

Neutralization of the Human Immunodeficiency Virus Type 1 Primary Isolate JR-FL by Human Monoclonal Antibodies Correlates with Antibody Binding to the Oligomeric Form of the Envelope Glycoprotein Complex

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To test whether antibodies that are neutralizing or nonneutralizing for human immunodeficiency virus type 1 (HIV-1) primary isolates can be distinguished by their affinities for the oligomeric envelope glycoproteins, we selected HIV-1_{JR-FL} as a model primary virus and a panel of 13 human monoclonal antibodies (MAbs) and evaluated three parameters: (i) half-maximal binding to recombinant monomeric envelope, gp120_{JR-FL}; (ii) half-maximal binding to oligomeric envelope of HIV-1_{JR-FL} expressed on the surface of transfected 293 cells; and (iii) neutralization of HIV-1_{JR-FL} in a peripheral blood mononuclear cell-based neutralization assay. Two conclusions can be drawn from these experiments. First, we confirm that antibody interactions with monomeric gp120 do not predict primary virus neutralization. Second, we show that neutralization correlates qualitatively with the relative affinity of an antibody for the oligomeric envelope glycoproteins, at least for HIV-1_{JR-FL}.

The rational development of a vaccine against human immunodeficiency virus type 1 (HIV-1) would be facilitated by an understanding of the immune responses that are most effective at countering this virus. Although the correlates of protection against HIV-1 remain unclear, induction of an antibody response capable of reducing the infectivity of a transmitted virus would be a desirable outcome of a vaccination regimen (20, 23). Preexisting antibodies that can neutralize HIV-1 may prevent the passage of HIV-1 across sexual mucosa or limit the subsequent systemic dissemination of the virus.

The types of HIV-1 strains most commonly transmitted during sexual contact with an infected individual have several distinct characteristics, including resistance to neutralization, incompetent replication and syncytium induction within permanent cell lines, and an ability to replicate efficiently in macrophages (77; reviewed in reference 41). These so-called macrophage-tropic primary strains also use the second receptor CCR-5 to enter permissive cells (1, 9, 13–15). The HIV-1_{JR-FL} strain possesses these same properties (6, 15, 39, 48, 49, 52) although it was originally isolated from the frontal lobes of an individual with neurological symptoms of HIV-1 infection (32). It is available as a well-characterized molecular clone (48), which facilitates precise studies of virus neutralization and its causes. We have used HIV-1_{JR-FL}, therefore, to understand the factors that impact on HIV-1 neutralization in vitro.

Early studies suggested that HIV-1 neutralization is mediated by antibodies that bind to certain continuous and discontinuous epitopes found on the envelope glycoproteins of HIV-1, the gp120-gp41 complex (22, 24–27, 29, 40, 60, 65). However, the HIV-1 strains used in these studies were adapted for growth in permanent cell lines (41). The structural changes in the envelope that mediated this adaptation created viruses

that were abnormally sensitive to neutralization and thus were unrepresentative of the more resistant primary (or field) isolates (5, 16, 38, 42, 50, 56, 70). Precisely why primary isolates are resistant to neutralization is uncertain, but it is clear that this phenomenon is not unique to HIV-1 and may be a general property of lentiviruses (3, 8, 11, 41, 74).

The envelope glycoproteins of HIV-1 form an oligomeric (almost certainly trimeric) glycoprotein complex of gp120 and gp41 (33). We have shown previously that for primary viruses, neutralizing and nonneutralizing antibodies cannot be distinguished by their affinities for the soluble, monomeric form of gp120 (39). In collaboration with Sattentau (55), we have also shown that neutralization of sensitive T-cell line-adapted (TCLA) HIV-1 strains does correlate with monoclonal antibody (MAb) reactivity with the native gp120-gp41 oligomer. The latter study used chronically infected H9 cell lines that expressed high levels of envelope glycoproteins, but an insignificant amount of the CD4 receptor, on the cell surface. Unfortunately, the same technique cannot be employed with primary viruses because they are altered when grown in cell lines (38, 41), and attempts to use HIV-1-infected primary CD4⁺ T cells for studies of surface-expressed oligomeric envelope glycoproteins were compromised by interfering, artifactual gp120-CD4 complexes (75). Vaccinia virus-driven overexpression of envelope yields aberrant structures on the surface of infected cells (54), and so we were reluctant to use this method. Finally, although measuring MAb interactions with cell-free virions is feasible (40, 42, 59), many primary isolates are difficult to culture in sufficient quantities for routine use in virion binding assays.

