

# Stimulation of HIV-1-neutralizing antibodies in simian HIV-IIIB-infected macaques

(AIDS/rhesus macaque/virus neutralization)

SYBILLE MÜLLER\*†, DAVID H. MARGOLIN‡, PETER L. NARAI¶, W. GREGORY ALVORD||, AND HEINZ KÖHLER\*\*

\*Immpheron, Inc., 5235 Athens–Boonesboro Road, Lexington, KY 50409-9491; †Beth Israel Deaconess Medical Center, Boston, MA 02215; ‡National Cancer Institute, Frederick, MD 21702-1201; and \*\*University of Kentucky, Lexington, KY 40536-0096

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**ABSTRACT** Previously we have discovered a public idiotope, designated 1F7, that is expressed on antibodies against HIV type 1 (HIV-1) in human and nonhuman primates. To test the potential of mouse monoclonal antibody (mAb) 1F7 as a therapeutic anti-clonotypic antibody in HIV-1-infected patients, we used the simian HIV-IIIB macaque infection model, which mimics several immunological and pathological characteristics of HIV-1 infection in humans. Four healthy simian HIV-infected rhesus monkeys (*Macaca mulatta*) expressing the 1F7 marker on anti-gp120 antibodies were selected for this study. Three monkeys of this group were immunized several times with the murine mAb 1F7 i.v., and one monkey received as control an isotype-matched antibody, TEPC183. No serious side effect or allergic reaction was encountered. Blood collected before and during the immunization and over several months afterward were analyzed for neutralizing antibodies. Significant increases in breadth and potency of HIV-1-neutralizing antibody titers to one or more virus strains were detected in all three of the 1F7-immunized monkeys, but not in the control monkey immunized with TEPC183. These results show that an antibody, recognizing a public idiotope associated with anti-HIV-1 antibodies can function in chronically infected primates as an anti-clonotypic immunogen to boost antibodies that neutralize homologous and heterologous virus strains. This study represents a first step toward the preclinical evaluation of 1F7 as a therapeutic AIDS vaccine.

The humoral immune response to HIV type 1 (HIV-1) infection is inadequate in part because of the narrow range of virus-neutralizing antibodies elicited by the initially infecting virus and the failure to recognize subsequently arising virus variants. The underlying mechanism for this immune defect has been termed “deceptive imprinting” (1) on basis of the “original antigenic sin” concept (2, 3). An effective therapeutic vaccine should broaden the neutralizing response in infected individuals so as to recognize viral variants present in the host quasispecies and to reduce the viral load.

The application of antibodies as immune-stimulating agents is based on two concepts: (i) antibodies can mimic epitopes, as demonstrated by so-called idiotope vaccines (4); and (ii) antibodies that recognize public B cell idiotopes associated with antibodies against related epitopes can function as anti-clonotypic antibodies, similar to anti-clonotypic T cell antibodies (5). mAb 1F7 selectively recognizes an idiotope expressed by a variety of monoclonal and polyclonal anti-HIV-1 antibodies against the viral glycoprotein gp120/160, the core protein p24, and reverse transcriptase (RT). mAb 1F7 recognizes the public idiotope on anti-HIV-1 antibodies in approximately 70% of HIV-1-infected individuals (6). Its wide prev-

alence and broad reactivity suggest that this anti-idiotope mAb should induce correspondingly broad perturbations in the idiotope network. With such broad reactivity, 1F7 is a candidate for an anti-clonotypic stimulatory antibody.

The potential of 1F7 to boost or induce *de novo* neutralizing immunities in infected individuals was tested in a nonhuman primate model (*Macaca mulatta*). The rhesus monkeys selected for this study were chronically infected with a chimeric virus expressing the HIV-1 HXBc2 envelope protein on a simian immunodeficiency virus (SIV) backbone (SHIV-HXBc2), and they had produced antibodies recognizing the viral envelope glycoprotein, HIV-1 gp120 (7).

The SHIV/macaque model is well suited to vaccine-related immunological studies. Macaque immunoglobulins are highly similar to their human homologues (8), and the infecting virus has been engineered and cloned so that all macaques used for this study have received identical inocula of virus. The envelope derives from HIV-1 isolates and therefore expresses serologically relevant neutralization epitopes. In recent studies we have shown that 1F7 idiotope is present on HIV-1 anti-gp120 antibodies produced by many SHIV-infected macaques (9).

In the present study we describe the changes in virus neutralizing antibody activity induced by “vaccination” with mAb 1F7 in four macaques chronically infected with chimeric simian human virus SHIV-HXBc2 (SHIV-IIIB). Two macaques vaccinated with 1F7 responded with a boost of antibodies neutralizing the infecting, homologous HIV-1 IIIB strain, and more significantly, all three 1F7-treated macaques responded with a significant increase of antibodies neutralizing the heterologous HIV-1 MN strain. Therefore, therapeutic “vaccination” with an anti-clonotypic antibody can increase the potency and broaden the breadth of virus neutralizing antibodies in a relevant nonhuman primate model for HIV-1 infection.

