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Postexposure immunoprophylaxis of primary isolates by an antibody to HIV receptor complex

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ABSTRACT **mAb B4 is a monoclonal antibody directed against HIV receptor complex. The antibody had broad neutralizing activity against HIV and provided postexposure prophylaxis to hu–peripheral blood leukocyte (PBL)–severe combined immunodeficient mice and chimpanzees. B4 recognized a complex receptor site for HIV on the T cell surface that includes CD4 and also may be influenced by interaction with HIV coreceptors. mAb B4 preferentially neutralized primary HIV-1 isolates compared with T cell line-adapted strains, including syncytium-inducing and non-syncytium-inducing phenotypes, representatives from HIV-1 subtypes A-G, as well as HIV-2, simian immunodeficiency virus, and chimeric simian/human immunodeficiency virus (SHIV). Neutralization was demonstrated in both pre- and postinfection models. The administration of mAb B4 after infectious challenge totally interrupted the infection of hu-PBL–severe combined immunodeficient mice by PBL-grown HIV-1 and the infection of chimpanzees by chimp-adapted HIV-1. This mode of protection suggested that the anti-HIV receptor antibody is efficacious for prophylaxis after exposure to HIV and for prevention of maternal transmission and may be an effective antiretroviral agent for treatment.**

Progress on HIV neutralizing antibodies for passive immunotherapy has been impeded by the relative resistance of most primary HIV isolates to neutralization by anti-HIV antibodies. Sera from infected individuals commonly have neutralizing activity against T cell line-adapted (TCLA) HIV-1 isolates such as IIIB/LAI and MN, but these sera only occasionally show potent neutralization of primary isolates (1–4). Moreover, most primary isolates are resistant to neutralization by the antibodies induced in volunteer vaccinees by envelope-derived candidate vaccines (4–6). Variable sensitivity to neutralization also remains as an obstacle to the development of antiviral antibodies and virally directed vaccinees with worldwide efficacy (1, 3, 7). Thus, targeting antibodies to a host cell site rather than the virus may facilitate both immunoprophylaxis and vaccine development by circumventing the needs for antibodies to act directly on neutralization-resistant phenotypes and confront the variability of the viral envelope.

A cell-directed approach for protection from HIV exposure was suggested by the ability of certain anti-CD4 monoclonal antibodies (mAbs) to block infection. Anti-CD4 mAb Leu3A blocked infection of cell cultures by primary isolates (8), and mAb P1 with a specificity similar to that of Leu3A broadly inhibited primary isolates of subtypes A, B, C, D, and E (9). However, the *in situ* receptor for HIV is a conformational complex of cell membrane and CD4 closely associated with a chemokine receptor as a coreceptor (10), predominantly CCR5 for M tropic, CXCR4 for T tropic, and both for dual tropic isolates (11–13). HIV env glycoprotein forms a complex with CD4 and the coreceptor that initiates fusion with the host cell membrane and the postentry steps of retrovirus replication (10, 12, 13). Antibodies directed to CD4 or to chemokine receptors have been shown to affect both binding and postbinding steps of HIV infection, and these antibodies neutralized virus-to-cell or cell-to-cell transmission of both syncytium-inducing (SI) and non-syncytium-inducing (NSI) strains of HIV (12–15). An antibody with specificity for the *in situ* receptor complex for HIV may be more broadly efficacious for passive immunotherapy than antibodies targeted to CD4 epitopes alone or to a selected chemokine receptor.

MATERIALS AND METHODS

Antibody Preparation. CD4-reactive mAbs B4, M2, D5, E2, and I26 were produced by hyperimmunization of BALB/c mice with HPB-ALL cells in PBS. Additional CD4-reactive mAbs E6, H5, E31, and J33 were produced by hyperimmunization with recombinant soluble CD4 (rsCD4) in Freund's complete adjuvant. "GP anti-rsCD4" is a high titer polyclonal anti-CD4 serum produced by hyperimmunization of guinea pigs with rsCD4 in Freund's complete adjuvant.

rsCD4 ELISA. ELISAs for binding to rsCD4 (Table 1) were done in microtiter plates coated with rsCD4 (American Biotechnologies, Columbia, MD) at 0.25 μg/ml. The plate coating and assay procedures were as described (16, 17).

