Neutralization of Primary Human Immunodeficiency Virus Type 1 Isolates by the Broadly Reactive Anti-V3 Monoclonal Antibody, 447-52D

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Human monoclonal antibody 447-52D binds to the V3 determinant of the human immunodeficiency virus type 1 (HIV-1) gp120 external glycoprotein. Its binding requires the expression of the GPxR sequence at the center of the V3 domain. HIV-1 variants that are adapted to replication in T-lymphoid cell lines and express this sequence motif are efficiently neutralized by the antibody (M. K. Gorny, A. J. Conley, S. Karwowska, A. Buchbinder, J.-Y. Xu, E. A. Emini, S. Koenig, and S. Zolla-Pazner, *J. Virol.* 66:7538–7542, 1992). In the present study, the antiviral activity of 447-52D was further defined with regard to its ability to mediate neutralization of primary HIV-1 clinical isolates. Again, the antibody was found to potently neutralize those isolates that expressed the binding sequence. We confirmed that this determinant is commonly expressed by virus isolates belonging to the subtype (clade) B sequence classification. As such, 447-52D may be useful for prophylactic and immunotherapeutic intervention. In addition, the study demonstrated that neutralization of primary HIV-1 isolates is possible if mediated by an appropriate antibody.

There are two primary reasons for characterizing the binding and virus neutralization properties of anti-human immunodeficiency virus type 1 (HIV-1) human monoclonal antibodies (hMAbs). The first is to identify antibodies with potent and broad neutralizing activity that may be used as prophylactic agents to prevent the establishment of HIV-1 infection. The second is to define the quality of the humoral antibody response that a successful vaccine immunogen should be designed to elicit. Accordingly, we have focused our efforts on establishing the antiviral characteristics of a number of hMAbs directed against different viral determinants. We performed these studies with primary HIV-1 clinical isolates, given the apparent inability of numerous antibodies to neutralize such isolates, although the same antibodies will neutralize T-lymphoid cell line-adapted variants (4).

This report describes the HIV-1 primary isolate neutralizing activity of hMAb 447-52D. This antibody binds to the V3 determinant of the viral gp120 surface glycoprotein. Chimpanzee studies have shown that antibody to this determinant, whether passively administered or actively elicited, is effective in preventing HIV-1 infection (1, 6, 7). The binding epitope of 447-52D requires the expression of the GPxR sequence at the center of the V3 domain. We previously reported that the antibody neutralizes all T-lymphoid cell line-adapted HIV-1 variants that express this sequence (8). In addition, 447-52D binds efficiently to recombinant phage that display the GPxR element (9).

We now demonstrate that 447-52D is capable of neutralizing a variety of primary HIV-1 isolates, including virus that was isolated from the infected person from whom the antibody was

derived. The ability of virus to be neutralized correlated exclusively with the expression of the GPxR sequence. These studies establish the potential of 447-52D as an anti-HIV-1 agent, given that the binding sequence is commonly expressed among the subtype (clade) B viruses prevalent in North America and Europe. In addition, we show that primary isolates can indeed be neutralized by an appropriate antibody.

MATERIALS AND METHODS

Preparation of hMAb 447-52D. The stable heterohybridoma clone 447-52D,10,1 (8) (herein referred to as 447-52D) was cultured in RPMI 1640 medium supplemented with 15% (vol/vol) fetal bovine serum (HyClone), 2 mM L-glutamine, penicillin (100 U/ml), and streptomycin (100 µg/ml). Cells were expanded in 225-cm² flasks at a concentration of 1×10^6 to 2×10^6 cells per ml. The supernatant medium was collected once each week, and the cells were resuspended in fresh complete medium. The supernatant medium was centrifuged immediately for separation of cells and cell debris and then was filtered through a 0.4-µm-pore-size filter (Nalgene). The ability of the antibody in the medium to bind to the strain MN V3 peptide was tested by enzyme-linked immunosorbent assay (ELISA) (8). The supernatant medium was stored at 4°C for 2 to 4 weeks, at which time 1 to 2 liters were collected. The immunoglobulin G (IgG) concentration in the medium was determined by ELISA (see below).

