JOURNAL OF VIROLOGY, Apr. 1995, p. 2271–2278 Vol. 69, No. 4
0022-538X/95/$04.00+0

Characterization of Neutralization Epitopes in the V2 Region of Human Immunodeficiency Virus Type 1 gp120: Role of Glycosylation in the Correct Folding of the V1/V2 Domain

Z. WU,[1] S. C. KAYMAN,[1] W. HONNEN,[1] K. REVESZ,[1] H. CHEN,[1] S. VIJH-WARRIER,[1] S. A. TILLEY,[1] J. McKEATING,[2] C. SHOTTON,[3] AND A. PINTER[1]*

Public Health Research Institute, New York, New York 10016,[1] and Microbiology Department, University of Reading, Reading RG6 2AJ,[2] and Hybridoma Unit, Institute of Cancer Research, Sutton SM2 5NG,[3] United Kingdom

Received 12 October 1994/Accepted 6 January 1995

A number of monoclonal antibodies (MAbs) with various levels of neutralizing activity that recognize epitopes in the V1/V2 domain of LAI-related gp120s have been described. These include rodent antibodies directed against linear and conformational epitopes and a chimpanzee MAb, C108G, with extremely potent neutralizing activity directed against a glycan-dependent epitope. A fusion glycoprotein expression system that expressed the isolated V1/V2 domain of gp120 in native form was used to analyze the structural characteristics of these epitopes. A number of MAbs (C108G, G3-4, 684-238, SC258, 11/68b, 38/66a, 38/66c, 38/62c, and CRA3) that did not bind with high affinity to peptides immunoprecipitated a fusion glycoprotein expressing the V1/V2 domain of HXB2 gp120 in the absence of other human immunodeficiency virus sequences, establishing that their epitopes were fully specified within this region. Biochemical analyses indicated that in the majority of V1/V2 fusion molecules only five of the six glycosylation signals in the V1/V2 domain were utilized, and the glycoforms were found to be differentially recognized by particular MAbs. Both C108G and MAbs directed against conformational epitopes reacted with large fractions of the fully glycosylated molecules but with only small fractions of the incompletely glycosylated molecules. Mutational analysis of the V1 and V2 glycosylation signals indicated that in most cases the unutilized site was located either at position 156 or at position 160, suggesting the occurrence of competition for glycan addition at these neighboring positions. Mutation of glycosylation site 160 destroyed the C108G epitope but increased the fraction of the molecules that presented the conformational epitopes, while mutation of the highly conserved glycosylation site at position 156 greatly diminished the expression of the conformational epitopes and increased expression of the C108G epitope. Similar heterogeneity in glycosylation was also observed when the HXB2 V1/V2 fusion glycoprotein was expressed without most of the gp70 carrier protein, and thus, this appeared to be an intrinsic property of the V1/V2 domain. Heterogeneity in expression of conformational and glycan-dependent epitopes was also observed for the natural viral *env* precursor, gPr160, but not for gp120. These results suggested that the closely spaced glycosylation sites 156 and 160 are often alternatively utilized and that the pattern of glycosylation at these positions affects the formation of the conformational structures needed for both expression of native epitopes in this region and processing of gPr160 to mature *env* products.

The human immunodeficiency virus type 1 (HIV-1) surface glycoprotein, gp120, is responsible for binding of the virus to its cellular receptor, the cell surface CD4 protein, and may play additional roles in the subsequent fusion events that lead to viral penetration. Most of the neutralizing antibody response to HIV-1 infection is directed against epitopes on gp120 (10, 15, 26, 29, 34), and thus, efforts to develop a protective vaccine have centered on gp120. Three major clusters of epitopes in gp120 that efficiently mediate viral neutralization have been identified. The V3 domain of gp120 has long been established as the principal neutralizing determinant of HIV-1 (reviewed in reference 18). Anti-V3 antibodies are believed to neutralize virus by inhibition of a late step in virus entry, since they do not interfere with gp120 binding to CD4 but do block HIV-induced syncytium formation (27, 28). A second group of neutralizing antibodies inhibit gp120 binding to CD4 (8, 17, 22, 25, 32). Potent monoclonal antibodies (MAbs) of this type recognize a cluster of highly conformational epitopes that are sensitive to mutations in multiple domains of gp120 and appear to overlap the binding site for CD4 (31). A third cluster of neutralizing epitopes involve the V2 domain (3, 16, 19, 33).

The V2 domain is part of a complex disulfide-bonded arm of gp120 that also includes the V1 region (14). Deletion of the V1/V2 domain has been shown to result in noninfectious virus (37), and point mutations in V2 have been shown to interfere with syncytium formation (30). Several studies have shown that this region cooperates with the V3 domain in determining the cellular tropism of HIV-1 (6, 12, 35). MAbs with virus-neutralizing activity were mapped to the V2 domain either by virtue of their binding to peptides corresponding to V2 sequences (3, 16, 33), by competition assays with previously mapped anti-V2 MAbs (7, 19, 33), or by mutational analyses (16, 19, 30). The conformational epitopes of two MAbs were more definitively assigned to the V1/V2 region by demonstrating MAb reactivity with chimeric glycoproteins consisting of the $V1/V2_{HXB2}$ domain fused to the N-terminal domain of the murine leukemia virus *env* protein, gp70 (16). C108G, a chimpanzee MAb with

* Corresponding author. Mailing address: Laboratory of Retroviral Biology, Public Health Research Institute, 455 First Ave., New York, NY 10016. Phone: (212) 578-0879. Fax: (212) 578-0804. Electronic mail address: pinter@phri.nyu.edu.

TABLE 1. Properties of MAbs used for the mapping of V1/V2 epitopes[a]

MAb (epitope)	Peptide epitope	Binding to:		Strain specificity	Reference(s)
		Denatured gp120	Deglycosylated gp120		
10/76b (V2)	162–170	+	+	HXB10	16
11/4c (V2)	162–170	+	+	HXB10	16
11/4b (V2)	162–170	+	+	HXB10	16
38/74 (V2)	/	+	/	HXB2	UD
38/12b (V2)	172–191	/	+	HXB2, MN, SF162	UD
38/60b (V2)	172–191	/	+	HXB2	UD
11/41e (V2)	162–170	/	+	HXB10	16
BAT085 (V2)	170–180	+	+	HXB2	3, 19
110.B (V1/V2)		−	/	BH10	19
684-238 (V1/V2)		−	+	BH10, MN, NL4-3	19
SC258 (V1/V2)		−	+	BH10, NL4-3, MN, RF, JR-CSF, ALA	19
11/68b (V1/V2)		−	+	HXB10	16
38/66a (V1/V2)		−	+	HXB2	UD
38/66c (V1/V2)		−	+	HXB2	UD
38/62c (V1/V2)	/	/	+	HXB2	UD
CRA3 (V1/V2)		−	−	HXB2	16, 19
G3-4 (V1/V2)	162–181[b]	−	−	BH10, RF, MN	3, 7, 19, 30
C108G (V2)	162–169[c]	+	−	LAI, Bal, HXB2	33, UD

[a] +, reactive; −, not reactive; /, not determined; UD, unpublished data.
[b] Weak binding (19).
[c] C108G bound to peptide 162–169 with an affinity at least 2,000-fold less than that with which it bound to recombinant gp160 (33).

particularly potent neutralizing activity, recognizes a glycan-dependent epitope that does not require intact disulfide bonds for high-affinity binding; this epitope has been assigned to V2 on the basis of binding competition studies and detection of low-affinity binding to V2 peptides (33). A fraction of HIV-infected humans also possess antibodies to epitopes in V1/V2, as evidenced by binding assays with peptides (16) or binding competition with MAbs (19). Studies with fusion glycoproteins expressing the V1/V2 region from multiple isolates demonstrated cross-reactive antibodies to this domain in many HIV-positive human sera (11), while peptide-binding assays using synthetic peptides identified a type-specific neutralizing response to the V1 domain in sera from two laboratory workers infected with HIV_{IIIB} (21). A human MAb directed against a conserved, conformational V2 epitope has been recently described (5).

