

Cross-Clade Neutralization of Primary Isolates of Human Immunodeficiency Virus Type 1 by Human Monoclonal Antibodies and Tetrameric CD4-IgG

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We have tested three human monoclonal antibodies (MAbs) IgG1b12, 2G12, and 2F5 to the envelope glycoproteins of human immunodeficiency virus type 1 (HIV-1), and a tetrameric CD4-IgG molecule (CD4-IgG2), for the ability to neutralize primary HIV-1 isolates from the genetic clades A through F and from group O. Each of the reagents broadly and potently neutralized B-clade isolates. The 2F5 MAb and the CD4-IgG2 molecule also neutralized strains from outside the B clade, with the same breadth and potency that they showed against B-clade strains. The other two MAbs were able to neutralize a significant proportion of strains from outside the B clade, although there was a reduction in their efficacy compared with their activity against B-clade isolates. Neutralization of isolates by 2F5 correlated with their possession of the LDKW motif in a segment of gp41 near the membrane-spanning domain. The other two MAbs and CD4-IgG2 recognize discontinuous binding sites on gp120, and so no comparison between genetic sequence and virus neutralization was possible. Our data show that a vaccine based on the induction of humoral immunity that is broadly active across the genetic clades is not impossible if immunogens that express the epitopes for MAbs such as 2F5, 2G12, and IgG1b12 in immunogenic configurations can be created. Furthermore, if the three MAbs and CD4-IgG2 produce clinical benefit in immunotherapeutic trials in the United States or Europe, they may also do so elsewhere in the world.

It is now clear that primary human immunodeficiency virus type 1 (HIV-1) isolates are relatively resistant to neutralization by antibodies as well as by CD4-based reagents (3, 18, 38, 40, 47, 73, 77, 81). Yet it is also apparent that primary isolates can be neutralized strongly by certain monoclonal antibodies (MAbs) and by some sera from HIV-1-infected humans (2, 11, 12, 16, 41, 43, 64, 76). The targets for the effective antibodies in human sera are not known, and the epitopes for active MAbs tend to be discontinuous and incompletely characterized (60, 65, 76). Consequently, the identity of the principal neutralizing determinant(s) on primary viruses is unresolved. What is also unknown is the breadth of neutralization shown by effective reagents. How conserved on primary HIV-1 isolates are the targets that are vulnerable to neutralizing antibodies?

These questions are relevant to the design of any HIV-1 vaccine that might be effective against HIV-1 isolates from multiple clades. This task is challenging, as it is not clear that the current generation of vaccine candidates will be able to protect humans from viruses closely related genetically to the immunogen (24, 38, 81). Furthermore, the correlates of protective immunity to field isolates of HIV-1 remain unknown, complicating rational vaccine design. However, it remains logical to assume that the induction of broadly neutralizing antibodies is a desirable feature of a vaccine candidate, on the grounds that preexisting antibodies may prevent the transmis-

sion of cell-free HIV-1 or limit the initial dissemination of a transmitted infection (7).

To address the issue of cross-clade neutralization, we have tested four reagents for their abilities to neutralize primary HIV-1 isolates from clades A through F and from group O. The test reagents selected were the human MAbs (HuMAbs) IgG1b12, 2G12, and 2F5 and the soluble CD4-based reagent tetrameric CD4-IgG (CD4-IgG2). In our experience, these are the broadest and most potently neutralizing reagents against clade B primary viruses described to date. We find that among the M group, strains from outside clade B are comparable to B-clade strains in terms of sensitivity to neutralization by CD4-IgG2 and 2F5. MAbs 2G12 and IgG1b12 are also able to neutralize a significant number of HIV-1 strains from outside clade B, although a greater proportion of non-B-clade strains than B-clade strains escape neutralization by these MAbs. These results suggest that cross-clade neutralizing antibodies exist; if immunogens can be designed to induce such antibodies efficiently, a broadly active HIV-1 vaccine based on humoral immunity might be an achievable goal.

MATERIALS AND METHODS

MAbs. All HuMAbs were isolated from individuals infected with clade B viruses. IgG1b12 is a human recombinant antibody initially isolated as a Fab fragment by screening against recombinant BRU gp120 (5, 11). It recognizes a discontinuous CD4-binding-site-related epitope on monomeric gp120 (65) but shows preferential reactivity with the oligomeric form of the envelope glycoproteins (65, 67). HuMAb 2G12 was isolated by screening against IIB, MN, and RF-infected cells and IIB gp160 (10); it recognizes a conformationally sensitive gp120 epitope unrelated to the V1, V2, or V3 loop or to the CD4-binding site (76). 2F5 is a gp41 HuMAb that has been mapped to the sequence ELDKWA (10, 16, 52, 53, 64), which is accessible on the oligomeric form of the envelope

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glycoproteins (69). Tetrameric CD4-IgG2 was prepared as described elsewhere (2). Dimeric CD4-IgG was obtained from Genentech Inc. (13).

Virus isolates. Virus isolates were collected from various regions of the world by three organizations: the World Health Organization (WHO) (4, 20, 22, 66, 80), the Henry M. Jackson Foundation for the Advancement of Military Medicine and the Military Medical Consortium for Applied Retroviral Research (HMJF/MMCARR) (34–37, 39), and the National Institute of Allergy and Infectious Diseases (NIAID) (42, 46). The F-clade strain R1 was a gift from Jay Levy (21), and the HIV-1 group O strains CA9 and MVP 5180 were provided by Guido van der Groen and Leo Gürtler (23, 58). Strain MVP 5180 had previously been passaged in H9 cells (23). Viruses designated by a code in the format exemplified by UG92/005 were provided by the WHO (32); viruses designated by a code in the format exemplified by DJ258 were from the HMJF/MMCARR repository. All other viruses were from the NIAID collection. All of the clade B isolates were collected in the United States except for 301593 (Haiti), BR92/019 (Brazil), and BK132 (Thailand). Isolates were expanded in mitogen-stimulated peripheral blood mononuclear cells (PBMC) (18, 37, 66), and culture supernatants containing infectious virus were stored at -80°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 (4, 19, 22, 34–37, 55, 80).

