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Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein

KASHI JAVAHERIAN*, ALPHONSE J. LANGLOIS†, CHARLENE MCDANAL†, KAREN L. ROSS*, LAWRENCE I. ECKLER*, CINDY L. JELLIS*, ALBERT T. PROFY*, JAMES R. RUSCHE*, DANI P. BOLOGNESI†, SCOTT D. PUTNEY*, AND THOMAS J. MATTHEWS†

*Repligen Corporation, One Kendall Square, Cambridge, MA 02139; and †Department of Surgery, Duke University Medical School, Durham, NC 27710

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ABSTRACT **The principal neutralizing determinant of human immunodeficiency virus type 1 (HIV-1) is located in the external envelope protein, gp120, and has previously been mapped to a 24-amino acid-long sequence (denoted RP135). We show here that deletion of this sequence renders the envelope unable to elicit neutralizing antibodies. In addition, using synthetic peptide fragments of RP135, we have mapped the neutralizing determinant to 8 amino acids and found that a peptide of this size elicits neutralizing antibodies. This sequence contains a central Gly-Pro-Gly that is generally conserved between different HIV-1 isolates and is flanked by amino acids that differ from isolate to isolate. Antibodies elicited by peptides from one isolate do not neutralize two different isolates, and a hybrid peptide, consisting of amino acid sequences from two isolates, elicits neutralizing antibodies to both isolates. By using a mixture of peptides of this domain or a mixture of such hybrid peptides the type-specificity of the neutralizing antibody response to this determinant can perhaps be overcome.**

Although not the only defense mechanism against viruses, neutralizing antibodies have played a major role in vaccine development. For human immunodeficiency virus (HIV) the principal targets for neutralizing antibody (1–5) are found in the external envelope protein, gp120 (5–7). gp120 also contains the binding sites for the CD4 receptor on helper CD4 cells (8). The major site of binding to CD4 lies in the COOH-terminal portion of gp120 (4, 9).

We have shown that a 180-amino acid recombinant protein (PB1) situated within gp120 contains the major neutralizing epitope of the complete envelope, gp160 (2, 3). By constructing a series of recombinant proteins and synthetic peptide fragments of PB1, we showed that the neutralizing determinant maps to a 24-amino acid peptide called RP135 (3). Independently, Palker *et al.* (10), Goudsmit *et al.* (11), Ho *et al.* (12), and Kenealy *et al.* (13) identified peptides that mapped to this region and induced neutralizing antibody. We proposed that the RP135 is contained within a disulfide loop in which the cysteines that flank RP135 (3) and are conserved in all sequenced HIV-1 isolates are disulfide cross-bridged (Fig. 1). The sequence Gly-Pro-Gly, a candidate for a polypeptide β-turn, which appears in the middle of RP135 is conserved in 80–90% of all sequenced HIV-1 envelopes, whereas the amino acid sequence flanking the Gly-Pro-Gly is hypervariable. Presence of the disulfide loop has been confirmed by disulfide analysis of gp120 (T. Gregory, personal communication).

In addition to eliciting antibodies that neutralize cell-free HIV and prevent fusion of virus-infected cells with uninfected CD4-bearing cells, RP135 can absorb all neutralizing antibodies from animals immunized with PB1, gp160, or from chimpanzees infected with human T-cell leukemia virus type III isolate B (HTLV-III$_B$) (3). This fact indicates that RP135 is the major neutralizing determinant. Because RP135 is from a region that is hypervariable in amino acid sequence, the sera elicited by this peptide are viral-variant specific (3). Several gp120-specific HIV-neutralizing monoclonal antibodies have been reported (15–17). All of these antibodies map to sequences in the RP135 determinant and are variant-specific. Antibody bound to the RP135 region does not prevent gp120 binding to CD4 (17, 18), suggesting that RP135-directed antibodies neutralize by interfering with a post-binding step in the infection process.

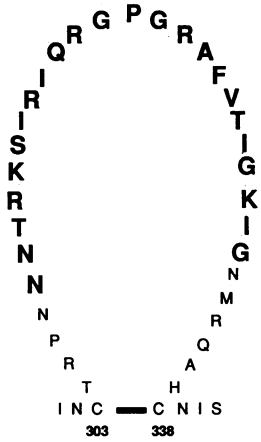

FIG. 1. Major neutralizing domain of HIV-1. The sequence is between amino acids 301–341 in one-letter code numbered according to a described system (14), and the cysteines are joined by a disulfide cross-bridge (T. Gregory, personal communication). The sequence of RP135 is denoted by boldface letters (NN–IG); this sequence is derived from the BH10 clone of the HTLV-III$_B$ isolate.

