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The Consequence of Passive Administration of an Anti-Human Immunodeficiency Virus Type 1 Neutralizing Monoclonal Antibody before Challenge of Chimpanzees with a Primary Virus Isolate

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The anti-gp41 virus neutralizing monoclonal antibody 2F5 was infused into chimpanzees, which were then given an intravenous challenge with a primary human immunodeficiency virus type 1 (HIV-1) isolate. In two control animals, the infection was established immediately, as evidenced by positive cell-associated DNA PCR and serum RNA PCR tests within 1 week, seroconversion by 4 weeks, and development of lymphadenopathy in this acute phase. Serum RNA PCR tests were negative in one of the two antibody-infused animals until week 8 and in the other antibody-infused animal until week 12; both animals seroconverted at week 14. The peak of measurable virus-specific serum RNA was delayed until week 16 in one antibody-infused animal. Virus-specific RNA in the other animal did not reach levels comparable to those in the other animals through 1 year of follow-up studies. Virus was isolated from the week 16 blood sample from one infused animal. Virus was not isolated from peripheral blood of the second animal but was isolated from lymph node cells taken at week 36. The infection of untreated chimpanzees with this primary isolate appears robust. Use of this isolate should widen the scope of possible experiments in the chimpanzee model. This antibody infusion study indicates that neutralizing antibody, when present at the time of challenge, affects the timing and level of infection and remains influential after it can no longer be detected in the peripheral circulation. It is possible that preexisting, neutralizing antibodies (passively administered or actively elicited) affect the course of acute-phase virus replication in humans. It remains to be established whether these immunologically mediated early effects will influence the course of HIV-1 disease.

Human anti-human immunodeficiency virus type 1 (HIV-1) monoclonal antibodies (MAbs) that neutralize primary HIV-1 isolates and the determinants bound by these antibodies have been the subjects of extensive research effort. Most of this work has concentrated on determinants located within the HIV-1 external glycoprotein, gp120, with a focus on antibodies that recognize the hypervariable V3 region, V1 and V2 regions, and the conformational determinants that comprise the CD4 receptor binding domain (10, 17–19, 23, 34). In addition to gp120, other determinants that were bound by neutralizing antibodies and mapped within the HIV-1 transmembrane glycoprotein, gp41, were described. One determinant recognized by a unique MAb, 2F5, comprises residues 660 to 665 and usually contains the sequence ELDKWA. This sequence is near the transmembrane domain, and current evidence suggests that it is part of the ectodomain of the viral envelope (31). Antibody 2F5 was described first by Muster et al. (24). This antibody recognizes most sequences that contain xLDK(R)WA(x) (where x represents any amino acid) (9, 24, 29). However, neutralization studies to date indicate that primary isolates must have the ELDKWA sequence to be neutralized in human peripheral blood mononuclear cell (PBMC) cultures in vitro (9). The gp41 sequence ELDKWA is present in isolates worldwide, although it appears more frequently in reported sequences of clade B, D, and E isolates (9, 14). The sequence does not vary much within sets of isolates obtained at different times from an infected human (14). The observed antiviral effect of MAb 2F5 and the widespread presence of its determinant warranted the in vivo evaluation of 2F5 activity.

Although chronic HIV-1 infection can be established in chimpanzees with primary isolates from infected humans (21, 33), primary isolates have not been used in vivo to assess antiviral properties of MAbs. For the passive protection study reported here, we chose a primary isolate, designated 5016, as a challenge virus. In this way, the challenge would be representative of isolates currently in the human population. Isolate 5016 establishes an acute infection in chimpanzees characterized by the development of lymphadenopathy and the presence of high levels of virus in serum. Isolate 5016 was passaged a limited number of times in vitro only in human PBMC cultures and most likely retains sequence heterogeneity. It was characterized by sequence analysis of its gp120 V3 region and gp41 sequence and evaluated by neutralization susceptibility to anti-gp120 and anti-gp41 MAbs. Thus, for all of these reasons, isolate 5016 was the choice for use in this study rather than challenge stocks prepared from laboratory strain IIIB (2), pre-

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cell culture-adapted SF-2 replicated only in PBMC culture (25), or DH12, a primary isolate demonstrated to replicate vigorously in chimpanzees but lacking the 2F5 determinant (35).

We report the preexposure passive protection study performed in the chimpanzee model by using antibody 2F5 and primary isolate 5016. This study characterizes the effects of the presence of virus neutralizing antibody in chimpanzees on the outcome of the challenge and the infection through 1 year postchallenge.

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MATERIALS AND METHODS

Source of MAb 2F5. MAb 2F5 was prepared from the bulk harvest of a standard stirred tank bioreactor culture (150 liters) of the hybrid cell line IAM 2F5 as previously described (24). The purification procedure (epitope affinity, anion-exchange, and cation-exchange chromatography) gave greater than 95% monomeric immunoglobulin G3. The antibody was formulated as an aqueous phosphate-buffered saline solution at 2 mg/ml with additional stabilizers consisting of clinical-grade human albumin (10 mg/ml), 0.8 mM acetyl-tryptophan, and 0.8 mM caprylic acid (pH 7.2). The material passed sterility testing, was negative for endotoxin, and was negative for skin hypersensitivity tests in the study animals.

Source of primary isolate 5016. Virus 5016 was isolated from a stimulated PBMC culture of cells from an HIV-1-positive individual in 1992. All additional passage stocks were prepared only in freshly prepared and stimulated donor PBMC cultures. Following the initial culture, a passage 1 stock of approximately 30 1-ml aliquots was made. After two additional passes in PBMC cultures, a stable and consistently high antigen output was found. A second passage 3 stock was made and designated the potential challenge stock. This stock was made by methods described previously that include collection of virus produced during the last 72 h of a 7-day incubation of infected PBMC cultures (8). The challenge stock had a p24 antigen content of 380 ng/ml. The challenge dose, a 1:10 dilution of the stock, was 30,000 50% tissue culture infective doses, as determined by retitration in human PBMC culture on the day of use. Sterile physiological saline containing 20% autologous, heat-inactivated, sterile serum was the diluent used to prepare the challenge virus for injection. Isolate 5016 was determined to have a typical clade B V3 region by sequence analysis and was neutralized in vitro in human PBMC cultures by the potent anti-gp120 V3 antibodies 447-52D and 19b (8, 23). The gp41 ectodomain region of isolate 5016 contains the ELDKWA motif, and the isolate also was neutralized in vitro by MAb 2F5 by using the infectivity reduction method (6, 9). The actual challenge dose was neutralized in vitro in infectivity reduction assays by all concentrations of MAb 2F5 greater than 62.5 μg/ml.