Neutralization assay. Virus neutralization was assessed by using phytohemagglutinin-stimulated PBMC as indicator cells, with determination of p24 antigen production as the endpoint. PBMC were stimulated with phytohemagglutinin for 48 h before removal of the mitogen by washing. Antibodies were adjusted to 100 $\mu\text{g}/\text{ml}$, and eight twofold serial dilutions were prepared. An aliquot (50 μl) of each dilution step was transferred to 4 replicate wells of a 96-well flat-bottom culture plate. The virus inoculum was adjusted to 200 50% tissue culture infective doses (TCID₅₀)/ml, and a 50- μl (10-TCID₅₀) aliquot was incubated with MAb (50 μl) for 1 h at 37°C . The calculated neutralization titers refer to the MAb concentrations present during this preincubation step. The MAb-virus mixture was then diluted twofold by addition of 100 μl of stimulated PBMC at a density of $4 \times 10^6/\text{ml}$, and the cultures were incubated for 7 days. The cultures were collected and treated with 1% Empigen detergent, and replicate wells were pooled before determination of the p24 antigen concentration by using an in-house enzyme-linked immunosorbent assay (ELISA) (47). As the virus-MAb inoculum was not washed away from the cells at any stage, we included in each assay 32 wells containing 50 μl of virus and 150 μl of medium to provide an estimate of the amount of input p24 antigen that carried over into the p24 antigen assay for virus production. This value was subtracted from all test results.

To ensure that an appropriate degree of virus infection of the culture had occurred in each experiment, the input virus was titrated to determine its TCID₅₀. The virus stock was serially diluted in fivefold steps, and a 50- μl aliquot of each dilution was added to 8 replicate wells of a 96-well plate. After addition of 50 μl of medium and 100 μl of cell suspension, the plates were incubated for 7 days. The number of cultures producing p24 antigen was estimated, and the TCID₅₀ was calculated by the method of Reed and Muench as described elsewhere (64). Virus production in the absence of MAb was measured from 32 wells containing 50 μl of virus, 50 μl of medium, and 100 μl of PBMC and was designated as equivalent to 100%. The ratios of p24 antigen production in MAB-containing cultures to p24 antigen production in these control cultures were estimated. The MAB concentrations (micrograms per milliliter) causing 50, 90, and 99% reduction in p24 antigen production were determined by linear regression analysis; these are the ID₅₀, ID₉₀, and ID₉₉ values. If the appropriate degree of virus neutralization was not achieved at the highest MAB input concentration tested (50 $\mu\text{g}/\text{ml}$), a value of >50 $\mu\text{g}/\text{ml}$ was recorded. The neutralization curves depicted in Fig. 1 are derived from a single experiment, while the tabulated data (Tables 1 and 2) are the mean values derived from two to four experiments with each MAB-virus combination. Greater weight should therefore be put on the tabulated data than on those in Fig. 1.

We consider 90% neutralization to be the minimum that is both experimentally and biologically significant in our neutralization assay. Ideally, a truly potent antibody should reduce infectivity by 99% or greater. However, we deem an ID₉₀ of ≤ 50 $\mu\text{g}/\text{ml}$ to be indicative of significant neutralization of a primary isolate by a MAB or CD4-IgG2, and it is this criterion which we use below when describing an isolate as “neutralized” without any qualifiers. We selected a MAB concentration of 50 $\mu\text{g}/\text{ml}$ as a cutoff because this is the upper range of estimates of the concentration of an antibody to the HIV-1 envelope glycoproteins that can be produced *in vivo* from an individual B-cell clone (25) and because 50 $\mu\text{g}/\text{ml}$ is close to the highest concentration of a MAB in plasma that could reasonably be achieved by passive infusion in humans.

MAB-gp120 binding assay. Infectious culture supernatants containing virus and free gp120 were treated with 1% nonionic detergent Nonidet P-40 to provide a source of gp120 (41, 42, 46). Binding assays for gp120 MABs and CD4-IgG2 were then performed essentially as described elsewhere (41, 46, 50). Briefly, gp120 from culture supernatants, diluted 1:2 to 1:16 in Tris-buffered saline containing 10% fetal calf serum and 1% Nonidet P-40, was captured onto solid phase via its carboxy terminus, using sheep polyclonal antibody D7324 (Aalto Bioreagents, Dublin, Ireland). CD4-IgG2, dimeric CD4-IgG, IgG1b12, or 2G12 was added in Tris-buffered saline containing 2% nonfat milk powder, 20% sheep serum, and 0.5% nonionic detergent Tween 20 at concentrations ranging from 4

ng/ml to 4 $\mu\text{g}/\text{ml}$, and bound MAB, CD4-IgG, or CD4-IgG2 was detected with alkaline phosphatase-conjugated goat anti-human IgG (41, 42, 46).

Statistical analysis. Wilcoxon rank sum tests were performed with the Splus package version 3.2 (Mathsoft, Inc., Seattle, Wash.). Tests were conducted either by including neutralization values of >50 $\mu\text{g}/\text{ml}$ as being equal to 50 $\mu\text{g}/\text{ml}$ or by excluding them from the data sets. Statistical differences in the neutralization parameters for B-clade and non-B-clade strains were observed only when the values of >50 $\mu\text{g}/\text{ml}$ were included.

RESULTS

Primary virus neutralization by MABs and CD4-IgG2.

Three PBMC-grown primary HIV-1 isolates from each of clades A, C, E, and F and 4 from clade D were selected for analysis, along with 12 clade B primary isolates (9 from the United States, 1 from Haiti, 1 from Thailand, and 1 from Brazil) for comparative purposes. The only criteria for inclusion of isolates in the test panel were their availability and their ability to grow to relatively high titers in PBMC ($\geq 10^{3.5}$ TCID₅₀/ml). The viral genetic sequences either were not known or were not a factor in the choice of test isolates. In addition, one group O primary isolate (CA9) and one group O isolate previously passaged in the H9 T-cell line (MVP 5180), but now grown in PBMC, were studied. However, data from experiments with the O-group isolates were analyzed separately, because of the divergence of these isolates from the other HIV-1 strains and because one of the two available O-group isolates had been passaged in a T-cell line.

