated with 4 cell culture infectious units became infected with HIV-1 allows us to assign a 50% chimpanzee infectious dose of the frozen virus stock of 4×10^3 infectious chimpanzee units per ml. As expected, the earliest response was seen in the animal that received 40 cell culture infectious units. Although the chimpanzee that received the lowest virus dose seroconverted to p24 and gp120 later than the animal receiving 40 cell culture infectious units, both animals had titers greater than 1:1,000 for core and envelope antigens within 10 weeks of virus inoculation.

Neutralization of HIV in infected chimpanzees. Sera taken 2 weeks following the fifth vaccination were compared with sera taken 6, 10, and 12 weeks after the challenge and were analyzed for their ability to neutralize HTLV-IIIB and HTLV-IIIRF (Fig. 2). Prior to challenge, sera from chimpanzees no. 1068 and 1125 neutralized HTLV-IIIB but not HTLV-IIIRF. Sera from no. 1068 neutralized 90% (V_n/V_0 = 0.1) of the input virus at titers between 1:8 and 1:16. Following infection, the neutralization titers increased in both animals and broadened to neutralize the HTLV-IIIRF variant.

T-cell stimulation of PBMC from HIV-infected chimpanzees. Proliferative T-cell responses of chimpanzee PBMC in both gp120-immunized chimpanzees and HIV-infected chimpanzees were examined. PBMC from gp120-alum-vaccinated chimpanzees showed a significantly greater incorporation of [^{3}H]thymidine on exposure to HIV proteins than did PBMC from unvaccinated controls (Table 4). Maximum responses were obtained with HTLV-IIIB. However, responses were also seen with HTLV-IIIRF and HTLV-IIIMN as well as purified gp120. After infection with HIV, proliferative responses were elevated in gp120-alum-immu-