Cell Binding Assay. For indirect immunofluorescence staining, 0.5×10^6 HPB-ALL cells per well were washed and incubated in 50 μl of murine CD4-reactive monoclonal antibody at 10 μg/ml (plateau concentration) or diluted guinea pig anti-rsCD4 serum, and bound antibody detected by FITC-conjugated goat anti-mouse IgG or FITC-conjugated goat anti-guinea pig IgG (Cappel). The stained cells were analyzed by fluorescence microscopy and cytofluorography (EPICS, Coulter). Cells were scored for percent of stained cells and for intensity of staining on a scale of 0 to +3 (Table 1).

Virus Stocks. HIV-1 stocks for neutralization and *ex vivo* and *in vivo* prophylaxis studies are listed in Table 2. IIIB was a gift of R. C. Gallo of the National Cancer Institute, and MN was a gift

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Abbreviations: SIV, simian immunodeficiency virus; SHIV, chimeric simian/human immunodeficiency virus; TCLA, T cell line-adapted; SI, syncytium-inducing; NSI, non-syncytium-inducing; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutin; PBL, peripheral blood leukocyte; SCID, severe combined immunodeficient.
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Table 1. Comparison of anti-cell CD4-reactive antibodies and anti-rsCD4 antibodies for binding characteristics and ability to neutralize a primary isolate of HIV

Antibody	Binding to rsCD4, A_{492}	Fluorescent staining of HPB-ALL		Antibody concentration at 50% neutralization
		Percent	Intensity*	
B4	1.424	>90%	3+	0.21 μg/ml
M2	0.871	>90%	3+	0.38 μg/ml
D5	1.930	>90%	1+	>10 μg/ml
E2	2.020	>10%	1+	>10 μg/ml
I26	0.793	0	0	>10 μg/ml
E6	2.007	>90%	2+	59 μg/ml
H5	1.984	>90%	1+	45.5 μg/ml
E31	0.936	>90%	1+	>100 μg/ml
J33	2.059	>90%	1+	>100 μg/ml
GP anti-rsCD4	$>10^{5}$†	>90%	3+	<1:10 dilution

B4, M2, D5, and I26 are anti-HPB-ALL. E6, H5, J33 and "GP anti-rsCD4" are anti-rsCD4.
*0, faint cell image, no bright spots; 1+, small bright spots, numerous; 2+, double sized bright spots; 3+, most of cell surface brightly fluorescent.
†Serum dilution endpoint at which $A_{492} > 0.154$.

of R. M. Hendry of the California Department of Health Services, Viral and Rickettsial Disease Laboratory, VRDL. Primary HIV-1 viruses VL135, VL114, VL172, VL069, and VL750 were isolated in 1992 from homosexual men participating in the San Francisco Men's Health Study (18). Subtype A isolate UG/92/029, subtype B isolate BR/92/014, and subtype F isolate BR/93/020 were acquired from the World Health Organization Network for HIV Isolation and Characterization. Subtype C isolate ZIM748 was a gift from D. Katzenstein (Stanford University). Subtype D isolate UG266, subtype E SI isolate TH32036, NSI isolates US1, US4, CM235, and CM237, subtype C NSI isolate ZB18, and subtype E NSI isolate CM238 were supplied by the U.S. Military HIV Research Program. Subtype E isolate TH 32036 also was received as a gift from J. Bradac, National Institute of Allergy and Infectious Diseases. DH-12, a patient isolate passaged in chimpanzee peripheral blood mononuclear cell (PBMC) (19) was supplied by the National Institute of Allergy and Infectious Diseases AIDS Research and Reference Reagent Program. Primary isolates 92HT593, 92US714, 92US727, 92US660, 91US056, 92US054, 92US657, and subtype G primary isolate JV1083 were supplied by the National Institute of Allergy and Infectious Diseases AIDS Research and Reference Reagent Program. M-tropic CVH was isolated at the California Department of Health Services, Viral and Rickettsial Disease Laboratory, from an infected infant. AD6 was isolated at The Aaron Diamond AIDS Research Center from a patient with primary infection (20). Primary strains W25798, P59423, V89872, and V67970 were isolated from progressor patients attending the AIDS clinic at Duke University Medical Center (1). Primary isolates were expanded in phytohemagglutin (PHA)-stimulated PBMC. TCLA strains IIIB and MN were grown in H9 cells, except for a sample of TCLA isolate MN passaged in PBMC.