Representative neutralization titration curves for one isolate from each of clades A through F are depicted in Fig. 1, and the mean ID₅₀s, ID₉₀s, and ID₉₉s derived from multiple neutralization titrations with each reagent against each isolate are listed in Table 1 (B-clade isolates) and Table 2 (non-B-clade isolates). Collated neutralization data for each reagent against the B- and non-B-clade isolates are presented in Table 3. The median ID₅₀s, ID₉₀s, and ID₉₉s recorded in this table were calculated by taking into account both neutralized and non-neutralized strains, but the arithmetic mean values were calculated only for those isolates for which the appropriate degree of infectivity reduction was achieved at ≤ 50 $\mu\text{g}/\text{ml}$. Also recorded is the proportion of isolates that were neutralized by each reagent by 50, 90, and 99% at a MAB concentration of ≤ 50 $\mu\text{g}/\text{ml}$ (Table 3).

As expected from our previous studies, each of the three MABs and CD4-IgG2 showed broad and potent neutralization of the B-clade primary isolates. Thus, of the 12 B-clade isolates, 11 were neutralized by CD4-IgG2, 10 were neutralized by IgG1b12, 9 were neutralized by 2G12, and 10 were neutralized by 2F5 (Table 1). The mean and median ID₉₀s for the group of B-clade isolates were in the range of 20 to 30 $\mu\text{g}/\text{ml}$ for CD4-IgG2, IgG1b12, and 2F5, but the mean and median ID₉₀s for 2G12 were significantly less than this range (8.9 and 4.8 $\mu\text{g}/\text{ml}$) (Table 3). This reflects the more extreme variation in the performance of 2G12 against primary isolates than shown by the other three MABs; when 2G12 neutralized a primary isolate, it usually did so at low concentrations (<5 $\mu\text{g}/\text{ml}$), but 3 of the 12 B-clade isolates tested were completely resistant to 2G12 (Tables 1 and 3). This probably reflects their lack of the 2G12 epitope. Each of the four reagents was also able to reduce the infectivity of several B-clade isolates by 99% at input concentrations of ≤ 50 $\mu\text{g}/\text{ml}$, the proportion of the 12 isolates that were sensitive at this level ranging from 4 to IgG1b12 and 2F5 to 7 to CD4-IgG2 (Table 3).

When tested against isolates from clades other than B, each of the four test reagents showed some neutralizing ability (Tables 2 and 3). Indeed, the neutralization activities shown by CD4-IgG2 and 2F5 against the non-B-clade isolates were es-

TABLE 1. Neutralization titers of MAbs and CD4-IgG2 against viruses of clade B^a

Isolate	IgG1b12			CD4-IgG2			2G12			2F5		
	ID ₉₉	ID ₉₀	ID ₅₀	ID ₉₉	ID ₉₀	ID ₅₀	ID ₉₉	ID ₉₀	ID ₅₀	ID ₉₉	ID ₉₀	ID ₅₀
301593	46	19	2.7	>50	19	4.8	5.8	0.3	0.1	23	8.6	3.2
301657	>50	32	9.9	45	14	1.8	>50	4.2	0.1	33	13	1.1
301660	>50	38	15	>50	22	7.7	5.9	0.5	0.1	36	14	0.1
301714	>50	19	6.0	11	4.7	1.0	>50	>50	22	>50	46	12
301717	23	9.4	2.9	46	29	7.6	>50	>50	>50	32	15	5.1
301727	>50	>50	26	37	22	9.9	11	2.1	0.1	>50	>50	>50
302054	>50	23	5.1	>50	36	11	>50	>50	>50	>50	36	13
302056	34	18	6.3	37	25	8.1	>50	7.5	1.8	>50	>50	>50
302073	43	12	2.9	27	14	3.6	11	1.8	0.3	>50	30	4.6
301712	>50	32	3.2	>50	16	3.1	>50	19	0.6	>50	34	0.8
BR92/019	>50	>50	17	>50	>50	23	>50	3.4	0.4	>50	26	3.7
BK132	>50	24	9.3	27	5.2	3.2	7.0	5.4	0.4	>50	27	9.6

^a ID₅₀, ID₉₀, and ID₉₉ values (micrograms per milliliter) are the means of values derived from two to four individual experiments. If the extent of infectivity reduction did not reach 50, 90, or 99%, a value of >50 µg/ml is recorded.

essentially indistinguishable from their activities against the group of B-clade isolates (Table 3); the differences in neutralization values for CD4-IgG2 and 2F5 against B-clade and non-B-clade strains were found not to be statistically significant with a nonparametric Wilcoxon rank test (data not shown). The conservation of neutralization potency across clades was, of course, to be expected for CD4-IgG2, as the structure of the CD4-binding site on gp120 is unlikely to vary significantly in a clade-dependent manner (46). However, the strong performance of 2F5 against non-B-clade strains was a less predictable result.

MAbs 2G12 and IgG1b12 were able to neutralize 8 and 9 of the 16 non-B-clade isolates, respectively (ID₉₀, ≤50 µg/ml). Conversely, eight and seven non-B-clade isolates were highly or totally resistant to 2G12 and IgG1b12. The variable efficacy of 2G12 and IgG1b12 is reflected in the high median ID₉₀s of

>50 and 44 µg/ml for these MAbs against the non-B-clade strains (Table 3). Yet whenever 2G12 and IgG1b12 were active against non-B-clade strains, it was with significant potency; the mean ID₉₀s for these MAbs against susceptible non-B-clade strains were <2-fold greater than the corresponding values for susceptible B-clade strains (Table 3). A nonparametric Wilcoxon rank test showed that there was a significant increase ($P = 0.04$) in the mean ID₉₀ for IgG1b12 against non-B-clade strains (35 µg/ml) compared with B-clade strains (22 µg/ml). For 2G12, the corresponding mean ID₉₀ increase (14 µg/ml compared with 8.9 µg/ml) was not statistically significant ($P = 0.13$). At the level of 99% neutralization, each of the four test reagents still possessed activity against non-B-clade isolates, although this was only rarely observed with IgG1b12 and 2G12 (Table 3).

