JOURNAL OF VIROLOGY, Sept. 1989, p. 3579–3585
0022-538X/89/093579-07$02.00/0

Vol. 63, No. 9

Generation and Characterization of Monoclonal Antibodies to the Putative CD4-Binding Domain of Human Immunodeficiency Virus Type 1 gp120

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Received 28 December 1988/Accepted 9 May 1989

A panel of seven monoclonal antibodies against the relatively conserved CD4-binding domain on human immunodeficiency virus type 1 (HIV-1) gp120 was generated by immunizing mice with purified gp120. These monoclonal antibodies reacted specifically with gp120 in an enzyme-linked immunosorbent assay and Western blots (immunoblots). By using synthetic peptides as antigens in the immunosorbent assay, the epitopes of these seven monoclonal antibodies were mapped to amino acid residues 423 to 437 of gp120. Further studies with radioimmunoprecipitation assays showed that they cross-reacted with both gp120 and gp160 of diverse HIV-1 isolates (HTLV-IIIB, HTLV-IIIRF, HTLV-IIIAL, and HTLV-IIIWMJ). They also bound specifically to H9 cells infected with HTLV-IIIB, HTLV-IIIRF, HTLV-IIIAL, HTLV-IIIZ84, and HTLV-IIIZ34 in indirect immunofluorescence studies. In addition, they blocked effectively the binding of HIV-1 to CD4$^+$ C8166 cells. Despite the similarity of these properties, the monoclonal antibodies differed in neutralizing activity against HTLV-IIIB, HTLV-IIIRF, and HTLV-IIIAL, as demonstrated in both syncytium-forming assays and infectivity assays. Our findings suggest that these group-specific monoclonal antibodies to the putative CD4-binding domain on gp120 are potential candidates for development of therapeutic agents against acquired immunodeficiency disease syndrome.

Human immunodeficiency virus (HIV) is the etiological agent of acquired immunodeficiency syndrome (AIDS) (1, 11). The major clinical manifestations of this syndrome, opportunistic infections and dementia, are attributable to the preferential tropism of HIV for CD4$^+$ T-helper lymphocytes as well as other CD4-bearing cells, such as monocytes/macrophages (12, 36), histiocytelike cells, and microglia in the brain (15). The tropism is dictated by specific interactions between the host-encoded CD4 receptor and the HIV envelope protein gp120 (4, 18, 26). The important role of CD4 as the HIV receptor is demonstrated from a study in which nonpermissive cell lines for HIV infection were converted into permissive cell lines after transfection with a human CD4 cDNA (23). Binding of HIV-1 to CD4 was reported to induce phosphorylation of CD4, which then activates protein kinase C. The HIV-induced CD4 phosphorylation can be blocked by a mouse monoclonal antibody (MAb) against CD4 and by a MAb against the HIV envelope protein gp120, suggesting the importance of the specific interaction between CD4 and gp120 (8).

Since the CD4 antigen was identified as the cell surface receptor for HIV type 1 (HIV-1), five independent laboratories have recently shown that soluble forms of CD4 antigen can block the infectivity of the virus (5, 9, 17, 38, 41). The soluble forms inhibit diverse variants of HIV-1, indicating that all of these viruses may share a relatively conserved CD4-binding region (2, 42). Taking advantage of the high affinity between the soluble form of gp120 and the recombinant cell surface CD4, Lasky et al. (21) have identified a gp120-specific murine MAb capable of inhibiting the interaction between gp120 and CD4. The epitope recognized by this MAb has been mapped to a conserved region C3 (27) within amino acid residues 413 to 456, where HIV-1 and distantly related HIV-2 share significant homology (28). By using in vitro mutagenesis, the deletion of 12 amino acids (426 to 437) from this region or an amino acid substitution at position 433 results in a complete loss of binding to CD4, suggesting that this region is directly involved in the binding of gp120 to CD4 receptor. This domain of gp120 also appears to be important in binding to CD4, as shown in several other studies (6, 19, 22). An immunological intervention directed toward the CD4-binding region of gp120 may be a promising approach to immunotherapy or vaccine development. However, the CD4-binding region of the envelope gp120 does not appear to be immunogenic in infected persons, since there is very weak antigenic cross-reactivity against envelope proteins from sera from patients infected with HIV-1 or HIV-2 (3, 44). If a high-affinity antibody specific for the CD4-binding region of gp120 can be produced, it is theoretically possible to block the infectivity of all isolates of HIV that use CD4 as the receptor. Here we report the generation of seven mouse MAbs against the CD4-binding region on gp120 and their neutralizing and virus-binding inhibitory activities against different isolates of HIV-1.

MATERIALS AND METHODS

MAb production. The envelope glycoprotein, gp120, of HTLV-IIIB was prepared from extracts of HTLV-IIIB-infected H9 extracts. HTLV-IIIB-infected H9 cells were lysed with a lysing buffer consisting of 10 mM Tris hydro-

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chloride (pH 7.5), 150 mM NaCl, 1 mM $MnCl_2$, 0.5% Triton X-100, and 0.1 mM phenylmethylsulfonyl fluoride. The extracts were heat inactivated for 1 h at 56°C and reacted with lentil-Sepharose (Sigma Chemical Co., St. Louis, Mo.). The bound fraction was eluted and incubated with Affigel-10 coupled with a murine MAb against gp120 (BAT123) (10). The viral gp120 fraction was eluted and used as the immunogen. Male BALB/c mice were immunized with 25 μg of protein in Freund complete adjuvant and three subsequent immunizations of 25 μg in the same adjuvant at 1-month intervals. Three days after the final booster immunization, the mice were sacrificed and spleen cells were isolated and fused with Sp2/0 myeloma cells as described by Fung et al. (10). Hybrids were selected by supplementing the growth medium with 0.1 mM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine. Two weeks later, supernatants were collected from the wells of the microdilution plates for consecutive screening by enzyme-linked immunosorbent assay (ELISA), with peptide T35S and gp120 as coating antigens. MAbs from hybridomas selected for further characterization were produced in mouse ascites fluid and purified by protein A affinity chromatography (7, 10).

