Antigenic implications of human immunodeficiency virus type 1 envelope quaternary structure: Oligomer-specific and -sensitive monoclonal antibodies

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ABSTRACT   A majority of monoclonal antibodies (mAbs) raised against soluble oligomeric human immunodeficiency virus type 1 isolate IIIB (HIV-1$_{\text{IIIB}}$) envelope (env) glycoprotein reacted with conformational epitopes within the gp120 or gp41 subunits. Of 35 mAbs directed against gp41, 21 preferentially reacted with oligomeric env. A subset of these mAbs reacted only with env oligomers (oligomer-specific mAbs). In contrast, only 1 of 27 mAbs directed against the gp120 subunit reacted more strongly with env oligomers than with monomers, and none were oligomer-specific. However, 50% of anti-gp120 mAbs preferentially recognized monomeric env, suggesting that some epitopes in gp120 are partially masked or altered by intersubunit contacts in the native env oligomer. Two mAbs to oligomer-dependent epitopes in gp41 neutralized HIV-1$_{\text{IIIB}}$ and HIV-1$_{\text{SF2}}$, and binding of these mAbs to env was blocked by preincubation with HIV-1-positive human serum. Thus, immunization with soluble, oligomeric env elicits antibodies to conserved, conformational epitopes including a newly defined class of neutralizing antibodies that bind to oligomer-specific epitopes in gp41, and may also minimize the production of antibodies that preferentially react with monomeric env protein.

The envelope (env) glycoprotein of human immunodeficiency virus type 1 (HIV-1) binds virus to cell surface receptors, mediates fusion between the viral envelope and cellular membrane, and contains the antigenic determinants to which neutralizing antibodies are directed (1–6). env consists of two subunits, gp120 and gp41, that arise by proteolytic cleavage of the precursor gp160 (7, 8). Most neutralizing antibodies fall into two categories: those that recognize determinants in the V3 loop of gp120 and those that block the gp120–CD4 interaction by binding to regions in gp120 conserved between different HIV strains (reviewed in ref. 9). Antibodies to the V3 loop generally recognize epitopes formed by a short continuous sequence. The anti-V3 loop antibodies are relatively independent of the tertiary or quaternary structure of gp120. While anti-V3 loop antibodies exhibit potent neutralizing activity against the strain to which they are raised, extensive antigenic variation in this region generally restricts their activity to closely related isolates (10–15). In contrast, antibodies that block CD4 binding recognize conserved, discontinuous, conformational epitopes in gp120 and are broadly neutralizing. These antibodies make up a significant fraction of the total neutralization activity present in HIV-1-positive human sera (16–23). Nevertheless, relatively few conformationally dependent, CD4-blocking monoclonal antibodies (mAbs) have been produced by immunization with env protein subunits. It is

therefore important to characterize the structural determinants in env that lead to the production of broadly cross-reactive, neutralizing antibodies so that they can be elicited more effectively by subunit vaccines.

The env glycoprotein forms noncovalently associated dimers shortly after synthesis. A higher-order structure, most likely a dimer of dimers, also forms (24–28). We recently described the isolation of a soluble, oligomeric env glycoprotein synthesized using recombinant vaccinia virus vPE12B. This genetically truncated form of the HIV-1$_{\text{IIIB}}$ env glycoprotein, containing all of gp120 and the entire gp41 ectodomain of this viral isolate, is secreted from cells largely in oligomeric form (29). This protein (termed gp140) was used to immunize mice and generate 138 mAbs. We found that nearly 60% of the mAbs recognized conformational epitopes; of these, 33 recognized epitopes in gp120 and 43 recognized epitopes in the gp41 ectodomain (29). In this paper we examine the effects of env quaternary structure on the reactivities of this unique panel of mAbs. We find that 21 of 35 mAbs directed against epitopes in gp41 react preferentially or exclusively with env oligomers. Oligomer-sensitive antibodies to at least two nonoverlapping epitopes were neutralizing. Furthermore, binding of these mAbs to env can be blocked by antibodies present in HIV-1-positive human serum. In contrast, nearly half of the tested mAbs directed against linear or conformational epitopes in gp120 react preferentially with monomeric env; only one reacts more strongly with oligomeric env. These results demonstrate that env quaternary structure has significant antigenic implications, not only in the ectodomain of gp41, but in gp120 as well.

