JOURNAL OF VIROLOGY, Dec. 1994, p. 8350–8364
0022-538X/94/$04.00+0

Vol. 68, No. 12

Exploration of Antigenic Variation in gp120 from Clades A through F of Human Immunodeficiency Virus Type 1 by Using Monoclonal Antibodies

JOHN P. MOORE,[1]* FRANCINE E. McCUTCHAN,[2] SHU-WING POON,[1] JOHN MASCOLA,[2] JOHN LIU,[1] YUNZHEN CAO,[1] AND DAVID D. HO[1]

Aaron Diamond AIDS Research Center, New York University School of Medicine, New York, New York 10016,[1] and Henry M. Jackson Foundation for Military Medicine and the Military Medical Consortium for Applied Retroviral Research, Rockville, Maryland 20850[2]

Received 19 July 1994/Accepted 14 September 1994

The reactivities of a panel of 14 monoclonal antibodies (MAbs) with monomeric gp120 derived from 67 isolates of human immunodeficiency virus type 1 of clades A through F were assessed by using an antigen-capture enzyme-linked immunosorbent assay. The MAbs used were all raised against gp120 or gp120 peptides from clade B viruses and were directed at a range of epitopes relevant to human immunodeficiency virus type 1 neutralization: the V2 and V3 loops, discontinuous epitopes overlapping the CD4-binding site, and two other discontinuous epitopes. Four of the five V3 MAbs showed modest cross-reactivity within clade B but very limited reactivity with gp120s from other clades. These reactivity patterns are consistent with the known primary sequence requirements for the binding of these MAbs. One V3 human MAb (19b), however, was much more broadly reactive than the others, binding to 19 of 29 clade B and 10 of 12 clade E gp120s. The 19b epitope is confined to the flanks of the V3 loop, and these sequences are relatively conserved in clade B and E viruses. In contrast to the limited reactivity of V3 MAbs, CD4-binding site MAbs were much more broadly reactive across clades, two of these MAbs (205-46-9 and 21h) being virtually pan-reactive across clades A through F. Another human MAb (A-32) to a discontinuous epitope was also pan-reactive. The CD4-binding site is strongly conserved between clades; but when considering the epitopes near the CD4-binding site, clade D gp120 appears to be the most closely related to clade B and clade E appears to be the least related. A tentative rank order for these epitopes is B/D-A/C-E/F. V2 MAbs reacted sporadically within and between clades, and no clear pattern was observable. While results from binding assays do not predict neutralization serotypes, they suggest that there may be antigenic subtypes related, but not identical, to the genetic subtypes.

The surface glycoprotein gp120 of human immunodeficiency virus type 1 (HIV-1) is responsible for mediating virus attachment to the CD4 receptor in the initial stage of HIV-1 infection of $CD4^+$ cells (31, 34, 42). It is also a major target for the human immune response to HIV-1 infection, and a considerable fraction of virus-binding and virus-neutralizing antibodies are directed against this protein (2, 41, 66, 73). Inspection of the primary sequences of gp120 from multiple isolates shows that it is a highly variable protein (36, 53, 72). This variation is manifested at several levels: within individuals (6, 18, 23, 61, 86), between isolates from individuals infected with HIV-1 within a localized geographical area (1, 5, 13, 24, 27, 29, 33, 50, 52, 57, 58, 63, 64, 71, 87), and between isolates identified in distinct areas of the world (11, 13, 14, 17, 20, 30, 53, 68, 78, 81). Early analyses of gp120 sequence variation within the predominant European/North American B clade allowed the division of the protein into conserved (C) and variable (V) regions (28, 36, 53, 72). The C- and V-region designations have mostly withstood the test of time, although the availability of more sequences has made it clear that revisions are needed: for example, segments of the C3 domain are more variable than the V3 loop, and the V5 domain is fairly conserved (53).

More-recent studies of HIV-1 isolates from throughout the world have enabled the identification of several clades of HIV-1 genotypes (8, 11, 14, 17, 20, 29, 30, 50, 53, 68, 78, 81). Assignment of a virus strain to a particular clade may be based on either *env* or *gag* sequences, and there is reasonable, but not exact, correlation between the different cladistic approaches (11, 19, 30, 53, 69, 81). Variation in the gp120 sequence between clades can be quite considerable, far in excess of the variation found within an individual or within a clade (11, 14, 17, 20, 29, 50, 53, 68, 78, 81). However, we possess very little information as to how primary sequence variation affects the tertiary structure of gp120 or its antigenicity (49). To date, exploration of antigenic variation in gp120 has been mostly limited to exploring the seroreactivity of peptides from the V3 region of different gp120 strains (8, 9, 24, 55, 59, 60, 63, 71, 87). This is important because of the significant contribution V3 antibodies make to virus neutralization, and the extensive sequence variation that is found in the V3 region (1, 5, 8, 9, 26, 27, 29, 35, 37, 38, 50, 52, 53, 59, 60, 64, 70, 71, 80, 81, 87). Yet a vaccine effective against multiple HIV-1 isolates will almost certainly require more antigens than V3 loop peptides alone; so, information on the antigenicity of gp120 from multiple clades and the way gp120 is presented to the immune system of infected individuals may be important for the rational design of HIV-1 vaccines.

In this study, we have used monoclonal antibodies (MAbs) raised against clade B viruses or fragments thereof to explore the degree of structural conservation between soluble gp120s derived from clade B viruses and soluble gp120s from clades A and C through F, using an assay to measure MAb-gp120

* Corresponding author. Mailing address: Aaron Diamond AIDS Research Center, New York University School of Medicine, 455 First Ave., New York, NY 10016. Phone: (212) 725-0018. Fax: (212) 725-1126.

binding. We conclude that V3 MAbs rarely cross-react with gp120s outside clade B, that epitopes around the CD4-binding site are generally well conserved within clade B viruses and can be conserved in other clades also, and that one MAb to a distinct, discontinuous epitope shows pan-reactivity across all clades.

MATERIALS AND METHODS

MAbs and gp120. All human MAbs (HuMAbs) were isolated from individuals infected with clade B viruses. CD4-immunoglobulin G (IgG) was obtained from Genentech, Inc. (South San Francisco, Calif.) (7). HuMAb 19b to a V3 epitope (47, 67, 70) and HuMAbs 21h and 15e to discontinuous epitopes overlapping the CD4-binding site (22, 76) have been described previously. HuMAbs 205-46-9 and 205-43-1 to discontinuous epitopes overlapping the CD4-binding site were obtained from M. Fung (Tanox Biosystems, Inc., Houston, Tex.) (40). HuMAb 48d to a discontinuous gp120 epitope whose exposure is enhanced by CD4 binding has been described elsewhere (67, 77), as has HuMAb A-32 to a separate, discontinuous epitope (40, 45, 67). MAbs loop 2 and IgG1b12 are human recombinant antibodies isolated as Fab fragments; loop 2 was screened against a cyclized V3 peptide based on the sequence SISGPGRAFYTG (3), whereas IgG1b12 was screened against recombinant BRU gp120 and recognizes a discontinuous CD4-binding-site-related epitope (4, 65). Murine MAbs 50.1, 58.2, and 83.1 were raised against an MN-V3 peptide (80), while the immunogen for murine MAbs SC258 and G3-4 was IIIB gp120. The last two MAbs recognize conformationally sensitive epitopes in the V2 loop (16, 21, 25, 45, 75, 85). Details of the epitopes for the MAbs in the test panel are summarized in Table 1. Purified gp120 from HIV-1 MN-infected virus cultures was a gift from Larry Arthur (National Cancer Institute, Frederick, Md.).

Viruses. Virus isolates were collected from various regions of the world by three organizations: the World Health Organization (WHO) (81), the Henry M. Jackson Foundation for the Advancement of Military Medicine and the Military Medical Consortium for Applied Retroviral Research (HMJF/MMCARR), and the National Institute of Allergy and Infectious Diseases (NIAID). Viruses were expanded in mitogen-stimulated peripheral blood mononuclear cells (PBMC) (12, 29, 30, 32, 68), and culture supernatants containing infectious virus were stored in central repositories at −140°C. The designation of viruses into clades was made on the basis of sequence information from the *gag* gene, from gp120 or gp160, from the C2-V5 region of gp120, or, in some cases, after heteroduplex mobility analysis (11, 17, 29, 30, 81). Sequences described in this paper were taken from primary publications that describe the isolates and methods used (9, 12, 17, 29, 59, 68, 81) or, for NIAID isolates, were provided by Fen Gao and Beatrice Hahn (University of Alabama at Birmingham). Several isolates have not yet been sequenced.

Viruses designated by a code in the format exemplified by UG 92/029 were provided by the WHO; viruses designated by a code in the format exemplified by DJ 258 were from the HMJF/MMCARR repository. All other viruses were from the NIAID collection. The countries of origin for the WHO and HMJF/MMCARR viruses are reflected in the code designation by the following abbreviations: UG, Uganda; RW, Rwanda; DJ, Djibouti; BR or BZ, Brazil; SG, Senegal; ZAM, Zambia; SM, Somalia; TH, BK, and CM, Thailand; and US, United States of America. The following NIAID viruses were obtained in the countries indicated: 8855, Malawi; 2818M, Rwanda; 301966, 301975, 301976, B06-003, and B06-015, Thailand; 301594, 301599, 301651, and 301652, Haiti; and 301711, 301712, 301714, 301715, 301716, 83017, 1045, 1064, and 20420-19, United States. Chinese isolates RYCA2 and RYCA4 were derived from samples provided by Y. Zeng.

MAb-gp120-binding assay. Infectious culture supernatants containing virus and free gp120 were treated with 1% Nonidet P-40 (NP-40) nonionic detergent to provide a source of gp120 (48). An appropriate volume of inactivated supernatant was diluted with a buffer consisting of Tris-buffered saline, 1% NP-40, and 10% fetal calf serum, and a 100-μl aliquot was added for 2 h at room temperature to microplate wells (Immulon II; Dynatech Ltd.) coated with sheep polyclonal antibody D7324. This antibody was raised to peptide APTKAKRRVVQREKR, derived from the C-terminal 15 amino acids of the clade B LAI isolate. Unbound gp120 was removed by washing with Tris-buffered saline, and bound gp120 was detected with CD4-IgG (1 μg/ml), or with MAb, diluted in TMTSS buffer essentially as described previously (43–48). The bound ligand was then detected with an appropriate alkaline-phosphatase-conjugated anti-IgG, followed by AMPAK (Dako Diagnostics). A_{492} (optical density at 492 nm [OD_{492}]) was read.

Each virus was tested against CD4-IgG in triplicate and against each MAb in duplicate. The average standard deviation of the triplicate CD4-IgG values was within 15% of the mean value. On the basis of our experience of the reactivities of these MAbs with gp120s from clade B viruses such as HIV-1 MN or BH10, we used the V3 MAbs at 0.3 μg/ml (58.2 at 1/30,000 dilution of ascites fluid), the CD4-binding site MAbs A-32 and 48d at 1 μg/ml, SC258 at 3 μg/ml, and G3-4 at 10 μg/ml. All OD_{492} values were corrected for nonspecific antibody binding in the absence of added gp120 (buffer blank). The mean, blank-corrected OD_{492} values for CD4-IgG and each MAb were then calculated, and the OD_{492} ratios of MAb to CD4-IgG were determined for each MAb. This procedure normalized the different amounts of gp120 captured onto the solid phase via antibody D7324 when the MAb reactivities with a panel of viruses were compared. Binding ratios of 0.50 or greater were deemed to represent strong MAb reactivity; ratios from 0.25 to 0.49 were considered indicative of weak reactivity; values of <0.25 were designated representative of essentially negative MAb reactivity.

The only exception to this procedure was made when HuMAb 19b was analyzed. We have noted that when saturating concentrations of 19b were reacted with gp120 from several clade B isolates, the OD_{492} values were two- to threefold lower than that expected from the extent of binding of other antibodies or CD4-IgG to these gp120s (47). This reduced binding was overcome by increasing, by three- to fivefold, the volume of culture supernatant used as the gp120 source in our assay. We believe that the reduced binding of 19b is due to proteolytic cleavage of the V3 loop on a fraction of the total gp120 molecules that occurs during the 5 to 7 days required to grow virus in PBMC cultures. Thrombin-mediated proteolysis of the V3 loop of purified MN gp120 destroys the 19b epitope but does not affect the binding of MAb 50.1 or 83.2 to other V3 epitopes (47). Because it was not always possible to increase the amount of culture supernatant added to the assay when studying viruses from clades other than clade B, in many cases we added 100 μl of culture supernatant, titrated HuMAb 19b, and determined the 19b concentration that resulted in 50% binding. A 50% binding value of <100 ng/ml was designated strong 19b reactivity; values from 100 to 250 ng/ml were deemed to represent weak 19b reactivity; values of >250 ng/ml were considered negative for 19b reactivity.