Virus isolates and persistently infected cells. H9 cells persistently infected with HTLV-IIIB, HTLV-IIIRF, HTLV-IIIMN, HTLV-IIIAL, HTLV-IIIWMJ, HTLV-IIIZ34, and HTLV-IIIZ84 were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Cell-free culture supernatants from H9 cells infected with HTLV-IIIB, HTLV-IIIRF, and HTLV-IIIAL containing high levels of reverse transcriptase activity were frozen in equal portions and used for neutralization assays.

Indirect immunofluorescence assay on live HIV-1-infected H9 cells. The method for immunofluorescence staining of HIV-1-infected H9 cells was described by Fung et al. (10). Portions (50 μl) of infected H9 cells at 5×10^6 cells/ml were added to 1.5-ml microfuge tubes. Protein A-purified MAb (50 μl; 5 μg/ml) was added, and the preparation was incubated for 30 min at room temperature (RT). The tubes were then centrifuged at 300 × *g* for 5 min, the supernatants were removed, and the cells were washed with RPMI 1640 containing 2% fetal bovine serum and 0.1% sodium azide. The tubes were tapped to loosen the cells. A 10-μl amount of fluorescein isothiocyanate-conjugated goat anti-mouse immunoglobulin G (Tago, Burlingame, Calif.) was added at a dilution of 1:200 in phosphate-buffered saline (PBS) and incubated for 30 min at RT. After the cells were washed, they were suspended in PBS, placed on slides, mounted, and examined with a fluorescence microscope. The controls were uninfected H9 cells and antibodies of irrelevant specificities.

ELISA. The screening and epitope mapping of MAbs were performed by ELISA. Wells of Immulon 2 microdilution plates (Dynatech, Chantilly, Va.) were coated overnight at RT with 100 μl of synthetic peptides (5 μg/ml; 0.5 μg/ml for T35S) or 100 μl of purified gp120 (0.1 μg/ml) in PBS. They were then incubated with 5% BLOTTO in PBS for 1 h at RT and washed with PBS-Tween 20 (0.05%). Next, 100 μl of culture medium, 100 μl of purified mouse MAb (diluted to 0.4 μg/ml with PBS-Tween 20), or 100 μl of diluted human serum (diluted 1:100 with BLOTTO buffer) was added, and the wells were incubated for 1 h at RT with the appropriate goat anti-mouse or goat anti-human immunoglobulin G conjugated with horseradish peroxidase (diluted with 5% BLOTTO in PBS). After another washing step, bound antibodies were visualized by reaction with 0.1% tetramethylbenzidine and 0.0003% hydrogen peroxide as substrates. The optical density (OD) of the reaction solution was read at 450 nm.

Generation of gp120 peptides. Peptide T35S was synthesized and characterized by Peninsula Laboratories (Belmont, Calif.). All other peptides used were synthesized by using the RaMPs peptide synthesis system (Du Pont Co., Wilmington, Del.) according to the 9-fluorenylmethoxycarbonyl synthesis protocol described in the manual. The purity of the RaMPS-synthesized peptides was characterized by high-performance liquid chromatography, and the predicted structure was assessed by fast-atom bombardment-mass spectrometry analysis. Amino acid residues and the sequences of the synthetic peptides were derived from the HXB2 clone of HIV-1 (28) (see Fig. 3 and 6). Peptides consisted of the following residues: T35S, 413 to 447; T15W, 413 to 427; I15P, 423 to 437; A15S, 433 to 447; I15D, 443 to 457; and Q11K, 422 to 432. Dynorphin A (residues 1 to 13), used as a negative control in assays of epitope mapping, was purchased from Peninsula Laboratories.

Immunoreactivities of patient sera to peptide T35S. The reactivities of HIV-1-seropositive patient sera to peptide T35S were tested by ELISA. Sera were collected randomly from 65 HIV-1-seropositive patients. Of these subjects, 30 had AIDS, 19 had AIDS-related complex (ARC), and 16 were asymptomatic. Sera from 10 HIV-1-seronegative donors were also tested as controls.

RIPA. The radioimmunoprecipitation (RIPA) procedure was carried out by an established protocol (14). Briefly, H9 cells (infected with HTLV-IIIB, HTLV-IIIRF, HTLV-IIIAL, and HTLV-IIIWMJ) were metabolically labeled for 4 h with [^{35}S]cysteine and [^{35}S]methionine (100 μCi/ml; ICN Pharmaceuticals Inc., Irvine, Calif.) and suspended in a RIPA lysing buffer (50 mM Tris hydrochloride [pH 8.0], 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride). Lysates were precleared with protein A-Sepharose bound to rabbit antiserum to mouse kappa light chain (κ-PAS) for 3 h at RT. RIPA was performed by adding 3 μg of purified mouse MAb and 0.2 ml of a 10% suspension of κ-PAS to 200 μl of labeled and clarified lysate. The samples were incubated for 18 h at 4°C, and the beads were washed with the RIPA lysing buffer. The pellets were suspended in electrophoresis sample buffer and boiled for 3 min. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by autoradiography.