To overcome these difficulties, we developed an alternative method for studying the interactions of 13 human MAbs with the envelope glycoproteins of a primary virus (HIV-1_{JR-FL}). We have used this assay to show that the affinity of antibody binding to the envelope glycoprotein oligomer is a good qualitative predictor of virus neutralization.

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MATERIALS AND METHODS

Plasmids. pSV7d-JRFL_{env} was provided by Tatjana Dragic, and pNL43 R+E- Luc (10) was provided by Nathaniel Landau (Aaron Diamond AIDS Research Center).

MABs, sCD4, recombinant gp120, and cell lines. The purified recombinant gp120 molecule that was derived from HIV-1_{JR-FL} and expressed in Chinese hamster ovary cells was a gift from Bill Olson, Graham Allaway, and Paul Maddon (Progenics Pharmaceuticals Inc., Tarrytown, N.Y.), as were CD4-IgG2 and soluble CD4 (sCD4) (2). MABs were obtained from the following sources: 447-52D (17, 19) and 697D (18), Cellular Products, Inc. (Buffalo, N.Y.); IgG1b12 (7, 53), Dennis Burton (Scripps Research Institute, La Jolla, Calif.); 2G12 (67, 68) and 3D6 (21), Hermann Katinger (IAM Pharmaceuticals, Inc. [Vienna, Austria]); and HT5, HT6, and HT7 (39), Ciba-Geigy AG (Basel, Switzerland). In addition, the following MABs were obtained from James Robinson: 19b (47, 58), 15e (25), 17b (64, 72), and 21h, F91, C11, 212A, A32, 2/11c, and 23A (46, 62, 64). The 293 cell line, a CD4-negative human kidney line, was obtained from the American Type Tissue Collection, Rockville, Md.

Oligomer binding assay. The binding of MABs to the oligomeric HIV-1 envelope was assessed as follows. Cells from the 293 line were transiently transfected with pSV7d-JRFL_{env} and pNL43 R+E- Luc. After 48 h, the cells were harvested, washed, and pelleted in microcentrifuge tubes (10^6 cells/tube). The cell pellets were resuspended in the appropriate dilution of MAB (where noted, also with sCD4 at 5 to 10 μ g/ml) in phosphate-buffered saline (PBS) containing 10% fetal calf serum and incubated for 2 h at 37°C. Unbound MABs were removed by two successive washes with 1 ml of PBS, and the cells were lysed with 100 mM octyl- β -glucoside (OGL; Boehringer Mannheim GmbH, Mannheim, Germany). In preliminary experiments, 100 mM OGL was found to optimally dissociate the oligomeric envelope complex. The MAB-gp120 complexes in the lysates were captured onto plastic (Immulon 2 microplates; Dynatech Ltd.) via adsorbed sheep antibody D7324 (Aalto BioReagents, Dublin, Ireland). D7324 is specific to the conserved C terminus of gp120. The bound antibody was detected via goat anti-human IgG-alkaline phosphatase (Accurate Chemical, Westbury, N.Y.). As a control for total envelope expression, HIV-1-positive human serum (QC256-alkaline phosphatase) (39) was used to assess the total level of envelope captured from lysed cells without antibody. 293 cells transfected with pNL43 R+E- Luc alone were used to control for antibody specificity. The half-maximal binding values were determined graphically for binding curves that reached saturation. For those that did not, half-maximal values were extrapolated by using Cricket Graph (version 1.3.2; Cricket Software, Malvern, Pa.).

Monomer binding assay. MAB binding to monomeric JR-FL gp120 was assessed by D7324 capture enzyme-linked immunosorbent (ELISA), in the absence of detergent, as described previously (40, 43). Inhibition of sCD4 binding to gp120 was also determined by ELISA (37, 40, 43).