## MATERIALS AND METHODS

**Simian/HIV Construction and Infection of Rhesus Monkeys.** The design and preparation of SHIV-HXBc2 (hereafter, SHIV-IIIB) stocks are described elsewhere (7). The rhesus monkeys (*Macaca mulatta*) used in this study were maintained at the New England Regional Primate Research Center in accordance with the guidelines of the Committee on Animals for the Harvard Medical School and the *Guide for the Care and Use of Laboratory Animals* (10). Juvenile macaques were infected by intravenous inoculation with 400 TCID<sub>50</sub> (tissue culture infectious dose yielding 50% positive cultures) of

Abbreviations: SHIV-HXBc2 (IIIB), simian HIV-HXBc2 (IIIB); HIV-1, HIV type 1; SIV, simian immunodeficiency virus; LAV, lymphadenopathy-associated virus; gp120/160, HIV-1 glycoprotein 120/160; p24, core protein 24.

†To whom reprint requests should be addressed. e-mail: Immpheron@aol.com.

¶Present address: Biological Mimetics, Inc., Frederick, MD 21701-4756.

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SHIV-IIIB. The animals selected were infected 2 years prior to the onset of this study. Infection of all animals was confirmed by serologic and virologic assay.

**Selection of SHIV-IIIB-Infected Macaques for 1F7 Inoculation.** Six macaques infected with SHIV-IIIB for 2 years were repeatedly monitored for anti-HIV gp120 antibodies and 1F7 idiotope expression during a period of 2 months (9). Four animals that showed consistent titer of anti-gp120IIIB antibodies and 1F7 idiotope expression over that time period were selected for *in vivo* inoculation with mouse mAb 1F7 or the isotype control mouse myeloma TEPC 183. The selected animals were assayed again for anti-gp120 antibodies and 1F7 idiotope expression before *in vivo* inoculation of mAb 1F7 or TEPC 183. The monkeys had low virus load throughout the study as determined by a commercial p27 antigen test at the threshold of detection level (data not shown). The animals selected for the study had a normal CD4<sup>+</sup>/CD8<sup>+</sup> cell ratio, which ranged from 1.2 to 1.6. No animals showed any signs of disease.

**Preparation and Purification of mAb 1F7 and TEPC 183.** The murine monoclonal anti-idiotypic antibody 1F7 (IgM,  $\kappa$ ) was generated previously by immunizing BALB/c mice with human pooled IgG from HIV-1-infected blood donors (HIVIG) as described (11). The 1F7 hybridoma protein product bound to human polyclonal and monoclonal antibodies derived from HIV-1-infected individuals and captured by HIV-1 antigen, but did not bind to Ig from HIV-1-seronegative donors (6, 11). The hybridoma was subcloned four times, and the subclone 1F7 was amplified as ascites in BALB/c mice and purified on a goat anti-mouse IgM-Sepharose 4B column as described earlier (6, 11). Some batches of monoclonal Ig were obtained by using a modified purification procedure for 1F7. The cells were injected intraperitoneally (i.p.) into SCID mice free of mouse Ig. The protein in ascites fluid was precipitated by 50% saturated (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and centrifuged at 10,000 rpm in a Sorval 1 RC-5B centrifuge for 30 min. The precipitate was dissolved in 10 ml of 0.01 M PBS, and aliquots were distributed into pyrogen-free, sterile plastic vials. Endotoxin concentrations were below the threshold pyrogenic dose (1 ng of endotoxin per kg of body weight) as determined in a commercial quantitative test kit (12).

Commercially obtained TEPC 183 (Sigma) was purified from ascites fluid as described above. TEPC 183 is a mouse myeloma protein and served as isotype control antibody (IgM,  $\kappa$ ) for 1F7.

**Anti-HIV-1 gp120 Antibody and 1F7 Idiotope Expression in Plasma of SHIV-IIIB-Infected Macaques.** Immunoglobulins in plasma recognizing HIV-1 envelope glycoproteins were detected by ELISA described in detail elsewhere (6, 9, 11), using HIV-1 recombinant gp120 IIIB or HIV-1 recombinant gp120 lymphadenopathy-associated virus (LAV) as antigen. Recombinant gp120 IIIB was purchased from Intracel (Cambridge, MA), and recombinant gp120 LAV was obtained through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases). Recombinant gp120 IIIB and recombinant gp120 LAV are closely related and are therefore treated in the present report as one antigen. HIV-1 gp120 LAV envelope protein was a full-length, glycosylated recombinant protein derived from the env gene of HIV-1, produced in insect cells by using the baculovirus expression system and purified under conditions designed to preserve biological activity and tertiary structure (MicroGeneSys, West Haven, CT).