Preexposure prophylactic study design. This study included one male and three female chimpanzees ranging in age from 6 to 8 years. Two chimpanzees received an intravenous infusion of MAb 2F5 at a dosage of 15 mg of antibody per kg of body weight over 50 to 60 min. On the following day, these chimpanzees and an uninfused control animal were challenged intravenously with the 5016 stock. The intravenous challenge of a second control chimpanzee preceded this procedure and was performed in the same manner. Blood samples were obtained from each chimpanzee on the day of challenge, at weekly intervals through week 3, biweekly through week 16, and then monthly through 1 year postchallenge. These samples were used for quantitation of antibody 2F5, T-cell subsets, mitogen responsiveness, and the presence and titer of anti-HIV-1 antibodies. PCR assays were performed to determine the presence of cell-associated HIV-1 DNA and quantitate serum RNA genomic copy equivalents. Three culture methods were used for virus isolation studies. Physical examinations were performed on these animals at the time of each sample collection.

Sequence analysis of viruses isolated from chimpanzees. To determine whether any changes occurred in vivo in the region of gp41 recognized by antibody 2F5, sequences of the *env* gene encompassing envelope residues approximately 630 to 675 were determined for each sample in question. Virus stocks were extracted, and the isolated RNA was transcribed with a GeneAmp kit (Perkin-Elmer/Cetus), using primer 5′-$(CAU)_4$CCTGCCTAACTCTATTCA CT-3′, in the reverse transcriptase reaction, and amplified after addition of the second primer, 5′-$(CUA)_4$CTGTGCCTTGGAATGCTAGT-3′, by methods described previously (9). PCR products were cloned by reacting with uracil DNA glycosylase while annealing to the pAMP vector plasmid from the CloneAMP system (Life Technologies). Cloned viral fragments were identified and sequenced as described previously (8, 9).

Measurement of anti-HIV-1 antibodies in chimpanzee serum. A peptide containing the sequence ELLELDKWASLWNC (custom synthesis; Multiple Peptide Systems) was coated on microtiter plate (Nunc) wells at 0.5 μg overnight at 4°C. Coated plates were washed and blocked with porcine skin gelatin (Sigma) as previously described (7, 14). Dilutions of chimpanzee sera were reacted, and bound antibodies were detected with alkaline phosphatase-conjugated goat anti-human antibody as described previously (7). Known standard solutions of MAb 2F5 were used in parallel control enzyme-linked immunosorbent assays (ELISAs). The dynamic range of MAb 2F5 reactivity in the peptide ELISA was 0.15 to 2.0 μg/ml, with a sensitivity 25-fold greater than that of a test using commercially available recombinant gp41. The concentration of 2F5 in serum samples was determined by comparison with the reactivity of the standards. HIV-1 Western blot (immunoblot) kits (Cambridge Biotech) were used to monitor the course of anti-HIV-1 antibody specificity development and seroconversion in all chimpanzees. Anti-HIV-1 antibody endpoint titers were determined by use of commercial ELISA kits (Immunodiagnostics or Vironostika HIV-1 MicroELISA system; Organon Teknika).

Peptide competition of 2F5 binding. Two peptides, NEQELLELDKWASL WNC and NEQKLLELDKWASLWNC, were obtained as custom syntheses (Multiple Peptide Systems). These differ from each other at a position three residues from the determinant for MAb 2F5 (both the variation position and the 2F5 determinant are underlined in the peptide sequences). Since the peptide used in ELISA (described above and in reference 9) bound MAb 2F5 efficiently, the peptides were used in the same ELISA format to confirm antibody binding to the level comparable to that for the ELLELDKWASLWNC peptide. Once binding capacity was confirmed, each peptide was used to coat separate plates for use in a binding comparison by solution competition. Each peptide was allowed to bind to a range of MAb 2F5 concentrations, using a constant peptide concentration in solution that was equivalent to the coated peptide concentration. From this experiment, it was possible to determine if one peptide was more efficient in competition. The concentrations of peptide solutions were verified by amino acid content analysis.

PBMC-associated viral DNA, serum virus-specific RNA, and isolation of virus from chimpanzee PBMC postchallenge. Virus-specific DNA in chimpanzee PBMC was determined by using methods described previously (12). Briefly, 10^7 cells were used to prepare genomic DNA. HIV-1-specific *gag* gene sequences in the 1- to 3-μg sample were amplified by PCR using the SK38/39 primer set.

Viral RNA in serum was quantitated commercially (Lab Corp.) by using a single-tube, internally standardized PCR procedure. Results are given as genome equivalents per milliliter. The method has a 4- to 5-log-unit dynamic range and a sensitivity of 200 genome equivalents per ml.

For virus isolation, three culture methods were used. Purified chimpanzee PBMC (10×10^6) were stimulated with phytohemagglutinin and cultivated either in medium with interleukin-2 (IL-2) or in medium without IL-2 or with IL-2 plus an equal number of separately mitogen-stimulated (3 days) human PBMC. Cultures were maintained and monitored for p24 antigen production for 1 month.

RESULTS

Isolate 5016 infection in control chimpanzees. An early feature of the infection in the two control chimpanzees (299 and 332) was the presence of greater than 1,000 RNA genome equivalents in the serum by week 1 postchallenge (Tables 1 and 2). Week 1 virus isolation cultures from chimpanzee 332 and week 2 cultures from chimpanzee 299 were positive. Serum viral RNA levels continued to rise and peaked at week 4 at 4.4 million in chimpanzee 299 and at week 3 at 4.3 million in chimpanzee 332. Both animals seroconverted in either week 3 or week 4 (Fig. 1A [data only for chimpanzee 299]). Anti-HIV-1 antibody titers rose and remained in the range of 1:25,000 to 1:100,000 through approximately week 36 postinfection. Antibody titers dropped slightly in chimpanzee 299 between weeks 36 and 52, with the lowest measurement at week 40. The post-week 36 titers in control chimpanzee 332 were maintained through week 52. Chimpanzee 299 samples had only one positive virus isolation culture post-week 12, while the majority of chimpanzee 332 cultures were positive for virus isolation through the entire year postchallenge.

