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Epitope Mapping of Two Immunodominant Domains of gp41, the Transmembrane Protein of Human Immunodeficiency Virus Type 1, Using Ten Human Monoclonal Antibodies

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Immunogenic regions of the gp41 transmembrane protein of human immunodeficiency virus type 1 (HIV-1) were previously mapped by examining polyclonal sera from HIV-infected patients and rodent polyclonal and monoclonal antibodies (MAbs) to peptides of gp41. To define the epitopes within these regions to which infected humans respond during the course of infection, the specificity of human MAbs to these regions had to be studied. Using 10 human MAbs identified initially by their reactivity to whole gp41 in HIV-1 lysates, the epitopes within the immunodominant region of gp41 and within a second immunogenic region of gp41 have been mapped. Thus, five MAbs (from five different patients) to the immunodominant domain of gp41 in the vicinity of the cysteines at positions 598 and 604 (hereinafter designated cluster I) reacted with a stretch of 11 amino acids from positions 590 to 600. Four of these five MAbs were reactive with linear epitopes, while one MAb required the conformation conferred by the disulfide bridge between the aforementioned cysteines. Three MAbs to cluster I revealed dissociation constants ranging from 10^{-6} to 10^{-8} M, depending on the MAb tested and the size of the synthetic or recombinant peptide used in the assay. Five additional MAbs reacted with a second immunogenic region between positions 644 and 663 (designated cluster II). Four of these five MAbs were specific for conformational determinants. Titration of sera from HIV-infected patients showed that there was about 100-fold more antibody to cluster I than to cluster II in patients' sera, confirming the immunodominance of cluster I.

The gp41 transmembrane protein of human immunodeficiency virus (HIV) serves as an anchor for the gp120 envelope protein and plays a critical role in infectivity (9, 16, 26). It is a highly immunogenic portion of the virus, inducing antibodies (Abs) which are usually demonstrable early after infection and persist into the terminal stages of disease (2, 23).

Several biologic and immunologic functions have been ascribed to gp41 and assigned to various regions of the molecule (Table 1). These include (i) the fusion domain at the N terminus of the molecule, which is responsible in part for infection of cells and for the cytopathic effects of fusion and cytolysis (8); (ii) the primary immunodominant domain in the region of the disulfide loop between cysteines at 598 and 604 (the amino acid-numbering system used throughout follows that of the HXB2 strain of HIV as designated by Myers et al. [21], and when peptides used in other studies are referred to, the amino acid positions are translated, where necessary, to the Myers system for consistency) (11, 14, 15, 22, 30); (iii) another immunogenic domain in a region just prior to the transmembrane domain (14, 15); (iv) the transmembrane domain, composed of highly hydrophobic, membrane-spanning amino acids (9); (v) a hydrophilic region just past the membrane-spanning region, which probably serves as an intracytoplasmic anchor (20), to which Abs with some neutralizing activity have been mapped (4, 5); (vi) a region of 5 amino acids which is highly homologous to a peptide at the N-terminal end of human leukocyte antigen class II molecules (12); and (vii) a 6-amino-acid region at the C terminus of gp41 which is homologous to an amino-terminal sequence in interleukin 2 (3).

While immunogenic regions of gp41 have been mapped by using human sera and peptides as small as 12 amino acids (11, 30), the conformation and location of epitopes within these regions could not be mapped by using polyclonal sera. Heretofore, only rodent monoclonal Abs (MAbs) have been available to map epitopes of HIV at the resolution of single or multiple amino acids, but these MAbs were induced experimentally with peptides (24, 25, 30) and thus do not reflect the humoral immune response of the natural host under infectious conditions.

In an effort to map the immunodominant epitopes of gp41 recognized by HIV-infected humans and to dissect the natural humoral immune response to the gp41 portion of HIV, the specificities and immunochemical characteristics of 10 human anti-gp41 MAbs were studied. These MAbs were derived from Epstein-Barr virus (EBV)-transformed lymphocytes from the blood of HIV-infected subjects and from heterohybridomas made from the aforementioned EBV-transformed cells fused to SHM-D33 mouse × human heteromyloma. The lines were selected for their abilities to bind to gp41 in whole virus lysates.

