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Characterization of Neutralizing Monoclonal Antibodies to Linear and Conformation-Dependent Epitopes within the First and Second Variable Domains of Human Immunodeficiency Virus Type 1 gp120

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A number of linear and conformation-dependent neutralizing monoclonal antibodies (MAbs) have been mapped to the first and second variable (V1 and V2) domains of human immunodeficiency virus type 1 (HIV-1) gp120. The majority of these MAbs are as effective at neutralizing HIV-1 infectivity as MAbs to the V3 domain and the CD4 binding site. The linear MAbs bind to amino acid residues 162 to 171, and changes at residues 183/184 (PI/SG) and 191/192/193 (YSL/GSS) within the V2 domain abrogate the binding of the two conformation-dependent MAbs, 11/68b and CRA-4, respectively. Surprisingly, a change at residue 435 (Y/H or Y/S), in a region of gp120 near the CD4 binding site (M. Kowalski, J. Potz, L. Basiripour, T. Dorfman, W. C. Goh, E. Terwilliger, A. Dayton, C. Rosen, W. Haseltine, and J. Sodroski, Science 237:1351–1355, 1987; L. A. Lasky, G. M. Nakamura, D. H. Smith, C. Fennie, C. Shimasaki, E. Patzer, P. Berman, T. Gregory, and D. Capon, Cell 50:975–985, 1987; and U. Olshevsky, E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski, J. Virol. 64:5701–5707, 1990), abrogated gp120 recognition by both of the conformation-dependent MAbs. However, both MAbs 11/68b and CRA-4 were able to bind to HIV-1 V1V2 chimeric fusion proteins expressing the V1V2 domains in the absence of C4, suggesting that residues in C4 are not components of the epitopes but that amino acid changes in C4 may affect the structure of the V1V2 domains. This is consistent with the ability of soluble CD4 to block 11/68b and CRA-4 binding to both native cell surface-expressed gp120 and recombinant gp120 and suggests that the binding of the neutralizing MAbs to the virus occurs prior to receptor interaction. Since the reciprocal inhibition, i.e., antibody inhibition of CD4-gp120 binding, was not observed, the mechanism of neutralization is probably not a blockade of virus-receptor interaction. Finally, we demonstrate that linear sequences from the V2 region are immunogenic in HIV-1-infected individuals, suggesting that the primary neutralizing response may be directed to both V2 and V3 epitopes.

The envelope glycoprotein of human immunodeficiency virus type 1 (HIV-1) is immunogenic and elicits most of the neutralizing activity found in human sera (28). The envelope gene (*env*) encodes a 160-kDa precursor protein (gp160) which is processed into the mature, external surface (SU), gp120, and the transmembrane, gp41, glycoproteins. The first step in the infection process involves a high-affinity interaction between gp120 and CD4 (29), the primary receptor for the virus (7, 23). This binding event is followed by a series of poorly characterized steps involving gp41, CD4 (4, 15), and possibly other, as-yet-undefined, cell surface proteins (5), probably resulting in the insertion of the amino-terminal fusion domain of gp41 (11) into the host cell membrane. The regions of the envelope glycoproteins involved in these processes are all potential targets for virus-neutralizing antibodies.

Neutralizing antibody appears to be an important component of the protective immune response against HIV-1 infection (1, 8, 9, 12). Antibodies capable of neutralizing a variety of divergent isolates have been termed group specific, and those restricted to a limited number of isolates have been termed type specific (61). Many reports have suggested that HIV-1-neutralizing activity is associated primarily with two gp120 regions: the third hypervariable domain (V3) (14, 18, 39, 49) and the CD4 binding site (CD4 bs) (20, 55). Generally, antibodies specific for V3 arise early after infection and exhibit the ability to neutralize a limited number of HIV-1 isolates. However, more recently a number of V3 monoclonal antibodies (MAbs) which recognize conserved features of the domain and exhibit a broader neutralization profile have been reported (13, 18, 41).

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Later in the course of viral infection, antibodies capable of neutralizing a wider range of HIV-1 isolates are detectable (36, 47, 61). The appearance of such group-specific neutralizing antibodies coincides with the detection of antibodies capable of blocking the gp120-CD4 interaction (17, 20, 34, 55). A number of neutralizing MAbs which block the gp120-soluble CD4 (sCD4) interaction have been identified, both from infected individuals (17, 21, 46, 51, 60) and from rats immunized with recombinant gp120 (rgp120) (6), suggesting that such antibodies may play a role in the group-specific neutralizing response. The epitopes recognized by these MAbs are affected by amino acid changes in each of the five conserved gp120 regions (C1 to C5), depending on the particular MAb (35, 58, 59). In addition, Ho and colleagues (16) reported a conformation-dependent MAb that was capable of blocking sCD4-gp120 interaction but which failed to cross-compete with MAbs overlapping with the CD4 bs. This MAb, G3-4, cross-competed with MAbs recognizing a linear V2 region (10) and has recently been shown to depend for binding on specific amino acids within the V2 domain (56a).

We have identified a number of linear and conformation-dependent MAbs which neutralize the LAI isolate of HIV-1. Epitope mapping studies demonstrate that the linear MAbs bind to amino acid (aa) residues 162 to 171 within the V2 domain and that the V1 and V2 domains are critical for gp120 recognition by the conformation-dependent MAbs. Some of these MAbs are as effective in neutralizing HIV infectivity as MAbs to the V3 domain and to the CD4 bs. We demonstrate that a linear epitope in V2, capable of binding neutralizing MAbs, is immunogenic in HIV-1-infected individuals. These results suggest that the primary neutralizing response in infected individuals may be directed against epitopes within the V2 and V3 domains.

MATERIALS AND METHODS

Sources of reagents. The following gp120 MAbs were used for neutralization and/or gp120-peptide binding studies: 39.13g, specific for a conserved conformation-dependent epitope involved in CD4 binding (6, 35); 38.1a, specific for aa 430 to 447 (6, 33); 8/19b, binding to a conformation-dependent epitope involving the C1 and C3 regions of gp120; 41.1, specific for a conformation-dependent epitope within V3; and MAbs 10/54, 10/36e, and 11/85b, mapping to aa 311 to 321 in V3. Peptides 740.13 (GEIKNCSFNISTSIRGKVQK), 740.14 (STSIRGKVQKEYAFFYKLDI), 740.40 (KQIINM WQKVGKAMYAPPIS), 794.1 consensus V2 (IRDKVQK EYALFYKLC), 794.2 LAI V2 (IRGKVQKEYAFFYKLC), 794.3 Mn V2 (IRDKMQKEYALLYKLC), and 794.4 RF V2 (RRDKTQKKYALFYKLC) and the full set of gp120 overlapping peptides were obtained from the Medical Research Council AIDS Directed Programme. V3 peptides were from the Mn strain of HIV-1 ADP 715 (RKRIHIGPGPAFYTT KN) and the HXB2 clone of LAI 740.28 (NTRKRIRI QRGPGRAFVTIG). The control Raf c peptide (IVQQFGY QRRASDDGKLTD) was a gift from C. Marshall (Institute of Cancer Research, London, United Kingdom).

MAb production. Both CBH/Cbi rats (via their Peyer's patches) and BALB/c mice (subcutaneously) were immunized with rgp120 (HIV-1 LAI:BH10 clone expressed in CHO cells and obtained from the Medical Research Council AIDS Directed Programme) emulsified in complete Freund's adjuvant. Hybridoma screening assays were screened for anti-gp120 activity with a solid-phase radio-binding assay as previously described (6).

Neutralization assays. HIV (10^3 50% tissue culture infective doses [$TCID_{50}$]) in a volume of 40 μl was incubated with 10 μl of a dilution of MAb under test at 37°C for 1 h. The virus-antibody mixture was then incubated with 100 μl of C8166 cells at a concentration of 2×10^5 cells per ml per well in a microtiter plate in triplicate. Five days postinfection, the wells were scored for the presence of syncytia, and the extracellular supernatant was collected from the wells by centrifugation, inactivated with 1% Empigen, and assayed for soluble p24 antigen as described previously (32). The lowest concentration of antibody resulting either in a complete blocking of syncytial formation or in a >90% reduction in p24 antigen produced was defined as the reciprocal neutralization titer.

(i) Peptide inhibition of MAb neutralization. Increasing concentrations of V2 (740.13 and 740.14) and C4 (740.40) peptides were incubated with an equal volume of test MAb, at a final concentration capable of resulting in 90% neutralization of 10^3 $TCID_{50}$ of HXB10, for 1 h at 37°C. MAb-peptide mixtures were then assessed for their ability to neutralize HXB10 as described above.