Viral RNA patterns in serum samples of control chimpanzees were similar until week 24, when a steep decline occurred for chimpanzee 332. Although levels in the latter samples were higher, 50,000 to 70,000 equivalents per ml, a pattern of decline was apparent. Viral RNA levels in the week 48 sample from chimpanzee 299 were near baseline but still measurable. Thus, anti-HIV-1 antibody and viral genomic RNA levels in

TABLE 1. Infection in control chimpanzee 299

Time post-challenge	Virus isolation	Anti-HIV-1 antibody		Virus genome copy no./ml of serum	DNA PCR
		ELISA titer	Western blot reactivity		
Day −1	ND[a]				
Wk					
0	−	<50	−	<200	−
1	−	<50	−	2.6×10^3	+
2	+	<50	−	2.0×10^5	+
3	−	<50	−	2.2×10^6	+
4	+	<50	+	4.4×10^6	+
6	+	3,200	+	1.0×10^6	−
8	+	51,200	+	2.9×10^5	−
10	+	102,400	+	5.5×10^4	+
12	+	51,200	+	6.3×10^4	+
14	−	51,200	+	3.6×10^4	+
16	−	25,600	+	5.4×10^4	+
20	+	25,600	+	7.5×10^4	+
24	−	51,200	+	7.0×10^4	+
28	−	102,400	+	1.7×10^4	+
32	−	51,200	+	9.1×10^3	−
36	−	51,200	+	9.9×10^3	
40	−	6400	+	1.8×10^3	
44	−	ND	+	3.0×10^3	
48	−	12,800	+	2.4×10^2	
52	−	12,800	+	8.8×10^2	

[a] ND, not done.

sera remained high in control chimpanzees through the acute phase of infection and up to year 1 postchallenge.

Levels of MAb 2F5 in experimental chimpanzees. To determine any possible relationship between level of antibody and virus load in antibody-infused chimpanzees, we developed an ELISA specific for MAb 2F5 which was based on reactivity to a peptide with the sequence ELLELDKWASLWNC. Experimental chimpanzee 318 had 293 μg of 2F5/ml of serum at the time of challenge (Table 3), with the last measurable quantity at 8.5 μg/ml in week 3 postchallenge serum. Experimental chimpanzee 325 had 432 μg of 2F5 per ml of serum at the time of challenge, and antibody remained detectable in the 1.0-μg/ml range as late as weeks 8 to 10 (Table 4). Measurement of 2F5 by peptide ELISA was consistent with the Western blot reactivity of these samples (Fig. 1B and C). Anti-gp41 reactivity was apparent, and this reactivity declined in parallel with the same decline observed by ELISA analysis. Thus, a 15-mg/kg of body weight infusion of MAb 2F5 gave levels in the peripheral circulation of 290 to 430 μg/ml of serum at day 1 postinfusion. This antibody's pharmacokinetic pattern was consistent with patterns observed for other passively transferred human antibodies (1, 36).

TABLE 2. Infection in control chimpanzee 332

Time post-challenge	Virus isolation	Anti-HIV-1 antibody		Virus genome copy no./ml of serum	DNA PCR
		ELISA titer	Western blot reactivity		
Day −1	−	<50	−	<200	
Wk					
0	−	<50	−	<200	−
1	+	<50	−	5.6×10^3	−
2	+	<50	−	1.2×10^6	+
3	+	<50	+/−	4.3×10^6	+
4	ND[a]	ND	ND	ND	ND
6	+	12,800	+	3.3×10^5	+
8	+	12,800	+	1.9×10^5	+
10	+	25,600	+	5.5×10^4	+
12	+	51,200	+	4.8×10^4	+
14	+	51,200	+	8.2×10^4	+
16	−	51,200	+	2.9×10^4	+
20	+	102,400	+	1.7×10^4	+
24	+	51,200	+	1.7×10^3	+
28	+	ND	+	1.4×10^4	+
32	−	102,400	+	5.4×10^4	+
36	+	25,600	+	1.3×10^4	+
40	−	25,600	+	6.2×10^3	
44	+	51,200	+	6.4×10^4	
48	+	51,200	+	7.6×10^3	
52	+	ND	+	3.7×10^3	

[a] ND, not done.

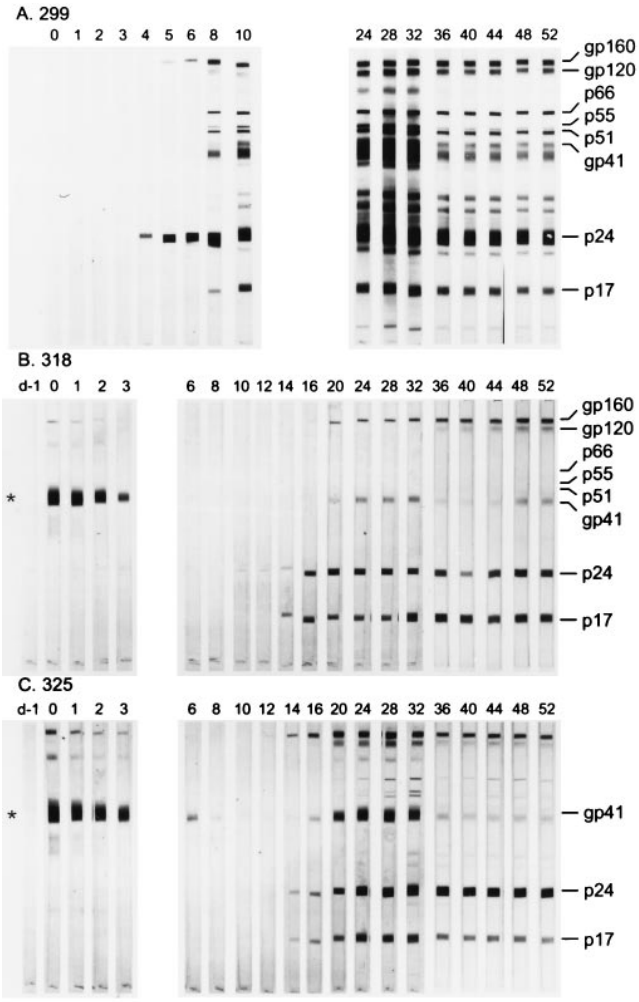

FIG. 1. Detection of seroconversion to HIV-1 antigen reactivity in chimpanzee sera by Western immunoblot analysis. (A) Reactivities of samples for control animal 299. The horizontal numbers refer to the week of the sample postchallenge. The vertical numbers on the right identify the positions of a select group of HIV-1 antigen positions in the strips. (B and C) Reactivities of samples from MAb 2F5-infused animals 318 (B) and 325 (C). The asterisks to the left of lanes d-1 (day −1) in panels B and C indicate the positions of the major reactive band (gp41) detected by the infused antibody. The infused antibody also detected gp160 and some possible gp160 degradation products that migrated more rapidly than gp160. The observed change in signal intensity that is seen between week 32 and week 36 samples is due to a change of lot of the Western blot kit (samples with the more intense signals give less intense signals when rerun with kits of the later lot).