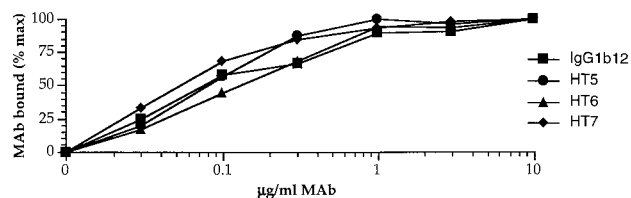
Neutralization assay. The capacity of each MAB to neutralize HIV-1_{JR-FL} was assessed by a neutralization assay using mitogen-stimulated peripheral blood mononuclear cells (PBMC), with extracellular p24 antigen production being used to record viral output, as an assay endpoint (12, 67). The MAB concentrations which reduced viral infectivity by 50 and 90% (ID_{50} and ID_{90} neutralization values) for each antibody were determined by linear regression. Statistical analysis (best fit of curve) was done with Cricket Graph.

RESULTS

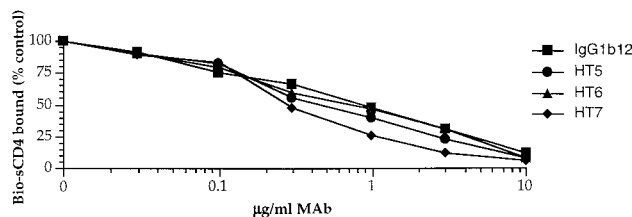
Neutralization of HIV-1_{JR-FL} is not predicted by MAB either binding to monomeric gp120 or inhibiting of sCD4-gp120 interaction. Previously, we showed that the binding of a MAB to monomeric gp120 is a poor predictor of primary virus neutralization (39). For some MABs, such as those that recognize epitopes in the N- and C-terminal domains of gp120, the explanation is reasonably clear: the epitope is inaccessible because it is in a region of gp120 that is associated with other components of the oligomeric complex (44, 46). With other MABs, such as those that recognize epitopes that overlap the CD4-binding site (CD4BS) (25, 53, 62, 63), the explanation of their inadequacy is less obvious (6, 12, 39, 42). This is exemplified by the experiment shown in Fig. 1.

Four MABs to CD4BS-related epitopes on gp120, IgG1b12, HT5, HT6, and HT7, mutually cross-compete for binding to monomeric gp120 (4). The pattern of amino acid substitutions in HxBc2 gp120 which disrupts the binding of HT5, HT6, and HT7 is characteristic of MABs to CD4BS-related epitopes (4). The IgG1b12 epitope has been described previously (53). Each MAB bound monomeric JR-FL gp120 (Fig. 1A), and all inhibited the JR-FL gp120-sCD4 interaction (Fig. 1B) equally well. Yet each MAB neutralized HIV-1_{JR-FL} to very different de-

A. Monomer Binding



B. Inhibition of CD4 Binding



C. Neutralization

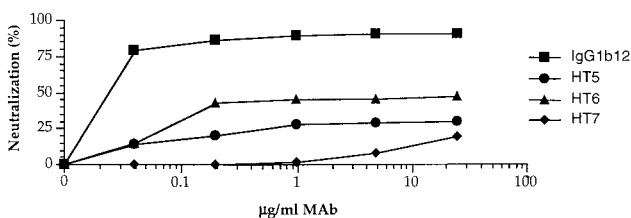


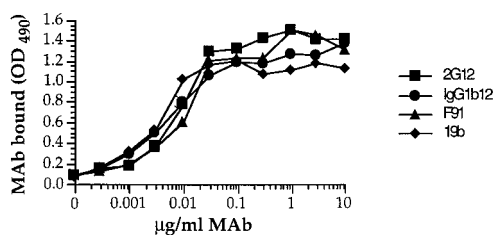
FIG. 1. MAB reactivity with monomeric JR-FL gp120 does not correlate with virus neutralization. (A) MAB binding to monomeric JR-FL gp120. Values are expressed as percentages of maximal MAB binding at saturation. (B) MAB inhibition of biotinylated sCD4 binding to monomeric JR-FL gp120. Values are expressed as a percentage of biotinylated sCD4 binding in the presence of MAB compared to that bound in the absence of MAB. (C) Neutralization of HIV-1_{JR-FL} by MABs.

grees, only IgG1b12 having any significant activity (Fig. 1C). Clearly, the potency of neutralization is not predicted by either the affinity of a MAB for monomeric gp120 or its ability to inhibit the sCD4-gp120 interaction.