Antibody binding to peptides, rgp120, and gp120 fusion proteins. (i) Peptide reactivity. Peptides were bound to a solid support (Immulon II 96-well plates; Dynatech) at 2.5 μg/ml in Tris-buffered saline (TBS) overnight. Plates were blocked with blocking buffer (4% Marvel–TBS) and were incubated with either MAbs at 10 μg/ml or human sera (1/200) diluted in 4% Marvel–20% sheep serum–TBS (TMSS) for 1 h at room temperature (RT). Unbound antibodies were removed by washing with TBS, and bound antibodies were detected with either anti-rat immunoglobulin G (IgG) or anti-human IgG conjugated to alkaline phosphatase (AP) (Seralabs, Crawley, United Kingdom) and visualized with the AMPAK substrate amplification system (Novo Biolabs, Cambridge, United Kingdom).

Sera were collected from 126 healthy Centers for Disease Control stage II and III seropositive males attending a sexually transmitted disease clinic as part of a longitudinal study of the natural history of HIV-1 infection (3, 22). These individuals had not received antiretroviral therapy at the time of sampling.

(ii) Synthesis of cellulose-membrane-bound overlapping peptides and SPOTscan. Overlapping nonapeptides were synthesized by F-moc chemistry on cellulose membranes, using a kit from Cambridge Research Biochemicals according to the manufacturer's specifications. SPOTscan analysis was performed as described by the manufacturers and is essentially as follows. The membrane was washed three times in TBS and blocked overnight in blocking buffer (5% sucrose, 10% concentrated membrane buffer [Cambridge Research Biochemicals], 0.05% Tween 20 in TBS). The membrane was washed in TBS and incubated with the test MAb diluted in blocking buffer for 3 h at RT. After washing of the membrane three times in TBS, bound MAb was detected with β-galactosidase-conjugated anti-rat IgG (Cambridge Research Biochemicals) diluted in blocking buffer for 2 h at RT. The membrane was finally washed three times in TBS and incubated with signal development solution: 4.9 mg of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside dissolved in 100 μl of dimethylformamide mixed with 1 mM magnesium chloride–3 mM potassium ferricyanide in phosphate-buffered saline (PBS, pH 7.4). The color reaction was allowed to develop for 10 to 40 min at RT.

(iii) gp120 reactivity. rgp120 was allowed to bind to the solid phase via a sheep antibody, D7324, to the C terminus of gp120, in the presence of 2% nonfat milk powder in TBS

(Marvel, Cadbury, United Kingdom) at an input concentration of 100 ng/ml. The ability of MAbs to bind to the captured gp120 was assessed in the same way as for the peptide reactivity assay (see above).

(iv) Binding of MAbs to gp120 mutants. Mutations were introduced into the HXBc2 *env* gene and mutant glycoproteins transiently expressed in COS-1 cells as described previously (43). Cells were lysed in the presence of 50 mM Tris–150 mM NaCl–1% Triton–0.1% sodium dodecyl sulfate (SDS), and the concentration of gp120 in the lysates was determined by twin-site enzyme-linked immunosorbent assay (ELISA) as previously described, with rgp120 as a calibrant (37). Mutant and wild-type envelope glycoproteins were allowed to bind to the solid-phase D7324 (see above) at an input concentration of 15 ng/ml. All MAbs were tested for their binding to the various mutant envelope glycoproteins at a predetermined saturating concentration of 10 μg/ml. Bound MAbs were detected with AP-conjugated anti-rat IgG (Seralabs). The results are expressed as a ratio of the optical density (OD) of the mutant protein to that of wild-type protein and are termed the relative binding index.

(v) Binding of MAbs to V1V2 chimeric proteins. The native V1-V2 domain of HIV-1 gp120 was expressed in the absence of other HIV sequences, using a eukaryotic expression system in which the N-terminal domain of the ecotropic murine leukemia virus (MuLV) SU protein serves as a carrier for heterologous sequences (21a). Briefly, a recombinant gene in which sequences coding for the signal peptide and the first 263 aa of the mature MuLV SU (42) are followed by GCTAGC (coding for an Ala-Ser linker dipeptide) and subsequently by sequences coding for aa 116 to 209 of the HXB2d clone of HIV-1 (52) was constructed (numbering system includes the 30 residues of the gp120 leader sequence). The expressed V1-V2 region contained three disulfide bonds and six N-linked glycosylation sites and included a disulfide-bonded arm of flanking constant domain sequence in addition to the V1V2 double loop structure (26). The recombinant V3 fusion protein was expressed in a similar way and contained aa residues 291 to 336 of the HXB2 sequence encompassing the complete V3 loop plus five residues N-terminal and five residues C-terminal to the loop. Included in the gp120-derived sequence were three N-linked glycosylation sites, one located within the V3 loop and one on each flanking sequence. The recombinant genes were expressed in NIH 3T3 cells from a retroviral vector derived from the FB29 isolate of MuLV (53). Radioimmunoprecipitations were performed as previously described (44) on supernatants from cells expressing fusion glycoproteins that had been labelled overnight with [^{35}S]cysteine and analyzed by SDS-polyacrylamide gel electrophoresis. Labelled supernatant from cells expressing the N-terminal domain of MuLV SU alone was used as a control.

A hybrid HIV-1 (LAI)–HIV-2 (Rod) glycoprotein in which the HIV-1 gp120 V1V2 sequence was exchanged for the HIV-2 gp105 sequence was constructed and expressed as previously reported (termed SF9,10) (40). Cell supernatants containing SF9,10, full-length HIV-1 gp120, and HIV-2 gp105 were tested for their ability to bind a panel of MAbs as previously described (37).

(vi) Removal of sialic acid and galactose from rgp120. Sequential removal of carbohydrates from rgp120, bound to microtiter plates, was carried out as previously described (2, 54). Briefly, polystyrene plates (Dynatech Laboratories) were coated with purified preparations of rgp120 (100 μl per well at 0.1 μg/ml in 0.1 M sodium carbonate buffer, pH 9.6) by incubation at 37°C for 24 h. After washing, the plates were treated with *Escherichia coli* β-galactosidase (Sigma, grade IX; in 0.1 M phosphate buffer–1 mM $MgCl_2$) at 10 U per well (54) at 37°C for 24 h. Alternatively, the plates were treated with 25 mM periodate at room temperature for 1 h (62), after sialidase digestion. The plates were subsequently incubated with MAbs to gp120. The effect of the combined sialidase-periodate treatment on the oligosaccharide side chains was monitored by the use of biotinylated *Ricinus communis* lectin (54). The reactions were visualized by addition of AP-conjugated avidin (lectin assay) or AP-conjugated goat anti-rat IgG with disodium *p*-nitrophenyl phosphate (Sigma) as substrate. The A_{405} was measured.

TABLE 1. Concentrations of V1V2 MAbs required to reach half-maximal binding to proteins[a]

MAb	Concn of MAb (μg/ml) resulting in half-maximal binding to protein:			
	rgp120	Cell surface gp120	740.13	740.14
10/76b	0.18	0.56	0.04	0.35
11/4c	0.40	0.77	0.12	0.90
11/41e	0.35	3.60	0.03	0.20
11/4b	0.25	0.60	0.06	0.45
11/68b	0.40	0.75	NDB[b]	NDB
CRA-4	0.15	0.56	NDB	NDB

[a] MAbs were tested for binding to either rgp120 (D7324 bound), HXB10-infected H9 cells (cell surface gp120), or peptides 740.13 (GEIKNCSFNISTSIRGKVQK) and 740.14 (STSIRGKVQKEYAFFIKLDI) bound to a solid support.
[b] NDB, no detectable binding.

(vii) In-solution peptide MAb-rgp120 competition assays. Various concentrations of V2 (740.13 and 740.14), C4 (740.40), and control (Raf c) peptides in TMSS were incubated with an equal volume of test MAb, at a concentration resulting in half-maximal binding to rgp120, for 1 h at RT. MAb-peptide mixtures were then assessed for their ability to bind to D7324-bound rgp120 (as described above).

(viii) MAb binding to sCD4-rgp120 complexes. D7324-bound rgp120 (as described above) was incubated with increasing concentrations of sCD4 (a kind gift of R. Ward, Genentech, Inc., San Francisco, Calif.) diluted in TMSS for 1 h at RT. Unbound sCD4 was removed by repeated (3×) washing with TBS, and the ability of MAbs to bind to rgp120 in the presence or absence of sCD4 was assessed as above. Bound sCD4 was detected with a polyclonal rabbit anti-sCD4 serum (rabbit 178) and AP-conjugated anti-rabbit IgG (Seralabs).

