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Two Immunodominant Domains of gp41 Bind Antibodies Which Enhance Human Immunodeficiency Virus Type 1 Infection In Vitro

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Four of eight human monoclonal antibodies (huMAbs) to gp41 were identified which could enhance human immunodeficiency virus type 1 (HIV-1) infection in vitro by complement-mediated antibody-dependent enhancement (C'-ADE). These enhancing huMAbs were mapped to two distinct domains on the HIV-1 gp41 transmembrane glycoprotein by using synthetic peptides. The first domain, amino acids 579 to 613 (peptide AA579-613), was recognized by three of the four enhancing huMAbs. The AA579-613 peptide blocked C'-ADE of HIV-1 infection in vitro whether it was mediated by these three huMAbs or by human polyclonal anti-HIV serum. The second domain, amino acids 644 to 663, bound the remaining enhancing huMAb. This peptide weakly blocked C'-ADE mediated by the huMAb and by an HIV immune globulin fraction but did not block C'-ADE mediated by a patient's serum. The patient's serum did react with the peptide in an enzyme immunoassay. The huMAbs to the two domains could interact in vitro to enhance HIV-1 infection in a synergistic manner. These two domains, which bind enhancing antibodies, are conserved between HIV-1 isolates as well as between HIV-2 and simian immunodeficiency virus isolates. These data demonstrate the existence of two conserved regions within the HIV-1 gp41 which bind enhancing antibodies; these two domains, amino acids 579 to 613 and 644 to 663, may prove important in HIV-1 vaccine development and in immunopathogenesis of HIV-1 infection.

In an effort to explore and identify functional domains within the structural proteins of human immunodeficiency virus type 1 (HIV-1), a number of laboratories have produced monoclonal antibodies (MAbs) against HIV-1. While most MAbs have been of rodent origin (3, 7, 10, 12, 18, 21, 26, 36, 38), a number of laboratories have had success in generating human MAbs (huMAbs) against HIV-1 envelope (1, 8, 14–16, 27, 37, 42) and core (11, 14, 16) antigens. These huMAbs offer certain advantages over more traditional murine MAbs (muMAbs), since huMAbs can be used therapeutically without inducing antihuman immunoglobulins, can interact with certain species-specific human proteins such as proteins of the alternative complement pathway, and represent epitopes to which individual patients have responded immunologically during a natural HIV infection.

Several antienvelope MAbs possessing biological activity have been described. These activities include the ability to neutralize HIV-1 infection in vitro and the ability to lyse HIV-1-infected cells in vitro via antibody-dependent cell-mediated cytotoxicity (ADCC). For example, several type-specific neutralizing MAbs to the V3 loop of HIV-1 have been reported (3, 15, 18, 21). This activity was mediated by eight different muMAbs (3, 18, 21) and two huMAbs (15). One report describes the isolation of a neutralizing muMAb directed against amino acids (AA) 728 to 745 of the HIV-1 transmembrane glycoprotein gp41 (7). The importance of this peptide is supported by the production of weakly neutralizing polyclonal antibodies in rabbits following immunization with a synthetic peptide homologous to AA 728 to 745 (4). An additional seven muMAbs mapping to AA 423 to 437 of the HIV-1 envelope gp120 glycoprotein were shown to have group-specific neutralizing activity (3). Several antienvelope MAbs have been shown to mediate ADCC in vitro. One of these was an muMAb directed against the V3 loop (3), while several huMAbs that bind to gp41 could also mediate ADCC (41). One huMAb mediating ADCC was shown to bind to AA 644 to 663 of the HIV-1 transmembrane glycoprotein gp41 (41), while another bound to AA 579 to 604 of gp41 (41).

Previous reports had demonstrated that complement-mediated antibody-dependent enhancement (C'-ADE) of HIV-1 infection required antibody to HIV-1 envelope glycoproteins (32) and proteins of the alternative complement pathway (31). Recently, it was shown that several huMAbs against two immunodominant regions within the HIV-1 gp41 transmembrane glycoprotein could mediate C'-ADE of HIV-1 infection in vitro (28). In the study reported here, it is demonstrated that enhancing huMAbs bind to two distinct domains in the HIV-1 transmembrane gp41. The first domain was previously demonstrated to bind enhancing antibodies (29), while the second represents a domain not previously known to generate enhancing antibodies. It is shown that enhancing huMAbs bind to these two domains and act synergistically in vitro. The synthetic peptides to which these huMAbs bind blocked the C'-ADE mediated by the huMAbs as well as by polyclonal anti-HIV serum. This blockade appeared to be specific, as peptides to those two domains blocked C'-ADE significantly while peptides to two other domains did not. Furthermore, neither antibody subclass nor ability to activate complement was a determinant. Finally, it is shown that these two domains are highly conserved between all HIV-1, HIV-2, and simian immunodeficiency virus (SIV) envelope proteins that have been sequenced to date.