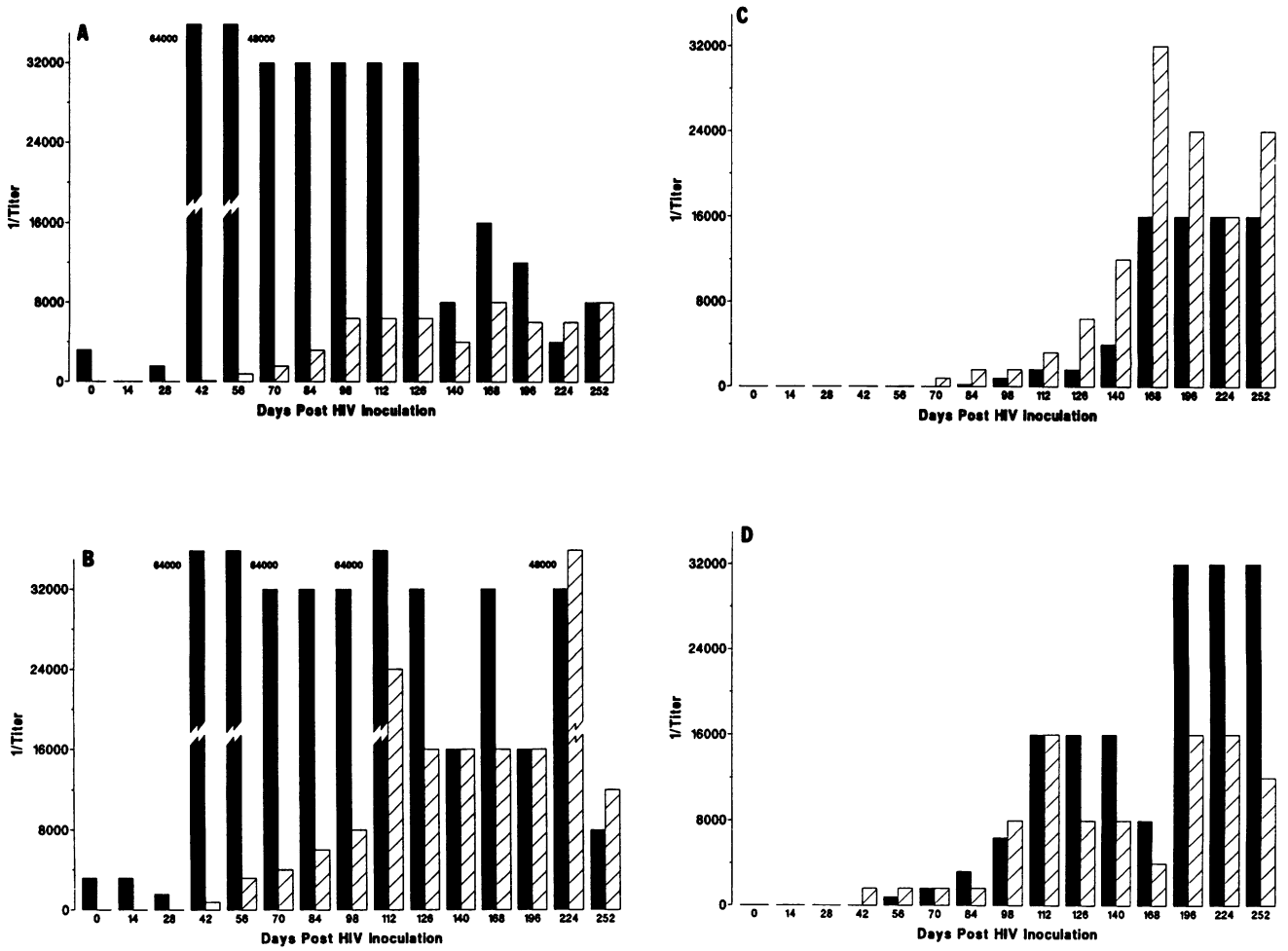

FIG. 1. Dilutions of serum samples from HTLV-IIIB-infected chimpanzees were examined for their ability to precipitate ^{125}I-labeled HTLV-IIIB gp120 (■) or ^{125}I-labeled HTLV-IIIB p24 (▨). Results are expressed as the highest dilution that precipitates threefold over background. Titer determinations are shown for serum from a gp120-alum-immunized chimpanzee (no. 1068) which received 40 cell culture infectious units (A), a gp120-alum-immunized chimpanzee (no. 1125) which received 400 cell culture infectious units (B), an unimmunized chimpanzee (no. 911) which received 40 cell culture infectious units (C), and an unimmunized chimpanzee (no. 854) which received 4 cell culture infectious units (D).

nized controls (Table 5). The PBMC taken 10 weeks following challenge from gp120-alum-immunized animals had stimulation indices of 80 and 26 when HTLV-IIIB was used as the antigen. Response to HTLV-IIIB, HTLV-IIIRF, HTLV-IIIMN, and gp120 were also greater with PBMC from vaccinated, HIV-infected animals than with PBMC from unvaccinated, HIV-infected controls (Table 5).

DISCUSSION

In this report, we document the failure of a gp120-alum vaccination to prevent infection of chimpanzees challenged with HIV-1. The challenge stock of HIV-1 is the HTLV-IIIB strain and has 10^4 cell culture infectious units/ml and a chimpanzee infectious titer of 4×10^3 infectious units/ml. This challenge stock of HIV has been stored for more than 2 years in liquid nitrogen with no detectable loss of infectivity. To enable investigators to limit the number of chimpanzees used, we have made this stock of virus available for chimpanzee infectivity experiments.

When characterizing this virus stock, it was clear that injection of extremely small numbers of infectious HIV would lead to infection in the chimpanzee. It should be emphasized that the virus used in this study was from unconcentrated cell culture fluid and that the chimpanzee receiving the largest dose of virus, 4,000 cell culture infectious units, was given only 400 μl of this virus stock. In preparing the stock, the unconcentrated cell culture fluid was diluted with equal volumes of fetal bovine serum, making a maximum inoculum equivalent of 200 μl of cell culture fluid. All three chimpanzees inoculated with 40 cell culture infectious units became infected; this was equivalent to only 40 μl of cell culture fluid. The fact that one of the two chimpanzees inoculated with 4 cell culture infectious units (0.2 μl of cell culture fluid) became infected will attest to the highly infectious potential of culture fluid from HIV-infected cells. This information highlights the need for continued evaluations of safe laboratory procedures when working with HIV-infected cells. Special emphasis should continue to be given to protection from parenteral exposure to infected cells and cell culture fluid.

