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Human Immunodeficiency Virus Type 1 Elite Neutralizers: Individuals with Broad and Potent Neutralizing Activity Identified by Using a High-Throughput Neutralization Assay together with an Analytical Selection Algorithm▽†

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The development of a rapid and efficient system to identify human immunodeficiency virus type 1 (HIV-1)-infected individuals with broad and potent HIV-1-specific neutralizing antibody responses is an important step toward the discovery of critical neutralization targets for rational AIDS vaccine design. In this study, samples from HIV-1-infected volunteers from diverse epidemiological regions were screened for neutralization responses using pseudovirus panels composed of clades A, B, C, and D and circulating recombinant forms (CRFs). Initially, 463 serum and plasma samples from Australia, Rwanda, Uganda, the United Kingdom, and Zambia were screened to explore neutralization patterns and selection ranking algorithms. Samples were identified that neutralized representative isolates from at least four clade/CRF groups with titers above prespecified thresholds and ranked based on a weighted average of their log-transformed neutralization titers. Linear regression methods selected a five-pseudovirus subset, representing clades A, B, and C and one CRF01_AE, that could identify top-ranking samples with 50% inhibitory concentration (IC_{50}) neutralization titers of ≥100 to multiple isolates within at least four clade groups. This reduced panel was then used to screen 1,234 new samples from the Ivory Coast, Kenya, South Africa, Thailand, and the United States, and 1% were identified as elite neutralizers. Elite activity is defined as the ability to neutralize, on average, more than one pseudovirus at an IC_{50} titer of 300 within a clade group and across at least four clade groups. These elite neutralizers provide promising starting material for the isolation of broadly neutralizing monoclonal antibodies to assist in HIV-1 vaccine design.

Since the identification of human immunodeficiency virus type 1 (HIV-1) as the cause of AIDS, one of the greatest challenges has been the development of a vaccine that will prevent infection and/or ameliorate disease progression (38, 43). Although over 100 phase I, II, and III vaccine clinical trials of different candidates have been conducted all over the world, only a few candidates have advanced to efficacy testing and none has yet to show any benefit in prevention or control of HIV-1 (HIV Vaccine Database; www.iavi.org). In other viral diseases (such as polio, influenza, and measles), neutralizing antibodies are generated as part of either the natural immune response to infection or the response to immunization, and their role in protective immunity is well established (10, 12, 15, 22, 37, 42, 45, 47, 49, 52). For HIV-1, studies in animal models indicate that both broadly neutralizing antibodies and cell-mediated responses may be required to provide vaccine pro-

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tection (7, 14, 16, 20, 29, 31, 33, 34, 39, 53). Unlike many other viruses, HIV-1 is highly variable, with multiple subtypes and recombinant forms circulating in different regions of the world. This high level of HIV-1 genetic variability, particularly in the envelope glycoproteins (gp120 and gp41), has been one of the greatest obstacles in development of a safe and effective HIV-1 vaccine and in particular in the elicitation of broadly neutralizing antibodies. In addition, HIV-1 has other mechanisms of immune escape preventing elicitation of broadly neutralizing antibodies, including the heavy glycosylation of the envelope glycoproteins, instability of such glycoproteins, and conformational masking of receptor-binding sites (6, 25, 32).

Despite the enormous diversity of HIV-1, a relatively small number of broadly neutralizing monoclonal antibodies (bnMAbs) have been isolated, providing evidence that broad neutralization by single antibody specificities can be achieved (3–5, 8, 9, 17, 21, 23, 24, 29, 35, 36, 40, 41, 44, 50, 51, 55). Structures for such bnMAbs have been determined in complex with HIV-1 Env (26, 54) and provide starting points for the design of immunogens capable of eliciting broadly neutralizing antibodies. However, since there are only a few such bnMAbs, we established a global program as part of International AIDS Vaccine Initiative's (IAVI's) Neutralizing Antibody Consortium (6), aimed at screening HIV-1$^+$ subjects with the goal of identifying individuals with broad and potent neutralizing activities as a potential source of novel bnMAbs, with an emphasis placed on individuals infected with non-clade B viruses. This paper describes the screening algorithm implemented to successfully identify HIV-1$^+$ subjects with broadly neutralizing antibodies, including a subset of individuals termed "elite neutralizers." These volunteers will be studied further to characterize the specificities of serum antibodies and will provide source materials for isolation of bnMAbs.

MATERIALS AND METHODS

Specimens. After obtaining written informed consent, sera and plasma were collected from HIV-1-infected volunteers in Australia, the United Kingdom, Rwanda, Zambia, Ivory Coast, Thailand, Kenya, Uganda, and the United States. Eligible participants were age 18 years or older, were HIV-1 infected for at least 3 years prior to the day of screening, were clinically asymptomatic, without evidence of progression to AIDS based on WHO stage III or IV criteria or a CD4 count of <200 cells/mm^3, and were not on antiretroviral therapy (ART) for at least the previous 1 year. In Rwanda, Zambia, and the United States, previously collected specimens from volunteers who met the eligibility criteria were included. In Australia, previously collected specimens from a smaller population of long-term nonprogressors with a Δ32CCR5 heterozygote mutation or who were infected with a Δnef virus were also included. In addition, the first 101 samples screened from Rwanda and Zambia were identified as long-term nonprogressors, defined in Zambia as individuals diagnosed with HIV-1 for at least 8 years with no clinical symptoms of AIDS and in Rwanda as individuals diagnosed with HIV-1 for at least 16 years who had CD4 counts of >500. The study was reviewed and approved by the Republic of Rwanda National Ethics Committee, Emory University Institutional Review Board, University of Zambia Research Ethics Committee, Charing Cross Research Ethics Committee, UVRI Science and Ethics Committee, University of New South Wales Research Ethics Committee, St. Vincent's Hospital and Eastern Sydney Area Health Service, Kenyatta National Hospital Ethics and Research Committee, University of Cape Town Research Ethics Committee, International Institutional Review Board, Mahidol University Ethics Committee, Walter Reed Army Institute of Research Institutional Review Board, and Ivory Coast Comité National d'Ethique des Sciences de la Vie et de la Santé.

Sera were obtained using standard serum separation tubes, and plasma was collected using acid citrate dextrose or EDTA tubes. All samples were aliquoted at either 500 μl or 1 ml and kept at −80°C. Although plasma samples were screened, <5% of all samples tested were from plasma. Previous work examining differences in neutralization activities between plasma and sera indicated that generally there are no nonspecific effects from the standard anticoagulants acid citrate dextrose and EDTA (T. Wrin, personal communication, March 2009). If anticoagulants were exercising a nonspecific inhibitory effect in a specific case, the specificity control, amphotrophic murine leukemia virus, would detect the effect and the inhibitory activity ascribed to the antibody would be adjusted accordingly.