Virus Neutralization Assays. The MT-2 microplaque assay (Tables 1, 2, and 3) was carried out as described (21) except that heat-inactivated sera were serially diluted in 50% high glucose DMEM with 15% FBS, antibiotics, 2% glutamine and bicarbonate buffer, and 50% pooled, defibrinated normal human plasma. Neutralization assays on mitogen-stimulated PBMC (Table 2) were done with HIV essentially as described (1, 5). B4 neutralizing activity was defined as the antibody concentration that provided the indicated percent (50–95%) reduction in virus as compared with controls containing no antibody. Antibody concentrations for the 50% and 90% endpoints were derived by interpolation between antibody dilutions. Neutralization of simian immunodeficiency virus (SIV) and recombinant simian/human immunodeficiency virus (SHIV) by B4 was observed by monitoring SIV_{mac} p27 gag antigen in culture supernatants from CEM × 174 cells infected by virus preincubated with B4 (22).

***Ex Vivo* Neutralization.** Non-leaky CB.17 severe combined immunodeficient (SCID)/SCID mice were maintained under specific pathogen-free conditions at The Aaron Diamond AIDS Research Center and were reconstituted by intraperitoneal injection (23). Reconstitution was confirmed by analysis for the presence of human Ig. HIV-1_{AD6} virus stocks were prepared from infected peripheral blood leukocytes (PBLs) (23, 24) and were titrated for infectivity in hu-PBL-SCID mice. Control antibody was a murine IgG_{2a} monoclonal antibody of unknown binding specificity from mouse myeloma cell line RPC5.4 (American Type Culture Collection No. TIB12). mAbs B4 and RPC5.4 were purified from mouse ascites fluids by Protein A affinity column chromatography (ImmunoPure Immobilized Recomb Protein A, Pierce) and were resuspended in sterile PBS at 2 mg/ml before use. Antibodies were administered once to the animals at a dose of 5 mg/kg at 1 hour prechallenge, at time of challenge, or at 1, 2, or 4 hours postchallenge, as shown in Table 4 [B4 at 1 hour prechallenge provided complete protection (data not shown)]. Three weeks after viral challenge, mice were killed, and cells were recovered from peritoneal lavage and spleens and were cocultured at 10-fold serial dilutions with PHA-stimulated human T cell blasts from seronegative donors in an endpoint dilution culture. Cocultures were monitored weekly for 4 weeks for the presence of HIV-1 p24 core antigen (23). The well of highest dilution still containing infected cells was taken as the endpoint.

Prophylaxis in Nonhuman Primates. mAb B4 for infusion was prepared as described above at 5 mg/ml. Chimpanzee X084 was infused with 5 mg/kg of mAb B4 1 hour before challenge with 100 $TCID_{50}$ of HIV-1_{DH-12}. X356 and X357 were treated 1 hour postchallenge. X259 was untreated. For Table 5, virus was detected in chimpanzee PBMC by virus isolation and by a DNA PCR assay to detect proviral DNA corresponding to *gag* (25). Virus production was evaluated by p24 antigen capture ELISA (Coulter). For Table 6, serial dilutions of 1×10^6 to 1×10^2 of chimpanzee PBMC and lymph node cells were prepared for coculture with 2×10^6 3-day-old PHA-stimulated blasts in IL-2 medium (25). The well of highest dilution that resulted in the production of p24 was taken as the endpoint. Chimpanzees were maintained at the Southwest Foundation for Biomedical Research in accordance with the National Research Council guidelines and with approval of the institutional IACUC. Rhesus macaques were maintained in accordance with the guidelines at the TSI Mason Laboratories Primate Center (Worcester, MA).

RESULTS

Antibody with Specificity for the *in Situ* Receptor. Anti-T cell monoclonal antibody mAb B4 was obtained by immunizing BALB/c mice with intact, uninfected HPB-ALL cells, an acute lymphoblastic leukemia human T cell line ($CD4^+$), suspended in