In natural HIV infection of humans, antibodies appear that exhibit a broad neutralization spectrum, and this cannot be easily accounted for by antibodies against this hypervariable region (19, 20). The most tangible hypothesis to explain this situation is that conserved regions of the envelope, perhaps noncontinuous determinants, are the targets for such antibodies. Such conserved, noncontinuous determinants may have been destroyed in the gp160 and PB1 immunogens used in our studies (2, 21) for several reasons, such as improper glycosylation, denaturing, and disulfide reduction during purification. The work presented herein shows that when the RP135 determinant is removed from gp160, the antiserum elicited by the purified protein does not neutralize HIV. In addition, we show that gp160 purified from insect cells under nondenaturing, nonreducing conditions does not elicit broadly neutralizing antibodies.

In this report, we also probe further into the nature of the RP135 region and the features that determine its specificity. To delineate the neutralizing domain within RP135, we have synthesized a number of overlapping peptides spanning RP135. Our data show that the major neutralizing epitope of RP135 is defined by 8 amino acids at the tip of the disulfide loop and contains a conserved Gly-Pro-Gly sequence. We

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Abbreviations: HIV-1, human immunodeficiency virus type 1; HTLV-III$_B$ and -III$_{RF}$, human T-cell leukemia virus type III isolates B and RF, respectively—now designated IIIB and RF of HIV.

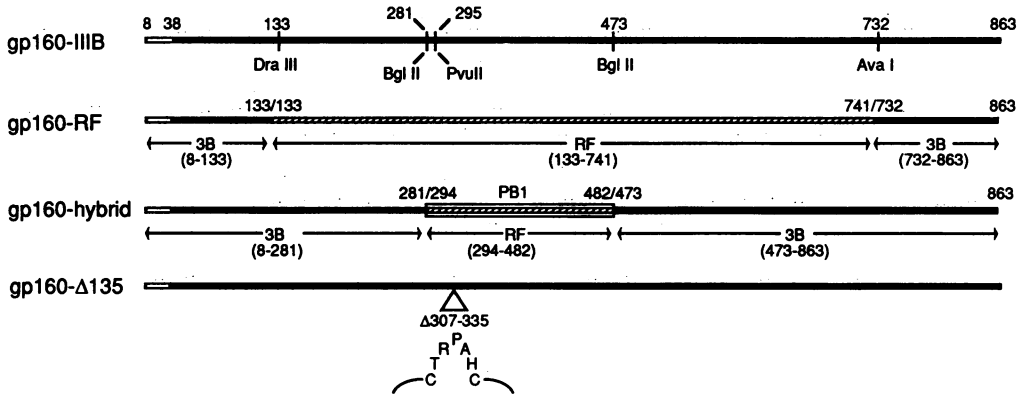

FIG. 2. Genomic representation of recombinant gp160 proteins. A restriction map of HTLV-III$_B$ (BH10) envelope protein (14) is shown at top. The secretion signal is amino acids 8–38. ▨, Segments inserted from the RF envelope of HIV.

also constructed a hybrid peptide of 14 amino acids that contains the tip of the loop from two viral isolates. This peptide elicits antisera that neutralize both isolates.

MATERIALS AND METHODS

Proteins. Recombinant gp160-IIIB and gp160-RF obtained from *S. frugiperda* cells by using a recombinant baculovirus have been described (3, 21). The envelope gene starts with an ATG (methionine) at the beginning of the secretion signal sequence [position +8 in the Ratner *et al.* (14) numbering system] and is 855 amino acids long. The protein is not processed to gp120 and gp41, and whether the secretion signal is removed after synthesis in the insect cells is unknown.