Post hoc comparisons of the MAb-binding patterns with

TABLE 1. Summary of epitopes for MAbs in test panel

MAb	Reference(s)	gp120 region	Epitope	Substitution(s)	
				Inhibitory[a]	Tolerated[b]
19b	47, 67, 70	V3 loop	-I----G--FY-T	-I---PG--FY-T S R YH LV L	RIHIGPGRAFYAT SVNLAW KTI T G PM L QSV R G VY V T
Loop 2	3	V3 loop	SISGPGRAFYTG		
50.1	80	V3 loop	RIHIG	A at any position	
83.1	80	V3 loop	I-IGPGR	A at any position	
58.2	80	V3 loop	HIGPGRAF	A at any position	HIGPGRAF Y A
205-46-9	40	CD4-binding site	Discontinuous	113 D/R (256 S/Y) 368 D/R (257 T/R) 368 D/T (477 D/V) 370 E/R	See reference 76
205-43-1	40	CD4-binding site	Discontinuous	368 D/R (314 G/W) 370 E/R (368 D/T) (384 Y/E) (421 K/L)	See reference 76
21h	22, 76	CD4-binding site	Discontinuous	113 D/R (368 D/R) 113 D/A	See reference 76
15e	22, 76	CD4-binding site	Discontinuous	113 D/R (257 T/A) 256 S/Y (257 T/G) 257 T/R (370 E/Q) 368 D/R (384 Y/E) 370 E/R (475 M/S) 427 W/V 427 W/S	See reference 76
Ig1b12	4, 65	CD4-binding site	Discontinuous	368 D/R (152/3 GE/SM) 368 D/T (191/3 YL/GS) 370 E/R (ΔV1/V2) 457 D/A (380 G/F) 457 D/R (386 N/Q) 477 D/V (420 I/R) (427 W/S) (470 P/L)	See references 4, 65
48d	67, 77	CD4-enhanced	Discontinuous	Multiple (see reference 77)	See reference 77
A-32	40, 44, 67	Unknown	Discontinuous	69 W/L (76 P/Y) 252 R/W (117 K/W) 256 S/Y (380 G/F) 262 N/T (381 E/P) 427 W/S (382 F/L) (384 Y/E) (420 I/R) (435 Y/S) (435 Y/M) (475 M/S) (485 K/V)	See reference 45
SC258	44	V2 loop (conformation sensitive)	KEYA--YKLD	Several outside epitope (see reference 45)	See reference 45
G3-4	21, 44, 75, 85	V2 loop (conformation sensitive)	KEYAF-YKLD	Several outside epitope (see reference 45)	See reference 45

[a] Amino acid substitutions indicated by prior studies to inhibit MAb binding to gp120 or a relevant peptide are listed below the epitope description for HuMAb 19b and are summarized for other MAbs, with the numbering system based on the HxBc2 clone of HIV-1 LAI. For MAbs 205-46-9 to A-32, substitutions that exhibit strong inhibition are listed, with those exhibiting weak inhibition given in parentheses.

[b] Amino acid substitutions indicated by prior studies not to affect MAb binding to gp120 or a relevant peptide are listed below the epitope description for HuMAb 19b and MAb 58.2. The panels of gp120 mutants used to analyze the binding of MAbs to discontinuous or V2 conformational epitopes are listed in the original reports (reference[s] cited); they should be consulted for information on amino acid substitutions that do not affect MAb binding. For HuMAbs 205-46-9, 205-43-1, IgG1b12, and A-32, the panels of mutants used were very similar to those used to analyze HuMAbs 21h, 15e and 48d.

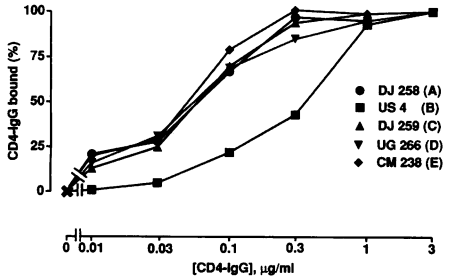

FIG. 1. Reactivity of CD4-IgG with gp120s from viruses of clades A through E. NP-40-treated culture supernatants were reacted with capture antibody D7324, and CD4-IgG was then titrated at the concentrations indicated before detection of gp120-bound CD4-IgG. The OD_{492} values were corrected for CD4-IgG absorption in the absence of gp120 (OD_{492} of <0.070) and then expressed relative to the value obtained at the highest input CD4-IgG concentration (defined as 100%). The volume of supernatant tested and 100% OD_{492} values, respectively, were 10 μl and 0.98 for clade A, 20 μl and 1.36 for clade B, 10 μl and 1.00 for clade C, 35 μl and 0.97 for clade D, and 50 μl and 1.21 for clade E.

available V2 and V3 sequences (see Fig. 3 and Tables 3 and 4) revealed a few results that appeared anomalous (see Fig. 2). To check these results, titration curves for selected MAbs and gp120s were plotted and a total of six changes were made in the data in Fig. 3 and Tables 3 and 4 to reflect the revisions.

RESULTS

Reactivities of clade A through F gp120s with CD4-IgG. For this study we accumulated viruses distributed among clades A through F, collected in a number of countries and stored in the form of infectious culture supernatants from PBMC cultures in repositories coordinated by the WHO, NIAID, and HMJF/MMCARR. We first confirmed that the CD4-binding site on gp120 was substantially conserved between clades by measuring CD4-IgG binding to NP-40-solubilized gp120 from representative clade A through E viruses, using the D7324-capture enzyme-linked immunosorbent assay (Fig. 1). The concentration of CD4-IgG giving half-maximal binding to each of the tested viruses was in the range 0.05 to 0.3 μg/ml (0.33 to 2 nM), with the clade B gp120 from isolate US 4 having a lower affinity for CD4-IgG than did the other gp120s. Half-maximal CD4-IgG binding values for 12 other clade B gp120s that we have studied fell in the range 0.06 to 0.22 μg/ml (39); so, US 4 gp120 is probably at the low end of a fairly narrow distribution of CD4-IgG-binding affinities. Thus, we could find no evidence for a major degree of variation in CD4-binding affinities between gp120s from different clades.

Each NP-40-treated virus stock was titrated (2 to 100 μl per well) to assess the amount of gp120 present that would react with capture antibody D7324. For this purpose, captured gp120 was detected with a saturating concentration of CD4-IgG (1 μg/ml). Each assay was internally calibrated by using a range of concentrations of MN gp120 (0.03 to 10 ng/ml). From the titration curves, we determined the amount of each culture supernatant that gave an OD_{492} value of 0.80 in the CD4-IgG-binding assay, which is equivalent to approximately 1 ng of MN gp120 per ml. For most clade B viruses, 5 to 15 μl of culture supernatant was sufficient to allow OD_{492} values in the above range, but for viruses from other clades, 50 to 100 μl of supernatant was more usually required. An OD_{492} value of 0.80 could not be obtained with several supernatants at the highest input of 100 μl per well, and about 25% of the cultures of isolates from clades other than B were essentially unreactive in our assay. This could be either because there was insufficient gp120 in the cultures or because of variation in the C5 epitope for capture antibody D7324 (see below). However, no clade was significantly less reactive than the others. Notwithstanding the above observations, we were able to constitute a panel of 67 gp120s from diverse isolates that were sufficiently reactive with antibody D7324 and CD4-IgG. The panel comprised clade A (8 isolates), clade B (29 isolates), clade C (8 isolates), clade D, (8 isolates), clade E (12 isolates), and clade F (2 isolates). Appropriate dilutions of the inactivated culture supernatants were stored at −40°C in microplate wells before use.

Reactivities of clade A to F gp120s with MAbs. Microplates containing previously diluted culture supernatants were unfrozen, and the contents were transferred to D7324-coated wells. After capture of gp120, CD4-IgG reactivity was measured in triplicate; the net, mean OD_{492} values were distributed around the desired value of 0.8, most falling in the range 0.5 to 1.1, although a few non-B gp120s produced OD_{492} values of 0.3 to 0.5 (data not shown). MAb reactivity was, in contrast, usually much more variable. Data representing MAb reactivities with gp120s from clades A through F are presented in Fig. 2.

All MAbs in our test panel were raised against gp120 or gp120-derived peptides from clade B isolates. In particular, the immunogen for three of the five V3 MAbs (50.1, 83.1, and 58.2) was a peptide from the V3 loop of HIV-1 MN (80), and a fourth V3 MAb (loop 2) was screened against a similar V3 peptide (3) (Table 1). It was, therefore, not surprising that the MAb panel showed greatest overall reactivity with clade B gp120s, which was especially apparent with most of the V3 loop MAbs (Fig. 2 and 3; Tables 2 and 3). There was also significant reactivity of the V3 MAb panel with clade F gp120s (Fig. 2; Table 3); although with only two isolates from this clade available for study, we are unsure of the generality of this observation. The reactivity of MAbs 50.1, 83.1, and 58.2 and loop 2 with gp120s from clades A, C, D, and E was uncommon and sporadic (Fig. 2). However, V3 HuMAb 19b did cross-react significantly with several E clade gp120s, and 19b also bound weakly to a few A, C, and F clade gp120s (Fig. 2). This pattern of reactivity is consistent with the relative conservation of the 19b epitope on the flanks of the V3 loop, compared with the more-extensive variation seen in the epitopes for the other V3 MAbs near the crown of the loop (Fig. 3). It was notable that the V3 MAbs tended to be poorly reactive with gp120s from Thai, Chinese, and Haitian clade B viruses compared with gp120s from American or Brazilian clade B viruses. For example, only a single Thai B gp120 (BK 130) was strongly reactive with V3 MAbs other than 19b (Fig. 2), although five of the seven Thai B gp120s do have the GPGR motif in their V3 loops that is typical of isolates predominant in the United States and Haiti (Fig. 3). The other Thai clade B gp120s (TH 92/026 and CM 237) have variant GPGQ and GPGK sequences at the crown of their V3 loops (17, 81), and neither reacted with any V3 MAbs (Fig. 2). Similarly, the Brazilian clade B isolate BR 92/021 with the variant GWGR motif at the V3 crown (29, 50, 81) was unreactive with all V3 MAbs except 19b, although BR 92/023 gp120 with the GPGK variant motif was weakly reactive with MAbs 50.1 and 83.1 as well as 19b (Fig. 2). A more detailed comparison of the V3 sequence and MAb reactivity data for the panel of gp120s is made below.

We tested five HuMAbs to related, but distinct, discontinuous epitopes that overlap the CD4-binding site of gp120. These CD4 site epitopes showed a much greater degree of conservation than the V3 loop epitopes, both within clade B and across clades. Some HuMAbs to CD4 site epitopes, notably 205-46-9

Clade B

Virus	V3					CD4BS					CD4 ↑	?	V2	
	19b	Loop 2	50.1	83.1	58.2	205-46-9	205-43-1	21h	15e	IgG-12	48d	A-32	SC258	G3-4
USA														
83017	(30)	0.53	0.20	0.54	0.83	1.16	0.95	0.87	0.25	1.29	0.51	1.26	1.27	0.39
1045	(15)	0.03	0.02	0.00	0.00	1.16	0.97	0.74	0.91	1.09	0.19	0.95	0.21	0.34
20420-19	(30)	0.24	0.05	0.30	0.34	1.21	0.99	0.78	0.81	0.89	0.19	0.55	1.00	0.00
1064	(10)	0.31	0.13	0.42	2.34	1.15	0.89	0.89	0.41	0.22	0.25	0.81	0.21	0.14
301711	(20)	0.21	0.00	0.00	0.89	1.43	1.02	0.90	0.96	0.00	0.00	0.72	0.00	0.76
301712	(10)	0.69	0.18	0.67	0.71	1.17	1.06	1.35	0.71	0.10	0.24	0.95	1.53	1.12
301714	(55)	0.06	0.00	0.00	0.00	1.14	0.99	1.04	1.07	0.61	0.26	0.74	0.20	0.30
301715	(25)	0.36	0.04	0.40	0.62	1.07	0.90	0.54	0.09	0.86	0.00	0.69	1.45	0.54
301716	(35)	0.27	0.20	0.66	0.68	1.12	0.97	0.76	0.27	0.97	0.15	0.72	0.02	0.00
US 1	(40)	0.83	0.71	0.71	0.83	1.00	0.99	0.84	0.85	1.07	0.76	0.51	0.25	0.20
US 4	(80)	0.58	1.01	1.00	0.88	1.04	1.03	0.69	0.18	0.02	0.02	0.54	0.00	0.12
Haiti														
301594	0.05	0.81	1.40	0.75	0.12	1.23	0.97	1.22	0.72	0.80	1.14	0.84	0.09	0.04
301599	0.08	0.00	0.00	0.00	0.00	0.05	0.81	0.83	0.09	0.35	0.00	0.63	0.00	0.00
301651	0.07	0.00	0.02	0.59	0.00	0.94	0.68	0.94	0.69	0.00	0.00	0.79	0.00	0.00
301652	(20)	0.15	0.02	0.40	0.00	0.87	0.58	0.74	0.45	0.00	0.12	0.44	0.35	0.00
Brazil														
BR 92/020	(10)	0.28	0.17	0.35	0.68	0.71	0.80	0.91	0.72	0.30	0.27	0.72	1.48	0.74
BR 92/021	(35)	0.10	0.00	0.00	0.00	0.88	0.95	0.75	0.79	0.08	1.19	0.81	0.00	0.00
BR 92/023	(80)	0.08	0.25	0.34	0.14	1.09	1.23	0.53	1.06	0.53	0.51	0.35	1.15	0.26
BZ 164	(15)	0.30	0.00	0.01	1.45	1.46	0.50	1.35	0.11	0.09	0.05	0.56	0.00	0.01
BZ 165	(35)	0.65	0.13	0.57	1.22	1.43	1.41	1.38	1.45	1.30	0.09	0.52	0.31	0.06
BZ 167	0.17	0.30	1.64	0.76	0.71	1.47	1.54	1.24	1.39	0.42	0.11	0.75	0.21	0.43
Thailand														
BK 130	(30)	0.81	0.90	0.86	0.88	1.08	1.15	0.90	0.34	1.10	0.89	0.53	0.00	0.00
BK 131	0.00	0.00	0.00	0.00	0.30	1.41	1.64	1.28	1.42	1.40	0.42	0.64	0.19	0.96
BK 132	(15)	0.00	0.00	0.01	0.01	1.46	1.71	1.42	0.22	0.22	0.49	0.62	1.15	0.10
CM 237	0.00	0.00	0.00	0.00	0.01	1.32	1.19	0.99	1.07	0.69	0.41	0.61	0.08	1.10
TH 92/014	0.06	0.00	0.00	0.02	0.04	1.35	1.10	1.07	0.83	0.54	0.51	1.14	0.67	1.39
TH 92/026	0.00	0.00	0.01	0.00	0.00	1.01	0.99	0.92	1.03	0.00	0.00	0.52	0.77	0.00
China														
RYC A2	0.02	0.00	0.19	0.00	0.00	1.05	1.38	0.87	1.07	0.14	1.02	0.52	0.19	1.14
RYC A4	0.03	0.00	0.02	0.00	0.04	1.38	1.59	0.88	1.01	0.08	0.04	0.55	0.00	0.01