Neutralization assay of HIV-1. Virus neutralization studies were performed by using two different assays. For the syncytium-forming assay with CEM-SS cells described by Nara et al. (29), twofold serial dilutions of MAbs were made in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum. A 50-μl sample of each diluted antibody was mixed with an equal volume of virus (100 syncytium-forming units [SFU]) and incubated for 1 h at RT. The mixtures were added to two poly-L-lysine-treated microdilution wells containing 5×10^4 DEAE-dextran-treated CEM-SS cells and incubated for 3 to 4 days. The number of syncytia formed was counted by using an inverted microscope. The neutralization titers were determined by using the 50% ($V_n/V_o = 0.5$) neutralization point, where V_n is the number of virus-induced SFU in the test wells and V_o is the total number of virus-induced SFU in the control when growth medium alone was added. A nonneutralizing anti-gp120 MAb (BAT496) was used as a negative control for specific inhibition of infection (10). The second neutralization assay, described by Ho et al. (14, 16), measured the

TABLE 1. Immunofluorescence staining of seven anti-CD4-binding-region antibodies with H9 cells infected with six HTLV-III strains

MAb	Relative intensity of immunofluorescence with given strain					
	B	RF	MN	AL	Z84	Z34
G3-42	+	++	++	++	+++	+
G3-211	+	++	++	++	+++	+
G3-299	+	+	++	+	++	+
G3-508	+	++	++	++	+++	+
G3-519	+	++	++	++	+++	+
G3-536	+	+	++	++	+++	+
G3-537	+	+	++	++	+++	+

TABLE 2. Radioimmunoprecipitation reactions of seven anti-CD4-binding-region antibodies with the gp120 of four HTLV-III strains

MAb	Relative intensity of gp120 protein band with given strain			
	B	RF	AL	WMJ
G3-42	++	+	+	+
G3-211	++	+	+	+
G3-299	++	+	+	+
G3-508	++	+	+	+
G3-519	+++	+	++	+
G3-536	+++	+	+	+
G3-537	+++	+	++	+

extent of the inhibition of HIV-1 infectivity in H9 cells. In this assay, 100 μl of virus inoculum (50 50% tissue culture infective doses) was preincubated with 100 μl of test antibodies of different concentrations for 1 h at 37°C before inoculation into 0.75×10^6 H9 cells in 1.5 ml of RPMI 1640 medium supplemented with 15% fetal bovine serum, 10 mM HEPES (*N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 250 U of penicillin per ml, 250 μg of streptomycin per ml, and 2 mM L-glutamine. On day 7, cell-free culture supernatants were collected for assay of HIV-specific p24 antigen by ELISA (Abbott Laboratories, North Chicago, Ill.).

HIV-1-binding inhibition assay. The binding inhibition assay was adopted from the method of McDougal et al. (26) and has been described previously (14). Briefly, a concentrated preparation of HIV-1 virions (10 μl) was pretreated with 10 μl of MAb (1 mg/ml) for 30 minutes at RT before incubation with C8166 cells (5×10^5, 30 min at 37°C). The cells were then washed and suspended in 25 μl of human anti-HIV conjugated to fluorescein isothiocyanate (diluted 1:50). After 30 min at 4°C, the cells were washed, fixed in 1% paraformaldehyde, and analyzed by flow cytometry.

RESULTS

Generation and characterization of gp120-specific MAbs. Affinity-purified gp120 was prepared from cell extracts of HTLV-IIIB-infected H9 cells. Five male BALB/c mice were used for immunization and fusion as described in Materials and Methods. In a typical fusion, equal numbers of spleen cells and Sp2/0 myeloma cells were fused, and 2×10^8 cells were plated in 10 96-well microdilution plates. On average, each well had about 10 hybrid colonies after 10 days. Culture supernatants from growing hybridomas (estimated to be 140,000) from five independent fusions were screened for antibodies first against gp120 by ELISA and then against T35S, a synthetic peptide containing the CD4-binding region, as described by Lasky et al. (21). Seven outgrowths giving the strongest positive reactions in both screening assays were single-cell cloned by limiting dilution, and supernatants were screened by ELISA, using peptide T35S as the coating antigen. These seven MAbs were designated as G3-42, -211, -299, -508, -519, -536, and -537; all were immunoglobulin G1. Recognition of gp120 of HTLV-IIIB by these antibodies was also demonstrated by Western immunoblots (results not shown). Their reactivities with other HIV-1 strains, such as RF, MN, AL, Z84, and Z34, were confirmed by indirect immunofluorescence staining of the surfaces of virus-infected cells (Table 1). By using metabolically labeled HTLV-IIIB-, HTLV-IIIRF-, HTLV-IIIAL-, and HTLV-IIIWMJ-infected H9 cell lysates, the MAbs were also examined by RIPA. All seven MAbs specifically precipitated both gp120 and gp160 of diverse HIV-1 isolates (Table 2). The results further indicated the broad reactivity of these antibodies to many strains of HIV-1.

Neutralization of HTLV-IIIB, HTLV-IIIRF, and HTLV-IIIAL infectivity. The neutralizing activities of these seven protein A-purified anti-CD4-binding-region MAbs were measured by two methods. In the syncytium-forming assay (30), 100 SFU of HIV-1 was mixed with various dilutions of the MAbs for 1 h at RT before inoculation onto CEM-SS cells in polylysine-coated microdilution wells. Syncytia were counted on day 3 or 4. To determine whether a group-specific neutralizing immune response was a feature of MAbs to the CD4-binding region, HTLV-IIIB and HTLV-IIIRF isolates, which differ by 21.4% in their predicted amino acid sequence in gp120 (28), were used in the neutralization assay. These seven MAbs clearly showed different neutralizing activities against both isolates (Fig. 1). The ID_{50} (50% inhibitory dose) of each MAb against both HTLV-IIIB and HTLV-IIIRF is listed in Table 3. The data in Fig. 1 and Table 3 suggest that G3-299 was strongest in neutralization of HTLV-IIIB, whereas G3-519 was the most potent in neutralization of HTLV-IIIRF. However, most of these MAbs required high doses (>10 μg/ml) to achieve 50% neutralization of the input HIV-1. In all of these assays, the nonneutralizing anti-gp120 MAb BAT496 did not inhibit infection even at 100 μg/ml (results not shown).