Neutralization assays. All samples were tested against one of four different pseudovirus screening panels (Tables 1, 2, and 3) at Monogram Biosciences using previously described methods (2). Briefly, pseudoviruses capable of a single round of infection were produced by cotransfecting HEK293 cells with a subgenomic plasmid, pHIV-1lucΔu3, that incorporates a firefly luciferase indicator gene and a second plasmid, pCXAS, that expresses HIV-1 Env libraries or clones. Following transfection, pseudoviruses were harvested and used to infected U87 cell lines expressing either the coreceptor CCR5 or CXCR4. Threefold dilutions of serum or plasma starting at either 1:50 or 1:100 were initiated (total ranges of 1:50 to 1:1,350 and 1:100 to 1:2,700, respectively), and for a few samples the dilution range was 1:100 to 1:4,106. Neutralization activity was assessed as positive if the inhibition of an HIV-1 isolate was >50% and at least 1.7 times higher than any inhibition of the specificity control virus, amphotrophic murine leukemia virus Env pseudotyped on an HIV-1 core. Data readouts for each HIV-1 pseudovirus indicated the highest dilution factor at which there was positive activity. All of the panels utilized consisted of envelopes from primary isolates that were either single clones or quasispecies, and the pseudoviruses selected were based on previously determined neutralization sensitivities to clade B sera and MAbs b12, 2G12, and 4E10.

Broadly neutralizing sera and polyclonal IgG. Immunoglobulin G (IgG) was extracted from the top 5% of samples identified by panel 3 by using protein A. Immunoglobulin in both the raw serum and the purified prep was quantified using a human IgG enzyme-linked immunosorbent assay (Zeptometrix). Both the serum and the IgG were then tested at starting concentrations equivalent to 100 μg/ml on panel 4.

Anti-CD4, anti-CCR5, and anti-CXCR4 activities. Raw serum and purified IgG from the top 5% of samples identified by panel 3 were evaluated for cell-binding activity through fluorescence-activated cell sorter (FACS) analysis. Target cells expressing CD4 alone, U87/CD4, or one or both of the HIV-1 coreceptor molecules U87/CD4/CCR5, U87/CD4/CXCR4 or U87/CD4/CCR5/CXCR4 were incubated with ~100 to 150 μg/ml of IgG at room temperature for 1 h. Cells were rinsed and incubated with labeled secondary antibody, R-PE-F(ab′)$_2$ goat anti-human IgG (Jackson Immunoresearch). Cells were stained separately for either CCR5 or CXCR4 directly with phycoerythrin-conjugated antibodies (Becton Dickinson). After 1 h of further incubation cells were rinsed, fixed with 2% paraformaldehyde, and evaluated for cell surface staining by FACS analysis (Becton Dickinson FACSCalibur).

Statistical analyses. For an individual sample, breadth was defined as the number of clade/CRF groups for which there were detectable titers against at least one pseudovirus in a given clade/CRF group. Breadth within clade was defined as the number of clade-specific pseudoviruses for which there were detectable titers. Potency of an individual sample was determined by its neutralization score, defined as a weighted average of log-transformed titers across the pseudoviruses of a given panel. Various weights for the pseudoviruses were considered that reflected a pseudovirus's relative sensitivity to neutralization. Seven scoring algorithms were examined to identify an algorithm that could best select individuals with broad and elite neutralizing activities. Details of the different methods explored are described in further detail in the supplemental material for this paper.

Spearman's rank correlation coefficient was used to assess the association between continuous variables, such as 50% inhibitory concentration (IC_{50}) titers, breadth of neutralization, and neutralization scores. Fisher's exact test and Pearson's chi-squared test were used to assess the association between categorical variables, such as the presence of detectable titer (yes/no), clade/CRF group of the panel pseudovirus, and site. Site was treated as a proxy for the clade of the HIV-1 infecting the serum/plasma donor. Estimates of the reported dominant clade group were obtained from the Los Alamos database and confirmed from either sequence data generated from random sampling of the cohorts from which the Protocol G volunteers were selected or from sequences directly derived from Protocol G volunteers (E. Hunter, P. Kaleebu, A. Pozniak, personal communication, March 2009) (18) (Table 4). Samples from Australia and the United Kingdom were from clade B-infected individuals. Samples from Rwanda were primarily from clade A, while samples from Zambia were primarily from clade C. Samples from Uganda represented a mix of clade A and D infections, with clade

TABLE 1. Neutralization profiles of HIV-1 isolates used in preliminary screening panels 1 and 2 (n = 101)

Virus	Clade	Origin, disease stage	Collection yr	Neutralization profile with:		Screening panel no(s).
				Clade B sera	MAbs	
94UG103	A	Uganda, chronic infection	1994	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive	1
92RW020	A	Rwanda, chronic infection	1992	Moderately resistant	b12 resistant; 2G12 sensitive; 4E10 sensitive	1
93RW029	A	Rwanda, chronic infection	1993	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive	2
93UG077	A	Uganda, chronic infection	1993	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive	2
92BR020	B	Brazil, chronic infection	1992	Moderately resistant	b12 sensitive; 2G12 sensitive; 4E10 borderline resistant	1
92HT593	B	Haiti, chronic infection	1992	Moderately sensitive	b12 very sensitive; 2G12 sensitive; 4E10 sensitive	1
AC10.0.29	B	United States, early infection	1998	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 very sensitive	2
TRJO4551.58	B	United States, early infection	2001	Moderately sensitive	b12 resistant; 2G12 resistant; 4E10 sensitive	2
QH0692.42	B	Trinidad, early infection	1994	Moderately resistant	b12 very sensitive; 2G12 sensitive; 4E10 sensitive	2
6535.3	B	United States, early infection	1995	Sensitive	b12 sensitive; 2G12 sensitive; 4E10 very sensitive	2
SC422661.8	B	Trinidad, early infection	1995	Moderately sensitive	b12 very sensitive; 2G12 sensitive; 4E10 very sensitive	2
PVO.4	B	Italy, early infection	1996	Moderately resistant	b12 resistant; 2G12 sensitive; 4E10 sensitive	2
TRO.11	B	Italy, early infection	1995	Moderately sensitive	b12 resistant; 2G12 very sensitive; 4E10 very sensitive	2
RHPA4259.7	B	United States, early infection	2000	Moderately resistant	b12 very sensitive; 2G12 resistant; 4E10 sensitive	2
THRO4156.18	B	United States, early infection	2000	Moderately resistant	b12 very sensitive; 2G12 resistant; 4E10 sensitive	2
REJO4541.67	B	United States, early infection	2001	Moderately resistant	b12 very sensitive; 2G12 resistant; 4E10 very sensitive	2
WITO4160.33	B	United States, early infection	2001	Moderately sensitive	b12 sensitive; 2G12 sensitive; 4E10 very sensitive	2
CAAN5342.A2	B	United States, early infection	2000	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive	2
JR-CSF	B	United States, chronic infection	1986	Moderately sensitive	b12 sensitive; 2G12 sensitive; 4E10 sensitive	1 and 2
NL4-3	B	United States, chronic infection	1985	Very sensitive	b12 very sensitive; 2G12 very sensitive; 4E10 very sensitive	1 and 2
93IN905	C	India, chronic infection	1993	Moderately sensitive	b12 sensitive; 2G12 resistant; 4E10 sensitive	1
93MW959	C	Malawi, chronic infection	1993	Moderately sensitive	b12 resistant; 2G12 resistant; 4E10 sensitive	2
98IN022	C	India, chronic infection	1998	Moderately sensitive	b12 very sensitive; 2G12 resistant; 4E10 very sensitive	2
92UG046	D	Uganda, chronic infection	1992	Moderately resistant	b12 very sensitive; 2G12 resistant; 4E10 sensitive	1
94UG114	D	Uganda, chronic infection	1994	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive	1
92UG024	D	Uganda, chronic infection	1992	Resistant	b12 borderline resistant; 2G12 very sensitive; 4E10 sensitive	2
92UG005	D	Uganda, chronic infection	1992	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive	2
VLGC G1	G	Unknown	2002	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive	
92TH021	CRF01_AE	Thailand, chronic infection	1992	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive	1
CMU02	CRF01_AE	Thailand, chronic infection	1993	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive	1
92TH022	CRF01_AE	Thailand, chronic infection	1992	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 very sensitive	2
92TH006	CRF01_AE	Thailand, chronic infection	1992	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive	2
98CN006	CRF08_BC	China, chronic infection	1998	Moderately sensitive	b12 sensitive; 2G12 resistant; 4E10 very sensitive	1
98CN009	CRF07_BC	China, chronic infection	1998	Moderately resistant	b12 very sensitive; 2G12 resistant; 4E10 sensitive	2
94KE105	A1C	Kenya, chronic infection	1994	Moderately resistant	b12 resistant; 2G12 sensitive; 4E10 sensitive	2