In humans, seroconversion to HIV-1 antigens is the primary method for detecting HIV-1 infection. Serological

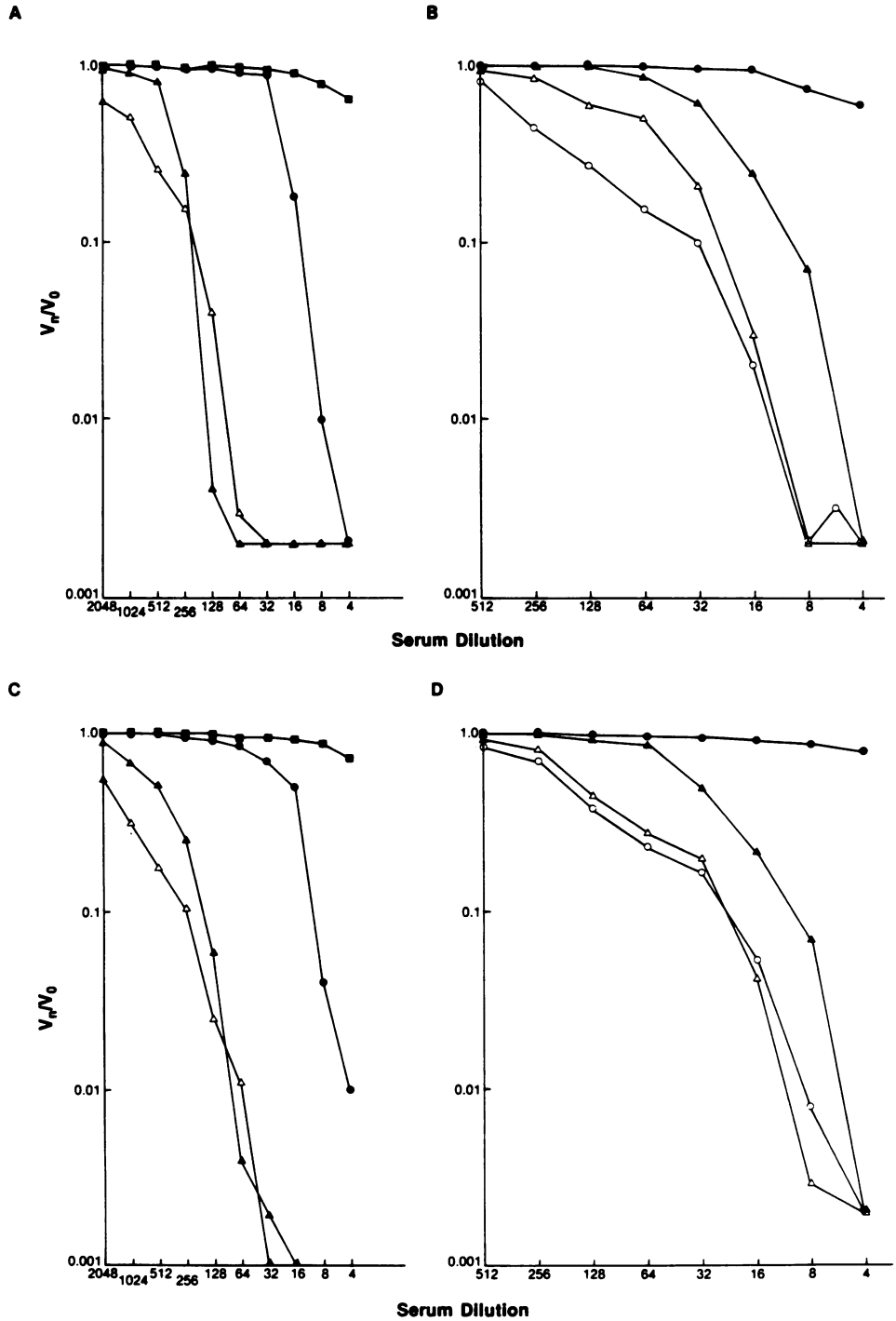

FIG. 2. Comparative sequential neutralization multiplicity curves for chimpanzees no. 1068 (A and B) and no. 1125 (C and D) following vaccination with gp120-alum and, subsequently, HTLV-IIIB challenge. Neutralization antibody for HTLV-IIIB (A and C) and HTLV-IIIRF (B and D) was determined by a microdilution syncytium-forming assay with CEM-SS cells. Data are expressed as the syncytium-forming virus neutralized (V_n) divided by the total number of input syncytium-forming viral units (V_0). Thus V_n/V_0 (representing the virus-surviving fraction) is plotted against serum dilution. The symbols represent different serum samples during the course of vaccination and virus challenge: ■, prebleed; ●, 2 weeks after the fifth vaccination, at the time of virus challenge; △, 6 weeks after challenge; ▲, 10 weeks after challenge; ○, 12 weeks after challenge.

