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Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection

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Abstract

Objective—Studies of neutralizing antibodies in HIV-1 infected individuals provide insights into the quality of the response that should be possible to elicit with vaccines and ways to design effective immunogens. Some individuals make high titres of exceptional broadly reactive neutralizing antibodies that are of particular interest; however, more modest responses may be a reasonable goal for vaccines. We performed a large cross-sectional study to determine the spectrum of neutralization potency and breadth that is seen during chronic HIV-1 infection.

Design—Neutralization potency and breadth were assessed with genetically and geographically diverse panels of 205 chronic HIV-1 sera and 219 Env-pseudotyped viruses representing all major genetic subtypes of HIV-1.

Methods—Neutralization was measured by using Tat-regulated luciferase reporter gene expression in TZM-bl cells. Serum-neutralizing activity was compared with a diverse set of human mAbs that are widely considered to be broadly neutralizing.

Results—We observed a uniform continuum of responses, with most sera displaying some level of cross-neutralization, and approximately 50% of sera neutralizing more than 50% of viruses. Titres of neutralization (potency) were highly correlated with breadth. Many sera had breadth comparable to several of the less potent broadly neutralizing human mAbs.

Conclusion—These results help clarify the spectrum of serum-neutralizing activity induced by HIV-1 infection and that should be possible to elicit with vaccines. Importantly, most people appear capable of making low to moderate titres of broadly neutralizing antibodies. Additional studies of these relatively common responses might provide insights for practical and feasible vaccine designs.

Conflicts of interest

There are no conflicts of interest.

Keywords

HIV-1; immunity; neutralizing antibodies; serum; vaccines

Introduction

Efforts to elicit broadly neutralizing antibodies (bnAbs) with HIV-1 vaccine immunogens face daunting challenges, owing to extraordinary genetic diversity of the gp120 and gp41 viral envelope glycoproteins (Env) and the poor immunogenicity of conserved Env epitopes [1]. For many years, only a small number of bnAbs were known; these recognized the CD4-binding site (CD4bs) (b12), a cluster of N-linked glycans on gp120 (2G12) and three adjacent epitopes in the membrane proximal external region (MPER) of gp41 (2F5, 4E10, Z13) [2–4]. Cautious optimism arose when it was shown that sera from some antiretroviral-naive chronically HIV-1 infected individuals neutralized a majority of viruses tested [5–9], suggesting it should be possible to design vaccines that elicit similar responses [10]. Since then, studies of infected individuals who possess the best bnAb activity have yielded a number of important advances. A plethora of new bnAbs were isolated and characterized, and together, these categories of antibodies explain much of the bnAb activity in HIV-1 sera. These new bnAbs permitted detailed analyses of additional epitopes in the CD4bs and MPER, as well as novel epitopes in the V1V2-glycan, V3/C3-glycan and C2-glycan regions of gp120 [11–13]. Some of the newer bnAbs are much better neutralizers than the earlier ones and are gaining more attention, although any of these bnAbs would be desirable to induce with HIV-1 vaccines.

Notably, the best bnAbs exhibit one or more unusual features, including high levels of somatic mutation in the complementary determining regions (CDRs) and framework regions (FWRs), long heavy-chain third CDRs (HCDR3 s) and polyreactivity, all of which may contribute to the relatively uncommon occurrence of such superior bnAbs during natural infection [12,14,15]. These features may also pose substantial potential barriers to the elicitation of similar bnAbs by vaccination. A more plausible vaccine approach might aim to elicit neutralizing antibodies that exhibit more moderate breadth and potency but are easier to induce because of their less restrictive maturation requirements. Polyvalent immunogens that induce several neutralizing antibody specificities, each with moderate breadth, could be used to generate a protective antibody response with increased breadth [16].

The feasibility of eliciting cross-reactive neutralizing antibodies via immunization would be better understood if more accurate information was available on the breadth of neutralization that is seen in the majority of infected individuals. Previous studies have estimated that only 10–25% of people can make antibodies that cross-neutralize many of the HIV-1 strains tested [5–9]; however, cross-reactive neutralizing antibodies with more modest potency and breadth may be a reasonable goal for HIV-1 vaccines. Due to the limited size and diversity of previous datasets, the level of breadth that is seen most often during chronic infection has remained unclear. We therefore assessed the neutralization profiles of a large multisubtyped panel of chronic sera from HIV-1 infected individuals assayed against an equally large multisubtype panel of Env-pseudotyped viruses. Our results demonstrate that broadly

neutralizing antibody responses of moderate potency are a common feature of HIV-1 infection.