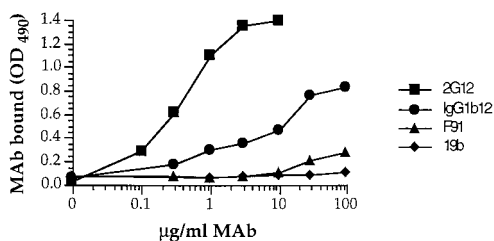
Binding of MABs to monomeric and oligomeric gp120. To determine whether neutralization of HIV-1_{JR-FL} was correlated with antibody binding to the oligomeric envelope of JR-FL, we needed an assay to measure the latter. For reasons outlined in the introduction, we used a cloned *env* gene (gp120-gp41) from the primary isolate HIV-1_{JR-FL} as a source of oligomeric envelope glycoproteins. This gene was expressed transiently in CD4-negative 293 cells, and the amount of MAB bound to the surface-expressed oligomer was assessed by lysing the cells and then measuring the amount of released MAB-gp120 complex by a gp120 capture ELISA. The ELISA endpoint was used to provide a simple, sensitive, and flexible assay for eventual use in larger-scale studies with HIV-1-positive sera and a range of primary isolates. Preliminary experiments demonstrated that the amount of gp120 expressed by 10^6 transiently transfected 293 cells was sufficient to saturate wells coated with the D7324 gp120 capture antibody (data not shown).

For our study, MABs were selected based on two criteria: (i) they were human derived, and so may represent part of the antibody repertoire that would be generated in a natural HIV-1 infection; and (ii) they recognized relatively conserved epitopes and/or neutralized a broad range of primary isolates

A. Monomer Binding



B. Oligomer Binding



C. Neutralization

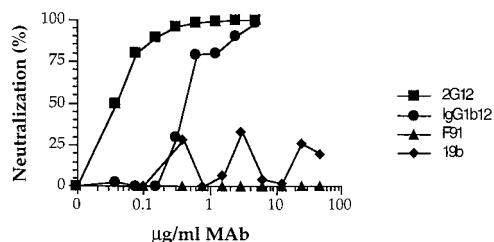


FIG. 2. Comparison of MAb binding to monomeric and oligomeric JR-FL gp120 with virus neutralization. (A) MAb binding to monomeric JR-FL gp120. (B) MAb binding to oligomeric JR-FL gp120 expressed on transfected 293 cells. The signal from MAb binding to 293 cells transfected with control plasmid pNL43 R+E- Luc alone was never above background (not shown). (C) Neutralization efficiency of MAbs against HIV-1_{JR-FL}.

in previous experiments (7, 17–19, 25, 39, 44–46, 53, 62–65, 67, 68, 72). (We also included the CD4-IgG2 molecule, because although it is not a MAb, it has many properties of one [2, 67]). The relative affinities of these MAbs for the monomeric and oligomeric forms of JR-FL gp120 were determined, exemplified by the MAb-*env* binding curves for four MAbs shown in Fig. 2A and B, respectively. Although saturating binding to the monomer was achieved with all of the antibodies, only MAb 2G12 bound saturably to the oligomer (Fig. 2 and data not shown), and there was clearly no correlation between MAb reactivity with the monomer and oligomer (cf. Fig. 2A and B). MAbs 447-52D and IgG1b12 and the CD4-IgG2 molecule bound to the JR-FL oligomer to varying but significant extents, while A32 and F91 reacted at minimal but detectable levels (Fig. 2B; Table 1). In contrast, although MAbs 19b, 697D, 15e, 21h, C11, 212A, 2/11c, and 23A bound strongly to the JR-FL gp120 monomer, we could measure no binding of them to the oligomer (Fig. 2; Table 1). The 3D6 gp41 MAb bound to neither the monomer nor the oligomer, as expected (Table 1).