Similar HIV-1 neutralization results were obtained for the seven MAbs in a different neutralization assay (14, 16) (Fig. 2 and Table 3). Again, the most potent antibodies for neutralization of HTLV-IIIB and HTLV-IIIRF were G3-299

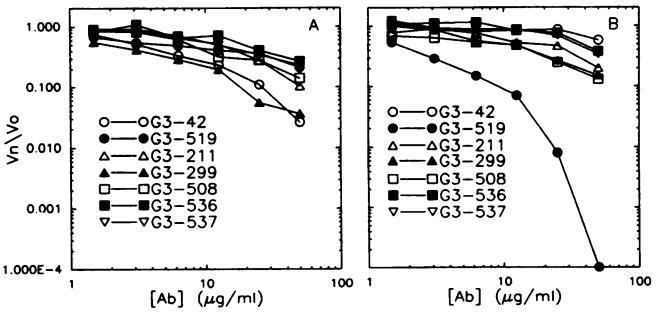

FIG. 1. Neutralization of HTLV-IIIB (A) and HTLV-IIIRF (B) isolates by seven anti-CD4-binding-region antibodies, determined by the syncytium-forming assay. V_n and V_o are defined in Materials and Methods. Ab, Antibody.

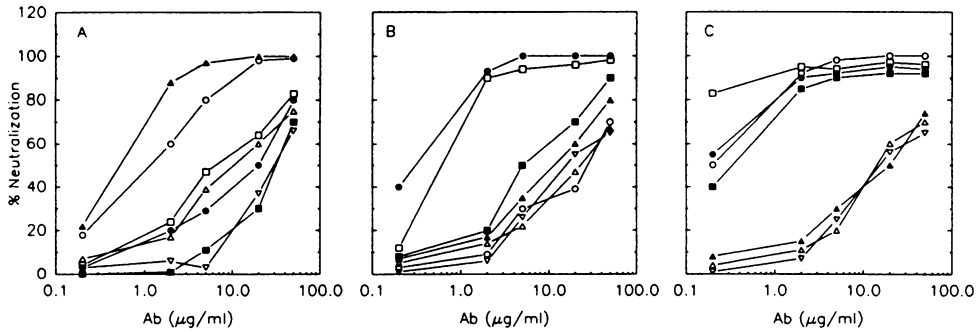

FIG. 2. Neutralization of HTLV-IIIB (A), HTLV-IIIRF (B), and HTLV-IIIAL (C) isolates by seven anti-CD4-binding-region antibodies, determined by an infectivity assay using H9 cells. The reduction in supernatant p24 antigen was compared with that of control cultures. Symbols as shown in Fig. 1. Ab, Antibody.

and G3-519, respectively. HTLV-IIIAL was particularly sensitive to neutralization by G3-508, G3-519, G3-42, and G3-536. The ID_{50}s of G3-508 and G3-519 were both less than 0.2 μg/ml (Table 3). Nevertheless, as observed with the syncytium-forming assay, several MAbs required doses of greater than 10 μg/ml to neutralize HIV-1 infectivity by 50%.

Delineation of the epitope in the CD4-binding region. To map more precisely the epitope location in the CD4-binding region on gp120, four peptides (T15W, I15P, A15S, and I15D), overlapping with each other by five amino acids, and a fifth peptide, Q11K, identified as the epitope of MAb 5C2E5 (21), were synthesized (Fig. 3). ELISA against this group of peptides was performed with the seven MAbs. All of the MAbs bound I15P (the critical region for interaction of gp120 with the CD4 receptor) very strongly (OD >1.0) but not the adjacent peptides (OD, <0.1) (Table 4) or the negative control peptide (dynorphin A). It is interesting that peptide Q11K, an 11-amino-acid peptide encompassed almost entirely by peptide I15P except for the N-terminal glutamine, did not exhibit appreciable reactivity to any of the seven MAbs under our experimental conditions.

Inhibition of HIV-1 binding to $CD4^+$ cells. All seven MAbs directed against amino acids 423 to 437 of gp120 exhibited significant inhibition of binding of HTLV-IIIB or HTLV-IIIRF to $CD4^+$ C8166 cells (Fig. 4). Despite the substantial differences in ability to neutralize HIV-1 (see above), the inhibitory activities of these MAbs on the specific binding of HIV-1 to the $CD4^+$ C8166 cells were comparable. In contrast, BAT123, a mouse MAb specific for the central hypervariable loop of gp120 (amino acid residues 300 to 330) (M. S. C. Fung et al., unpublished results), had no effect on HIV-1 binding to the target cells.

Serum reactivity of the CD4-binding-region peptide T35S. Serum samples from patients with AIDS or ARC, asymptomatic HIV-1-seropositive individuals, and normal healthy donors were analyzed by ELISA for reactivity with peptide T35S. For each serum sample, an OD value was calculated as the difference between the average absorbance of two peptide-coated wells and two PBS-treated wells. The cutoff OD (0.075) represented the mean OD plus 2 standard deviations for 10 seronegative serum samples. The results of the ELISA (Fig. 5) indicated that the immunoreactivities exhibited by sera from patients with AIDS or ARC were not significantly different from those of normal control samples. Among the 30 AIDS and 19 ARC patients studied, no detectable antibodies to peptide T35S were found. Only serum samples from 2 of the 16 asymptomatic seropositive individuals gave signals significantly above the cutoff point.