PBS. (HPB-ALL cells were determined to be 100% positive for surface-expressed CD4 and CXCR4, and negative for CCR5.) Hybridomas were produced and selected for antibodies reactive with the HPB-ALL surface by indirect immunofluorescent staining followed by cytofluorographic analysis. Five anti-T cell surface murine monoclonal antibodies were found to bind recombinant soluble CD4 (rsCD4). An additional four monoclonal antibodies were produced by immunization of mice with rsCD4. Of these nine rsCD4-binding antibodies, only anti-HPB-ALL monoclonal antibodies B4 and M2 displayed potent neutralizing activity against HIV-1 primary isolate VL135 (Table 1). In comparison to the other seven monoclonal antibodies, the two neutralizing antibodies were intensely reactive with the HPB-ALL cell surface, although recognition of rsCD4 by B4 and M2 was not remarkable in comparison to the three other CD4-reactive anti-cell monoclonal antibodies, the four anti-rsCD4 monoclonals, or the guinea pig polyclonal anti-rsCD4. The polyclonal anti-rsCD4, generated by hyperimmunization of guinea pigs with rsCD4, strongly stained the HPB-ALL surface and had high affinity for rsCD4 but lacked neutralizing activity (Table 1). The preferential recognition by B4 and M2 of the cell surface relative to recognition of rsCD4 is indicative of binding sites that are better presented by the intact cell membrane than by the recombinant protein.

The non-neutralizing anti-cell and anti-rsCD4 monoclonal and polyclonal antibodies displayed moderate to strong reactivities for CD4 peptides. In contrast, the neutralizing antibodies B4 and M2 were at best weakly reactive for several CD4 peptides, suggesting that the B4 and M2 epitopes are related to the overall conformation of the complex (17).

B4 was further studied for possible interaction with sites found on chemokine receptors. ELISA binding studies using solid-phase chemokine receptor peptides predicted no direct interaction between mAb B4 and β chemokine receptors. However, interaction between rsCD4 and chemokine receptor peptides enhanced binding. For example, solid-phase rsCD4 required at least 15× as much available mAb B4 as was required by solid-phase rsCD4/CCR5 amino acid 168–199 complex to produce equivalent binding. The observations of enhanced affinity suggest a promiscuous nature for the conformation of the B4 binding site because CD4 is perturbed by contact with any of several chemokine receptors (17). The data do not distinguish whether the coreceptors directly contact mAb B4 or whether they participate indirectly in B4 binding by induction of conformational changes to the membrane-bound CD4 receptor complex.

Binding studies with virus and recombinant gp120 favored distinct prebinding and postbinding modes of action for B4. Prior binding of B4 to HPB-ALL cells blocked the subsequent binding of HIV or recombinant gp120 (data not shown). On the other hand, prior binding of gp120 to the cells did not block the subsequent binding of B4, and previously bound gp120 was not dislodged (17). These properties mark the separation of the gp120 binding site on CD4 from the B4 recognition site, similar to the binding site of anti-CD4 monoclonal antibody 5A8 and in con-

Table 2. Neutralization by B4 of PBMC-grown primary isolates and TCLA isolates of HIV-1

Virus strain	Virus subtype	Virus host	Assay host	Percent neutralization	mAb B4 concentration, μg/ml	Phenotype	Coreceptor
IIIB	B	H9	MT-2	90%	>100	TCLA	X4
MN	B	H9	MT-2	90%	67	TCLA	X4
VL069*	B	PBMC	MT-2	90%	>100	SI	
VL135	B	PBMC	MT-2	90%	0.84	SI	
VL114	B	PBMC	MT-2	90%	0.56	SI	
VL172	B	PBMC	MT-2	90%	0.39	SI	
VL750	B	PBMC	MT-2	90%	2.0	SI	
UG/92/029	A	PBMC	MT-2	90%	3.9	SI	
DH12	B	PBMC	MT-2	90%	1.9	SI	
BR/92/014	B	PBMC	MT-2	90%	1.2	SI	R5X4
ZIM 748	C	PBMC	MT-2	90%	1.5	SI	
UG266	D	PBMC	MT-2	90%	18	SI	
TH 32036	E	PBMC	MT-2	90%	1.8	SI	
BR/93/020	F	PBMC	MT-2	90%	15	SI	R5X4
92HT593	B	PBMC	PBMC	90%	0.29	SI	R5X4
92US714	B	PBMC	PBMC	90%	0.34	NSI	R5
92US727	B	PBMC	PBMC	90%	0.13	NSI	R5
92US660	B	PBMC	PBMC	90%	0.45	NSI	R5
91US056	B	PBMC	PBMC	90%	0.12	NSI	R5
91US054	B	PBMC	PBMC	90%	9.0	SI	R5
92US657	B	PBMC	PBMC	90%	3.0	NSI	R5
CVH	B	PBMC	PBMC	90%	2.0	NSI	
AD-6	B	PBMC	PBMC	50%	0.25	NSI	
VL135	B	PBMC	PBMC	90%	0.63	SI	
US4	B	PBMC	PBMC	90%	0.16	NSI	
DH12	B	PBMC	PBMC	90%	6.0	SI	
CM238	E	PBMC	PBMC	50%	0.12	NSI	
JV1083	G	PBMC	PBMC	90%	0.80	SI	R5
MN	B	PBMC	PBMC	90%	>100	TCLA	X4
CM237	B	PBMC	PBMC	90%	0.43	NSI	R5
US1	B	PBMC	PBMC	90%	1.2	NSI	R5
ZB18	C	PBMC	PBMC	90%	2.3	NSI	
CM235	E	PBMC	PBMC	90%	0.95	NSI	R5
W25798	B	PBMC	PBMC	92%	1.0	NSI	R5
P59423	B	PBMC	PBMC	65%	1.0	NSI	R5
V89872	B	PBMC	PBMC	83%	1.0	SI	R5X4
V67970	B	PBMC	PBMC	95%	1.0	SI	R5X4