(ix) sCD4 binding to MAb-gp120 complexes. MAbs were evaluated for their ability to block D7324-bound rgp120 interacting with sCD4 as described previously (6).

(x) MAb cross-competition analysis for rgp120 binding. Three linear V2 MAbs (11/41e, 11/4c, and 10/76b), one linear C4 MAb (38.1a), one conformation-dependent MAb mapping to the CD4 bs (39.13g), and one conformation-dependent gp120-specific MAb (8/19b) were tested for their ability to compete with iodinated preparations of MAbs 11/68b and CRA-4 for binding to D7324-bound rgp120. The MAbs, at a concentration sufficient to saturate the gp120 (10 μg/ml), were mixed with an equal volume of 11/68b or CRA-4, at a concentration resulting in half-maximal binding (Table 1), and the mixture was incubated with gp120 for 1 h. Amounts of 11/68b or CRA-4 that bound to gp120 in the presence or absence of the test MAb were determined.

Cell surface MAb binding. (i) MAb recognition of HIV-1-

infected cells. Infected H9 cells were washed twice in wash buffer (WB) (complete PBS–1% fetal calf serum–0.05% sodium azide), resuspended at a concentration of 10^7 cells per ml, and chilled on ice. Cells were incubated for 1 h in the presence or absence of sCD4 (10 μg/ml) on ice, to prevent gp120 shedding (39), at a final cell concentration of 5 × 10^6/ml. Unbound sCD4 was removed by repeated washing (2×) with prechilled WB. MAbs were diluted in WB, and 100 μl was mixed with 100 μl of cells (10^6 cells) and incubated in a V-bottomed microtiter plate for 30 min on ice. Cells were washed with WB by centrifugation, and the bound MAb was detected with a 1/40 dilution of fluorescein isothiocyanate-conjugated anti-rat IgG (Seralabs) in WB. Bound sCD4 was detected with a CD4-specific MAb, L120 (Medical Research Council AIDS Directed Programme Repository), and fluorescein isothiocyanate–anti-mouse IgG (Seralabs). After a 30-min incubation on ice with the conjugate, the cells were washed three times with WB and inactivated by resuspension in 500 μl of 1% paraformaldehyde in PBS at 4°C overnight. Cells were analyzed on a FACSTAR (Becton Dickinson) with Lysis software. The results shown are the mean fluorescence intensities obtained from one experiment. However, all experiments were repeated three times.

(ii) MAb recognition of $SupT_1$-bound rgp120. $SupT_1$ cells were washed twice in WB and resuspended at a concentration of 10^7/ml, and 10^6 cells (100 μl) were incubated with increasing concentrations of rgp120 on ice for 1 h. Unbound gp120 was removed by washing with WB, and the cell surface-bound rgp120 was detected with a range of gp120-specific MAbs. All MAbs were used at a concentration of 10 μg/ml, previously shown to saturate 10^6 HXB10-infected H9 cells. Bound MAb was detected as described above.

RESULTS

Neutralization and gp120-peptide binding. Hybridoma culture supernatants were screened for their ability to neutralize LAI (HXB10) infection of C8166 cells and for their reactivity with rgp120 and a mutant gp120 with both the V1 and V2 domains deleted (ΔV1V2, deletion of aa 119 to 205). Six MAbs which neutralized HXB10 and which failed to recognize the ΔV1V2 protein were identified and were chosen for second-round cloning and purification. The new MAbs were compared for their ability to neutralize HXB10 with a number of other rat gp120 MAbs: 10/54, 10/36e, 11/85b, and 41.1, mapping to the V3 domain (31); and 39.13g, binding to an epitope overlapping the CD4 bs (6, 35) (Table 2). MAbs 10/76b, 11/4c, 11/4b, 11/68b, and CRA-4 failed to neutralize the RF, Mn, and SF-2 strains of HIV-1 but neutralized HXB10 with titers ranging from 0.60 to 2.50 μg/ml, comparable to those for MAbs 39.13g, 10/36e, and 11/85b. MAb 11/41e resulted in only 50% neutralization at a concentration of 20.0 μg/ml. The type-specific neutralization observed for MAbs 10/76b, 11/4c, 11/41e, 11/4b, 11/68b, and CRA-4 is in agreement with their ability to bind only to HXB10-infected H9 cells (Table 1) but not to Mn-, RF-, or SF-2-infected cells by fluorescence-activated cell sorter (FACS) (data not shown).

All six MAbs, 10/76b, 11/4c, 11/41e, 11/4b, 11/68b, and CRA-4, bound to gp120 in the absence of any detergent, with half-maximal binding at concentrations of 0.15 to 0.40 μg/ml (Table 1). All of the MAbs bound to HXB10-infected H9 cells with maximal fluorescence intensities greater than that achieved with either the V3 or CD4 bs MAbs (Table 3). However, the concentration of MAb 11/41e required to result in 50% maximal binding to the cell surface gp120 was 3.6 μg/ml, up to 6.4-fold greater than that for the other MAbs, which may account for its reduced neutralization efficiency (Table 1).

TABLE 2. Neutralization titers for various MAbs[a]

MAb	Isotype	Epitope	Neutralization titer (μg/ml) for strain:			
			HXB10	RF	SF-2	Mn
10/76b	IgG2a	V2	0.9	NDN[b]	NDN	NDN
11/4c	IgG2a	V2	1.5	NDN	NDN	NDN
11/41e	IgG1	V2	20.0[c]	NDN	NDN	NDN
11/4b	IgG2a	V2	2.5	NDN	NDN	NDN
11/68b	IgG1	V1V2	0.8	NDN	NDN	NDN
CRA-4[d]	IgG1	V1V2	0.6	NDN	NDN	NDN
10/54	IgG1	V3	8.3	NDN	NDN	NDN
10/36e	IgG2a	V3	1.3	NDN	NDN	NDN
11/85b	IgG2b	V3	1.1	NDN	NDN	NDN
41.1	IgG2a	V3	0.1	NDN	NDN	NDN
39.13g	IgG2b	CD4 bs	0.7	1.8	0.9	0.1

[a] Concentrations of MAb resulting in a 90% reduction in soluble p24 antigen production, 5 days postinfection, are classified as neutralizing.
[b] NDN, no detectable effect on p24 antigen production in the presence of MAb.
[c] MAb 11/41e only reduced p24 antigen production by 50%.
[d] CRA-4 is a murine MAb; the other MAbs in the table are derived from rats.

The MAbs were tested for their ability to bind to a denatured and unglycosylated *Saccharomyces cerevisiae*-expressed gp120 (56). Four MAbs, 10/76b, 11/4c, 11/41e, and 11/4b, were able to bind to the denatured gp120, suggesting that the MAbs were recognizing linear epitopes. We therefore tested the ability of these MAbs to bind to a series of overlapping peptides encompassing the HIV-1 HXB10 gp120 sequence. All of the MAbs bound to two overlapping peptides, GEIKNCSFNISTSIRGKVQK (ADP 740.13, aa 152 to 171) and STSIRGKVQKEYAFFYKLDI (ADP 740.14, aa 162 to 181), in the V2 region of gp120, suggesting that residues common to both peptides (STSIRGKVQK) comprised the epitope(s). MAbs 10/76b, 11/4c, 11/41e, and 11/4b bound with an average 7.6-fold-greater affinity to ADP 740.13 than to ADP 740.14, giving half-maximal binding values at 0.04, 0.12, 0.03, and 0.06 μg/ml, respectively

TABLE 3. Antibody recognition of cell surface-bound rgp120[a]

MAb	Epitope	Mean FI[b]				
		H9		$SupT_1$		
		Uninfected	HXB10	Uninfected	rgp120	rgp120-sCD4
10/54	V3	5.4	54.4	6.0	37.8	5.6
11/85b	V3	4.8	48.7	8.6	39.2	8.6
39.13g	CD4 bs	5.2	45.3	7.2	7.7	6.9
38.1a	CD4 bs	5.5	35.2	7.3	7.8	7.2
10/76b	V2	5.1	60.4	7.2	52.3	7.9
11/4c	V2	5.3	86.5	8.3	43.5	8.3
11/68b	V2	6.3	80.5	8.0	10.3	9.4
CRA-4	V2	5.4	76.5	7.4	8.5	8.2

[a] MAbs were tested for their abilities to bind to gp120 expressed at the surface of H9 cells either uninfected or infected with HXB10 and to recognize rgp120, previously incubated with sCD4 or not, bound to the surface of $SupT_1$ cells. See text for details.
[b] FI, fluorescence intensity.

