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Improved Elicitation of Neutralizing Antibodies against Primary Human Immunodeficiency Viruses by Soluble Stabilized Envelope Glycoprotein Trimers

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Human immunodeficiency virus (HIV-1) envelope glycoprotein subunits, such as the gp120 exterior glycoprotein, typically elicit antibodies that neutralize T-cell-line-adapted (TCLA), but not primary, clinical isolates of HIV-1. Here we compare the immunogenicity of gp120 and soluble stabilized trimers, which were designed to resemble the functional envelope glycoprotein oligomers of primary and TCLA HIV-1 strains. For both primary and TCLA virus proteins, soluble stabilized trimers generated neutralizing antibody responses more efficiently than gp120 did. Trimers derived from a primary isolate elicited antibodies that neutralized primary and TCLA HIV-1 strains. By contrast, trimers derived from a TCLA isolate generated antibodies that neutralized only the homologous TCLA virus. Thus, soluble stabilized envelope glycoprotein trimers derived from primary HIV-1 isolates represent defined immunogens capable of eliciting neutralizing antibodies that are active against clinically relevant HIV-1 strains.

The human immunodeficiency virus (HIV-1) glycoproteins are initially synthesized as a polyprotein precursor that undergoes posttranslational modifications including glycosylation, oligomerization, and proteolytic cleavage between the gp120 and gp41 subunits (2, 22, 23, 56, 77, 82). The mature envelope glycoproteins are transported to the cell surface, where they are incorporated into the virus as an oligomeric complex. The preponderance of evidence indicates that the mature oligomer consists of and functions as a trimer of gp120-gp41 heterodimers (11, 24, 39, 55, 69, 78). The envelope glycoprotein complex promotes viral entry into host cells by binding cellular receptors and mediating the fusion of the viral and cellular membranes (1, 13, 16, 17, 19, 20, 25, 34, 43). The gp120 exterior envelope glycoprotein binds the CD4 molecule, which facilitates the interaction of gp120 with a second receptor (typically, the chemokine receptors CCR5 or CXCR4) (74, 81). The interactions between gp120 and the cellular receptor molecules are believed to trigger conformational changes in the envelope glycoprotein complex important for the membrane fusion process (12, 64, 68).

Most antibodies elicited against the HIV-1 envelope glycoproteins during natural infection or after vaccination are incapable of neutralizing HIV-1 infectivity in vitro (3, 4, 14, 29, 33, 41, 42, 54, 57, 75, 76, 79). Neutralizing antibodies that are elicited often are restricted to a limited number of HIV-1 strains. These antibodies recognize variable structures on the surface of the gp120 exterior glycoprotein, in particular the gp120 variable loops (18, 45, 47, 49, 52, 54, 58, 60). Only after several months of natural HIV-1 infection are more broadly neutralizing antibodies directed against the conserved receptor-binding regions of gp120 elicited (5, 10, 31, 32, 35, 53, 54, 61, 65, 70–73, 82, 83). These antibodies have been difficult to elicit with subunit vaccine candidates (3, 4, 14, 28, 29, 41, 42, 57, 75, 76).

A further problem confronting the elicitation of protective antibody responses against HIV-1 infection is the relative resistance to antibody-mediated neutralization of primary, clinical HIV-1 isolates compared with T-cell-line-adapted (TCLA) HIV-1 strains (8, 15, 27, 40–42, 46, 48, 50, 51, 59, 79, 80, 86). Considerably higher concentrations of most neutralizing antibodies are required to inhibit the infection of primary HIV-1 strains, some of which are even enhanced by subneutralizing concentrations of antibodies (62, 63, 66, 67).

To date, most recombinant HIV-1 glycoproteins tested as vaccine candidates have been gp120 monomers. The antibody responses to gp120 are not usually effective in neutralizing primary HIV-1 isolates (3, 4, 6, 14, 28, 29, 41, 57, 76, 79). To attempt to mimic the native HIV-1 envelope glycoprotein oligomer, soluble gp140 glycoproteins containing gp120 and the gp41 ectodomain have been created (9, 22). When the gp120-gp41 junction is modified to reduce proteolytic cleavage, these soluble gp140 glycoproteins assemble into dimers and tetramers in addition to the monomeric forms (9, 21, 22). The elicitation of neutralizing antibodies by oligomeric forms of soluble gp140 has been disappointing, perhaps because these oligomers do not fully resemble the biologically relevant envelope glycoprotein trimers (6, 21, 33, 75).