TABLE 3. Infection in MAb-2F5 infused chimpanzee 318

Time post-challenge	Virus isolation	Anti-HIV-1 antibody		MAb 2F5, peptide ELISA titer (μg/ml)	Virus genome copy no./ml of serum	DNA PCR
		ELISA titer	Western blot reactivity			
Day −1	−	<50	−	0	<200	
Wk						
0	−	<50	Anti-gp41 (+)	293	<200	ND[a]
1	−	<50	Anti-gp41 (+)	95	<200	−
2	−	<50	Anti-gp41 (+)	27.5	<200	−
3	−	<50	Anti-gp41 (+)	8.5	<200	−
4	ND	ND	ND	ND	ND	ND
6	−	<50	−	0	<200	−
8	−	<50	−	0	7.2×10^2	−
10	−	<50	−	0	4.8×10^3	−
12	−	<50	−	0	4.2×10^3	+
14	−	50	+/−	0	5.6×10^4	−
16	+	200	+	0	3.6×10^6	+
20	−	ND	+		3.0×10^5	+
24	−	6,400	+		7.8×10^3	+
28	−	ND	+		2.4×10^3	+
32	−	6,400	+		4.8×10^3	
36	−	6,400	+		7.6×10^3	
40	−	3,200	+		2.5×10^3	
44	−	12,800	+		4.3×10^3	
48	−	12,800	+		1.9×10^3	
52	−	ND	+		1.3×10^3	

[a] ND, not done.

Isolate 5016 infection in chimpanzees infused with antibody. In contrast to the immediate virus production in the control 5016-infected chimpanzees, no virus replication was observed from weeks 0 through 8 in the antibody-infused chimpanzees (Tables 3 and 4). Thus, when antiviral neutralizing antibody was present in the peripheral circulation, there was no detectable replication of the primary isolate virus. The first indication that isolate 5016 infection was established in chimpanzee 318 was the presence of virus-specific RNA in the serum as determined by the RNA PCR test. The level rose from an initial low of 720 copies per ml at week 8 to a peak of 3.6 million copies per ml at week 16. Compared with the appearance of RNA in

TABLE 4. Infection in MAb 2F5-Infused chimpanzee 325

Time post-challenge	Virus isolation	Anti-HIV-1 antibody		MAb 2F5, peptide ELISA titer (μg/ml)	Virus genome copy no./ml of serum	DNA PCR
		ELISA titer	Western blot reactivity			
Day −1	−	<50	−	0	<200	
Wk						
0	−	<50	Anti-gp41 (+)	432	<200	−
1	−	<50	Anti-gp41 (+)	151	<200	−
2	−	<50	Anti-gp41 (+)	89	<200	+
3	−	<50	Anti-gp41 (+)	52	<200	−
4	ND[a]	ND	ND	ND	ND	ND
6	−	<50	Anti-gp41 (+)	7	<200	−
8	−	<50	−	1	<200	−
10	−	<50	−	<1	<200	−
12	−	<50	−	0	<200	−
14	−	50	+	0	6.4×10^2	+
16	−	200		0	2.2×10^2	−
20	−	ND	+		2.6×10^4	+
24	−	6,400	+		1.2×10^4	+
28	−	ND	+		4.7×10^3	−
32	−	1,600	+		5.8×10^3	
36	−[b]	400	+		4.7×10^3	
40	−	1,600	+		2.3×10^3	
44	−	6,400	+		1.9×10^3	
48	−	3,200	+		1.0×10^4	
52	−	ND	+		4.8×10^2	

[a] ND, not done.
[b] Coculture of 10^6 cells from week 36 lymph node biopsy with donor cells was virus positive. Both left and right inguinal lymph nodes were biopsied at this time. The right node material was negative in the DNA PCR test and in virus culture, while the left node material was positive for both tests.

5016 Virus Source	Sequence	Total Found/ Number Sequenced
Challenge stock	MEWEREIN NYTGLIYTLI EESQNQQEKN EQ**ELLELDKWA** SLWNWFDITN WLWYI	
animal no. 299	....GLIYTLI EESQNQQEKN EQ**ELLELDKWA** SLWNWFDITN WLWYI	5/5
animal no. 332	.....IN NYTGLIYTLI EESQNQQEKN EQ**ELLELDKWA** SLWNWFNITN WLWYI	4/4
animal no. 315 (wk. 16)	MEWEREIN NYTGLIYTLI EESQNQQEKN EQ**KLLELDKWA** SLWNWFDITN WLWYI	9/12
	-------- ---------- ---------- ---------- --*------- -----	2/12
	V - . .---------- ------N--- -----	1/12
animal no. 325 (lymph node)	MEWEREIN NYTGLIYTLI EESQNQQEKN EQ**ELLELDKWA** SLWNWFDITN WLWYI	9/9

FIG. 2. Amino acid sequences of the HIV-1 envelope glycoprotein region encompassing the 2F5 determinant for the challenge stock of 5016 and virus isolates from the chimpanzees used in this study. The sequences were derived from DNA sequence analysis of genes from these viruses. The residues of the 2F5 determinant are in boldface, as are the positions of the differing residue from animal 318 and the colinear positions in the other isolates. Three additional changes in this region that were observed are underlined. The asterisk indicates the position and presence of stop codons in clones derived from animal 318. Dots indicate that no sequence was generated; dashes indicate that the residues are identical to the first sequence listed in the set.

the control chimpanzee sera, the interval from initial time of appearance to the peak was delayed (8 weeks versus 2 to 3 weeks). Serum anti-p17 and anti-p24 antibodies were detected starting at week 14, although diversity in the positive Western blot pattern remained restricted for the time points tested through week 52 (Fig. 1B). The corresponding total anti-HIV-1 antibody titer was $\leq$1:12,800 for these time points. Only the week 16 culture of peripheral cells from chimpanzee 318 yielded a virus-positive result. These observations reflect how the course of virus 5016 infection in antibody-infused animals differed from that in control animals in the initial phase.