Briefly, microplate wells were coated with 2  $\mu$ g/ml HIV-1 recombinant gp120 IIIB or LAV and incubated at 4°C overnight. After washing three times with PBS/Tween 20, the wells were blocked for 2 hr with 120  $\mu$ l of 3% BSA per well. Plates were washed three times as above and 100  $\mu$ l per well of diluted duplicates of macaques' plasma specimens were added and incubated for another 2 hr. After an additional three washes, 1:4000 diluted goat anti-rhesus IgG coupled to horseradish

peroxidase (Southern Biotechnology) was added, and the mixture was incubated for 2 hr. The binding antibodies were visualized by adding 50  $\mu$ g of well substrate solution (*o*-phenylenediamine; Sigma) to each well. The enzyme/substrate reaction was stopped by 30  $\mu$ l of 2 M sulfuric acid. All incubations were at room temperature unless otherwise noted. Optical density was measured at 490 nm.

1F7 idiotope expression on antibodies against HIV-1 gp120 was detected with a modified sandwich ELISA (6, 9, 11). Briefly, microplate wells were coated with recombinant gp120 and blocked as described above. After washing three times, 100  $\mu$ l of diluted plasma specimens was added per well, and the plate was incubated for 2 hr. Plates were washed three times and 200  $\mu$ g per well mAb 1F7 or TEPC 183 was added at 2  $\mu$ g/ $\mu$ l and incubated for another 2 hr. After washing three times, 1:4000 diluted goat anti-mouse IgM-horseradish peroxidase was added and incubated for 2 hr. mAb 1F7 or TEPC 183 binding was visualized as described above. The mean of duplicate OD readings  $\pm$  SEM is used for data presentation in the graphs.

**Immunization of the Monkeys with Murine mAbs.** Three monkeys were immunized with purified, sterile mAb 1F7 *i.v.* Two monkeys (149-92 and 337-91) received twice-weekly injection of 5 mg of mAb 1F7, resulting in a total dose of 20 mg of 1F7. A third monkey (42-93) received four injections with the isotype-matched antibody control mouse mAb TEPC 183 (IgM,  $\kappa$ ), following the same protocol of injections twice a week. A fourth monkey (441-92) was injected twice-weekly with 5 mg of 1F7, delivering a total dose of 30 mg of 1F7. Blood samples were collected immediately prior to each injection and then weekly, biweekly, and monthly. Plasma was obtained by centrifugation (400  $\times$  g) of EDTA-anticoagulated venous blood samples and was stored frozen at -20°C until use. Prior to ELISA, plasma samples were heat-inactivated (56°C for 40 min) to kill residual virus.

**Determination of Neutralizing Activity of Antibodies Against HIV-1 IIIB and MN.** A quantitative syncytium-forming microassay was employed for detection of the virus-neutralizing antibody response (13). Neutralization titers were determined by using two HIV-1 strains, HIV-1 IIIB and HIV-1 MN. A virus-syncytial-sensitive clone of CEM cells (CEM-SS) develops quantifiable, adherent syncytia (syncytium-forming units; SFUs) on a background of confluent, normal CEM monolayer in 4–6 days. Total infectious virus can be accurately determined in this assay; results using standard HIV-1 stocks showed a close association with the p24 antigen test (13, 14).

Serial dilutions of plasma specimens taken at time points before and after injection with 1F7 mAb or TEPC 183 from the four SHIV-IIIB-infected macaques were screened for antibody neutralizing activity by means of inhibition of syncytium formation. Briefly, CEM-SS cells are plated as described (13) and incubated with duplicates of various preincubated virus/plasma dilutions (50  $\mu$ l per well) at 37°C, in a 5% CO<sub>2</sub> atmosphere, for 60 min. Neutralization was calculated from the reduction of SFUs and represented as the number of syncytia induced by HIV-1 ( $V_n$ ) in the presence of serial 2-fold dilutions of serum divided by the number of total added virus-induced SFUs ( $V_0$ ).

**Statistical Analysis of Antibody Responses.** Viral antigen-binding antibodies and HIV-1 IIIB- and MN-neutralizing antibody titers were subjected to statistical analysis to determine the significance of changes observed after vaccination with mAb 1F7 or control mAb TEPC 183.

Plasma neutralization activity was determined from viral neutralization curves for all animals on given days. Daily curves were fit with a quadratic regression model,  $y = b_0 + b_1x + b_2x^2$ , in which  $y = V_n/V_0$ , the viral surviving fraction, and  $X =$  the log of the plasma dilution. The  $b_0$  parameter describes the  $y$ -intercept of the graph that is the most diluted plasma tested (no neutralizing antibody activity, *i.e.*,  $V_n/V_0 = 1$ ). The  $b_1$  parameter describes the 'slope' of the curve (how rapidly the curve is falling). The  $b_2$  parameter describes how much the curve levels out at higher