A predominating among the cohort from which Protocol G volunteers were identified. Stepwise linear regression was utilized to determine a suitable subset of pseudoviruses from panel 3 that explained at least 90% of the variation in the neutralization score that gave equal weight to each panel pseudovirus. Linear mixed effects models were applied to the data on IC_{50} titers from panel 3 to assess the effect of the clade/CRF group of a given panel pseudovirus and the effect of site from which the sample was obtained on the magnitude of the antibody response. These models included fixed effects for clade/CRF group of

TABLE 2. Neutralization profiles with final panel selected (panel 3) for evaluation of samples from clade A, B, C, or D HIV-1 epidemics (n = 463)

Virus	Clade	Origin, disease stage	Collection yr	Neutralization profile with:	
				Clade B sera	MAbs
94UG103	A	Uganda, chronic infection	1994	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive
92RW020	A	Rwanda, chronic infection	1992	Moderately resistant	b12 resistant; 2G12 sensitive; 4E10 sensitive
92BR020	B	Brazil, chronic infection	1992	Moderately resistant	b12 sensitive; 2G12 sensitive; 4E10 borderline resistant
APV13	B	United States, early infection	2001	Resistant	b12 resistant; 2G12 sensitive; 4E10 sensitive
APV17	B	United States, early infection	2002	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive
APV6	B	United States, early infection	2003	Sensitive	b12 resistant; 2G12 sensitive; 4E10 sensitive
JR-CSF	B	United States, chronic infection	1986	Moderately sensitive	b12 sensitive; 2G12 sensitive; 4E10 sensitive
NL4-3	B	United States, chronic infection	1985	Very sensitive	b12 very sensitive; 2G12 very sensitive; 4E10 very sensitive
93IN905	C	India, chronic infection	1993	Moderately sensitive	b12 sensitive; 2G12 resistant; 4E10 sensitive
IAVI_C3	C	Africa, unknown infection stage	2001	Moderately sensitive	b12 sensitive; 2G12 resistant; 4E10 sensitive
IAVI_C18	C	Africa, unknown infection stage	2003	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 resistant
IAVI_C22	C	Africa, unknown infection stage	2005	Resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive
94UG114	D	Uganda, chronic infection	1994	Moderately resistant	b12 resistant; 2G12 resistant; 4E10 sensitive
92TH021	CRF01_AE	Thailand, chronic infection	1992	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive
CMU02	CRF01_AE	Thailand, chronic infection	1993	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive

the pseudovirus, site from which the sample was obtained, and all two-way interaction terms between clade and site. A random effect for each sample was included to account for the correlation among titer values for a given sample. All analyses were implemented with the software program R, version 2.7.1, or SAS version 9.1.

RESULTS

Demographics of all volunteers screened. A total of 1,798 samples from HIV-1-infected individuals were collected from Australia (n = 81), the United Kingdom (n = 196), Rwanda (n = 207), Kenya (n = 201), Uganda (n = 247), Zambia (n = 205), Ivory Coast (n = 200), Thailand (n = 200), South Africa (n = 170), and the United States (n = 91). Of these, 101 were screened on panels 1 and 2, 463 on panel 3, and 1,234 on panel 4 (Tables 1 to 3). Overall, 36% of participants were male and 57% were female; 7% of samples did not have available data on gender. Heterosexual transmission was reported in 78% of volunteers, while men who have sex with men accounted for 11% of the study population. Intravenous drug use accounted for 3% of volunteers, primarily from Thailand, and "other" constituted 3% of the study population and included transmission via blood transfusion or unknown transmission route as self-reported. Five percent of samples did not have available data on mode of HIV-1 transmission (Table 4).

Reduction of initial HIV-1 screening panels. Between November 2005 and April 2006, 101 samples from Rwanda and Zambia were initially screened against panel 1, which consisted of 12 HIV-1 pseudoviruses representing clades A, B, C, and D and CRF08_BC and CRF01_ AE (Table 1). Four of these samples were excluded from further analysis due to suspected antiretroviral use as determined by neutralization patterns on the screening panel. Of the 97 samples evaluated, 18 (19%) had no inhibition ($IC_{50} \leq 50$) of any of the clade groups, 44 (45%) had an IC_{50} titer of ≥150 to one to three clades, and 35 (36%) had IC_{50} titers of ≥150 to four or more clades (Fig. 1). Of the 35 samples exhibiting broad neutralization, 22 were selected for screening against panel 2. Panel 2 was chosen for further screening of individuals that had been preliminarily identified as having broad neutralization activity on panel 1, because panel 2 represented more recently transmitted viruses, with 26 pseudoviruses from clades A, B, C, D, and G and CRF07_BC and CRF01_AE (Table 1). In addition, 12 of the 14 clade B pseudoviruses on the panel represented *env* clones from acute and early subtype B infections previously characterized by Montefiori et al. and are frequently used as a standard reference panel for neutralization screening (28). All 22 samples tested continued to show both breadth and potency of neutralization against the larger panel with an average geometric mean titer (GMT) of 130 (Fig. 2). Spearman's rank correlation analyses of the natural log-transformed maximum titers of panel 1 and 2 pseudoviruses indicated a strong correlation between the titers of the 12 clade B pseudoviruses from acute and early infection and the two clade B pseudoviruses from chronically infected individuals (R = 0.74; $P < 0.001$). Similar patterns of correlation between panels 1 and 2 were also seen for clade A (R = 0.44; $P < 0.05$), clade C (R = 0.83; $P < 0.001$), clade D (R = 0.67; $P < 0.001$), CRF07_BC or CRF08_BC (R = 0.57; $P < 0.05$), and CRF01_AE (R = 0.70; $P < 0.001$). Clades G and A1C were not examined, since isolates from the same clade group were not included on the earlier panel.