The appearance of HIV-1 in chimpanzee 325 was delayed to a greater extent. RNA was found in serum in the week 14 sample and remained below 10^3 copies per ml until week 20, when a peak of 2.6×10^4 copies per ml was observed. (The DNA PCR signal observed at week 2 is interpreted to be extraneous and may have arisen from sample contamination.) This low peak was unlike that seen in the other chimpanzees and suggested a difference in the course of the acute infection in chimpanzee 325. Virus was not isolated from the PBMC cultures from this animal (Table 4). To characterize the virus established in chimpanzee 325, a lymph node biopsy was performed at week 36 postinfection. Cocultivation of 10^6 cells from the lymph node gave a positive culture. Total anti-HIV-1 antibody titers remained low through the first year postinfection ($\leq$1:6,400). Also, the sera from this animal had a less diverse Western blot recognition pattern compared with the control, infected chimpanzee sera.

Sequence of the 2F5 determinant in HIV-1 5016-infected chimpanzees. It was important to sequence the gp41 region of the week 16 virus isolate from chimpanzee 318 and the virus from the week 36 lymph node biopsy from chimpanzee 325. A comparison of these sequences with those of the 5016 challenge stock and isolates from the control animals is shown in Fig. 2. Viruses from control animals and the isolate from the lymph node biopsy of chimpanzee 325 had sequences identical to that of the challenge stock virus. In contrast, a sequence variant was present in the week 16 virus stock from animal 318. A Glu-to-Lys change was found at a position three amino acid residues to the amino side of the 2F5 determinant. We hypothesized that such a dramatic mutational change might alter the local conformation in gp41 and influence MAb 2F5 binding to the nearby determinant, thereby changing the antiviral effect. To examine this possibility, we performed infectivity reduction tests with the isolate and found a sensitive pattern similar to that of the challenge stock virus (data not shown). Further, we demonstrated that MAb 2F5 could bind to this mutant sequence by comparing binding to two colinear peptides that differed from each other only in the above-described mutation. Both peptides were equally capable of binding the antibody. However, in peptide ELISA competition experiments, the peptide representing the wild-type sequence (...ELLELDKWA...) was a more efficient competitor of antibody binding to either the wild-type or the mutant peptide (Fig. 3). This observation was consistent whether competition was with the wild type or the mutant (Fig. 3). Thus, the variant (...KLLELDKWA...) from chimpanzee 318 was not an obvious MAb-resistant mu-

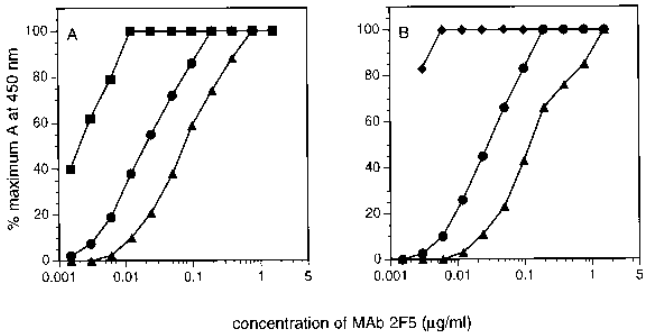

FIG. 3. Reactivities of MAb 2F5 with the indicated peptides. The data are expressed relative to the maximum A_{450} value obtained in the experiment. The 100% A_{450} value was 4.2 in both experiments. Peptide H30-20 contains the ...KLLELDKWA... variant, while peptide H30-21 contains the normal ...ELLELDKWA... sequence. MAb 2F5 bound both peptides as it did peptide E33-35, the peptide with the wild-type sequence previously reported (9). (A) Peptide on plate is H30-21 (normal sequence). ■, no competing peptide; ●, H30-20 ...KLLELDKWA... competitor in solution; ▲, H30-21 ...ELLELDKWA... competitor in solution. (B) Peptide on plate is H30-20 (variant sequence). ◆, no competing peptide; ●, H30-20 ...KLLELDKWA... competitor in solution; ▲, H30-21 ...ELLELDKWA... competitor in solution. Competition reactions were performed with 0.5 μg, which represents an equimolar amount of the bound peptide in each well.

tant but might be a variant selected in vivo in the presence of a high level of virus neutralizing antibody during the acute phase of the infection.

DISCUSSION

This study addresses issues regarding anti-HIV-1 neutralizing antibody and HIV-1 infection in the chimpanzee model. We wanted to determine whether MAb 2F5, an antibody to gp41, had any prophylactic antiviral activity in vivo and whether the presence of the antibody would alter the course of virus replication if an infection was established. We used a preexposure antibody infusion protocol and numerous sample collections for quantitation of virus replication to establish the course of virus infection. Importantly, we used a primary HIV-1 isolate of limited in vitro passage, since the data presented below suggest that use of either strain IIIB or strain SF-2 stock would not have been the optimum possible challenge.

Strain IIIB and SF-2 stocks give reproducible infections in chimpanzees (2, 4, 11). However, as a laboratory-adapted strain, IIIB produced from chronically infected immortalized human T cells (2) can be neutralized in vitro by infected human sera and by experimental antisera generated in a variety of ways by using diverse immunogens (2, 7, 38). Chimpanzees immunized with IIIB-specific immunogens have been protected from the IIIB challenge (3, 13, 15) and indeed protected from the SF-2 challenge (16). Chimpanzees infused with a IIIB-type-specific anti-gp120 V3 region MAb were protected from IIIB infection as well (12), indicating the protective nature of antibodies against this determinant. However, IIIB-specific antibodies appear to have no antiviral effect on primary isolates in vitro in human PBMC cultures. The antiviral property of an antibody can be obtained from neutralization tests in vitro by using primary isolates. It seems to be the most discriminatory in vitro test that can estimate the protective potential of an antibody or antiserum in vivo. The SF-2 challenge stock presents other problems in the chimpanzee model. First, in vitro infectivity determinations and antigenic mass compared with that of the challenge stock are reported to be low (4, 16). Second, in vivo measurements that are available suggest that this virus does not replicate to the levels that have been observed for IIIB (37), acute-phase human virus (22), or primary isolate DH12 or 5016 (6, 35). SF-2 replication usually peaks near 10^2 to 10^3 copies per ml, and that level is not sustainable above the background level of sensitivity much after the peak. Conversely, the other primary isolates reach levels of 10^5 to $>10^6$ copies per ml in vivo either in humans or in chimpanzees. Finally, once infection of SF-2 is established in chimpanzees, antigen recognition patterns observed in Western blots postseroconversion have limited diversity, which is different from the case for infected humans and suggests that continual virus replication is reduced (11). Data reported here show that isolate 5016 replicates efficiently in the chimpanzee, as would be predicted for a typical clade B primary isolate. Measurements of both virus production and anti-HIV-1 antibody show robust replication and an early, strong immune response, in contrast to the patterns for the SF-2 challenge stock. Although envelope sequence variation among these isolates is not an issue for MAb 2F5 and its gp41 binding sequence, neither IIIB nor SF-2 is representative of clade B isolates in regard to the sequence of the gp120 V3 region, which is known to contain a number of determinants that can bind antibodies capable of neutralizing primary isolates (17, 23).