In the present study, fusion proteins expressing the isolated V1/V2 region of gp120 were used to characterize a panel of MAbs believed to recognize epitopes in this region. The epitopes of these MAbs were shown to be fully specified by the V1/V2 region, and the distribution of these epitopes among distinct virus isolates was determined. Specific N-linked glycans were shown to regulate folding of the V1/V2 domain into the conformation recognized by MAbs against disulfide bond-dependent epitopes in this region, and the epitopes recognized by two MAbs were shown to require the presence of particular glycans. Viral gPr160 was also shown to be heterogeneous for V1/V2 epitopes, and the correctly folded and glycosylated V1/V2 domain was found to be necessary for processing of gPr160 into mature *env* proteins.

MATERIALS AND METHODS

MAbs and polyclonal sera. The following MAbs (Table 1) were used for the characterization of V1/V2 epitopes. Rat MAbs 10/76b, 11/4c, 11/41e, 11/4b, 11/68b, 38/12b, 38/60b, 38/74, 38/66a, 38/66c, and 38/62c were raised against BH10 gp120. 10/76b, 11/4c, 11/41e, and 11/4b have been shown to react with synthetic peptides bearing the sequence from position 162 to 170 at the N terminus of the V2 loop (16); 38/12b and 38/60b reacted with a peptide with the sequence from position 172 to 191 (data not shown), and 11/68b recognized a conformational epitope mapped to the V1/V2 domain by reactivity with V1/V2 fusion proteins and by sensitivity to point mutations in V2 (16). Murine MAbs 684-238 and SC258, which have been shown to recognize conformational epitopes sensitive to point mutations in the V2 region of gp120 (19), were provided by G. Robey of Abbott Laboratories. CRA3 (ADP-324), a murine MAb raised against recombinant BH10 gp120 by Mark Page and colleagues that is sensitive to point mutations in V2 (19), was obtained from the United Kingdom Medical Research Council ADP Program. G3-4 (7), a murine MAb that recognizes a conformational epitope that is sensitive to point mutations in V2 (19, 30) was provided by Tanox, Inc. C108G, an extremely potent neutralizing MAb with high affinity for native gp120, was isolated from a chimpanzee infected with IIIB virus and subsequently immunized with $rgp160_{MN}$. This MAb reacts with reduced but not deglycosylated gp120 and shows low-affinity binding to synthetic peptides that include residues 162 to 169 of HIV_{HXB2} (33). Goat antiserum to Rauscher gp70 was obtained from Quality Biotech, Camden, N.J. Polyclonal chimpanzee antisera from animals experimentally infected with $HIV\text{-}1_{IIIB}$ (4) were provided by E. Muchmore at the Laboratory of Experimental Medicine and Surgery in Primates (LEMSIP), Tuxedo, N.Y.

Construction of vectors and cell lines. Construction of vectors and cell lines used to express gp(1-263):V1/V2 fusion proteins has been described elsewhere (11) except for the SF162 V1/V2 gene fragment that was taken from p162/3′4.5 (1), provided by Cecelia Cheng-Mayer. Fusion proteins containing mutated glycosylation sites were generated from mutant viral genomes (13), provided by Tun-Hou Lee. These all contain Asn-to-Gln mutations at the signal asparagine residues. Many of the fusion proteins were engineered to contain a His-6 affinity tag near the N terminus of the gp70 sequence to allow their purification on Ni^{2+}-nitrilotriacetic acid affinity columns (11). The vector for expression of the gp(1-20):V1/V2 fusion glycoprotein was constructed by PCR overlap mutagenesis (9). It incorporates the His-6 affinity tag, and the glycosylation signal normally present at residue 12 of gp70 was removed by mutating Thr-14 to Val. Stably expressing NIH 3T3 cell lines were maintained in supplemented Dulbecco modified Eagle medium containing 10% bovine serum (Whittaker). Whenever available, cloned cell lines were used for radioactive labelling experiments. In some cases uncloned cell cultures that contained a contaminating murine leukemia virus gp70 detected in precipitations with anti-gp70 serum were used.

Metabolic radiolabelling of cells. Proteins were metabolically labelled at 37°C for 18 h with ^{35}S-cysteine (ICN) at 100 μCi/ml of culture medium. Proteins containing only endo-B-*N*-acetylglucosaminidase (endo H)-sensitive, high-mannose oligosaccharides were prepared by labelling in the presence of 2 mM 1-deoxymannojirimycin (MMN) (Bohringer Mannheim). MMN inhibits alpha-mannosidase I, which is required for trimming of Man9-containing N-linked glycans (2).

Endo H deglycosylation. Supernatant media containing fusion glycoproteins radioactively labelled in the presence of MMN were deglycosylated by digestion with 10^5 U of recombinant endo H (endo H_f [New England BioLab]) per ml at 37°C for 60 min. This was sufficient to completely remove all N-linked glycans. In

order to minimize any possible perturbation to protein conformation, deglycosylation of native proteins was carried out in the absence of detergents and denaturants. Deglycosylation ladders to count the number of N-linked glycans carried by fusion glycoproteins were generated with serial fivefold dilutions of endo H_f.

Immunoassays. Radioimmunoprecipitation and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses were performed essentially as previously described (23). MAbs and polyclonal antisera were used at predetermined saturating concentrations. For experiments using deglycosylated proteins, antibody binding reactions were carried out at 4°C overnight to minimize proteolysis. All samples were analyzed by 10 or 15% linear SDS-PAGE under reducing conditions. Enzyme-linked immunosorbent assays (ELISAs) were performed with appropriate alkaline phosphatase-coupled secondary antibodies and wash buffers containing 0.05% Tween by using fusion glycoproteins that had been partially purified on Ni^{2+}-nitrilotriacetic acid columns as previously described (11).

Sequential immunoprecipitations. ^{35}S-cysteine radiolabelled V1/V2 fusion protein produced in the presence of MMN was immunoprecipitated three times with MAbs and then with polyclonal anti-gp70 serum at a 1:100 dilution. For each precipitation, MAbs and polyclonal sera were incubated with the culture supernatant at 37°C for 60 min followed by addition of staph A (Pansorbin; Calbiochem). For the rat MAbs, rabbit anti-rat immunoglobulin G or M (Zymed) was added at 10 μg/ml, and the mixtures incubated at 37°C for 30 min prior to addition of staph A. Sequential immunoprecipitations of intracellular HIV-1 *env* proteins were performed by treating cell lysates of HIV-1_{HXB2}-infected H9 cells labelled with ^{35}S-cysteine for 30 min or 4 h with the MAb followed by staph A. This was repeated three times with fresh addition of the MAb and once just with addition of staph A. The lysates were then immunoprecipitated twice with a chimp polyclonal antiserum from an HIV_{IIIB}-infected animal to detect residual antigen.