TABLE 4. SPOTscan analysis with V1V2 MAbs

Spot	M_r	Peptide sequence[a]	Color reaction with MAb used as substrate[b]			
			10/76b	11/4c	11/41e	11/4b
1	975	NISTSIRGK	++	++	+	++
2	960	ISTSIRGKV	+++	+++	++	++
3	975	STSIRGKVQ	++++	++++	++++	++++
4	1,016	TSIRGKVQK	+	+	−	+
5	1,044	SIRGKVQKE	+	+	−	+
6	1,120	IRGKVQKEY	+	+	−	+

[a] Peptides, which are each 9 aa and have a single amino acid overlap, cover aa residues 160 to 170.
[b] − to ++++, no color reaction to very intense color reaction.

(Table 1). SPOTscan analysis using six peptides with a single amino acid stagger was used to further delineate the amino acid residues recognized by the MAbs (Table 4). MAbs 10/76b, 11/4c, 11/41e, and 11/4b bound to peptides 1 to 3, inclusive, confirming that residues STSIRGKVQ comprise their epitope. MAbs 11/68b and CRA-4 failed to bind to any of the gp120 peptides (data not shown), suggesting that they recognize conformation-dependent epitopes.

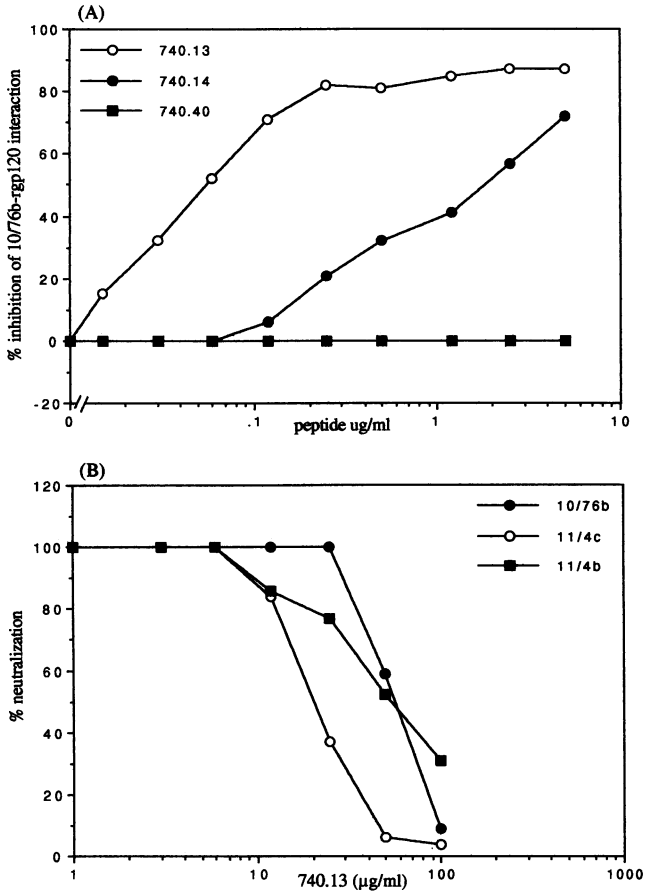

FIG. 1. Ability of peptides to inhibit MAb-mediated neutralization of HXB10 and interaction with rgp120. (A) Peptide inhibition of 10/76b-rgp120 interaction. Increasing concentrations of peptides 740.13, 740.14, and 740.40 were incubated with MAb 10/76b, at a final concentration of 0.56 μg/ml, and were tested for their ability to inhibit the MAb's binding to rgp120. (B) Peptide inhibition of neutralization. Increasing concentrations of peptide 740.13 were incubated with MAbs 10/76b, 11/4c, and 11/4b at final concentrations of 1.80, 3.0, and 5.0 μg/ml, respectively. The peptide-MAb mixtures were then tested for their ability to neutralize 10^3 $TCID_{50}$ of HXB10 infection of C8166 cells.

TABLE 5. Concentrations of peptides able to inhibit V1V2 MAb interaction with rgp120 by 50%

MAb[a]	Concn of peptide (μg/ml) inhibiting MAb-rgp120 binding by 50%[b]			
	740.13	740.14	740.40	Raf c
10/76b	0.12	3.60	>10.0	>10.0
11/4c	0.07	2.00	>10.0	>10.0
11/41e	1.20	—[c]	>10.0	>10.0
11/4b	0.06	2.80	>10.0	>10.0
11/68b	>10.0	>10.0	>10.0	>10.0
CRA-4	>10.0	>10.0	>10.0	>10.0

[a] MAbs were used at the concentrations resulting in half-maximal binding to rgp120 listed in Table 1.
[b] Control peptides used were 740.40 (KQIINMWQKVGKAMYAPPIS) and Raf c (IVQQFGYQRRASDDGKLTD).
[c] Peptide 740.14, at a final concentration of 10.0 μg/ml, inhibited 11/41e-rgp120 interaction by 30%.

We previously reported that MAbs mapping to the fourth conserved region of gp120 (C4) exhibit negligible binding for peptides bound to a solid support, whereas they bind with high affinity to the same peptides in solution (33). These data suggest that the number of conformations available for tethered peptides to adopt is limited. We therefore investigated the ability of the soluble peptides, 740.13 and 740.14, to inhibit both the MAb-gp120 binding reaction and the neutralization of HXB10. Both peptides were able to inhibit the binding of MAbs 10/76b (Fig. 1A), 11/4c, 11/41e, and 11/4b to gp120 (Table 5); however, at a given concentration, peptide 740.13 was on average 35-fold more effective at blocking the MAb-gp120 interaction than peptide 740.14 (Table 5). Both peptides were less efficient at inhibiting the binding of MAb 11/41e to gp120 than they were at inhibiting that of the other MAbs, despite 11/41e having an affinity for the peptides, when bound to a solid support, comparable to that of the other MAbs (Table 1). A final concentration of peptide 740.13 of 0.5 μg/ml inhibited the binding of MAbs 10/76b, 11/4c, 11/4b, and 11/41e to gp120 by 81, 69, 89, and 41%, respectively. In agreement with these data, peptide 740.13 was able to block neutralization by MAbs 10/76b, 11/4c, and 11/4b of HXB10 at 55.0, 20.0, and 52.0 μg/ml, respectively (Fig. 1B). Control peptide KQIINMWQKV GKAMYAPPIS (ADP 740.40, aa 421 to 440) had no detectable effect in both assays (Tables 5 and 6). Peptide 740.14 failed to block neutralization by all of the MAbs at up to 100 μg/ml; this is consistent with the observation that 740.14 was up to 35-fold less efficient at inhibiting the antibody-rgp120 interaction. These findings suggest that the epitope recognized by MAbs 10/76b, 11/4c, 11/4b, and 11/41e is present on both peptides but that it may be presented more effectively on peptide 740.13.

Since the V1V2 domains of gp120 are glycosylated, we analyzed the effect of removing (β1-4)-linked galactose from N-linked sugars on MAb gp120 recognition. As a control, *Ricinus communis* lectin, which binds to exposed galactose, and MAb 4c11.D8, which binds to an epitope whose availability is enhanced by NeuAc-Gal removal (2), were used to monitor the presence of galactose. gp120 recognition by MAbs 11/68b, CRA-4, and 11/41e was partially affected by removal of peripheral monosaccharides (Fig. 2). In contrast,

TABLE 6. Concentrations of peptides required to block neutralization of HXB10 by V1V2 MAbs

MAb[a]	Concn of peptide (μg/ml) inhibiting neutralization of HXB10[b]		
	740.13	740.14	740.40
10/76b	55.0	>100.0	>100.0
11/4c	20.0	>100.0	>100.0
11/4b	52.0	>100.0	>100.0
11/68b	>100.0	>100.0	>100.0
CRA-4	>100.0	>100.0	>100.0

[a] Final concentrations of MAbs were as follows (micrograms per milliliter): 10/76b, 1.80; 11/4c, 3.0; 11/4b, 5.0; 11/68b, 1.6; and CRA-4, 1.2.
[b] 10^3 $TCID_{50}$ of HXB10 was used in all assays.

binding of MAb 38.1a, which maps to the C4 region of gp120, was not affected by either sialidase–β-galactosidase or periodate treatment.