*VL069 has phenotypic similarities to IIIB, such as rapid adaptation to growth in H9 cells.

trast to the recognition site of Leu3A, for which Leu3A and gp120 displace each other in binding CD4 (14, 26).

Broad Neutralization Activities. The *in vitro* neutralizing activity of B4 against H9-grown TCLA isolates is compared in the first group of strains listed in Table 2 with activity for PBMC-grown SI primary isolates. In the MT-2 microplaque assay, the primary isolates of this group were generally more sensitive to neutralization by B4 than were the TCLA isolates HIV-1_{IIIB} and HIV-1_{MN}. This pattern reverses the order typically seen for anti-env neutralizing antibodies as exemplified by mAb IgG1b12, for which laboratory-adapted MN displayed exceptional sensitivity whereas PBMC-grown isolates were less sensitive (24, 27). The lack of neutralization of HIV-1_{IIIB} and HIV-1_{VL069} by B4 (Table 2) shows that B4 neutralizing activity cannot be attributed to subtle cytotoxic interference by B4 in the MT-2 microplaque assay.

In Table 3 (also the second group in Table 2), B4 neutralized a broad range of primary SI isolates originating from six countries and representing HIV-1 subtypes A, B, C, D, E, and F, as well as the chimpanzee-adapted HIV-1 strain DH-12 (19). In keeping with a cell-directed mode of action, and in imitation of a vaccination/challenge trial, preincubation of B4 with the host cells was as effective in these assays as the more conventional preincubation of antibody with the virus (Table 3, columns 3 and 4). Table 3 also shows that the antireceptor complex antibody is more potent and more broadly neutralizing on primary isolates than the well studied anti-gp120 antibody, IgG1b12 (24, 27).

Neutralization by anti-cell mAb B4 was shown to include HIV-1 of both the SI and the NSI phenotypes by neutralization assays on PHA-stimulated PBMC indicator cells (Table 2). In a PBMC-based neutralization assay (5), the infectivity of HIV stocks of both the SI and NSI phenotypes were reduced to the 90% endpoint (Table 2, third group), including the dual tropic DH-12 adapted to chimpanzee PBMC (19) and JV1083, a subtype G isolate of the SI phenotype. The concentrations of neutralizing antibody for AD6 (20) and for subtype E NSI virus CM238 are shown at the 50% endpoint because 90% neutralization was not reached. However, these viruses were definitely neutralized by B4, and B4 was protective against infection by AD6 of hu-PBL-SCID mice (Table 4). TCLA strain MN remained resistant to neutralization by mAb B4, as it was on the MT-2 indicator cells. Consistent with the previous MT-2 neutralization results, M-tropic NSI isolate CVH and T-tropic SI isolate VL135 were inhibited by 10 μg/ml of B4 in the PBMC-based infectivity reduction assay regardless of whether the antibody was first added to the cells or first added to the virus.