The MAb was purified by precipitation with a 50% saturated ammonium sulfate solution, subsequent dissolution of the precipitate in phosphate-buffered saline (PBS), and dialysis against several changes of PBS at 4°C. The MAb was purified further by protein A chromatography (HiTrap Protein A [Pharmacia], equilibrated in 20 mM sodium phosphate [pH 7.0] buffer). About 20 mg of IgG was applied to the column,

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TABLE 1. Primary HIV-1 isolates used in infectivity reduction tests with hMAb 447-52D and their gp120 V3 region apex sequences

Class	Isolate	gp120 V3 apex sequence summary		
		Patient plasma	Producer cells	Virus stock
Non-GPGR	NIH225	ND ^a	ND	APGR (5/7 ^b)
	An201	ND	ND	APGS (1/7)
	UAB430	GPGR (9/9)	GRGR (9/9)	GGGR (7/7)
	UAB112	GVPGS (3/6)	GVPGS (8/12)	GRGR (6/6)
		GPGR (3/6)	GIPGD (4/12)	GVPGS (6/6)
GPGR present	NYU CM28	ND	ND	GPGR (4/7)
GPGR	UAB116	ND	8/8 ^c	17/17
	UAB120	ND	6/6	ND
	UAB421	8/8	6/6	ND
	UAB422	6/6	8/8	5/5
	UAB428	ND	5/5	11/11
	UAB442	ND	2/2	ND
	MR452	22/22	20/20	18/18
	060 ^d	ND	ND	11/11
	5001	ND	5/5	6/6
	5003	ND	4/4	7/7
	5005	ND	9/9	7/7
	5010	ND	6/6	7/7
	5016	ND	6/6	7/7
	NIH205	ND	ND	13/13
	NYUGK25	ND	ND	12/12
	NYUPS29	ND	ND	10/10

^a ND, not done or not available.^b Number of clones of a particular sequence identified/total number sequenced.^c Values for all clones of isolates in this class represent number with GPGR/total number sequenced.^d Derived from a patient whose plasma is designated S219 (see Fig. 4 for susceptibility of infectious plasma S219).

since the binding capacity of this column for human IgG was limited. The column was washed with 5 to 10 ml of buffer, and the MAb was eluted with 2.7 ml of 0.1 M glycine-HCl (pH 2.7); 0.3 ml of 1 M Tris-HCl (pH 9.0) was then added, resulting in a final pH of the solution of about 7.0. The IgG concentration in the sample was determined by ELISA for its ability to bind to the strain MN V3 peptide.

The IgG quantitation was performed by ELISA in the following manner. An Immulon 2 plate (Dynatech) was coated overnight at 4°C with affinity-purified goat anti-human IgG that was diluted 1:500 (gamma specific; Organon-Cappel, Malvern, Pa.). After being washed five times, the plate was incubated for 1.5 h at 37°C with medium or MAb solutions that were diluted from 1:10 to 1:10,000 (depending on expected concentration) in complete medium. The bound IgG was detected by incubation with alkaline phosphatase-labeled goat anti-human IgG (gamma specific; Organon-Cappel) for 1.5 h at 37°C. Color was developed by incubation for 10 min with the substrate *p*-nitrophenyl phosphate in 10% (vol/vol) diethanolamine. The reaction was terminated with 100 µl of 1 M NaOH, and the A₄₀₅ was read in an automated ELISA reader (MR600 microplate reader; Dynatech). Affinity-purified human IgG (Cappel) was used to produce standard curves. Standards were diluted from IgG stock (1 mg/ml) at seven dilutions: 5, 10, 25, 50, 75, 100, and 125 µg/ml.