Epitope mapping of conformation-dependent MAbs 11/68b and CRA-4. To identify amino acids important for recognition of gp120 by MAbs 11/68b and CRA-4, their reactivity with a set of mutants altered in conserved gp120 residues was examined. These mutant glycoproteins have been previously characterized with respect to gp160 precursor processing, gp120-gp41 association, and CD4 binding ability (43). The wild-type and mutant gp120s were tested for their ability to bind a saturating concentration of the following: 11/68b; CRA-4; four linear V2 MAbs; and two control linear MAbs, 11/85b, binding to the V3 loop (31), and 38.1a, binding to C4 (aa 430 to 447) (31). The results are expressed as a ratio of MAb bound to the mutant gp120 in comparison with the wild-type protein (binding index) (Fig. 3). The V3 MAb, 11/85b, bound equivalently to all the mutants, with binding indices ranging from 0.82 to 1.08 (data not shown), serving as an internal control for gp120 levels. Deletion of the V1V2 (Δ119-205) domains abrogated the binding both of MAbs 11/68b and CRA-4 and of all the linear MAbs: 10/76b, 11/4c, 11/41e, and 11/4b, mapping to aa residues 162 to 171 (Fig. 3 and data not shown). A change in residue 166 from arginine to leucine (within linear peptides 740.13 and 740.14) abrogated the binding of all the linear V2 MAbs (Fig. 3D and data not shown). Mutants 183/184 PI/SG and 191/192/193 YSL/GGS containing amino acid changes within the V2 domain exhibited decreased binding of 11/68b and CRA-4, respectively (Fig. 3). The latter amino acid changes reduced the binding index of 11/68b to 0.36. Surprisingly, changes in the C4 residue 435 Y/H or Y/S (data not shown) abrogated the binding of both 11/68b and CRA-4 MAbs. The same changes in residue 435, plus adjacent residues, affected the binding of the MAb 38.1a (Fig. 3A).

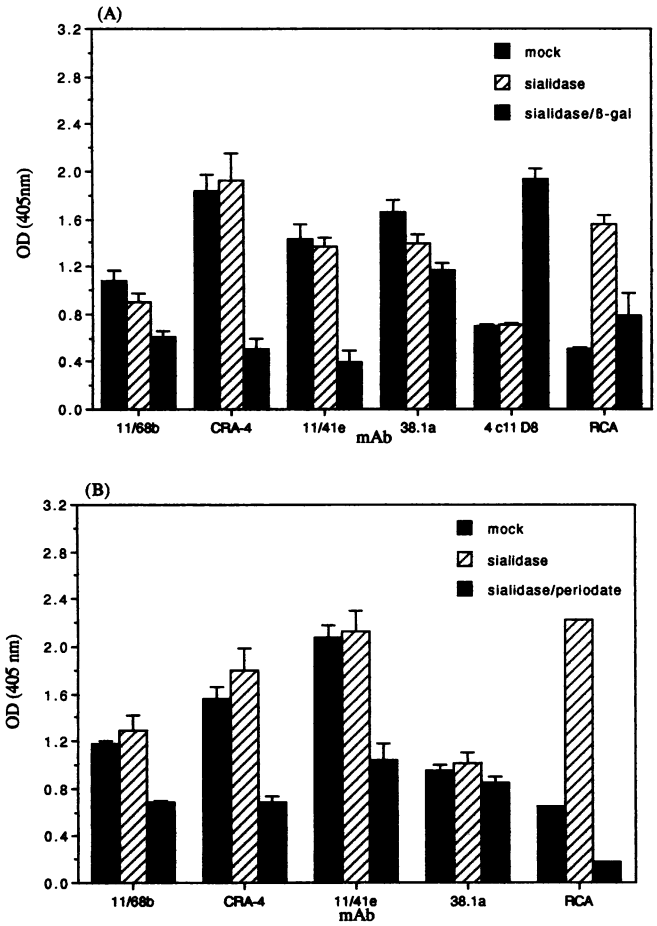

FIG. 2. Sequential removal of carbohydrates from gp120: effect on MAb recognition of gp120. Shown are the effects of sialidase (A and B) and combined sialidase–β-galactosidase (A) and sialidase-periodate (B) treatments on the reactivity of MAbs 11/68b, CRA-4, 11/41e, 38.1a (specific for HIV-1 gp120), and 4c11D8 (2) and the reporter lectin *Ricinus communis* lectin with gp120.

This result could be interpreted in two ways. Residues in V2 and C4 may be proximal on the native molecule and may contribute to the formation of these epitopes. Alternatively, the amino acids identified as affecting MAb recognition may do so indirectly and may not constitute the epitope. Since the change at residue 435 Y/H affects both the 11/68b and CRA-4 MAbs and the linear C4 MAb 38.1a, the antibodies should compete with each other for gp120 binding if the 435 residue is a component of the epitope recognized by all the MAbs. We therefore compared the abilities of several MAbs, 11/41e, 11/4c, 10/76b, 8/19b, 38.1a, and 39.13g (see Materials and Methods and Fig. 4 for MAb specificities), to compete with radiolabelled 11/68b or CRA-4 for binding to gp120. MAbs 11/68b and CRA-4 cross-competed with each other; however, none of the linear V2 MAbs, 8/19b, 39.13g, or 38.1a, competed with either MAb, suggesting that the 435 residue is not a component of the epitope recognized by 11/68b or CRA-4 (Fig. 4). The ability of MAbs 11/68b and CRA-4 to bind to two chimeric proteins both expressing the V1V2 domains of HIV-1 LAI in the absence of C4 (Table 7) confirms the interpretation of the MAb competition results (Fig. 4). The first of these constructs consists of the HIV-1 V1 and V2 domains as a fusion protein with the N-terminal domain of the MuLV gp70 protein; the second is a baculovirus-expressed chimeric HIV-2 envelope, in which the HIV-2 Rod V1V2 domains were replaced with those of HIV-1 HXB10 (SF9,10) (40). All of the linear V2 MAbs bound both to MuLV-V1V2 and to SF9,10 chimeric proteins, while a control V3 MAb, 41.1, only recognized the MuLV-V3 protein (Table 7).

Ability of MAbs to recognize rgp120-CD4 complexes. Given that the amino acid change in C4, a region which has been reported to be a component of the CD4 bs (24, 25, 43), abrogates the binding of both 11/68b and CRA-4, we investigated the effects of sCD4 on MAb-gp120 recognition. sCD4 (10 μg/ml) was found to inhibit the binding of both 11/68b and CRA-4 to rgp120 in ELISA by 40 and 55%, respectively (Fig. 5A), and to native gp120 expressed at the cell surface by 73