In an alternative PBMC-based antigen reduction assay (1, 2), the PHA-stimulated PBMC indicator cells were incubated with each of four primary SI and NSI isolates that had been preincubated with mAb B4 at 1 μg/ml. These viruses had been taken from progressor patients and were generally resistant to neutralization by patient sera (1, 2). Values for p24 production by SI isolates V67970 and V89872 and NSI isolates W25798 and P59423 were reduced by 65–95% (Table 2, fourth group). The sensitivity to B4 of these neutralization-resistant isolates was also unaffected by CCR5 versus dual coreceptor usage (Table 2). Viability of PBMC was not affected by mAb B4 as monitored by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide cell viability assay (28). In a postexposure mode, mAb B4 at 20 μg/ml was able to completely inhibit the formation of plaques by HIV-1_{VL135} when added up to 24 hours after infection of MT-2 cells, and it significantly inhibited production of p24 antigen when added up to 72 hours postinfection (17).

B4 neutralization also was shown to extend to other immunodeficiency viruses. In the MT-2 microplaque assay, B4 achieved 90% neutralization of HIV-2_{ROD} at 14.7 μg/ml in the presence of complement and at 8.5 μg/ml in its absence. In contrast, a pool of HIV-1 positive human plasmas was able to neutralize only 50% of the input HIV-2, and only in the presence of complement.

Table 4. Postexposure prophylaxis of HIV-1_{AD6} infection of hu-PBL-SCID mice by mAb B4

Experimental groups, 5 mg/kg	HIV-1 recovery from hu-PBL-SCID mice: week 4 coculture		
	Peritoneal lavage	Spleen	Culture end-point
Murine IgG2a (RPC 5.4) 0 hours after challenge			
4630	+	+	5×10^5
4634	+	+	5×10^1
4647	+	+	5×10^1
4652	+	+	5×10^5
4664	+	+	5×10^1
B4 0 hours after challenge			
4636, 4639			
4640, 4665	0/5	0/5	0/5
4666			
B4 1 hour after challenge			
4643, 4644			
4645, 4646	0/5	0/5	0/5
4648			
B4 2 hours after challenge			
4642, 4649			
4650, 4653	0/5	0/5	0/5
4656			
B4 4 hours after challenge			
4651, 4654			
4657, 4658	0/5	0/5	0/5
4659			

Table 3. Neutralization by MAb B4 and MAb IgG2b12 of a panel of HIV-1 primary isolates from subtypes A-F, by MT-2 microplaque assay

Virus strain	Subtype	mAb B4 concentration at 90% neutralization in replicate experiments, μg/ml								mAb IgG1b12 concentration at 90% neutralization, μg/ml
		Preincubate B4 with virus				Preincubate B4 with cell				Preincubate b12 with virus
UG/92/029	A	6.8	1.0			3.4	3.7			38.5
DH 12	B	2.1	1.7			4.3	3.0			ND
VL 135	B	1.5	1.2	0.27	0.77	0.85	0.54	0.84	1.1	ND
BR/92/014	B	1.6	0.75			ND				>50
ZIM 748	C	2.8	0.23			0.35	0.34			>50
UG 266	D	25	11			38	31			>50
TH 32036	E	3.3	0.25			1.1	0.52			>50
BR/93/020	F	15				18				ND
MN	B	66.7				ND				0.91

Antibody was preincubated with either virus or with cells as indicated. The multiple numbers in columns 3 and 4 represent 90% neutralization titers determined in independent assays. The primary isolates originated in various regions of the world: VL135 (North America), UG/92/029 (Uganda), BR/92/014 and BR/93/020 (Brazil), ZIM748 (Zimbabwe), UG266 (Uganda), and TH32036 (Thailand). ND, not determined.

Table 5. Postexposure prophylaxis of HIV-1_{DH12} infection of chimpanzees by mAb B4

	X259 (control)		X084		X356		X357	
Week	VI	PCR	VI	PCR	VI	PCR	VI	PCR
0	−	−	−	−	−	−	−	−
1	+	+	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−
3	+	+	−	−	−	−	−	−
4	+	+	−	−	−	−	−	−
6	+	+	−	−	−	−	−	−
8	+	+	−	−	−	−	−	−
10	−	+	−	−	−	−	−	−
12	+	+	−	−	−	−	−	−
14	+	+	−	−	−	−	−	−
16	−	+	−	−	−	−	−	−
18	+	+	−	−	−	−	−	−
20	−	+	−	−	−	−	−	−
24	−	ND	−	ND	−	ND	−	ND
28	+	ND	−	ND	−	ND	−	ND
32	−	ND	−	ND	−	ND	−	ND

Control chimp X259 received no antibody. Animal X084 received B4 1 hour prechallenge with HIV, and X356 and X357 were infused with B4 1 hour post-challenge. All chimps were intravenously challenged with HIV-1_{DH-12} at time 0. VI, virus isolation; ND, not determined.