Source of viruses, virus stock preparation, and cell preparation. The designations of the HIV-1 isolates used in this study are given in Table 1. Viruses with the prefixes MR and NYU were from New York City patients, those with the prefix UAB were from southeastern U.S. patients, isolates with the 5xxx numbers were from European patients, and those with the prefix NIH and virus isolate 060 were from Washington, D.C.,

area subjects; AN201 and AN608 were Brazilian isolates. Initial virus isolation of NYU CM28, NYU GK25, NYU PS29, NYU SJ31, NYU CB24, NYU CJ48, and NYU MP49 was performed by coculture of peripheral blood mononuclear cells (PBMC) from the seropositive individual with PBMC from donors after each cell culture was stimulated independently for 3 days with phytohemagglutinin (PHA) at 5 µg/ml. The ratio of infected subject PBMC to uninfected donor PBMC during this cocultivation, in medium with interleukin-2, was 1:3. Viral p24 antigen in the culture supernatant medium was monitored by antigen capture ELISA (by using either a system from Coulter, Hialeah, Fla., or an antigen capture method previously described [10]). From 10 to 20 days postinfection, aliquots of the cell culture supernatant media were collected every 2 days and frozen at -70°C. These aliquots were used in an antibody-mediated neutralization assay as described below.

For the remaining primary isolates listed in Table 1 or 2 as well as those described above, virus stocks for in vitro infectivity reduction assays were prepared by infecting freshly prepared PHA-stimulated PBMC with a low-PBMC culture passage primary isolate by the method described previously (3). In all methods described here, PBMC were PHA stimulated before use. In brief, this method involved infection at time zero, collection of cells by centrifugation and resuspension in fresh medium on day 1, expansion with additional uninfected PBMC on day 2, and another cell collection, resuspension, and expansion with additional PBMC on day 4. These high-titered virus stocks consisted of virus that accumulated in culture medium between 5 and 7 days postinfection. These virus stocks were subsequently stored in vapor-phase liquid nitrogen and were used in the infectivity reduction assay described below within 4 days of preparation.

The third method of virus preparation for infectivity reduction was the identification of an infected individual whose plasma, as a cell-free preparation, could initiate infection of donor PBMC cultures. From a survey of approximately 25 plasma samples, only reference S219 plasma was capable of infection consistently. This patient also had virus present in the range of 6,000 to 20,000 ng of p24 antigen per ml. A virus stock derived from a PBMC culture of this plasma was designated 060 (Tables 1 and 2). This plasma was used in ex vivo infectivity reduction assay as described below.

Neutralization assays using hMAb 447-52D. (i) **Antibody neutralization assay.** A concentration range of 447-52D from 3 to 100 µg/ml was chosen for testing against 100 50% tissue culture infective doses (TCID₅₀) of virus that had been titered on human PBMC. The virus and MAb were mixed and were added to PBMC cultures in 96-well culture plates. These PBMC had been previously stimulated for 3 days with PHA at 5 µg/ml. The cultures were incubated for 7 days, after which the level of virus replication was determined by sampling p24 antigen in culture supernatant medium. Specificity of neutralization was monitored by examining virus replication in the presence of an hMAb that was specific for human cytomegalovirus as well as by examining replication in the absence of antibody.

(ii) **Infectivity reduction assay.** In this method, the ability of 447-52D to neutralize primary isolates was determined by testing a constant MAb concentration of 100 µg/ml against a serial dilution of primary virus stocks. This method was described previously (3). Briefly, the mixed antibody and the virus dilutions were incubated for 1 h and then added to PBMC cultures. Virus replication was measured after 7 days, and the reduction in infectivity was determined by comparison of the replication of each input concentration of virus in the presence and the absence of MAb 447-52D. Independent infectivity reduction tests were performed simultaneously with a constant amount of a standard HIV-1-seropositive human serum for comparison and for determination of reproducibility.

(iii) **Ex vivo infectivity reduction assay.** This technique consisted of incubation of infectious plasma dilutions with either 50 or 25 µg of 447-52D per ml for 1 h at 37°C. The mixtures were added to PHA-stimulated PBMC cultures and incubated for 10 to 14 days. Culture supernatant media were collected, and infectivity reduction was determined by comparison with the plasma dilution's infectivity in the absence of 447-52D. A non-HIV-1 MAb (1308, a humanized version, that was specific for respiratory syncytial virus and isotype matched) also was used for control of specificity and nonspecific events.