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MATERIALS AND METHODS

Cells and virus. A clone of the human T-cell lymphotropic virus type I (HTLV-I)-transformed T-lymphoblastoid cell line MT-2, expressing high levels of both CD4 and complement receptor type 2 (CR2) (30), was generously donated by D. D. Richman (San Diego, Calif.). H9 cells chronically infected with the HTLV-III$_B$ isolate of HIV-1 (HIV$_{3B}$) was obtained from R. C. Gallo's laboratory (National Institutes of Health, Bethesda, Md.). All cell lines were maintained in RPMI-1640 supplemented with 12% fetal calf serum and 50 μg of gentamicin per ml (growth medium). Virus was obtained from culture supernatants clarified of cells by low-speed centrifugation followed by filtration (0.45-μm pore size). For all infectivity assays, HIV-1 was inoculated at a multiplicity of infection of >1.

Peptides. Peptides 140 (AA 579 to 613), 110 (AA 488 to 510), and 120 (AA 597 to 613) were purchased from IAF Biochem (Natick, Mass.). Peptides were confirmed to be 99% pure by reverse-phase high-pressure liquid chromatography and amino acid analysis. The other HIV-1 gp41 peptides, AA540-564, AA579-604, AA611-627, AA644-663, AA661-683, and AA703-721, were gifts from T. Palker (Duke University, Durham, N.C.).

huMAbs. The huMAbs were obtained as described before (14). Briefly, lymphocytes from HIV-1 antibody-positive donors were isolated on Ficoll-Hypaque and were then transformed with Epstein-Barr virus. Transformed lymphocytes were subjected to limiting-dilution cloning, and clones were screened for reactivity against HIV-1 with a commercially available HIV-1 lysate (ElectroNucleonics, Silver Spring, Md.). Positive clones were subcloned, and supernatant fluids were used for epitope mapping experiments. Lines designated with the suffix -D were obtained by fusion of the Epstein-Barr virus-transformed lymphoblastoid lines with the SHM-D33 human-mouse heteromyeloma (40).

Peptide ELISA. For the data reported in Table 2, 1 μg of synthetic peptide in 100 μl of coating buffer (0.1 M bicarbonate buffer [pH 9.6]) was added to each well of Immulon 2 microtiter plates and incubated at 37°C for 2 h. The wells were next washed three times in phosphate-buffered saline (PBS) containing 0.05% Tween 20, and then undiluted culture supernatants containing huMAbs were added. After incubation for 2 h at 37°C, the wells were washed three times with washing buffer. Next, goat anti-human immunoglobulin G (IgG; heavy- and light-chain specific) coupled to horseradish peroxidase was added at a dilution of 1:1,000 and incubated for another hour at 37°C. After the wells were washed five times with PBS containing 0.05% Tween 20, 2,2′-azino-bis(3-ethylbenzthiazoline sulfonate) (ABTS) was added as the substrate and incubated for 30 min at room temperature. The optical density (OD) of each well was read in an enzyme-linked immunosorbent assay (ELISA) reader at 410 nm. Each assay was repeated at least three times with at least two different aliquots of huMAb-containing supernatants.

For the ELISA results reported in Table 3, a commercially available peptide derived from the envelope glycoprotein of HIV$_{3B}$ (IAF Biochem, Natick, Mass.), was coated onto Immulon plates with 2 μg of peptide in 0.1 M bicarbonate buffer (pH 9.6) per well. After overnight incubation at 4°C, nonspecifically reactive binding sites were blocked with 1% bovine serum albumin (BSA) in PBS (PBS-BSA) for 2 h at 37°C. The BSA was removed, and the wells were washed three times with PBS-BSA. HuMAbs were added at concentrations ranging from 0.9 to 460 ng/ml in PBS-BSA and incubated for 2 h at 37°C. Each well was washed three times with PBS containing 0.1% Tween 20 (PBS-Tween), and then horseradish peroxidase-conjugated goat anti-human IgG (Cappel) diluted 1:200 in PBS-Tween was added to each well and incubated for 1 h at 37°C. Wells were washed three times with PBS-Tween, and the substrate, *O*-phenylaminediamine, was added to each well. Reactions were stopped 10 min later by addition of an equal volume of 0.5 M H_2SO_4 to each well. The A_{492} was quantitated with a Flow Titertek-II.