MATERIALS AND METHODS

Generation of lymphoblastoid lines and heterohybridomas producing human MAbs to gp41. Lymphoblastoid lines producing human MAbs to gp41 were obtained as described previously (13). Briefly, peripheral blood mononuclear cells

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TABLE 1. Identified regions of HIV type 1 transmembrane protein

Function and designation[a]	Amino acid sequence	Reference
Fusion domain	512–527	8 9
Immunodominant domain, gp120 anchor, E4 domain	583–609	22 15 11 14
E5 and E6 domains	644–668	15 14
Transmembrane domain	684–705	9
Hydrophilic cytoplasmic anchor	728–745	4
MHC class II homolog	824–830	12
Interleukin 2 homolog	852–857	3

[a] MHC, major histocompatibility complex.

obtained by Ficoll-Hypaque centrifugation of blood from HIV-seropositive volunteers were transformed with EBV. The EBV-infected cells were cultured for 4 weeks in microtiter plates, and their supernatants were screened by enzyme-linked immunosorbent assays (ELISA) and radioimmunoprecipitation assays for reactivity to HIV gp41. Each line was cloned at least three times at 100 or 10 cells per well. Some lines, designated by the suffix D, were derived by fusing EBV-transformed cells with the SHM-D33 mouse × human heteromyeloma generously provided by N. Teng (32). Briefly, the SHM-D33 cells were mixed with the lymphoblastoid cells at a ratio of 1:3, centrifuged, and treated with 1 ml of 50% polyethylene glycol 1300-1600 (Sigma Chemicals, St. Louis, Mo.). After gradual dilution with Iscove's medium (GIBCO, Grand Island, N.Y.), the cells were gently centrifuged, suspended, and plated in medium at 8×10^4 cells per well. After 24 h, 10^4 mouse peritoneal cells were added as feeder cells and the cultures were incubated in the presence of 0.5 mM hypoxanthine, 0.2 μM aminopterin, 16 μM thymidine, and 1 μM ouabain. After 2 to 3 weeks, wells were screened for Ab production, and those wells which were positive were expanded and sequentially cloned at 100, 10, and 1 cell per well.

Immunoglobulin assays. Ab subclasses were determined by ELISA. Briefly, Immulon 2 (Dynatech, Alexandria, Va.) plates were coated with HIV viral lysate (4 μg/ml; ENI Diagnostic, Inc., Silver Spring, Md.) and incubated with culture supernatants. The subtype of the MAb was detected by using alkaline phosphatase-labeled mouse MAb against the four subclasses of human immunoglobulin G (IgG; Zymed Laboratories, Burlingame, Calif.).

The light-chain type of each MAb was analyzed by ELISA using microplates coated with rabbit anti-human kappa-chain or rabbit anti-human lambda-chain Abs (Dakopatts, Copenhagen, Denmark). The developing Abs used were alkaline phosphatase-coupled goat anti-human kappa chain or goat anti-human lambda chain (Sigma), respectively.

IgG quantitation was also performed by ELISA. The plates were coated with goat anti-human IgG (gamma-specific) Abs and incubated with serially diluted culture supernatants. Bound IgG was detected with alkaline phosphatase-labeled goat anti-human IgG (gamma-specific) Abs. Affinity-purified human IgG (Organon-Teknika Cappel, Malvern, Pa.) was used as a standard. Plates were read and standard curves were generated by using an automated MR700 Microplate Reader (Dynatech, Chantilly, Va.).