The gp160-RF expression vector was made by replacing the *Dra* III–*Ava* I segment of the gp160-IIIB gene (encoding amino acids 126–724) with the *Dra* III–*Ava* I segment of the gp160-RF gene. The gene encoding gp160-RF is thus a hybrid consisting of the secretion signal and the NH_2-terminal 94 amino acids of the mature gp160-IIIB protein, a 609-amino acid portion encoded by the HAT-3 molecular clone of the RF isolate of HIV (22) (from amino acids 124–733), and finally the COOH-terminal end of the gp160-IIIB protein from amino acids 724–855. This protein is, therefore, 866 amino acids long (Fig. 2). The gp160 hybrid expression vector was made by replacing the *Bgl* II–*Bgl* II segment of the gp160-IIIB gene (encoding amino acids 273–466) with the *Dra* III–*Ava* I segment of the gp160-RF gene. The *Bgl* II segment is only slightly larger than the *Pvu* II–*Bgl* II segment that encodes the 180-amino acid long PB1 and, therefore, encompasses the PB1 region. The protein gp160-hybrid (IIIB-RF) (Fig. 2) therefore consists of the secretion signal and the NH_2-terminal 242 amino acids of the mature gp160-IIIB gene, a 187-amino acid portion encoded by the HAT-3 clone of the HTLV III$_{RF}$ isolate extending from amino acids 287–474 and the COOH-terminal end of the gp160-IIIB gene from amino acids 466–855 (Fig. 2).

Protein gp160-Δ135 is a construction of gp160-IIIB from which the RP135 disulfide loop was removed. This protein is expressed from a gene encoding gp160-IIIB, in which the sequence encoding the amino acids (in one-letter code) . . . EINCTRPNNNTRKSIRIQRGPGRAFVTIGKIGNMRQAHCNIS . . . is replaced by the sequence . . . EINCTRPAHCNIS . . . . Nucleotides encoding the underlined amino acids were deleted by oligonucleotide mutagenesis.

Peptides. Synthesis and purification of peptides, conjugation to carrier keyhole-limpet hemocyanin (all peptides were conjugated to keyhole-limpet hemocyanin), animal immunizations, and fusion inhibition and neutralization assays were as described (3). For the fusion-inhibition assay, CEM cells chronically infected with the virus were mixed with uninfected cells. The number of syncytia were determined in duplicate wells after 24 hr. Neutralization assays were done by mixing antiserum with virus and incubating with target cells; after 5–7 days, reverse transcriptase activity was measured. Two types of animals were used for immunization with peptides—goats and guinea pigs; in general, two goats and three guinea pigs were immunized with each antigen.

RESULTS

Recombinant gp160 and gp160 Hybrid Proteins Elicit Antibodies That Neutralize Cell-Free HIV and Inhibit Fusion of Infected Cells. Immune sera elicited by various forms of the HIV envelope were used to identify the location of antigenic epitopes. Four recombinant gp160 proteins were expressed in insect cells (Fig. 2). These proteins are gp160-IIIB (from the HTLV-III$_B$ isolate), gp160-RF (a hybrid containing ≈85% of gp160 from HTLV-III$_{RF}$), a gp160 that contains PB1 from RF isolate and the remainder of gp160 from IIIB [gp160-hybrid (IIIB/RF)] and gp160-Δ135, which is gp160-IIIB lacking the RP135 determinant. By using denaturing and reducing conditions the gp160-IIIB protein was purified to >90% purity judging from a Coomassie blue-stained polyacrylamide gel (Fig. 3). gp160-RF and gp160-Δ135 were purified by using the same scheme (data not shown). The gp160-hybrid (IIIB/RF) was purified from a polyacrylamide gel as described (21).

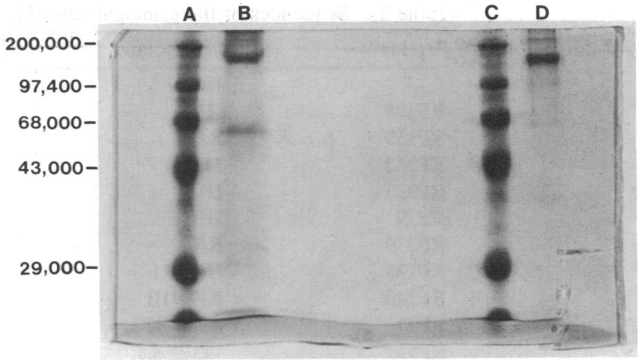

FIG. 3. Purification and 9% SDS/polyacrylamide gel of recombinant gp160-IIIB envelope protein from infected *S. frugiperda* insect cells. Briefly, the cells were lysed and extracted with 8 M urea followed by affinity purification using lentil lectin resin. For the final separation step, a sizing column (Sepharose CL-4B) was used that employed urea, 2-mercaptoethanol, and SDS. Soluble gp160 was purified essentially as in the above scheme, except no urea, 2-mercaptoethanol, or SDS were used. Lanes: A and C, protein M_r markers; B, purified soluble gp160; D, gp160, purified with urea, SDS, and 2-mercaptoethanol.