Attempts to produce HIV-1 envelope glycoprotein trimers for structural and immunologic analysis have been frustrated by the lability of these glycoprotein complexes. Both the intersubunit interactions that promote trimer formation and the association between gp120 and gp41 are labie (26, 44). Modifications of the gp120-gp41 cleavage site and introduction of cysteine cross-links between gp120 and gp41 have been employed to address the latter problem (7, 9, 22). The addition of

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trimeric motifs from the GCN4 transcription factor (30) to the carboxyl terminus of soluble HIV-1 envelope glycoproteins has been successfully used to overcome the instability of the oligomeric associations and the tendency of the envelope glycoprotein ectodomains to form dimers and tetramers (84, 85). Here we test the hypothesis that these soluble stabilized HIV-1 envelope glycoprotein trimers will elicit virus-neutralizing antibodies more effectively than monomeric gp120 will.

MATERIALS AND METHODS

Plasmids. Details of the plasmids expressing soluble, stabilized trimers have been previously reported (84, 85). Briefly, all plasmids were derivatives of the pSVIIIenv vector (66). For production of soluble gp120 monomers, a stop codon was introduced into the *env* gene of the pSVIIIenv plasmid, resulting in termination after arginine 508 (amino acid residues are numbered as in the HXBc2 prototype). The gp120-gp41 proteolytic cleavage site was modified in the soluble, stabilized trimers by altering the arginines at residues 508 and 511 to serines. The GCN4 trimeric motif (MKQIEDKIEEILSKIYHIENEIARIKKLIGEV) (30) was positioned after leucine 593 in the gp130(−/GCN4) construct, after lysine 675 in the gp140Δ675(−/GCN4) construct, and after lysine 683 and a pair of glycine residues in the gp140Δ683(−/GCN4) construct. The open reading frames of these constructs were sequenced in their entirety to confirm that only the desired changes had been introduced.

Protein expression and purification. Envelope glycoproteins were produced by transfection of 40 100-mm plates of 293T cells with the pSVIIIenv plasmid and another plasmid expressing the HIV-1 Tat protein, using Effectene reagents (Qiagen). The envelope glycoproteins were purified from the pooled supernatants using an F105 antibody affinity column as described previously (36, 81). The protein preparations were evaluated for purity and quantified by comparison with serial dilutions of bovine serum albumin (BSA) after resolution on sodium dodecyl sulfate–7.5% polyacrylamide gels. The purified envelope glycoproteins were stored in aliquots at −20°C.

Immunization and serum preparation. The amounts of each envelope glycoprotein in the inoculum were adjusted so that each animal received the same molar quantity of the gp120 moiety, which is the major target for neutralizing antibodies (5, 29, 42, 54, 65, 75, 76, 82, 83). Thus, the following amounts of each protein were added to 200 μl (final volume) of a solution containing 1× Ribi adjuvant (Sigma): 6.8 μg of YU2 gp120, 7.8 μg of YU2 gp130(−/GCN4), 9.0 μg of YU2 gp140Δ675(−/GCN4) or gp140Δ683(−/GCN4), 6.5 μg of HXBc2 gp120, and 9.0 μg of HXBc2 gp140Δ675(−/GCN4). As controls, 9.0 μg of bovine serum albumin (BSA) or phosphate-buffered saline (PBS) alone was inoculated. Groups of at least six BALB/c female mice (Taconic) were inoculated subcutaneously with 200 μl of the immunogen solutions at three separate sites. Inoculations were administered at the ages of 9, 13, and 17 weeks. Eye bleeding was performed at 7 and 14 days after the third injection. Following clot formation for 24 h at 4°C, the samples were centrifuged at 13,000 × *g* for 10 min at room temperature and the sera were harvested in a sterile manner. The two serum samples from each mouse were pooled and incubated at 55°C for 1 h to inactivate complement. The sera were then stored at 4°C.