MATERIALS AND METHODS

Cells and Viruses. Recombinant vaccinia virus vPE12B was used to produce soluble, noncleaved gp140 HIV-1$_{\text{IIIB}}$ env protein, while recombinant virus vCB-14 expressed soluble, cleaved gp140 (29). HIV-1$_{\text{IIIB}}$ and HIV-1$_{\text{SF2}}$ infectious supernatants were harvested from acutely infected H9 cell cultures. The median tissue culture infective dose (TCID$_{50}$) of clarified supernatants was determined on H9 cells. The following reagents were obtained through the AIDS Research and Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health: HIV-1$_{\text{SF2}}$ from Jay Levy (30) and HTLV-IIIB/H9 from Robert Gallo (31).

Neutralization Assays. For neutralization assays, dilutions of mAbs were preincubated with 200 TCID$_{50}$ of HIV-1$_{\text{IIIB}}$ or HIV-1$_{\text{SF2}}$ for 1 h at room temperature. H9 target cells pretreated with Polybrene (2 μg/ml) for 30 min at room temperature were added and incubated for 16–18 h at 37°C.

Abbreviations: HIV-1, human immunodeficiency virus type 1; mAb, monoclonal antibody; TCID$_{50}$, median tissue culture infective dose.

The cells were washed once and treated with trypsin to remove the unbound virus and antibody. The cells were then suspended in 2 ml of complete culture medium supplemented with one-fifth the original concentration of mAb and incubated at 37°C in a 24-well plate for 6 days (HIV-1$_{IIIB}$) or 12 days (HIV-1$_{SF2}$). The cultures were split 1:2 on days 4 and 8. The amount of virus released into the medium each day in the absence of mAb was determined by measuring the amount of p24 in the medium by ELISA (Coulter) to determine the point of exponential viral production. The extent of neutralization was then determined for these periods by comparing the amount of viral p24 produced in the presence of antibody to that produced in the absence of antibody or in the presence of a nonspecific murine IgG.

Antibodies. Members of a panel of 123 murine mAbs raised against soluble oligomeric, noncleaved gp140 env and 15 mAbs raised against the monomeric form were used in this study (29). The rabbit polyclonal serum 2018, raised against gp120, was used for immunoblotting. Human sera pooled from 9 HIV-1-positive individuals (kindly provided by James Hoxie) or from one HIV-1-negative individual were incubated for 1 h at 56°C followed by mixing 1:1 with phosphate-buffered saline containing 0.5% Triton X-100. mAb 0.5β (12) was obtained from the AIDS Research and Reference Program, National Institute of Allergy and Infectious Diseases.

Production and Immunoprecipitation of Dimeric and Monomeric env. BS-C-1 cells were infected with vCB-14 at a multiplicity of infection of 10. After 4 h, the virus inoculum was replaced with methionine-free minimal essential medium containing 5% dialyzed serum and 250 μCi (1 Ci = 37 GBq) of [35S]methionine per ml and incubated overnight. The medium was then collected, clarified by low-speed centrifugation, and concentrated 10-fold with a microconcentrator (Amicon). The proteins were separated by sucrose velocity gradient sedimentation through a 5–20% sucrose gradient at 40,000 rpm for 20 h at 4°C in an SW40Ti rotor (Beckman) (24) to separate monomeric and dimeric forms of gp140. After fractionation, the distribution of gp140 across the gradient was determined and peak fractions containing dimeric gp140 were pooled. To minimize contamination of monomeric gp140 with dimeric protein, monomeric gp140 was pooled from the fractions immediately above the monomer peak (indicated in Fig. 1). Aliquots of the pooled fractions were incubated overnight at 4°C with a panel of mAbs. Antibody–antigen complexes were recovered by precipitation with protein A-Sepharose and with goat anti-mouse IgG when needed. The amount of dimeric and monomeric protein immunoprecipitated was analyzed by SDS/PAGE, visualized by fluorography, and quantitated by scanning densitometry. The ratio of dimeric gp140 precipitated relative to monomeric gp140 and gp120 was calculated (gp120 was

excluded from calculations for mAbs directed against gp41 epitopes).