Materials and methods

HIV-1 positive sera

Serum samples were obtained from chronically infected individuals who were antiretroviral drug-naive and infected with HIV-1 subtypes A ($n = 8$), B ($n = 59$), C ($n = 58$), D ($n = 3$), CRF01-AE ($n = 15$), CRF07_BC ($n = 16$), CRF02_AG ($n = 2$), CRF10_CD ($n = 1$), AC ($n = 4$), AD ($n = 3$) and ABCD ($n = 2$); 34 were infected with subtypes that could not be determined due to low levels of plasma viraemia (Supplementary Tables 1–3, <http://links.lww.com/QAD/A426>). When possible, sets of sera collected from particular sites were prescreened to exclude sera with little or no neutralizing activity, as at the time of collection (prior to 2009), there was a concern that such sera might be frequent and would be noninformative. In such cases, six Env-pseudotyped viruses commonly used in neutralization assay panels, mostly from clades B and C, were used to select 88 sera after prescreening for neutralization activity. The other 117 sera were collected from sites where no prescreening was performed. Prescreening would bias the data set towards individuals with higher levels of neutralizing activity, and one of our goals was to estimate the frequency of individuals with varying levels of neutralizing activity in the infected population, including low levels. Thus, for such estimates, we excluded prescreened sample sets. However, we were also interested in exploring the relationship between serum potency and breadth, and for this comparison, the 88 prescreened samples were a useful addition. HIV-1 genetic subtypes in the samples were determined by single genome amplification and sequencing of a single serum gp160 gene as described [17].

Env-pseudotyped viruses

Molecularly cloned *env-rev* cassettes containing full-length gp160 were used to produce Env-pseudotyped viruses by cotransfection with an Env-defective backbone plasmid (pSG3 *env*) in 293T cells as described [17]. The panel of 219 Env-pseudotyped viruses included subtypes A ($n = 10$), B ($n = 54$), C ($n = 67$), D ($n = 5$), G ($n = 8$), CRF01_AE ($n = 21$), CRF02_AG ($n = 16$), CRF06 ($n = 1$), CRF07_BC ($n = 14$), AC ($n = 6$), AD ($n = 5$), ACD ($n = 1$), BC ($n = 4$), BG ($n = 1$) and CD ($n = 6$). Assay stocks of Env-pseudotyped viruses were titrated in TZM-bl cells. None of the viruses included in this panel were among the readily neutralizing tier 1 category [18].

Neutralization assay

Neutralization was measured in 96-well culture plates by using Tat-regulated firefly luciferase (Luc) reporter gene expression to quantify reductions in virus infection in TZM-bl [17]. Heat-inactivated (56°C, 1 h) serum samples were assayed at three-fold dilutions starting at 1 : 20. Human mAbs were assayed at three-fold dilutions starting at 25–50 µg/ml. Neutralization titres are either the serum dilution (ID50) or mAb concentration (IC50) at which relative luminescence units (RLUs) were reduced by 50% compared with RLU in virus control wells after subtraction of background RLU in cell control wells. Titres that reduced 80% of the virus signal were also recorded.

Data analyses/statistical methods

R (version 2.15.2) was used to analyse and visualize neutralization data. Spearman's nonparametric correlation test was used with α equal to 0.05 on each of two tests of independent data. Cumulative distribution of 117 serum neutralization breadths were compared with a uniform distribution with the one-sample Kolmogorov test, though tied values prevent computing an exact P value. Serum percentile scores were computed as the complement of the cumulative distribution function from neutralization breadths of 117 nonprescreened sera (Serum percentile = 100% - Cumulative breadth). All analyses were repeated with neutralization data for 80% neutralization titres and, as expected, this more stringent criterion yielded fewer quantified results within current assay sensitivity limits (57.2% of 44 758 assays quantified by ID50s versus 23.2% from ID80s). When most assay results fell below the sensitivity limit, the censored data caused intractable difficulties for statistical inference when using the ID80s. To mitigate this, and because we think they are most relevant to the discussion at hand, we emphasize analysis of 50% neutralization titres, but still report salient 80% neutralization results in Table 1.