RESULTS

The V1/V2 region of gp120 contains linear, conformational, and glycan-dependent epitopes. The epitopes recognized by a number of MAbs that react with gp120 from HIV_{IIIB} but show little or no reactivity with synthetic peptides have been previously assigned to the V2 region, in most cases on the basis of binding competition studies and analyses with gp120 proteins containing point mutations (16, 19, 30, 33). These approaches do not eliminate the possibility of involvement of residues from other regions of gp120 in the presentation of these epitopes, and, in fact, gp120 binding by some of these MAbs (11/68b, CRA3, CRA4, and G3-4) was also affected by mutations in C1 and/or C4. Therefore, a fusion glycoprotein, gp(1-263):–V1/$V2_{HXB2}$, expressing residues 116 to 209 (numbered from the N terminus of mature gp120), encompassing the V1/V2 domain of HXB2 gp120 in the absence of other gp120 regions, was used to determine whether the V1/V2 region by itself contained sufficient information for expression of these epitopes. This fusion glycoprotein has been used previously to localize the epitopes of 11/68b and CRA4 to the V1/V2 domain (16). Radioimmunoprecipitations of gp(1-263):V1/$V2_{HXB2}$ are shown in Fig. 1 (upper panel). MAbs 10/76b and 38/12b, which react with linear V2 region epitopes within residues 162 to 171 (16) and 172 to 191 (data not shown), respectively, served as positive controls in these experiments. The V1/V2 fusion protein was precipitated by a number of MAbs recognizing disulfide bond-dependent epitopes (684-238, SC258, G3-4, 11/68b, 38/66a, and CRA3) and by C108G, a glycan-dependent MAb that shows low-affinity binding to V2 peptides (33). The differences between the efficiencies of precipitation by these MAbs were due in large part to the presence of multiple glycoforms and conformers, only some of which expressed particular epitopes (see below). These data confirmed the localization of the linear, conformational, and glycan-dependent epitopes recognized by these MAbs to the V1/V2 domain.

The V1/V2 domain contains epitopes that are conserved among independent HIV-1 isolates. All of the MAbs used in these studies were generated by immunization of animals with purified HIV *env* protein derived from LAI-related clones, except for the chimpanzee MAb C108G, which was isolated from an animal experimentally infected with HIV_{IIIB}. Whereas the V1/V2 domain is a highly variable region of gp120 and many MAbs directed to this region are type specific, cross-reactivity has been reported for G3-4, SC258, and other MAbs not included in this study that recognize linear epitopes in V2 (3, 7, 19). To determine the distribution of the epitopes recognized by the antibodies included in this study, six additional gp(1-263):V1/V2 fusion glycoproteins were prepared from the following clade B sequences: NL4-3 (a recombinant genome with an LAI-related *env* gene that differs slightly from HXB2 in the V1/V2 region); MN and SF2 (two other T-cell-tropic isolates); and JR-CSF, JR-FL, and SF162 (three primary isolate viruses, the latter two of which are macrophage tropic). The seven different V1/V2 fusion proteins were purified on Ni^{2+}-chelating resin via their His-6 affinity tag, and their immunoreactivities were determined by ELISA. Reactivity with the hyperimmune anti-gp70 serum was used to normalize the amount of each fusion protein used in these assays. All the MAbs recognized gp(1-263):V1/$V2_{HXB2}$, and there was generally good agreement between the intensity of the ELISA signal and the efficiency of immunoprecipitation for a given MAb. MAbs that recognize disulfide bond-dependent epitopes were generally more cross-reactive than other MAbs. SC258 was the most broadly cross-reactive, reacting strongly with six of the seven gp(1-263):V1/V2 fusion glycoproteins, while G3-4 recognized five constructs, CRA3 and 684-238 recognized four constructs, and 38/66c recognized two constructs (Table 2). Although 684-238 did not recognize gp(1-263):V1/$V2_{MN}$, which incorporated the sequence from the MN-ST clone, this MAb did recognize gp120 from the uncloned culture of HIV_{MN} currently in use in this laboratory (data not shown). 11/68b was the only MAb with a disulfide bond-dependent epitope that was strictly type specific among these gp(1-263):V1/V2 fusion glycoproteins, while the low level of reactivity obtained with 38/62a precluded evaluation of its cross-reactivity. By the criteria of cross-reactivity, the disulfide bond-dependent conformational epitopes in the V1/V2 region fell into five distinct classes, with most identified by a single MAb. Of MAbs recognizing linear epitopes, only 38/12b, which bound to three of the gp(1-263):V1/V2 fusion glycoproteins, was at all cross-reactive. Surprisingly, gp(1-263):V1/$V2_{NL43}$, which carries a sequence that was the most similar to the HXB2 sequence, was recognized by fewer MAbs than was JR-CSF or JR-FL, two primary viruses whose amino acid sequences in the V1/V2 region are much more distantly related to that of

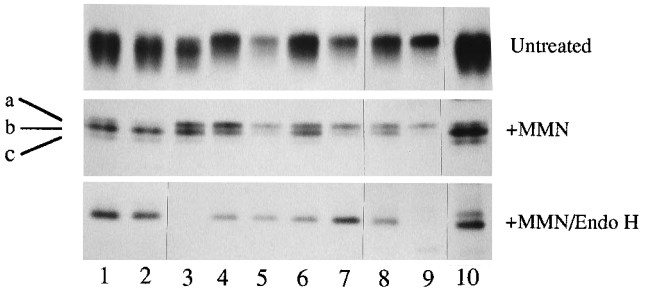

FIG. 1. SDS-PAGE analysis of the reactivity of MAbs with native and deglycosylated gp(1-263):V1/$V2_{HXB2}$ fusion glycoprotein. NIH 3T3 cells expressing the fusion protein were metabolically labelled overnight with ^{35}S-cysteine with or without the presence of 2 mM MMN, as indicated. Supernatants of the MMN-treated cells were either directly precipitated with MAbs or deglycosylated with endo H under native conditions and then precipitated. The precipitates were analyzed on SDS gels after reduction. Lanes: 1, 10/76b; 2, 38/12b; 3, C108G; 4, 11/68b; 5, 38/66a; 6, 684-238; 7, SC258; 8, G3-4; 9, CRA3; 10, anti-gp70 polyclonal serum.

TABLE 2. Cross-reactivities of anti-V1/V2 MAbs

Antibody[a]	A_{405}[b]						
	HXB2	JR-CSF	NL4-3	JR-FL	SF162	MN	SF2
SC258	**0.918**	**1.335**	**1.186**	**0.850**	**1.025**	**1.219**	0.043
G3-4	**1.051**	**2.366**	**1.915**	**1.518**	**2.021**	0.015	0.050
CRA3	**0.316**	**0.160**	**0.674**	**0.485**	0.071	0.005	0.013
684-238	**1.338**	**1.118**	**1.635**	**1.147**	0.030	0.055	0.055
38/12b	**1.225**	0.012	−0.015	−0.013	**0.945**	**0.544**	−0.005
38/66a	**0.424**	**0.092**	0.036	−0.003	0.043	0.000	−0.010
10/76b	**1.350**	−0.003	−0.057	−0.046	−0.035	−0.047	−0.052
11/4c	**1.069**	0.033	−0.003	0.000	0.000	−0.002	−0.004
C108G	**0.672**	0.007	0.003	0.004	0.005	0.000	0.002
11/68b	**0.513**	0.025	0.020	0.012	0.007	0.005	0.005
Anti-gp70	**1.127**	**1.371**	**1.398**	**0.894**	**0.827**	**1.575**	**0.967**

[a] Assays were performed with purified antibodies used at 5 μg/ml except for 38/12b, 11/68b, and CRA3, for which culture supernatants were used at a 1/10 or 1/5 dilution or undiluted, respectively. The hyperimmune anti-gp70 serum was used at a 1/400 dilution.

[b] ELISAs were performed on partially purified gp(1-263):V1/V2 fusion glycoproteins expressing sequences matching those of the indicated HIV-1 isolates. The data are averages from duplicate samples after subtraction of background values, which were between 0.150 and 0.350, depending on the antibody. A_{405} values greater than or equal to the average absolute value of all negative values plus 3 standard deviations [0.0205 + 3(0.0192) = 0.078] were taken to indicate positive reactivity and are boldfaced.