FIG. 2. Reactivities of MAbs with gp120s from clades A through F. The data presented are MAb/CD4-IgG binding ratios (see Materials and Methods). Dark shading indicates strong MAb reactivity (ratio of ≥0.5), light shading denotes weak MAb reactivity (ratio of 0.25 to 0.49), and no shading represents negative reactivity (ratio of <0.25). For HuMAb 19b, the values in parentheses represent the 19b concentrations giving 50% binding (see Materials and Methods). CD4BS, CD4-binding site.

and 21h, were strongly cross-reactive; 205-46-9 bound to 65/67 test viruses from clades A through F, failing to bind to only a single gp120 from each of clades B and C (Fig. 2; Table 2). HuMAb 21h was pan-reactive among gp120s from clades A through D and F but bound to only a minority of clade E gp120s, which distinguishes it from HuMAb 205-46-9 (Table 2). HuMAb 205-43-1 was strongly reactive with all B, D, and F clade gp120s but was only sporadically reactive with A and C gp120s and bound to only a single gp120 from clade E (Fig. 2). The other two MAbs to the CD4 site epitopes, 15e and IgG1b12, were unreactive with a significant fraction of B clade gp120s, and this reduced reactivity was also manifested with gp120s from clades other than B (Table 2). However, 15e bound strongly to all eight D clade gp120s (Fig. 2). Taken together, the CD4 site MAbs indicate that the degree of relatedness of the complex epitopes in this area to those on clade B gp120s is B/D-A/C-E/F, although with only two F clade viruses available any judgement must be tentative (Table 3). No clear distinctions in respect of MAb reactivity could be drawn between clade B isolates from different geographic locales. With the exception of the V3 MAb reactivity pattern noted above, clade B viruses appear relatively homogeneous in terms of their overall antigenicity (Fig. 2).

HuMAbs 48d and A-32 recognize discontinuous gp120 epitopes that do not overlap the CD4-binding site in that the binding of these antibodies to soluble gp120 is not inhibited by prior binding of CD4-IgG. Indeed, the binding of either CD4-IgG or A-32 causes a gp120 conformational change that better exposes the epitope for 48d, resulting in increased 48d binding (40, 67, 77). The binding of A-32 to gp120 from the clade B virus HxBc2 is disrupted by only a few amino acid substitutions in the C1, C2, and C4 domains (Table 1) and is probably located exclusively within conserved regions of the protein (40, 45). Consistent with this, the A-32 epitope was strongly conserved across clades A through F, only a single A clade gp120 being unreactive with this MAb (Table 2). In contrast, the 48d epitope is very sensitive to multiple amino acid substitutions scattered throughout the conserved domains

Clades A, C

Virus	MAb	V3					CD4BS					CD4 ↑	?	V 2	
		19b	Loop 2	50.1	83.1	58.2	205-46-9	205-43-1	21h	15e	IgG-12	48d	A-32	SC258	G3-4
	Clade A														
Virus	UG 92/029	0.29	0.00	0.56	0.00	0.00	1.01	0.29	0.63	0.41	0.98	0.78	0.71	0.00	0.07
Virus	UG 92/031	0.00	0.00	0.00	0.00	0.00	0.95	0.13	0.79	0.16	0.00	0.00	0.50	0.00	0.00
Virus	RW 92/008	0.00	0.00	0.00	0.00	0.00	0.52	0.30	0.74	0.24	0.44	0.00	0.58	0.00	0.00
Virus	RW 92/009	0.00	0.00	0.00	0.00	0.00	1.08	0.65	0.64	0.26	0.00	0.00	0.55	0.00	0.00
Virus	DJ 258	(220)	0.00	0.00	0.00	0.00	0.95	0.12	0.81	0.66	0.39	0.02	0.54	0.00	0.28
Virus	DJ 263	(150)	0.01	0.00	0.03	0.01	1.04	0.21	0.85	0.47	0.06	0.04	0.58	0.00	0.76
Virus	UG 273	0.06	0.00	0.00	0.00	0.00	1.11	0.06	0.86	0.04	0.17	0.12	0.39	0.03	0.00
Virus	UG 276	0.13	0.00	0.00	0.00	0.03	0.93	0.22	0.66	0.17	0.70	0.09	0.05	0.00	0.00
	Clade C														
Virus	BR 92/025	0.09	0.00	0.39	0.00	0.00	0.00	0.01	1.06	0.16	0.00	0.00	0.52	0.00	0.14
Virus	SG 364	0.03	0.00	0.00	0.18	0.00	0.88	0.72	0.81	0.46	0.70	0.00	0.71	0.46	0.98
Virus	ZAM 18	0.07	0.04	0.64	0.04	0.00	0.67	0.52	0.90	0.17	0.39	0.08	0.57	0.26	0.00
Virus	ZAM 20	0.04	0.00	0.70	0.03	0.02	1.05	0.92	1.08	0.13	0.05	0.05	0.64	0.02	0.62
Virus	SM 145	0.08	0.00	0.02	0.02	0.02	0.65	0.45	0.67	0.61	0.19	0.00	1.16	0.00	0.11
Virus	UG 268	0.18	0.00	0.00	0.08	0.06	1.08	0.64	0.87	0.48	1.19	0.03	0.59	0.00	0.90
Virus	DJ 259	(65)	0.00	0.02	0.01	0.00	1.02	0.54	0.90	0.95	0.06	0.02	0.57	1.01	0.00
Virus	8855	0.15	0.08	0.00	0.05	0.08	0.63	0.83	1.10	0.64	0.56	0.10	0.78	0.15	0.32

Clades D, E, F

Virus	MAb	V3					CD4BS					CD4 ↑	?	V 2	
		19b	Loop 2	50.1	83.1	58.2	205-46-9	205-43-1	21h	15e	IgG-12	48d	A-32	SC258	G3-4
	Clade D														
Virus	UG 92/001	0.08	0.00	0.00	0.00	0.00	1.21	1.05	0.93	0.77	0.39	0.20	0.79	0.00	0.00
Virus	UG 92/005	0.05	0.02	0.20	0.80	0.44	1.09	0.91	0.64	0.68	0.37	0.85	0.82	0.00	0.00
Virus	UG 92/021	0.05	0.00	0.00	0.00	0.00	0.98	0.91	0.86	0.62	0.33	0.18	0.77	0.49	0.00
Virus	UG 92/024	0.12	0.00	0.00	0.00	0.00	1.11	0.82	0.88	0.82	0.02	0.09	0.92	2.11	0.03
Virus	UG 266	0.03	0.00	0.00	0.00	0.00	0.90	1.35	1.04	0.76	0.28	0.03	0.26	0.11	0.00
Virus	UG 270	0.04	0.01	0.00	0.00	0.04	1.08	1.30	1.19	0.79	1.61	0.05	0.56	0.00	0.00
Virus	UG 274	0.00	0.01	0.00	0.01	0.03	1.03	1.48	1.20	1.22	1.58	0.03	0.40	0.06	0.00
Virus	SG 365	0.05	0.00	0.00	0.02	0.05	0.98	1.20	0.97	0.55	0.73	0.80	0.30	0.09	0.00
	Clade E														
Virus	TH 92/009	0.78	0.00	0.00	0.00	0.00	1.03	0.22	0.45	0.26	0.00	0.15	1.05	0.00	0.59
Virus	TH 92/022	0.08	0.00	0.00	0.00	0.00	0.78	0.04	0.10	0.17	0.00	0.00	0.73	0.00	0.12
Virus	TH 92/023	0.34	0.00	0.00	0.00	0.00	0.65	0.05	0.13	0.25	0.00	0.00	0.86	0.00	0.00
Virus	CM 235	(200)	0.00	0.00	0.01	0.02	1.01	0.05	0.10	0.06	0.15	0.02	0.41	0.00	0.08
Virus	CM 238	(60)	0.01	0.00	0.00	0.02	0.89	0.20	0.46	0.12	0.12	0.03	0.56	0.00	0.65
Virus	CM 240	(85)	0.02	0.00	0.03	0.00	0.98	0.02	0.21	0.10	0.05	0.25	0.52	0.00	0.17
Virus	CM 244	(75)	0.00	0.00	0.01	0.00	0.99	0.00	0.51	0.07	0.02	0.00	0.52	0.01	0.11
Virus	301966	0.11	0.02	0.00	0.05	0.03	1.10	1.06	0.95	0.28	0.35	0.04	1.27	0.07	0.87
Virus	301975	(300)	0.00	0.00	0.02	0.00	0.89	0.06	0.20	0.16	0.07	0.06	1.02	0.00	0.43
Virus	301976	(25)	0.01	0.10	0.07	0.00	0.74	0.00	1.23	0.25	0.11	0.01	0.99	0.02	0.90
Virus	B06-003	(30)	0.07	0.06	0.02	0.05	0.84	0.11	0.28	0.12	0.10	0.08	0.79	0.05	0.14
Virus	B05-015	(200)	0.00	0.02	0.02	0.06	1.01	0.00	0.91	0.09	0.20	0.09	1.02	0.00	0.08
	Clade F														
Virus	BZ 162	(100)	0.55	0.40	0.56	1.47	0.40	0.68	0.40	0.15	0.08	0.10	0.54	0.00	0.00
Virus	BZ 163	(150)	0.05	0.01	0.21	0.35	0.45	0.88	0.52	0.05	0.04	0.03	0.64	0.10	0.27