TABLE 3. ID_{50}s for seven MAbs in two different neutralization assays against HTLV-IIIB, HTLV-IIIRF, and HTLV-IIIAL[a]

MAb	ID_{50} (μg/ml)				
	Syncytium-forming assay		Infectivity assay		
	HTLV-IIIB	HTLV-IIIRF	HTLV-IIIB	HTLV-IIIRF	HTLV-IIIAL
G3-42	3.4	>50.0	1.2	25.0	0.2
G3-211	13.4	18.8	10.0	22.0	12.0
G3-299	2.1	11.1	0.5	10.0	20.0
G3-508	8.0	10.4	6.0	0.6	<0.2
G3-519	5.7	1.8	20.0	0.3	<0.2
G3-536	21.4	42.1	30.0	5.0	0.3
G3-537	12.5	38.6	30.0	15.0	12.0

[a] Results were obtained from experiments described in Fig. 1 and 2. ID_{50} is defined as the concentration of MAb at which the infection of target cells by HIV-1 is inhibited by 50%.

DISCUSSION

gp120, the exterior envelope protein of HIV-1, has been shown to be the major target of neutralizing antibodies (34). Recombinant gp120 or gp160 expressed in bacteria (32), mammalian cells (20), insect cells (35), or yeast cells (39) also elicits neutralizing antibodies in immunized animals. The epitopes involved in virus neutralization appear to be located in both hypervariable and conserved regions of gp120 (16, 43). Animal antisera against purified gp120 derived from HTLV-IIIB were able to neutralize infection of target cells by the homologous virus isolate but failed to neutralize the divergent HTLV-IIIRF isolate (25, 30). The production of

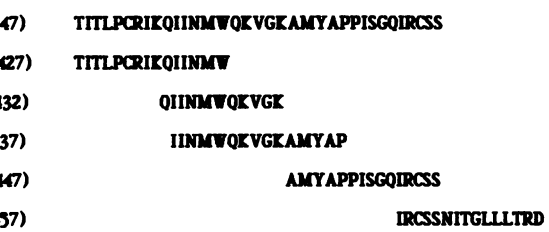

FIG. 3. Location and sequence of synthetic peptides within the CD4-binding region of gp120 of HTLV-IIIB. Amino acid residues and sequences are from Myers et al. (28).

TABLE 4. Reactivities of seven MAbs to HIV-1 envelope gene-encoded synthetic peptides in ELISA[a]

Peptide	Reactivity						
	G3-42	G3-211	G3-299	G3-508	G3-519	G3-536	G3-537
T35S	+	+	+	+	+	+	+
T15W	−	−	−	−	−	−	−
Q11K	−	−	−	−	−	−	−
I15P	+	+	+	+	+	+	+
A15S	−	−	−	−	−	−	−
I15D	−	−	−	−	−	−	−

[a] The ELISA procedure is described in Materials and Methods. Reactions of the MAbs were considered negative (−) when the OD was less than 0.1 and positive (+) when the OD was greater than 1.0.

type-specific antisera in goats, rabbits, and chimpanzees appears to be largely due to the presence of an immunodominant but hypervariable epitope in the V3 region (amino acids 307 to 321). This neutralizing epitope was hypothesized to be a loop between two cysteine residues connected by a disulfide bridge (13). The high variability of HIV envelope protein gp120 may contribute to the lack of success of initial trials of vaccines in monkeys (31). The immunodominance of this hypervariable neutralizing epitope can also account for the type specificity of several mouse MAbs against HIV-1 isolates reported by Matsushita et al. (24), Thomas et al. (40), and our laboratory (unpublished observation).

The ability of soluble forms of CD4 to block the infectivity of several diverse HIV-1 isolates implies that the CD4-binding region of gp120 should be well conserved (Fig. 6) and that MAbs interacting with this region may be able to block the infectivity of various HIV-1 isolates. Kowalski et al. (19) have identified mutations at amino acids 363, 419, and 473 that disrupt CD4 binding. The second mutant (Δ419) is contained in the conserved region previously defined by Lasky et al. as important for CD4 binding (21). However, truncation of the C-terminal region of gp120 (amino acids 467 to 511) greatly reduces its binding to CD4-positive cells (22), and deletion of the N-terminal 164 amino acids of gp120 results in a total loss of CD4 binding (6). Nevertheless, several MAbs against these regions do not affect gp120-CD4 binding, suggesting their potential role in maintaining the proper conformation of the CD4-binding site (6, 22). One mouse MAb interacting with a gp120 peptide containing amino acids 413 to 456 is reported to be able to block the

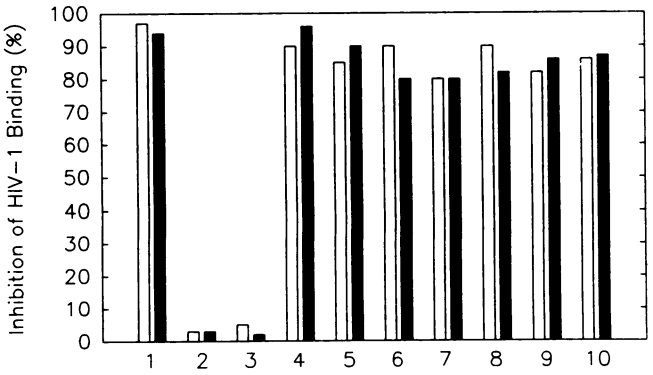

FIG. 4. HIV-1-binding inhibition studies of HTLV-IIIB (□) and HTLV-IIIRF (■), using AIDS serum (1), normal human serum (2), BAT123 (3), G3-519 (4), G3-508 (5), G3-42 (6), G3-211 (7), G3-299 (8), G3-536 (9), and G3-537 (10).