HXB2. This suggested that a subtle relationship exists between the primary sequence in this region and immunoreactivity. None of the MAbs recognized gp(1-263):V1/$V2_{SF2}$, a finding similar to what was observed for a panel of human sera containing cross-reactive anti-V1/V2 activity (11). These observations suggested that the SF2 V1/V2 sequence is immunologically distinct from the others.

N-linked glycans are important to formation, and in some cases, maintenance, of nonlinear V1/V2 epitopes. Additional information about the structural requirements of the epitopes recognized by these MAbs was obtained by examining their reactivities with gp(1-263):V1/$V2_{HXB2}$ synthesized in the presence of MMN, an inhibitor of glycan processing. Glycoproteins made in this way are more homogeneous because all of their N-linked glycans are retained in the high-mannose form. The use of MMN allowed resolution of the broad band of gp(1-263):V1/$V2_{HXB2}$ obtained in the absence of the inhibitor (Fig. 1, upper panel) into three major components (a, b, and c), whose differences in mobility corresponded to differences in molecular mass of about 2 kDa (Fig. 1, middle panel). Digestion with endo H, an endoglycosidase that removes high-mannose N-linked glycans, converted these components into a single band (Fig. 1, lower panel), indicating that they represent gp(1-263):V1/$V2_{HXB2}$ glycoforms containing different numbers of N-linked glycans. Limited digestion with endo H generated a ladder of nine discrete bands (data not shown), indicating that the largest band (band a in Fig. 1) carried eight N-linked glycans. This corresponded to fully glycosylated material, since gp(1-263):V1/$V2_{HXB2}$ contains six signals for N-linked glycan addition in the HIV sequence and two signals in the gp70 carrier domain sequence. As indicated by the relative band intensity of samples immunoprecipitated with the hyperimmune anti-gp70 serum (lane 10), the majority of secreted gp(1-263):V1/$V2_{HXB2}$ carried only seven N-linked glycans (band b, the −1 glycoform). MAbs that recognize linear epitopes in V2 (lanes 1 and 2) preferentially recognized the −1 glycoform, suggesting that these epitopes may be less accessible in the fully glycosylated molecules. All of the MAbs against nonlinear epitopes (lanes 3 to 9) reacted as well or preferentially with the largest band, representing fully glycosylated fusion protein. This suggested that N-linked glycans were required either for the formation or for maintenance of these epitopes.

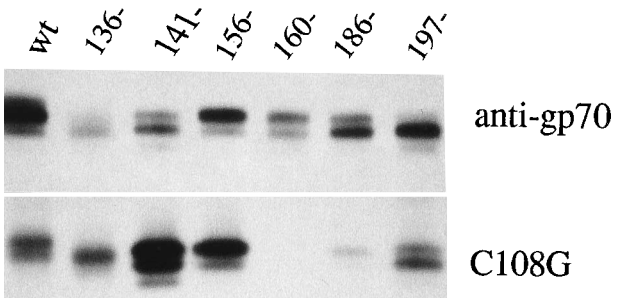

FIG. 2. Glycan dependence of antibody reactivity with the V1/V2 fusion glycoproteins. NIH 3T3 cells expressing the gp(1-263):V1/$V2_{HXB2}$ fusion proteins in which individual N-linked glycosylation sites had been mutated were metabolically labelled with ^{35}S-cysteine in the presence of 2 mM MMN, and supernatants were immunoprecipitated with the indicated MAbs. wt, wild-type fusion glycoprotein.

The possible requirement of N-linked glycans for maintenance of the nonlinear epitopes recognized by these MAbs was explored by using enzymatically deglycosylated V1/V2 fusion protein. The high-mannose glycans present on the proteins made in the presence of MMN were readily cleaved by endo H under nondenaturing conditions. Most of the MAbs that recognize nonlinear epitopes in the V1/V2 region were able to precipitate endo H-digested gp(1-263):V1/$V2_{HXB2}$ (Fig. 1, lower panel). Therefore, these epitopes did not require N-linked glycans, beyond the single Glc–*N*-acetyl that remained at each glycan attachment site after endo H digestion. Two MAbs failed to react with endo H-digested gp(1-263):V1/$V2_{HXB2}$: C108G, which has been reported to recognize a glycan-dependent epitope (33), and CRA-3, for which glycan dependence has not been previously described (19). G3-4 binding to gp120 was previously reported to be sensitive to digestion with endo H (7), but in this study it bound gp(1-263):V1/$V2_{HXB2}$ from which all N-linked glycans had been removed with endo H.

To evaluate the contribution of specific glycans to the formation and maintenance of nonlinear epitopes in V1/V2, fusion glycoproteins in which single N-linked glycosylation sites had been removed by point mutation were prepared and labelled in the presence of MMN. Each of the mutant proteins and wild-type gp(1-263):V1/$V2_{HXB2}$ were immunoprecipitated with anti-gp70 serum and analyzed by SDS-PAGE to allow resolution of glycoforms (Fig. 2, upper panel). In all cases, the largest of the glycoforms for the mutant proteins comigrated with the −1 glycoform of the wild-type protein, an observation consistent with the loss of one N-linked glycan on the fully glycosylated mutant proteins. Four of the mutant proteins ($gs136^-$, $gs141^-$, $gs186^-$, and $gs197^-$) had a distribution of material among glycoforms that was similar to the distribution seen for wild-type protein, with the −1 glycoform being the major component. For the $gs156^-$ and $gs160^-$ proteins, however, the fully glycosylated material was the major component, suggesting that underutilization of these two glycosylation sites was the primary cause of heterogeneity in the other gp(1-263):V1/$V2_{HXB2}$ proteins. The efficient formation of fully glycosylated isoforms for the $gs156^-$ and $gs160^-$ mutants but not for the other glycosylation site mutants and wild-type proteins suggested that in the gp(1-263):V1/$V2_{HXB2}$ fusion glycoproteins these two sites compete for glycan addition.

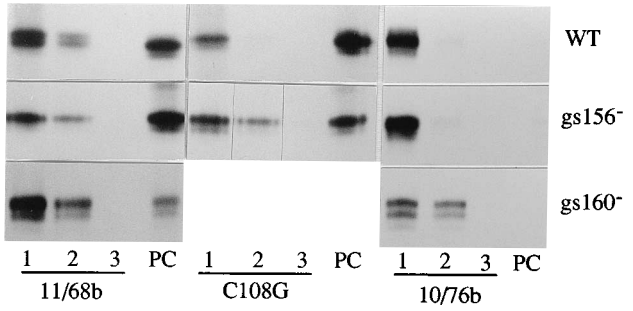

FIG. 3. Sequential immunoprecipitation of gp(1-263):V1/V2$_{HXB2}$ fusion glycoproteins by MAbs. Cell culture supernatants containing the MMN-treated, ^{35}S-cysteine labelled wild-type (WT) fusion protein, gs156$^-$ mutant fusion protein, and gs160$^-$ mutant fusion protein were immunoprecipitated sequentially three times with MAbs and then once with polyclonal anti-gp70 serum and analyzed on SDS gels under reducing conditions. Lanes: 1 to 3, sequential precipitation with MAbs; PC, anti-gp70 polyclonal serum. Sequential precipitations for C108G were not performed for the gs160$^-$ mutant, because of the lack of reactivity of this mutant with C108G.

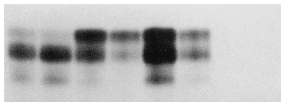

FIG. 4. Multiple glycoforms are also present in the V1/V2 fusion glycoprotein expressed in the absence of most of the murine leukemia virus *env* carrier domain. NIH 3T3 cells expressing the fusion glycoprotein were metabolically labelled overnight with ^{35}S-cysteine in the presence of 2 mM MMN, and the supernatant was precipitated with MAbs and analyzed on 15% SDS gels under reducing conditions. Lanes: 1, 11/4c; 2, 38/12b; 3, 11/68b; 4, 38/66a; 5, 684-238; 6, G3-4; 7, 38.1a (an anti-C4 rat MAb) (negative control); 8, 2M3 (an anti-V3 mouse MAb) (negative control).