Extraction of RNA from plasma, sera, or virus stocks and sequence determination. The gp120 V3 region sequences of the viruses used in this study were determined. In some cases when available, as indicated in Table 1, gp120 V3 region sequences were determined on RNA extracted from patient plasma or serum that was taken at the time of initial virus isolations. gp120 V3 region sequence determination of provirus derived from infected virus stock producer cells was performed essentially as described previously (3). For the RNA extraction method, samples were mixed with an equal volume of 5 M guanidine thiocyanate–0.5% *N*-laurylsarcosine–0.1 M 2-mercaptoethanol–25 mM sodium citrate (pH 7.0). The solution was made 0.2 M with sodium acetate; 100 µg of tRNA was added and extracted with an equal volume of water-saturated phenol containing 10% chloroform. Phases were separated by centrifugation, and the nucleic acid in the aqueous phase was precipitated with isopropanol (50%, final concentration). The *env* V3 region was amplified by using the GeneAmp RNA PCR kit (Perkin-Elmer Cetus, Norwalk,

TABLE 2. Summary of gp120 V3 region apex sequences among all patient and isolate materials sequenced in this study

Group	No. of individuals	V3 apex sequence characteristics ^a
1	22	GPGR
2	3	GPGK/GPGR
3	3	ΔPGR/GPGR
4	5	GPGR mixed/GPGR majority
5	2	GPGR mixed/GPGR minority
6	3	GPGK/GPGK
7	5	ΔPGR/ΔPGR
8	4	xPGS ^b
9	4	Divergent

^a Underlining indicates difference from the most conserved apex sequence.

^b x = G, A, or V.

Conn.). The primer used in the reverse transcriptase reaction was the oligomer 5'-CAU CAU CAU CAAATTCTG GGTCCCCTCCTGAGG-3', where the 13th nucleotide represents nucleotide pair number 1092 of the *env* gene consensus (12). The second primer used in the PCR mix addition was 5'-CUA CUA CUA CUA GCTAAAACCATAATAGTACA GCTG-3', where the 13th nucleotide represents nucleotide pair number 826 of the *env* gene consensus. The PCR conditions were an initial 2 min at 94°C followed by 35 cycles of 1.5 min at 94°C, 1.5 min at 55°C, and 1.5 min at 72°C, with an 8.5-min incubation at 72°C. The PCR products were cloned by reacting with uracil DNA glycosylase while annealing to the pAMP vector plasmid of the CloneAMP System (Life Technologies, Inc., Gaithersburg, Md.). Competent *Escherichia coli* DH5α was transformed with the annealed vector. Plasmid DNA was purified by using the Magic MiniPrep DNA purification system (Promega, Madison, Wis.). Inserts were verified by *Bam*HI-*Eco*RI digestion followed by electrophoresis in 1.5% agarose gels. Positive clones were sequenced by using the SP6 promoter primer (Life Technologies) by a modification of the U.S. Biochemical Sequenase version 2.0 protocol using 35-Sequettide (NEN-DuPont) and electrophoresed in 6% sequencing gels at 70 W for approximately 2 h. The gels were dried and exposed to X-ray films overnight at –70°C.

RESULTS

Classification of gp120 V3 region sequences. hMAb 447-52D may be useful if its epitope occurs frequently among the gp120 V3 determinants of circulating HIV-1 isolates. To obtain the proper perspective in regard to hMAb 447-52D, HIV-1 gp120 V3 region sequence analysis was performed on patient plasma (when available), virus isolates, and DNA of PBMC cultures that were used to produce virus stocks. Materials were obtained from, or derived from, 51 HIV-positive individuals. A total of 690 clonal gp120 V3 sequences were generated from these materials. When possible, comparative numbers of sequences were obtained from individual samples. In agreement with previously reported data, the apex sequence GPGR was found most frequently, 432 times (approximately 63%) (11, 12). The next two most common variant apex sequences were ΔPGR and GPGK. Potential relationships in variation at the V3 apex among the viruses in individual HIV-1-infected persons were observed after classification according to virus gp120 V3 sequence and patient (Table 2). Most individuals had viruses that had GPGR at the apex of V3, group 1. This group included 22 individuals. Furthermore, groups 2 and 3 contained viruses with V3 apex sequences in which either GPGK or ΔPGR was found. These variant apex sequences were more