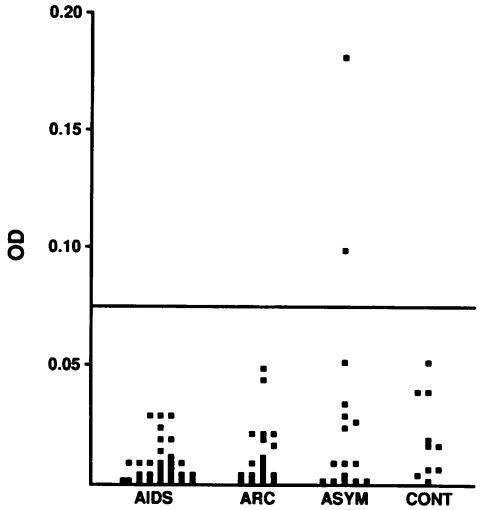

FIG. 5. Immunoreactivity of human sera from AIDS (n = 30), ARC (n = 19), and asymptomatic HIV-1-seropositive (ASYM; n = 16) patients and from seronegative controls (CONT; n = 10) to peptide T35S. The cutoff OD was taken as the mean OD plus 2 standard deviations for 10 seronegative samples.

binding of gp120 to CD4, and further studies map its epitope to the peptide segment Q11K (amino acids 422 to 432) (21). A second mouse MAb with an epitope residing in amino acid residues 409 to 424 of gp120 also inhibits CD4-gp120 binding and thus confirms the importance of residues 413 to 456 for binding (6). Our seven MAbs also bind specifically to this putative CD4-binding region, and their epitopes are mapped to peptide I15P (amino acids 423 to 437). These antibodies block the specific binding of both HTLV-IIIB and HTLV-IIIRF to $CD4^+$ cells with higher potency (Fig. 4) than that of MAb BAT123, which probably mediates a distinctive mechanism of neutralization. These findings strongly support the role of peptide I15P in gp120 binding to CD4.

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        413                                                  457
HXB2    T  ITLPCRIKQIINMWQKVGKAMYAPPISGQIRCSSNITGLLLTRD
BRU     ------------F-----E--------------------------
MN      -------Q-K---------E----------E----------------
SC      ------------E------E----------K--VK------------
SF2     -----I------------E----------G---S------------
NY5     -----II---------SGR-+++
CDC4    -GDI-----------R--V-------L--K-L---------------
WMJ2    -L----------------G----------Q-----------------
RF      -------------V----E-------------K-I-----------
MAL     S-------------------T---------A-V-N-L------I----
ELI      ------Q-------K-VAGR --I-----ERN-L------------
Z6      KL-----Q----------G----------E---N-------------
Z3      -GN----------VVRT--G--Q-------E-T---------------
Z321    -----I---------V----R--Q-------K-V-K-V-----I-----
JY1      ----K------------G----------E-L-K-T-----------
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FIG. 6. Amino acid sequences of the putative CD4-binding domain of gp120 of different HIV-1 strains (28). Amino acid sequences are given in single-letter codes. Homologous sequences are represented by broken lines, deletions by spaces, and undetermined sequences by +.

All of our seven MAbs neutralize the infectivity of HIV-1 in vitro, though with various levels of activity. Their neutralizing activities against HTLV-IIIB and HTLV-IIIRF were first demonstrated by the syncytium-forming assay (29) and then independently confirmed against HTLV-IIIB, HTLV-IIIRF, and HTLV-IIIAL in an infectivity assay (14, 16). In contrast, the MAb 5C2E5 reported by Lasky et al., with the epitope mapped to peptide Q11K (residues 422 to 432), to which our seven MAbs did not bind (Table 4), failed to neutralize HIV-1 (21). It is still unclear why these MAbs differ significantly in neutralizing activity despite having the same isotype, similar epitope, and comparable effects on gp120-CD4 binding. The answer probably lies with antibody affinity and avidity, studies of which are now in progress.

Our data clearly show a lack of antibodies to the CD4-binding region of gp120 peptide (T35S) in AIDS and ARC patient sera. In addition, only two serum samples from 16 asymptomatic HIV-1-seropositive individuals showed positive reactions with this peptide. Whether the absence or decrease of antibodies to the CD4-binding region is related to disease progression remains unclear. In this context, Lasky et al. (21) attributed the low titers of antibodies directed against the CD4-binding region to the hypothesized cleft shape of this domain, which made the generation of antibodies difficult or impossible. Better understanding of the conformation of the CD4-binding site on gp120 from various HIV-1 isolates will provide important information on the neutralization activities of the antibodies. Another possibility is raised by the findings of Siliciano et al. (37) that uninfected $CD4^+$ T lymphocytes and antigen-presenting cells expressing class II molecules of the major histocompatibility complex are killed by HIV-1-specific cytotoxic T cells after exposure to soluble gp120 and presentation of part of the CD4-binding-region sequence LPCRIKQIINMWQK (amino acids 416 to 429). Thus, HIV-1 may be able to escape immunosurveillance in part because of the inability of the human host to generate neutralizing antibodies against this conserved site. Our data that only seven monoclonal antibodies specific for this region are isolated from 140,000 fusion hybrids support the notion that that CD4-binding region of gp120 is immunosilent.

Sera from most HIV-1-infected patients have neutralizing antibodies (16, 33) that in some cases are group specific and can cross-neutralize up to seven different HIV-1 isolates, indicating that there are conserved antigenic epitopes for neutralizing antibodies (43). Antibodies to these conserved regions are potential candidates for targeting cytotoxic agents to infected cells. It is our goal to generate mouse MAbs against critical conserved sites and to engineer such MAbs into human-mouse chimeric antibodies of an appropriate isotype while retaining their neutralizing activity and ability to mediate antibody-dependent accessory functions. Such chimeric antibodies may be less immunogenic and thus better suited for therapeutic or prophylactic use. Our current results represent a first step in this endeavor.