Antibody Competition Experiments. Purified mAbs were biotinylated using a 20-fold excess of NHS-LC-Biotin (Pierce) for 1 h at room temperature. Unreacted biotin was removed by overnight dialysis. To determine if binding of a given mAb to HIV-1$_{IIIB}$ env could be blocked by prior addition of HIV-1-positive human serum, aliquots of a cell lysate containing full-length env (produced by vPE16) were incubated with different volumes of pooled HIV-1-positive human serum. After 1 h at 4°C, 5 μl of a 40 μg/ml stock of biotinylated mAb was added for an additional hour followed by precipitation with 10 μl of avidin-agarose. After two washes with buffer (10 mM sodium phosphate, pH 7.2/1 mM EDTA/150 mM NaCl/1% Triton X-100), precipitated proteins were analyzed by SDS/PAGE. env was detected by immunoblotting with a mixture of mAbs to linear epitopes in gp120 and gp41 and visualized by chemiluminescence (DuPont).

Epitope Mapping. Aliquots of soluble, metabolically labeled gp140 produced by vPE12B were incubated with 2.0 or 0.2 μg of one purified mAb. A second mAb, biotinylated as described above, was then added for an additional hour. The gp140 was then immunoprecipitated by addition of avidin-agarose, separated by SDS/PAGE, and quantitated with a Molecular Dynamics PhosphorImager.

RESULTS

Reactivities of mAbs to Monomeric and Oligomeric env. To determine if antigenic differences exist between monomeric and oligomeric env glycoprotein, a panel of 62 mAbs raised against uncleaved gp140 (29) were screened for their ability to selectively immunoprecipitate metabolically radiolabeled dimeric or monomeric gp140. For this screening, we synthesized a cleavable form of gp140 which contained all of gp120 and the gp41 ectodomain. This protein, made by recombinant vaccinia virus vCB-14, was subjected to velocity gradient sedimentation. During centrifugation, gp120 dissociated from the gp41 ectodomain and was recovered exclusively in monomeric form (24). Thus, noncleaved, dimeric gp140 was clearly resolved from monomeric gp140, gp120, and the gp41 ectodomain (Fig. 1).

Sucrose gradient fractions containing metabolically labeled dimeric gp140 or monomeric gp120 and gp140 were pooled (Fig. 1), and aliquots were immunoprecipitated with excess amounts of the mAbs. To determine the total amount of immunoprecipitable material, R160 (a rabbit polyclonal anti-gp160 antiserum), 9b2 (an anti-V3 loop mAb), and D20 (an anti-gp120 mAb) were also used. The relative amounts of monomeric and dimeric env immunoprecipitated by each mAb were calculated by scanning densitometry of autora-
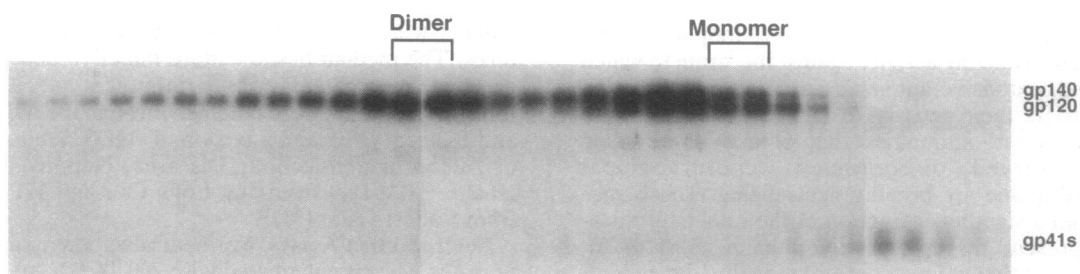
FIG. 1.   Isolation of dimeric and monomeric env glycoprotein by gradient centrifugation. BS-C-1 cells infected with vCB-14 (expressing soluble, cleaved gp140) were labeled overnight with [35S]methionine at 250 μCi/ml. The culture medium was then concentrated and the proteins were separated on a 5–20% sucrose gradient. Aliquots of each gradient fraction were immunoprecipitated with rabbit polyclonal anti-env antibody and analyzed by SDS/PAGE to determine the distribution of env across the gradient. The fractions containing dimeric or monomeric env were pooled and used in immunoprecipitation experiments with the panel of mAbs. Due to dissociation of gp120 during centrifugation (24), the dimeric env pool contained only gp140 while the monomer pool contained both gp140 and gp120.