FIG. 2—*Continued.*

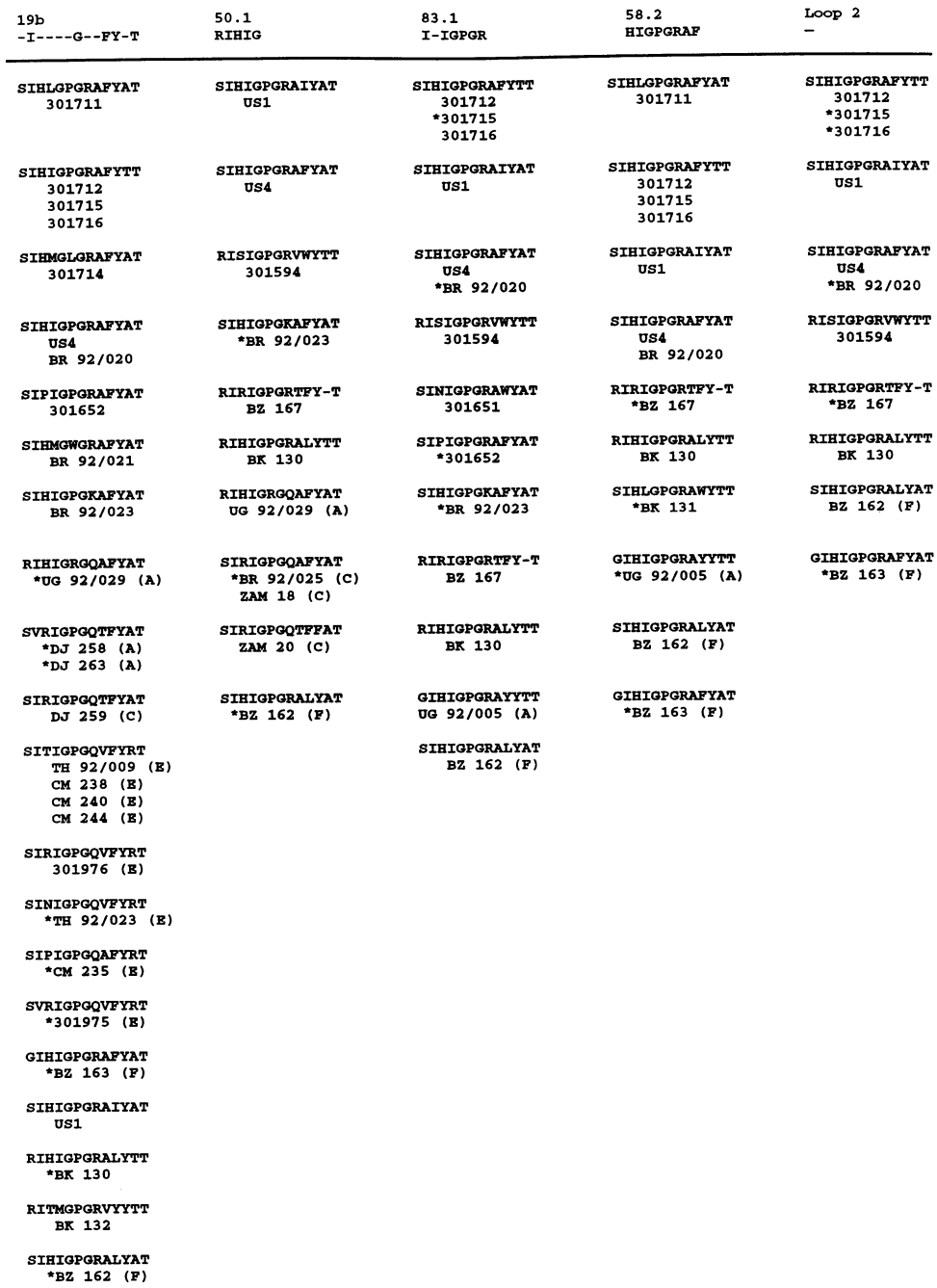

FIG. 3. Partial V3 sequences of gp120s reactive with V3 MAbs. MAb designations and estimated epitopes based on prior studies (47, 70, 80) are shown at the top. In each column, partial V3 sequences from MAb-reactive gp120s are listed; a weak reaction is denoted by an asterisk. Each gp120 is from clade B unless otherwise indicated in parentheses. For MAbs 19b, 83.1, and 58.2 and loop 2, there were 8, 4, 5, and 4 reactive isolates, respectively, for which sequences are not available.

(77). The clade B and cross-clade reactivities of 48d were correspondingly much more restricted than those of A-32. Thus, outside clade B, only a very few gp120s possessed the 48d epitope (Table 2). No test was made of 48d reactivity with clade A through F viruses in the presence of sCD4 or CD4-IgG.

Two V2-reactive MAbs, G3-4 and SC258, were also tested for cross-clade reactivity. Both MAbs recognized conformationally sensitive epitopes involving sequences in the central section of the V2 loop (21, 25, 45, 75, 85). Neither G3-4 nor SC258 showed appreciable breadth of reactivity, either within clade B or across clades, although both MAbs were able to bind sporadically to gp120s from clades other than B (Fig. 2; Tables 4 and 5). On the basis of our limited analysis using only two V2 MAbs, the degree of antigenic relatedness in the V2 domain among the clades is B/C-E/F-A/D (Table 3).

Relationship between V3 MAb binding and V3 sequence. We analyzed the sequences of the gp120s able or unable to bind

TABLE 2. Summary of MAb reactivity with international clade isolates[a]

Epitope and MAb	No. of isolates showing reactivity						Subtotal for clade B (n = 29)	No. of isolates showing reactivity				Total (n = 67)
	A (n = 8)	B						C (n = 8)	D (n = 8)	E (n = 12)	F (n = 2)	
		U.S. (n = 11)	Haiti (n = 4)	Brazil (n = 6)	Thai (n = 6)	China (n = 2)						
V3												
19b	0, 3, 5	11, 0, 0	1, 0, 3	5, 0, 1	1, 1, 4	0, 0, 2	18, 1, 10	1, 0, 7	0, 0, 8	6, 4, 2	0, 2, 0	25, 10, 32
Loop 2	0, 0, 8	4, 3, 4	1, 0, 3	1, 3, 2	1, 0, 5	0, 0, 2	7, 6, 16	0, 0, 8	0, 0, 8	0, 0, 12	1, 1, 0	8, 7, 52
50.1	1, 0, 7	2, 0, 9	1, 0, 3	1, 1, 4	1, 0, 5	0, 0, 2	5, 1, 23	2, 1, 5	0, 0, 8	0, 0, 12	0, 1, 1	8, 3, 56
83.1	0, 0, 8	5, 3, 3	2, 1, 1	2, 2, 2	1, 0, 5	0, 0, 2	10, 6, 13	0, 0, 8	1, 0, 7	0, 0, 12	1, 0, 1	12, 6, 49
58.2	0, 0, 8	8, 1, 2	0, 0, 4	3, 1, 2	1, 1, 4	0, 0, 2	12, 3, 14	0, 0, 8	0, 1, 7	0, 0, 12	1, 1, 0	13, 5, 49
CD4BS												
205-46-9	8, 0, 0	11, 0, 0	3, 0, 1	6, 0, 0	6, 0, 0	2, 0, 0	28, 0, 1	7, 0, 1	8, 0, 0	12, 0, 0	0, 2, 0	63, 2, 2
205-43-1	1, 2, 5	11, 0, 0	4, 0, 0	6, 0, 0	6, 0, 0	2, 0, 0	29, 0, 0	4, 3, 1	8, 0, 0	1, 0, 11	1, 1, 0	44, 6, 17
21h	8, 0, 0	11, 0, 0	4, 0, 0	6, 0, 0	6, 0, 0	2, 0, 0	29, 0, 0	8, 0, 0	8, 0, 0	2, 5, 5	1, 1, 0	56, 6, 5
15e	1, 3, 4	6, 4, 1	2, 1, 1	5, 0, 1	4, 1, 1	2, 0, 0	19, 6, 4	3, 2, 3	8, 0, 0	0, 4, 8	0, 0, 2	31, 15, 21
IgG1b12	2, 2, 4	7, 1, 3	1, 1, 2	2, 2, 2	4, 0, 2	0, 0, 2	14, 4, 11	3, 1, 4	3, 4, 1	0, 1, 11	0, 0, 2	22, 12, 33
Complex												
48d	1, 0, 7	2, 2, 7	1, 0, 3	2, 1, 3	2, 3, 1	1, 0, 1	8, 6, 15	0, 0, 8	2, 0, 6	0, 1, 11	0, 0, 2	11, 7, 49
A-32	6, 1, 1	11, 0, 0	3, 1, 0	5, 1, 0	6, 0, 0	2, 0, 0	27, 2, 0	7, 1, 0	5, 3, 0	11, 1, 0	1, 1, 0	57, 9, 1
V2												
SC258	0, 0, 8	4, 1, 6	0, 1, 3	2, 1, 3	2, 1, 3	0, 0, 2	8, 4, 17	1, 2, 5	1, 1, 6	0, 0, 12	0, 0, 2	10, 7, 50
G3-4	1, 1, 6	3, 3, 5	0, 0, 4	1, 2, 3	3, 0, 3	1, 0, 1	8, 5, 16	3, 1, 4	0, 0, 8	4, 1, 7	0, 1, 1	16, 9, 42

[a] The data are collated from the results presented in Fig. 2 and are presented as the number of gp120s (n) with which each MAb shows strong, weak, or negative reactivity, respectively, for each clade.

the MAbs in our test panel, to see if there were any simple relationships between gp120 sequence and MAb recognition, both within clade B and across clades. Characteristics of the epitopes for several of the MAbs are summarized in Table 1, on the basis of previous studies of these antibodies. It should be noted that because of the difficulty in interpreting the influence of amino acid substitutions on discontinuous or conformationally sensitive epitopes, only the epitopes for the V3 MAbs are known with any precision. We have therefore restricted our present analyses to the V3 and V2 MAbs; the sequence requirements for binding of the CD4-binding site MAbs, A-32 and 48d, will be discussed when more information is available concerning the epitopes of these MAbs.

The epitopes of the murine anti-MN V3 peptide MAbs 50.1, 83.1, and 58.2 have been previously defined only by studying the effects on MAb binding of alanine substitution of residues in the immunogenic peptide (80); this technique does not allow the identification of tolerated substitutions at important residues and cannot predict, with certainty, effects of amino acid changes on the conformation of a MAb epitope in the context of the folded gp120 protein (37, 38, 47). Nonetheless, the published information provides a useful scaffold for further analysis. In general, the V3 sequences of isolates able to bind each V3 MAb correlated reasonably well with the predicted presence of the MAb epitope, independently of the isolates' clades. The epitope for HuMAb 19b has been defined as -I----G--FY-T (47, 70). All 27 of the sequenced isolates that were able to bind 19b possessed that canonical epitope or one with conservative substitutions at one of two positions: a valine substitution for the isoleucine (DJ 258, DJ 263, and 301975) or an isoleucine, leucine, or tyrosine substitution for the phenylalanine (US 1, BK 130, BZ 162, and BK 132). These substitutions appear to be tolerated for 19b binding at least in the context of certain overall V3 sequences, albeit often with some loss of affinity (Fig. 3). An additional clade B isolate (ACP-1) with a valine residue at this position (VYTT motif) is also able to bind 19b (47). However, a tryptophan residue does not appear to be an acceptable substitute for the phenylalanine, accounting for the failure of 19b to recognize the four Thai B isolates BK 131, CM 237, TH 92/014, and TH 92/026 that possess the WYTT motif (Fig. 2). Three clade C isolates (BR 92/025, ZAM 18, and UG 268) and one clade E isolate (TH 92/022) failed to bind 19b, despite the presence of the canonical 19b epitope. This may be attributable to proteolytic destruction of the epitope, as the OD_{492} signals were generally weak in experiments using these isolates, and one of the cultures of ZAM 18 did contain some 19b-reactive gp120. Alternatively, variation in other regions of gp120 from these isolates may indirectly influence the presence of the 19b epitope.

Alanine substitutions indicate that the epitope for MAb 50.1 is centered on the RIHIG sequence on the amino terminal flank of the MN V3 loop (80). Only 11 isolates, 6 from clade B,

TABLE 3. Antigenic relatedness of gp120 epitope clusters among clades A through F

Epitope cluster (no. of MAbs)	Antigenic relatedness in clade[a]:					
	A (n = 8)	B (n = 29)	C (n = 8)	D (n = 8)	E (n = 12)	F (n = 2)
V3 (n = 5)	0.06	0.42	0.09	0.04	0.13	0.55
CD4BS (n = 5)	0.59	0.86	0.70	0.93	0.33	0.40
V2 (n = 2)	0.09	0.35	0.35	0.09	0.19	0.13

[a] The data depicted in Fig. 2 were processed as follows: a strong, weak, or negative reaction between a MAb and a gp120 was allocated a score of 2, 1, or 0, respectively. For each epitope cluster (V3, CD4BS, and V2), the total score within each clade was determined and its ratio to the maximum possible score was calculated and presented here. For example, there are five V3 MAbs and eight clade A isolates, giving a maximum score of 80; the observed reactivity was scored as 5 (one strong and three weak reactions), and the ratio of 5/80 was 0.06.