Assays for inhibition of binding of MAbs to HIV lysate. Human MAbs were biotinylated by a modification of the method of Liu and Green (19), which was previously described (28). Assays to measure the abilities of unlabeled MAbs or patients' sera to block the binding of biotinylated MAb to HIV lysate were also performed as previously described (28). For these assays, Immulon 2 plates were coated with 0.5 μg of HIV lysate in 100 μl of coating buffer (0.05 M sodium carbonate buffer, pH 9.6), and 100 μl of culture supernatants or 100 μl of human serum diluted in phosphate-buffered saline (pH 7.2) containing 10% normal goat serum and 0.5% bovine serum albumin was added to each well. After incubation at room temperature overnight, wells were washed and 100 μl of titrated biotinylated MAb was added. After a further incubation at 37°C for 2 h and washing, 1 drop of the VECTASTAIN ABC Reagents (avidin and biotinylated horseradish peroxidase; Vector Laboratories, Burlingame, Calif.) was added for 30 min at 37°C. After wells were washed, 50 μl each of 2,2'-azino-bis[3-ethylbenzthiazoline sulfonate] peroxidase substrate and peroxidase solution (ABTS-H_2O_2; KPL, Gaithersburg, Md.) was added per well, and the color was read in an ELISA reader at 405 nm.

The following formula was used to determine the percent inhibition of binding: $(A_{max} - A_x)/(A_{max} - A_{min}) \times 100$, where A_{max} is the absorbance in the presence of diluent, A_x is the absorbance of the sample being tested, and A_{min} is the absorbance in the presence of sufficient amounts of the MAb homologous to the biotinylated MAb used in the assay to give maximum inhibition (usually 0.3 to 5.0 μg/ml).

To titrate the 50% inhibition titer of human sera, the assay described above was performed with dilutions of patients' sera ranging from 1:10 to $1:5 \times 10^6$. Simultaneously, an inhibition curve was determined to test the inhibitory capacity of the homologous MAb. The 50% inhibition titer of a given serum specimen was identified as the highest dilution of serum which gave at least 50% inhibition of the binding of the biotinylated MAb, as defined above.

Binding of MAb to synthetic and recombinant peptides by ELISA. Synthetic peptides were prepared on an Applied Biosystems, Inc. (Foster City, Calif.), 430A Peptide Synthesizer according to instructions supplied by the manufacturer. Peptides were deprotected and cleaved from the supporting resin with hydrogen fluoride. Amino acid analysis was performed (at Immunodynamics, Inc., La Jolla, Calif.) on all peptides to monitor content; all peptides employed from this source had the expected amino acid content.

Two peptides were purchased from commercial suppliers. The recombinant peptide p121, an 82-mer made in *Escherichia coli* that spans amino acids 560 to 641 encoded by the *env* gene, was purchased from Centacore (Malvern, Pa.). This peptide was used at a concentration of 4 μg per well for coating ELISA plates. Peptide 6140 was purchased from American International Chemical, Inc. (Natick, Mass.). This synthetic peptide spans amino acids 579 to 613 and contains a disulfide bridge between the two cysteines at positions 598 and 604. This peptide was used at a concentration of 0.25 μg per well for the coating of ELISA plates. Peptide 6140 was also reduced and alkylated to test its reactivity after the cleavage of the disulfide bond. The peptide was suspended to a concentration of 2.5 μg/ml in coating buffer containing 100 mM dithiothreitol. After incubation for 1 h at 37°C, an equal volume of 220 mM iodoacetamide in coating buffer was added. After another incubation for 1 h at 37°C, the reduced and alkylated peptide was used to coat ELISA plates at 0.25 μg per well.