HIV-1 neutralization assay. The HIV-1-neutralizing activity of the serum samples was tested using a single-round virus entry assay. Recombinant HIV-1 expressing the firefly luciferase gene was produced by transfecting 293T cells with the pCMV Gag-Pol packaging construct and the pHIV-luc vector, along with a pSVIIIenv plasmid expressing the envelope glycoproteins of different HIV-1 strains (66; M. Koch, P. D. Kwong, P. Kolchinsky, L. Wang, W. Hendrickson, J. Sodroski, and R. Wyatt, submitted for publication). Two days after transfection, the cell supernatants were harvested and frozen in aliquots as viral stocks.

To create target cells, 2.5×10^6 Cf2Th canine thymocytes were transfected using Lipofectamine PLUS (Gibco Lifetech, Inc.) with 5 μg each of plasmids expressing human CD4 and either human CCR5 or CXCR4, as appropriate for the infecting virus (13). After being cultured overnight, the transfected cells were detached from the plates using 10 mM EDTA–PBS. After being washed in PBS, 6×10^3 cells were distributed into each well of a 96-well cell culture plate (Dynex). After overnight incubation, the cells were used for infection.

To quantify the infectivity of each viral stock, different amounts of the stocks were diluted to 200 μl using growth medium and incubated at 37°C for 1 h. Growth medium was thoroughly removed from the target cells, and 50 μl of the virus suspension was added to triplicate wells. The virus-cell mixture was incubated at 37°C in 5% CO_2 for 2 h, after which the medium was aspirated and the cells were washed once with 200 μl of prewarmed growth medium per well. After aspiration of the medium, another 200 μl of growth medium was added, and the cells were cultured for 2 days. At this time, luciferase activity was measured using the luciferase assay system (Pharmingen). Any values more than 200% above or less than 50% below the median value of triplicates were excluded from calculation of the mean infectivity titer. In practice, less than 10% variation was observed for the infectivity of viral stocks within an experiment. The linear range of the assay extended from 50 to 2×10^5 arbitrary luciferase units (data not shown).

In the neutralization assays, an amount of viral stock sufficient to result in luciferase activity of approximately 10^5 units was diluted to 50 μl in Dulbecco modified Eagle medium–10% fetal bovine serum. The mouse sera were diluted in the same medium, and the final volume was adjusted to 150 μl. The virus and sera were then mixed, briefly vortexed, and incubated in a 5% CO_2 incubator at 37°C for 1 h. The residual viral infectivity was then measured in the single-round infection assay as described above. The reported serum titers represent the dilution of the serum in the final virus-serum mixture that resulted in either 50 or 90% neutralization, compared with the infectivity of viruses incubated with medium alone.

Measurement of anti-gp120 reactivity of sera. To quantitate anti-gp120 reactivity in the sera, 15 ng of the YU2 or HXBc2 gp120 glycoprotein produced in *Drosophila* cells (36, 81) was adsorbed onto the well of an enzyme-linked immunosorbent assay (ELISA) plate (Costar) overnight at 4°C. After blocking the plates, 100 μl of serially diluted serum from mice immunized with envelope glycoproteins were applied to each well for 1 h. After consecutive incubation with biotinylated anti-mouse immunoglobulin G (IgG) (Sigma) and streptavidin-horseradish peroxidase (Pierce), the plates were vigorously washed and developed with the TMB peroxidase substrate kit (Bio-Rad). A well was classified as positive if the value was greater than 200% of the average values observed in the four wells that were incubated with the dilution buffer only. These negative controls exhibited a standard deviation of no more than 20% of the mean value.