TABLE 2. Neutralization titers of MAbs and CD4-IgG2 against viruses of clades A, C, D, E, and F and group O

Clade or group	n	Isolate	IgG1b12			CD4-IgG2			2G12			2F5		
			ID ₉₉	ID ₉₀	ID ₅₀	ID ₉₉	ID ₉₀	ID ₅₀	ID ₉₉	ID ₉₀	ID ₅₀	ID ₉₉	ID ₉₀	ID ₅₀
Clade A	3	RW92/026	>50	>50	>50	>50	29	9.0	>50	13	0.4	37	22	5.8
		DJ258	>50	49	35	>50	35	16	4.5	1.1	0.4	>50	34	5.2
		UG273	>50	38	10	>50	13	2.6	18	2.5	0.4	>50	19	4.3
Clade C	3	DJ259	>50	>50	29	>50	27	14	>50	>50	31	>50	34	17
		SG364	>50	17	6.4	15	3.7	0.6	>50	>50	30	>50	>50	>50
		ZAM20	>50	>50	>50	>50	18	0.7	>50	0.4	0.4	>50	25	4.5
Clade D	4	UG270	14	5.2	0.4	7.3	3.4	0.4	>50	42	7.3	>50	29	13
		UG93/070	>50	>50	26	>50	>50	32	>50	>50	>50	>50	>50	>50
		SG365	37	33	12	17	8.4	4.9	7.0	3.4	0.4	>50	46	26
		UG92/005	49	40	24	42	5.3	0.4	>50	46	12	>50	22	3.9
Clade E	3	CM235	>50	>50	20	39	20	13	>50	>50	44	>50	23	4.6
		TH92/001	>50	33	19	15	9.2	2.4	>50	>50	>50	13	3.3	1.3
		CM238	>50	32	6.4	>50	18	5.0	>50	>50	10	29	6.0	2.5
Clade F	3	BZ162	>50	>50	29	21	6.4	1.1	>50	>50	>50	36	22	6.2
		R1	>50	35	6.2	23	13	4.6	>50	>50	37	>50	15	5.5
		BZ163	>50	>50	49	>50	27	5.8	>50	3.0	0.7	>50	33	8.7
Group O	2	CA9	>50	>50	17	25	16	7.5	>50	>50	37	>50	>50	22
		MVP 5180	>50	>50	>50	3.3	0.4	0.4	>50	>50	26	>50	>50	>50

^a ID₅₀, ID₉₀, and ID₉₉ values (micrograms per milliliter) are the means of values derived from two to four individual experiments. If the extent of infectivity reduction did not reach 50, 90, or 99%, a value of >50 µg/ml is recorded.

TABLE 3. Neutralization of B and non-B (clade A, C, D, E, and F) isolates by MAbs and CD4-IgG2^a

Antibody and isolate type	ID ₉₉		% Neutralized viruses	ID ₉₀		% Neutralized viruses	ID ₅₀		% Neutralized viruses
	Mean	Median		Mean	Median		Mean	Median	
IgG1b12									
B	36	>50	33	22	23	83	8.8	6.1	100
Non-B	33	>50	19	31	44	56	19	22	88
CD4-IgG2									
B	33	46	58	19	20	92	7.0	6.2	100
Non-B	22	>50	50	16	15	94	7.0	4.8	100
2G12									
B	8.0	>50	42	8.9	4.8	75	2.6	0.4	83
Non-B	9.8	>50	19	14	>50	50	13	11	81
2F5									
B	31	>50	33	25	28	83	5.3	4.8	83
Non-B	29	>50	25	24	24	88	7.7	5.7	88

^a For each reagent, arithmetic mean and median ID₅₀s, ID₉₀s, and ID₉₉s (micrograms per milliliter) for B-clade ($n = 12$) and non-B-clade ($n = 16$) strains were estimated. The median values take both neutralized and nonneutralized strains into account. Arithmetic mean values were calculated only for the neutralized strains. The percentages of viruses neutralized by each reagent at the 50, 90, and 99% levels are also recorded.

As only three or four isolates from each of clades A, C, D, E, and F were tested, we cannot conclude with any certainty whether isolates from any one of these clades are more or less sensitive to the test reagents than isolates from the other clades. It may be noted, however, that 2G12 strongly neutralized all three clade A isolates against which it was tested but was almost completely ineffective against the three clade E strains (Table 2). We note that 2G12 binding to monomeric gp120 from HIV-1 LAI is sensitive to, inter alia, amino acid substitutions in the V4 loop (48, 76); E-clade strains have particularly unusual V4 sequences, with an additional pair of cysteine residues (55). Whether this contributes to the inactivity of 2G12 against E-clade strains remains to be determined.

Overall, 15 of the 16 isolates from clades A, C, D, E, and F had their infectivities reduced by $\geq 90\%$ by at least one of the test reagents at an input concentration $\leq 50\% \mu\text{g/ml}$; neutralization by this criterion was observed with 46 of the 64 (72%) combinations of 16 viruses and four reagents (Table 2). However, ID₉₀s of $\leq 10 \mu\text{g/ml}$ were much rarer (14 of 64; 22%), as were ID₉₉s of $\leq 50 \mu\text{g/ml}$ (18 of 64; 28%) (Table 2). For comparison, $\geq 90\%$ neutralization at $\leq 50 \mu\text{g/ml}$ was observed with 40 of 48 (83%) combinations of the test reagents with clade B isolates, ID₉₀s of $\leq 10 \mu\text{g/ml}$ were seen with 12 of 48 combinations (25%), and ID₉₉s of $\leq 50 \mu\text{g/ml}$ were seen with 20 of 48 (42%) (Tables 1 and 3). Thus, taking the data together, we found little difference between B-clade and non-B-clade isolates in terms of sensitivity to the four test reagents, CD4-IgG2, IgG1b12, 2G12, and 2F5, with the possible exception that ID₉₉s of $\leq 50 \mu\text{g/ml}$ were more frequent among B-clade isolates. Omitting CD4-IgG2 data from these calculations did not change this conclusion (not shown).