TABLE 4. Partial V2 sequences of gp120s reactive and unreactive with V2 MAb SC258[a]

Isolate	Clade	Sequence[b]
Reactive		
301652	B	**K**LKKE**YALFYKLD**VVQI
301712	B	**K**MQRE**YALFYKLD**VIQI
US1	B	**K**VERE**YALFYKLD**VVPM
BK 132	B	**K**LQKE**YALFYKLD**VVPI
ZAM 18	C	**K**KQRV**YALFYKLD**IVPL
UG 92/021	D	**K**KKQA**YALFYKLD**VVQM
SG 364	C	**K**RRNE**YALFYRLD**IVPL
DJ 259	C	**K**RQKA**YALFYRLD**IVPL
BR 92/020	B	**K**EKKE**YALFYNLD**VVQI
TH 92/014	B	**K**VKD-**YALFYKLD**VVPI
TH 92/026	B	**K**VKD-**YALFYKSD**VVPI
301715	B	**K**VQKEF**ALFYKLD**VVPI
Unreactive		
SM 145	C	KKQKVYALFYRLDIVPL
301976	E	KKQKVYALFYKLDIVQI
301711	B	KVKKEYALFY**SLN**VIPI
301714	B	KMQKEYALFYK**F**DVVPI
301599	B	KIQKEYALF**S**KLDVLPI
301716	B	KMQKEYAL**L**YKLDVIPI
SG 365	D	KRQNVY**S**LFYRLDLVPI
US4	B	KVQKEY**S**LFYKLDVVPI
UG 273	A	KTRRVY**S**LFYKLDVVQI
UG 92/031	A	KKQKIY**S**LFYRLDVVPI
RW 92/009	A	KKQRVY**S**LFYRLDIVQI
301651	B	KVTKQ**S**ALFYRLDVVPI
DJ 258	A	KQQKM**S**ALFYRLDVVQI
301594	B	KTTKE**H**ALFYKLDVVPI
ZAM 20	C	KTSKV**H**ALFYR**V**DIVPL
UG 268	C	KKQTA**H**ALFYKLDVVSL
BR 92/025	C	KREKV**H**ALFYRLDIVPL
CM 235	E	KKQKV**H**ALFYKLDIVPI
CM 238	E	KKQKV**H**ALFYKLDIVQM
CM 240	E	KKQKV**H**ALFYKLDIVQI
CM 244	E	KKQKV**H**ALFYKLDIVPI
301966	E	KKQKV**H**ALFYKLDLVQI
301975	E	KKQKV**H**ALFYKLDLVQM
TH 92/022	E	KQQKV**H**ALFYRLDIVQM
BZ 163	F	KQQKV**H**ALFYRLDIVPI
BZ 162	F	KQKRV**H**ALFYKLDIVPI

[a] Isolates from clades A through F are grouped on the basis of their reactivity or unreactivity with MAb SC258. Sequences from the central section of V2 from these isolates are listed, with common sequences indicated in boldface for reactive isolates and divergent residues indicated in boldface for unreactive isolates. V2 sequences from other isolates in the test panel were not available.

[b] For reactive isolates, tolerated residues were as follows:

```
K----YALFYKLDVVPI
     F    RS IIQl
          N     M
```

TABLE 5. Partial V2 sequences of gp120s reactive and unreactive with V2 MAb G3-4[a]

Isolate	Clade	Sequence
Reactive		
301711	B	**K**VKKEY**ALFY**SLNVIPI
301712	B	**K**MQREY**ALFY**KLDVIQI
301714	B	**K**MQKEY**ALFY**KFDVVPM
301715	B	**K**VQKEF**ALFY**KLDVVPI
CM 238	E	**K**KQKVH**ALFY**KLDIVQM
301966	E	**K**KQKVH**ALFY**KLDLVQI
301975	E	**K**KQKVH**ALFY**KLDLVQM
301976	E	**K**KQKVY**ALFY**KLDIVPL
BZ 163	F	**K**QQKVH**ALFY**RLDIVPI
DJ 258	A	**K**QQKMS**ALFY**RLDVVQI
SG 364	C	**K**RRNEY**ALFY**RLDIVPL
ZAM 20	C	**K**TSKVH**ALFY**RVDIVPL
UG 268	C	**K**KQTAH**ALFY**KLDVVSL
BR 92/020	C	**K**EKKEY**ALFY**NLDVVQI
TH 92/014	B	**K**VKD-Y**ALFY**KLDVVPI
Unreactive		
US4	B	KVQKEY**S**LFYKLDVVPI
UG 273	A	KTRRVY**S**LFYKLDVVQI
SG 365	D	KRQNVY**S**LFYRLDLVPI
UG 92/031	A	KKQKIY**S**LFYRLDVVPI
RW 92/009	A	KKQRVY**S**LFYRLDIVQI
301716	B	KMQKEYAL**L**YKLDVIPI
301599	B	KIQKEYALF**S**KLDVLPI
301652	B	KLKKEYALFYKLDVVQI
BK 132	B	KLQKEYALFYKLDIVPI
US1	B	KVEREYALFYKLDVVPM
ZAM 18	C	KKQRVYALFYKLDIVPL
SM 145	C	KKQKVYALFYRLDIVPL
DJ 259	C	KRQKAYALFYRLDIVPL
UG 92/021	D	KKKQAYALFYKLDVVQM
CM 235	E	KKQKVHALFYKLDIVPI
CM 240	E	KKQKVHALFYKLDIVQI
CM 244	E	KKQKVHALFYKLDIVPI
BZ 162	F	KQKRVHALFYKLDIVPI
TH 92/022	E	KQQKVHALFYRLDIVQM
301651	B	KVTKQSALFYRLDVVPI
BR 92/025	C	KREKVHALFYRLDIVPL
301594	B	KTTKEHALFYKLDVVPI

[a] Isolates from clades A through F are grouped on the basis of their reactivity or unreactivity with MAb G3-4. Sequences from the central section of V2 from these isolates are listed, with common sequences indicated in boldface for reactive isolates and divergent residues indicated in boldface for unreactive isolates. V2 sequences from other isolates in the test panel were not available.

bound 50.1 (Table 2), and only two of those isolates (BK 130 and UG 92/029) possessed the RIHIG motif (Table 3). Other 50.1-reactive isolates contained the motifs SIHIG (four), SIRIG (three), RISIG, and RIRIG at the corresponding position (Fig. 3). However, four unreactive isolates also had the SIHIG motif (301712, 301715, 301716, and BR 92/020) and two possessed the SIRIG sequence (DJ 259 and 301976). This suggests that the 50.1 epitope may be subject to significant conformational constraints, such that more-distant residues unpredictably affect MAb binding. Consistent with this, there was considerable variation in the affinities of 50.1-reactive gp120s for this MAb. Thus, antibody titrations showed that half-maximal 50.1 binding occurred at 1 ng/ml for BZ 167 (RIRIG), at 20 ng/ml for US 4 (SIHIG), at 40 ng/ml for BK 130 (RIHIG), at 100 ng/ml for US 1 (SIHIG), and at 500 ng/ml for BZ 162 (SIHIG). It is also possible that the 50.1-reactive material in some cultures may constitute only a minor fraction of the total gp120s that is unrepresented by the bulk sequence.

The epitope for MAb 83.1 is reported to be I-IGPGR (80). Eighteen isolates, 16 of them from clade B, bound 83.1. All but one of those sequenced possessed the above motif, with H, S, N, P, or R being present at the second position (Fig. 3). The exception, BR 92/023, had an I-IGPGK motif but bound 83.1 weakly, suggesting that a conservative R to K substitution can be tolerated to some extent in the 83.1 epitope. However, many 83.1-unreactive isolates possessed the I-IGPGQ motif (data not shown), which implies that the R to Q substitution was not acceptable. Other selected isolates unreactive with 83.1 contained the following motifs: IHLGPGR (301711), IHMGLGR (301714), IHMGWGR (BR 92/021), ITMGPGR (BK 132), and TPIGVGR (UG 92/021), with substitutions in the canonical 83.1 epitope indicated by boldface. Of the two Brazilian F clade isolates, only one (BZ 162) bound 83.1 (Fig.

4), despite both possessing the canonical 83.1 epitope (Table 3). Half-maximal 83.1 binding to BZ 162 occurred at approximately 200 ng/ml, whereas most reactive isolates bound 83.1 half-maximally in the range of 30 to 75 ng/ml (data not shown). A marginal level of binding of 83.1 to BZ 163 was consistently detectable (Fig. 4 and data not shown).

MAb 58.2 reportedly recognizes the HIGPGRAF sequence (80). Eighteen isolates, 15 of which were from clade B, were recognized by this MAb. Among the thirteen sequenced isolates binding 58.2, seven had the above HIGPGRAF motif, while the others contained closely related sequences (Table 3). These included IGPGRAI (US 1), HIGPGRAL (BK 130 and BZ 162), HIGPGRAY (UG 92/005), HLGPGRAF (301711), HLGPGRAW (BK 131), and RIGPGRTF (BZ 167). Thus, an arginine residue may substitute for histidine at the first position (both are positively charged); a leucine or an isoleucine residue is acceptable at the second position; a threonine may replace alanine at the penultimate residue; degeneracy is permitted at the final position, provided that a bulky, hydrophobic residue is present (I, L, Y, F or W). Note, however, that divergence in more than one position from the canonical HIGPGRAF sequence was often associated with reduced MAb binding (Fig. 2). Thus, antibody titrations showed that relative to the degree of affinity (defined as 100) of 58.2 for gp120 from 301712 (HIGPGRAF), the degrees of affinity of other gp120s were as follows: 210, 301711; 100, US 1; 60, BK 130; 10, BZ 167; and 4, BK 131. Among 58.2-unreactive isolates, the following motifs were present: PIGPGRAF (301652), NIGPGRAW (301651), and HIGPGKAF (BR 92/023), consistent with the above conclusions. However, the unreactive isolate TH 92/014 possessed the same HLGPGRAW motif as the weakly reactive isolate BK 131; variations in the presentation of the same sequence in the context of different gp120s may account for this.

Little prior information is available on the nature of the epitope for HuMAb loop 2. Inspection of the reactivity patterns in Fig. 2 indicated that loop 2 most closely resembled MAb 83.1. This was confirmed when the sequences of 11 of the 15 loop 2-reactive isolates were examined; all 11 isolates contained the I-IGPGR motif, where the variant residue could be H, S, or R (Fig. 3). Among unreactive isolates, motifs present included IHIGPGK (BR 92/023) and IRIGPGQ (RW 92/008), indicating the importance of the final arginine residue to the loop 2 epitope, and INIGPGR (301651) and IPIGPGR (301652), demonstrating that not all residues are acceptable at the second position. The only isolate apparently unreactive with loop 2 that might have been expected to be reactive on the basis of the sequence was the clade D isolate UG 92/005 (GIHIGPGRAYYTT).

Relationship between V2 MAb binding and V2 sequence. The epitopes for the V2 MAbs SC258 and G3-4 are sensitive to gp120 conformation and may even be discontinuous in nature (21, 25, 45, 75, 85). This factor complicates analysis of the gp120 sequences of isolates reactive and unreactive with these MAbs, because it is uncertain precisely where on the V2 domain the MAbs bind. Previous studies have, however, indicated that the identity of several amino acids near the central section of the V2 domain of HIV-1 HxB2 influences the binding of SC258 and G3-4 (45, 75, 85). We therefore focused on this region and compared the sequences of V2 MAb-reactive and -unreactive isolates (Tables 4 and 5).

Isolates able to bind SC258 shared a common sequence motif that was generally absent from unreactive isolates (Table 4). All the SC258-reactive isolates possessed the motif K----YALFY-LD---- or one divergent in only a single residue (301715, TH 92/014, and TH 92/026). SC258-unreactive isolates generally had changes in one or more of the residues in the above motif, except for SM 145 and 301976. The inability of 301976 gp120 to bind SC258 was confirmed by performing an antibody titration (data not shown). Isolates SM 145 and 301976 may have distant-site changes in V2 or elsewhere that affect the presentation of the SC258 epitope. Of note is that SC258 was unable to bind to gp120s containing a histidine residue in place of the first tyrosine, although the rare phenylalanine substitution appears to be acceptable in isolate 301715. The inability to accommodate a histidine in its epitope accounts for the failure of SC258 to react with any gp120s from clade E, which have a consensus sequence K----HALFYKLD----.

Inspection of the V2 sequences of isolates reactive and unreactive with MAb G3-4 did not, however, yield a definitive explanation of the reactivity pattern (Table 5). All G3-4-reactive isolates possessed the motif K-----ALFY-------, and isolates with changes in this sequence did not bind G3-4 (Table 5). However, many G3-4-unreactive isolates had no changes in the above motif, which is highly conserved. Presumably, the G3-4 epitope is influenced by the overall conformation of the V2 domain in a way that we are unable to predict from inspection of the primary sequence.

DISCUSSION

It is relevant to the design and development of any vaccine that might be effective in preventing infection by multiple clades of HIV-1 to determine whether there are antigenic and neutralization serotypes of HIV-1. This paper focuses on antigenic serotypes. Previous approaches to understanding global HIV-1 variation have focused on analyzing the primary sequence of gp120, either by direct sequencing or by use of the heteroduplex mobility assay. These genetic techniques have enabled the multiple clades of HIV-1 to be identified (8, 11, 14, 17, 20, 29, 30, 50, 53, 68, 81). However, because of our current lack of understanding of gp120 structure, relating primary sequence variation in gp120 to its antigenicity is difficult, if not impossible (49). There have been explorations of the patterns of inter- and intraclade neutralization of global HIV-1 strains (10, 32, 79), but it is premature to draw any definitive conclusion as to whether there are neutralization serotypes or how many there might be. Antigenic variation in HIV-1 has also been studied by V3 peptide-based techniques, which involve analysis of the variations in the primary and secondary structures of a small, albeit serologically important, segment of gp120 (1, 8, 9, 26, 27, 35, 37, 38, 52, 53, 59, 60, 64, 70, 71, 80, 87). To complement these approaches, we have used MAbs raised against clade B viruses to explore variation in the tertiary structure of soluble gp120 derived from nonionic detergent-treated cultures of PBMC-grown primary viruses from clades A through F. These studies provide a background for future work analyzing the interactions of HIV-1-positive sera with gp120s from multiple clades.