Two of the MAbs in the test panel (17b and A32) bind to epitopes that are better exposed on gp120-sCD4 complexes than they are on gp120 alone (CD4-induced [CD4i] epitopes), at least for gp120 from the TCLA strains HIV-1_{HXB2} and HIV-1_{BH-10} (64, 72). We assessed whether the CD4i epitopes were also present on JR-FL gp120 and induced by CD4, focusing on

TABLE 1. MAb reactivities with monomeric and oligomeric JR-FL gp120 and virus neutralization

Epitope cluster ^a	Antibody	Oligomer (μg/ml) ^b	Monomer (μg/ml) ^c	ID ₉₀ (μg/ml) ^d	ID ₅₀ (μg/ml) ^d
V3	447-52D	40	0.001	47.5	0.9
	19b	...	0.007	—	—
V2	697D	...	0.145	—	—
	CD4BS	...	0.008	—	—
	21h	...	0.173	—	—
	F91	...	0.011	—	—
	IgG1b12	30	0.010	1.0	0.3
	CD4-IgG2	80	0.035	2.0	0.4
	sCD4	nd	nd	48	15
CD4i	17b	...	0.265	—	—
	17b/sCD4	220	0.006	40	8.2
C1-C5 (D)	C11	...	0.283	—	—
	212A	...	0.300	—	—
C1-C4 (D)	A32	...	0.032	—	—
	A32/sCD4	240	0.010	ND	ND
C3-V4 (D)	2/11C	...	0.060	—	—
	2G12	1	0.019	0.15	0.04
C5	23A	...	1.50	—	—
gp41	3D6	...	—	—	—

^a The epitope cluster recognized by each test MAb (46).

^b Half-maximal MAb concentration for binding to the oligomeric JR-FL gp120. . . ., the half-maximal binding values are greater than the highest concentration of MAb used (100 μg/ml). Values above 100 μg/ml were derived by extrapolation (see Materials and Methods).

^c Half-maximal MAb concentration for binding to the monomeric JR-FL gp120.

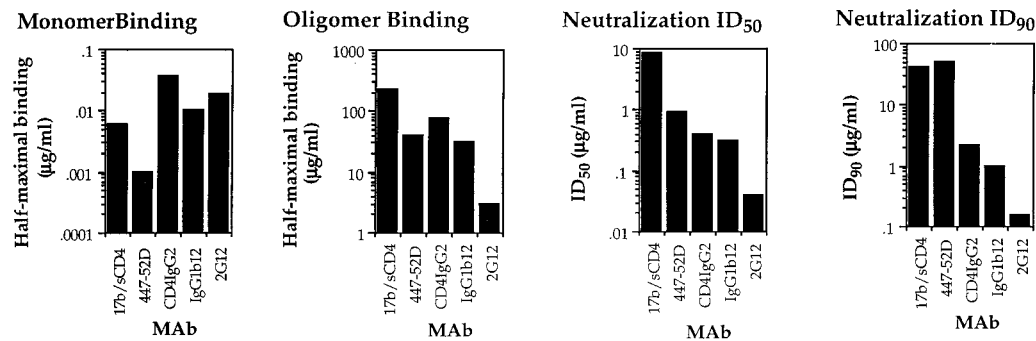
^d Determined by mitogen-stimulated PBMC-based neutralization assay (see Materials and Methods). —, value is greater than the highest concentration of MAb used (50 μg/ml); ND, not determined.