Disulfide bond reduction. The ELISA plates were coated with antigen (peptide AA579-613) as described above at a concentration of 2 μg per well. Wells were then treated for 1 h at 37°C with 100 μl of dithiothreitol (500 pmol per well) in 0.01 carbonate buffer at pH 9.6. This and all subsequent steps were performed under a nitrogen atmosphere. Next, dithiothreitol was removed, and the wells were treated with 100 μl of iodoacetic acid (500 pmol per well) in 0.01 M carbonate buffer, pH 9.6, for 1 h at 37°C. Then, all wells were washed six times with 200 μl of 2 mM acetate buffer at pH 4.5. Wells were then blocked with 1% BSA in PBS, and ELISA was performed as described above except that all steps were done under N_2.

Assay for blockade of C′-ADE. In 96-well microdilution plates, twofold dilutions of each peptide were made in growth medium supplemented with huMAb 50-69 (80 ng/ml), huMAb 120-16 (90 ng/ml), or serum number 200 (1:500 dilution) and 1:20 human complement. HIV-1 was added and incubated at 37°C for 1 h, and then 2×10^5 MT-2 cells were added to each well. The cells were incubated for 2.5 days at 37°C in sealed modular incubators containing 5% CO_2 and 95% air. Cells were harvested for determination of cytopathic effect as described before (23), and viable cells were quantitated with Finter's neutral red dye. The A_{540} was measured with a Flow Titertek-II microcolorimeter. Controls contained HIV-1 and complement plus MT-2 cells (100% viable) or HIV-1, antibody, complement, and MT-2 cells (0% viable). The 100% viable cells represent nonenhanced HIV-1 infections, while the 0% viable cells represent maximally enhanced HIV-1 infection. The range for these two controls was 0.6 OD unit.

TABLE 1. Characteristics of huMAbs

HuMAb	Specificity	IgG subclass	Enhancing concn[a] (ng/ml)	Neutralizing concn[b] (ng/ml)
120-16	gp41	IgG2(κ)	7	>8,100
50-69	gp41	IgG2(κ)	12	>18,000
240-D	gp41	IgG1(κ)	22	>20,000
246-D	gp41	IgG1(κ)	20	>25,000
181-D	gp41	IgG2(κ)	>49,800	>49,800
98-6	gp41	IgG2(κ)	>9,000	>9,000
126-6	gp41	IgG2(κ)	>3,100	>3,100
126-50	gp41	IgG2(κ)	>2,000	>2,000
71-31	p24	IgG1(λ)	>2,000	>2,000
98-4.3	p24	IgG1(λ)	>2,000	>2,000

[a] Enhancing concentration was determined as described before (30). Enhancement was defined as a statistically significant increase in virus-induced cytopathic effect compared with control HIV-1 infections. A > symbol indicates no enhancement at any concentration of huMAb tested, and the number after the symbol represents the highest concentration of antibody tested.

[b] Neutralizing titer was determined as described before (23). Neutralization was defined as the lowest antibody concentration giving 50% or greater protection against HIV-1-induced cytopathic effect. A > symbol indicates no neutralization at any concentration of antibody tested, and the number after the symbol indicates the highest concentration of antibody tested.

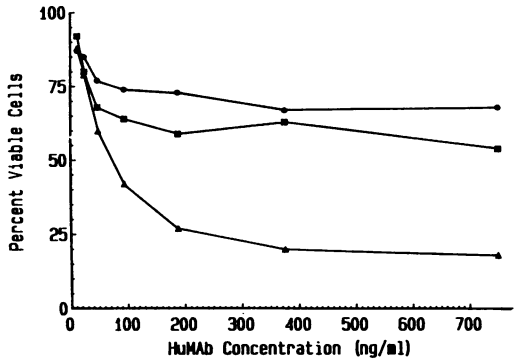

FIG. 1. Interaction between huMAbs in vitro. C'-ADE of HIV-1 infection was tested for huMAb 120-16 (■) and 50-69 (●) alone and both in combination (▲) for the ability to enhance HIV-1 infection in vitro. The percentage of viable cells is relative to the number of viable cells in a control with MT-2 cells plus HIV-1 and human complement (100% viable) and a blank (0% viable).