FIG. 1. Virus infectivity reduction by hMAb 447-52D. Infectivity reduction of these primary HIV-1 isolates was tested in human PBMC cultures in the approximate input range of 0.02 to 15 ng of p24 antigen. ■, virus infectivity titration in the absence of antibody; ●, virus infectivity in the presence of 100 µg of hMAb 447-52D per ml; ▲, virus infectivity in the presence of a 1:50 dilution of HIV-seropositive human serum 498. (A) Virus UAB112; (B) virus UAB430; (C) virus AN201; (D) virus NIH225. The apex V3 sequence for each isolate is indicated.

common in patient plasma, and usually GPGR apex sequences were present. Analysis of the corresponding virus stocks yielded only clones with GPGR apex sequences. This situation may reflect a selection for GPGR-containing viruses in early passage in human PBMC in vitro. On the other hand, groups 6 and 7 consisted of GPGK and APGR sequence-containing variants, respectively, in which no evidence of a GPGR apex V3 sequence was found. Group 4 and 5 contained virus isolates in which V3 GPGR sequences were mixed with another, less frequently occurring non-APGR or GPGK variant. Groups 4 and 5 differed according to the proportion of GPGR-containing clones that were derived as virus stock. Clones with GPGR apex sequences were found predominantly in the five individuals of group 4 and were in the minority among the two individuals from group 5. Group 8 contained four individuals in whom sequences were found to contain the apex sequence xPGS, where x = G, A, or V. Lastly, group 9 contained four individuals with viruses that were classified as containing divergent V3 apex sequences. Two of these four, with the apex sequences GGGR and GWGR, were Brazilian in origin. The apex sequences GPGT and GRGR were present in the remaining two individual's virus sequences.

Infectivity reduction of primary isolates in human PBMC cultures. Primary isolates representing three sequence classes were used in infectivity reduction assays with hMAb 447-52D. These consisted of 16 isolates that expressed GPGR at the V3 apex, 4 isolates that did not contain GPGR, and 1 isolate that contained the apex sequence GPGQ (Table 1). The four primary isolates with non-GPGR V3 apex sequences showed a marked insensitivity to the action of 447-52D (Fig. 1). For these four non-GPGR-containing primary isolates, the titrations of infectivity in the presence and absence of hMAb

FIG. 2. Virus infectivity reduction by hMAb 447-52D as indicated in the legend to Fig. 1. These four primary isolates are listed in Table 3 as class 1, most sensitive isolates. ■, virus infectivity titration in the absence of antibody; ●, virus infectivity in the presence of 100 µg of hMAb 447-52D per ml; ▲, virus infectivity in the presence of a 1:50 dilution of HIV-seropositive human serum 498. (A) Virus UAB421; (B) virus UAB116; (C) virus UAB120; (D) virus 060.

447-52D were nearly identical. In addition, three of the four isolates also appeared unaffected in infectivity by an HIV-positive human serum. The fourth isolate, AN201 (Fig. 1C), although not affected by 447-52D, was reduced in infectivity by the HIV-positive human serum.

Figure 2 illustrates the patterns of infectivity reduction by 447-52D among a group of isolates that contain GPGR at the apex of the gp120 V3 region. These four examples belong to a class of primary isolates that were among the most susceptible to the neutralizing action of hMAb 447-52D (Table 3). For this susceptibility pattern, termed pattern 1, there existed a greater than 30-fold difference between the neutralization endpoint (90% or greater) observed with hMAb 447-52D and the endpoint of the virus stock titration in the absence of antibody. In addition, isolates UAB421 and UAB116 were moderately sensitive to the neutralizing activity present in this HIV-1-positive human serum (Fig. 2A and C, respectively), while isolate UAB116 was able to replicate to only a low output at the higher virus input in the presence of HIV-1-positive human serum. Isolate 060 (Fig. 2D) was relatively insensitive to the action of the human serum. Thus, there appeared to be a variety of susceptibility patterns of these primary isolates to neutralization by this HIV-positive serum despite their sensitivity to neutralization by hMAb 447-52D.