SIV_{mac251} passaged in rhesus PBLs and SIV_{mac239} passaged in a human T cell line were 80% neutralized by 1–2 μg/ml of B4. Consistent with the preference of B4 for primary HIV-1 isolates, SHIV constructed with the TCLA envelope of HIV_{IIIB} was 50% neutralized by 0.68 μg/ml whereas only 0.09 μg/ml of B4 was required to neutralize 50% a dual tropic SHIV containing the primary $HIV_{89.6}$ *env* (29).

***Ex Vivo* Postexposure Neutralization.** Engrafted CB.17 *scid/scid* mice were divided into groups of four to six mice. The mice were challenged intraperitoneally with 10 MID_{50} of PBMC-grown HIV-1_{AD6}. Two preliminary trials established that this challenge dose resulted in infection of five of five hu-PBL-SCID mice and that a dose range for mAb B4 of 5–50 mg/kg provided optimum protection (data not shown). The hu-PBL-SCID mice were administered 5 mg/kg of mAb B4 or an IgG_{2a} control antibody at intervals from 1 hour prechallenge to 4 hours postchallenge. Three weeks after challenge, the mice were killed, and cells were recovered from peritoneal lavage and spleens. Administration of 5 mg/kg at 0, 1, 2, or 4 hours after challenge provided complete protection whereas all five animals given the control mAb became infected (Table 4).

Pre- and Post-Exposure Prophylaxis in Nonhuman Primates. mAb B4 was infused intravenously at 5 mg/kg to chimpanzees. Chimpanzee X084 was treated with B4 1 hour prechallenge, and chimpanzees X356 and X357 were treated 1 hour postchallenge. These three and a control animal (X259) were intravenously challenged with 100 $TCID_{50}$ of HIV-1_{DH-12} taken from a virus stock previously prepared and titered in chimpanzee PBMC at the Southwest Foundation for Biomedical Research (25). The susceptibility of the animals to infection with DH-12 stock was determined by *in vitro* infection of their PBMC before treatment and infection. All cultures were infected within 3 days of exposure to virus (data not shown).

Establishment of infection in the chimpanzees was monitored by detection of plasma viremia, cell-associated viral load, and immune response to HIV by using DNA PCR amplification of *gag* sequence, co-culture, p24 capture ELISA, and immunoblot (25). No markers of infection could be detected in any of the three animals treated with antibody during 32 weeks of follow-up (Tables 5 and 6). In contrast, virus was readily isolated from PBMC of X259 (control) beginning at week 1 postinfection (Table 5) and from plasma by week 2. Virus also was isolated from lymph node cells of X259 biopsied at weeks 4 and 20. Infected cells in the PBMC and lymph node compartments were detected at dilutions that ranged from 1×10^4 to 1×10^6 (Table 6). Seroconversion occurred in animal X259 by week 4.

mAb B4 was rapidly depleted from circulation. $CD4^+$ and $CD8^+$ subsets from the treated chimpanzees were monitored over 20 weeks postinfusion with no evidence of $CD4^+$ depletion. There was no suppression of the proliferative response of chimpanzee PBMC to mitogens (PHA, Pokeweed mitogen, and Concanavalin A) through week 32 (data not shown).

The pharmacokinetics was monitored more closely in a similar study in four rhesus macaques administered mAb B4 at 4 mg/kg (17). Endpoint titers of 1:250 to 1:290 for 90% inhibition of HIV were determined by the neutralization assay on MT-2 cells on sera collected from the macaques at 1 hour postinfusion. The B4 concentration in serum at that interval was 36 μg/ml and was reduced to 4 μg/ml by 24 hours as determined by quantitative rsCD4 ELISA. The pharmacoki-

Table 6. Quantitation of infected PBMC and lymph node cells by infectious cell dilution