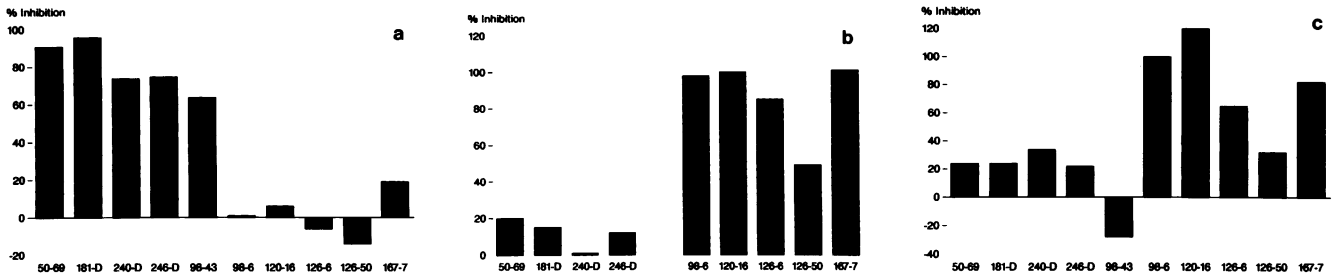

FIG. 1. Blocking of 3 human MAbs to gp41 (MAb 50-69 [a], MAb 120-16 [b], and MAb 98-6 [c]) by 10 human MAbs. Each of three MAbs was biotinylated, and the abilities of homologous and heterologous unlabeled anti-gp41 human MAbs to block the binding of the labeled MAb to HIV lysate was tested by ELISA. The ordinate shows the percent inhibition calculated as explained in Materials and Methods.

To identify regions of gp41 that bind human anti-gp41 MAbs, 1 μg of synthetic peptides in 100 μl of coating buffer was added to each well of Immulon 2 microtiter plates and incubated at 37°C for 2 h. The wells were then washed three times in phosphate-buffered saline containing 0.05% Tween 20 (washing buffer), and undiluted culture supernatants containing MAbs were added. After incubation for 2 h at 37°C, the wells were washed three times with washing buffer. Goat anti-human IgG1 coupled to horseradish peroxidase (Bio-Rad, Richmond, Calif.) was added at a dilution of 1:1,000 and incubated for another hour at 37°C. After five washes, ABTS-H_2O_2 was added as substrate, and after 30 min, color was detected at 405 nm. Each assay was repeated at least three times with at least two different aliquots of MAb-containing supernatants.

Fine mapping of the epitopes of these MAbs was performed by using the Epitope Mapping Kit (Cambridge Research Biochemicals, Valley Stream, N.Y.), which utilizes the method developed by Geysen et al. (10) to synthesize hexapeptides on polyethylene pins. Each MAb was tested against a set of 21 hexapeptides which spans amino acids 579 to 604, each hexapeptide overlapping its neighbor by 5 amino acids. The hexapeptides, bound to the polyethylene pins on which they were synthesized, were reacted with undiluted supernatant fluids of the heterohybridoma. The color reaction was developed by using goat anti-human IgG coupled to horseradish peroxidase with ABTS-H_2O_2 as substrate.

Determination of dissociation constants of MAbs. To determine the dissociation constants (K_d) for the MAbs, the method of Friguet et al. was used (7). Briefly, culture supernatants of 181-D, 240-D, and 246-D were tested at concentrations of 36 to 480 ng/ml; when 240-D was tested against the 26-mer (peptide AA 579-604), it was tested at 7.1 μg/ml. The peptides used in these experiments AA 579-604 and AA 560-641) were dissolved in coating buffer at 1 mg/ml, diluted in phosphate-buffered saline (pH 7.2), and tested at concentrations of 1.1×10^{-6} to 1.1×10^{-9} and 0.7×10^{-7} to 0.7×10^{-9} M, respectively. Equal volumes of supernatant and peptide were mixed, and after 16 h, the mixture was added to plates coated with the homologous peptide (1 μg/ml) and the amount of unbound MAb was measured by ELISA. Data were plotted according to the Friguet modification of Klotz (7) to determine the K_d.