TABLE 3. Dose-dependent binding of enhancing huMAbs to peptide AA579-613

huMAb	Concn (ng/ml)	A_{492}
120-16	90	0.043
	9	0.040
	0.9	0.035
50-69	80	1.446
	8	0.231
	0.8	0.038
240-D	250	0.940
	25	0.297
	2.5	0.063
246-D	460	1.055
	46	0.126
	4.6	0.048
98-6 (nonenhancing *env*)	120	0.036
	12	0.036
	1.2	0.038
98-4.3 (*gag*)	90	0.033
	9	0.032
	0.9	0.034

RESULTS

Enhancing huMAbs. Previously, it had been demonstrated that huMAb 120-16 enhanced HIV-1 infection in vitro from concentrations of 12.5 μg/ml to 7 ng/ml. As illustrated in Table 1, three new huMAbs have recently been identified which enhance HIV-1 infection in vitro, huMAbs 50-69, 240-D, and 246-D. Two of the three are of the IgG1 kappa chain immunoglobulin subclass, while 50-69 is of the IgG2 kappa subclass. These huMAbs enhanced HIV-1 infection in vitro to minimum concentrations of 12 to 22 ng/ml. Enhancing huMAbs were identified in an in vitro cytopathic effect-based assay of HIV-1 infectivity described previously (30).

Two of the huMAbs, 120-16 and 50-69, were tested in in vitro assays for the ability to synergize and thereby produce greater C'-ADE than either huMAb alone. These results are shown in Fig. 1. Although both 120-16 and 50-69 enhanced HIV-1 infection alone, they were much more effective together. Furthermore, when mixed dose-effect analyses were done by the method of Chou and Talalay (6), these antibodies were synergistic. Synergy is defined by combination indices of less than 1. The combination indices for 120-16 plus 50-69 were 0.2 to 0.002 at all huMAb concentrations tested. For these analyses, $R = 0.9941$, $m = 1.021$, and $D_m = 39.7$ ng/ml, where R is the linear regression correlation coefficient, m is the slope, and D_m is the median-effect dose. For tissue culture-derived data, R should be greater than 0.95 for the Chou and Talalay analyses to be valid; $m > 1$ denotes a sigmoidal curve. These results suggest that the two antibodies recognize different, noncompeting epitopes of HIV-1.

Peptide mapping of enhancing domains. With standard ELISA methods, huMAbs were screened undiluted at concentrations ranging from 9 to 45 μg/ml against several peptides homologous to the HIV-1 41-kDa transmembrane glycoprotein. As shown in Table 2, huMAb 120-16 mapped to AA 644 to 663, while 240-D and 246-D mapped to AA 579 to 604. None of the huMAbs bound to other peptides, as indicated by an OD not significantly above background. The peptide AA579-604 is a truncation of the first enhancing domain, AA 579 to 613, and has been shown to bind several other huMAbs which enhance HIV-1 infection in vitro (29). The specificity of the huMAbs for AA 579 to 613 was confirmed by dose-dependent binding of the huMAbs to the AA579-613 peptide (Table 3). Domain AA 579 to 613 contains an intrachain disulfide linkage between AA 598 and 604; peptide AA579-604 does not have an intact disulfide linkage. As illustrated in Table 4, the binding of huMAb 50-69 to peptide AA579-613 required an intact disulfide linkage, as reduction and alkylation of the cysteines led to decreased binding. Neither huMAb 240-D nor huMAb 246-D required such an intact linkage for binding. The requirement of a disulfide bond for huMAb 50-69 to bind was supported by the finding that 50-69 did not bind to a truncated version of the peptide (AA579-604) lacking an intact disulfide linkage (Table 2).

TABLE 2. Reactivity of enhancing huMAbs with various domains of gp41

huMAb	Concn (μg/ml)	A_{405} with peptide[a]:						A_{405} in blank[b]
		1	2	3	4	5	6	
120-16	45.0	0.191	0.177	0.185	**0.610**	0.213	0.228	0.098
50-69	9.8	0.182	0.185	0.182	0.186	0.197	0.187	0.098
240-D	49	0.192	**>2.0**	0.153	0.147	0.159	0.150	ND[c]
246-D	16.8	0.173	**>2.0**	0.221	0.295	0.232	0.175	0.156
98-6	9.0	0.200	0.179	0.195	0.180	0.207	0.202	0.112

[a] Values in boldface were scored as positive. Peptides coated on plates were: 1, AA540-564; 2, AA579-604; 3, AA611-627; 4, AA644-663; 5, AA661-683; 6, AA703-721.