RESULTS

Soluble stabilized envelope glycoprotein trimers. To create soluble forms of the HIV-1 envelope glycoproteins, the proteins were truncated at various locations within the gp41 ectodomain. In addition, the natural cleavage site between the gp120 and gp41 glycoproteins was altered to minimize proteolytic processing at this site (Fig. 1). Although these two modifications result in soluble envelope glycoproteins, such proteins exhibit considerable heterogeneity, forming monomers, dimers, tetramers, and other oligomers (21, 22). To promote the formation of soluble trimers, a sequence from the GCN4 transcription factor that was modified to form trimeric coiled coils (30) was appended to the carboxyl terminus of the soluble envelope glycoproteins (84, 85). Three constructs that differ in the location of the carboxyl terminus, gp 130(−/GCN4), gp140Δ675(−/GCN4), and gp140Δ683(−/GCN4), were studied. All three soluble glycoproteins assemble into relatively homogeneous, stable trimers (84, 85). The antibody-accessible surface of the last two trimers closely resembles that expected for the virion envelope glycoprotein complex, although subtle differences between these two molecules were observed (85).

Soluble, stabilized trimers derived from two different clade B HIV-1 strains were expressed in a human cell line and purified to greater than 95% homogeneity. The two strains were chosen to represent the extremes of phenotypic variation exhibited by HIV-1 isolates. The YU2 primary strain of HIV-1 was not passaged in tissue culture prior to molecular cloning (38). This CCR5-using virus is one of the most difficult HIV-1 isolates to neutralize with antibodies or soluble forms of the CD4 receptor, and it often demonstrates significant enhancement by these ligands (66, 67). The TCLA HXBc2 strain of HIV-1, by contrast, utilizes CXCR4 as a coreceptor and is extremely sensitive to antibody-mediated neutralization (66).

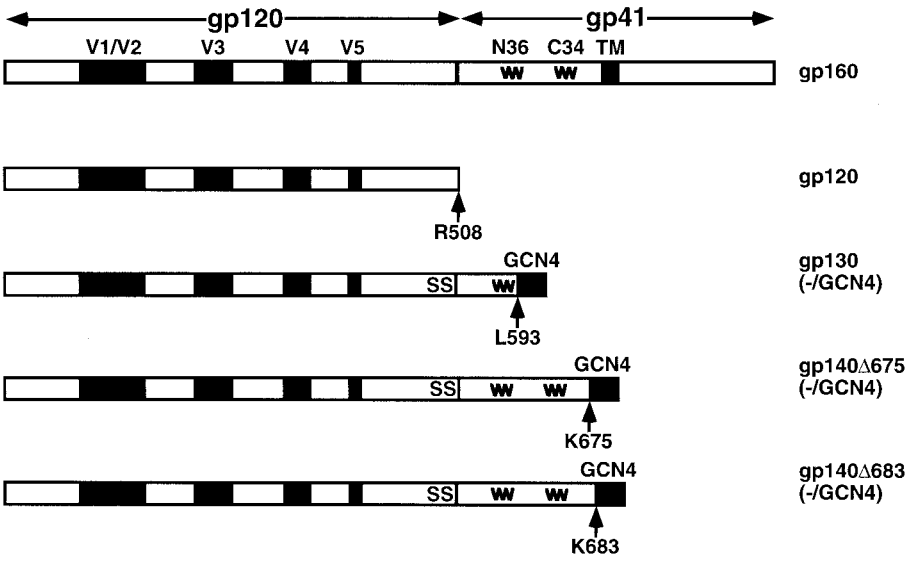

FIG. 1. HIV-1 envelope glycoprotein immunogens used in this study. The linear map of the HIV-1 gp120 and gp41 envelope glycoproteins is shown in the top diagram. The gp120 variable regions (V1 to V5) and the gp41 α-helical regions (N36 and C34) and transmembrane region (TM) are indicated. The soluble gp120 envelope glycoprotein was produced by a plasmid in which a stop codon was introduced into the HIV-1 *env* gene (from either the YU2 or HXBc2 strain) such that the encoded protein was truncated immediately after arginine 508. In the gp130 and gp140 constructs, the gp120-gp41 cleavage site was altered by the introduction of serine substitutions for arginines at positions 508 and 511 (indicated by SS) (84, 85). The GCN4 trimeric motif (30) was added to the carboxyl terminus of some of the constructs (indicated by GCN4). The GCN4 motif was added immediately after the indicated gp41 amino acid residue, except in the gp140Δ683(−/GCN4) construct, where two glycines separate the gp41 terminus and the GCN4 sequences.