RESULTS

Blocking experiments between anti-gp41 MAbs reveal two groups of MAbs. Each MAb was biotinylated and tested for its ability to bind to HIV lysate in the presence of unlabeled MAbs to gp41. The results of three such experiments are shown in Fig. 1, in which biotinylated MAbs 50-69, 120-16, and 98-6 were tested against a panel of 10 unlabeled MAbs to gp41. In these and all similar experiments, the human MAbs fell into two groups on the basis of their inhibitory characteristics. Thus, MAbs 50-69, 98-43, 181-D, 240-D, and 246-D constitute a single group in which each member is able to inhibit the other members. Similarly, MAbs 98-6, 120-16, 126-6, 126-50, and 167-7 constitute a second group related by Ab specificity. In Fig. 2, the ability of two MAbs from group 1 (246-D and 50-69) and of one MAb from group 2 (126-6) to inhibit the binding of a group 1 biotinylated MAb (50-69) over 4 orders of magnitude is shown. Inhibition of biotinylated 50-69 by itself or by 246-D is detectable at concentrations as low as 0.006 μg/ml, whereas a MAb from the heterologous group (126-6) does not inhibit at a concentration 500-fold higher.

Peptide mapping of anti-gp41 MAbs. In an initial attempt to locate the epitopes with which these two groups of MAbs were reactive, each MAb was tested for its ability to bind to a commercially available recombinant peptide, p121, which encompasses 82 amino acids from the extracellular domain of gp41 (AA 560-641). Those MAbs which were categorized as belonging to the first group of cross-inhibitory MAbs all bound to p121, whereas those MAbs which belonged to the second group of cross-inhibitory MAbs could not bind to p121 (data not shown).

To further localize the binding of the MAbs, they were tested for reactivity against six synthetic peptides which span approximately 60% of the extracellular portion of gp41. Table 2 shows that four MAbs (98-43, 181-D, 240-D, and 246-D) react with the peptide representing amino acids 579 to 604. All four of these MAbs had been categorized as cross-

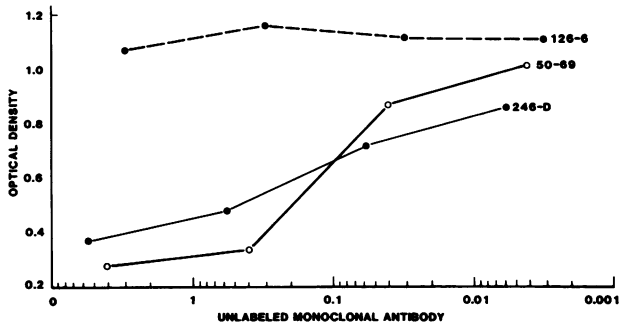

FIG. 2. Blocking of binding of biotinylated MAb 50-69 to HIV lysate in an ELISA by homologous and heterologous MAbs to gp41 used at concentrations spanning 4 orders of magnitude. Unlabeled MAbs (μg/ml) tested included MAbs 50-69, 246-D, and 126-6.

TABLE 2. Summary of peptide mapping of human MAbs to gp41

MAb	IgG subclass	Reactivity to gp41 peptide: AA 540-564	AA 579-604	AA 611-627	AA 644-663	AA 661-683	AA 703-721
50-69	2(κ)	−	−	−	−	−	−
98-6	2(κ)	−	−	−	−	−	−
98-43	2(κ)	−	+	−	−	−	−
120-16	2(κ)	−	−	−	+	−	−
126-6	2(κ)	−	−	−	−	−	−
126-50	2(κ)	−	−	−	−	−	−
167-7	1(λ)	−	−	−	−	−	−
181-D	2(κ)	−	+	−	−	−	−
240-D	1(κ)	−	+	−	−	−	−
246-D	1(κ)	−	+	−	−	−	−

inhibitory on the basis of the blocking experiments shown in Fig. 1. Thus, four of the five MAbs in group 1 were defined as being specific for linear sequences in the region of the extracellular disulfide loop of gp41, a region previously defined as a major immunodominant region (11, 14, 22). A single MAb (120-16) reacted with a peptide representing amino acids 644 to 663. This latter MAb had previously been categorized as a member of the second group of MAbs. Thus, only one of five MAbs of the second group was to a linear sequence, and the rest appeared to be specific for conformational or discontinuous epitopes.