The reactivity of each of these glycosylation site mutants with several MAbs was determined by radioimmunoprecipitation. The ability of MAbs that recognize linear epitopes to precipitate the fusion glycoproteins was not affected by the absence of any of the glycans (data not shown). Reactivity with C108G was completely abolished by mutation of gs160 but not by mutation of any of the other glycosylation signals (Fig. 2). MAbs directed against conformational epitopes recognized all of the glycosylation site mutants to different extents. As illustrated for SC258 in Fig. 2, these MAbs generally showed reduced reactivity for the gs156$^-$ and the gs197$^-$ proteins but similar or enhanced reactivity for the gs160$^-$ protein. CRA3 was unique in that in addition to reduced reactivity with the gs156$^-$ protein its reactivity was abolished by mutation of glycosylation site 197 (data not shown). It has been previously reported that this antibody recognizes a conformation-dependent epitope (19), and the present data indicate that the CRA3 epitope is also glycan dependent.

Conformational heterogeneity of the V1/V2 domain is affected by glycosylation at the gs156 and gs160 sites. In view of the fact that MAbs that recognize conformational epitopes reacted with completely deglycosylated V1/V2 fusion proteins (Fig. 1), the reduction of reactivity of these MAbs with the gs156$^-$ mutant was of interest. This could be accounted for either by a reduced affinity of these antibodies for the mutant protein or by an effect of the gs156 glycan on the fraction of material that folds into the native conformation recognized by these MAbs. To determine which of these explanations was correct, all material immunoreactive with a given MAb was depleted by several sequential immunoprecipitations with that MAb, and then immunoprecipitation with a broadly reactive polyclonal anti-gp70 serum was used to quantitate the amount of residual material. Wild-type, gs156$^-$, and gs160$^-$ fusion glycoproteins made in the presence of MMN were used in this experiment (Fig. 3).

In all cases, most of the material reactive with a MAb was brought down in the first precipitation, and by the third precipitation there was no longer any fusion glycoprotein left that the MAb could recognize, even on longer film exposures than that shown. This demonstrated that the MAbs were present at close to saturating levels and that their affinities for the immunoreactive components of the wild-type and mutant proteins were similar. MAb 10/76b, which reacts with a linear V2 epitope, precipitated essentially 100% of all glycoforms of each of the three fusion glycoproteins. MAb 11/68b, directed against a conformational epitope, precipitated essentially all of the fully glycosylated wild-type molecules (the upper band of the doublet precipitated by this MAb) but only a fraction of the −1 glycoforms. Similar results were obtained for a second MAb directed against a different conformational V2 epitope, 38/66a (data not shown). C108G also precipitated most of the fully glycosylated molecules but only a small fraction of the incompletely glycosylated molecules. For both 11/68b and C108G, the residual wild-type antigen recognized by the polyclonal antisera corresponded mostly to the −1 glycoform. These results indicated that the fully glycosylated wild-type fusion protein was homogeneous with respect to the epitopes of these MAbs, whereas the incompletely glycosylated molecules were heterogeneous.

For the gs156$^-$ mutant, a relatively small fraction of the fully glycosylated molecules was recognized by 11/68b and other MAbs to conformational epitopes, even in the presence of saturating levels of antibody. This indicated that the majority of these molecules did not express the conformational epitopes recognized by these MAbs, presumably because of incorrect folding of these proteins. A different effect was observed for the gs160$^-$ mutation. 11/68b precipitated the large majority of both the fully glycosylated and the −1 glycoforms of this mutant protein, showing that the absence of the gs160 glycan caused an increase in the fraction of molecules that folded correctly to form the conformational epitopes recognized by anti-V2 MAbs. This may be directly due to the presence of the 156 glycan on an increased fraction of the mutant fusion protein. If this is the case, then the efficient recognition of the −1 glycoform of the gs160$^-$ protein presumably means that these molecules were missing a glycan at a site other than position 156; alternatively, it is possible that in the absence of the 160 glycan the correct folding of these molecules did not require the 156 glycan. C108G immunoprecipitated a larger fraction of the gs156$^-$ fusion protein than that of the wild-type protein, suggesting that a larger fraction of these mutant molecules carried a glycan at position 160. However, C108G still recognized only about half of the fully glycosylated gs156$^-$ protein, indicating that its epitope had structural requirements beyond the primary sequence and the gs160 glycan.

To rule out the possibility that the conformational and glycosylation heterogeneity described above was an artifact due to the presence of the large, cysteine-containing carrier domain of the gp70 region of the fusion glycoprotein, the V1/V2 region of HXB2 gp120 was expressed in a fusion protein by utilizing only the first 20 amino acids of gp70 (plus the His-6 residue affinity tag) as a carrier. This fragment of gp70 is free of cysteine residues, and the glycosylation signal normally present at residue 12 of gp70 was removed from this construct by introduction of a point mutation. As shown in Fig. 4, this fusion glycoprotein, gp(1-20):V1/V2$_{HXB2}$, yielded a triplet pattern of

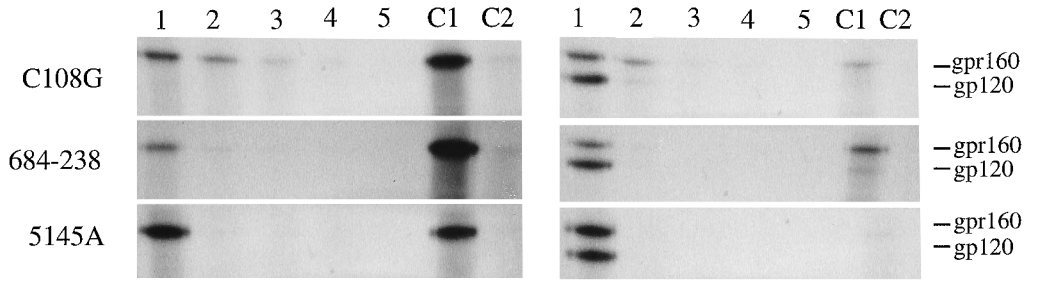

FIG. 5. Sequential immunoprecipitations of lysates of HIV-infected cells. H9 cells infected with HIV-1_{HXB2} were metabolically labelled with ^{35}S-cysteine for either 30 min or 4 h, and the cells were solubilized with a radioimmunoprecipitation buffer containing 20 mM Tris (pH 7.4), 500 mM NaCl, 5 mM EDTA, and 0.5% Nonidet P-40. The cell lysates were precipitated sequentially four times with MAbs and then once with staph A alone and twice with HIV-positive chimpanzee serum and analyzed on SDS gels under reducing conditions. Lanes: 1 to 4, sequential precipitation with MAbs; 5, precipitation with staph A alone; C1 and C2, sequential immunoprecipitation with polyclonal HIV-positive chimpanzee serum.

glycoforms similar to that seen for the gp(1-263):V1/$V2_{HXB2}$ fusion protein and presented both linear and nonlinear V1/V2 epitopes. As was the case for the larger V1/V2 fusion proteins, the −1 glycoforms were preferentially precipitated by MAbs to linear epitopes (lanes 1 and 2), while MAbs to conformational epitopes preferred the fully glycosylated molecules (lanes 3 to 6).