screening is currently the only method used to identify HIV-1-positive blood and prevent it from being used for transfusions. Although it is evident that serological screening has greatly decreased the incidence of HIV-1 infection due to blood transfusion (32), information pertaining to the length of time between infection, the presence of infectious virus, and seroconversion is useful in assessing the utility of serological screening as a means of detecting HIV-1-contam-

TABLE 5. Proliferative T-cell responses of chimpanzee PBMC toward three different HIV-1 virion preparations and toward gp120 envelope glycoprotein[a]

Chimpanzee no.	Status	Response (cpm) to following antigen[b]:				
		None	HTLV-IIIB	HTLV-IIIRF	HTLV-IIIMN	gp120
911	Unimmunized control	300	1,300 (4.3)	400 (1.3)	850 (2.8)	2,300 (7.7)
854	Unimmunized control	280	2,500 (8.9)	880 (3.1)	2,000 (7.1)	3,100 (11.1)
1125	gp120-alum vaccinated	110	8,800 (80)	5,400 (49.1)	6,300 (57.3)	2,950 (26.8)
1068	gp120-alum vaccinated	220	5,800 (26.4)	2,200 (10.0)	3,700 (16.8)	3,900 (17.7)

[a] Chimpanzee PBMC were taken 10 weeks after challenge. Both controls and vaccinated animals were infected following challenge with HTLV-IIIB.
[b] Stimulation indices are in parentheses. The stimulation index was calculated by dividing the cpm with antigen by the cpm without antigen.

inated blood. All the chimpanzees infected with HTLV-IIIB seroconverted to p24-positive status 4 to 8 weeks after virus inoculation (Table 2). Antibodies to p24 were detected in all animals within 4 weeks of virus isolation. A commercially available whole-virus enzyme-linked immunosorbent assay also detected seroconversion at the same time as the p24 assay (data not shown). Our observation that seroconversion occurs shortly after virus infection confirms the supposition that in humans, infection of recipients of HIV-1 antibody-negative blood is possible if the donor was in the analogous "window" period when virus was present but before detectable antibody was present.

This study shows that immunization with gp120 formulated in alum is not effective in preventing primary infection by HIV. Alum was used as the adjuvant because it presently is the only adjuvant approved by the Food and Drug Administration and our initial experiments were designed to allow extension into humans as rapidly as possible if protection was achieved. Similar results of low antibody response to recombinant gp120 formulated in alum and lack of protection of immunized chimpanzees have been reported (3). A number of characteristics of HIV may make development of a protective vaccine difficult. Most important, perhaps, is the heterogenicity of HIV isolates with respect to gp120 (7, 12, 33). Distinct HIV isolates can differ from each other by 20% or more in the amino acid sequence of the gp120. This heterogeneity is most apparent in neutralization assays involving the use of sera generated by vaccination with gp120 or subunits which normally neutralize only the HIV isolate from which the immunogen was prepared (20, 30, 34). An epitope involved in the type-specific neutralization has been identified as a 24-amino-acid peptide which represents amino acids 307 to 330 of gp120 (32). To determine whether the gp120 heterogeneity will affect vaccinations, protection against homologous virus challenge must be achieved. Heterologous HIV challenge could then be analyzed. An immune response comparable to that seen in the chimpanzees immunized with gp120 in alum and challenged with HTLV-IIIB (Fig. 1) would be a desirable goal prior to homologous challenge. With this goal in mind, we have examined adjuvants other than alum and have found that formulation of gp120 into immunostimulatory complexes (22, 23) is highly immunogenic in rhesus monkeys (S. W. Pyle, B. Morein, J. W. Bess, Jr., L. Akerblom, P. L. Nara, S. M. Nigida, Jr., N. W. Lerche, W. G. Robey, P. J. Fischinger, and L. O. Arthur, Vaccine, in press). Experiments are in progress to determine whether immunizations with gp120 that give high, persistent immune responses will be protective.

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