Mouse immunization and neutralization assay. The immunogenicities of the soluble, stabilized trimers and gp120 monomers were compared in mice. Previous studies have demonstrated that gp120 induces neutralizing antibodies active against clinical HIV-1 isolates only after multiple immunizations, and then only at very low titers and with limited breadth (3, 4, 6, 14, 28, 29, 41, 57, 76, 79). Nonetheless, since no other defined immunogen has proven consistently superior to gp120 in this respect, the use of gp120 as a point of comparison was reasonable (6, 33, 41, 75). In this study, we utilized a conservative immunization protocol consisting of a priming inoculation followed by two boosts. Sera were collected from each immunized mouse at 1 and 2 weeks following the last boost, pooled, and assessed for virus-neutralizing activity. For this purpose, recombinant HIV-1 strains containing different envelope glycoproteins and expressing firefly luciferase were incubated with the sera and then used to infect canine thymocytes expressing CD4 and the appropriate chemokine receptor. The efficiency of this single round of infection was assessed by measurement of luciferase activity in the target cells 2 days after infection. This neutralization assay is quantitative, reproducible and relatively resistant to nonspecific effects of animal sera on the target cells. Three clade B HIV-1 envelope glycoproteins, YU2, ADA, and HXBc2, were incorporated into the recombinant viruses used in the assay. The ADA primary isolate, like YU2, is resistant to neutralizing antibodies, whereas HXBc2 is quite sensitive to neutralizing antibodies (66). This is reflected in the amounts of a highly potent neutralizing antibody, lgG1b12, required to inhibit recombinant viruses containing the three envelope glycoproteins (10). Whereas 90% neutralization of the HXBc2 virus was observed in the presence of only 1.25 μg of lgG1b12 per ml, the same degree of neutralization of the ADA and YU2 viruses could not be achieved by 10 and 20 μg of lgG1b12 per ml, respectively. Approximately 50% neutralization of the ADA and YU2 viruses was observed at 2.5 and 5 μg of lgG1b12 per ml, respectively (data not shown).

Neutralizing antibodies elicited by primary HIV-1 envelope glycoproteins. Sera collected at 1 and 2 weeks following the second boost were pooled and examined for gp120 reactivity and neutralizing activity. The immune responses to the YU2 envelope glycoprotein variants are summarized in Table 1. All of the YU2 envelope glycoproteins elicited roughly comparable titers of antibodies reactive with the homologous YU2 gp120 glycoprotein captured on an ELISA plate. The titers of antibodies recognizing the HXBc2 gp120 glycoprotein were not higher in the sera of mice immunized with the trimeric YU2 glycoproteins than in the sera of gp 120-immunized animals (data not shown). None of the sera from mice immunized with the YU2 gp120 glycoprotein exhibited neutralizing activity against any of the viruses. By contrast, the soluble, stabilized YU2 trimers elicited neutralizing antibodies. The YU2 gp130 (−/GCN4) and gp140Δ675 (−/GCN4) glycoproteins generated serum responses that in some cases neutralized the heterologous viruses but did not neutralize the homologous YU2 virus. This pattern of neutralization probably reflects the relative ease of neutralization of the three viruses (66). The sera of several mice immunized with the YU2 gp140Δ683(−/GCN4) glycoprotein neutralized all three HIV-1 strains. Four of six sera from this group of immunized mice mediated 90% neutralization of the YU2 virus at dilutions of 1:20 or greater; even 20 μg of the lgG1b12 antibody per ml could not achieve this level of neutralization in this assay. These results indicate that the soluble, stabilized YU2

TABLE 1. Immune responses following immunization with primary HIV-1 envelope glycoproteins