Table 1. Fusion inhibition and neutralization activities of different gp160 proteins

Protein	Fusion-inhibition titer		Neutralization titer		ELISA titer	
	IIIB	RF	IIIB	RF	PB1-IIIB	PB1-RF
gp160-IIIB	80	—	2560	—	8,000	800
gp160-RF	—	40	—	2560	900	2900
gp160-IIIB (soluble)	40	—	2560	—	12,000	400
gp160-hybrid (IIIB/RF)	—	90	—	320	800	3600
gp160-Δ135	—	—	—	—	3,900	800

The fusion-inhibition titer is the reciprocal of the dilution that reduces the number of syncytial foci by 90% (3). Neutralization titers are the reciprocal of the dilution that reduces reverse transcriptase activity by 50% (2). ELISA titers, done on plates coated with the noted PB1 protein, are the serum dilution where the immune serum is 0.21 OD unit above the preimmune serum at A_{410} with 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) as substrate. For gp160 antigens, two goats were immunized with each antigen; each goat was given four immunizations, and the sera used were collected 2 weeks after the last immunization. gp160-IIIB and gp160-Δ135 were purified as described for Fig. 3; the immunization dose was 1 mg. gp160-RF and gp160-hybrid were purified directly from a polyacrylamide gel (21). The doses per immunization for gp160-RF and gp160-hybrid were 100 μg and 200 μg, respectively. gp160-IIIB (soluble) was prepared as described for Fig. 3 and was given at doses of 100, 150, 400, and 250 μg during the immunizations sequence. —, Titer of <10.

Immune sera from goats immunized with these recombinant gp160s were assayed for the ability to neutralize cell-free virus and to prevent syncytia formation (Table 1). Both gp160-IIIB and gp160-RF elicit antibodies that neutralize or inhibit fusion of only the homologous isolate. In general, the neutralization titer of an antiserum is 5- to 10-fold higher than fusion-inhibition titers, indicating that neutralization is a more sensitive assay. Antibodies elicited by the gp160 hybrid (IIIB/RF), in which the PB1 region from RF is substituted, neutralize only RF and inhibit fusion of cells infected with RF (Table 1). This result is consistent with the observation that the principal neutralizing determinant lies within PB1.

Our inability to elicit antibodies with broad neutralization potential with full-length gp160 raised the possibility that noncontinuous neutralization determinants, composed of conserved regions of the envelope, may have been destroyed by the denaturing and reducing procedures of purification. Such determinants may cause the multi-HIV isolate neutralization capacity of antisera taken from seropositive individuals (19, 20). In an attempt to preserve the secondary and tertiary domains associated with gp160, we purified gp160-IIIB under nondenaturing and nonreducing conditions (Fig. 3). PAGE of the purified protein showed the purity of gp160 to be ≈50% and the presence of a number of minor contaminants [negative by gp160 sera on a Western (immunologic) blot]. The results included in Table 1 show that the serum elicited by this protein is still virus specific.

gp160-Δ135 Elicits Antisera That Do Not Neutralize the IIIB Virus. To further determine the significance of the RP135 sequence for production of neutralizing antibodies, gp160 lacking the RP135 sequence was prepared (see Fig. 2). By this process 29 amino acids (307–335) were deleted, and only 7 amino acids from the base of the loop were retained [Cys-Thr-Arg-Pro-Ala-His-Cys (CTRPAHC)]. This recombinant protein was purified as described for Fig. 3. The expression and the purity of gp160-Δ135 are similar to gp160-IIIB. Serum elicited by this protein shows neither fusion inhibition nor neutralization activity against either the IIIB or RF isolates, even though the protein elicits PB1-IIIB binding antibody as determined by ELISA (Table 1). These data confirm that the principal neutralization epitope lies within RP135 because upon removal of this domain the envelope loses the ability to elicit neutralizing antibodies.