Study approval

This study utilized preexisting, de-identified specimens and was conducted under the approval of the Duke University Medical Center Institutional Review Board. The data were analysed anonymously.

Results and discussion

The neutralization profiles of 205 chronic sera from HIV-1 infected individuals were assessed against a panel of 219 Env-pseudotyped viruses that exhibit a tier 2 neutralization phenotype resembling most circulating strains [18] (Fig. 1). Both the viruses and sera were sampled globally from diverse geographic locations and together represented five HIV-1 M-group subtypes (A, B, C, D and G), and three common circulating recombinant forms (CRFs 01, 02 and 07), as well as additional unique recombinants (Supplementary Tables 1–3, <http://links.lww.com/QAD/A426>). Neutralization was assessed with molecularly cloned Env-pseudotyped viruses expressing the entire gp160 of the designated strain. Among the serum samples, 117 out of 205 were selected randomly from chronically infected individuals, and these were analysed separately in Fig. 2a, enabling an assessment of the range of breadth of neutralizing activity found among randomly selected HIV-1 chronically infected individuals. Essentially, all sera could neutralize at least a few of the tier 2 pseudoviruses, and most had activity against a substantial proportion of the diverse Envs that were tested in our panel (Fig. 1a, Fig. 2a). In hindsight, because of the unexpected high frequency of positive neutralization seen in the randomly selected group, prescreening was not necessary. The cumulative distribution of neutralization breadths for the 117 randomly selected sera in this large panel revealed a strikingly uniform continuum of the response (Fig. 2c). Although only a small fraction of these sera neutralized most viruses (10% neutralized 90% of viruses), 50% of them neutralized 50% of the virus panel. Neutralization potency (geometric mean ID50 titre) was highly correlated with breadth; this was true of both the randomly selected (Fig. 2a) and prescreened samples (Fig. 2b).

This activity was compared with the breadth of a set of 10 human mAbs selected because they are considered broadly neutralizing and represent the known major neutralization epitopes on tier 2 viruses (Fig. 2d). We included the CD4bs bnAbs VRC01 [19,20], CH01 [16,21] and b12 [2], the glycan-dependent V1V2 bnAbs PG9 [22–24] and PGT145 [23], the V3/C3 glycan-dependent bnAb PGT128 [23], the glycan-specific bnAb 2G12 [3] and the MPER bnAbs 2F5, 4E10 [4] and 10E8 [25]. This includes the older set of bnAbs that have lower potency and breadth but are still considered broadly neutralizing (b12, 2G12, 2F5, 4E10), as well as some of the more potent recently isolated bnAbs (VRC01, PGT128, 10E8). The bnAbs were tested against a panel of 119 tier 2 viruses, 103 of which were also in the panel of 219 used to evaluate the sera (Fig. 1b). At the highest concentration tested (50µg/ml), 4E10, 10E8, VRC01, PG9 and PGT145 demonstrated the greatest breadth (75.7–97.3% of viruses), corresponding to 2.6–19.7% of sera using 50% neutralization cut-off (Fig. 2d–f, Table 1). Other bnAbs (e.g. PGT128, 2F5, CH01, b12, 2G12) neutralized 20.2–59.1% of the virus panel and this level of breadth was seen among 33.3–83.8% of the sera. Indeed, 77% of the sera exhibited greater breadth than the prototypic CD4bs bnAb, b12. The sera also exhibited substantial breadth when compared with several bnAbs using more stringent 80% neutralization values, especially when compared with more moderate concentrations of 1–5µg/ml of the bnAbs (Table 1).

Notably, our results with b12 and 2G12 suggest that these two bnAbs may not be as broadly neutralizing as previously thought. We found that b12 (50µg/ml) neutralized only 29.4% of all 119 viruses tested, including 57.1 and 33% of subtype B and C viruses, respectively. A previous large study estimated these values to be 50% (all), 72% (subtype B) and 67% (subtype C) [26]. We also found that 2G12 (50µg/ml) neutralized 20.2% of the 119 viruses tested, including 57.1 and 7.7% of subtype B and C viruses, respectively, whereas previous estimates were 41, 72 and 0%. These differences may reflect greater genetic diversity among the viruses in our panel than in previous panels.