Correlation of V1/V2 epitope expression with processing of viral gPr160. In order to determine whether heterogeneity in expression of conformational and glycan-dependent V1/V2 epitopes also occurred for natural viral *env* proteins, sequential precipitations with C108G and 684-238 were performed on HIV-1 gp120 and gPr160 from infected cells (Fig. 5). In these experiments, four sequential precipitations with a MAb were followed by two precipitations with a broadly reactive polyclonal chimpanzee antiserum. Parallel immunoprecipitations were performed with 5145A, a human MAb directed against a highly conserved conformational epitope that overlaps the CD4 binding site (22). After a 30-min pulse-label, the major *env*-related protein detected was the precursor protein, gPr160. The newly synthesized gPr160 was clearly heterogeneous for both the C108G and the 684-238 epitopes in that these MAbs precipitated only a small fraction of the gPr160 molecules present in the cells at this time point. Less heterogeneity was detected for the CD4 binding site epitope, which was present on about half of the gPr160 molecules. After a 4-h labelling period, gPr160 and gp120 were present in cell lysates in approximately equal amounts. This more mature gPr160 remained heterogeneous in its expression of nonlinear V1/V2 epitopes: most of these molecules were precipitated by C108G, while only about half of the molecules present at this time point were recognized by 684-238. Both anti-V2 MAbs precipitated almost all of the gp120 present in the 4-h lysate, and 5145A immunoprecipitated all of the gPr160 and gp120 present at this time point. These data indicated that, as was the case for the V1/V2 fusion proteins, intracellular gPr160 in virus-infected cells was heterogeneous with respect to conformation and presumably glycosylation in the V1/V2 region and that only the fraction of gPr160 with a fully glycosylated and correctly folded V1/V2 domain was processed into gp120.

DISCUSSION

The V1/V2 domain of gp120 contains linear, conformational, and glycan-dependent epitopes. Many anti-V1/V2 MAbs possess virus-neutralizing activity, ranging from the extremely high potency of C108G with a 50% neutralization end point in the low nanograms-per-milliliter range (33) to antibodies with 50% neutralization end points in the micrograms-per-milliliter range (3, 7, 16, 19), demonstrating the presence of these V1/V2 epitopes in native gp120 and their importance in *env* protein function. The studies described in this article further define structural characteristics of a number of neutralization epitopes in the V1/V2 region of gp120, and they document a critical role for glycosylation in the proper folding of this domain.

At least six nonlinear epitopes for neutralizing MAbs in the V1/V2 domain were distinguished by their distribution among different HIV isolates, their sensitivity to disulfide bond reduction, and their glycan dependence. SC258, 684-238, 38/66a, and 11/68b each identified different disulfide bond-dependent, glycan-independent epitopes; CRA3 identified a disulfide bond-dependent epitope that requires the gs197 glycan; and C108G identified a nonlinear epitope that is not dependent on disulfide bonds but requires the glycan at position 160. Present data do not exclude the possibility that the MAbs recognizing distinct conformational epitopes recognize the same general structure within the V1/V2 region but are differentially dependent on specific residues in this structure. In general, the conformational epitopes in V1/V2 are more broadly conserved than are the linear epitopes; this is in agreement with observations that the cross-reactive anti-V1/V2 components in natural HIV-positive immune sera (11) recognize primarily disulfide bond-dependent epitopes (unpublished results). Some of these epitopes were quite broadly distributed in North American HIV-1 isolates, particularly the SC258 epitope that was found in six of the seven sequences examined. Cross-reactivity data reported in previous studies for SC258 (19), CRA-3 (19), and G3-4 (7) are consistent with the results reported here. Earlier work failed to detect 684-238 reactivity with JR-CSF gp120 (19), although reactivity with gp(1-263):V1/$V2_{JR-CSF}$ was detected here.

The most potent linear epitope in V1/V2 currently identified is located between amino acids 162 and 171 (STSIRGKVQK), a relatively conserved sequence present at the N terminus of the V2 region. A group of MAbs that react with synthetic peptides containing this sequence were derived from rats immunized with BH10 rgp120 (16); these antibodies are type specific for a subset of LAI-related viruses. C108G, which binds with low affinity to the 162-169 peptide (33), has similar type specificity. Neither the rat MAbs nor C108G recognized V1/V2 fusion protein from the related clone, NL4-3, which differs in this region only by substitution of a D for G-167, and these MAbs were also nonreactive with the equivalent peptides containing this substitution (33; unpublished results). Other than IIIB-derived isolates, only one published sequence, Ba-L, contains a G at position 167 (20), and C108G recognized the

Ba-L isolate. Thus, this polymorphism presumably accounts for the type specificity of these antibodies.

Other than C108G and CRA3, all of the anti-V1/V2 MAbs used in this study reacted with V1/V2 fusion glycoprotein that had been deglycosylated with endo H. Although a single Glc–*N*-acetyl remains at each glycan attachment site following endo H digestion, these MAbs also recognized all mutant fusion glycoproteins in which single glycosylation sites had been modified to prevent glycan addition. Together, these data argue that maintenance of these epitopes did not require any N-linked carbohydrate. The glycan requirement of C108G was mapped to gs160 and that of CRA3 was mapped to gs197 by using the fusion proteins carrying glycosylation site mutations. Although the high-affinity epitope for C108G does not require disulfide bonds for its maintenance either in gp120 (33) or in gp(1-263):V1/V2$_{HXB2}$ (data not shown), sequential immunoprecipitation experiments using gp(1-263):V1/V2$_{HXB2}$ proteins with uniform glycan content showed that a fraction of these molecules were not immunoprecipitated by C108G (Fig. 3), demonstrating that additional structural requirements exist for expression of the high-affinity C108G epitope. The CRA3 epitope was unique in its dependence on both the presence of N-linked glycans and intact disulfide bonds. Whereas the reactivity of G3-4 with gp120 was previously reported to be sensitive to glycan removal by endo H (7), in this study G3-4 recognized the fully deglycosylated V1/V2 fusion protein. This discrepancy may indicate that the loss of a glycan or glycans outside the V1/V2 region causes masking or destruction of the G3-4 epitope in full-length gp120.

Although not required for maintenance of most of the disulfide bond-dependent epitopes studied, glycan addition appeared to be important for the formation of these epitopes. Studies using individual glycosylation site mutant proteins indicated that glycans at positions 197 and particularly 156 promoted proper folding (Fig. 2). It was also observed that one glycosylation site was not utilized in the majority of gp(1-263):V1/V2$_{HXB2}$ fusion proteins, and gs156 and gs160 were shown to be the major contributors to this underglycosylation. This indicated that these two sites were often alternatively used, suggesting that competition occurred between these signals for recognition by the cellular glycan addition machinery (see Fig. 6). This might be due to the proximity of these two sites resulting in steric effects that suppress their simultaneous usage. Such an effect would have functional ramifications in view of the importance of the gs156 glycan for the efficient folding of the V1/V2 fusion protein into the native conformation recognized by many of the MAbs and the essential role of the gs160 glycan in the C108G epitope. gs156 is immediately adjacent to Cys-157, which is involved in the formation of a disulfide bond that is presumably required for the conformational epitopes. The model presented in Fig. 6 depicts how differential glycosylation at these two sites may affect the global structure of the V1/V2 domain. Occasionally, both gs156 and gs160 are modified, and these molecules fold correctly. Usually a glycan is added at only one of the two sites. When the 156 glycan is present Cys-157 forms the correct disulfide bond and the molecule folds properly, whereas when there is no glycan at this position most of the molecules form aberrant disulfide bonds. In this regard, it is of note that gs156 is the most highly conserved glycosylation signal in the V1/V2 domain (20), an observation consistent with its playing an important role in folding.