TABLE 3. Neutralization profiles with the high-throughput screening panel (panel 4)

Virus	Clade	Origin, disease stage	Collection yr	Neutralization profile with:	
				Clade B sera	MAbs
94UG103	A	Uganda, Chronic Infection	1994	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive
92BR020	B	Brazil, Chronic Infection	1992	Moderately resistant	b12 sensitive; 2G12 sensitive; 4E10 borderline resistant
JRC-CSF	B	USA, Chronic Infection	1986	Moderately sensitive	b12 sensitive; 2G12 sensitive; 4E10 sensitive
NL4-3	B	USA, Chronic Infection	1985	Very sensitive	b12 very sensitive; 2G12 very sensitive; 4E10 very sensitive
93IN905	C	India, Chronic Infection	1993	Moderately sensitive	b12 sensitive; 2G12 resistant; 4E10 sensitive
IAVI_C22	C	Africa, Unknown Infection Stage	2005	Resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive
92TH021	CRF01_AE	Thailand, Chronic Infection	1992	Moderately resistant	b12 sensitive; 2G12 resistant; 4E10 sensitive

TABLE 4. Baseline demographics of volunteers screened on panels 1, 2, 3, and 4[a]

Panel(s) used and site no.	Country (*n*)	Gender [% of study population (*n*)]			Mode of transmission [% of study population (*n*)]					Dominant HIV-1 clade(s)
		M	F	Unknown	Heterosexual	IDUs	MSM	Other	Unknown	
Screened on panels 1 and 2										
Site 1	Rwanda (24)	0	100 (24)		100					A
Site 2	Zambia (77)	40.3 (31)	59.7 (46)		100					C
All sites	All (101)	30.7 (31)	69.3 (70)		100					A, C
Screened on panel 3										
Site 1	Australia (81)	97.5 (79)	2.5 (2)				96.3 (78)	2.7 (3)		B
Site 2	Rwanda (83)	53.7 (44)	46.3 (38)		100					A
Site 3	Uganda (189)	23.3 (44)	76.7 (145)		100					A, D
Site 4	United Kingdom (58)	94.8 (55)	5.2 (3)		5.2 (3)	1.7 (1)	91.4 (53)	1.7 (1)		B
Site 5	Zambia (52)	34.6 (18)	65.4 (34)		100					C
All sites	All (463)	51.8 (240)	48.2 (223)		70.63 (327)	<1 (1)	28.2 (131)	<1 (4)		A, B, C, D
Screened on panel 4										
Site 1	Rwanda (100)	49 (49)	51 (51)		100					A
Site 2	Uganda (58)	18.96 (11)	81.04 (47)		100					A, D
Site 3	United Kingdom (138)	34.06 (47)	3.6 (5)	62.32 (86)	5.8 (8)		27.53 (38)		66.67 (92)	B
Site 4	United States (91)	0	100 (91)		47.25 (43)	16.48 (15)		36.27 (33)		B
Site 5	Zambia (76)	34.2 (26)	65.8 (50)		100					C
Site 6	Thailand (200)	56 (112)	44 (88)		62 (124)	23 (46)	11 (22)	4 (8)		CRF01_AE, B
Site 7	Ivory Coast (200)	40.5 (81)	59.5 (119)		100					CRF02_AG
Site 8	South Africa (170)	7.1 (12)	89.9 (107)	30 (51)	100					C
Site 9	Kenya (201)	14.93 (30)	85.07 (171)		100					A
All sites	All (1,234)	29.82 (368)	59.08 (729)	11.1 (137)	79.42 (980)	4.94 (61)	4.86 (60)	3.32 (41)	7.46 (92)	A,B,C, D, CRF01_AE, and CRF02_AG
Screened on panels 1, 2, 3, and 4										
All sites	All (1,798)	35.54 (639)	56.84 (1,022)	7.62 (137)	78.31 (1,408)	3.45 (62)	10.62 (191)	2.50 (45)	5.12 (92)	A, B,C, D, CRF01_AE, and CRF02_AG

[a] M, male; F, female; MSM, men who have sex with men; IDU, intravenous drug users.

Preliminary data indicated that samples identified as having neutralization titers of ≥100 to at least four out of five clades consistently had breadth within a clade group and across clades no matter how many different pseudoviruses they were screened against. Therefore, there was no value in having two separate screening panels. Due to the strong correlation in neutralization titers between the pseudoviruses within each clade group and across clade groups, a new panel of 15 pseudoviruses (panel 3) was formed by selecting a subset of viruses from the first and second screening panels. Panel 3 consisted of pseudoviruses from clades A, B, C, and D and CRF01_AEs derived from either acute or chronic infections; it included nine of the previous pseudoviruses tested along with three new acute clade B isolates (APV6, APV13, and APV17), which represented more recently transmitted viruses (2001 to 2003) than the previous acute viruses, and also three new clade C African isolates (IAVIC3, IAVIC18, and IAVIC22) collected between 2001 and 2005.

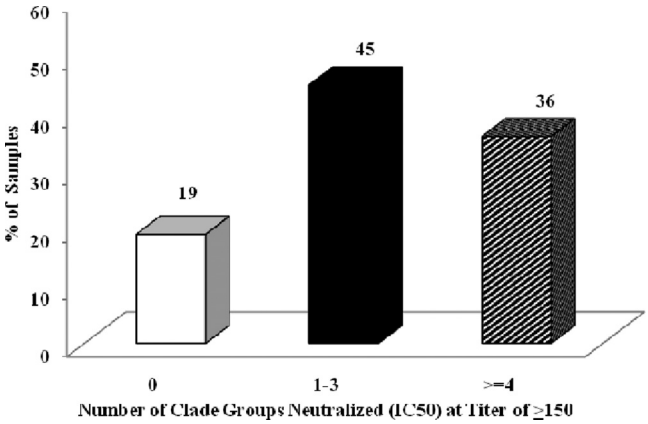

FIG. 1. Frequency of neutralization breadth, defined as an IC_{50} titer of ≥150 to at least one pseudovirus from four out of five clade/CRF groups, in 97 serum samples from Rwanda and Zambia.

Samples with cross-clade activity and neutralization sensitivities. Of the 463 samples evaluated by panel 3, 67 (15%) showed no inhibition (IC_{50} titer, <100) of pseudoviruses from any of the clade/CRF groups; 237 (51%) had an IC_{50} titer of ≥100 against pseudoviruses from one to three groups; and 159 (34%) had an IC_{50} titer of ≥100 against four or more groups (Fig. 3). There was a significant association between the site of origin and a sample showing no inhibition [$\chi^2(4) = 41.8$; $P < 0.001$]. Twenty-eight (35%) of 81 samples from Australia had no detectable titers. Fourteen of the 81 volunteers from Australia were either a CCR5 heterozygote or were infected with

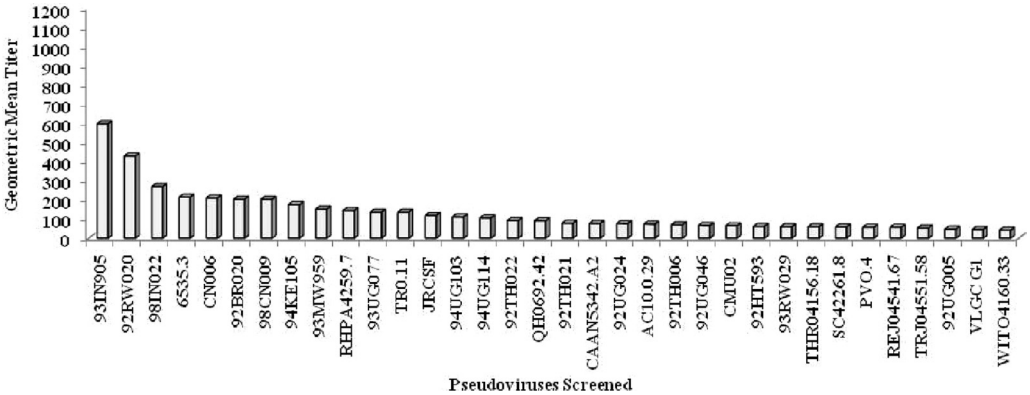

FIG. 2. Distribution of neutralization sensitivities of early/chronic HIV-1 Env clones on panel 2 among HIV-1-infected individuals previously identified as having broad neutralizing activity on panel 1. Each bar represents the geometric mean IC_{50} titer of 22 serum and plasma samples from Rwanda and Zambia. Among individuals with broad neutralizing activity, pseudovirus sensitivities varied.

a Δnef virus, although only 8 of the 14 had no detectable titers while 2 samples had very high titers to multiple clade/CRF groups. Table 5 displays the geometric mean neutralization titers for each pseudovirus by the site of sample origin.