RP135 Peptides from Other HIV Isolates Elicit Antisera That Neutralize the Homologous Virus. To investigate whether the RP135 determinant, responsible for neutralization of IIIB, is the neutralization determinant of other viral isolates, peptides analogous to RP135 of the RF and MN viral isolates were synthesized. The sequence of these peptides, along with the neutralization and fusion-inhibition assays, are presented in Tables 2 and 3. Peptides RP135 (IIIB) or RP139 (RF) can completely abrogate the cell fusion activity of antibodies elicited by the homologous PB1 protein (Table 3). Because these proteins completely abrogate the cell fusion inhibition, the RP determinant must contain the amino acid sequence to which all the cell fusion-inhibiting antibodies elicited by the larger PB1 immunogen are directed. The concentration at which RP139 blocks the PB1-RF-elicited antibodies is 10-fold lower (0.01 μm) than that required for RP135 to block PB1-IIIB-elicited antibodies (0.1 μM). Possibly conforma-

Table 2. Sequences of the principal neutralizing domain peptides

Peptide	Viral isolate	Sequence
—	IIIB	CTRPNNNTRKSIRIQRGPGRAFVTIGK IGNMRQAHC
RP135	IIIB	NNTRKSIRIQRGPGRAFVTIGK IG(C)
RP139	RF	——— TK——VIYAT-QI—(C)
RP142	MN	Y-K—R-H- ———Y-TKNI—(C)
RP335	IIIB	————(C)
RP79	IIIB	————(C)
RP339	RF	-TK——VIY(C)
RP138	IIIB-RF	————VIYAT— —(C)
RP140	RF-IIIB	——— TK———— —(C)
RP73	(IIIB-RF)	————VIY(C)
RP55		AHCNIS
RP57		INCTRPAHCNIS
RP59		IGDIRQAHCNIS
RP60		INCTRPNNNTRKSI

The disulfide loop sequence is shown (in one-letter code) at top, and the sequences of the peptides are indicated beneath. A cysteine (C) was added to the COOH-terminal end of each peptide to allow conjugation to the carrier and is not part of the HIV sequence. Sequence of RP135 is from the BH10 molecular clone of the IIIB isolate (14), and sequence of RP139 is from the HAT-3 molecular clone of the RF isolate (22). The sequence of peptides corresponding to the base of the loop is at bottom (RP55, -57, -59, and -60); these peptides were synthesized on the basis of the most conserved sequences among the IIIB, RF, and MN isolates. —, Identity with RP135.

tion of the RP139 peptide more closely resembles the determinant of the RF virus than RP135 resembles this determinant in the IIIB virus.

Besides competing for cell fusion-inhibiting antibodies, RP135 (IIIB), RP139 (RF), and RP142 (MN) elicit sera that neutralize and inhibit fusion of cells infected with the homologous isolate. The results indicate that the determinant analogous to RP135 for each of these isolates is a variant-specific neutralizing epitope.

The Neutralizing Determinant Maps to the Tip of the Disulfide Loop. In an attempt to localize more precisely the amino acids of the RP135 determinant that elicit neutralizing and cell fusion-inhibiting antibodies, we synthesized a series of hybrid peptides (containing sequences from both the IIIB and RF isolates) and a series of RP135 fragments (Table 2). Peptide RP138 is a hybrid peptide that has the same number of amino acids as RP135 but has the amino acid sequence of IIIB on the left and one similar to RF on the right side of the Gly-Pro-Gly. In the competition fusion assay, RP138 completely abrogates the fusion-inhibiting antibodies elicited by both PB1-IIIB and PB1-RF (Table 3), although the peptide concentration required is 10-fold different. The amount of RP138 required to block PB1-IIIB serum is the same as that required for RP135 to block the same serum (0.1 μM), indicating that the neutralizing determinant in RP138 is the same as in RP135. On the other hand, the blockade of PB1-RF serum by RP138 is achieved only at a concentration 100 times greater than with RP139 (0.01 μM for RP139 and 1 μM for RP138). RP138 is therefore less efficient in binding the RF fusion-inhibiting antibodies, suggesting that some of the RF-specific amino acids composing the neutralizing determinant of PB1-RF are not present in RP138. The result with RP339 (see below) shows that these amino acids are those immediately to the left of the Gly-Pro-Gly. Hybrid peptide RP140 with the RF sequence on the left and the IIIB sequence on the right of Gly-Pro-Gly is unable to block either PB1-IIIB or PB1-RF antiserum.