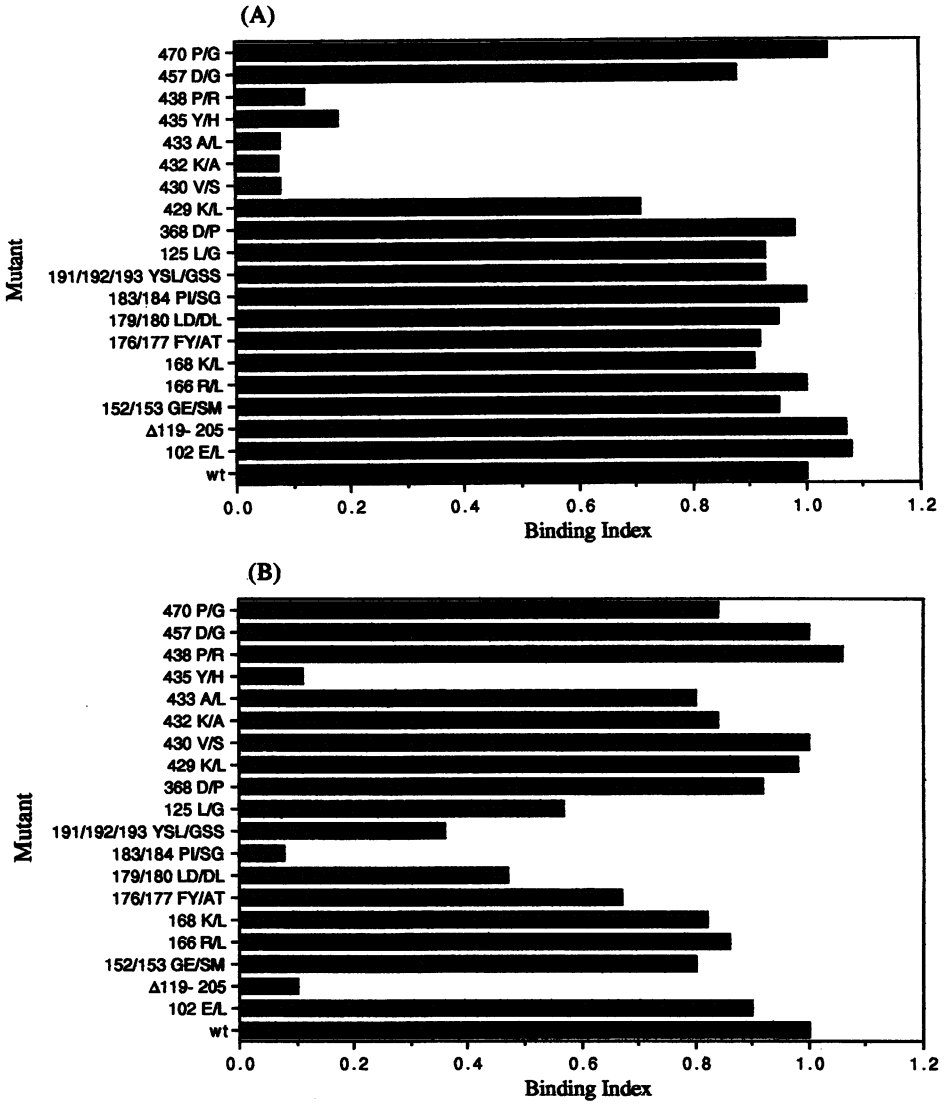

FIG. 3. Binding of selected gp120 mutants to MAbs 38.1a, 11/68b, CRA-4, and 10/76b. MAbs 38.1a (A), 11/68b (B), CRA-4 (C), and 10/76b (D) at a concentration of 10 μg/ml were monitored for their ability to bind to a panel of gp120 mutants. Additional gp120 mutants not shown here were tested (43) but gave binding indices of >0.7 for all the MAbs tested. MAb 11/85b, mapping to the V3 loop, bound to all the mutants listed above, with binding indices in the range of 0.82 to 1.08. The results represent the means of triplicate wells for which the standard deviations were within 15% for all samples. These results are from one experiment, but similar results were obtained in two separate experiments. wt, wild type.

and 69%, respectively (Fig. 5B). Recognition of gp120 by MAbs 10/76b, 11/4c, 11/41e, and 11/4b was not significantly affected by sCD4 in either assay. MAb 11/85b, specific for the V3 loop, demonstrated a 50% increase in binding to cell surface gp120 in the presence of sCD4 (Fig. 5B). gp120 recognition by two control MAbs, 39.13g and 38.1a, mapping to epitopes involved in CD4 binding, was inhibited (Fig. 5B). Neither MAb 11/68b nor CRA-4 affected sCD4 binding to rgp120 or to cell surface-expressed gp120 (data not shown).

In order to determine whether cellular CD4 could similarly inhibit 11/68b and CRA-4 recognition of rgp120, we assessed the ability of these, and a number of other MAbs, to recognize cell-bound rgp120. We monitored the binding of rgp120 to $SupT_1$ cells by FACS with a V3 MAb (11/85b) to detect cell surface-bound rgp120. gp120 concentrations of 1.0 μg/ml and greater were able to saturate cellular CD4 at 4°C (data not shown). Prior incubation of the rgp120 with a 3 M excess of sCD4 inhibited its binding to the $SupT_1$ cells, confirming that the positive binding signal was CD4 dependent (Table 3). A number of MAbs, mapping to V2, V3, and the CD4 bs, were compared for their ability to recognize cell surface-bound rgp120. As a control, the MAbs were also monitored for their ability to bind to gp120 expressed at the surface of infected H9 cells (Table 3). All of the MAbs bound to HXB10-infected H9 cells, with the V2 MAbs giving the greatest mean fluorescence intensity (Table 3). The MAbs 39.13g, 38.1a, 11/68b, and CRA-4 failed to recognize cell-bound rgp120, suggesting that their epitopes are masked by cellular CD4. In contrast, MAbs specific for linear epitopes in V2 or V3 bound to cell-associated rgp120 (Table 3).

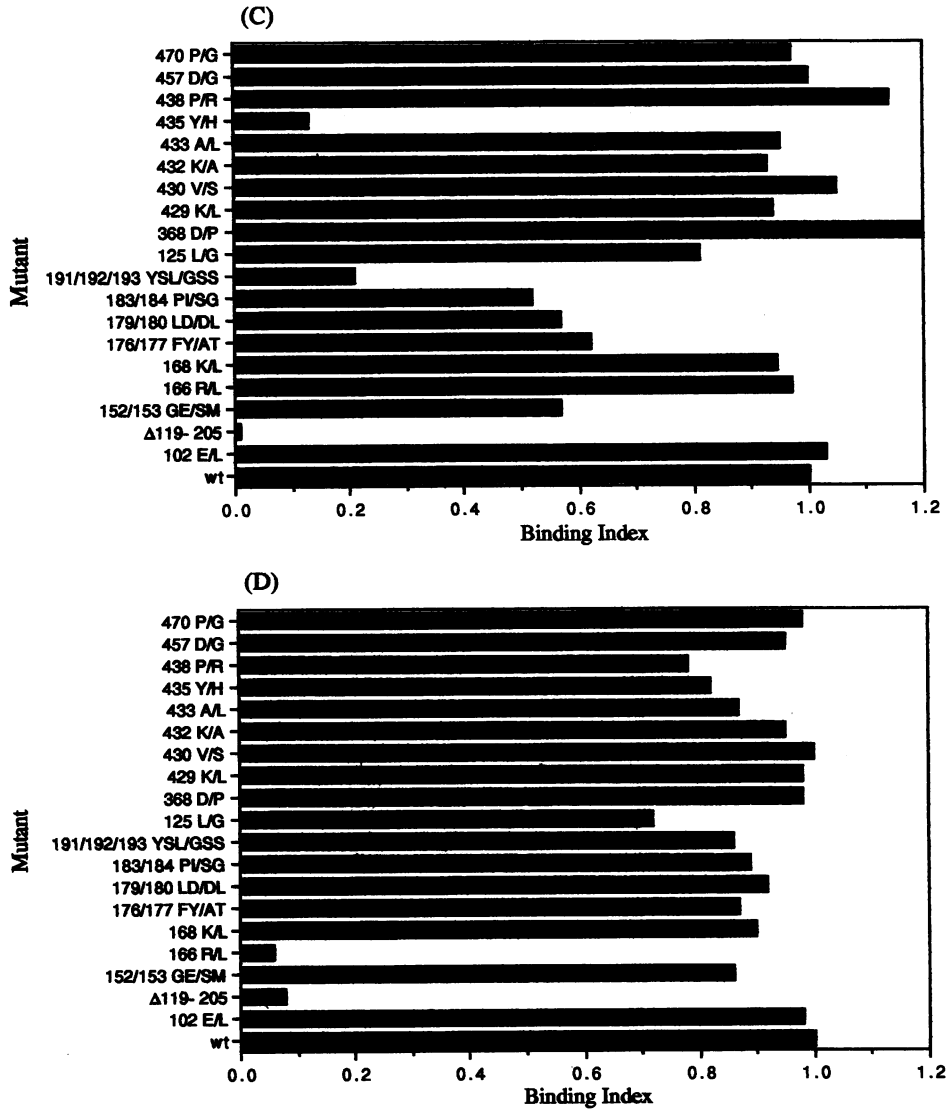

FIG. 3—*Continued.*

Human serum reactivity to linear V2 peptides. We screened sera from 126 individuals diagnosed as being HIV-1 seropositive for reactivity to a number of peptides: a consensus group B (North American and European) V2 peptide (794.1), corresponding to aa residues 164 to 180, recognized by a number of neutralizing linear MAbs (10, 29a); a V3 Mn peptide (aa 309 to 325); and an irrelevant peptide, Raf c. In order to determine a cutoff value for positivity, sera from 50 individuals known to be seronegative were included in the assays. A seropositive serum giving an OD greater than the mean OD plus 3 standard errors of the seronegative sera was scored as positive for the peptide under test. All of the sera were tested at a single dilution of 1/200 in the absence of detergent. The results are expressed as the ratio of the serum OD to the cutoff OD (i.e., a value of ≥ 1.0 is considered positive). A total of 26 of 126 (21%) HIV-1-infected individuals were found to be reactive to the V2 consensus peptide, and 78 of 126 (62%) were reactive with the V3 Mn peptide (Fig. 6A). None of the sera tested bound to the control Raf c peptide (data not shown). Of the 26 of 126 serum samples identified which bound to the consensus V2 peptide (794.1), 24 of 26 serum samples recognized the equivalent peptide from the Mn strain of HIV-1 (794.3), 11 of 26 recognized the LAI V2 peptide (794.2), and none bound to the RF V2 peptide (794.4) (Fig. 6B and data not shown). Of the individual sera identified which bound to the LAI V2 peptide (794.2), all reacted with the peptide 740.13 (aa 152 to 172) (data not shown). The temporal development of antibodies to the V2 (794.1) and V3 (Mn) linear peptides was monitored by measuring the peptide reactivity of sera taken sequentially from 20 individuals from the time of seroconversion. All individuals developed an antibody response to rgp120, and 5 of 20 developed reactivity to both the V2 (794.1) and V3 (Mn) peptides simultaneously (data not shown).