[b] Blank represents background when no huMAb was incubated in the plate prior to addition of enzyme-linked second antibody.

[c] ND, not determined.

TABLE 4. Binding of huMAb to reduced peptide AA579-613 versus binding to nonreduced peptide[a]

Serum or huMAb	Binding (OD_{492})	
	Reduced AA579-613	Nonreduced AA579-613
HIV-negative serum	0.150	0.227
HIV-positive serum	3.717	3.859
120-16	0.046	0.014
50-69	0.677	1.446
240-D	1.875	1.852
246-D	3.806	3.470
126-6	0.052	0.033
126-50	0.010	0.033
98-4.3	0.012	0.003

[a] AA579-613 was bound to Immulon plates, reduced with dithiothreitol, alkylated with iodoacetic acid, and then washed, and ELISA reactions were carried out under N_2. The ELISAs were performed as described in Materials and Methods.

TABLE 5. Activity of synthetic HIV-1 gp41 peptide AA651-670 against huMAbs and anti-HIV-1 serum[a]

Peptide concn (ng/ml)	Viable cells (% of control)			
	HuMAb 50-69	HuMAb 120-16	HIV immune globulin	Serum 200
4,000	0 ± 2	26 ± 4	10 ± 7	0 ± 6
2,000	0 ± 3	21 ± 3	17 ± 5	0 ± 3
1,000	1 ± 3	10 ± 2	13 ± 4	8 ± 6
500	1 ± 6	11 ± 1	12 ± 4	0 ± 4
250	0 ± 4	10 ± 4	17 ± 6	0 ± 2
125	2 ± 7	14 ± 2	0 ± 0	0 ± 3
63	1 ± 3	10 ± 3	5 ± 8	0 ± 3
32	0 ± 2	11 ± 3	6 ± 6	0 ± 5
16	0 ± 1	11 ± 2	0 ± 2	0 ± 6
8	0 ± 3	5 ± 2	2 ± 4	2 ± 3
4	2 ± 4	0 ± 3	3 ± 6	0 ± 4

[a] Assays were performed as described in Materials and Methods. Antibodies were incubated with peptide for 1 h prior to addition of virus. Values from triplicate experiments are percentages of viable cells in control infections (±1 standard deviation). Values of zero represent no protection from C'-ADE, while a value of 100% would represent complete protection from C'-ADE.

Synthetic peptides compete with HIV-1 for biological activity mediated by huMAbs. To confirm that the peptides recognized by the enhancing huMAbs, as determined by ELISA, were biologically active, enhancing huMAbs 50-69 and 120-16 were first incubated with peptide before the addition of HIV-1. As illustrated in Fig. 2, C'-ADE mediated by 50-69 was completely inhibited by the peptide AA579-613. Peptides AA488-510, AA597-613, and AA644-663 were completely incapable of inhibiting this enhancing activity. In similar experiments, peptide AA644-663 could inhibit the interaction between huMAb 120-16 and HIV-1, as illustrated by 26% inhibition of C'-ADE (Table 5); peptide AA579-613 could not inhibit C'-ADE mediated by huMAb 120-16 (data not shown). Inhibition of C'-ADE denotes an increase in viable cells compared with the number in the C'-ADE control infection; i.e., protection from enhancing antibodies. None of these peptides had any effect on HIV-1 infection in the absence of C'-ADE (data not shown).

Synthetic peptides inhibit C'-ADE mediated by human anti-HIV-1 polyclonal serum. The ability of peptides AA579-613 and AA644-663 to block C'-ADE mediated by HIV-1 antibody-positive serum was also tested. As was previously described, the peptide AA579-613 could block C'-ADE mediated by a pooled HIV-1 immunoglobulin fraction (33) (Fig. 3). As illustrated in Fig. 3, peptide AA579-613 could also block C'-ADE mediated by serum from an HIV-1 antibody-positive individual. Inhibition in this study was approximately 30% for a 1:400 dilution of anti-HIV-1 serum 200 (a potent HIV-1 infection-enhancing serum); furthermore, this peptide could inhibit HIV-1 infection to a minimum concentration of 125 ng/ml. The AA644-663 peptide could also partially block enhanced HIV-1 infection mediated by the immune globulin but had no effect on the HIV-1-positive serum (Table 5). This peptide was less effective at blocking C'-ADE than was the AA579-613 peptide.