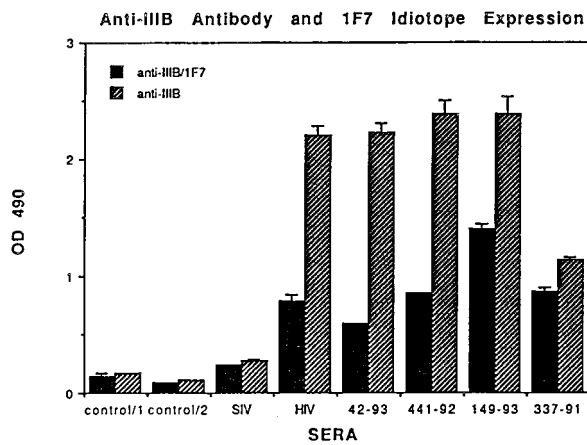


FIG. 1. SHIV-IIIB-infected rhesus monkeys express 1F7 idiotope on anti-gp120 antibodies. Plasma specimens from normal noninfected rhesus monkeys (control/1 and control/2) and SIV-infected rhesus monkey (SIV) together with serum from HIV-1-infected human (HIV) are shown in the first four column pairs. The following pairs show ELISA data from the four SHIV-infected rhesus monkeys selected for mAb vaccination. Anti-gp120 IIIB antibodies reacting with 1F7 (anti-IIIB/1F7, solid bars) and antibodies binding to recombinant gp120 IIIB (anti-IIIB, hatched bars) were detected in independent sandwich ELISAs. A replicate assay yielded similar results.

concentration. Two (or more) curves can be compared by determining whether these parameters across curves are the same or different statistically. If the  $b_0$ ,  $b_1$ , and  $b_2$  parameters do not differ significantly across the curves (i.e.,  $P > 0.05$ ), the curves will look similar. On the other hand, if one or more of the parameters across the curves differ significantly ( $P < 0.05$ ), the graphs will look different. For each animal, we compared daily curves to

baseline ("prebleed") curves (or each other) with the analysis of covariance (ANCOVA). In this framework, hypotheses regarding significant differences in  $b_0$ ,  $b_1$ , and  $b_2$  parameters were performed on the basis of the "extra sums of squares" principle (15) or the principle of "conditional error" (16, 17). In practice, we found that all hypotheses for significant differences in neutralization curves reduced to determining whether there existed significant differences in the  $b_0$  parameters (intercepts) of the two curves being compared.

**RESULTS**

**Expression of 1F7-Positive Antibodies in SHIV-IIIB-Infected Macaques.** In a recent study we screened six SHIV-infected rhesus macaques for the presence of anti-envelope antibodies (LAV and IIIB) in blood (9). We found that two-thirds of the SHIV-IIIB-infected monkeys express the 1F7 marker on anti-HIV-IIIB or -LAV antibodies. Four of these animals were selected for *in vivo* treatment (immunization) with mAb 1F7. All four monkeys contained anti-HIV-1 gp120 IIIB antibodies which were even 1F7 idiotope positive prior to immunization with mAb 1F7 (Fig. 1). As a negative control for the assays, plasma from an SIV-infected macaque that does not contain antibodies against HIV-1 gp120 IIIB is presented. Binding of 1F7-idiotope-expressing serum antibodies directed against HIV-1 gp120 IIIB derived from a human HIV-1-positive individual is shown as positive control to demonstrate the specificity of the assay measuring anti-gp120 IIIB antibody. Furthermore, normal noninfected human and macaque serum showed no reactivity in the 1F7/IIIB ELISA (Fig. 1). In Fig. 1 antibodies binding to gp120 IIIB are shown together with the amount of 1F7 idiotope detected from monkeys 149-93, 42-93, and 441-92 assayed 3 months before the vaccination. Because the ELISAs for anti-envelope and 1F7