Virus replication eventually began in both antibody-infused chimpanzees, indicating that a single dose of neutralizing antibody did not prevent eventual infection by this highly replicative primary isolate. This conclusion leads to the question of why the infection occurred. The infection proceeded immediately in both control animals but was delayed in antibody-infused animals. Thus, it appears that as long as this neutralizing antibody was present, HIV-1 replication was prevented. We hypothesize that if the levels of neutralizing antibody achieved during the first 3 weeks postinfusion were maintained in the animals by additional infusions, then isolate 5016 might not have replicated. From a practical view, if neutralizing anti-HIV-1 MAbs are used for prophylaxis in humans, methods should be devised to maintain circulating levels for longer periods of time.

In addition to total antibody dose and the eventual outcome postchallenge, the other results of this study may provide hints for how immunoprotection against HIV-1 may be achieved. First, it is important to know where the challenge virus remained during weeks 0 to 8 in chimpanzee 318 and during weeks 0 to 14 in chimpanzee 325, when no replication was detected. Second, it is important to know why a virus with a sequence variation near but not in the determinant was selected in chimpanzee 318. Finally, understanding the unique early time point reduction in virus output in chimpanzee 325 could provide some insight as to mechanisms (possibly antibody mediated) that may control replication in the acute phase.

From the amount of measured antibody at the time of challenge and our in vitro studies of binding and infectivity reduction with MAb 2F5, it seems probable that MAb 2F5 neutralized the entire challenge mass immediately upon infusion. However, when virus-antibody complex particles passed through lymph nodes, they may have been localized to the processes of follicular dendritic cells. Such complexes have been shown to be infectious in vitro (20). It is possible that the virus-antibody complex eventually established the infection within a nearby T-helper cell. We hypothesize that if this was the case, then the infection did not begin while antibody was present and that the time delay observed was brought about by normal reduction in concentration of neutralizing antibody. It is important to note that the amount of antibody infused in a previously successful passive protection study with chimpanzees was over twice the amount used in this study (12). Thus, we further suggest that the amount and/or timing of delivery of a MAb are critical factors for complete immunoprotection against a challenge of isolate 5016. Alternative ways of making active antibodies with longer half-lives or enhancing their ability to reach all necessary cell compartments after infusion should be explored (27).

Once a challenge virus enters susceptible cells, a passively administered neutralizing MAb may not be able to prevent the establishment of a persistent infection. Likewise, nonnucleoside reverse transcriptase inhibitors appear to efficiently suppress detectable viral replication in the chimpanzee model but cannot prevent the latency that leads to eventual infection (32). Enzyme inhibitor antiviral agents do not prevent the entry of virus into susceptible cells; thus, we suggest that the best prophylactic regimens (and potentially therapeutic anti-HIV-1 regimens) will include combinations of both small-molecule antivirals and effective immunological agents.

The presence of the ...KLLELDKWA... variant as an apparently homogenous sequence population in chimpanzee 318 may be correlated to the delay in virus replication until week 8 in this animal. Whether the variant was a preexisting member of the 5016 stock quasispecies is not known. However, variation at this position is highly unusual among field isolate sequences (9, 14). This position, underlined above, is normally

occupied by either Asp or Glu, and to our knowledge, only one infrequently occurring species with Lys at this position has ever been found in a determined gp41 sequence set (14). This sequence change, and the possible change in interaction of 2F5 with the gp41 virion ectodomain, probably influenced the establishment of this variant in animal 318. The variant from chimpanzee 318 is an example of a uniquely derived and unpredicted subtle HIV-1 variant, differing from what would have been predicted in vivo on the basis of in vitro 2F5 assays and in vitro models (5, 26). In light of this variant selection, we hypothesize that a neutralizing antibody, in monotherapy or prophylaxis, would have the same practical limitations as seen in treating HIV-1 infection with small-molecule antiretroviral monotherapy.

Finally, the delay and reduced virus production in chimpanzee 325 suggest that the infusion of MAb 2F5 was involved in altering the course of the acute phase of infection. It has been proposed that acute-phase replication of HIV-1 in humans is reduced largely by emergence of specific $CD8^+$ cytotoxic lymphocytes and virus-binding antibodies (30). The course of virus production in control chimpanzees presented here is representative of a recently proposed model suggesting that the peak of virus production and initial decline in serum virus do not require evocation of an immune response (28). In the control chimpanzees, the initial rise of total anti-HIV-1 antibodies coincided with the decline of circulating viral RNA, and clearly an immune response was elicited (Tables 1 and 2; Fig. 1). Interestingly, our initial studies showed no neutralization of the challenge stock when naive control chimpanzee sera were tested in vitro in PBMC cultures by infectivity reduction (6). The quality of the early immune response in HIV-1-infected chimpanzees should be explored in further detail. However, in chimpanzee 325, the pattern of virus replication does not follow the pattern proposed in the model. Perhaps in this animal, a cellular response that was elicited early could account for the lack of a representative peak of virus replication. In this regard, El-Amad et al. found in an immunized and challenged chimpanzee a lymphoproliferative response to a nonvaccine viral antigen that successfully cleared what may have been a transient infection (11). Since antibody in that chimpanzee was actively elicited rather than passively transferred, sufficient antibody may have been present and sufficient levels may have been maintained in the circulation to act in concert with a developing cellular response and clear the infection. In the case of chimpanzee 325, the eventual decline in MAb 2F5 concentration may have allowed the infection to be established. We previously showed in the simian immunodeficiency virus-rhesus monkey model that a cellular response alone was not sufficient to prevent the establishment of infection (39). It is plausible that effective anti-HIV-1 neutralizing antibodies could be used, or elicited, in the context of developing effective anti-HIV-1 cellular responses.

We continue to examine the course of isolate 5016 infection in these chimpanzees and to study the quality and type of immune responses in these now chronically infected animals.