Homology between HIV-1, HIV-2, and SIV isolates. Previous results had demonstrated that enhancing huMAbs were directed against immunodominant regions of gp41 when competition studies with human HIV-1 antibody-positive sera were performed (28). Since the four enhancing huMAbs were mapped to two domains, sequence comparisons be-

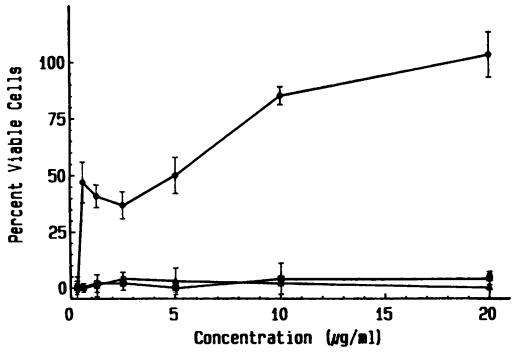

FIG. 2. Ability of synthetic peptides to block C'-ADE mediated by huMAb 50-69. Peptides were incubated with huMAb 50-69 and complement for 1 h prior to addition of HIV-1 and MT-2 cells. Peptides tested included AA579-613 (●), AA597-613 (■), and AA488-510 (▲). Not shown is peptide AA644-663, which also gave 0% viable cells at all concentrations. Results are percent viable cells (±1 standard deviation) relative to the number of viable cells in a control with MT-2 plus HIV-1 and complement (100% viable) and MT-2 plus HIV-1 plus huMAb 50-69 (80 ng/ml) plus complement (0% viable).

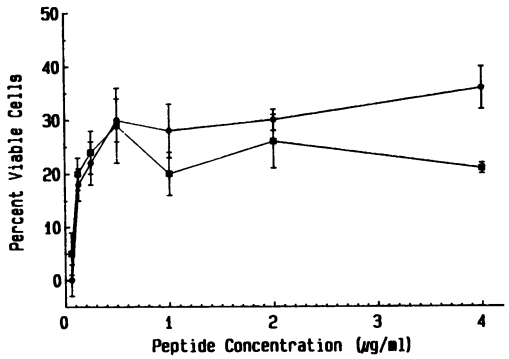

FIG. 3. Ability of peptide AA579-613 to block C'-ADE mediated by human polyclonal anti-HIV serum. Peptide was incubated with serum 200 (●) (1:400 dilution) or HIV immune globulin (■) (1:1,000, 50 µg/ml) for 1 h prior to addition to HIV-1 and MT-2 cells. Results are percent viable cells (±1 standard deviation) relative to the number in a control with MT-2 plus HIV-1 and complement (100% viable) and MT-2 plus the appropriate anti-HIV antibody (serum 200 at 1:400; anti-HIV IgG at 1:1,000) plus complement (0% viable).