Linear mixed effects models of the impact of site and clade/CRF group of the panel pseudovirus on the magnitude of the antibody titers showed that with the exception of the Rwandan samples neutralizing antibody titers were often highest toward strains that matched the presumed clade of the HIV-1 infecting the donor. For the Australian samples, the highest titers were exhibited against the clade B pseudoviruses; these titers were significantly higher on average ($P < 0.001$) than those against clades A, D, and AE, but not C. For the United Kingdom samples, the highest titers were again against the clade B pseudoviruses; these titers were significantly higher than those against clades C and AE, but not A or D. While Rwanda is experiencing a clade A epidemic, samples from this country showed higher titers against the pseudoviruses from clade C. Titers against the clade C pseudoviruses were higher on average than those of the other clade/CRF groups as well. Serum samples from Uganda had highest titers against pseudoviruses from clade A, which were significantly higher than those against clade B, D, and AE, but not C. Zambian samples displayed the highest titers against clade C strains; these titers were significantly higher than those from all other clade/CRF groups. Of note, the Zambian titers for the clade B strains did not appear to be different than those against clade B observed in the Australian and United Kingdom samples. Results from Spearman's rank correlation analyses examining the association between breadth within a clade B panel versus a clade C panel indicated that for the combined Australian and United Kingdom samples there was a strong positive association ($R = 0.66$; $P < 0.001$). Samples that neutralized multiple B pseudoviruses tended to neutralize multiple C pseudoviruses. For Zambia the correlation between clade B and C was $R = 0.72$ ($P < 0.0001$). Other clade groups were not examined, since ≤2 pseudoviruses per clade were available for comparison. In addition, Fig. 4 and 5 display the overall correlations between breadth across clade/CRF groups and overall potency of response within the 463 samples screened on panel 3. As breadth across clade/CRF groups increased, the overall probability of neutralization within a clade group also increased along with the overall potency of response.

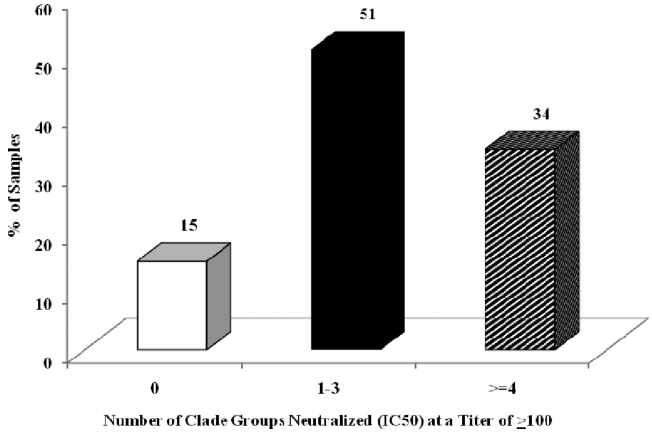

FIG. 3. Frequency of breadth, defined as an IC_{50} titer of ≥100 to at least four out of five clade/CRF groups, from 463 samples from Rwanda, Zambia, the United Kingdom, Australia, and Uganda.

TABLE 5. Geometric mean titers against panel 3 by site ($n = 463$)

Virus	Clade	GMT against panel 3				
		Clade A, Rwanda	Clades A and D, Uganda	Clade C, Zambia	Clade B, United Kingdom	Clade B, Australia
94UG103	A	58	54	60	49	36
92RW020	A	86	100	130	62	37
92BR020	B	84	77	67	81	52
APV13	B	37	37	62	41	36
APV17	B	40	35	38	36	35
APV6	B	131	96	119	94	83
JR-CSF	B	52	47	59	62	38
NL4-3	B	379	448	555	588	329
93IN905	C	172	110	391	81	60
IAVI_C3	C	114	64	155	43	42
IAVI_C18	C	38	39	40	33	34
IAVI_C22	C	77	73	179	55	40
94UG114	D	37	44	62	52	36
92TH021	CRF01_AE	51	48	52	39	36
CMU02	CRF01_AE	44	40	53	94	37

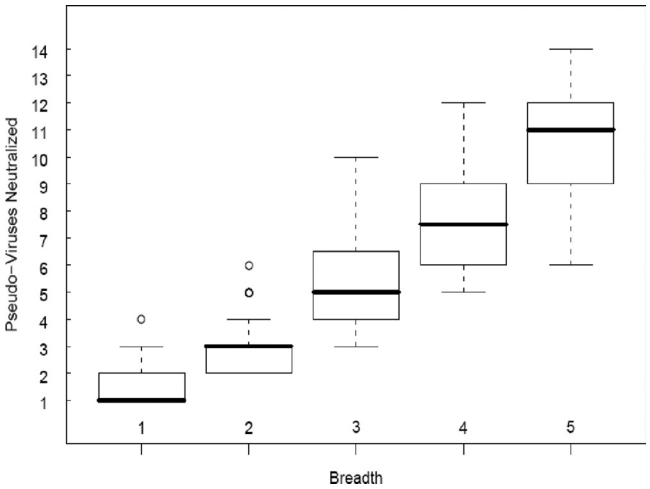

FIG. 4. Correlation between breadth of neutralization and number of pseudoviruses neutralized, representing the 463 samples screened on panel 3. As breadth increases, the overall probability of having a response to more than one pseudovirus both within and across clade/CRF groups also increases.

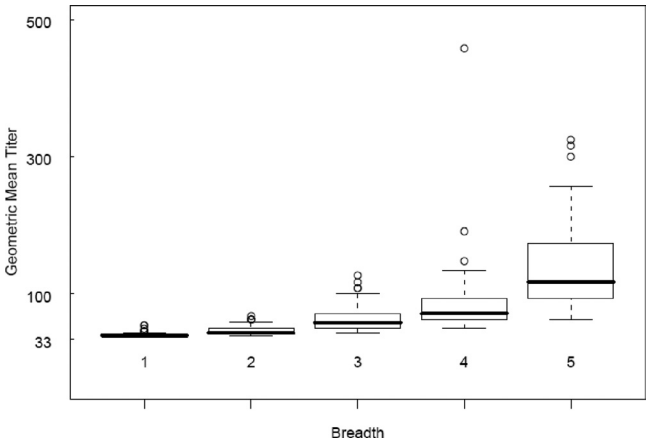

FIG. 5. Correlation between breadth of neutralization to different clade groups representing the 463 samples screened on panel 3 and overall geometric mean IC_{50} neutralization titer. As breadth increases the overall probability of having a more potent response to the pseudoviruses on the screening panel also increases.