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diographs. Four different patterns of antibody reactivity were observed: (*i*) antibodies that exclusively reacted with dimeric env, (*ii*) antibodies that preferentially reacted with dimeric env, (*iii*) antibodies that reacted equally well with monomeric and dimeric env, and (*iv*) antibodies that reacted more strongly with monomeric env than with dimeric env (Fig. 2). Five mAbs to conformation-dependent epitopes in gp41 (T4, T6, T9, T10, and T35) were oligomer specific. These five antibodies efficiently immunoprecipitated oligomeric gp140 (Fig. 2), but did not immunoprecipitate monomeric gp140 (Fig. 2), monomeric gp120 (Fig. 2), or monomeric gp160 (not shown), isolated under identical conditions. Thus, we concluded that these five mAbs recognize epitopes present only in oligomeric env protein.

In addition to the five oligomer-specific mAbs, a larger number of mAbs consistently immunoprecipitated oligomeric env 2 to 7 times more efficiently than they precipitated the native, monomeric form. mAbs that immunoprecipitated dimeric env at least twice as efficiently as the monomeric form were defined as oligomer-sensitive. This class of mAbs recognizes epitopes that are influenced by, but not strictly dependent upon, env quaternary structure (for example, mAbs D4 and D12, Fig. 2). Most of these mAbs bound to conformational epitopes in gp41. Of the 27 mAbs directed against conformational epitopes in gp41 tested thus far, 18 were oligomer-sensitive or -specific (Table 1), suggesting that the antigenic structure of gp41 is strongly influenced by quaternary interactions. In contrast, only 1 of 22 conformation-dependent mAbs to gp120 tested was oligomer-sensitive and none was oligomer-specific. Instead, 50% of the anti-gp120 mAbs reacted equally well with oligomeric and monomeric env (Fig. 2). We term these mAbs oligomer-independent. The remaining anti-gp120 mAbs reacted somewhat more strongly (2 to 4 times) with monomeric env, suggesting that their epitopes are partially masked or altered in the oligomeric molecule. By contrast, none of the gp41 mAbs tested exhibited this property (Table 1).

Influence of Quaternary Structure on Linear Epitopes. Antibodies to continuous epitopes such as the V3 loop can also exhibit some conformation dependence (34). We therefore tested a panel of apparently conformation-independent mAbs—i.e., those that react with reduced, denatured env in Western blots, for oligomer dependence. We found that 3 of 8 of these mAbs directed against gp41 were oligomer-sensitive, whereas 4 of 5 conformation-independent mAbs directed against gp120 reacted more strongly with native, monomeric env (Table 1). None of these mAbs were oligomer-specific. Thus, the reactivities of antibodies directed against linear epitopes recognized on Western blots may also be influenced by quaternary structure.

Epitope Mapping. We performed antibody blocking experiments with the oligomer-specific and -sensitive mAbs to

Table 1. Influence of HIV-1 env quaternary structure on mAb reactivity

Epitope type	Number of mAbs				
	Total	D ≫	D > M	D = M	M > D
Conformational					
gp120	22	0	1	11	10
gp41	27	5	13	9	0
Linear					
gp120	5	0	0	1	4
gp41	8	0	3	5	0
All anti-gp120 mAbs	27	0	1	12	14
All anti-gp41 mAbs	35	5	16	14	0
Total of all mAbs	62	5	17	26	14

A total of 62 mAbs have been tested for their ability to recognize monomeric and oligomeric forms of env by immunoprecipitation, SDS/PAGE, and quantitative densitometry (see Fig. 2). The percent of dimer and monomer immunoprecipitated by each mAb was determined relative to the total amount of env available as defined by quantitative immunoprecipitation with polyclonal and monoclonal antibodies. Oligomer-specific mAbs are indicated under the column D ≫. Antibodies that recognized dimeric env at least twice as efficiently as monomeric protein were termed oligomer-sensitive (D > M); antibodies that reacted with monomeric protein at least twice as efficiently as oligomeric env were termed monomer-sensitive (M > D). All other antibodies recognized monomeric and oligomeric env equally well (D = M).