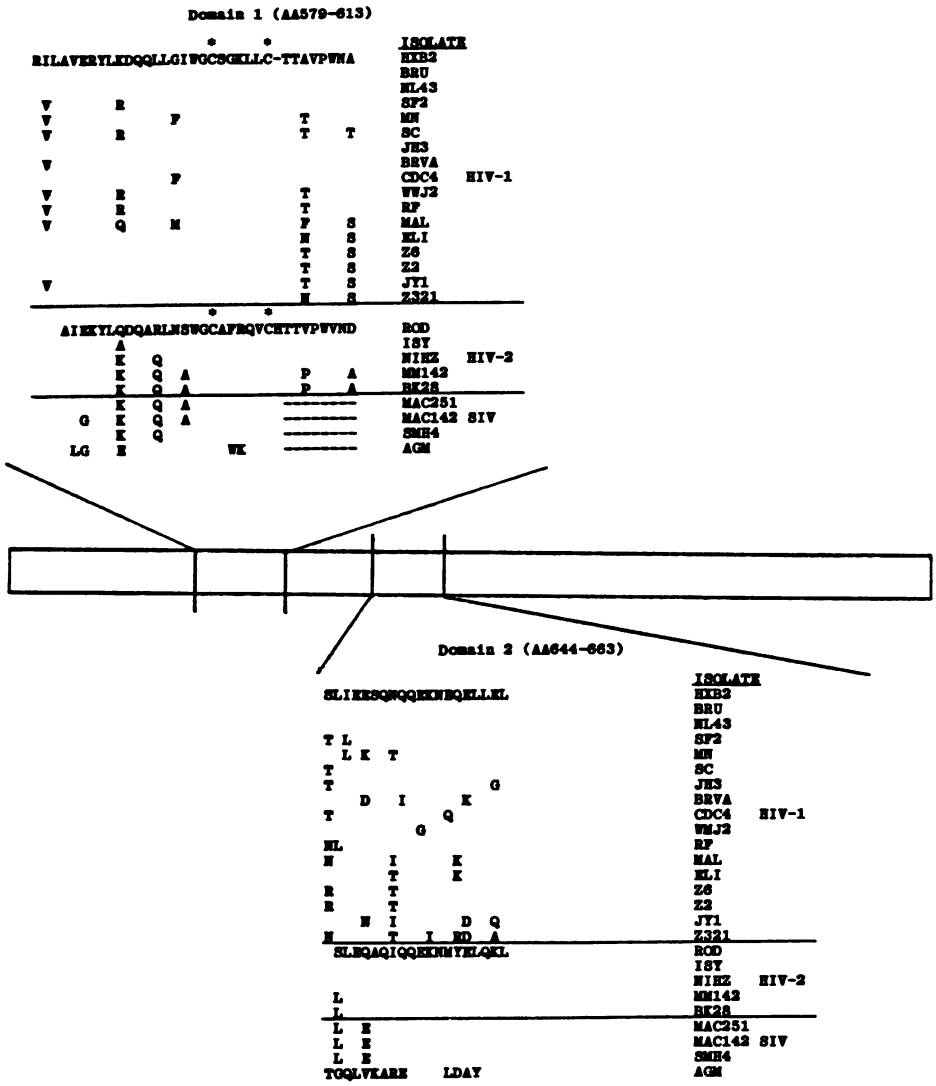

FIG. 4. Location of enhancing domains within the HIV gp41 transmembrane glycoprotein. Sequences of peptides and amino acid homologies between HIV-1, HIV-2, and SIV are indicated for each domain. Amino acid numbers are for the BH-2 isolate of HTLV-IIIB and are according to the Los Alamos Sequence Data Base (25). Letters are the standard single-letter amino acid code. *, intrachain disulfide linkage; —, amino acid deletion.

tween HIV-1, HIV-2, and SIV isolates were performed. As illustrated in Fig. 4, both domains are highly conserved among the isolates listed in the Los Alamos sequence directory (25).

DISCUSSION

Data from experiments on C'-ADE with huMAbs demonstrate that two immunogenic domains in the HIV-1 transmembrane glycoprotein gp41 are important for in vitro enhancement of HIV-1 infection. The first of these two domains, AA 579 to 613, binds five enhancing huMAb (29; this study). This peptide is strongly reactive with HIV antibody-positive serum and has been demonstrated to be the primary immunodominant domain of gp41 (5, 13, 19, 34). A portion of this domain, AA 579 to 586, has been demonstrated to be a cytotoxic T-cell epitope (34). Furthermore, at least one of the enhancing huMAbs which recognizes this domain, 50-69, has also been shown to mediate ADCC (41). The second domain, AA 644 to 663, has been identified as an immunodominant domain (19) and has been shown to bind antibodies that mediate ADCC in vitro (41).

The ability of these two domains to stimulate the in vivo formation of antibodies that confer C'-ADE of HIV-1 infection was demonstrated in several ways. First, huMAbs which mediate C'-ADE were directed toward these two domains. Second, the peptides blocked C'-ADE mediated by both polyclonal human anti-HIV serum and the enhancing huMAbs. Third, the primary requirement for an antibody to mediate enhancement was the ability of the huMAb to bind to one of the two immunogenic domains; neither immunoglobulin subclass nor ability to activate complement was a major factor (28, 29; this study), although these may play a minor role. Finally, the ability of huMAbs 120-16 and 50-69 to synergize in vitro and to enhance HIV-1 infection to a level approaching that found in polyclonal anti-HIV serum (Fig. 1) makes it appear that there are at most a few

enhancing epitopes in the HIV-1 envelope glycoproteins which can stimulate the production of enhancing antibodies. The magnitude of this enhancement is illustrated in Fig. 1 and in references 28, 29, and 30.