Although it was not possible to directly compare the inhibitory concentration of monoclonal bnAbs with the inhibitory dilution of sera, we note that many serum neutralization titres were moderate in magnitude, often near or below the threshold shown to prevent infection after experimental challenge with R5, tier 2 simian HIV (SHIV) in nonhuman primates [27,28]. Nonetheless, our results demonstrate the potential to elicit measurable cross-neutralizing activity in most people at levels that exceed the best responses seen in HIV-1 vaccine efficacy trials [29,30]. Although it is not known what titres will be needed to protect against natural HIV-1 transmission, relatively low serum neutralization titres in the range of 1 : 20 to 1 : 50, which were often exceeded here, are protective against other viruses [31] and have been shown to impede HIV-1 replication and drive neutralization escape in humans [32], suggesting that they may have adequate potency for vaccines that aim to prevent HIV-1 infection.

We have not determined whether the serum neutralization breadth seen here is monoclonal or polyclonal in nature. In most cases examined, broad serum neutralization is explained by the presence of one or very few antibody specificities, each of which exhibits substantial breadth against tier 2 viruses [16,19,22,23,25,33–35]. Strain-specific neutralizing antibodies to other epitopes are also present in these sera; however, polyvalent mixtures of such strain-

specific antibodies exhibit little breadth [36]. Thus, detection of even moderate breadth against tier 2 viruses usually indicates the presence of antibodies to one or more of a small number of conserved vulnerable regions on the virus.

We note that moderate cross-neutralizing activity generally is not observed prior to 3 years of HIV-1 infection [37]. Because we do not know the length of time of infection in our study participants, some individuals might have been infected for less than 3 years, which could have resulted in an underestimation of neutralization breadth. Also, serum neutralization potency and breadth can fluctuate over time during chronic infection. In a study of 155 chronically infected individuals sampled at two time points and tested against a panel of 12 tier-2 viruses, 30% had geometric mean titres that varied more than two-fold, and 6% had geometric mean titres that varied between three and six-fold (unpublished data). This dynamic nature of the bnAb response suggests that cross-sectional studies based on single samples may underestimate the potential of infected individuals to make bnAbs at some point during infection.

Overall, these observations provide a more accurate picture of the spectrum of neutralizing antibody responses that are possible in HIV-1 infected individuals. Sera with greatest breadth of neutralization are certainly of particular interest for detailed study. Still, most people are capable of making antibodies with more moderate breadth of coverage that would be deemed of value if elicited in a vaccine context. Studies of the relatively common HIV-1 infected individuals who have moderate breadth of neutralization could be valuable for understanding how to elicit similar responses via vaccination. An example of particular interest is the recent description of a clonal lineage of CD4bs-specific bnAbs that exhibits less breadth of neutralization but is also less mutated than other CD4bs bnAbs, and that may serve as a template to identify suitable immunogens that will re-elicite this lineage and generate neutralizing antibodies with similar breadth [38].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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D.C.M. designed and led the CAVD Neutralization Serotype Discovery Project (NSDP) that provided the data for analysis. B.T.K. and P.H. designed and conducted the data analyses. All three wrote the initial draft of the manuscript. M.S.S., R.T.B. and J.R.M. supervised the majority of NSDP neutralization assays and assisted with the interpretation of results and manuscript writing.