In this study, gp120 was captured via an immobilized polyclonal antibody, D7324, to its C terminus. Considerable variation was observed in the amount of D7324-reactive gp120 in different virus preparations, but we noted that clade B virus stocks tended to contain 5- to 10-fold more of this material than most stocks of viruses from the other clades. In principle, this could be due to lower absolute levels of gp120 in the non-B virus cultures, but measurement of p24 values indicated that this explanation was improbable. Instead, we favor the explanation that variation in the C5 epitope for the capture antibody D7324 was responsible. This polyclonal antibody was raised to peptide APTKAKRRVVQREKR from the clade B LAI isolate. This sequence is very strongly conserved throughout clade

B gp120s. However, there are minor sequence variations in the corresponding regions of gp120s from other clades; the most common sequence found in clade A, C, D, and E gp120s is APTRAKRRVVEREKR (53), in which the two changes from the clade B sequence are indicated in boldface. Thus, it is reasonable to believe that sequence variation in the capture antibody epitope accounts for the reduced capture of non-B gp120s onto the solid phase. Notwithstanding this problem, the reactivity of D7324 with non-B viruses was demonstrably adequate for the purposes of our assay, although we are now evaluating a sheep antiserum to the above non-B consensus peptide in an attempt to improve assay performance.

Interpretation of some of the MAb binding data in terms of gp120 sequence variation can be relatively straightforward; specifically, much of the V3 MAb binding data can be related to the presence or absence of known antigenic amino acids in the primary V3 sequences, on the basis of prior analyses of the epitopes for these MAbs (3, 47, 80), coupled with post hoc comparisons of the V3 sequences of V3 MAb-reactive and -unreactive isolates (Fig. 3). To a lesser degree, V2 MAb binding is also comprehensible from sequence analysis (45, 75, 85), although there are clearly unpredictable influences of distant-site residues on the epitopes for both V2 and V3 MAbs (Fig. 3 and Tables 4 and 5). However, predicting whether a MAb will bind to a discontinuous epitope, for example those around the CD4-binding site, is not as easy. This is because of our lack of understanding of the tertiary structure of gp120 and the way that amino acid changes in one region of the protein can affect the conformation of a separate region, which they demonstrably can (15, 44–46, 49, 51, 54, 56, 74–77, 82–84). For example, we have previously shown that very minor sequence variations between molecular clones of the same LAI clade B isolate can drastically influence the binding of HuMAbs such as 15e and 48d from our present panel (49), and similar results were observed in studies with molecular clones of HIV-1 MN (54). For these reasons, we have not yet attempted a systematic comparison of gp120 sequences from CD4BSAb-reactive and -unreactive isolates. This must await more knowledge of the natures of the discontinuous epitopes for these MAbs.

It is important to realize that assays, such as the one used here, that measure antibody binding to soluble gp120 do not readily predict virus neutralization. Any correlation between data derived from binding and neutralization assays is, at best, inexact (39). This is especially true for the highly prevalent antibodies to the complex gp120 epitopes that lie near the CD4-binding site (39, 41). What can, however, be said with reasonable confidence is that if a gp120 molecule lacks the epitope for an antibody, that antibody will not neutralize the virus (although there may be rare exceptions). For example, a MAb such as 83.1 to a clade B gp120 that is dependent on the GPGR motif is very unlikely to bind to or neutralize viruses of any clade that have sequences such as GPGK, GPGQ, or GLGQ (80). Furthermore, amino acid changes both within and outside V3 MAb epitopes can influence the degree of reactivity of the MAb with gp120. Our data on the binding of V3 MAbs to gp120 are generally fairly consistent with the V3 sequences of the test viruses, combined with knowledge of the epitopic requirements for binding of these MAbs (Fig. 3), although we did note a few exceptions. Thus, we suggest that cross-clade neutralization via V3 will be improbable given the considerable divergence in key antigenic sites and that cross-neutralization via V3 within a clade may only be possible with viruses that are genetically homogenous in areas of the V3 loop containing antigenically important residues. Nonetheless, broadly reactive V3 antibodies can be made by humans, as exemplified by HuMAb 19b. This antibody can bind to at least one gp120 from all clades other than clade D, although it has a very limited ability to reduce the infectivity of viruses from outside clade B (47). To some extent, the most critical antigenic variation in the V2 and V3 regions, both within and between clades, involves changes in only a few key amino acids that are critical for antibody epitopes. The inter- and intraclade prevalence of key groups of amino acids can, to some extent, be predicted from primary sequence analysis, leading to first-approximation estimates of the probability of cross-clade reactivity of V3 (and perhaps some V2)-directed antibodies if their epitopes are known with any precision.

In principle, MAbs could be used in a binding assay such as ours to allocate gp120s into clades, if a sufficient number of MAbs to multiple clades were available. A high degree of imprecision would have to be tolerable, however. Thus, a cocktail of V3 MAbs raised to clade B MN-like viruses with GPGR motifs should be able to pick out related clade B viruses with reasonable precision but would probably fail to detect Thai GPGQ, American GPGK, or Brazilian GWGR B-clade variants or to discriminate between these variants. Similarly, a clade C V3 MAb would probably not distinguish clade A viruses from clade C viruses, because of the similarity between the V3 regions of gp120s from these clades. A MAb-based assay could, in principle, be used to detect the presence of isolates with unusual sequences within a cohort from a single geographical area. Thus, only one of the American, Haitian, and Brazilian clade B isolates (301599) in our test panel was unreactive with every V3 MAb (Fig. 2). The V3 sequence of this isolate, SVHSGHIGGGRTLFTT, is highly divergent, containing a 3-amino-acid insertion. However, a MAb-based clading system is probably impractical because of the necessity of growing large volumes of viruses as antigen sources for MAb-gp120-binding assays. The speed and utility of the heteroduplex mobility assay render it the system of choice for virus clading, at least in preliminary form (11), or for detecting the spread of unusual variants into a localized area, such as North America.

Further development of clading systems based on serum antibody reactivities does, however, seem warranted, for it is important for vaccine design to determine how many immunotypes or serotypes of HIV-1 may need to be countered. We have used MAbs to probe the antigenicity of gp120 across clades, whereas others have focused on the polyclonal antibody response to continuous V3 epitopes. It is the pattern of V3 sequence variation between clades, combined with the knowledge that the V3 region is both a reasonably immunodominant epitope and a target for neutralizing antibodies, that underlies the use of V3 peptides for determining HIV-1 serotypes (1, 8, 9, 26, 27, 35, 37, 38, 52, 53, 59, 60, 64, 70, 71, 80, 87). However, the inexact division of V3 sequences between clades will tend to obscure the patterns of reactivity observed and limit the utility of peptide-based assays in complex situations. For example, the strongly antigenic residues IGPGQTF are quite common in both clades A and C; so, discrimination between A and C viruses is difficult with a V3 peptide-based assay (9, 59). Conversely, a peptide-based assay can discriminate between the B consensus HIGPGRAFY and the E consensus SITIGPGQVFY (9, 59). Because the V3 region is strongly antigenic, these considerations would also contribute to the discriminatory powers of assays such as ours that are based on the complete gp120 molecule.

It is not immediately obvious that approaches to serotyping based on other isolated segments of gp120, notably the V1 and V2 domains, offer any advantage over V3-based serology. The V1 domain of gp120 is perhaps the most variable region of this molecule (53), and the extensive variation within the V1

domain of clade B viruses has precluded to date the identification of any cross-reactive V1 antibodies. The V2 domain is poorly represented by short, linear peptides (45), and the use of more complex constructs expressing conformationally correct V2 segments may well be subject to interpretive problems (25); understanding how sequence variation affects antigenicity is difficult for the relatively simple V3 peptide-based assays and may be much more difficult when considering the interactions of antibodies with more-complex epitopes (Table 6) (45, 46, 49, 54, 56, 76, 77). It is, however, notable that the sporadic reactivity of V2 MAb SC258 with gp120s from clades B, C, D, and F (but not A and E) can often be predicted from the primary V2 sequence. Thus, the SC258 epitope appears to encompass the sequence K----YALFYKLD---- (Table 4). Within this motif, the boldfaced tyrosine and alanine residues show the greatest variation between clades; the clade E V2 consensus sequence has a histidine in place of the tyrosine, and the clade A consensus has a serine in place of the alanine (Table 4). Both of these changes are presumably incompatible with SC258 binding. However, the same changes are also commonly found in gp120s from other clades, notably clade C, rendering the clade-predictive value of the SC258 reactivity pattern negligible. An even more complex, and essentially uninterpretable, pattern of reactivity was found with the second V2 MAb G3-4 (Table 5). On the basis of our limited analysis with only these two MAbs, the V2 domain of clade C isolates has the closest antigenic resemblance to that of clade B (Table 3). However, we conclude that the antigenic properties of the V2 domain are such that it is not a good candidate for determining the clade of an unknown isolate.

The CD4-binding site is absolutely conserved functionally between clades (Fig. 1). However, when considering the discontinuous epitopes near the CD4-binding site, clade D gp120 appears to be the most closely related to clade B, and clade E appears to be the least related (Table 3). A tentative rank order for these epitopes is B/D-A/C-E/F, although only two F clade isolates were included in our analysis. No data are yet available to assess the relative positions of other clades. Several of the CD4-binding site antibodies are strongly reactive with gp120 from clades A to F, as is MAb A-32. For example, HuMAb 205-46-9 is virtually pan-reactive, and 21h fails to react with only clade E gp120s. A HuMAb that reacts with both clade B and African isolates has been previously described as recognizing a "unique" CD4-binding site epitope (62); our data suggest that such cross-reactivity is actually quite common and can even, in the case of 205-46-9, extend to clade E gp120s. Yet, there is diversity among the epitopes overlapping the CD4-binding site, because HuMAb 205-43-1 reacts with all clade B and D gp120s but only sporadically with clade A and C gp120s and with only a single gp120 from clade E. HuMAbs 15e and IgG1b12 are more restricted in their patterns of reactivity, as is 48d to the CD4-enhanced epitope; none of these MAbs reacts with all clade B gp120s. Of note is that both IgG1b12 and 48d are sensitive to amino acid changes in the variable regions of HxBc2 gp120 (65, 77).

As the epitopes near the CD4-binding site are strongly represented in the polyclonal anti-gp120 component of the human immune response to HIV-1 infection, at least by clade B viruses (41), it might be anticipated that the above rank order would be reflected in patterns of data derived from gp120-binding assays using HIV-1-positive sera. Antibodies to other epitopes will, of course, also contribute to the overall serological pattern according to their relative abundance in the polyclonal antibody response. Our preliminary results from serology studies are, however, in accord with the above rank order (data not shown), and others have reached a similar conclusion on the basis of V3 peptide serology studies (9, 59). Taken together, our observations using MAbs support the conclusions of these serological studies: there are a limited number of serotypes that are fewer in number than the genotypes.

But how might serotypes based on binding assays relate to neutralization serotypes? Disappointingly, we do not know; for while we are struggling to relate sequence to antigenicity, neutralization is yet another degree of complexity removed from antigenicity. If there are neutralization serotypes, the patterns may be even less distinct than the antigenic serotypes, which are themselves more obscure than the genotypes. Put simply, sequence analysis depends on the primary structure of gp120, V3-peptide-based assays depend on its secondary structure, and gp120-based assays depend on its tertiary structure. In contrast, virus neutralization is probably a function of gp120/gp41 quaternary structure. Furthermore, neutralization assays using HIV-1-positive sera may be influenced by even higher orders of complexity, such as the presence of enhancing antibodies. Thus, as we move from the purity of genetics to the fog of biology, an obfuscation of patterns might be expected. Yet perversely, it is also possible that some of the complexities might turn out to be mere eddies in the mist that shields the presence of just a limited number of neutralization serotypes for HIV-1.

ACKNOWLEDGMENTS

We are very grateful to the staff of the WHO and their collaborators who have provided HIV-1 isolates to the international repository, to those who similarly assist the HMJF/MMCARR and NIAID in collecting international isolates, and to Y. Zeng for provision of Chinese samples. We thank Don Burke for his support. We also appreciate the cooperation of the repository staffs who expand and produce virus stocks, particularly Helga Rübsamen-Waigmann and Harvey Holmes. We thank Beatrice Hahn and Fen Gao for sequence information on the NIAID isolates, and we thank the donors of MAbs to our test panel: Denis Burton and Carlos Barbas III for loop 2 and IgG1b12; Jim Robinson for 21h, 15e, A-32, and 48d; Kate Hanham for 50.1, 58.2, and 83.1; Michael Fung for 205-43-1, 205-46-9, and G3-4; and Gerry Robey for SC258. We are also grateful to Larry Arthur for the gift of MN gp120 and to Genentech, Inc., for CD4-IgG. We thank Jamie Franklin for assistance with the graphics.

This work was supported by NIAID contract NO1 AI35168, "Antigenic Variation of HIV-1 and Related Lentiviruses," and by grant AI 25541.