The peptide from the second domain, AA644-663, was much less capable of competing with HIV-1 than was the peptide from the primary immunodominant domain, AA579-613. This was evidenced by the weak inhibition of C'-ADE mediated by huMAb 120-16 by peptide AA644-663 (Table 5) compared with the complete inhibition of C'-ADE mediated by huMAb 50-69 (Fig. 2) by peptide AA579-613. Reasonable explanations include the possibility that AA644-663 is a much less antigenic domain than is AA579-613, a hypothesis supported by quantitation of antibody in patient sera to these two epitopes (41a). It is also possible that antibodies to this domain bind more effectively to native gp41 than to the synthetic peptide. This is supported by the finding that the synthetic peptide blocked C'-ADE mediated by huMAb 120-16 by only 30% and barely blocked C'-ADE mediated by human anti-HIV serum.

The two domains described herein are highly conserved between HIV-1, HIV-2, and SIV isolates (Fig. 4). Although both domains have been identified as immunogenic (5, 13, 19, 34), there are relatively few nonconservative amino acid substitutions between these three lentiviruses. Considering the high degree of mutability inherent in the HIV-1 glycoproteins and the selective pressure that should occur in vivo, this would suggest that these domains play an important role in the pathogenesis of both HIV and SIV infections. In vitro, human polyclonal antibodies against HIV-2 and monkey polyclonal antibodies against SIV both enhance HIV-1 infection (unpublished data). Therefore, the conserved nature of this region is mirrored by the immune response to these domains. One area of relatively high variability exists in the disulfide loop between AA 598 and 604. The disulfide loop itself is present in all isolates of HIV and SIV. The amino acids in the loop, however, are completely different. Thus, the architecture of a seven-amino-acid loop may be more important than the actual amino acid sequence of the loop. Alternatively, the lack of identity between amino acids within the loop may be important for the lack of cross-reactivity between the enhancing anti-HIV huMAbs and SIV (data not shown).

Although the in vitro mechanism of C'-ADE has been described (30, 33) and has been shown to require cells bearing both CD4 and complement receptor type 2 (30, 33), the in vivo relevance of enhancing antibodies remains to be determined. Nevertheless, there is a growing body of evidence to suggest that enhancing antibodies are important in the pathogenesis of lentivirus infection. In the SIV model of HIV-1 infection, the levels of enhancing antibodies rise as animals progress toward death, with peak enhancing antibody titers observed immediately prior to death (22). For HIV, it has been demonstrated by using Fc receptor-mediated ADE that patients' antibodies initially can enhance but not neutralize their own viral isolate. Furthermore, later in infection, when antibodies that neutralize a viral isolate from a previous timepoint are detectable, newly isolated virus is subject only to enhancement, not to neutralization (17). These results suggest that selective pressures are present which favor the maintenance of an enhancing serotype.

In vaccine trials, there is additional evidence of enhancement of both HIV-1 infection and SIV infection in vivo. Despite successful immunizations of rhesus macaques with whole, inactivated SIV (9, 24, 39), there have been several noteworthy failures. For example, macaques immunized with inactivated SIV and subsequently mucosally challenged with SIV were not protected and may have shown increased illness (39). Macaques which were immunized with a vaccinia virus-SIV envelope recombinant virus and were subsequently boosted with whole inactivated SIV, thus developing high humoral responses to the SIV envelope, were not protected from SIV challenge, although the same whole inactivated SIV had protected other animals (9). Finally, in a recently published study in which recombinant gp120 protected two chimpanzees from subsequent challenge with HIV-1, it was shown that two additional chimpanzees which had received recombinant gp160 were not protected (2). This gp160 preparation contained the two domains to which enhancing antibodies bind. In fact, there was some evidence that HIV-1 infection was enhanced, since humoral responses against the HIV-1 p24 core antigen were apparent much earlier in the gp160-immunized animals than in the control animal (2).

Although there is still much debate as to the importance of ADE of HIV-1 infection in HIV-1 pathogenesis, there is some evidence that ADE may be important. The identification and mapping of enhancing huMAbs provide tools which can be used to determine the in vivo importance of C'-ADE. Additional studies should be performed to determine whether anti-SIV mAbs (20) can enhance SIV infection in vitro. If so, epitopes which bind SIV-enhancing antibodies could be mapped directly. Future studies should involve the immunization of macaques with the SIV equivalent of both domains to which enhancing antibodies bind and the subsequent challenge of immunized and control macaques with the same isolate of SIV. Only these types of experiments, in which animals are challenged in the presence of "pure" enhancement, will allow the determination of whether enhancement is important in vivo. Nevertheless, the identification of two domains which bind enhancing antibodies in gp41 should alert vaccine developers to the possible benefits of deleting such domains from future vaccine candidates.

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