DISCUSSION

We have identified four neutralizing MAbs mapping to linear epitopes within the V2 domain and two conformation-dependent MAbs mapping within the V1V2 domains. The MAbs exhibit a type-specific binding and neutralizing activity for the HXB10 clone of LAI and are generally, with the

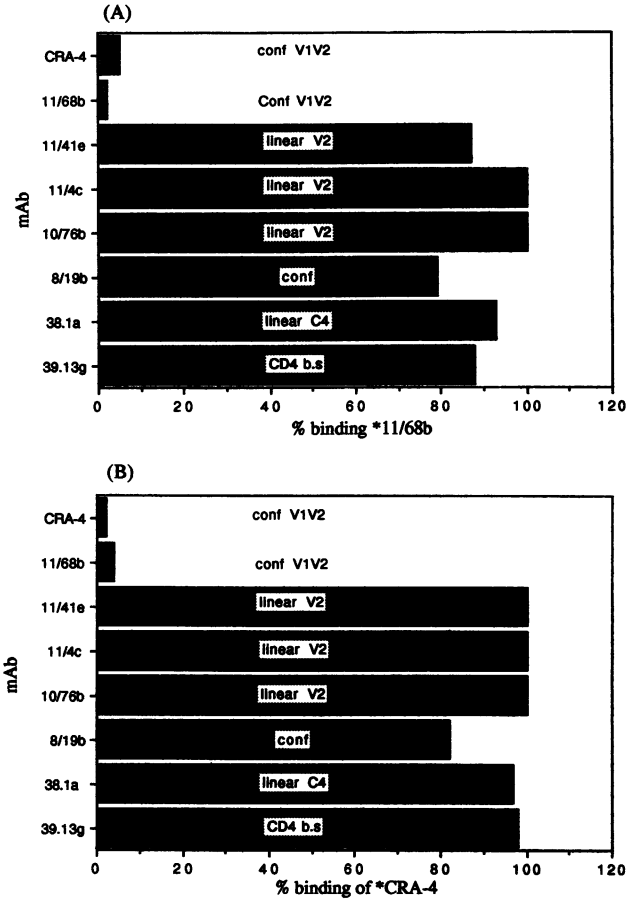

FIG. 4. Cross-competition analysis for MAbs 11/68b and CRC-4 binding to rgp120. MAbs (at a saturating concentration of 10 μg/ml) mapping to linear V2 epitopes (11/41e, 11/4c, and 10/76b), a linear C4 epitope (38.1a), a conformation-dependent CD4 bs (39.13g), and a conformation-dependent epitope involving the C1 and C3 regions of gp120 (8/19b) were tested for their ability to compete with iodinated preparations of 11/68b (A) and CRA-4 (B) for binding to rgp120. The results represent the means of triplicate wells, for which the standard deviations were within 10% of the mean for all samples. These results are from one experiment, but similar results were obtained in two separate experiments.

exception of MAb 11/41e, as efficient at neutralizing HXB10 infection of C8166 cells as MAbs mapping to the V3 loop (31) and CD4 bs (6, 35) (Table 2). MAb 11/41e binds to native gp120 at the cell surface with the lowest affinity of the four linear MAbs tested, which may explain its low neutralizing activity. All of the linear MAbs bind to two overlapping peptides, GEIKNCSFNISTSIRGKVQK (aa 152 to 171) and STSIRGKVQKEYAFFYKLDI (aa 162 to 181), suggesting that the shared residues, STSIRGKVQK (aa 162 to 171), comprise the epitope(s), a conclusion confirmed by SPOTscan analysis (Table 4). Alteration of residue 166 in gp120 from arginine to leucine abrogates the binding of all the linear MAbs (Fig. 3).

MAbs 10/76b, 11/4c, 11/41e, and 11/4b bind to peptide 740.13 with an average sevenfold-greater affinity than to peptide 740.14, suggesting that the epitope(s) may be more accessible on peptide 740.13 and may therefore be influenced by amino acids flanking the minimal epitope. The ability of peptide 740.13 to block both the MAb-gp120 interaction and

TABLE 7. MAb recognition of V1V2 chimeric proteins

MAb[a]	Result with V1V2 chimeric protein:				
	MuLV-V1V2[b]	MuLV-V3[b]	SF9,10[c]	gp105[c]	gp120[c]
10/76b	+	−	+++	−	+++
11/4c	+	−	+++	−	+++
11/41e	+	−	+++	−	+++
11/4b	ND	ND	+++	−	+++
11/68b	+	−	++	−	+++
CRA-4	+	−	++	−	++
41.1	−	+	−	−	+++
28/45b	ND	ND	+++	+++	−

[a] MAbs 10/76b, 11/4c, 11/41e, and 11/4b map to linear epitopes within V2; MAbs 11/68b and CRA-4 map to conformation-dependent epitopes within V1V2; MAb 41.1 binds to a conformation-dependent epitope within V3; and MAb 28/45b binds to an undefined HIV-2 Rod gp105 epitope.

[b] MAb recognition of MuLV-V1V2 and MuLV-V3 chimeric proteins was tested by radioimmunoprecipitation assay. + and −, recognition and no recognition, respectively; ND, not done.

[c] MAb recognition of HIV-2 (Rod) gp105, HIV-1 (LAI) gp120, and HIV-2–HIV-1 V1V2 (SF9,10) was tested by ELISA. OD values at 492 nm are as follows: +++, 0.6 to 1.0; ++, 0.4 to 0.6; +, 0.1 to 0.3; and −, <0.1.

neutralization lends further support to this interpretation of the data. Fung and colleagues (10) reported two neutralizing murine MAbs mapping to aa 169 to 183 in the V2 domain, one of which demonstrated an LAI type-specific neutralization and the other of which cross-neutralized the RF strain of HIV-1, suggesting that further epitopes exist in the V2 domain.

Mapping of the two conformation-dependent MAbs, 11/68b and CRA-4, with a panel of site-directed gp120 mutants showed that deletion of the V1V2 domains (Δ119-205) abrogated binding of both of the MAbs and that substitutions of residues 183/184 PI/SG and 191/192/193 YSL/GGS within the V2 domain also affected binding of the MAbs. In addition, two changes at residue 435 in C4, from tyrosine to histidine or to serine, also abrogated both 11/68b and CRA-4 binding. However, changes at other C4 residues, 430, 432, 433, and 438, had no effect. Furthermore, a sCD4-selected escape mutant with a single amino acid change at residue 434, M/T, was resistant to neutralization by both 11/68b and CRA-4 (data not shown) (30). These results could be interpreted to suggest that the V1V2 and C4 domains may be components of a discontinuous epitope and therefore be proximal on the native molecule. This region of C4 is likely to be near the discontinuous CD4 bs (25, 43), and MAbs such as 38.1a, binding to a linear epitope covering these residues, are capable of blocking the gp120-sCD4 interaction and of neutralizing HIV infection (6, 35, 57). However, the C4 linear MAb, 38.1a, did not cross-compete with either 11/68b or CRA-4 for gp120 binding, suggesting that residues in C4 are not components of their epitope(s) (Fig. 4). The ability of MAbs 11/68b and CRA-4 to bind to the V1V2 domain expressed as a chimeric protein either with MLV envelope gp70 (21a) or with HIV-2 gp105 (Table 7) (40) further supports the argument against residues in C4 comprising part of their epitope.