determine the minimum number of nonoverlapping epitopes. Aliquots of gp140 were incubated with different mAbs for 1 h, after which a biotinylated mAb was added followed by precipitation with avidin-agarose. A representative experiment demonstrating epitope blocking between a set of mAbs and biotinylated T9 is shown in Fig. 3. The mAbs were judged to block an epitope if binding of the biotinylated mAb was inhibited by preincubation of gp140 with a nonbiotinylated mAb. We found that the 5 oligomer-specific mAbs (T4, T6, T9, T10, and T35) all competed with one another, but not with three oligomer-sensitive mAbs (D4, D12, and D40), indicating that there are at least two distinct, nonoverlapping sites in the gp41 ectodomain sensitive to env quaternary structure.

Neutralization Activity of Oligomer-Specific mAbs. Because of their novel properties, we asked whether representative oligomer-sensitive and -specific mAbs were capable of neutralizing HIV-1. The mAbs were incubated with virus at the indicated concentrations (Table 2) for 1 h and then incubated with target H9 cells overnight. Unbound virus was removed, and the infection was allowed to proceed for 6 (HIV-1$_{IIIB}$) or 12 (HIV-1$_{SF2}$) days in the presence of antibody. The amount of viral core antigen (p24) in the supernatant was then measured by ELISA. A potent neutralizing antibody to the V3 loop, 0.5β (12), neutralized HIV-1$_{IIIB}$. Oligomer-specific mAb T4 and oligomer-sensitive mAb D12 exhibited signifi-
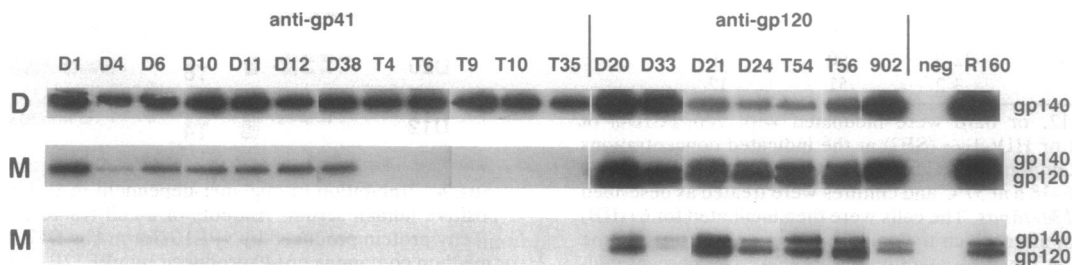

FIG. 2. Immunoprecipitation of monomeric and dimeric env glycoprotein with mAbs. Aliquots of monomeric or dimeric gp140 shown in Fig. 1 were incubated with the indicated mAbs overnight at 4°C. Antibody–antigen complexes were recovered by precipitation and analyzed by SDS/PAGE. Immunoprecipitation of dimeric env (D) and monomeric env (M) are shown in the upper and middle rows, respectively. A shorter exposure of the M panel is shown in the lower row to demonstrate the separation between gp140 and gp120. Representative anti-gp41 mAbs are shown on the left and anti-gp120 mAbs on the right as indicated. R160 is a control, rabbit polyclonal serum raised against gp160 (32) while 902 is an anti-V3 loop mAb (33).
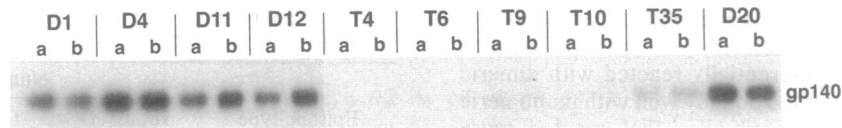
FIG. 3. Epitope mapping of mAbs. Metabolically labeled gp140 was incubated with 2 (lane a) or 0.2 (lane b) μg of purified mAbs D1, D4, D11, D12, T4, T6, T9, T19, T35, or D20 for 1 h, after which biotinylated T9 was added for an additional hour. The gp140 was then precipitated with avidin-agarose and analyzed by SDS/PAGE.

cant neutralization activity in a concentration-dependent manner (Table 2). These antibodies also neutralized HIV-1$_{SF2}$ (Table 2) and therefore must react with an epitope that is conserved between these divergent strains. Similar levels of neutralization were obtained when the mAb was not maintained in the culture. However, results from these experiments were more variable.