Experiments were next performed to identify the fine specificity of MAb 50-69, a MAb of group 1 which does not react with any linear peptides. This MAb reacts with a peptide that spans amino acids 579 to 613 and contains a disulfide bridge between amino acids 598 and 604. To determine the role of the disulfide bridge in this reactivity, the peptide was reduced and alkylated. The results of a representative ELISA using this peptide in unreduced and reduced states are shown in Fig. 3. Since MAb 50-69 reacted only with the unreduced form of the peptide and not with the reduced form and since it did not react with peptide AA 579-604 (Table 2), which lacks the disulfide bond, the specificity of MAb 50-69 was shown to map to an area of gp41 affected by the conformation conferred by the two cysteines at amino acids 598 and 604.

Three MAbs reacting with peptide AA 579-604 were studied further to identify their fine specificities. They were tested against a set of hexapeptides which spans this region,

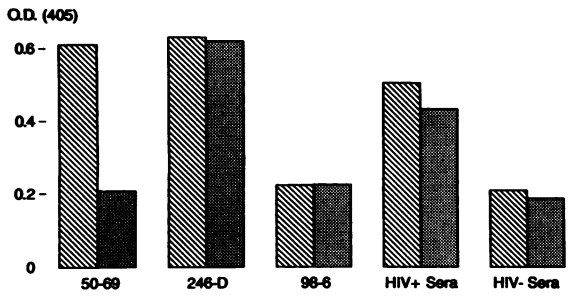

FIG. 3. Epitope mapping of MAb 50-69 with reduced (▨) and unreduced (▧) peptide AA 579-613. In the unreduced form, this peptide contains a disulfide bond between cysteines at positions 598 and 604. Data from a representative ELISA are shown. O.D. (405), optical density at 405 nm.

each hexapeptide overlapping its neighbor by 5 amino acids. The hexapeptides, synthesized in situ on pins of the Epitope Mapping Kit, were reacted with culture supernatants of lines producing MAbs 181-D, 240-D, and 246-D which contained 2 to 50 μg of MAb per ml. The results, summarized in Fig. 4, show that MAb 181-D reacts with two overlapping hexapeptides, QLLGIW and LLGIWG. These data suggest that the epitope to which MAb 181-D is directed is qLLGIWg, where the capital letters of the amino acid code represent the core of the epitope and the lowercase letters represent flanking amino acids which also probably contribute to the binding of the epitope. Similarly, MAbs 240-D and 246-D bind to four and three overlapping peptides, identifying the epitopes to which these MAbs react as 11gIWGcsg and qqLLGIwg, respectively.

Determination of MAb dissociation constants. The K_d of three MAbs which react with linear epitopes in the 579- to 604-amino acid region were determined by using the method of Friguet et al. (7). These MAbs (181-D, 240-D, and 246-D) were tested for their binding activities to a synthetic 26-mer peptide (AA 579-604) and to a recombinant 82-mer peptide (AA 560-641). Dissociation constants ranged from $\geq 1 \times 10^{-6}$ to 9.1×10^{-8} M for MAbs reacting with the 26-mer and from 2.2×10^{-8} to 3.6×10^{-8} M for MAbs reacting with the 82-mer (Table 3). The affinity of 240-D for the 26-mer was insufficient to meet the minimum requirements of this particular assay; i.e., even at a fivefold concentration of supernatant (35 μg of MAb per ml), there was insufficient reactivity with the 26-mer to give an optical density reading of 1.0 when the method described above was used. The K_d for 240-D, therefore, exceeds the maximum calculated for other MAbs, i.e., $>10^{-6}$ M. Noting that the K_d for the larger peptide was in all cases lower than that for the smaller peptide, it might be expected that the K_d of the MAbs for the native gp41 and gp160 molecules will be lower yet by virtue of contributions of conformation of the whole molecule to the epitopes to which the MAbs react.