Ranking of samples with cross-clade and potent neutralizing activities. The seven scoring methods for assessing potency were applied to the 463 samples evaluated by panel 3. All methods were highly correlated with one another and essentially selected the same top 5% of samples with the exception of the score that included only sensitive and moderately sensitive pseudoviruses. These findings suggest that the neutralization sensitivity of a pseudovirus may not be informative for identifying samples with high potency. Panel 3, however, had more pseudoviruses deemed resistant or moderately resistant than sensitive or moderately sensitive. Also, the resistance profiles were previously determined in clade B sera, and reexamination of these sensitivities found that not all classifications were the same in non-clade B sera (data not shown). Therefore, the score that gave equal weight to the pseudoviruses of all viruses on the panel excluding NL4-3 (score 1) was judged suitable for identifying samples with both broad and potent neutralization activities (further details are described in the supplemental material).

Table 6 displays the titer values for the top 5% of samples identified by score 1, while Fig. 6 is the histogram of score 1 for all 463 samples. Figure 7 illustrates the relationship between score 1 and the GMT for a given sample evaluated by panel 3. Figure 8 presents the GMT for each pseudovirus on panel 3 for the top 5% of samples. Again there was some suggestion of an association between region and having a high neutralization score [$\chi^2(3) = 4.89$; $P = 0.18$]. Only 5 (4%) of 139 samples from clade B regions were selected, while 6 (11%) of 54 samples from Zambia were selected.

Identification of a reduced panel of predictive cross-clade pseudoviruses and elite neutralizers. Stepwise linear regression analyses were performed to identify which pseudoviruses in panel 3 explained at least 90% of the variation in the neutralization score that gave equal weight to all pseudoviruses (score 1) and therefore could reliably predict the neutralization activity previously seen in the larger pseudovirus panel. One analysis which used an exhaustive search found three five-virus subsets that explained 93% of the variation in the neutralization score. The first subset contained 94UG103, 92BR020, 93IN905, IAVI C22, and 92TH021, which includes one resistant, three moderately resistant, and one moderately sensitive pseudovirus and represents four clade/CRF groups. The second subset replaces 93IN905, a moderately sensitive clade C pseudovirus, with IAVIC3, a moderately resistant clade C pseudovirus. The third subset replaces 94UG103, a moderately resistant clade A pseudovirus, in the second subset with 92RW020, another moderately resistant clade A pseudovirus. A forward selection procedure using the Akaike information criterion selected the first subset of 94UG103, 92BR020, 93IN905, IAVIC22, and 92TH021, while a backward elimination procedure chose the second subset of 94UG103, 92BR020, IAVIC3, IAVIC22, and 92TH021. The first subset along with NL4-3 and JR-CSF were selected for screening of future samples, since these pseudoviruses represented envelopes from the dominant HIV-1 clade groups and also regions in which the study was being conducted. For each new sample, a neutralization score was computed by calculating the average of the log-transformed titers for these pseudoviruses, excluding NL4-3. All samples with a score greater than or equal to 1.5 were retained for further possible studies and samples with scores greater than or equal to 2.5 were identified as elite neutralizers and were prioritized for isolation of bnMAbs. The neutralization scores and geometric mean titers of the elite neutralizers are displayed in Table 7. The majority of elite neutralizers identified in Table 7 were selected using panel 4 ($n = 1{,}234$), with only 6 elite neutralizers identified during the initial screening of 463 individuals on panel 3. Displayed in Table 7 for these six individuals are confirmatory neutralization activity results on the reduced panel at the time of peripheral blood mononuclear cell collection for the purpose of monoclonal antibody identification. The proportion of elite neutralizers by site is displayed in Fig. 9, with South Africa, Zambia, and the Ivory Coast accounting for the greatest proportion of elite neutralizers, 22%, followed by the United Kingdom and Uganda with 11% and then Rwanda and Zambia

TABLE 6. Neutralization profiles of top 5% of samples selected on panel 3 (n = 26)

			Clade A		Clade B						Clade C				Clade D	CRF01_AE	
Rank	Score1	Country	92RW020	94UG103	92BR020	APV6	APV13	APV17	JRCSF	NL43	IN905	IAVIC3	IAVIC18	IAVIC22	94UG114	92TH021	CMU02
1	2.387	Rwanda	4106	100	1190	345	100	345	1190	345	1190	1190	100	4106	4106	<100	<100
2	2.071	Rwanda	300	300	300	300	300	100	300	300	900	900	100	300	300	900	300
3	2.047	Zambia	345	345	345	100	100	345	1190	1190	1190	345	345	1190	100	345	100
4	2	Zambia	900	300	300	300	300	<100	300	900	900	900	300	900	300	300	<100
5	1.857	Uganda	900	100	900	300	100	100	300	900	900	300	100	900	900	100	<100
6.5	1.786	Australia	300	100	300	300	300	300	300	900	300	300	100	300	300	300	100
6.5	1.786	Uganda	2700	900	300	300	100	100	900	900	300	300	100	300	100	<100	100
8.5	1.714	Uganda	900	300	300	900	100	100	300	900	900	100	100	300	100	100	100
8.5	1.714	Zambia	100	300	<100	300	<100	<100	900	2700	2700	900	<100	300	300	2700	100
10.5	1.643	Australia	100	300	300	900	300	100	300	2700	300	300	100	300	100	100	100
10.5	1.643	UK	300	300	300	300	100	100	300	900	300	100	<100	300	300	300	300
12	1.591	UK	1190	345	345	345	100	100	345	1190	345	100	<100	1190	345	<100	<100
13	1.573	Zambia	1190	345	100	100	100	100	345	1190	345	345	100	345	100	345	<100
15	1.571	Rwanda	300	300	300	900	<100	100	300	900	900	300	<100	300	100	100	100
15	1.571	Uganda	900	300	300	900	300	300	900	2700	100	300	<100	<100	100	<100	100
15	1.571	Uganda	100	300	100	300	<100	<100	2700	300	2700	900	100	<100	100	900	<100
17	1.501	Zambia	1190	100	100	100	<100	100	100	1190	1190	100	100	1190	1190	<100	100
19	1.5	UK	300	300	300	300	100	<100	100	900	900	300	<100	900	100	100	100
19	1.5	Rwanda	300	100	300	300	<100	300	<100	900	900	300	100	300	300	100	100
19	1.5	Uganda	900	100	300	100	300	<100	100	300	900	300	100	900	100	<100	100
21.5	1.492	Zambia	345	100	345	100	100	100	100	1190	1190	345	100	1190	100	100	<100
21.5	1.492	Uganda	1190	1190	345	100	100	<100	100	1190	345	345	100	100	100	100	100
24.5	1.429	Rwanda	300	100	300	100	<100	100	300	900	900	900	<100	300	100	300	<100
24.5	1.429	Rwanda	900	300	300	300	100	<100	300	900	300	300	<100	300	100	100	<100
24.5	1.429	Rwanda	300	300	300	300	100	<100	300	900	300	300	<100	100	300	100	100
24.5	1.429	Uganda	300	100	300	300	100	100	<100	900	300	300	<100	900	300	100	100

with 6%. Elite neutralizers represent the top 1% of all samples screened and on average have a mean IC_{50} titer of 1:500 to the majority of the pseudoviruses on the screening panel. In addition, all elite neutralizers have a response to at least four clade/CRF groups.