	X259		X084		X356		X357	
Week	PBMC	LN	PBMC	LN	PBMC	LN	PBMC	LN
0	−	ND	−	ND	−	ND	−	ND
1	−	ND	−	ND	−	ND	−	ND
2	$1{:}10^5$	ND	−	ND	−	ND	−	ND
3	$1{:}10^4$	ND	−	ND	−	ND	−	ND
4	$1{:}10^6$	$1{:}10^5$	−	−	−	−	−	−
6	$1{:}10^6$	ND	−	ND	−	ND	−	ND
8–18	−	ND	−	ND	−	ND	−	ND
20	−	$1{:}10^6$	−	−	−	−	−	−
24	−	ND	−	ND	−	ND	−	ND
28–32	ND	ND	−	ND	−	ND	−	ND

Animals are as described in Table 5. LN, lymph node cells; ND, not determined.

netics in macaques was consistent with a rapid transfer of the antibody from the plasma to the B4-targeted cells. The passively immunized macaques also were administered an intravenous challenge with 10 AID_{50} of SIV_{mac251} 1 hour after infusion with mAb B4. Two control animals not given B4 were persistently infected whereas three of the four infused and challenged animals remained uninfected through the 1 year of monitoring. The fourth animal of this preexposure trial became infected by day 15, as shown by p27 antigenemia, seroconversion, and virus isolation from PBMC and plasma (17).

DISCUSSION

The characterization of the B4 recognition site was consistent with a conformational epitope. The site was preferentially presented by the cell surface receptor complex involving human CD4 rather than recombinant soluble CD4, and B4 displayed poor recognition of CD4 peptides. It may be influenced by a promiscuous interaction of CD4 and any of several chemokine receptors. The B4 site is distinct but near the binding site for gp120, as shown by viral and gp120 binding studies. In a separate study of B4 treatment in HIV-infected chimpanzees, the B4 binding site has been characterized by sequential immunostaining of PBMC and flow cytometry. B4 blocked binding by Leu3A but not the reverse, in agreement with gp120 binding. Thus, the B4 recognition site was distinguished from the site for the HIV-neutralizing anti-CD4 antibody Leu3A (26).

The binding of mAb B4 prevents the HIV envelope from binding to the cell receptor. B4 binding also appears to interrupt infection by already bound virus and has the capability for postexposure protection. B4 inactivated 32 of 33 primary HIV-1 strains of seven subtypes, including T-tropic and M-tropic isolates irrespective of SI and NSI phenotype, dual or CCR5 coreceptor usage, as well as HIV-2, SIV, and SHIV. Complement was shown to have a negligible effect on the neutralizing activity of mAb B4, as shown here for HIV-2 and confirmed for HIV-1 by additional experiments (data not shown).

In the chimpanzee trial, HIV infection by a virulent primary isolate was aborted by the administration of mAb B4 within a short interval after exposure. The transferred immunity was sterilizing with no evidence of a transient, reduced, or delayed viremia. Complete protection was evident, despite the rapid clearance of an anti-CD4 antibody from plasma, by being sequestered on $CD4^+$ cells in the peripheral blood and lymphoid tissue (30). mAb B4 provided slightly less protection in the rhesus macaque SIV_{mac251} infection model, consistent with mAb B4 having a better specificity for the more humanlike receptor complex of the chimpanzee. Macaque CD4 differs from human CD4 by 36 amino acids whereas chimpanzee CD4 differs from human CD4 by only four amino acids (31). The differences in sequence and in greater protective efficacy of B4 for chimpanzees suggest that the macaque is a less suitable model for immunoprophylaxis directed at the host cell.

Applications for Post-Exposure Prophylaxis by mAb B4. U.S. Public Health Service Guidelines recommend postexposure prophylaxis by antiretroviral drugs for a healthcare worker after an accidental exposure (32). However, a recent report of treatment failure with a four-drug regimen immediately after exposure,†† and the growing risk to healthcare workers given the increasing utilization of combination antiretroviral therapy and the concomitant increase in the number of patients harboring drug-resistant viruses (33) suggest a need for more effective postexposure prophylaxis. Moreover, the U.S. Public Health Service Guidelines (32) express reservations regarding the toxicity of the drugs that are presently available for postexposure prophylaxis. That B4 was not toxic to either the cells used in the *in vitro* neutralization assays nor to the hu-PBL-SCID mice and produced no marked immunosuppression in chimpanzees indicated that it will be well tolerated. The potential of B4 for low toxicity and to be more broadly active than antiretroviral drugs points to its consideration for postexposure prophylaxis and for prevention of vertical transmission of HIV. mAb B4 also may be proven to complement antiretroviral therapy for HIV viremia.

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