Binding of Oligomer-Dependent mAbs Is Blocked by HIV-1-Positive Human Serum. To determine if antibodies in HIV-1-positive human serum bind to oligomer-dependent epitopes in gp41, aliquots of a cell lysate containing HIV-1$_{IIIB}$ env protein or culture medium containing gp140 were incubated with different dilutions of pooled, HIV-1-positive human sera prior to addition of a biotinylated mAb and precipitation with avidin-agarose. Inhibition of mAb binding was monitored by comparing the amount of env precipitated by the biotinylated mAb with and without prior human serum incubation. As shown in Fig. 4A, the pooled HIV-1-positive human sera did not inhibit binding of a V3 loop mAb, D47. This was not surprising, since most HIV-1-positive humans are not infected with IIIB-like strains (35). However, complete blocking of mAb D20, which recognizes a conformation-dependent epitope associated with the CD4 binding site in gp120 (29), was obtained (Fig. 4A). Indeed, HIV-1-positive human serum has been shown to contain significant levels of broadly cross-reactive antibodies to the CD4 binding site (13, 21–23). Similar levels of blocking were seen with three oligomer-sensitive or -specific mAbs: D12 (Fig. 4A), T4, and T6 (Fig. 4B). No blocking was observed with HIV-1-negative human serum (not shown). That significant blocking was achieved

using HIV-1$_{IIIB}$ env as the antigen suggests that HIV-1-positive human serum may contain significant levels of broadly cross-reactive antibodies to oligomer-sensitive epitopes in the gp41 ectodomain.

DISCUSSION

Like other viral membrane proteins, HIV-1 env forms an oligomeric complex that is essential for its structure and function. Interactions between adjoining subunits involve the ectodomain of gp41 as well as adjoining gp120 subunits (24, 28, 36–38). That this may translate into differences in antigenic structure has been shown for a number of proteins, notably the trimeric influenza hemagglutinin (39, 40). We recently reported that immunization with soluble, oligomeric env glycoprotein effectively elicits antibodies to conformation-dependent epitopes in both gp120 and the gp41 ectodomain (29). Here we have shown that quaternary interactions make significant contributions to the antigenic structure of env, particularly in gp41. Of 52 gp41 mAbs, 82% recognized conformational epitopes and 66% preferentially or specifically reacted with env oligomers. Other antibodies sensitive to gp41 oligomeric structure have been reported. Poumbourios *et al.* (41) reported that antibodies purified from HIV-1-positive human serum by adsorption with a gp41 peptide reacted more strongly with oligomeric gp41, and Pinter *et al.* (26) identified several human mAbs that reacted exclusively or preferentially with oligomeric gp41 by Western blotting. We consider it significant that these antibodies, as well as all of the oligomer-specific and all but one of the oligomer-sensitive mAbs reported here, recognize epitopes in the ectodomain of gp41. That the antigenic structure of this region seems to be particularly sensitive to quaternary interactions is consistent with its role in oligomerization (24, 28, 29, 36).

The antigenic structure of gp120 was also influenced by env quaternary interactions, though in a different way than gp41. Only one of 27 anti-gp120 mAbs (both linear and conforma-