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REFERENCES

1. **Arizono, H., S. Ishii, T. Nagao, S. Kudo, S. Sasaki, S. Kondo, and M. Kiyoki.** 1994. Pharmacokinetics of a new human monoclonal antibody against cytomegalovirus. Arzneim. Forsch. **44:**890–898.
2. **Arthur, L. O., J. W. Bess, Jr., D. J. Waters, S. W. Pyle, J. C. Kelliher, P. L. Nara, K. Krohn, W. G. Robey, A. J. Langlois, R. C. Gallo, and P. J. Fischinger.** 1989. Challenge of chimpanzees (*Pan troglodytes*) immunized with human immunodeficiency virus envelope glycoprotein gp120. J. Virol. **63:** 5046–5053.
3. **Berman, P. W., T. J. Gregory, L. Riddle, G. R. Nakamura, M. A. Champe, J. P. Porter, F. M. Wurm, R. D. Hershberg, E. K. Cobb, and J. W. Eichberg.** 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. Science **345:**622–625.
4. **Berman, P. W., K. K. Murthy, T. Wrin, J. C. Vennari, E. K. Cobb, D. J. Eastman, M. Champe, G. R. Nakamura, D. Davison, M. F. Powell, J. Bussiere, D. P. Francis, T. Matthews, T. J. Gregory, and J. F. Obijeski.** 1996. Protection of MN-rgp120-immunized chimpanzees from heterologous infection with a primary isolate of human immunodeficiency virus type 1. J. Infect. Dis. **173:**52–59.
5. **Cao, J., L. Bergeron, E. Helseth, M. Thali, H. Repke, and J. Sodroski.** 1993. Effects of amino acid changes in the extracellular domain of the human immunodeficiency virus type 1 gp41 envelope glycoprotein. J. Virol. **67:**2747–2755.
6. **Conley, A. J.** Unpublished results.
7. **Conley, A. J., P. Conard, S. Bondy, C. A. Dolan, J. Hannah, W. J. Leanza, S. Marburg, M. Rivetna, V. K. Rusiecki, E. E. Sugg, F. Van Middlesworth, S. A. Warne, J. T. Ulrich, J. A. Rudbach, R. L. Tolman, and E. A. Emini.** 1994. Immunogenicity of synthetic HIV-1 gp120 V3-loop peptide-conjugate immunogens. Vaccine **12:**445–451.
8. **Conley, A. J., M. K. Gorny, J. A. Kessler II, L. J. Boots, M. Ossorio-Castro, S. Koenig, D. W. Lineberger, E. A. Emini, C. Williams, and S. Zolla-Pazner.** 1994. Neutralization of primary human immunodeficiency virus type 1 isolates by the broadly reactive anti-V3 monoclonal antibody, 447-52D. J. Virol. **68:**6994–7000.
9. **Conley, A. J., J. A. Kessler II, L. J. Boots, J.-S. Tung, B. A. Arnold, P. M. Keller, A. R. Shaw, and E. A. Emini.** 1994. Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, an anti-gp41 human monoclonal antibody. Proc. Natl. Acad. Sci. USA **91:**3348–3352.
10. **Ditzel, H. J., J. M. Binley, J. P. Moore, J. Sodroski, N. Sullivan, L. S. W. Sawyer, R. M. Hendry, W.-P. Yang, C. F. Barbas III, and D. R. Burton.** 1994. Neutralizing recombinant human antibodies to a conformational V2- and CD4-binding site-sensitive epitope of HIV-1 gp120 isolated by using an epitope-masking procedure. J. Immunol. **154:**893–906.
11. **El-Amad, Z., K. K. Murthy, K. Higgins, E. K. Cobb, N. L. Haigwood, J. A. Levy, and K. S. Steimer.** 1995. Resistance of chimpanzees immunized with recombinant $gp120_{SF2}$. AIDS **9:**1313–1322.
12. **Emini, E. A., W. A. Schleif, J. H. Nunberg, A. J. Conley, Y. Eda, S. Tokiyoshi, S. D. Putney, S. Matsushita, K. E. Cobb, C. M. Jett, J. W. Eichberg, and K. K. Murthy.** 1992. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. Nature (London) **355:**728–730.
13. **Fultz, P. N., P. Nara, F. Barre-Sinoussi, A. Chaput, M. L. Greenberg, E. Muchmore, M.-P. Kieny, and M. Girard.** 1992. Vaccine protection of chimpanzees against challenge with HIV-1-infected peripheral blood mononuclear cells. Science **256:**1687–1690.
14. **Geffin, R. B., G. B. Scott, M. Melenwick, C. Hutto, S. Lai, L. J. Boots, P. M. McKenna, J. A. Kessler II, and A. J. Conley.** Association of antibody reactivity to ELDKWA, a gp41 neutralization epitope, with disease progression in children perinatally infected with human immunodeficiency virus type 1. Submitted for publication.
15. **Girard, M., M.-P. Kieny, A. Pinter, F. Barre-Sinoussi, P. Nara, H. Kolbe, K. Kusumi, A. Chaput, T. Reinhart, E. Muchmore, J. Ronco, M. Kaczorek, E. Gomard, J.-C. Gluckman, and P. N. Fultz.** 1991. Immunization of chimpanzees confers protection against challenge with human immunodeficiency virus. Proc. Natl. Acad. Sci. USA **88:**542–546.
16. **Girard, M., B. Meignier, F. Barre-Sinoussi, M.-P. Kieny, T. Matthews, E. Muchmore, P. L. Nara, Q. Wei, L. Rimsky, K. Weinhold, and P. N. Fultz.** 1995. Vaccine-induced protection of chimpanzees against infection by a heterologous human immunodeficiency virus type 1. J. Virol. **69:**6239–6248.
17. **Gorny, M. K., A. J. Conley, S. Karwowska, A. Buchbinder, J.-Y. Xu, E. A. Emini, S. Koenig, and S. Zolla-Pazner.** 1992. Neutralization of diverse human immunodeficiency virus type 1 variants by an anti-V3 human monoclonal antibody. J. Virol. **66:**7538–7542.
18. **Gorny, M. K., J. P. Moore, A. J. Conley, S. Karwowska, J. Sodroski, C. Williams, S. Burda, L. J. Boots, and S. Zolla-Pazner.** 1994. Human anti-V2 monoclonal antibody that neutralizes primary but not laboratory isolates of human immunodeficiency virus type 1. J. Virol. **68:**8312–8320.
19. **Gorny, M. K., J.-Y. Xu, V. Gianakakos, S. Karwowska, C. Williams, H. W. Sheppard, C. V. Hanson, and S. Zolla-Pazner.** 1991. Production of site-selected neutralizing human monoclonal antibodies against the third variable domain of the human immunodeficiency virus type 1 envelope glycoprotein. Proc. Natl. Acad. Sci. USA **88:**3238–3242.
20. **Heath, S. L., J. G. Tew, J. G. Tew, A. K. Szakal, and G. F. Burton.** 1995. Follicular dendritic cells and human immunodeficiency virus infectivity. Nature (London) **377:**740–744.
21. **Johnson, B. K., G. A. Stone, M. S. Godec, D. M. Asher, D. C. Gajdusek, and C. J. Gibbs, Jr.** 1993. Long-term observations of human immunodeficiency