The ability of hMAb 447-52D to neutralize diverse primary isolates may be influenced by both the presence of the epitope in V3 and the surrounding residues in the gp120 V3 loop of a given isolate. The four isolates shown in Fig. 2 were pattern 1 neutralization examples with sequence variation around the hMAb 447-52D core epitope (Table 3). Examples of primary isolates that had endpoints in the presence of 447-52D that were 10- to 20-fold lower than in the absence of MAb are presented in Fig. 3A and B. Examples of infectivity reduction

TABLE 3. gp120 V3 region sequences representing HIV-1 subtype B primary isolates that can be neutralized by hMAb 447-52D

Relative susceptibility pattern	Isolate designation	V3 apex sequence
1 ^a	5016	-T R K S I H I G P G R A F Y-
	UAB421	-T R K S I H I G P G R A F Y-
	UAB116	-T R R G I H I G P G R A F Y-
	5001	-T R R G I H I G P G R A F Y-
	5003	-T R R G I H I G P G R A F Y-
	UAB120	-T R K S I N I G P G R A F Y-
2 ^b	060	-T R R S I H L G P G R A L F-
		Q H I Y H
	NIH205	-T R K S I H I G P G R A F Y-
	MR452	-T R K G I H I G P G R A F Y-
	5010	-T R R S I H I G P G R A F Y-
	5005	-T R K S I P I G P G R A F Y-
3 ^c	NYU PS29	-T R K S I H I G P G R A F Y-
		(P)
	UAB422	-T R K S I P I G P G R A F Y-
	UAB428	-T R K S I P I G P G R A F Y-
	UAB442	-T R K S I P I G P G R A F Y-
	NYUGK25	-T R K S I P M G P G R A F Y-
	NYUCM28	-T R R S I H L G P G Q S F Y-

^a Greater than 30-fold difference between the 90% neutralization endpoint achieved by 100 μ g of 447-52D per ml and the endpoint of the virus stock titration.

^b Difference of 10- to 20-fold between the 90% neutralization endpoint and the endpoint of the virus stock titration.

^c Difference of two- to eightfold between the 90% neutralization endpoint and the endpoint of the virus stock titration.

for two primary isolates with susceptibility patterns in the two- to eightfold range are given in Fig. 3C and D.

A significant observation was that the extent of an isolate's susceptibility was influenced by the presence of a proline residue in V3 at the sixth residue position noted in Table 3. This position and positional variation from His to Pro was noted for primary isolates previously, and it is considered an important residue position that can influence antibody affinity (8, 9, 17). In addition to isolates UAB422 and UAB442 (Fig. 3C and D), isolates NYU GK25 and UAB428 (Table 3) had infectivity reduced in the range of two- to eightfold by hMAb 447-52D. However, isolate 5005, which has a proline residue at that position in V3, had infectivity reduced to a greater extent by 447-52D (Fig. 3B). Thus, there exist primary isolates with susceptibility to 447-52D in both the pattern 2 and pattern 3 ranges with a proline residue at the first contact position. Finally, virus NYU CM28 was susceptible to hMAb 447-52D in the pattern 3 range; both a non-GPGR sequence, GPGQ, and GPGR were found by sequence analysis of this isolate's gp120 V3 region (Table 3).

Ex vivo infectivity reduction. To test the ability of hMAb 447-52D to neutralize an uncultured primary isolate, we screened HIV-1-positive subjects and identified one whose plasma reproducibly transmitted infection to human PBMC in culture. The infectious plasma, designated S219, was incubated with hMAb 447-52D to determine ex vivo infectivity reduction (Fig. 4). 447-52D was able to dramatically reduce infectivity over the entire range of plasma dilutions that were tested and to specifically reduce output greater than 10^4 -fold at all tested plasma dilutions greater than 1:30. A non-HIV-1 humanized MAb, 1308, had no effect on the infectivity of this S219 plasma. Sequence analysis of virus stock (virus 060; Fig. 2D, Table 1, and Table 3) derived from this sample and of RNA directly

FIG. 3. Virus infectivity reduction by hMAb 447-52D as indicated in the legend to Fig. 1. ■, virus infectivity in the absence of antibody; ●, virus infectivity in the presence of 100 μ g of hMAb 447-52D per ml; ▲, virus infectivity in the presence of a 1:50 dilution of HIV-seropositive human serum 498. Shown are results for two isolates classified in Table 3 as class 2, sensitive isolates, virus 5010 (A) and virus 5005 (B), and two isolates classified in Table 3 as class 3, sensitive isolates, virus UAB422 (C) and virus UAB442 (D).

from S219 plasma yielded the apex sequence ...SIHLG PGRALF... This sequence is consistent with susceptibility to neutralization by hMAb 447-52D.