MAb 17b. The closely related (but not identical) MAb 48d binds relatively weakly to monomeric JR-FL gp120, and so we did not assess this MAb further (4). We compared the half-maximal binding of 17b and A32 to that of monomeric and oligomeric JR-FL gp120 in the presence and absence of a saturating concentration of sCD4. Inclusion of sCD4 induced a 40-fold increase in 17b binding to the monomer but only at least a threefold increase in its binding to the oligomer (Table 1). The addition of sCD4 increased A32 binding to the gp120 monomer by 3-fold and to the oligomer by at least 2.5-fold. Thus, the CD4i epitopes are present and inducible on JR-FL gp120, but even in the presence of sCD4, the binding of MAbs 17b and A32 to the oligomeric envelope glycoprotein complex may be less than optimal.

MAb binding to oligomeric gp120 parallels neutralization efficiency. We evaluated the capacity of each of the MAbs to neutralize of HIV-1_{JR-FL}, using mitogen-stimulated PBMC as target cells and a p24 antigen assay as an endpoint (12, 67). This assay was used because it allows a rigorous assessment of the neutralizing capacity of MAbs (39). The ID₅₀ and ID₉₀ values were recorded. Only 2G12, IgG1b12, CD4-IgG2, and 447-52D reduced the infectivity of HIV-1_{JR-FL} by 50% at concentrations below 50 μg/ml (Fig. 2C; Table 1). The combination of sCD4 with 17b or A32 was also effective, but only slightly better than sCD4 alone (Table 1).

Virus neutralization efficiency was compared with MAb binding to monomeric and oligomeric forms of JR-FL gp120 (Fig. 3). The MAbs are aligned according to their ID₅₀ neutralizing values (weakest neutralizer on the left and strongest on the right) in Fig. 3A. Visual inspection of the three data sets indicates that the rank order for neutralization efficiency closely parallels that for oligomeric gp120 binding but that

A. Neutralizing MAbs



B. Non-neutralizing MAbs

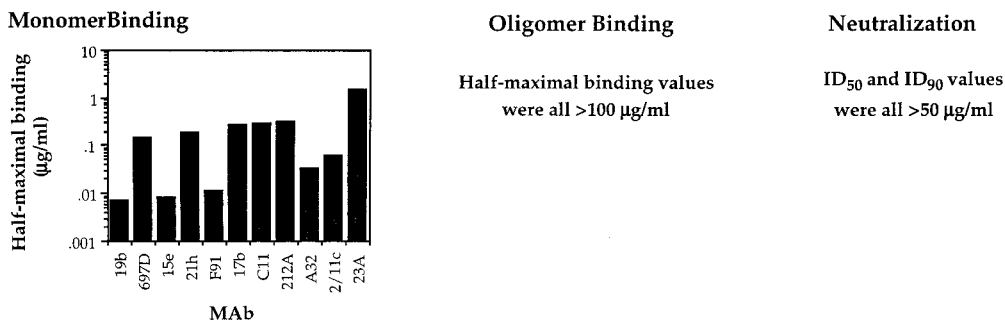


FIG. 3. Comparison of MAb binding to monomeric and oligomeric JR-FL gp120 with neutralization efficiency. (A) Comparison of MAb concentrations giving half-maximal binding to monomeric and oligomeric JR-FL gp120 with ID_{50} and ID_{90} values for MAbs that neutralized HIV-1_{JR-FL}. The MAbs are aligned according to their neutralizing efficiency. (B) Comparison of MAb concentrations giving half-maximal binding to monomeric and oligomeric JR-FL gp120 with ID_{50} and ID_{90} values for MAbs that did not neutralize HIV-1_{JR-FL}.

there is no relationship between monomer binding and virus neutralization.

The nonneutralizing MAbs are represented in Fig. 3B. The neutralization and oligomer binding assays do not yield numerical endpoints for most of the MAbs that we tested, because there is a practical limit to how much MAb can be used in these assays (50 $\mu\text{g/ml}$ in the neutralization assay and 100 $\mu\text{g/ml}$ in the oligomer binding assay). This complicates a rigorous statistical analysis of the data sets. However, visual inspection of the monomer binding data for neutralizing and nonneutralizing sets of MAbs again reveals no correlation between monomer binding and virus neutralization (cf. Fig. 3A and B). We also consider it of significance that none of the MAbs which neutralized HIV-1_{JR-FL} at <50 $\mu\text{g/ml}$ bound detectably to the JR-FL gp120 oligomer (Fig. 3B; Table 1).