Among the non-B-clade strains, examples of particularly potent neutralization were UG273 (clade A) by 2G12 (Fig. 1a),

BK132 (clade B) by CD4-IgG2 and 2G12, ZAM20 (clade C) by 2G12 (Table 2), UG270 (clade D) by CD4-IgG2 (Fig. 1d), TH92/001 (clade E) by 2F5 (Fig. 1e), and BZ163 (clade F) by 2G12. The group O strain MVP 5180 was also extremely sensitive to CD4-IgG2, but this almost certainly reflects the passage history of this strain in a T-cell line. Some viruses, for example, the D-clade isolate UG 93/070 and the E-clade isolate CM235, were particularly refractory to neutralization by the test reagents. Both group O isolates were resistant to all three of the MAbs (Table 2). Whether this reflects a general resistance of these isolates to all group M strain-derived neutralizing agents or a specific resistance to just our test panel is not yet known.

HuMAb 2G12 caused significant ($>90\%$, often $>99\%$) reductions in the infectivity of several isolates at relatively low concentrations, but increasing the 2G12 concentration further caused little or no additional loss of infectivity. These isolates include UG273 (Fig. 1a), BR92/019, ZAM20, and BZ163. We presume that this phenomenon reflects the presence of a significant population of viral variants resistant to 2G12 because of sequence changes within or around the epitope, forming a nonneutralizable fraction. The other reagents seem less prone to this problem, their neutralizing ability being much more a concentration-dependent, all-or-none event. However, despite the presence of nonneutralizable fractions in some isolates, 2G12 was highly potent against the neutralizable fractions of most of those isolates against which it had any significant activity.

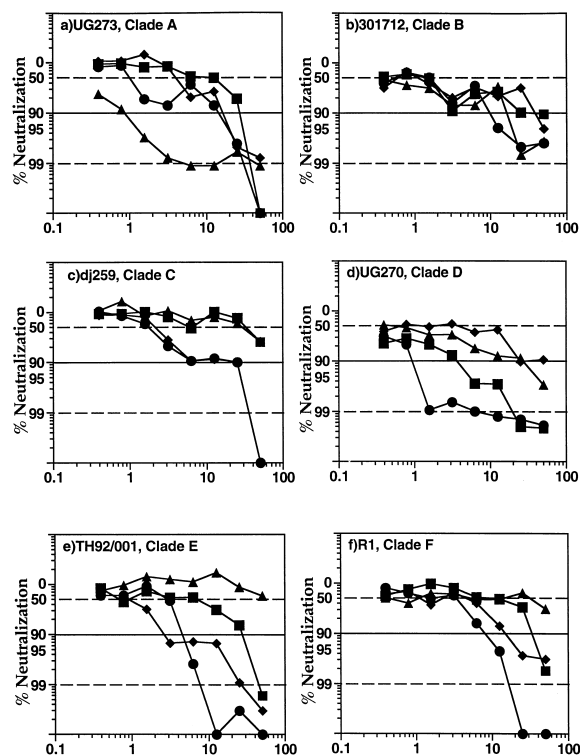


FIG. 1. Neutralization of primary viruses of clades A, B, C, D, E, and F with CD4-IgG2 and MAbs IgG1b12, 2G12, and 2F5. CD4-IgG2 or MAbs were incubated with 100 TCID₅₀ of the indicated viruses per ml. The extent of virus replication was monitored by measurement of p24 antigen after 7 days. The percentage of neutralization recorded is plotted against the reagent concentration present. Each titration curve shown represents data from a single experiment; data from two to four experiments on each MAb-virus combination were used to derive the mean values presented in Tables 1 and 2. The test reagents were IgG1b12 (■), CD4-IgG2 (●), 2G12 (▲), and 2F5 (◆).

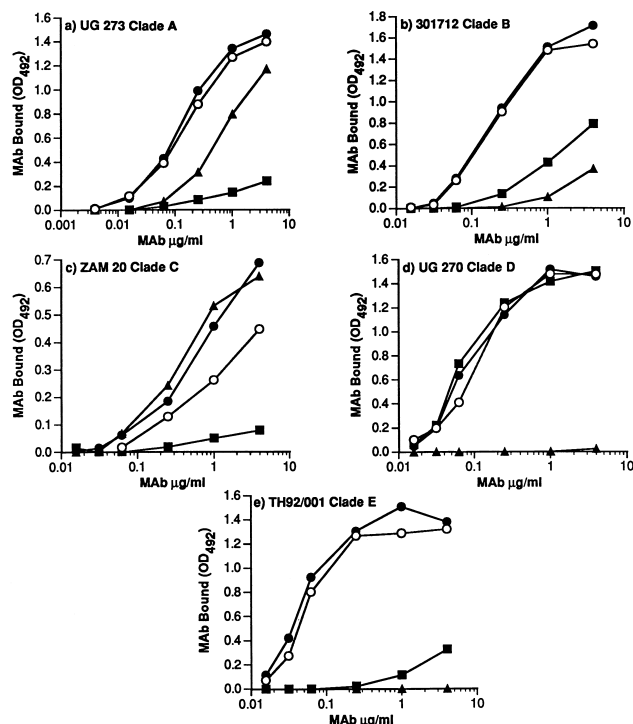


FIG. 2. Binding of CD4-IgG2 and MABs to detergent-solubilized gp120. CD4-IgG2, CD4-IgG, or MABs were incubated with antibody D7324-captured gp120 derived from PBMC-grown cultures of HIV-1 strains from clades A through E, and bound reagent was detected. The reagents were IgG1b12 (■), CD4-IgG2 (●), 2G12 (▲), 2F5 (◆), and CD4-IgG (○). OD₄₉₂, optical density at 492 nm.