Confirmation that broadly neutralizing activity is attributable to the IgG fraction of serum. IC_{50} titers based on the extracted IgG samples from 22 of the 26 top-ranking serum samples in panel 3 were measured against each of the pseudoviruses on the reduced panel. The association between serum and IgG neutralization for each pseudovirus was assessed by calculation of the Spearman rank correlation coefficient. IC_{50} titers greater than 100 μg/ml were left censored at the upper limit of detection. Table 8 displays the correlation coefficients along with their levels of significance. All correlation coefficients were significant at the 0.05 level with correlation coefficients ranging from 0.46 for pseudovirus 92TH021 to 0.94 for 92BR020. The relatively low correlation for 92TH021 is due in part to a significant number of samples with titers censored at the upper limit of detection. Overall, these results suggest that neutralization activity is attributable primarily to IgG. Furthermore, there was no evidence of anti-CD4, anti-CCR5, or anti-CXCR4 activity based on the comparison of differences between staining of plasma known to be negative for CCR5 and CXCR4 antibodies and donors identified as having broadly neutralizing activities in both the U87 CCR5 and U87 CXCR4 cell lines (data not shown).

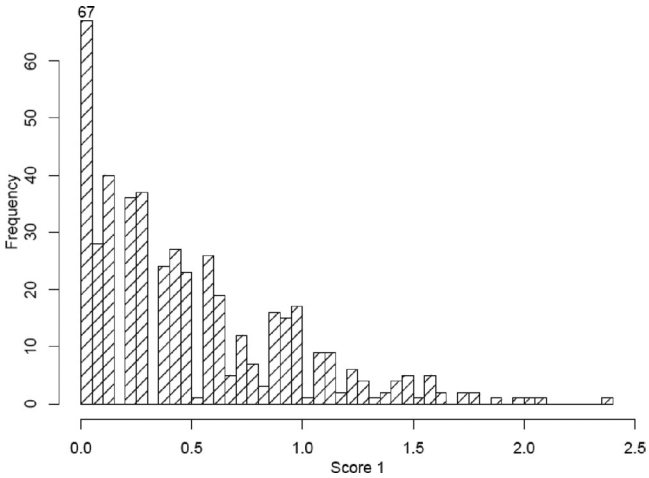

FIG. 6. Scoring distribution of all 463 samples screened on panel 3 using scoring method 1. Each bar represents the number of samples that fell within each scoring range. A score of 1.5 and above represents the top 10% of the overall distribution.

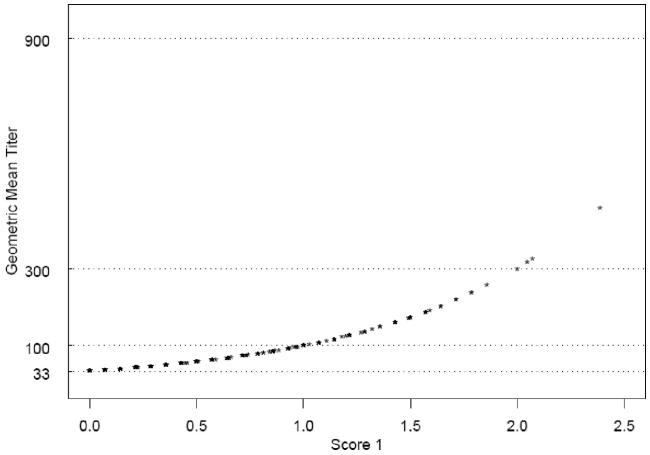

FIG. 7. Correlation plot between scoring method 1 and geometric mean IC_{50} titers. Each data point represents the geometric mean IC_{50} titer of all 463 samples tested on panel 3 against all 15 pseudoviruses evaluated. Samples that were scored between 1.5 and 2 had an average IC_{50} titer of 1:100 to 1:300. Samples that were scored between 2 and 2.4 had an average IC_{50} titer between 1:300 and 1:500. Samples with scores greater than or equal to 2.5 have an average IC_{50} titer greater than 1:500.

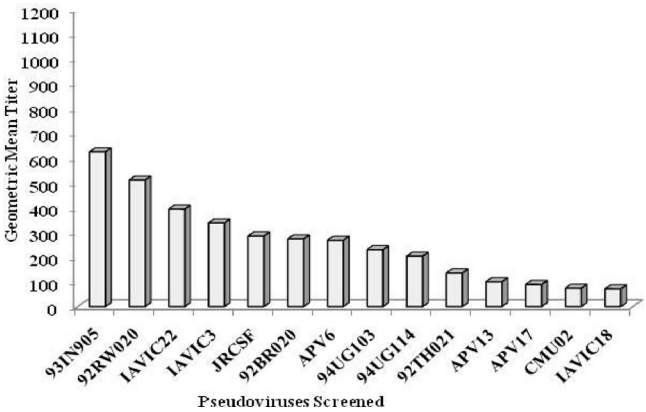

FIG. 8. Distribution of neutralization sensitivities of early/chronic HIV-1 Env clones on panel 3 among the top 5% of samples from HIV-1-infected individuals screened on panel 1. Each bar represents the geometric mean IC_{50} titer of 26 serum and plasma samples from Rwanda, Zambia, Uganda, Australia, and the United Kingdom.

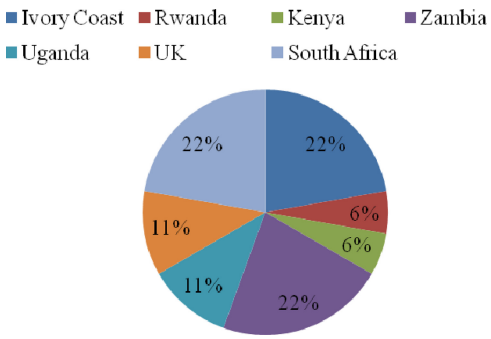

FIG. 9. Proportion of elite neutralizers with neutralization scores of ≥2.5 by site. Percentages represent the top 1% (n = 18) of 1,798 samples screened on panels 1, 2, 3, and 4.

DISCUSSION

This study represents the largest screening and evaluation of neutralization patterns to date in individuals infected with non-clade B HIV-1. The creation of an analytical selection algorithm and reduced virus screening panel to assess serum neutralization activity against multiple HIV-1 isolates representing several clade groups have enabled us to rapidly identify and prioritize HIV-1^+ individuals who have a combination of both potency and breadth in neutralization. Our results confirm and extend observations that broad neutralizing serum activity is not uncommon in chronically HIV-1-infected individuals, with 34% of volunteers from the first 463 screened possessing broad neutralizing activity, defined as an IC_{50} titer of ≥100 to at least one pseudovirus from four clade groups (11, 46). One percent of all volunteers from approximately 1,800 screened exhibited elite activity, defined as the ability to neutralize on average more than one pseudovirus at an IC_{50} titer of ≥300 within a clade group and across at least four clade groups. For all elite neutralizers the average geometric mean IC_{50} titer was 500 (Table 7). While it is not precisely known what level of Abs are required for protection against HIV-1 infection, recent work examining the efficacy of low b12 antibody titers against low-dose repeated pathogenic simian-human immunodeficiency virus challenge in macaques indicates that high concentrations of antibodies may not be needed to provide protective benefit (19). The individuals identified as elite neutralizers in this study represent a new resource for the HIV vaccine community for the identification of novel monoclonal antibodies that are both broad and potent against HIV-1 and offers new hope for the creation of immunogens capable of eliciting bnMAbs at titers which could be effective in protection against HIV-1. By extension, these findings and the methods employed may also be useful in rational vaccine design for the identification of novel broad and potent neutralizing antibodies for other viral diseases, such as hepatitis C, for which hypervariability within