The data with RP138 and RP140 indicate that the neutralizing epitope in PB1-IIIB is composed of the Gly-Pro-Gly plus amino acids on the left and the neutralizing epitope in PB1-RF is the Gly-Pro-Gly plus amino acids on the right. A shorter hybrid peptide, RP73 (14 amino acids) with a sequence arrangement similar to RP138 (IIIB sequence on the left and RF sequence on the right) blocks both PB1-IIIB and PB1-RF sera at roughly the same concentration as RP135, showing that the sequence within RP73 defines neutralizing epitopes for both isolates.

Table 3. Fusion and neutralization activities of the disulfide loop peptides

Peptide	Fusion-inhibition titer			Neutralization titer			Peptide blockade of serum, μM	
	IIIB	RF	MN	IIIB	RF	MN	PB1-IIIB	PB1-RF
RP135 (G)	40	—	—	1500	—	—	0.1	—
RP139 (G)	—	320	—	—	2560	—	—	0.01
RP142 (G)	—	—	20	—	—	280	—	—
RP335 (GP)	160	—	—	2560	—	ND	—	—
RP79 (GP)	10	—	—	190	—	—	—	—
RP339 (G)	—	90	—	—	1300	—	—	0.01
RP138 (G)	40	40	—	340	1250	—	0.1	1
RP140 (G)	—	—	—	—	—	ND	—	—
RP73 (G)	20	40	—	1600	1300	ND	0.5	5
RP55 (G)	—	—						
RP57 (G)	—	—						
RP59 (G)	—	—						
RP60 (G)	—	—						

The peptides are those in Table 2. In the peptide blockade assay, the indicated concentration is the least peptide necessary to completely block inhibition of a 10-fold dilution of the corresponding PB1 serum when used in the cell fusion-inhibition assay. Either guinea pigs (GP) or goats (G) were used to generate the immune sera; values for each peptide are those from the animal responding with the highest titer. Only fusion inhibition data are given for the sera elicited by peptides at the base of the loop. —, Titers of <10 for fusion-inhibition and neutralization assays or the 40 μM peptide could not completely block the fusion-inhibition antibodies elicited by PB1 proteins. ND, not done.

The minimum neutralization determinants for IIIB and RF were defined by competition experiments using RP335 and RP339, peptides with 9 or 10 amino acids from the tip of the loop. RP339 contains a COOH-terminal sequence identical to RP73 and three additional RF amino acids on the left of Gly-Pro-Gly. RP339 completely blocks PB1-RF at the same concentration as RP139 (0.01 μM), indicating that the neutralization determinant of RP139 is contained in RP339. This shows that the amino acids Ile-Thr-Lys on the NH_2 terminus of RP339 contribute to the RF neutralizing epitope in PB1-RF. RP335, a peptide of nine amino acids with a IIIB-specific amino acid sequence is not able to block PB1-IIIB sera, even at 40 μM. Additional amino acids on the NH_2 terminus (in addition to the Ile-Gln-Arg present in RP335) must, therefore, be present in the minimum neutralizing epitope in PB1-IIIB.

The ability of these peptides to elicit neutralizing antibody was also studied. Peptides, RP335 and RP339 elicit high-titer neutralizing and cell-fusion-inhibiting antibody to the homologous isolate (Table 3). RP79, which is one amino acid shorter than RP335 [Gln-Arg-Gly-Pro-Gly (QRGPG. . .)] rather than Ile-Gln-Arg-Gly-Pro-Gly (IQRGPG. . .)] also elicits neutralizing antibody, albeit at ≈10-fold lower titer. Probably the isoleucine on the NH_2 terminus of RP335 is important for eliciting higher titer neutralizing antibody. RP335 elicits neutralization and fusion-inhibition titers as large as those elicited by the entire envelope, gp160.