Heterogeneity in expression of the C108G epitope and conformational V1/V2 and CD4 binding-site epitopes was also observed for gPr160 but not gp120 (Fig. 5), suggesting that correct folding of both the V1/V2 domain and the CD4 binding-site epitopes is required for processing of gPr160 molecules into gp120. A significant fraction of gPr160 expressed the 5145A epitope but not the conformational V1/V2 epitope, indicating that the CD4 binding-site domain can be formed even in molecules with an incorrectly folded V1/V2 domain. This is consistent with information showing that deletion of V1/V2 does not prevent binding of gp120 to CD4 (24, 37). A larger fraction of newly synthesized gPr160 molecules reacted with 5145A than with MAbs to native V1/V2 epitopes, and conformational heterogeneity of the V1/V2 domain persisted in gPr160 labelled after 4 h, while that of the CD4 binding-site did not. These observations indicated that folding of the CD4 binding domain is more efficient than that of the V1/V2 domain and suggested either that the misfolding of the CD4 binding site is more readily reversible than that of the V1/V2 domain or that gPr160 with a misfolded CD4 binding site is degraded more rapidly. The heterogeneity for the C108G epitope was likely to reflect a lack of glycosylation at gs160, an irreversible defect, and thus, the rapid loss of gPr160 molecules lacking the C108G epitope suggests that such improperly glycosylated molecules are efficiently degraded. This is consistent with a previous report that <15% of gPr160 is converted to gp120 in infected cells (36) and suggests that the formation of incorrect V1/V2 structures may be a major cause of the inefficient processing of HIV-1 *env* protein. It will be of interest to analyze various forms of recombinant gp120 proteins for correct folding of the V1/V2 domain and to determine whether there is a correlation between the fraction of correctly folded molecules and the abilities of these proteins to induce neutralizing antibodies directed against native epitopes in the V1/V2 domain.

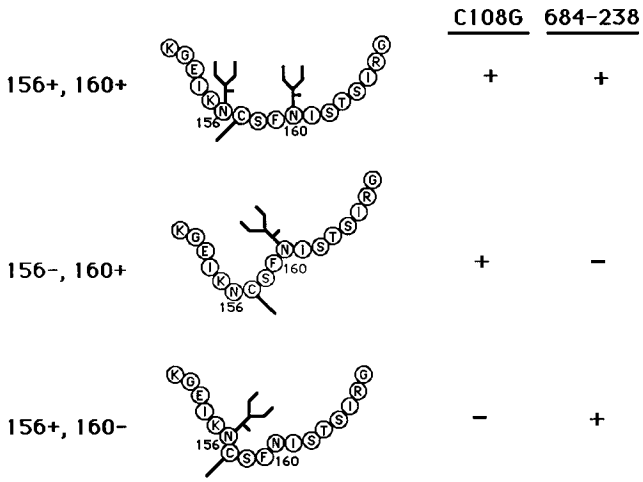

FIG. 6. Model of competition between the 156 and 160 glycosylation sites and the involvement of the gs156 glycan in proper folding. Because of the proximity of gs156 and gs160, competition for glycan addition at these two sites frequently occurs, and the proximity of Cys-157 with the bulky N-linked glycans at these positions affects the formation of the disulfide bond between Cys-157 and its correct partner (Cys-131). Molecules with glycan at gs156 generally formed the correct disulfide bond, while molecules with glycan at gs160 but not at gs156 usually formed an aberrant disulfide bond, which did not express the conformational V2 epitopes.

ACKNOWLEDGMENTS

This work was supported by PHS grants AI23884 and AI34217 to A.P. and AI26081 to S.A.T. and grants from the Aaron Diamond Foundation to A.P. and S.A.T. Z.W. was a Fellow of the Aaron Diamond Foundation, and S.C.K. is a Scholar of the American Foundation for AIDS Research.