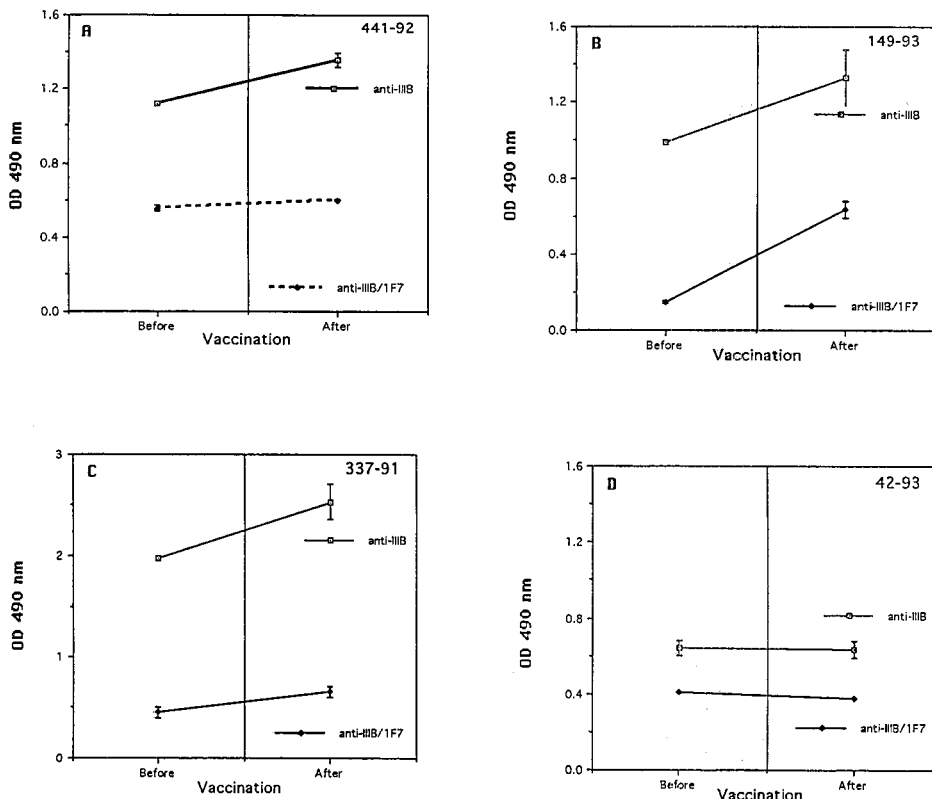


FIG. 2. Sera from selected monkeys 441-92, 149-93, 337-91, and 42-93 were analyzed by ELISA for antibodies binding to gp120 IIIB and 1F7 expression. Macaques 441-91, 149-93, and 331-91 displayed in A, B, and C were inoculated with mAb 1F7; macaque 42-93 was inoculated with isotype control mAb TEPC 183 (D). Prevaccination bleeds and sera from the peak response after vaccination are compared for each monkey as described for Fig. 1. Determinations of triplicate ELISA readings are shown as mean and SD.

idiotope use different reagents in part, the OD values are not quantitatively comparable.

These results indicate that the four selected monkeys produced significant amounts of 1F7-idiotope-expressing anti-envelope antibodies and were therefore suitable for anti-clonotypic stimulation using the mAb 1F7 as therapeutic vaccine.

**Immunization of Three Monkeys with mAb 1F7 and One Monkey with Control Antibody TEPC 183.** Three 1F7-idiotope-positive infected monkeys were injected with mAb 1F7 *i.v.* Monkeys 149-93 and 337-91 each received *i.v.* injections of 5 mg of mAb 1F7 twice a week, resulting in four injections and a total dose of 20 mg of 1F7. Monkey 441-92 was injected with 5 mg of mAb 1F7 twice weekly, delivering a total dose of 30 mg of 1F7. The fourth monkey, 42-93, received four injections with the isotype-matched antibody control mouse mAb TEPC 183 (IgM,  $\kappa$ ), following the same protocol of twice-weekly injections.

**Analysis of Antibodies Against HIV-1 Envelope.** Blood samples were obtained from all monkeys prior to injection and afterward, as specified below. All animals were observed for up to 9 months after the vaccination. No allergic or toxic reactions to the murine mAbs were observed.

Sera were assayed in ELISA against recombinant gp120 IIIB and for 1F7 expression on anti-gp120 IIIB antibodies as described (6, 9, 18). The data from the prebleed are compared with readings of the peak response from each monkey (see Fig. 2). All monkeys vaccinated with 1F7 showed an increase of anti-gp120 titers (Fig. 2 A-C), while the control TEPC 183-vaccinated monkey produced no increase of anti-IIIB titer (Fig. 2D). The 1F7-positive anti-IIIB titers also increased after 1F7 vaccination, although the increase was not as pronounced as in the anti-IIIB titer.

**Analysis of Virus Neutralization Potency.** Blood samples from the three monkeys injected with mAb 1F7 and the control

monkey injected with TEPC 183 were analyzed before, during, and after the vaccination regimen. Serial dilution of plasma specimens collected at different time points during the observation period were analyzed for virus-neutralizing antibody activity by using a quantitative syncytia-forming microassay (13) and HIV-1 strains IIIB and MN. The neutralization activity for HIV-1 IIIB and for HIV-1 MN was derived from five plasma dilutions at day 0 (prebleed), and four times after day 0 for each macaque. To assess the significance of mAb 1F7 inoculation, neutralization curves were constructed on different days and compared with baseline (prebleed) according to the procedures outlined in the statistical analysis section.

(i) **Monkey 441-92.** Monkey 441-92 was vaccinated six times, each with 5 mg of mAb 1F7. After 1F7 vaccination, monkey 441-92 showed increased virus neutralization of both IIIB (Fig. 3A) and MN (Fig. 4A) strains. With respect to virus IIIB, neutralization curves were significantly different from baseline (prebleed) on days 17 ( $P = 0.0012$ ), 35 ( $P = 0.0019$ ), and 94 ( $P = 0.0009$ ). With respect to virus MN, neutralization curves were significantly different from baseline on days 7 ( $P = 0.0044$ ), 17 ( $P = 0.016$ ), 28 ( $P = 0.015$ ), and 94 ( $P = 0.013$ ).

(ii) **Monkeys 149-93 and 337-91.** Monkeys 149-93 and 337-91 were both immunized four times with 5 mg of mAb 1F7 antibody, resulting in a total dose of 20 mg of mAb 1F7 per macaque.

Antibody neutralization activity for HIV-1 IIIB increased significantly after four inoculations of 1F7 in monkey 149-93 as determined at day 31 ( $P = 0.028$ ), Fig. 3B) and 37 ( $P = 0.024$ , Fig. 3B). A transient decrease, however, was detected prior to the increase at day 2 of IIIB virus neutralization (Fig. 3B). Enhanced antibody neutralization activity for HIV-1 MN almost achieved statistical significance for day 31 ( $P = 0.062$ , Fig. 4B) and day 37 ( $P = 0.066$ , Fig. 4B). Monkey 337-91 showed no change in IIIB virus neutralization activity (Fig.