DISCUSSION

Multiple mechanisms may contribute to the neutralization resistance shown by primary HIV-1 strains relative to TCLA variants (reviewed in references 30 and 41). These mechanisms may or may not include the density of glycoprotein spikes on virions (28, 30); the stability of these spikes (28, 30, 38, 69); whether antibody binding activates, reversibly inhibits, or irreversibly inhibits the fusogenic potential of the spikes (31, 34, 36, 51, 57, 61); the degree of exposure of antibody epitopes on the virion (5, 35, 55, 59, 61); and differential second receptor usage by primary (CCR-5) and TCLA (fusin, now renamed CKCR-4) HIV-1 strains (1, 9, 13–15, 66, 71). Whether all these factors are relevant, and which are dominant in any particular

situation, is not readily apparent. However, irrespective of the mechanism of neutralization sensitivity or resistance, it is obvious that neutralization cannot occur without an initial binding reaction between antibody and virion. Studies of the interactions of antibodies with a form of the viral envelope glycoproteins found on the virion surface (i.e., oligomeric forms) are therefore relevant to understanding neutralization resistance.

The oligomer binding assay that we developed for this study utilized transient transfection of 293 cells as a source of oligomeric envelope and a gp120 capture ELISA endpoint. We used the cloned *env* gene from HIV-1_{JR-FL}, and so the results are not influenced by any other viral protein or by virus or cell culture conditions, which have been suggested to influence HIV-1 neutralization (56, 76). Note, however, that neither MAbs which are gp120 oligomer specific (recognize discontinuous epitopes formed exclusively by amino acids on different gp120 subunits within the oligomeric complex) nor MAbs to gp41 (e.g., 3D6 [Table 1]) can be detected by this assay. It is also necessary to understand the limitations of the assay and how to interpret the results it generates. Although the MAbs bind to the oligomeric form of the envelope on the cell surface, the quantity of MAb bound is assessed later in an ELISA format, using a capture antibody which recognizes an epitope exposed on the gp120 C terminus after detergent disruption of the cells and the associated MAb-oligomer complexes. In principle, this procedure is similar to that used previously to show that Fab b12 (which is related to MAb IgG1b12) preferentially binds the oligomeric form of envelope glycoproteins (53, 55). Various detergent lysis procedures were carefully assessed to

ensure that the one selected (100 mM OGL) caused optimal dissociation of the oligomeric envelope.

The initial binding of the MAb to the oligomer occurs in antibody excess. However, a new equilibrium is established after lysis of the cells and during D7324 capture of the MAb-gp120 complex, for free MAb is washed away. MAbs with rapid off rates (low affinities) will dissociate disproportionately during this step, and there will be a decrease in the ELISA signal. This dissociation will shift the binding curves to the right, suggesting that the MAb has a lower affinity than it actually has. For this reason, the oligomer binding assay cannot be used either to make quantitative predictions of neutralization potencies or to compare directly the affinities of a MAb for the monomer and the oligomer. In particular, the values listed in Table 1 for half-maximal binding of MAbs to oligomeric gp120 are not affinity constants; they are presented to facilitate comparisons between antibodies, not as indications of the absolute affinity of a MAb for its oligomeric ligand. However, that there was no correlation between the affinity of a MAb for monomeric gp120 and its binding to the oligomer in this assay indicates that the above-specified factors do not have a major qualitative impact on the results obtained. As all of the MAbs in the test panel bind to the gp120 monomer, the discrimination observed in the oligomer binding assay must reflect the relative affinity of MAbs for epitopes that are exposed on the oligomeric form of gp120 during the MAb-cell reaction stage. We believe that this assay therefore has qualitative value and is especially useful for high-affinity MAbs, which are arguably the most important *in vivo*.