Infectivity reduction during short exposure of virus to antibody. To further assess the neutralizing properties of 447-52D, an experiment was performed to compare the levels

FIG. 4. Infectivity reduction of virus present in plasma S219 by hMAb 447-52D. Plasma dilutions were incubated with 50 μ g of hMAb 447-52D per ml (●), with a nonrelevant, non-HIV-1 human MAb, 1308 (▲), or with no MAb (■) and used to infect human PBMC in the ex vivo neutralization procedure described in the text. For this neutralization test, only a recombinant version designated (r) 447-1 was used. The virus, 060, derived from plasma of this infected individual was equally susceptible to both the recombinant version and the heterohybridoma-derived antibody. Both 447-52D and the control, 1308, were gamma 1 isotype matched for this study.

FIG. 5. Infectivity reduction assay of virus 5016 by hMAb 447-52D during short virus-antibody exposure and continuous virus-antibody exposure. ■, virus infectivity in the absence of antibody; ●, virus infectivity in the presence of antibody. (A) One hour after addition of antibody-virus dilution mixtures to human PBMC cultures, the cells were washed, collected by centrifugation, and resuspended in fresh medium with no additional antibody. (B) The same washing process performed on an identical antibody-virus dilution cell mixture at 18 h postinfection. (C) An additional infectivity reduction assay for virus 5016 in which no cell washing occurred during the 7 days of incubation of the infected culture.

of primary isolate neutralization sensitivity upon continuous and short-term MAb exposure in PBMC culture. With primary isolate 5016, nearly identical infectivity reduction by 447-52D was obtained whether the MAb was continuously present or removed 18 h postinfection (Fig. 5B and C). Removal of 447-52D at 1 h, which also necessitated the removal of any unadsorbed virus, led to a fourfold reduction in the infectivity endpoint (Fig. 5A). Despite that reduction, significant infectivity reduction by hMAb 447-52D was still observed.

Potency of hMAb 447-52D on primary isolate infectivity. The potency of a virus neutralizing MAb is a major factor in determining its usefulness and in determining potential prophylactic levels. This property of hMAb 447-52D was examined for primary isolates. For NYU CJ48 and NYU MP49, a single input of 100 TCID₅₀ was tested against antibody concentrations from 3 to 100 µg/ml (Fig. 6A). Both of these primary isolates were neutralized. The 90% or greater endpoint for NYU CJ48 was approximately 10 µg/ml, and that for NYU MP49 approximately 30 µg/ml. Both isolates were unaffected by an anti-human cytomegalovirus hMAb (data not shown). In addition, the potency of 447-52D was tested against primary isolate 5016 at three different virus inputs over the same hMAb 447-52D concentration range, 3 to 100 µg/ml (Fig. 6B). The potency of 447-52D was proportional to the amount of input virus. The endpoints were approximately 3 µg/ml for 15-pg input, approximately 12 µg/ml for 31-pg input, and approximately 50 µg/ml for 63-pg input. Thus, the potency of 447-52D appeared consistent among sensitive primary isolates and appeared proportional to the amount of virus when tested against one individual isolate.

DISCUSSION

Primary HIV-1 viruses that have been isolated from locations where subtype (clade) B HIV-1 is prevalent have GPGR as the predominant gp120 V3 region apex sequence. The second and third most common V3 apex sequence variations include APGR and GPGK, respectively. Primary HIV-1 viruses that contain GPGR are susceptible to neutralization by hMAb 447-52D in vitro in human PBMC cultures. The susceptibility can be demonstrated with infectious virus prepared by different methods from infected human PBMC cultures and in different experimental formats, including an ex vivo neutralization. hMAb 447-52D is avid, since neutralization does not