**Virus: III B**

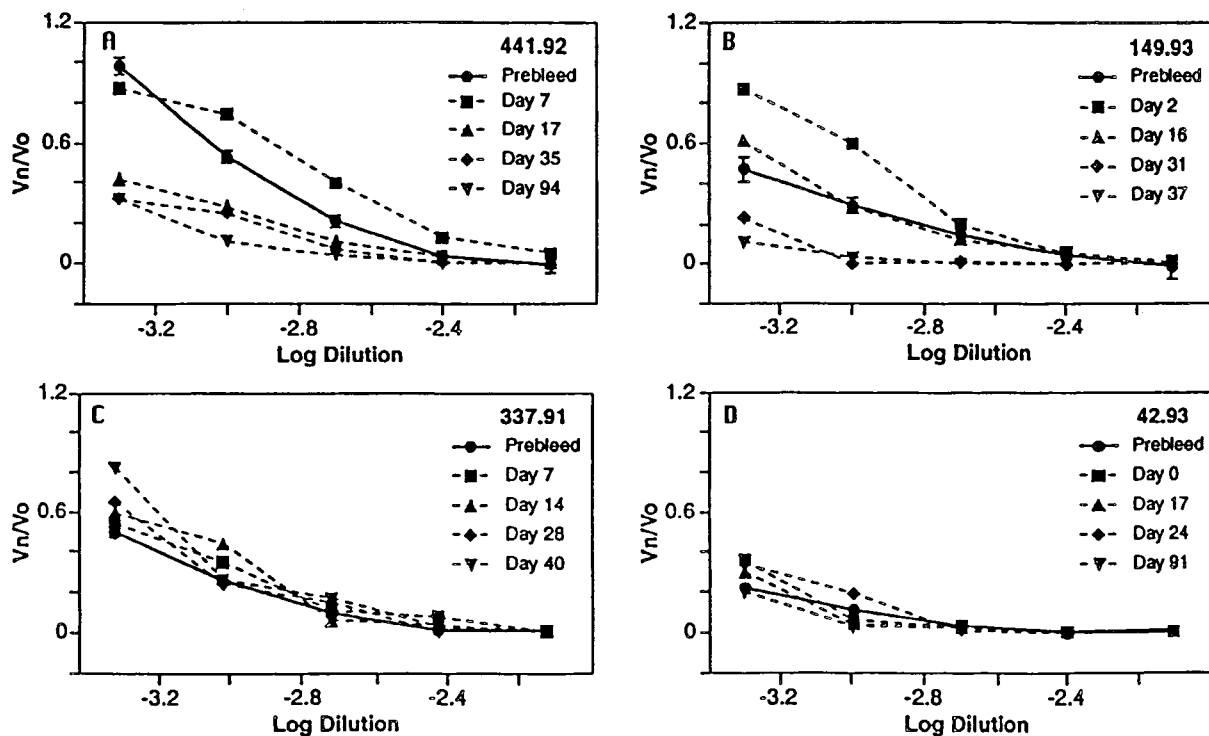


FIG. 3. Effect of 1F7 or TEPC 183 injections on the neutralization response against HIV-1 IIIB in four macaques. Data are shown for the four monkeys as in Fig. 2. Macaques 441-91, 149-93, and 337-91 displayed in A, B, and C were inoculated with mAb 1F7; macaque 42-93 was inoculated with isotype control mAb TEPC 183 (D). Selected time points (prebleed and days after the first mAb inoculation) are represented as serial serum dilutions (*x* axis) versus the reduction in the virus surviving fraction ( $V_n/V_0$ ) on the *y* axis. The vertical bars indicate the standard error of the predicted means from the fitted regression curve (17).