REFERENCES

1. **Cheng-Mayer, C., M. Quiroga, J. W. Tung, D. Dina, and J. A. Levy.** 1990. Viral determinants of human immunodeficiency virus type 1 T-cell or macrophage tropism, cytopathogenicity, and CD4 antigen modulation. J. Virol. **64:**4390–4398.
2. **Fuhrmann, U., E. Bause, G. Legler, and H. Ploegh.** 1984. Novel mannosidase inhibitor blocking conversion of high mannose to complex oligosaccharides. Nature (London) **307:**755–758.
3. **Fung, M. S. C., C. R. Y. Sun, W. L. Gordon, R. S. Liou, T. W. Chang, W. N. C. Sun, E. S. Daar, and D. D. Ho.** 1992. Identification and characterization of a neutralization site within the second variable region of human immunodeficiency virus type 1 gp120. J. Virol. **66:**848–856.
4. **Girard, M., M. P. Kieny, A. Pinter, F. Barre-Sinoussi, P. Nara, H. Kolbe, K. Kusumi, A. Chaput, J. I. Mullins, E. Muchmore, J. Ronco, M. Kaczorek, E. Gomard, J.-C. Gluckman, and P. Fultz.** 1991. Immunization of chimpanzees confers protection against challenge with HIV. Proc. Natl. Acad. Sci. USA **88:**542–546.
5. **Gorny, M. K., J. P. Moore, A. J. Cloney, S. Karwowska, J. Sodroski, C. Williams, S. Burda, L. J. Boots, and S. Zolla-Pazner.** 1994. Human anti-V2 monoclonal antibody that neutralizes primary but not laboratory isolates of human immunodeficiency virus type 1. J. Virol. **68:**8312–8320.
6. **Groenink, M., R. A. M. Fouchier, S. Broersen, C. H. Baker, M. Koot, A. B. van Wout, H. G. Huisman, F. Miedema, M. Tersmette, and H. Schuitemaker.** 1993. Relation of phenotype evolution of HIV-1 to envelope V2 configuration. Science **260:**1513–1516.
7. **Ho, D. D., M. S. C. Fung, Y. Cao, X. L. Li, C. Sun, T. W. Chang, and N. C. Sun.** 1991. Another discontinuous epitope on glycoprotein gp120 that is important in human immunodeficiency virus type 1 neutralization is identified by a monoclonal antibody. Proc. Natl. Acad. Sci. USA **88:**8949–8952.
8. **Ho, D. D., J. A. McKeating, X. L. Li, T. Moudgil, E. S. Daar, N.-C. Sun, and J. E. Robinson.** 1991. Conformational epitope on gp120 important in CD4 binding and human immunodeficiency virus type 1 neutralization identified by a human monoclonal antibody. J. Virol. **65:**489–493.
9. **Ho, S. N., H. D. Hunt, R. M. Horton, J. K. Pullen, and L. R. Pease.** 1989. Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene **77:**51–59.
10. **Javaherian, K., A. J. Langlois, C. McDanal, K. L. Ross, L. I. Eckler, C. L. Jellis, A. T. Profy, J. R. Rusche, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1989. Principal neutralizing domain of the human immunodeficiency virus type 1 envelope protein. Proc. Natl. Acad. Sci. USA **86:**6768–6772.
11. **Kayman, S. C., Z. Wu, K. Revesz, H.-C. Chen, R. Kopelman, and A. Pinter.** 1994. Presentation of native epitopes in the V1/V2 and V3 domains of HIV-1 gp120 by fusion glycoproteins containing fragments of gp120. J. Virol. **68:** 400–410.
12. **Koito, A., G. Harrowe, J. A. Levy, and C. Cheng-Mayer.** 1994. Functional role of the V1/V2 region of human immunodeficiency virus type 1 envelope glycoprotein gp120 in infection of primary macrophages and soluble CD4 neutralization. J. Virol. **68:**2253–2259.
13. **Lee, W. R., W.-J. Syu, B. Du, M. Matsuda, S. Tan, A. Wolf, M. Essex, and T.-H. Lee.** 1992. Nonrandom distribution of gp120 N-linked glycosylation sites important for infectivity of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA **89:**2213–2217.
14. **Leonard, C. K., M. W. Spellman, L. Riddle, R. J. Harris, J. N. Thomas, and T. J. Gregory.** 1990. Assignment of intrachain disulfide bonds and characterization of potential glycosylation sites of the type 1 recombinant human immunodeficiency virus envelope glycoprotein (gp120) expressed in Chinese hamster ovary cells. J. Biol. Chem. **265:**10373–10382.
15. **Matthews, T., A. Langlois, W. Robey, N. Chang, R. Gallo, P. Fischinger, and D. Bolognesi.** 1986. Restricted neutralization of divergent human T-lymphotropic virus type III isolates by antibodies to the major envelope glycoprotein. Proc. Natl. Acad. Sci. USA **83:**9709–9713.
16. **McKeating, J. A., C. Shotton, J. Cordell, S. Graham, P. Balfe, N. Sullivan, M. Charles, M. Page, A. Blomstedt, S. Olofsson, S. C. Kayman, Z. Wu, A. Pinter, C. Dean, J. Sodroski, and R. A. Weiss.** 1993. 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. J. Virol. **67:**4932–4944.
17. **McKeating, J. A., M. Thali, C. Furman, S. Karwowska, M. K. Gorny, J. Cordell, S. Zolla-Pazner, J. Sodroski, and R. A. Weiss.** 1992. Amino acid residues of the human immunodeficiency virus type 1 gp120 critical for the binding of rat and human monoclonal antibodies that block the gp120-sCD4 interaction. Virology **190:**134–142.
18. **Moore, J. P., and P. L. Nara.** 1991. The role of the V3 loop of gp120 in HIV infection. AIDS **5:**S21–S33.
19. **Moore, J. P., Q. J. Sattentau, H. Yoshiyama, M. Thali, M. Charles, N. Sullivan, S.-W. Poon, M. S. Fung, F. Traincard, M. Pincus, G. Robey, J. E. Robinson, D. D. Ho, and J. Sodroski.** 1993. Probing the structure of the V2 domain of human immunodeficiency virus type 1 surface glycoprotein gp120 with a panel of eight monoclonal antibodies: human immune response to the V1 and V2 domains. J. Virol. **67:**6136–6151.
20. **Myers, G., B. Korber, J. Berzofsky, R. F. Smith, and G. N. Pavlakis.** 1992. Human retroviruses and AIDS database. Los Alamos Laboratory, Los Alamos, N. Mex.
21. **Pincus, S. H., K. G. Messer, P. L. Nara, W. A. Blattner, G. Colclough, and M. Reitz.** 1994. Temporal analysis of the antibody response to HIV envelope protein in HIV-infected laboratory workers. J. Clin. Invest. **93:**2505–2513.
22. **Pinter, A., W. J. Honnen, M. E. Racho, and S. A. Tilley.** 1993. A potent, neutralizing human monoclonal antibody against a unique epitope overlapping the CD4-binding site of HIV-1 gp120 that is broadly conserved across North American and African virus isolates. AIDS Res. Hum. Retroviruses **9:**985–996.
23. **Pinter, A., W. J. Honnen, S. A. Tilley, C. Bona, H. Zaghouani, M. K. Gorny, and S. Zolla-Pazner.** 1989. Oligomeric structure of gp41, the transmembrane protein of human immunodeficiency virus type 1. J. Virol. **63:**2674–2679.
24. **Pollard, S. R., M. D. Rosa, J. D. Rosa, and D. C. Wiley.** 1992. Truncated variants of gp120 bind CD4 with high affinity and suggest a minimum CD4 binding region. EMBO J. **11:**585–591.
25. **Posner, M. R., T. Hideshima, T. Cannon, M. Mukherjee, K. H. Mayer, and R. A. Byrn.** 1991. An IgG human monoclonal antibody that reacts with HIV-1/gp120, inhibits virus binding to cells, and neutralizes infection. J. Immunol. **146:**4325–4332.
26. **Robert-Guroff, M., M. Brown, and R. C. Gallo.** 1985. HTLV-III neutralizing antibodies in patients with AIDS and AIDS-related complex. Nature (London) **316:**72–74.
27. **Rusche, J. R., K. Javaherian, C. McDanal, J. Petro, D. L. Lynn, R. Grimaila, A. Langlois, R. C. Gallo, L. O. Arthur, P. J. Fischinger, D. P. Bolognesi, S. D. Putney, and T. J. Matthews.** 1988. Antibodies that inhibit fusion of human immunodeficiency virus-infected cells bind a 24-amino acid sequence of the viral envelope, gp120. Proc. Natl. Acad. Sci. USA **85:**3198–3202.
28. **Skinner, M. A., R. Ting, A. J. Langlois, K. J. Weinhold, H. K. Lyerly, K. Javaherian, and T. J. Matthews.** 1988. Characteristics of a neutralizing monoclonal antibody to the HIV envelope glycoprotein. AIDS. Res. Hum. Retroviruses **4:**187–197.
29. **Steimer, K. S., C. J. Scandella, P. V. Skiles, and N. L. Haigwood.** 1991. Neutralization of divergent HIV-1 isolates by conformation-dependent human antibodies to gp120. Science **254:**105–108.
30. **Sullivan, N., M. Thali, C. Furman, D. D. Ho, and J. Sodroski.** 1993. Effect of amino acid changes in the V1/V2 region of the human immunodeficiency virus type 1 gp120 glycoprotein on subunit association, syncytium formation, and recognition by a neutralizing antibody. J. Virol. **67:**3674–3679.
31. **Thali, M., C. Furman, D. D. Ho, J. Robinson, S. Tilley, A. Pinter, and J. Sodroski.** 1992. Discontinuous, conserved neutralization epitopes overlapping the CD4 binding region of the human immunodeficiency virus type 1 gp120 envelope glycoprotein. J. Virol. **66:**5635–5641.
32. **Tilley, S. A., W. J. Honnen, M. E. Racho, M. Hilgartner, and A. Pinter.** 1991. A human monoclonal antibody against the CD4 binding site of HIV-1 gp120 exhibits potent, broadly neutralizing activity. Res. Virol. **142:**247–259.
33. **Warrier, S., A. Pinter, W. J. Honnen, M. Girard, E. Muchmore, and S. A. Tilley.** 1994. A novel glycan-dependent epitope in the V2 domain of human immunodeficiency virus type 1 gp120 is recognized by a highly potent neutralizing chimpanzee monoclonal antibody. J. Virol. **68:**4636–4642.
34. **Weiss, R. A., P. R. Clapham, R. Cheingsong-Popov, A. G. Dalgleish, C. A. Carne, I. V. D. Weller, and R. S. Tedder.** 1985. Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. Nature (London) **316:**69–72.
35. **Westervelt, P., D. B. Trowbridge, L. G. Epstein, B. M. Blumberg, Y. Li, B. H. Hahn, G. M. Shaw, R. W. Price, and L. Ratner.** 1992. Macrophage tropism determinants of human immunodeficiency virus type 1 in vivo. J. Virol. **66:**2577–2582.
36. **Willey, R. L., J. S. Bonafacino, B. J. Potts, M. A. Martin, and R. D. Klausner.** 1988. Biosynthesis, cleavage, and degradation of the human immunodeficiency virus 1 envelope glycoprotein gp160. Proc. Natl. Acad. Sci. USA **85:**9580–9584.
37. **Wyatt, R., N. Sullivan, M. Thali, H. Repke, D. Ho, J. Robinson, M. Posner, and J. Sodroski.** 1993. Functional and immunologic characterization of human immunodeficiency virus type 1 envelope glycoproteins containing deletions of the major variable regions. J. Virol. **67:**4557–4565.