Antigen	Mouse	Neutralizing antibody titer[a]			Anti-gp120 reactivity with YU2 gp120[b]
		YU2	ADA	HXBc2	
BSA	34	−	−	−	−
	35	−	−	−	−
	36	−	−	−	−
	37	−	−	−	−
	38	−	−	−	−
	39	−	−	−	−
YU2 gp120	1	−	−	−	+++
	2	−	−	−	++++
	3	−	−	−	+++
	4	−	−	−	+++
	5	−	−	−	++
	6	−	−	−	+++
	53	−	−	−	+
	54	−	−	−	++
YU2 gp130(−/GCN4)	7	−	−	1:20	+++
	8	−	−	−	+++
	9	−	−	1:40	+++
	10	−	−	−	+++
	11	−	1:10	1:10	+++
	12	−	1:10	1:10	+++
YU2 gp140Δ675(−/GCN4)	13	−	1:40	1:10	+++
	14	−	−	1:20 (1:20)	+++
	15	1:20	1:20	1:20	++++
	16	−	1:20	1:20	+++
	17	−	−	1:10	++++
	18	−	1:20	1:10	++
YU2 gp140Δ683(−/GCN4)	55	>1:40 (>1:40)	−	>1:20 (1:10)	++++
	56	−	−	−	++
	57	1:20 (1:20)	1:40	1:20 (1:10)	++++
	58	1:40	1:40	1:10	+++
	59	1:20 (1:20)	1:40	1:10	+++
	60	1:40 (1:20)	1:40	1:20	+++

[a] Sera collected at 7 and 14 days following the second boost were pooled and assessed for neutralizing activity. The dilution of serum that resulted in at least 50% neutralization of recombinant viruses containing the indicated HIV-1 envelope glycoproteins is shown. A minus sign indicates that less than 50% inhibition was observed at a 1:10 dilution of the serum tested. The dilution of serum that resulted in at least 90% neutralization of the recombinant viruses is indicated in parentheses, when such neutralization was achieved.

[b] The reactivity of the serum with the YU2 gp120 glycoprotein captured on an ELISA plate is indicated. The scale is as follows: −, no signal at 1:2,000 dilution; +, signal at 1:2,000 dilution; ++, signal at 1:10,000 dilution; +++, signal at 1:50,000 dilution; ++++, signal at 1:250,000 dilution.

trimers, particularly the gp140Δ683(−/GCN4) glycoprotein, can elicit antibodies that neutralize primary HIV-1 isolates. In this respect, soluble, stabilized YU2 trimers appear to be significantly more effective than the monomeric YU2 gp120 glycoprotein.

To evaluate the breadth of neutralizing antibody responses elicited by the soluble, stabilized YU2 trimers, the sera were tested against recombinant viruses containing the envelope glycoproteins of primary HIV-1 isolates from clade B as well as clades C, D, and E. Due to limitations in the amount of sera available, these experiments were performed at only one dilution (1:20). Again, the three soluble, stabilized YU2 trimers induced better neutralizing antibodies against the 89.6 and JR-FL viruses, two clade B HIV-1 strains, than the gp120 glycoprotein did (data not shown). This neutralizing activity was weaker than that seen for viruses with the YU2 and ADA envelope glycoproteins. The gp140Δ683(−/GCN4) glycoprotein was not significantly different from the other two trimeric forms in the elicitation of neutralizing antibodies against the 89.6 and JR-FL strains. No neutralizing activity was observed against recombinant viruses containing the envelope glycoproteins from primary isolates outside of clade B (data not shown).

Antibodies elicited by TCLA HIV-1 envelope glycoproteins. To examine whether the strain of the envelope glycoprotein immunogen can influence the results, mice were immunized with the gp120 and gp140Δ683(−/GCN4) glycoproteins derived from the HXBc2 TCLA HIV-1 strain. The HXBc2 trimers elicited more consistent neutralizing antibody responses than the HXBc2 gp120 glycoprotein did (Table 2). However, this neutralizing activity was almost exclusively restricted to the homologous HXBc2 virus and did not inhibit infection by the primary viruses, ADA and YU2. These results suggest that the strain from which the envelope glycoprotein components of soluble, stabilized trimers are derived can influence the efficiency with which neutralizing activity against clinical HIV-1 isolates is generated.