This study suggests that nonneutralizing and neutralizing antibodies can be distinguished by their relative affinities for the oligomeric envelope; nonneutralizing MAbs bind poorly or not at all. This begs the question as to whether there is such a thing as a nonneutralizing antibody that can actually bind strongly to the oligomer; in other words, are there epitopes accessible anywhere on the functional oligomeric gp120-gp41 complex to which an antibody can bind without hindering the function of that complex and thereby reducing the infectivity of the virion? This question is unresolved, but it may be inherently unlikely, with the following exception: activation of *env*-mediated fusion by MAbs requires that a MAb binds to the oligomer without causing neutralization (31, 36, 57, 61). However, we did not observe the binding of nonneutralizing MAbs to the JR-FL oligomer, or the activation of HIV-1_{JR-FL} infectivity, with the panel that we studied. Among these MAbs, there are probably different explanations for a failure to bind and neutralize HIV-1_{JR-FL}. Some MAbs are simply directed against epitopes that are constitutively occluded on all HIV-1 strains, and they lack significant neutralizing activity against primary and TCLA strains; in this category are antibodies which bind to epitopes around the N and C termini that are likely to be occluded by gp41, such as D7324 (43, 45). However, other MAbs, particularly those like 15e and 21h which recognize epitopes around the CD4BS, neutralize TCLA strains quite potently (24, 25, 62, 63, 65). Why do they then fail to bind to and neutralize primary viruses, despite the presence of their epitope, at least on the gp120 monomer? We can only suppose that these MAbs do not bind the oligomeric envelope glycoproteins of primary viruses because the accessibility of their epitopes on oligomers varies subtly between primary and TCLA strains of HIV-1 (5, 35, 41, 59, 61). And this variation must indeed be subtle, for the MAbs in question cross-block one another's binding to the monomeric gp120s of both primary and TCLA strains (4, 46). Perhaps clarification of some of these issues may be provided by a greater understanding of the interactions of the HIV-1 envelope glycoproteins with CD4

and the CCR5 or CXCR4 second receptors and of the way that neutralizing antibodies interfere with this (66, 71).

Of those MAbs with significant neutralization potency against HIV-1_{JR-FL}, one (IgG1b12) is to a CD4BS-related epitope (7, 53) (as of course is the CD4-IgG2 molecule [2]), another (2G12) is to an unrelated conformationally sensitive, conserved epitope (68), while a third (447-52D) is to the V3 loop (17, 19). The CD4-IgG2 molecule as well as MAbs 2G12 and IgG1b12 possess broad and potent neutralizing activity (67), whereas 447-52D does not (39); HIV-1_{JR-FL} is one of the few primary viruses truly sensitive to the actions of this MAb.

It has been suggested that primary virus neutralization resistance is largely an artifact of assay conditions (76). This is a misguided perspective (73), and there are rational explanations for the phenomenon, even if all of the details remain unresolved. In this study, we demonstrate that for at least one neutralization-resistant primary virus, there is a correlate of the resistance that is apparent at the level of the cloned *env* gene and thus independent of any conditions that may or may not be used to assay viral infectivity and its neutralization.

Our conclusions from this study are twofold: first, we confirm that antibody interactions with monomeric gp120 do not predict virus neutralization; second, we show that antibody binding to the oligomeric envelope glycoproteins is of predictive value, at least for HIV-1_{JR-FL}, broadly consistent with the observations of others (5, 35, 59, 61). There are implications of these findings for vaccine development. One is that studies of the immunogenicity of stable, oligomeric forms of the envelope glycoproteins seem worth pursuing. Another is that many antibodies raised to gp120 *in vivo* (human MAbs) lack the ability to recognize primary viruses; if these MAbs were raised against gp120 monomers which had dissociated from virions or infected cells (as is likely but not proven), then monomeric gp120 subunit vaccines will also induce many antibodies that are functionally inert. This factor might contribute to the disappointing results of vaccination of humans with these proteins (20, 23). It is important to note, however, that irrespective of whether relevant antibody epitopes are present on gp120 monomers or oligomers, eliciting antibodies to them in the context of a practical vaccine is not a trivial matter. To fully exploit the immunogenicity of subunit vaccines based on the HIV-1 envelope glycoproteins, in whatever form, is likely to require a greater knowledge of the way in which these antigens are best presented to the human immune system than we yet possess.

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