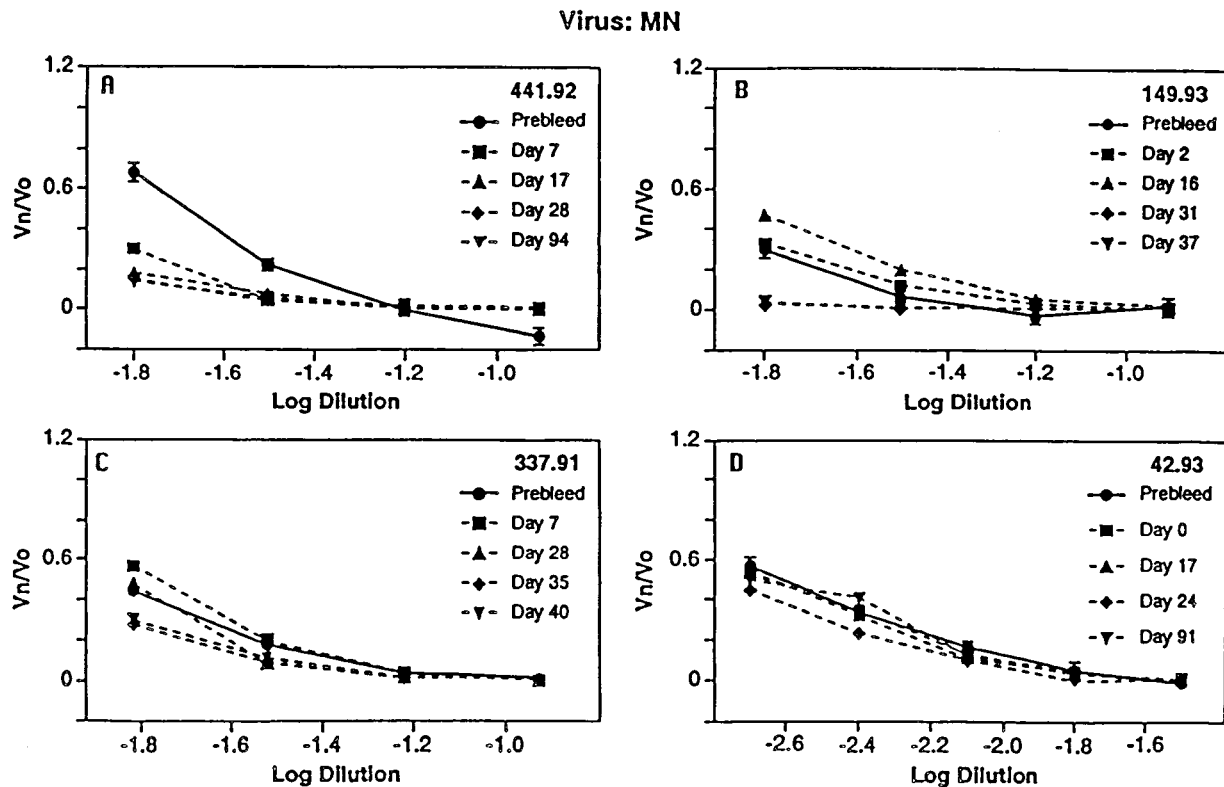


FIG. 4. Effect of 1F7 or TEPC 183 injections on the neutralization response against HIV-1 MN in four macaques. Data are presented as in Fig. 3.

3C), but had significant increases in MN virus neutralization activity on days 35 ( $P = 0.0075$ ) and 40 ( $P = 0.0067$ ) (Fig. 4C).

(iii) **Monkey 42-93 (Control).** To determine whether HIV-1-neutralizing antibodies increased in response to clonotypic stimulation or in a nonspecific reaction to murine mAbs, one SHIV-IIIIB-infected monkey was immunized with the mouse mAb TEPC 183, an isotype matched for 1F7. Monkey 42-93 was immunized four times with 5 mg of mAb, resulting in a total dose of 20 mg of mAb TEPC 183. As shown in Figs. 3D and 4D, the IIIIB and MN neutralizing antibodies did not change before, during, or after the immunization with the isotype control antibody during up to 3 months of follow-up.

In conclusion, the relative changes with respect to the prevaccine neutralizing antibody titers for all four monkeys can be summarized as follows: In monkey 441-92 antibodies neutralizing IIIIB and MN increased after 1F7 vaccination beginning at day 7 and remained higher compared with the prevaccine virus-neutralizing antibody titer. Monkey 149-93 showed an initial decrease of neutralization potency at days 2 and 16 for IIIIB and MN. The neutralization titer, however increased significantly over prevaccine levels by days 31 and 37. In monkey 337-91, no increase of IIIIB neutralization was evident, whereas a statistically significant increase of antibodies neutralizing the nonhomologous MN strain was observed. The control TEPC 183-injected monkey did not show significant changes in either IIIIB or MN neutralization.

These data show that immunization with four and six, *i.v.* doses of 5 mg of mAb 1F7 did induce significant changes, lasting up to 3 months, in the potency of endogenous antibodies mediating neutralization of HIV-1.

## DISCUSSION

Infection with HIV elicits strong immune responses by B lymphocytes and T lymphocytes that appear to be directed at nonprotective, immunodominant epitopes and fail to eradicate

viral infection. This occurs, in part, because the immune system is unable to control the virus variants that arise because of error-prone viral replication and selection pressures, including immunity. The humoral response to viral proteins in HIV-1-infected human and nonhuman primates is characterized by restriction of clonal heterogeneity that is stable in magnitude and antibody composition over time and characteristic for each patient, a sort of "immune fingerprinting" (18–20). Detection of oligoclonal spectrotypes (characterized by a few clusters of bands) of anti-gp120 and -p24 antibodies in sera and cerebrospinal fluid of HIV-1-infected patients has been interpreted as evidence that during infection a limited number of HIV epitope-specific B cell clones are expanded. Similar stability of clonal composition was observed with T cells in HIV-1-infected patients (21). The restriction of the HIV-specific antibody repertoire in HIV-1 and SIV infection is also apparent when the monoclonal anti-idiotypic antibody 1F7 is used (9). The 1F7 mAb recognizes antibodies reactive with several HIV-1 and SIV antigens in the majority of infected subjects (6, 9, 20). Thus, 1F7 functions as public idiotope marker in HIV-1 infection and provides an opportunity to manipulate the anti-viral response in infected individuals.