Titration of serum Abs from HIV-infected patients to epitopes in peptides AA 579-604 and AA 644-663. Several studies have noted the nearly universal appearance of Abs to the region of gp41 in the vicinity of the cysteines at amino acids 598 and 604 (11, 14, 22, 30), hereafter designated cluster I. A much lower percentage of patients' sera react with the region that maps from approximately 644 to 663, i.e., cluster II (14, 15, 23). To study the relative amounts of serum Abs to epitope clusters I and II in patients' sera, inhibition assays were performed in which serum samples from seven HIV-infected patients were tested for their abilities to inhibit by 50% the binding to HIV lysate of biotinylated MAbs specific for cluster I (MAb 50-69) or cluster II (MAb 120-16). The sera tested were derived from patients at diverse stages of disease progression, as previously defined (37). One hundred percent inhibition of binding of the homologous labeled MAbs was achieved with ≥300 ng of MAb 50-69 per ml and with ≥1,375 ng of MAb 120-16 per ml. Sera from seronegative volunteers gave no significant inhibition at a dilution of 1:10. The 50% inhibitory titers of the patients' sera are shown in Table 4. The geometric mean titer for 50% inhibition of binding of 50-69 (MAb to cluster I) by sera from HIV-infected volunteers was 1:220,220. The geometric mean titer for 50% inhibition of binding of 120-16 (MAb to cluster II) by sera from HIV-infected volunteers was 1:2,200. Thus, the epitopes of cluster I appear to be approximately 100-fold more immunogenic than those of cluster II.

FIG. 4. Scan of MAb reactivities by ELISA with overlapping hexapeptides homologous with the region of amino acids 579 to 604 in Env. The reactivity of each hexapeptide with supernatants from heterhyberdomas 181-D (a), 240-D (b), and 246-D (c) is shown on the ordinate, and each hexapeptide is designated by the single-letter code of its N-terminal residue and the subsequent 5 amino acids. Thus, the sequence appearing on the abscissa is the sequence of peptide AA 579-604. O.D., optical density.

DISCUSSION

The gp41 transmembrane protein of HIV is relatively invariant, with greater than 80% conservation at the amino acid level and no regions of hypervariability (20). The N-terminal half of the molecule is displayed on the surface of the virus particle as well as on the surface of infected cells, and gp41 is the antigen which is most consistently recognized by the immune systems of HIV-infected individuals (2, 14, 23).

gp41 contains approximately 345 amino acids and many B-cell epitopes. Initial attempts to identify immunogenic regions were based on computer programs that analyzed the secondary structure predictions, hydrophilicity, surface probability, and flexibility of the molecule. These analyses led to the identification of four predicted B-cell epitopes (11, 20, 29). More recent analyses of sera from HIV-infected patients with a variety of peptides have demonstrated Abs to at least 11 sites in gp41 (11, 14, 15, 22, 23, 30, 36).

The specificities of the human MAbs to HIV gp41 described in this report extend the serologic findings with human polyclonal antisera, especially with respect to two epitopes or epitope clusters. Thus, five of the MAbs described here react in the region encompassed by amino acids 579 to 604 (designated cluster I). Another five MAbs react with the region from amino acids 644 to 663 (designated cluster II). Two additional human MAbs, one to each of these clusters, were previously described by other groups (1, 31).

The nearly equal number of lines producing MAbs to clusters I and II probably does not reflect the distribution of Ab-forming cells in the blood of infected donors, since each of the MAbs to cluster I comes from a separate patient but four of the five MAbs to cluster II come from a single patient, suggesting a vigorous and perhaps preferential response by that particular patient to cluster II. The frequency of lines synthesizing MAbs to a particular antigen or determinant is probably a function of many factors, including the frequency of Ab-producing B cells to the given determinant in the blood, the differential efficiency of EBV transformation of B cells derived from various donors, the relative expression of the antigen or antigenic determinant in the antigen preparation used for the screening procedure, etc.

A better measure of relative immunogenicity is given by the titration of Abs in patients' sera to different determinants, as shown in Table 4. These data suggest the immunodominance of cluster I, which is shown to induce a level of Abs in the serum of infected subjects that is 100-fold higher than the level of Abs to cluster II. This corroborates the findings of Neurath et al. (23) that polyclonal Abs from patients' sera to peptides spanning the approximate epitopes designated here as clusters I and II differ in immunogenicity, according to Ab titers, by more than 2 orders of magnitude.