REFERENCES

1. **Albert, J., L. Franzén, M. Jansson, G. Scarlatti, P. K. Kataaka, E. Katabira, F. Mubiro, M. Rydåker, P. Rossi, U. Pettersson, and H. Wigzell.** 1992. Ugandan HIV-1 V3 loop sequences closely related to the US/European consensus. Virology **190**:674–681.
2. **Allan, J. S., J. E. Coligan, F. Barin, J. Sodroski, C. A. Rosen, W. A. Haseltine, T. H. Lee, and M. Essex.** 1985. Major glycoprotein antigens that induce antibodies in AIDS patients are encoded by HTLV-III. Science **228**:1091–1094.
3. **Barbas, C. F., III, T. A. Collet, P. Roben, J. Binley, W. Amberg, D. Hoekstra, D. Cababa, T. M. Jones, R. A. Williamson, G. R. Pilkington, N. L. Haigwood, A. C. Satterthwait, I. Sanz, and D. R. Burton.** 1993. Molecular profile of an antibody response to HIV-1 as probed by combinatorial libraries. J. Mol. Biol. **230**:812–823.
4. **Barbas, C. F., III, D. Hu, N. Dunlop, L. Sawyer, D. Cababa, R. M. Hendry, P. L. Nara, and D. R. Burton.** 1993. *In vitro* evolution of a neutralizing human antibody to human immunodeficiency virus type 1 to enhance affinity and broaden strain cross-reactivity. Proc. Natl. Acad. Sci. USA **91**:3809–3813.
5. **Bobkov, A., M. M. Garaev, A. Rzhaninova, P. Kaleebu, R. Pitman, J. N. Weber, and R. Cheingsong-Popov.** 1994. Molecular epidemiology of HIV-1 in the former Soviet Union: analysis of *env* V3

sequences and their correlation with epedemiologic data. AIDS **8**:619–624.

6. **Bruce, C., C. Clegg, A. Featherstone, J. Smith, and J. Oram.** 1993. Sequence analysis of the gp120 region of the *env* gene of Ugandan human immunodeficiency proviruses from a single individual. AIDS Res. Hum. Retroviruses **9**:357–363.
7. **Capon, D. J., S. M. Chamow, J. Mordenti, S. A. Marsters, T. Gregory, H. Mitsuya, R. A. Byrn, C. Lucas, F. M. Wurm, J. E. Groopman, and D. H. Smith.** 1989. Designing CD4 immunoadhesins for AIDS therapy. Nature (London) **337**:525–531.
8. **Cheingsong-Popov, R., A. Bobkov, M. M. Garaev, P. Kaleebu, D. Callow, A. Rziminova, S. R. Saukhat, N. P. Burdajev, N. D. Kolomijets, and J. N. Weber.** 1993. Identification of human immunodeficiency virus type 1 subtypes and their distribution in the Commonwealth of Independent States (former Soviet Union) by serologic V3-peptide-binding assays and V3 sequence analysis. J. Infect. Dis. **168**:292–297.
9. **Cheingsong-Popov, R., S. Lister, D. Callow, P. Kaleebu, S. Beddows, J. N. Weber, and the WHO Network for HIV Isolation and Characterization.** Serotyping HIV-1 through antibody binding to the V3 loop: relationship to viral genotype. AIDS Res. Hum. Retroviruses, in press.
10. **Cheng-Mayer, C., J. Homsy, L. A. Evans, and J. A. Levy.** 1988. Identification of human immunodeficiency virus subtypes with distinct patterns of sensitivity to serum neutralization. Proc. Natl. Acad. Sci. USA **85**:2815–2819.
11. **Delwart, E. L., E. G. Shpaer, J. Louwagie, F. E. McCutchan, M. Grez, H. Rübsamen-Waigman, and J. I. Mullins.** 1993. Genetic relationships determined by a DNA heteroduplex mobility assay: analysis of HIV-1 *env* genes. Science **262**:1257–1261.
12. **de Wolf, F., E. Hogervorst, J. Goudsmit, E.-M. Fenyö, H. Rübsamen-Waigmann, H. Holmes, B. Galvao-Castro, E. Karita, C. Wasi, S. D. K. Sempala, E. Baan, F. Zorgdrager, V. Lukashov, S. Osmanov, C. Kuiken, M. Cornelissen, and the WHO Network for HIV Isolation and Characterization.** Syncytium inducing (SI) and non-syncytium-inducing (NSI) capacity of human immunodeficiency virus type 1 (HIV-1) subtypes other than B: phenotypic and genotypic characteristics. AIDS Res. Hum. Retroviruses, in press.
13. **Dietrich, U., M. Grez, H. von Briesen, B. Panhans, M. Giessendörfer, H. Kühnel, J. Maniar, G. Makambre, W. B. Becker, M. L. B. Becker, and H. Rübsamen-Waigmann.** 1993. HIV-1 strains from India are highly divergent from prototypic African and US/European strains, but are linked to a South African isolate. AIDS **7**:23–27.
14. **Dumitrescu, O., M. L. Kalish, S. C. Kliks, C. I. Bandea, and J. A. Levy.** 1994. Characterization of human immunodeficiency virus type 1 isolates from children in Romania: identification of a new envelope subtype. J. Infect. Dis. **169**:281–288.
15. **Freed, E. O., and M. A. Martin.** 1994. Evidence for a functional interaction between the V1/V2 and C4 domains of human immunodeficiency virus type 1 envelope glycoprotein gp120. J. Virol. **68**:2503–2512.
16. **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.
17. **Gao, F., L. Yue, S. Craig, C. L. Thornton, D. L. Robertson, F. E. McCutchan, J. A. Bradac, P. M. Sharp, B. H. Hahn, and the WHO Network for HIV Isolation and Characterization.** Genetic variation of HIV-1 in four WHO-sponsored vaccine evaluation sites: generation of functional envelope (gp160) clones representative of sequence subtypes A, B, C, and E. AIDS Res. Hum. Retroviruses, in press.
18. **Groenink, M., A. C. Andeweg, R. A. M. Fouchier, S. Broersen, R. C. M. van der Jagt, H. Schuitemaker, R. E. Y. de Goede, M. L. Bosch, H. G. Huisman, and M. Tersmette.** 1992. Phenotype-associated *env* gene variation among eight related human immunodeficiency virus type 1 clones: evidence for in vivo recombination and determinants of cytotropism outside the V3 domain. J. Virol. **66**:6175–6180.
19. **Guo, H.-G., M. S. Reitz, R. C. Gallo, Y. C. Ko, and K. S. S. Chang.** 1993. A new subtype of HIV-1 *gag* sequence detected in Taiwan. AIDS Res. Hum. Retroviruses **9**:925–927.
20. **Gürtler, L. G., P. H. Hauser, J. Eberle, A. von Braun, S. Knapp, L. Zekeng, J. M. Tsague, and L. Kaptue.** 1994. A new subtype of human immunodeficiency virus type 1 (MVP-5180) from Cameroon. J. Virol. **68**:1581–1585.
21. **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.
22. **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.
23. **Holmes, E. C., L. Q. Zhang, P. Simmonds, C. A. Ludlam, and A. J. Leigh Brown.** 1992. Convergent and divergent sequence evolution in the envelope glycoprotein of human immunodeficiency virus type 1 within a single infected patient. Proc. Natl. Acad. Sci. USA **89**:4835–4839.
24. **Ichimura, H., S. C. Klins, S. Visrutaratan, C.-Y. Ou, M. L. Kalish, and J. A. Levy.** 1994. Biological, serological, and genetic characterization of HIV-1 subtype E isolates from Northern Thailand. AIDS Res. Hum. Retroviruses **10**:263–269.
25. **Kayman, S. C., Z. Wu, K. Revesz, H. Chen, R. Kopelman, and A. Pinter.** 1994. Presentation of native epitopes in the V1/V2 and V3 regions of human immunodeficiency virus type 1 gp120 by fusion glycoproteins containing isolated gp120 domains. J. Virol. **68**:400–410.
26. **Korber, B. T. M., R. M. Farber, D. H. Wolpert, and A. S. Lapedes.** 1993. Covariation of mutations in the V3 loop of human immunodeficiency virus type 1 envelope protein: an information theoretic analysis. Proc. Natl. Acad. Sci. USA **90**:7176–7180.
27. **LaRosa, G. J., J. P. Davide, K. Weinhold, J. A. Waterbury, A. T. Profy, J. A. Lewis, A. J. Langlois, G. R. Dreesman, R. N. Boswell, P. Shadduck, C. H. Holley, M. Karplus, D. P. Bolognesi, T. J. Matthews, E. A. Emini, and S. D. Putney.** 1990. Conserved sequence and structural elements in the HIV-1 principal neutralizing determinant. Science **249**:932–935.
28. **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.
29. **Louwagie, J., E. L. Delwart, J. I. Mullins, F. E. McCutchan, G. Eddy, and D. S. Burke.** 1994. Genetic analysis of HIV-1 isolates from Brazil reveals presence of two distinct genetic subtypes. AIDS Res. Hum. Retroviruses **10**:561–567.
30. **Louwagie, J., F. McCutchan, M. Peeters, T. P. Brennan, E. Sanders-Buell, G. Eddy, G. van der Groen, K. Fransen, G.-M. Gershy-Damet, R. DeLeys, and D. S. Burke.** 1993. Comparison of gag genes from seventy international HIV-1 isolates provides evidence for multiple genetic subtypes. AIDS **7**:769–780.
31. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and brain. Cell **47**:333–348.
32. **Mascola, J. R., J. Louwagie, F. E. McCutchan, C. L. Fischer, P. A. Hegerich, K. F. Wagner, A. K. Fowler, J. G. McNeil, and D. S. Burke.** 1994. Two antigenically distinct subtypes of HIV-1: viral genotype predicts neutralization serotype. J. Infect. Dis. **169**:48–54.
33. **McCutchan, F. E., P. A. Hegerich, T. P. Brennan, P. Phanuphak, P. Singharaj, A. Jugsudee, P. W. Berman, A. M. Gray, A. K. Fowler, and D. S. Burke.** 1992. Genetic variants of HIV-1 in Thailand. AIDS Res. Hum. Retroviruses **8**:1887–1895.
34. **McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. Nicholson.** 1986. Binding of HTLV-III/LAV to T4+ T cells by a complex of the 110K viral protein and the T4 molecule. Science **231**:382–385.
35. **Milch, L., B. Margolin, and R. Swanstrom.** 1993. V3 loop of the human immunodeficiency virus type 1 env protein: interpreting