TABLE 2. Immune responses following immunization with TCLA HIV-1 envelope glycoproteins

Antigen	Mouse	Neutralizing antibody titer[a]			Anti-gp120 reactivity[b]	
		YU2	ADA	HXBc2	HXBc2 gp120	YU2 gp120
HXBc2 gp120	22	−	−	1:10	++	++
	23	−	−	1:80 (1:20)	++	++
	24	−	−	−	++	+
	25	−	−	−	−	+
	26	−	−	−	−	+
	27	−	−	−	+	+
HXBc2 gp140Δ675(−/GCN4)	28	−	−	1:80 (1:40)	++	++
	29	−	−	1:20 (1:20)	++	+
	30	−	1:10	1:80 (1:40)	++	+
	31	−	−	1:40 (1:10)	+	++
	32	−	−	1:10 (1:10)	+	+
	33	−	−	1:20 (1:10)	++	+

[a] Neutralizing antibody titers in pooled sera collected at 1 and 2 weeks following the second boost are reported as in Table 1.
[b] The reactivity of the serum with the HXBc2 and YU2 gp120 glycoproteins captured on ELISA plates is indicated. The scale is the same as that in Table 1. The sera from mice immunized with BSA did not exhibit reactivity with the captured gp120 glycoproteins (data not shown).

DISCUSSION

The development of an HIV-1 vaccine has been frustrated in part by the difficulty of eliciting neutralizing antibodies active against clinical HIV-1 isolates (3, 4, 6, 14, 28, 29, 41, 42, 57, 76, 79). To date, no defined immunogen has proven better than the gp120 glycoprotein, which generates primary virus-neutralizing activity only after an aggressive immunization protocol involving many boosts (3, 4, 14, 28, 29, 41, 42, 57, 76). Immunization of specific transgenic mice with mixtures of cells expressing envelope glycoproteins and receptors has been reported to yield antibodies able to neutralize a broad range of primary HIV-1 isolates (37); however, despite extensive efforts, the relevant immunogen has not been defined and the reproducibility and general applicability of the results have not been demonstrated. Our results indicate that soluble, stabilized trimers are more effective than gp120 at eliciting antibodies that neutralize HIV-1. Such trimers may represent more faithful mimics of the functional envelope glycoprotein complex, may retain relevant conformations more stably in vivo, and may present multiple, cross-linked epitopes to responding B lymphocytes.

Our results suggest that the HIV-1 strain from which the soluble, stabilized trimers are derived can influence the elicitation of primary virus-neutralizing activity. The trimers derived from the primary YU2 isolate generated better neutralizing responses against clinical HIV-1 isolates than did trimers from a TCLA virus. This suggests that some primary virus trimers can elicit antibodies that recognize structures common to several primary isolates and at least one TCLA HIV-1 isolate. The neutralizing antibodies generated by the TCLA virus trimer, although quite potent against the homologous TCLA isolate, were not generally active against the primary isolates tested. Despite the similarity of recognition of the YU2 and HXBc2 trimers by antibodies directed against conserved epitopes (85), the neutralizing antibody response to the TCLA virus trimer appears to be dominated by reactivity with more strain-specific elements. The properties that render soluble, stabilized trimers from certain HIV-1 strains more effective as immunogens merit further investigation.

The YU2 gp140Δ683(−/GCN4) trimers, which contain the complete HIV-1 envelope glycoprotein ectodomains, raised antibodies that inhibited the YU2 virus, one of the primary HIV-1 isolates most resistant to antibody-mediated neutralization. The neutralizing activity of this sera, at 1:20 and 1:40 dilutions, was comparable in our assay to that of 5 to 20 μg of the lgG1b12 antibody per ml, one of the most potent HIV-1-neutralizing antibodies identified to date (10). Thus, with the appropriate immunogen, neutralizing active against even relatively refractory primary HIV-1 isolates is achievable using a conservative immunization protocol.

Although soluble, stabilized trimers appeared to elicit some qualitatively desirable neutralizing antibody responses, efforts to improve the titer and breadth of these responses are clearly merited. The titers of primary-virus-neutralizing antibodies observed in our immunized mice were low. Furthermore, the breadth of neutralization was limited to a subset of clade B viruses. These limitations may reflect intrinsic immunogenic properties of the HIV-1 envelope glycoprotein complexes. Alternatively, the soluble stabilized trimers may imperfectly mimic the functional virion envelope glycoprotein spikes. Further modifications of the immunogens and methods of antigen presentation to the immune system may lead to improved neutralizing antibody responses.

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