Affinities of the MAbs studied here fall in the midrange of affinities previously noted for MAbs and for Abs in polyclonal sera (7, 18). The finding that two of three MAbs had higher affinities for longer peptides suggests that the conformation of the epitope contributes to the binding affinity of MAbs specific for linear sequences, confirming a similar suggestion by Fieser et al. (6). In this context, it is important to view the tri-, tetra-, and pentapeptides identified as the targets of specificity (Fig. 4) as the epitope core and not necessarily representative of the entire epitope.

As demonstrated by the epitope-mapping data, some MAbs to gp41 are also reactive with discontinuous and/or entirely conformational determinants. In this context, the reactivity of MAb 50-69 is particularly interesting in that it depends on the presence of a conformation which is maintained by an intrachain disulfide bond (Fig. 3). This MAb is similar to rodent MAbs induced with peptides of gp41 which require the intramolecular disulfide bridge (25) and may represent those Abs found in polyclonal sera which react with unreduced but not with reduced proteins in viral lysates (17). It should be noted, however, that MAb 50-69 can react with the rather large recombinant peptide AA 560-641, which

TABLE 3. Determination of K_d for anti-gp41 human MAbs against two peptides of different lengths

MAb	K_d (M) vs:	
	AA 579-604 (synthetic)	AA 560-641 (recombinant)
181-D	1.1×10^{-6}	2.2×10^{-8}
240-D	$\geq 1 \times 10^{-6}$	3.6×10^{-8}
246-D	9.1×10^{-8}	2.6×10^{-8}

TABLE 4. Titration of patients' sera for Abs to epitope cluster I (amino acids 579 to 604) and cluster II (amino acids 644 to 663)

Patient no.	Serum titer for 50% inhibition of biotinylated MAb:	
	50-69[a]	120-16[b]
1	1:100,000	1:1,000
2	1:100,000	1:5,000
3	1:500,000	1:5,000
4	1:500,000	1:10,000
5	1:1,000,000	1:10,000
6	1:100,000	1:1,000
7	1:100,000	1:1,000

[a] Geometric mean titer, 1:220,220.
[b] Geometric mean titer, 1:2,200.

does not contain the intrachain disulfide bond. The data suggest, therefore, that with a large enough peptide, in this case one with 82 amino acids, sufficient conformation exists to mimic the shape of the native protein, whereas in a relatively short synthetic peptide, e.g., AA 579-613, the disulfide bridge is necessary to maintain the three-dimensional structure required by the MAb combining site.

The human MAbs described herein have also been characterized for biological function. While none of these MAbs neutralize virus infectivity, all anti-gp41 human MAbs tested to date mediate Ab-dependent cellular cytotoxicity (35). Preliminary data suggest that MAbs to cluster II may be somewhat more effective in this regard than MAbs to cluster I. In addition, of 10 MAbs tested, 4 mediate complement-mediated, Ab-dependent enhancement (C'-ADE) of viral infection (27, 28). Thus, both MAb 120-16 (cluster II) and MAbs 50-69, 240-D, and 246-D (cluster I) mediate C'-ADE. These findings demonstrate that C'-ADE can be induced by MAbs to epitope clusters I and II and that the phenomenon is not restricted to a particular subclass of IgG (Table 2). Finally, human MAbs to epitope clusters I and II, when coupled to the deglycosylated A chain of ricin, can serve as immunotoxins (33, 34).

Thus, the epitopes of gp41 can induce a variety of Abs with a multitude of immunologic functions, and even a single epitope, or "epitope cluster," can give rise to Abs with apparently contradictory biologic functions (Ab-dependent cellular cytotoxicity and C'-ADE). The continued study of these monoclonal reagents should lead to a clearer definition of those aspects of the natural human immune response to HIV which are deleterious or protective to the host, which could be targeted for vaccine development, and which could be used for immunotherapeutic intervention.

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