gp120 and gp160 binding assays. We have previously shown that the resistance of primary HIV-1 isolates from clade B to neutralization by MABs and soluble CD4 is not usually due to the absence of the MAB epitope from the gp120 monomer (41, 43, 50). Furthermore, we have demonstrated that many gp120 MABs to discontinuous epitopes, including IgG1b12, show broad, cross-clade reactivity with monomeric gp120 (46). We therefore determined whether CD4-IgG2, IgG1b12, and 2G12 were able to bind to gp120 molecules derived from the viruses used in the neutralization experiments (Fig. 2; Table 4). For comparison with previous data sets (46), we also measured the binding of the dimeric CD4-IgG molecule. Note that the highest MAB or CD4-IgG2 concentration tested was 4 µg/ml. Furthermore, it was not possible to test the F-clade strains for MAB reactivity, as some cultures did not produce sufficient gp120 or the gp120 produced was inadequately reactive with the anti-gp120 C-terminal antibody that we use in our ELISA procedure (46).

Every solubilized gp120 that could be tested bound CD4-IgG and CD4-IgG2 with high affinity, half-maximal binding occurring at 0.1 to 1 µg of these reagents per ml (Fig. 2). However, isolates BR92/019 and UG93/070 were highly resistant to neutralization by CD4-IgG2 (Tables 1 and 2), exemplifying the point that gp120 binding does not predict virus neutralization by soluble CD4-based reagents (3, 18, 40, 47, 49). The binding of IgG1b12 to gp120 was sporadic, as we observed previously (46), with the most consistent reactivity of this MAB being with D-clade strains. It was notable, however, that IgG1b12 bound well to gp120 from the D-clade strain UG93/070 (Table 4) but did not neutralize this virus (Table 2). This finding is reminiscent of the observations with CD4-IgG2.

Conversely, gp120 from several isolates that were neutralizable by IgG1b12, including UG273, BK132, SG364, TH92/001, and CM238, reacted poorly with this MAB, possibly because of the lower concentrations of IgG1b12 used in the binding assays than in the neutralization tests (maxima of 4 and 50 µg/ml, respectively). Alternatively, the IgG1b12 epitope, which is influenced by envelope glycoprotein oligomerization (65, 67), may be more sensitive to nonionic detergent on some strains than on others.

There was a much stronger correlation between MAB 2G12 binding to gp120 and virus neutralization (Tables 1, 2, and 4). Thus, 2G12 bound strongly to gp120 from strains RW92/026, DJ258, UG273, BR92/019, BK132, ZAM20, and SG365 and potently neutralized the corresponding isolates. 2G12 also bound weakly to gp120 from 301712, an isolate neutralized only moderately by 2G12. No 2G12 binding to gp120 from strain DJ259, SG364, UG270, UG93/070, UG92/005, CM235, TH92/001, or CM238 could be detected, and these isolates either were not neutralized by 2G12 or were only weakly neutralized (UG270 and UG92/005). Assays of MAB binding to solubilized gp120 appear, therefore, to have some value for predicting qualitatively virus neutralization by MAB 2G12, although not by other reagents such as IgG1b12 or CD4-IgG2.

Sequence analysis. We were not able to devise a gp41 antibody ELISA of sufficient breadth of reactivity with non-B-clade gp41 molecules to assess in binding assays the reactivity of 2F5 reactivity with gp41. However, previous studies have shown that the 2F5 epitope is centered around the linear sequence ELDKWA near the transmembrane segment of gp41 (16, 52, 53). We therefore compared available gp41 sequences from isolates neutralized or nonneutralized by 2F5 to determine if there were any correlations between viral sequence and neutralizability (Table 5). Although gp41 sequences were available from only 15 of the 30 HIV-1 strains that we tested, a pattern was clearly observed; all 13 2F5-sensitive, sequenced strains possessed the LDKW central motif, and the 2 insensitive, sequenced strains (SG364 and 5180) had amino acid

TABLE 4. Binding of MABs and CD4-IgG2 to soluble gp120 of primary viruses from clades A, B, C, D, and E^a

Isolate	IgG1b12		CD4-IgG2		2G12	
	1 µg/ml	4 µg/ml	1 µg/ml	4 µg/ml	1 µg/ml	4 µg/ml
RW92/026	0.11	0.12	1.07	0.68	0.43	0.48
DJ258	0.14	0.38	1.08	1.03	1.42	1.34
UG273	0.11	0.17	1.06	1.05	0.62	0.84
301712	0.51	0.29	1.02	1.11	0.07	0.24
BR92/019	0.10	0.14	1.01	1.07	1.24	1.21
BK132	0.06	0.11	0.89	0.92	1.35	1.18
DJ259	0.03	0.14	1.05	1.16	0.00	0.00
SG364	0.16	0.24	0.80	0.75	0.00	0.00
ZAM20	0.19	0.17	1.75	1.54	2.03	1.43
UG270	0.96	1.02	1.02	0.99	0.00	0.02
UG93/070	0.63	0.72	1.05	0.96	0.00	0.00
SG365	0.22	0.48	0.93	0.99	1.39	1.39
UG92/005	0.22	0.42	1.20	1.06	0.00	0.01
CM235	0.04	0.16	1.33	1.17	0.00	0.00
TH92/001	0.08	0.25	1.17	1.05	0.00	0.00
CM238	0.00	0.11	0.85	0.79	0.00	0.00

^a Ratio of OD₄₉₂ value obtained with IgG1b12, CD4-IgG2, or 2G12 to the OD₄₉₂ value obtained with dimeric CD4-IgG (1 or 4 µg/ml) at MAB or CD4-IgG2 concentrations of 1 and 4 µg/ml.