virus-infected chimpanzees. AIDS Res. Hum. Retroviruses **9:**375–378.
22. **Kahn, J. O., K. S. Steimer, J. Baenziger, A.-M. Duliege, M. Feinberg, T. Elbeik, M. Chesney, N. Murcar, D. Chernoff, and F. Sinangil.** 1995. Clinical, immunologic, and virologic observations related to human immunodeficiency virus (HIV) type 1 infection in a volunteer in an HIV-1 vaccine clinical trial. J. Infect. Dis. **171:**1343–1347.
23. **Moore, J. P., A. Trkola, B. Korber, L. J. Boots, J. A. Kessler II, F. E. McCutchan, J. Mascola, D. D. Ho, J. Robinson, and A. J. Conley.** 1995. A human monoclonal antibody to a complex epitope in the V3 region of gp120 of human immunodeficiency virus type 1 has broad reactivity within and outside clade B. J. Virol. **69:**122–130.
24. **Muster, T., F. Steindl, M. Purtscher, A. Trkola, A. Klima, G. Himmler, F. Rüker, and H. Katinger.** 1993. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. J. Virol. **67:**6642–6647.
25. **Murthy, K. K., K. E. Cobb, Z. El-Amad, D. R. Lee, W. Satterfield, M. Kalish, R. C. Kennedy, K. Steimer, A. Schultz, and J. Levy.** 1993. Titration of an HIV-1 SF2 virus stock in chimpanzees, poster 80. Presented at the Conference on Advances in AIDS Vaccine Development, Sixth Annual Meeting of the National Cooperative Vaccine Development Groups for AIDS, Alexandria, Va.
26. **Neurath, A. R., N. Strick, K. Lin, and S. Jiang.** 1995. Multifaceted consequences of anti-gp41 monoclonal antibody 2F5 binding to HIV type 1 virions. AIDS Res. Hum. Retroviruses **11:**687–696.
27. **Pardridge, W. M., J. Buciak, J. Yang, and A. Diagne.** 1994. Treatment of human immunodeficiency virus-infected lymphocytes with cationized human immunoglobulins. J. Infect. Dis. **170:**563–569.
28. **Phillips, A. N.** 1996. Reduction of HIV concentration during acute infection: independence from a specific immune response. Science **271:**497–499.
29. **Purtscher, M., A. Trkola, G. Gruber, A. Buchacher, R. Predl, F. Steindl, C. Tauer, R. Berger, N. Barrett, A. Jungbauer, and H. Katinger.** 1994. A broadly neutralizing human monoclonal antibody against gp41 of human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses **10:**1651–1658.
30. **Safrit, J. T., and R. A. Koup.** 1995. The immunology of primary HIV infection: which immune responses control HIV replication? Curr. Opin. Immunol. **7:**456–461.
31. **Sattentau, Q. J., S. Zolla-Pazner, and P. Poignard.** 1995. Epitope exposure on functional, oligomeric HIV-1 gp41 molecules. Virology **206:**713–717.
32. **Schleif, W. A., K. A. Murthy, V. V. Sardana, C. L. Schneider, V. W. Byrnes, K. E. Cobb, E. Roth, J. A. Wolfgang, J. M. Hoffman, A. M. Smith, K. M. Gelotte, and E. A. Emini.** 1994. Attempted prophylaxis of human immunodeficiency virus type 1 infection in chimpanzees with a nonnucleoside reverse transcriptase inhibitor. AIDS Res. Hum. Retroviruses **10:**107–110.
33. **Schuitemaker, H., L. Meyaard, N. A. Kootstra, R. Dubbes, S. A. Otto, M. Tersmette, J. L. Heeney, and F. Miedema.** 1993. Lack of T cell dysfunction and programmed cell death in human immunodeficiency virus type 1-infected chimpanzees correlates with absence of monocytotropic variants. J. Infect. Dis. **168:**1140–1147.
34. **Scott Jr., C. F., S. Silver, A. T. Profy, S. D. Putney, A. Langlois, K. Weinhold, and J. E. Robinson.** 1990. Human monoclonal antibody that recognizes the V3 region of human immunodeficiency virus gp120 and neutralizes the human T-lymphotropic virus type III_{MN} strain. Proc. Natl. Acad. Sci. USA **87:**8597–8601.
35. **Shibata, R., M. D. Hoggan, C. Broscius, G. Englund, T. S. Theodore, A. Buckler-White, L. O. Arthur, Z. Israel, A. Schultz, H. C. Lane, and M. A. Martin.** 1995. Isolation and characterization of a syncytium-inducing, macrophage/T-cell line-tropic human immunodeficiency virus type 1 isolate that readily infects chimpanzee cells in vitro and in vivo. J. Virol. **69:**4453–4462.
36. **Stephens, S., S. Emtage, O. Vetterlein, L. Chaplin, C. Bebbington, A. Nesbitt, M. Sopwith, D. Athwal, C. Novack, and M. Bodmer.** 1995. Comprehensive pharmacokinetics of a humanized antibody and analysis of residual anti-idiotypic responses. Immunology **85:**668–674.
37. **ten Haaft, P., M. Cornelissen, J. Godsmit, W. Koornstra, R. Dubbes, H. Niphuis, M. Peeters, C. Thiriart, C. Bruck, and J. L. Heeney.** 1995. Virus load in chimpanzees infected with human immunodeficiency virus type 1: effect of pre-exposure vaccination. J. Gen. Virol. **76:**1015–1020.
38. **Wang, C. Y., D. J. Looney, M. L. Li, A. M. Walfield, J. Ye, B. Hosein, J. P. Tam, and F. Wong-Staal.** 1991. Long-term high-titer neutralizing activity induced by octameric synthetic HIV-1 antigen. Science **254:**285–288.
39. **Yasutomi, Y., S. Koenig, R. M. Woods, J. Madsen, N. M. Wassef, C. R. Alving, H. J. Klein, T. E. Nolan, L. J. Boots, J. A. Kessler, E. A. Emini, A. J. Conley, and N. L. Letvin.** 1995. A vaccine-elicited, single viral epitope-specific cytotoxic T lymphocyte response does not protect against intravenous, cell-free simian immunodeficiency virus challenge. J. Virol. **69:**2279–2284.