Hybrid peptides RP138 and RP73 elicit antibodies that neutralize both IIIB and RF isolates, whereas antisera elicited by RP140 does not neutralize either isolate (Table 3). RP138 and RP73 have the IIIB-specific sequence to the left of the Gly-Pro-Gly and RF-specific sequence to the right, whereas in RP140 this order is reversed. This fact further supports the finding of the competition experiments that the neutralizing epitope of IIIB consists of the Gly-Pro-Gly plus amino acids to the left and that the neutralizing epitope of RF is Gly-Pro-Gly plus the amino acids to the right. These results show that hybrid peptides can be designed to elicit antibodies that neutralize more than one virus isolate.

Separately, a series of peptides corresponding to the conserved sequences of the base of the loop were synthesized (RP55, -57, -59, and -60). None of these peptides elicited neutralizing antibodies (Tables 2 and 3). All these sera showed reasonable ELISA titers against the corresponding peptides (data not shown).

DISCUSSION

These results provide further evidence that the RP135 determinant located in the third variable region (22) of gp120 is the principal neutralizing determinant of HIV-1. Removal of this determinant results in a loss of the ability of gp160 to elicit neutralizing antibody, and antibody elicited by this determinant from each of three different HIV-1 isolates (IIIB, RF, and MN) neutralizes the homologous virus isolate. Our data do not prove, however, that RP135 is the only neutralizing determinant on the envelope and, in fact, other determinants have been reported to elicit neutralizing antibody (12, 23–26).

In further work we have been able to absorb out from some human HIV seropositive antisera most neutralizing antibody to one or another virus isolate with a peptide of the RP135 determinant of that isolate (A.T.P., unpublished data). However, with other human sera neutralizing antibody to a

particular isolate could not be removed with the homologous peptide, an indication that neutralizing epitopes other than RP135 are present. These neutralizing epitopes may be masked or invisible on gp160 when presented as a subunit immunogen but may be recognized as smaller fragments or synthetic peptides, as reported by others (23, 24). During natural infection determinants may arise after association of the HIV envelope with other molecules that can elicit antibodies of broad cross-neutralizing activity.

Our data show that, at least with the two virus isolates studied (IIIB and RF), the neutralizing determinant is defined by ≈10 amino acids located at the tip of the disulfide loop. Our data with hybrid peptides RP138 and RP140 indicate that the neutralizing epitopes in the IIIB and RF isolates do not occur at the same place relative to the Gly-Pro-Gly tripeptide, and it is likely that neutralizing antibodies against different HIV isolates will bind to different positions at the tip of this loop. All data accumulated to date, however, support the notion that an antibody bound to the RP135 determinant will neutralize virus infectivity and inhibit fusion of infected cells.

It is significant that antibodies bound to the RP135 determinant can both neutralize infectivity of free virus particles and prevent fusion of virus-infected cells. Because it is not known whether the predominant route of initial virus infection in humans is by cell-free or cell-associated virus, it is important that any vaccine based on the RP135 determinant be able to block both infectious pathways. Antibodies bound to the RP135 determinant do not block binding of gp120 to CD4, an indication that they prevent infection by interfering with a post-binding event in the infection pathway (17, 18). Because RP135 antibodies bind to a different site in gp120 than does CD4, antibodies to the RP135 determinant do not have to compete for gp120 with CD4 that has a high association constant for the external envelope (3×10^9) (4).

To develop a vaccine for HIV by using the RP135 determinant, the type specificity of the antibody response to this determinant must be overcome. One approach to this problem is to immunize with a mixture of peptides consisting of the RP135 determinant from several different isolates. Another approach is to immunize with a mixture of hybrid peptides. To accomplish this goal, HIV-1 strains must be grouped into serotypes based on the sequence of the RP135 determinant. Because the Gly-Pro-Gly is highly conserved, it may serve as an anchor around which to design such hybrid peptides. For example, hybrid peptides could be constructed with the amino acid sequence of serotype A on the left of the Gly-Pro-Gly and with serotype B on the right. Such a peptide should elicit antibodies able to neutralize members of both serotype classes in the same manner as antibodies elicited by RP138 and RP73, which are hybrids of IIIB and RF, can neutralize these two isolates. If defined serotype classes can be shown to exist and their number is not prohibitive, the use of mixtures of the RP135-neutralizing epitope may be an important constituent of vaccine strategies.

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