TABLE 5. Presence of the 2F5 epitope in tested virus isolates^a

Clade or group	Isolate	Sequence	Neutralized
Clade A		<u>ALDkWan</u>	
	RW92/026	—	+
	DJ258	ALDKWAG	+
	UG273	ALDKWAS	+
Clade B		<u>eLdkWas</u>	
	301593	ELDKWAS	+
	301657	ELDKWAS	+
	301660	—	+
	301714	ELDKWAG	+
	301717	—	+
	301727	—	—
	302054	—	+
	302056	—	—
	302073	—	+
	301712	ELDKWAS	+
	BR92/019	—	+
	BK132	ELDKWAS	+
Clade C		<u>ALDsW?N</u>	
	DJ259	ALDKWQN	+
	SG364	ALDNWNN	—
	ZAM20	ALDKWNI	+
Clade D		<u>eLDKWAS</u>	
	UG270	—	+
	UG93/070	—	—
	SG365	ALDKWAS	+
	UG92/005	—	+
Clade E		<u>eLDKWAS</u>	
	CM235	ELDKWAS	+
	TH92/001	—	+
	CM238	ELDKWAS	+
Clade F		<u>ALDKWAS</u>	
	BZ162	—	+
	R1	—	+
	BZ163	ALDKWAS	+
Group O		<u>ELDEWAS</u>	
	CA9	—	—
	MVP 5180	ELDEWAS	—

^a The 30 isolates used in this study are listed by clade or group, along with the gp41 sequence corresponding to the 2F5 epitope and the consensus sequence (underlined) for each clade (55). The unavailability of a sequence is indicated (—). Also shown is whether each virus is (+) or is not (–) neutralized by 2F5 (ID₅₀ of ≤50 µg/ml).

changes in this motif (LDNW and LDEW). Some promiscuity appears to be tolerable in the residues flanking this core motif, suggesting that the 2F5 epitope is a/eLDKWa/q/n.

DISCUSSION

A globally effective HIV-1 vaccine should be able to counter all subtypes of HIV-1, not just locally circulating variants related genetically to the immunogen. The variation of HIV-1 within an individual, between individuals, and between strains circulating in different parts of the world has always been a major obstacle to vaccine design (7, 28, 30, 31, 54, 70). For example, the current subunit gp120 or gp160 vaccine candidates are of a fixed genetic composition, and so there has been a concern about the breadth, as well as the potency, of the humoral immune responses that they are able to generate (24,

38, 81). Thus, it has been considered that cocktails of immunogens might be necessary to counter HIV-1 diversity or that vaccines might need to be tailored to match the predominant, locally circulating strains in each geographic area (7).

It is clear that HIV-1 strains circulating worldwide can be segregated into at least eight genetic subtypes or clades (1, 4, 6, 19, 21, 22, 28, 33, 51, 61, 70, 80), along with a second major category, the O group (23, 55, 58, 78). Variation in the antigenic structure of HIV-1 gp120 does not strictly correlate with the genetic clades (46), although clade-dependent variations in the antigenicity of gp120, or fragments of it, can be discerned (15, 42, 46, 62). HIV-1 anti-gp120 antibody serotypes also do not correspond precisely to the genetic clades, but once again, there are some clade-dependent patterns of serological responses to HIV-1 (15, 42, 62). More important for vaccine design than antibody serotypes are neutralization serotypes, and here again there does not appear to be a consistent correlation between HIV-1 neutralization patterns and the genetic clades (42, 57, 79). In general, primary strains from all clades of HIV-1 are relatively resistant to neutralization by serum antibodies, irrespective of the clade of the strain infecting the serum donor (37, 42, 57). However, some HIV-1 primary isolates from several clades are fairly neutralization sensitive, and some sera are able to neutralize multiple isolates from several clades (42, 57). Sera obtained from long-term survivors of HIV-1 infection are especially effective at HIV-1 neutralization (12), and the observation of cross-clade neutralization by selected sera (42, 57) provides a useful clue as to the potential of the humoral immune system for suppressing HIV-1 replication if the right antibodies are induced.

The viral targets for serum antibodies that show broad neutralization of primary HIV-1 strains are presently uncertain. Up until recently, the major focus of HIV-1 neutralization studies has been the V3 loop of gp120, as this region contains the principal neutralizing determinant (PND) for T-cell line-adapted strains such as HIV-1 LAI and HIV-1 MN (27). Overcoming the variation in V3 sequences has, therefore, been at the forefront of vaccine design strategies (7, 15, 50, 62, 63). However, it is now widely accepted that adaptation of primary isolates to growth in T-cell lines selects for variants that are abnormally neutralization sensitive, not only to HIV-1-positive sera but also to soluble CD4 and to MABs against a wide range of gp120 or gp41 epitopes (3, 38, 40, 43, 45, 47, 59, 71, 73, 77, 81). Thus, what has been learned from studies with adapted strains of HIV-1 cannot necessarily be applied in totality to studies of primary HIV-1 strains. In particular, the relative inaccessibility of the gp120 V3 region on virions of primary HIV-1 strains renders it uncertain whether the V3 loop is the PND for primary HIV-1 isolates (8, 43, 71, 73). Indeed, whether primary strains have a PND as such and what it is are currently unknown.

Despite their relative resistance to neutralization, it is clear that primary HIV-1 isolates can be neutralized by at least a subset of human MABs. We have therefore used MABs to investigate the conservation of neutralization epitopes on primary isolates from six clades (A through F) of HIV-1 and within the HIV-1 O group. Lacking definitive information on the identity of the PND of primary isolates, we selected for study three HuMABs (IgG1b12, 2G12, and 2F5) and the CD4-IgG2 molecule, on the basis of our experience that these reagents showed greater breadth and potency against clade B primary isolates than any other MABs described to date (2, 11, 16, 43, 64, 76).

The three MABs used were isolated from individuals infected with B-clade strains. Despite this fact, each MAB was able to neutralize selected isolates from clades other than B.