Amino acid changes in C4 may therefore affect the structure of the V1V2 domain recognized by conformation-dependent MAbs. This is consistent with the ability of sCD4 to inhibit binding of 11/68b and CRA-4 both to rgp120 and to cell surface-expressed gp120 (Fig. 5) and its inability to affect the binding of the linear V2 MAbs. Our data are consistent with that of Fung and colleagues (10), who reported that sCD4 inhibited the binding to rgp120 of a neutralizing

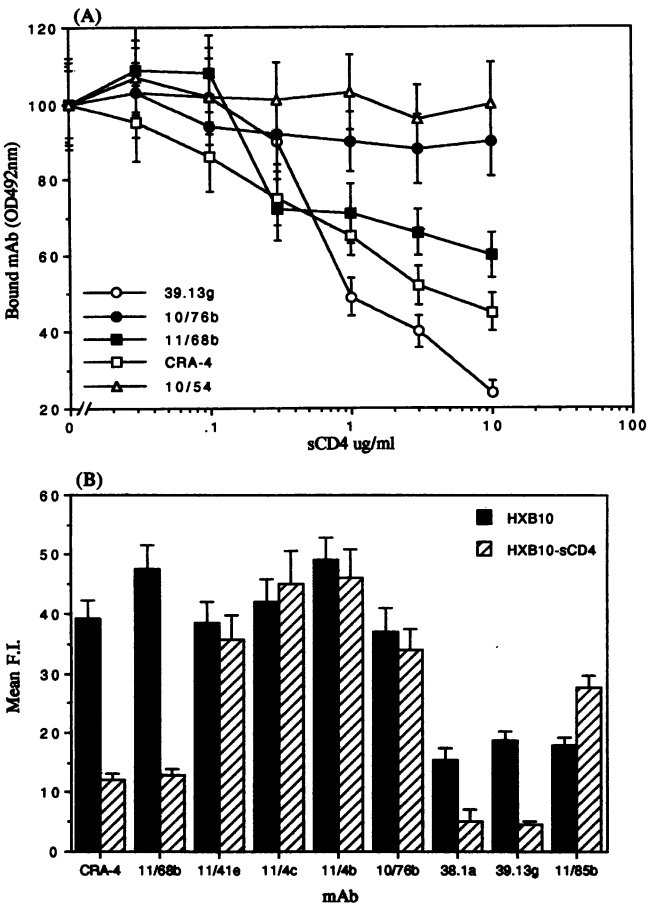

FIG. 5. Effect of sCD4 on MAb recognition of native cell surface-expressed gp120 and rgp120. (A) MAbs 39.13g, 10/76b, 11/68b, CRA-4, and 10/54 at a saturating concentration of 10 μg/ml were tested for their ability to bind to rgp120 in the presence or absence of sCD4. (B) A range of MAbs mapping to conformation-dependent epitopes within V1 and V2 (CRA-4 and 11/68b), linear V2 epitopes (11/41e, 11/4c, 11/4b, and 10/76b), a linear C4 epitope (38.1a), a linear V3 epitope (11/85b), and a conformation-dependent epitope overlapping the CD4 bs (39.13g) were tested for their ability to bind to HXB10-infected H9 cells with or without prior incubation with sCD4 (10 μg/ml). F.I., fluorescence intensity.

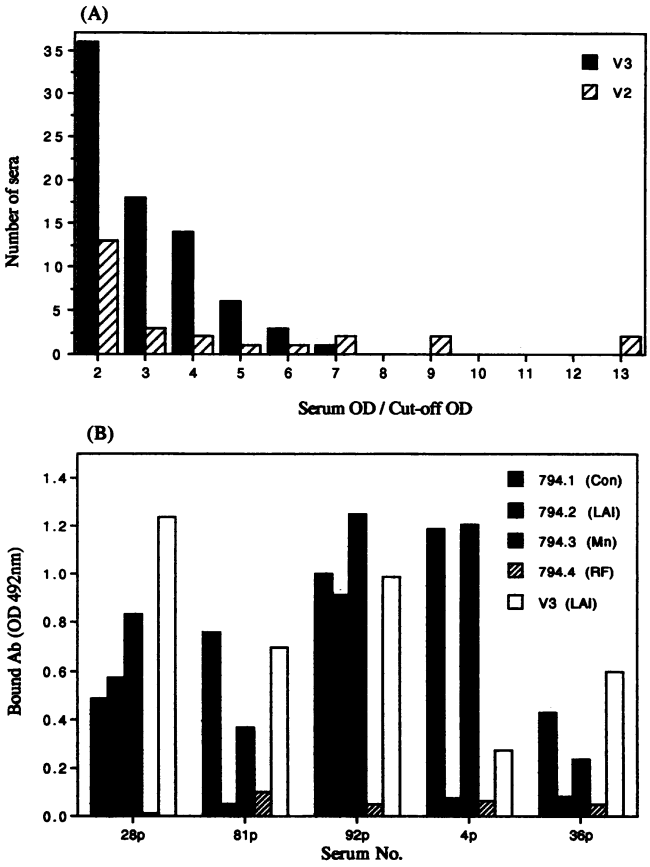

FIG. 6. Reactivity of human sera from HIV-1-infected individuals with V2 and V3 peptides. (A) Sera from 126 HIV-1-infected individuals were tested for their reactivity to a consensus group B (North American and European) V2 peptide (aa 162 to 180), a consensus Mn strain V3 peptide (aa 309 to 325), and a control Raf c peptide (data not shown). Fifty HIV-1-seronegative serum samples were also tested for peptide reactivity, and the mean OD plus 3 standard errors was defined as the cutoff value. The results are expressed as a ratio of the test serum OD to the cutoff value. (B) Serum samples from five individuals were tested for their reactivity to the following peptides: consensus V2 (794.1), LAI V2 (794.2), Mn V2 (794.3), RF V2 (794.4), and LAI V3. The results are shown as the mean OD value obtained at a single serum dilution of 1/200.

conformation-dependent MAb, G3-4. Cross-competition gp120 binding experiments between linear V2 MAbs and G3-4 led Fung et al. to conclude that MAb G3-4 recognized an epitope within V2. This has been confirmed by recent analysis of G3-4 recognition of HIV-1 gp120 mutants (56a). Given the ability of sCD4 to block the MAb-gp120 interaction, one might expect the MAbs to block sCD4 interaction with gp120. However, we could not demonstrate any reduction in sCD4 binding either to rgp120 or to gp120 at the surface of infected cells in the presence of saturating concentrations of 11/68b and CRA-4 (data not shown). In the same assays, control MAbs to the CD4 bs, 39.13g and 38.1a, did block the interaction. This is consistent with reports that a gp120 protein deleted in the V1V2 region binds sCD4 with an affinity equivalent to that for the full-length native protein (43, 45). MAbs to the deleted region might therefore be expected to have no effect on the sCD4-gp120 interaction.

Both the linear V2 and conformation-dependent V1V2 MAbs bind well to gp120 expressed at the surface of infected cells (Table 3), suggesting that this region of the envelope is well exposed on the cell or virion surface. However, neither MAb 11/68b nor CRA-4 recognized rgp120 when bound to cellular CD4 (Table 3), suggesting that binding of rgp120 to either soluble or cellular CD4 results in a masking of their epitopes. In contrast, a number of human MAbs demonstrate enhanced recognition of gp120 and neutralizing activity in the presence of sCD4 (19, 48). Interaction of viral gp120 with sCD4 has been reported to lead to increased exposure of epitopes within gp41 and the V3 domain (31, 50). These observations suggest that conformational changes occur in the envelope as a result of its interaction with CD4. One may speculate on the potential mechanism of neutralization of MAbs 11/68b and CRA-4. The results presented suggest that the antibody may bind to the virus prior to its attachment to cellular CD4 and may therefore block subsequent CD4-induced conformational changes in gp120 required for fusion to take place. Both MAbs 11/68b and CRA-4 are inefficient at neutralizing virus once bound to the

cell surface (data not shown), probably because the epitopes for these MAbs are inaccessible after virus binding. In contrast, V3 MAbs neutralize viral infection equally efficiently whether the virus is cell surface bound or not (unpublished data and reference 27).

Peptides corresponding to defined linear neutralization epitopes within V2 (10, 29a) are recognized by human sera (Fig. 6A), suggesting that the primary, type-specific neutralization response observed early after infection may not be entirely directed to the V3 domain (14, 18, 39, 49). The estimated frequency of V2 peptide reactivity of 21%, based on the random screening of individuals, may be an underestimate given the variability of this domain (Fig. 6B). Furthermore, we and others (10) have characterized neutralizing MAbs which recognize conformation-dependent epitopes within the V1V2 domains, raising the possibility that similar antibodies may be present in HIV-1-infected individuals. Future studies will investigate the occurrence and the temporal development of both linear and conformation-dependent antibodies to the V1V2 domains in infected individuals.

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