ACKNOWLEDGMENTS

We thank Mary Song and Tarsem Moudgil for excellent technical assistance and W. T. Moore and R. Caprioli of the Department of Analytical Chemistry, University of Texas Health Science Center, Houston, for fast-atom bombardment-mass spectrometry analysis.

LITERATURE CITED

1. **Barré-Sinoussi, F., J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Brun-Vézinet, C. Rouzioux, W. Rozenbaum, and L. Montagnier.** 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). Science **220**:868–871.
2. **Clapham, P. R., J. N. Weber, D. Whitby, K. McIntoch, A. G. Dalgleish, P. J. Maddon, K. C. Dean, R. W. Sweet, and R. A. Weiss.** 1989. Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. Nature (London) **337**:368–370.
3. **Clavel, F., D. Guetard, F. Brun-Vézinet, S. Chamaret, M.-A. Rey, M. O. Santos-Ferreira, A. G. Laurent, C. Dauguet, C. Katlama, C. Rouzioux, D. Klatzmann, J. L. Champalimaud, and L. Montagnier.** 1986. Isolation of a new human retrovirus from West African patients with AIDS. Science **233**:343–346.
4. **Dalgleish, A. G., P. C. L. Beverley, P. R. Clapham, D. H. Crawford, M. F. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–767.
5. **Deen, K. C., J. S. McDougal, R. Inacker, G. Folena-Wasserman, J. Arthos, J. Rosenberg, P. J. Maddon, R. Axel, and R. W. Sweet.** 1988. A soluble form of CD4 (T4) protein inhibits AIDS virus infection. Nature (London) **331**:82–84.
6. **Dowbenko, D., G. Nakamura, C. Fennie, C. Shimasaki, L. Riddle, R. Harris, T. Gregory, and L. Lasky.** 1988. Epitope mapping of the human immunodeficiency virus type 1 gp120 with monoclonal antibodies. J. Virol. **62**:4703–4711.
7. **Ey, P. L., S. J. Prowse, and C. R. Jenkin.** 1978. Isolation of pure IgG1, IgG2a, and IgG2b immunoglobulins from mouse serum using protein A-Sepharose. Immunochemistry **15**:429–436.
8. **Fields, A. P., D. P. Bednarik, A. Hess, and W. S. May.** 1988. Human immunodeficiency virus induces phosphorylation of its cell surface receptor. Nature (London) **333**:278–280.
9. **Fisher, R. A., J. M. Bertonis, W. Meier, V. A. Johnson, D. S. Costopoulos, T. Liu, R. Tizard, B. D. Walker, M. S. Hirsch, R. T. Schooley, and R. A. Flavell.** 1988. HIV infection is blocked *in vitro* by recombinant soluble CD4. Nature (London) **331**: 76–78.
10. **Fung, M. S. C., C. Sun, N. C. Sun, N. T. Chang, and T. W. Chang.** 1987. Monoclonal antibodies that neutralize HIV-1 virions and inhibit syncytium formation by infected cells. Biotechnology **5**:940–946.
11. **Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, P. Foster, and P. O. Markham.** 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science **224**: 500–503.
12. **Gartner, S., P. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M. Popovic.** 1986. The role of mononuclear phagocytes in HTLV-III/LAV infection. Science **233**:215–219.
13. **Goudsmit, J., C. Debouck, R. H. Meloen, L. Smit, M. Bakker, D. M. Asher, A. V. Wolff, C. J. Gibbs, Jr., and C. Gajdusek.** 1988. Human immunodeficiency virus type 1 neutralization epitope with conserved architecture elicits early type-specific antibodies in experimentally infected chimpanzees. Proc. Natl. Acad. Sci. USA **85**:4478–4482.
14. **Ho, D. D., J. C. Kaplan, I. E. Rackauskas, and M. E. Gurney.** 1988. Second conserved domain of gp120 is important for HIV infectivity and antibody neutralization. Science **239**:1021–1023.
15. **Ho, D. D., R. J. Pomerantz, and J. C. Kaplan.** 1987. Pathogenesis of infection with human immunodeficiency virus. N. Engl. J. Med. **317**:278–283.
16. **Ho, D. D., M. G. Sarngadharan, M. S. Hirsch, R. T. Schooley, T. R. Rota, R. C. Kennedy, T. C. Chanh, and V. L. Sato.** 1987. Human immunodeficiency virus neutralizing antibodies recognize several conserved domains on the envelope glycoproteins. J. Virol. **61**:2024–2028.
17. **Hussey, R. E., N. E. Richardson, M. Kowalski, N. R. Brown, H. C. Chang, R. F. Siliciano, T. Dorfman, B. Walker, J. Sodroski, and E. L. Reinherz.** 1988. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. Nature (London) **331**:78–81.
18. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier.** 1984.

T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) **312**:767–768.