In HIV-1 infected individuals, circulating antibodies antibodies typically are inefficient in neutralizing the infecting virus. An increase in neutralizing immunity is one aim of both preventive vaccine and immune therapies of HIV-1 infection.

The concept of stimulating the immune response in infected patients is well established, and such a therapeutic vaccine approach has been recently proposed for AIDS by Salk (22). The classical vaccine therapy approach relies on using virus-derived material, such as inactivated virus (22), to boost and broaden the anti-HIV-1 immune response in infected individuals. An alternative approach uses antibodies that either mimic HIV-1 epitopes or function as clonotypic immunogens. Our approach, described here, takes advantage of the oligoclonal nature of the antibody response in HIV-1 infection and the availability of an anti-idiotypic antibody (1F7) that reacts with

antibodies against env and gag viral epitopes (6). mAb 1F7 presumably also reacts with the antigen receptors on B cells capable of producing these antibodies. By analogy with anti-clonotypic antibodies against T cell receptors, 1F7 may also be able to stimulate B cells with the corresponding receptor idiotype. Because 1F7 is not an anti-idiotypic antibody that mimics antigen, the optimal administration is without adjuvant. Thus, using antibodies against public idiotypes becomes an alternative strategy to classical virus-based formulations for developing therapeutic vaccines.

To test the effect of a clonotypic vaccine we have employed the SHIV-infected macaque model. This nonhuman primate model is well suited to evaluate immunostimulatory mechanisms because the chimeric virus expresses human viral envelope protein and induces a 1F7-reactive clonotypically restricted anti-viral response, similar to human HIV-1 infection (9, 20). The pig-tailed monkey (*Macaca nemestrina*) infected with HIV-1 may be an alternative suitable model (23); however, it is not known whether their antibodies' response is 1F7 positive.

This study was conceived as an preclinical pilot trial using a nonhuman primate infection model that shares several immunological characteristics with the human HIV-1 infection, including the variability of the infection and its immune response. Four SHIV-infected macaques were selected that had anti-virus antibodies expressing the public 1F7 idiotype marker. Three monkeys were treated with 1F7, while one monkey was injected with an irrelevant isotype-matched control antibody. Our primary endpoint measure was the potency and strain specificity of virus-neutralizing antibodies during up to 3 months follow-up.

The effect of 1F7 injections on the neutralization profiles and potency of standardized HIV-1 laboratory strains was evaluated. The neutralization curves for all three animals receiving mAb 1F7 shared a significant shift at one or more time points for one or both viruses. Animals 441-92 and 149-93 demonstrated the greatest shifts in their curves for both HIV-1 IIIB and HIV-1 MN. The initial decrease of neutralization on days 2 and 16 in monkey 149-93 might be explained by transient suppression of antiviral antibodies by mAb 1F7 or by immune complex formation with the injected mAb.

It should be noted that the monkey with the strongest increase of neutralizing antibodies (monkey 441-92) had received the largest vaccine dose. This suggests that vaccine dose escalation could enhance the therapeutic effects. The TEPC 183 isotype control antibody-injected animal (42-93) demonstrated no shifts in the neutralization curves for either virus either before, during, or after injections, demonstrating the overall stability of the neutralization response both *in vivo* and *in vitro* over time. Significant shifts in the neutralization curves as compared with "prebleeds" occurred for all animals within 7–35 days after the first injection of 1F7. In two animals (441-92 and 149-93) for both viruses a significant shift in the neutralization curves was noted out to 94 days.

An increase and a broadening of antibodies against the envelope were also detected in solid-phase binding assays (unpublished data). The changes in titer and specificity of anti-gp120 antibodies in 1F7-vaccinated macaques did not exactly parallel the observed changes in neutralizing activities, which is not surprising because it is known that not all envelope-binding antibodies are also virus-neutralizing. A detailed study of the anti-gp120 titer kinetics by using gp120 proteins from various strains (unpublished data) revealed also boosting and broadening effects induced by 1F7 vaccination.

These results demonstrate that 1F7 is capable of modifying an existing virus-neutralizing response and also of inducing operationally "de novo" neutralizing antibodies against the noninfecting MN virus. Regarding the underlying mechanisms, two hypotheses will be tested in follow-up studies: (i) induction of neutralizing antibodies by direct clonotypic stimulation of 1F7-positive B cells; and (ii) suppression of dominant 1F7-positive B

cells, allowing noncommitted B cell precursors to respond to escape virus epitopes as proposed earlier (1, 2).

These increases in potency and breadth of neutralizing activities may have a favorable impact on the virus load and eventually also disease progression. In this study, using the nonpathogenic SHIV-IIIB virus, the virus load is below detectability with standard assays and therefore could not be monitored. In addition, the infected animals remain healthy for years and changes in disease progression were unlikely to occur during the time of observation. Because it has been established that mAb 1F7 can increase and broaden the virus-neutralization activity in chronically infected, healthy monkeys, a follow-up study with animals infected with a more virulent strain is warranted prior to a trial with HIV-1-infected humans.

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