The two group O strains were resistant to the three MABs, although they could be neutralized by CD4-IgG2, presumably reflecting the extreme genetic divergence of the O group from strains in the HIV-1 M group. Antibodies targeted at the HIV-1 envelope glycoproteins can, therefore, have broad, inter- and intraclade neutralizing activities against primary isolates. This suggests that should passive immunotherapy with IgG1b12, 2G12, 2F5, or CD4-IgG2 be of clinical benefit in trials in Europe or North America, where B-clade strains are endemic, there is a reasonable chance that such therapy would also be effective elsewhere in the world. For immunotherapeutic purposes, it is an obvious advantage that, except for IgG1b12 and CD4-IgG2, the reagents are to nonoverlapping epitopes on the viral envelope and so might reasonably be expected to be more effective in combination than alone.

Against B-clade strains, the three MABs and CD4-IgG2 were each of comparable and considerable breadth and potency, neutralizing from 75 to 92% of the 12 isolates with ID_{50} s of ≤ 50 μ g/ml. Neutralization at the 90% level by MAB and CD4-IgG2 concentrations of ≤ 10 μ g/ml was less common but was observed in a significant number of cases (Table 1). Furthermore, the breadth and potency of neutralization shown by 2F5 and CD4-IgG2 against non-B-clade strains were indistinguishable from their activities against B-clade strains. Neutralization by 2F5 was strongly correlated with the presence of the ELDKWA epitope, or a close homolog, in the gp41 sequence of susceptible strains. MABs IgG1b12 and 2G12 were less effective against non-B-clade isolates than against those from the B clade, presumably because of increased, clade-dependent amino acid sequence divergence in the gp120 proteins; while there is an obvious necessity to conserve the CD4-binding site on all HIV-1 isolates, this is not necessarily true of all MAB epitopes (46). However, a significant number of non-B-clade isolates were still sensitive to IgG1b12 and 2G12. The discontinuous nature of the IgG1b12 and 2G12 epitopes renders it difficult to correlate gp120 amino acid sequences with virus neutralization. The gp120-binding data for these MABs were in one sense broadly correlated with virus neutralization, in that if the MAB failed to bind to solubilized gp120, the corresponding isolate was usually not neutralized. However, some exceptions to this were noted with IgG1b12. But, as we have shown previously (43, 47), the converse is not true, for the binding of an antibody or a CD4-based reagent to monomeric gp120 does not necessarily predict that the corresponding primary virus will be neutralized. This was especially true in the present study for CD4-IgG2 and IgG1b12, although binding of 2G12 to monomeric gp120 was, in general, reasonably predictive of virus neutralization.

Overall, the findings of our neutralization studies with MABs support those from our study using HIV-1-positive sera, which showed that cross-clade neutralization was possible, if rare (42). Thus, a vaccine based on the induction of humoral immunity that would be effective against multiple HIV-1 isolates is not impossible. However, it will not be simple to induce antibodies with the properties of those that we have studied here by vaccination with the present generation of subunit vaccines. Of the reagents that we used, 2F5 recognizes the simplest epitope, represented by the continuous amino acid sequence ELDKWA in gp41 (16, 52, 53). Antibodies to this peptide could not, of course, be induced by gp120 subunit vaccines but might be raised against gp160 immunogens or those containing the gp41 ectodomain linked to gp120. The gp120 epitopes for the other two HuMABs are discontinuous, as is the binding site on gp120 for CD4 (CD4-IgG2) (60, 65, 76). These binding sites are incompletely characterized, although studies with mutants of HxB2 monomeric gp120 have

revealed some of their characteristics (60, 65, 76). No antibodies, human or otherwise, that have the same gp120-mutant binding profile as soluble CD4 have been identified (60, 65, 74, 75). This suggests that the exact CD4-binding site on gp120 might not be immunogenic and that antibodies to it might not be inducible by a gp120-based vaccine (49). However, antibodies to epitopes overlapping the CD4-binding site are very common in HIV-1-positive human sera (14, 25, 26, 44, 72) and have been raised in animals immunized with gp120 subunits (17, 29, 56). These CD4-binding-site-related epitopes are often highly conserved within and across clades (46), but unfortunately, most HuMABs of this type have negligible neutralizing activity against primary HIV-1 isolates (43). HuMAB IgG1b12, which does neutralize primary isolates strongly (6, 43), has a gp120-mutant binding profile that is subtly different from binding of other HuMABs to similar epitopes and shows better recognition of oligomeric envelope glycoproteins than gp120 monomers (65). Crucially, binding of IgG1b12 to the oligomeric form of the HIV-1 envelope on the surface of infected cells correlates with virus neutralization (67). Thus, it is possible that induction of antibodies like IgG1b12 will require immunogens more sophisticated than monomeric gp120. The epitope for HuMAB 2G12 is clearly present on monomeric gp120, although the antibody was selected by a procedure that involved screening against oligomeric envelope glycoproteins on the surface of infected cells (10). However, 2G12 is unique in our experience in forming a gp120-MAB competition group with no other members (48, 76). This finding suggests that the 2G12 epitope is not particularly immunogenic or else that most antibodies to this site cannot be identified by screening against monomeric gp120. It is therefore uncertain whether subunit gp120s or gp160s can be used to raise 2G12-like antibodies.

Virus neutralization is dependent on antibody interactions with the oligomeric forms of the viral envelope glycoproteins (67, 68, 73). This phenomenon, discussed elsewhere (45, 68), accounts for the wide variation in the neutralization potency of CD4-IgG2 against primary strains despite the strong, cross-clade conservation of the CD4-binding site on monomeric gp120 (46). Monomeric gp120 subunits may not be adequate mimics of the more complex structures present on virions (9, 43, 67, 68, 71, 73). The rational design of a vaccine effective against multiple HIV-1 isolates from different clades that relies, wholly or in part, on neutralizing antibody induction would therefore be facilitated by more information on the structure and immunogenicity of the native forms of the HIV-1 envelope glycoproteins. But our overall conclusion is encouraging: if an immunogen can be designed to elicit significant neutralizing activity against a representative set of primary HIV-1 strains from one clade, it may also be able to induce cross-clade neutralizing activity. Thus, we suggest that the problem of HIV-1 genetic variation for vaccine design, while important, is superseded by the problem of developing an immunogen effective for countering any primary HIV-1 strains.

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