FIG. 6. Determination of the potency of neutralization of hMAb 447-52D on primary HIV-1 isolates by MAb titration for different isolates. (A) and by MAb titration for different inputs of a singular primary isolate (B). For panel A, viruses NYU CJ48 ■ and NYU MP49 ●, a virus infectivity input of 100 TCID₅₀ was tested against the variety of MAb 447-52D concentrations as indicated. For panel B (virus 5016), virus inputs of 15 ■, 31 ●, and 63 ▲ pg were tested against the indicated range of hMAb 447-52D concentrations. The discontinuity indicates that the one particular point in the titration had an output that exceeded the maximum output for virus 5016 in this case. Maximum output for virus NYU MP49 was 250.6 ng/ml, that for NYU CJ48 was 60 ng/ml, and that for 5016 ranged from 213 ng/ml for 15-pg input to 192 ng/ml for 60-pg input.

require continual exposure of infected PBMC cultures to antibody. Although only a limited number of virus isolates have been studied in potency tests, hMAb 447-52D can neutralize to greater than 90% neutralization endpoints at low antibody concentrations (below 10 µg/ml).

The identification of an immune effector function(s) that can prevent the establishment of a persistent HIV-1 infection remains an important objective guiding HIV-1 vaccine research. Successful protection of chimpanzees from infection by the laboratory-adapted strain IIB has been accomplished by immunization with homologous gp120 formulations, and the protection was best correlated with the elicitation of anti-V3 antibody (1, 7). Further, passive administration of an anti-V3 MAb can protect chimpanzees from a homologous challenge (6). This objective now needs to be extended to experimental vaccines that elicit antibodies that can neutralize primary HIV-1 isolates rather than laboratory-adapted strains. However, the general inability of sera from vaccinees to neutralize HIV-1 primary isolates (16) has led to the proposal that vaccine-elicited, potent neutralization of primary HIV-1 isolates in human PBMC may not be possible. The evidence presented in this report demonstrates that HIV-1 isolates obtained directly from infectious human plasma or produced from human PBMC cultures can be neutralized by an antibody which was part of the normal human response to HIV-1. The implication of these observations is that vaccines that have been designed and tested to date have not elicited potent virus-neutralizing antibodies, exemplified by 447-52D, which by definition can be made by appropriately stimulated individuals. Most HIV-1-positive sera do not have much of this or a similar antibody (4, 8). The additional implication of this observation, notwithstanding V3 region variability, is that potent anti-V3 antibodies capable of neutralizing primary isolates can be elicited by an appropriate immunogen.

It has been suggested that a high titer of potent antibody that recognizes consensus gp120 V₃ sequences may correlate with a lack of maternal-fetal transmission. Since transmission is most likely multifactorial, an interpretation of the protective role of maternal antibodies may be confounded by reliance on sero-

logical analysis alone (5, 13, 15). Sequence analysis of maternal and infant pair virus isolates clearly identifies infectious viruses that are transmitted and that would need to be neutralized by a prophylactic antibody. In this regard, Scarlatti et al. (14) reported that of 10 virus pairs, a GPGR V3 region virus was established in nine infants. For the 10th non-GPGR pair, the virus was transmitted from a maternal virus population that consisted solely of one type of gp120 V3 apex sequence, GPGR. Likewise, one donor with an identified GPGR gp120 V3 apex sequence transmitted virus both horizontally and by maternal-fetal transmission (2). Virus from both infected recipients had GPGR V3 apex sequences. Thus, from these studies, the transmissibility of GPGR-containing viruses strongly supports a role for anti-GPGR agents such as hMAb 447-52D.

The studies reported here suggest that vaccines that elicit antibody identical or equivalent to 447-52D may have value in preventing the establishment of a persistent HIV-1 infection. Since the target of 447-52D is the most conserved gp120 V3 element in subtype (clade) B isolates, an immunogen that would elicit similar antibodies could overcome some of the problems associated with gp120 hypervariability. The identification of such an immunogen is the subject of ongoing study. Finally, the role of hMAb 447-52D in protection can be addressed in two ways: first in a direct chimpanzee challenge experiment using as a challenge a primary HIV-1 isolate that would be representative of isolates present in the human population, and second by the use and resultant analysis of 447-52D as an agent of passive immunoprophylaxis in humans.

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