TABLE 7. Neutralization profiles of all samples with scores of ≥2.5 (elite neutralizers; n = 18)

			Clade A	Clade B		Clade C		CRF01_AE	
Rank	Score 1	Country	94UG103	92BR020	JRCSF	IAVI C22	93IN905	92TH021	GeoMean
1	3.67	Ivory Coast	900	900	2700	2700	2700	2700	1872
2	3	Zambia	300	300	2700	300	2700	2700	900
5	2.83	Ivory Coast	300	300	900	300	2700	2700	749
5	2.83	Ivory Coast	300	900	2700	900	2700	100	749
5	2.83	Kenya	300	900	900	900	2700	300	749
5	2.83	South Africa	300	900	900	2700	2700	100	749
5	2.83	Rwanda	300	2700	900	2700	2700	<100	748
8	2.69	Zambia	345	345	1190	1190	1190	345	641
10	2.67	UK	300	900	900	2700	900	100	624
10	2.67	Zambia	900	900	900	300	2700	100	624
10	2.67	Uganda	900	900	900	2700	900	<100	623
15	2.5	Ivory Coast	300	900	300	900	900	300	520
15	2.5	South Africa	100	300	300	2700	900	900	520
15	2.5	South Africa	300	300	300	2700	2700	100	520
15	2.5	UK	300	900	300	900	900	300	520
15	2.5	South Africa	2700	100	300	2700	2700	<100	519
15	2.5	Uganda	900	900	900	900	900	<100	519
15	2.5	Zambia	300	<100	900	300	2700	2700	519

TABLE 8. Spearman rank correlation of the association between serum and IgG neutralization by pseudoviruses[a]

Pseudovirus	R	R^2	P value
94UG103	0.85	0.73	0.0001
92BR020	0.94	0.88	<0.0001
93IN905	0.87	0.76	0.0001
IAVI.C22	0.94	0.88	<0.0001
92TH021	0.46	0.22	0.0334

[a] IC_{50} titers greater than the upper limit of detection were assigned a value of 100 units, equal to the upper limit of detection.

the envelope is a major issue in vaccine design (13, 27, 48). In addition, our results suggest that neutralizing activity across multiple geographic regions, with different spectra of circulating HIV-1, can be reliably assessed using a small panel of pseudoviruses. Such a panel can be easily incorporated into a high-throughput selection protocol for screening additional human sera and potentially for immunogen screening. Previous recommendations for the design and use of standard neutralization screening panels suggested the use of a multitier approach to screen potential vaccine immunogens (30). The recommended multitier approach included the incorporation of vaccine strains and neutralization-sensitive viruses (tier 1), heterologous viruses matching the genetic subtype(s) of the vaccine (tier 2), and a multiclade panel comprised of six tier 2 viruses of each genetic subtype, excluding the genetic subtype(s) evaluated in tier 2 (tier 3). In addition, it was suggested that only viruses representing recently transmitted isolates or viruses collected within the first year of infection should be evaluated in this multitier approach to account for viral and antigenic drift (30).

We found that the inclusion of NL4-3, a tier 1 virus representing a laboratory-adapted strain of HIV-1, provided no benefit in helping to predict the overall sera potency to other pseudoviruses representing primary HIV-1 isolates. While NL4-3 is a representative tier 1 neutralization-sensitive virus, by extension our findings suggest that tier 1 viruses may provide little to no information regarding neutralization activity to primary isolates and should only be used as an assay control. Our results also indicate that there is no difference in neutralization patterns between viruses isolated during chronic or acute infection or viruses that represent recently transmitted HIV-1 isolates. Individuals whose sera possessed both broad and potent activity, as determined by score 1, neutralized both chronic and acute viruses and more recently transmitted isolates at similar frequencies and potencies (Fig. 8). These findings indicate that although significant HIV-1 envelope variation occurs within an individual over the course of the infection, broadly neutralizing antibodies that can recognize divergent epitopes both within an individual and across HIV-1 epidemics are generated. Therefore, the selection of pseudoviruses for incorporation in standardized neutralization screening panels probably need not take into account collection year and stage of HIV-1 infection but should focus more on the overall resistance profile of the pseudovirus as measured in serum and against the current identified monoclonal antibodies, b12, 4E10, 2F5, and 2G12.

In addition, our findings suggest that screening panels which primarily include heterologous viruses within a clade group and not across clade groups (tier 2) may overestimate the overall breadth and potency of a potential immunogen across multiple HIV-1 epidemics. We observed that volunteers' sera tended to preferentially neutralize at higher potency pseudoviruses that were closer in phylogenetic origin to the infecting strain and that having a response to viruses that represented the presumed infecting clade group only correlated with a response to other clade groups 66% of the time. It should be noted that this preferential neutralization does not suggest clade-specific serotypes, as many of the volunteers were also found to have a high prevalence of broadly neutralizing antibodies to pseudoviruses from other clades, although at low titers (Table 5). Therefore, a panel that only includes heterologous viruses matching the genetic subtype(s) of either the vaccine candidate or the virus of the HIV-1-infected individual may only be useful in measuring change in breadth within a clade group and not across clade groups. This may be of benefit if one is trying to identify immunogens that possess neutralization characteristics that are more clade restricted, such as 2G12 and 2F5, but not immunogens that elicit antibodies that are more cross-clade, such as b12 and 4E10. Collectively, these findings suggest that the best method to rapidly identify bnMAbs that are both broad and potent against primary HIV-1 isolates from diverse regions would be by using a panel of heterologous isolates of a moderately resistant profile crossing the spectrum of clades. Although our current reduced panel meets this criterion, it should be noted that all of the pseudoviruses identified in the regression analysis were by chance somewhat b12 sensitive. We are currently examining all individuals identified as having either broad or elite neutralizing activity on the previously identified panel 3, including b12-resistant pseudoviruses, to ensure that sample selection was not skewed toward donors with a greater contribution of "b12-like" (CD4b) antibodies to neutralization.

One limitation of the current study is the lack of understanding of the specificities accounting for the broadly neutralizing activity seen within both our broad and elite neutralizers and also the host factors responsible for generating and maintaining such a response. Recent work done by Connors, Binley, and Stamatatos et al. found that although CD4-binding site antibodies are found in individuals with broadly neutralizing antibodies, cross-neutralization activity and epitope specificity in many of their volunteers could not be mapped to currently known neutralization epitopes. In addition, time since infection and the presence of low to moderate viremia have been suggested as strong clinical predictors for the development of broadly neutralizing antibodies(1, 11, 46). Examination of each of these findings in order to identify possible new mechanisms and epitopes that are responsible for the production of broadly neutralizing activity primarily in elite neutralizers is currently under way with our study population.

In summary, this study represents the establishment of a high-throughput screening method for the identification of elite neutralizers and is the first study to date to identify elite neutralizers representative of diverse HIV-1 clade regions. Identification of the specificities responsible for the broad and potent neutralization activity seen in these elite neutralizers may provide new insight and guidance for rational HIV-1 vaccine design.

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