sequence variability. J. Virol. **67**:5623–5634.
36. **Modrow, S., B. H. Hahn, G. M. Shaw, R. C. Gallo, F. Wong-Staal, and H. Wolf.** 1987. Computer-assisted analysis of envelope protein sequences of seven human immunodeficiency virus isolates: prediction of antigenic epitopes in conserved and variable domains. J. Virol. **61**:570–578.
37. **Moore, J. P.** 1993. The reactivities of HIV-1+ human sera with solid-phase V3 loop peptides can be poor predictors of their reactivities with V3 loops on native gp120 molecules. AIDS Res. Hum. Retroviruses **9**:209–219.
38. **Moore, J. P., Y. Cao, A. J. Conley, R. Wyatt, J. Robinson, M. Gorny, S. Zolla-Pazner, D. D. Ho, and R. A. Koup.** 1994. Studies with monoclonal antibodies to the V3 region of HIV-1 gp120 reveal limitations to the utility of solid-phase peptide binding assays. J. Acquired Immune Defic. Syndr. **7**:332–339.
39. **Moore, J. P., Y. Cao, L. Qing, Q. J. Sattentau, J. Pyati, R. Koduri, J. Robinson, C. F. Barbas III, D. R. Burton, and D. D. Ho.** Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. J. Virol., in press
40. **Moore, J. P., M. Fung, J. Robinson, and J. Sodroski.** Unpublished data.
41. **Moore, J. P., and D. D. Ho.** 1993. Antibodies to discontinuous or conformationally-sensitive epitopes on the gp120 glycoprotein of human immunodeficiency virus type 1 are highly prevalent in sera of infected humans. J. Virol. **67**:863–875.
42. **Moore, J. P., B. A. Jameson, R. A. Weiss, and Q. J. Sattentau.** 1993. The HIV-cell fusion reaction, p. 233–289. *In* J. Bentz (ed.), Viral fusion mechanisms. CRC Press, Boca Raton, Fla.
43. **Moore, J. P., J. A. McKeating, I. M. Jones, P. E. Stephens, G. Clements, S. Thomson, and R. A. Weiss.** 1990. Characterisation of recombinant gp120 and gp160 from HIV-1: binding to monoclonal antibodies and sCD4. AIDS **4**:307–315.
44. **Moore, J. P., Q. J. Sattentau, R. Wyatt, and J. Sodroski.** 1994. Probing the structure of the surface glycoprotein gp120 of human immunodeficiency virus type 1 with a panel of monoclonal antibodies. J. Virol. **68**:469–484.
45. **Moore, J. P., Q. J. Sattentau, H. Yoshiyama, M. Thali, M. Charles, N. Sullivan, S.-W. Poon, M. S. Fung, F. Traincard, M. Pinkus, G. Robey, J. E. Robinson, D. D. Ho, and J. Sodroski.** 1993. Probing the structure of the V2 domain of the human immunodeficiency virus type 1 surface glycoprotein gp120 with a panel of eight monoclonal antibodies: the human immune response to the V1 and V2 domains. J. Virol. **67**:6136–6151.
46. **Moore, J. P., M. Thali, B. A. Jameson, F. Vignaux, G. K. Lewis, S.-W. Poon, M. S. Fung, P. J. Durda, L. Åkerblom, B. Wahren, D. D. Ho, Q. J. Sattentau, and J. Sodroski.** 1993. Immunochemical analysis of the gp120 surface glycoprotein of human immunodeficiency virus type 1: probing the structure of the C4 and V4 domains and the interaction of the C4 domain with the V3 loop. J. Virol. **67**:4785–4796.
47. **Moore, J. P., A. Trkola, B. Korber, L. J. Boots, J. A. Kessler II, F. E. McCutchan, J. Mascola, D. D. Ho, J. Robinson, and A. J. Conley.** A human monoclonal antibody to a complex epitope in the V3 region of gp120 of human immunodeficiency virus type 1 has broad reactivity within and outside clade B. J. Virol., in press.
48. **Moore, J. P., L. A. Wallace, E. A. C. Follett, and J. A. McKeating.** 1989. An enzyme-linked immunosorbent assay for antibodies to the envelope glycoproteins of divergent strains of HIV-1. AIDS **3**:155–163.
49. **Moore, J. P., H. Yoshiyama, D. D. Ho, J. E. Robinson, and J. Sodroski.** 1993. Antigenic variation in gp120s from molecular clones of HIV-1 LAI. AIDS Res. Hum. Retroviruses **9**:1179–1187.
50. **Morgado, M. G., E. C. Sabino, E. G. Shpaer, V. Bongertz, L. Brigido, M. D. C. Guimaraes, E. A. Castilho, B. Galvão-Castro, J. I. Mullins, R. M. Hendry, and A. Mayer.** 1994. V3 region polymorphisms in HIV-1 from Brazil: prevalence of subtype B strains divergent from North American/European prototype and detection of subtype F. AIDS Res. Hum. Retroviruses **10**:569–576.
51. **Morrison, H. G., F. Kirchhoff, and R. C. Desrosiers.** 1993. Evidence for the cooperation of gp120 amino acids 322 and 448 in SIV_{mac} entry. Virology **195**:167–174.
52. **Murphy, E., B. Korber, M.-C. Georges-Courbot, B. You, A. Pinter, D. Cook, M.-P. Kieny, A. Georges, C. Mathiot, F. Barré-Sinoussi, and M. Girard.** 1993. Diversity of V3 region sequences of human immunodeficiency virus type 1 from the Central African Republic. AIDS Res. Hum. Retroviruses **9**:997–1006.
53. **Myers, G., B. Korber, J. A. Berzofsky, R. F. Smith, and G. N. Pavlakis.** 1991. Human retroviruses and AIDS: a compilation and analysis of nucleic acid and amino acid sequences. Los Alamos National Laboratory, Los Alamos, N. Mex.
54. **Nakamura, G. R., R. Byrn, D. M. Wilkes, J. A. Fox, M. R. Hobbs, R. Hastings, H. C. Wessling, M. A. Norcross, B. M. Fendly, and P. W. Berman.** 1993. Strain specificity and binding affinity requirements of neutralizing monoclonal antibodies to the C4 domain of gp120 from human immunodeficiency virus type 1. J. Virol. **67**: 6179–6191.
55. **Okuda, K., H. Bukawa, S. Kawamoto, M. Imai, T. Saito, P. Phanuphak, and K. Hamajima.** 1994. A serologic analysis of the V3 region of human immunodeficiency virus from carriers in Bangkok. J. Infect. Dis. **169**:227–228.
56. **Olshevsky, U., E. Helseth, C. Furman, J. Li, W. Haseltine, and J. Sodroski.** 1990. Identification of individual human immunodeficiency virus type 1 gp120 amino acids important for CD4 binding. J. Virol. **64**:5701–5707.
57. **Orloff, G. M., M. L. Kalish, J. Chiphangwi, K. E. Potts, C.-Y. Ou, G. Schochetman, G. Dallabetta, A. I. Saah, and P. G. Miotti.** 1993. V3 loops of HIV-1 specimens from pregnant women in Malawi uniformly lack a potential N-linked glycosylation site. AIDS Res. Hum. Retroviruses **9**:705–706.
58. **Ou, C.-Y., Y. Takebe, B. G. Weniker, C.-C. Luo, M. L. Kalish, W. Auwanit, S. Yamazaki, H. D. Gayle, N. L. Young, and G. Schochetman.** 1993. Independent introduction of two major HIV-1 genotypes into distinct high-risk populations in Thailand. Lancet **341**: 1171–1174.
59. **Pau, C.-P., M. Kai, D. L. Holloman-Candal, C.-C. Luo, M. L. Kalish, G. Schochetman, B. Byers, J. R. George and the WHO Network for HIV Isolation and Characterization.** Antigenic variation and serotyping of HIV-1 from four WHO-sponsored HIV vaccine sites. AIDS Res. Hum. Retroviruses, in press.
60. **Pau, C.-P., S. Lee-Thomas, W. Auwanit, J. R. George, C.-Y. Ou, B. S. Parekh, T. C. Granade, D. L. Holloman, S. Phillips, G. Schochetman, N. L. Young, Y. Takebe, H. D. Gayle, and B. G. Weniger.** 1993. Highly specific V3-peptide enzyme immunoassay for serotyping HIV-1 specimens from Thailand. AIDS **7**:337–340.
61. **Pedroza Martins, L., N. Chenciner, and S. Wain-Hobson.** 1992. Complex intrapatient variation in the V1 and V2 hypervariable regions of the HIV-1 gp120 envelope sequence. Virology **191**:837–845.
62. **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.
63. **Potts, K. E., M. L. Kalish, C. I. Bandea, G. M. Orloff, M. St. Louis, C. Brown, N. Malanda, M. Kavuka, G. Schochetman, C.-Y. Ou, and J. L. Heyward.** 1993. Genetic diversity of human immunodeficiency virus type 1 strains in Kinshasa, Zaire. AIDS Res. Hum. Retroviruses **9**:613–618.
64. **Potts, K. E., M. L. Kalish, T. Lott, G. Orloff, C.-C. Luo, M. A. Bernard, C. B. Alves, R. Badaro, J. Suleiman, O. Ferreira, G. Schochetman, W. D. Johnson, Jr., C.-Y. Ou, J. L. Ho, and the Brazilian Collaborative AIDS Research Group.** 1993. Genetic heterogeneity of the V3 region of the HIV-1 envelope glycoprotein in Brazil. AIDS **7**:1191–1197.
65. **Roben, P., J. P. Moore, M. Thali, J. Sodroski, C. F. Barbas III, and D. R. Burton.** 1994. Recognition properties of a panel of human recombinant Fab fragments to the CD4 binding site of gp120 showing differing ability to neutralize human immunodeficiency virus type 1. J. Virol. **68**:4821–4828.
66. **Robey, W. G., B. Safai, S. Oroszlan, L. O. Arthur, M. A. Gonda, R. C. Gallo, and P. J. Fischinger.** 1985. Characterization of envelope and core structural gene products of HTLV-III with sera from AIDS patients. Science **228**:593–595.
67. **Robinson, J. E., H. Yoshiyama, D. Holton, S. Elliott, and D. D. Ho.**

1992. Distinct antigenic sites on HIV gp120 identified by a panel of human monoclonal antibodies. J. Cell. Biochem. **1992**(Suppl. 16E):71.

68. **Rübsamen-Waigmann, H., H. von Briesen, H. Holmes, A. Björndal, B. Korber, R. Esser, S. Ranjbar, P. Tomlinson, B. Galvao-Castro, E. Karita, S. Sempala, C. Wasi, S. Osmanoz, E. M. Fenyö, and the WHO Network for HIV Isolation and Characterization.** Standard conditions of virus isolation reveal biological variability of HIV-1 in different regions of the world. AIDS Res. Hum. Retroviruses, in press.
69. **Salminen, M., A. Nykänen, H. Brummer-Korvenkontio, M. L. Kantanen, K. Litsola, and P. Leinikki.** 1993. Molecular epidemiology of HIV-1 based on phylogenetic analysis of *in vivo gag* p7/p9 direct sequences. Virology **195:**185–194.
70. **Scott, C. F., Jr., S. Silver, A. T. Profy, S. D. Putney, A. Langlois, K. Weinhold, and J. E. Robinson.** 1990. Human monoclonal antibody that recognizes the V3 region of human immunodeficiency virus gp120 and neutralizes the human T-lymphotropic virus type IIIMN strain. Proc. Natl. Acad. Sci. USA **87:**8597–8601.
71. **Smith, J. D., C. B. Bruce, A. S. R. Featherstone, R. G. Downing, B. Biryahawaho, J. C. S. Clegg, J. W. Carswell, and J. D. Oram.** 1994. Reactions of Ugandan antisera with peptides encoded by V3 loop epitopes of human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses **10:**577–583.
72. **Starcich, B. R., B. H. Hahn, G. M. Shaw, P. D. McNeely, S. Modrow, H. Wolf, W. P. Parks, S. F. Josephs, R. C. Gallo, and F. Wong-Staal.** 1986. Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell **45:**637–648.
73. **Steimer, K. S., C. J. Scandella, P. V. Stiles, and N. L. Haigwood.** 1991. Neutralization of divergent HIV-1 isolates by conformation-dependent human antibodies to gp120. Science **254:**105–108.
74. **Stomatatos, L., and C. Cheng-Mayer.** 1993. Evidence that the structural conformation of envelope gp120 affects human immunodeficiency virus type 1 infectivity, host range, and syncytium-forming ability. J. Virol. **67:**5635–5639.
75. **Sullivan, N., M. Thali, C. Furman, D. Ho, and J. Sodroski.** 1993. Effect of amino acid changes in the 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.
76. **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.
77. **Thali, M., J. P. Moore, C. Furman, M. Charles, D. D. Ho, J. Robinson, and J. Sodroski.** 1993. Characterization of conserved human immunodeficiency virus type 1 gp120 neutralization epitopes exposed upon gp120-CD4 binding. J. Virol. **67:**3978–3988.
78. **Vanden Haesevelde, M., J.-L. Cecourt, R. J. De Leys, B. Vanderborght, G. van der Groen, H. van Heuverswijn, and E. Saman.** 1994. Genomic cloning and complete sequence analysis of a highly divergent African human immunodeficiency virus isolate. J. Virol. **68:**1586–1596.
79. **Weiss, R. A., P. R. Clapham, J. N. Weber, A. G. Dalgleish, L. A. Lasky, and P. W. Berman.** 1986. Variable and conserved neutralisation antigens of HIV. Nature (London) **324:**572–575.
80. **White-Scharf, M. E., B. J. Potts, L. M. Smith, K. A. Sokolowski, J. R. Rusche, and S. Silver.** 1993. Broadly neutralizing monoclonal antibodies to the V3 region of HIV-1 can be elicited by peptide immunization. Virology **192:**197–208.
81. **WHO Network for HIV Isolation and Characterization.** HIV-1 variation in WHO-sponsored vaccine-evaluation sites: genetic screening, sequence analysis and preliminary biological characterization of selected viral strains. AIDS Res. Hum. Retroviruses, in press.
82. **Willey, R. L., and M. A. Martin.** 1993. Association of human immunodeficiency virus type 1 envelope glycoprotein with particles depends on interactions between the third variable and conserved regions of gp120. J. Virol. **67:**3639–3643.
83. **Willey, R. L., E. K. Ross, A. J. Buckler-White, T. S. Theodore, and M. A. Martin.** 1989. Functional interaction of constant and variable domains of human immunodeficiency virus type 1 gp120. J. Virol. **63:**3595–3600.
84. **Wyatt, R., M. Thali, S. Tilley, A. Pinter, M. Posner, D. Ho, J. Robinson, and J. Sodroski.** 1992. Relationship of the human immunodeficiency virus type 1 gp120 third variable loop to elements of the CD4 binding site. J. Virol. **66:**6997–7004.
85. **Yoshiyama, H., H.-M. Mo, J. P. Moore, and D. D. Ho.** 1994. Characterization of mutants of human immunodeficiency virus type 1 that have escaped neutralization by monoclonal antibody G3-4 to the gp120 V2 loop. J. Virol. **68:**974–978.
86. **Zhu, T., H. Mo, N. Wang, D. S. Nam, Y. Cao, R. A. Koup, and D. D. Ho.** 1993. Genotypic and phenotypic characterization of HIV-1 in patients with primary infection. Science **261:**1179–1181.
87. **Zwart, G., T. F. W. Wolfs, R. Bookelman, S. Hartman, M. Bakker, C. A. B. Boucher, C. Kuiken, and J. Goudsmit.** 1993. Greater diversity of the HIV-1 V3 neutralization domain in Tanzania compared with The Netherlands: serological and genetic analysis. AIDS **7:**467–474.