Table 2. Neutralization of HIV-1 by oligomer-specific and -sensitive mAbs

mAb	Conc., μg/ml	% neutralization	
		IIIB	SF2
D12	100.0	96	96
	20.0	74	67
	4.0	64	53
	0.8	36	0
T4	185.0	86	100
	37.0	78	88
	7.4	66	85
	1.5	64	26
	0.3	6	36
0.5β	32.0	90	0
	3.2	51	12

mAbs T4, D12, or 0.5β were incubated with 200 TCID$_{50}$ of HIV-1$_{IIIB}$ (IIIB) or HIV-1$_{SF2}$ (SF2) at the indicated concentrations for 1 h at room temperature. The virus/antibody mixture was added to H9 cells for 16–18 h at 37°C and cultures were treated as described in *Materials and Methods*. The cells were then incubated for 6 (IIIB) or 12 (SF2) days, after which the amount of viral p24 protein in the medium was determined by quantitative ELISA. The percent neutralization was determined relative to the amount of viral p24 protein present in the medium of infected cells in the absence of neutralizing antibody. D12 is an oligomer-sensitive mAb to gp41; T4 is an oligomer-specific mAb to gp41, and 0.5β is an anti-V3 loop mAb that neutralizes IIIB. A pool of HIV-1-positive human sera was used as a positive control for the SF2 neutralization experiments (not shown). All assays were performed in duplicate.
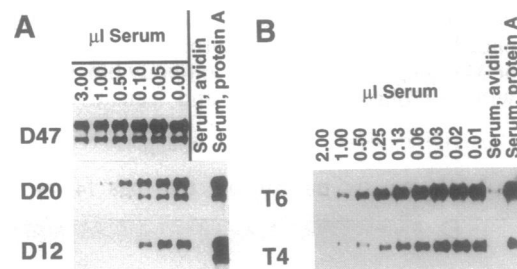

FIG. 4. Inhibition of oligomer-dependent mAb binding by HIV-1-positive human serum. Aliquots of a cell lysate containing HIV-1$_{IIIB}$ env protein produced by vPE16 (for mAbs D47, D20, and D12) or medium containing gp140 produced by vPE12B (for mAbs T4 and T6) were incubated with different amounts of pooled human sera (from nine HIV-1-positive individuals) as indicated. The following biotinylated mAbs were then added: (A) D47 (anti-V3 loop), D20 (conformation-dependent, CD4 blocking), D12 (oligomer-sensitive, anti-gp41). (B) T4 or T6 (oligomer-specific, anti-gp41). After 1 h, env was precipitated by the addition of avidin-agarose and analyzed by SDS/PAGE and Western blotting.

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tional epitopes) were oligomer-sensitive, and none were oligomer-specific (Table 1). However, half of the gp120 mAbs tested reacted with monomeric protein 2 to 4 times more efficiently than with env oligomers, suggesting that some epitopes in gp120 can be partially masked or altered during the assembly process. Whether this epitope masking is due to interactions with gp41 or with adjoining gp120 subunits is not yet known, but the observation illustrates the importance of intersubunit interactions for the molecule's antigenic structure. Finally, env quaternary structure may increase the reactivity of some mAbs that can also react with denatured gp120 and gp41. These findings are consistent with studies that show that human mAbs to the V3 region sometimes react more strongly with native than denatured gp120 (34). Thus, the presentation of an apparently linear epitope can be influenced by the molecule's overall conformation, including its quaternary structure.

The results described here have several implications for the development of subunit vaccines. We have evidence for two conformational epitopes in the gp41 ectodomain to which oligomer-dependent neutralizing antibodies can be directed, and we have found that antibodies in HIV-1-positive human serum inhibit binding of mAbs to these regions. More generally, we have shown that immunization with native, oligomeric env yields a high proportion of conformation-dependent antibodies (29) and have demonstrated that a large fraction of these are influenced by env quaternary structure. The strong influence of quaternary interactions on the conformation of gp41 and the presence of oligomer-dependent neutralizing epitopes suggests that the antigenic potential of this subunit needs to be more carefully evaluated. Immunization with monomeric env, either native or denatured, is unlikely to efficiently yield oligomer-sensitive or -specific antibodies to gp41. Another implication from our results is that immunization with gp120 by itself, even if the molecule retains the native structure, may result in the production of antibodies that preferentially react with monomeric env and so may react more weakly with native oligomeric env on the surface of virions or infected cells. The fact that gp41 is more highly conserved than gp120 raises the possibility that potent, broadly neutralizing antibodies can be generated against this region provided that native, oligomeric env protein is used as an immunogen. In summary, these results show that quaternary interactions have significant effects on the antigenic structure of HIV-1 env and, as a consequence, the oligomeric state of env should be taken into account in the design of potential subunit vaccines.

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