19. **Kowalski, M., J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski.** 1987. Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. Science **237**:1351–1355.
20. **Lasky, L. A., J. E. Groopman, C. W. Fennie, P. M. Benz, D. J. Capon, D. J. Dowbenko, G. R. Nakamura, W. M. Nunes, M. E. Renz, and P. W. Berman.** 1986. Neutralization of the AIDS retrovirus by antibodies to a recombinant envelope glycoprotein. Science **233**:209–212.
21. **Lasky, L. A., G. M. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. J. Capon.** 1987. Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. Cell **50**:975–985.
22. **Linsley, P. S., J. A. Ledbetter, E. Kinney-Thomas, and S. L. Hu.** 1988. Effects of anti-gp120 monocloncal antibodies on CD4 receptor binding by the *env* protein of human immunodeficiency virus type 1. J. Virol. **62**:3695–3702.
23. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348.
24. **Matsushita, S., M. Robert-Guroff, J. Rusche, A. Koito, T. Hattori, H. Hoshino, K. Javaherian, K. Takatsuki, and S. Putney.** 1988. Characterization of a human immunodeficiency virus neutralizing monoclonal antibody and mapping of the neutralizing epitope. J. Virol. **62**:2107–2114.
25. **Matthews, T. J., A. J. Langlois, W. G. Robey, N. T. Chang, R. C. Gallo, P. J. Fischinger, and D. P. Bolognesi.** 1986. Restricted neutralization of divergent human T-lymphotropic virus type III isolates by antibodies to the major envelope glycoprotein. Proc. Natl. Acad. Sci. USA **83**:9709–9713.
26. **McDougal, J., J. Nicholson, G. Cross, S. Cort, M. Kennedy, and A. Mawie.** 1986. Binding of the human retrovirus HTLV/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition and potential for idiotypic mimicry. J. Immunol. **137**:2937–2944.
27. **Modrow, S., B. H. Hahn, G. M. Shaw, R. C. Gallo, F. Wong-Staal, and H. Wolf.** 1987. Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: prediction of antigenic epitopes in conserved and variable regions. J. Virol. **61**:570–578.
28. **Myers, G., S. F. Josephs, A. B. Rabson, T. F. Smith, and F. Wong-Staal (ed.).** 1988. Human retroviruses and AIDS. Los Alamos National Laboratory, Los Alamos, N.M.
29. **Nara, P. L., W. C. Hatch, N. M. Dunlop, W. G. Robey, L. O. Arthur, M. A. Gonda, and P. J. Fischinger.** 1987. Simple, rapid, quantitative, syncytium-forming microassay for the detection of human immunodeficiency virus neutralizing antibody. AIDS Res. Human Retroviruses **3**:283–302.
30. **Nara, P. L., W. G. Robey, S. W. Pyle, W. C. Hatch, N. M. Dunlop, J. W. Bess, Jr., J. C. Kelliher, L. O. Arthur, and P. J. Fischinger.** 1988. Purified envelope glycoproteins from human immunodeficiency virus type 1 variants induce individual, type-specific neutralizing antibodies. J. Virol. **62**:2622–2628.
31. **Newmark, P.** 1988. Receding hopes of AIDS vaccines. Nature (London) **333**:699.
32. **Putney, S. D., T. J. Matthews, W. G. Robey, D. L. Lynn, M. Robert-Guroff, W. T. Mueller, A. J. Langlois, J. Ghrayeb, S. R. Petteway, Jr., K. J. Weinhold, P. J. Fischinger, F. Wong-Staal, R. C. Gallo, and D. P. Bolognesi.** 1986. HTLV-III/LAV-neutralizing antibodies to an *E. coli*-produced fragment of the virus envelope. Science **234**:1392–1395.
33. **Robert-Guroff, M., M. Brown, and R. C. Gallo.** 1985. HTLV-III-neutralizing antibodies in patients with AIDS and AIDS-related complex. Nature (London) **316**:72–74.
34. **Robey, W. G., L. O. Arthur, T. J. Matthews, A. Langlois, T. D. Copeland, N. W. Lerche, S. Oroszlan, D. P. Bolognesi, R. V. Gilden, and P. J. Fischinger.** 1986. Prospect for prevention of human immunodeficiency virus infection: purified 120-kDa envelope glycoprotein induces neutralizing antibody. Proc. Natl. Acad. Sci. USA **83**:7023–7027.
35. **Rusche, J. R., D. L. Lynn, M. Robert-Guroff, A. J. Langlois, H. K. Lyerly, H. Carson, K. Krohn, A. Ranki, R. C. Gallo, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1987. Humoral immune response to the entire human immunodeficiency virus envelope glycoprotein made in insect cells. Proc. Natl. Acad. Sci. USA **84**:6924–6828.
36. **Salahuddin, S. Z., R. M. Rose, J. E. Groopman, P. D. Markham, and R. C. Gallo.** 1986. Human T lymphotropic virus type III infection of human alveolar macrophages. Blood **68**:281–284.
37. **Siliciano, R. F., T. Lawton, C. Knall, R. W. Karr, P. Berman, T. Gregory, and E. L. Reinherz.** 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for $CD4^+$ cell depletion. Cell **54**:561–575.
38. **Smith, D. H., R. A. Byrn, S. A. Marsters, T. Gregory, J. E. Groopman, and D. J. Capon.** 1988. Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. Science **238**:1704–1707.
39. **Steimer, K. S., G. V. Nest, D. Dina, P. J. Barr, P. A. Luciw, and E. T. Miller.** 1987. Genetically engineered human immunodeficiency virus envelope glycoprotein gp120 produced in yeast is the target of neutralizing antibodies. Vaccines **87**:236–241.
40. **Thomas, E. K., J. N. Weber, J. McClure, P. R. Clapham, M. C. Singhal, M. K. Shriver, and R. A. Weiss.** 1988. Neutralizing monoclonal antibodies to the AIDS virus. AIDS **2**:25–29.
41. **Traunecker, A., W. Luke, and K. Karjalainen.** 1988. Soluble CD4 molecules neutralize human immunodeficiency virus type 1. Nature (London) **331**:84–86.
42. **Weiss, R. A.** 1988. Receptor molecule blocks HIV. Nature (London) **331**:15.
43. **Weiss, R. A., P. R. Clapham, J. N. Weber, A. G. Dalgleish, L. A. Lasky, and P. W. Berman.** 1986. Variable and conserved neutralization antigens of human immunodeficiency virus. Nature (London) **324**:572–575.
44. **Weiss, R. A., P. R. Clapham, J. N. Weber, D. Whitby, R. S. Tedda, T. O'Connor, S. Chamaret, and L. Montagnier.** 1988. HIV-2 antisera cross-neutralize HIV-1. AIDS **2**:95–100.