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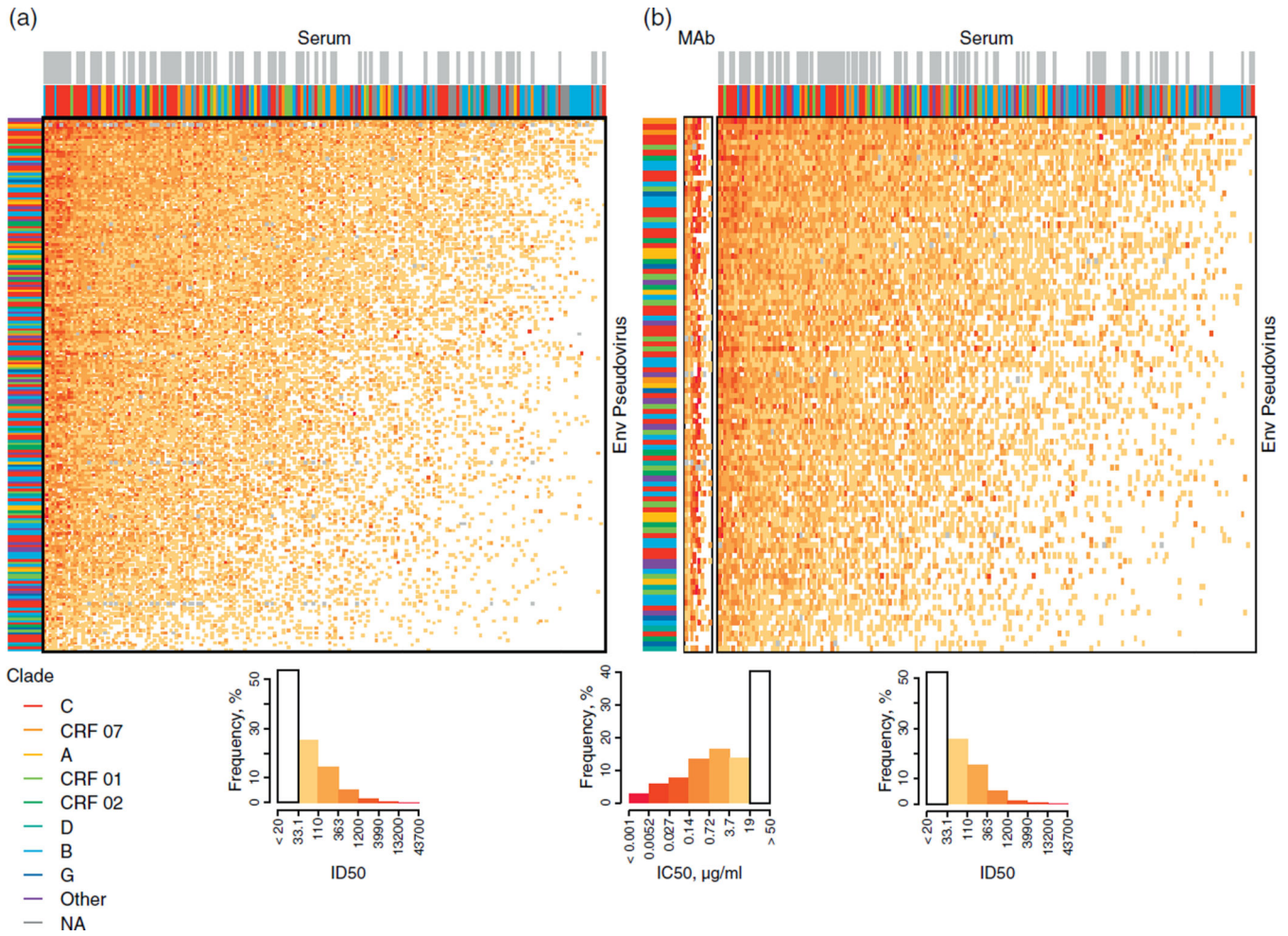


Fig. 1. Variation in serum neutralization potency and virus susceptibility

(a) Heatmap displaying ID50 titres from 205 chronic donor sera (columns) tested against 229 phylogenetically diverse Env-pseudotyped viruses (rows). (b) Heatmaps comparing 10 bnAbs tested against 103 diverse viruses, and the 205 chronic donor sera assayed with the same set of 103 viruses. Columns in both heatmaps are ordered with greatest potencies to the left. Rows are ordered with greatest virus susceptibilities at the top. Neutralization titres of bnAbs and sera are presented separately because their different units are not directly comparable (i.e. concentration for bnAbs, dilution for sera). For bnAbs, lower values represent more potent neutralization, whereas for serum samples, higher values represent more potent neutralization. The column order of bnAbs follows that of columns in Table 1 (10E8 to 2G12 from left to right). Colour-key histograms (left and right histograms are for serum samples, middle histogram is for bnAbs) summarize the percentage of the total number of reactions that fell within a given range of neutralization titres. Annotation bands indicate virus clades (left column), serum clades (coloured bars, immediately above heatmaps for all 205 samples) and the 88 prescreened sera (grey bars above the colored bars). An 'x' indicates missing data.

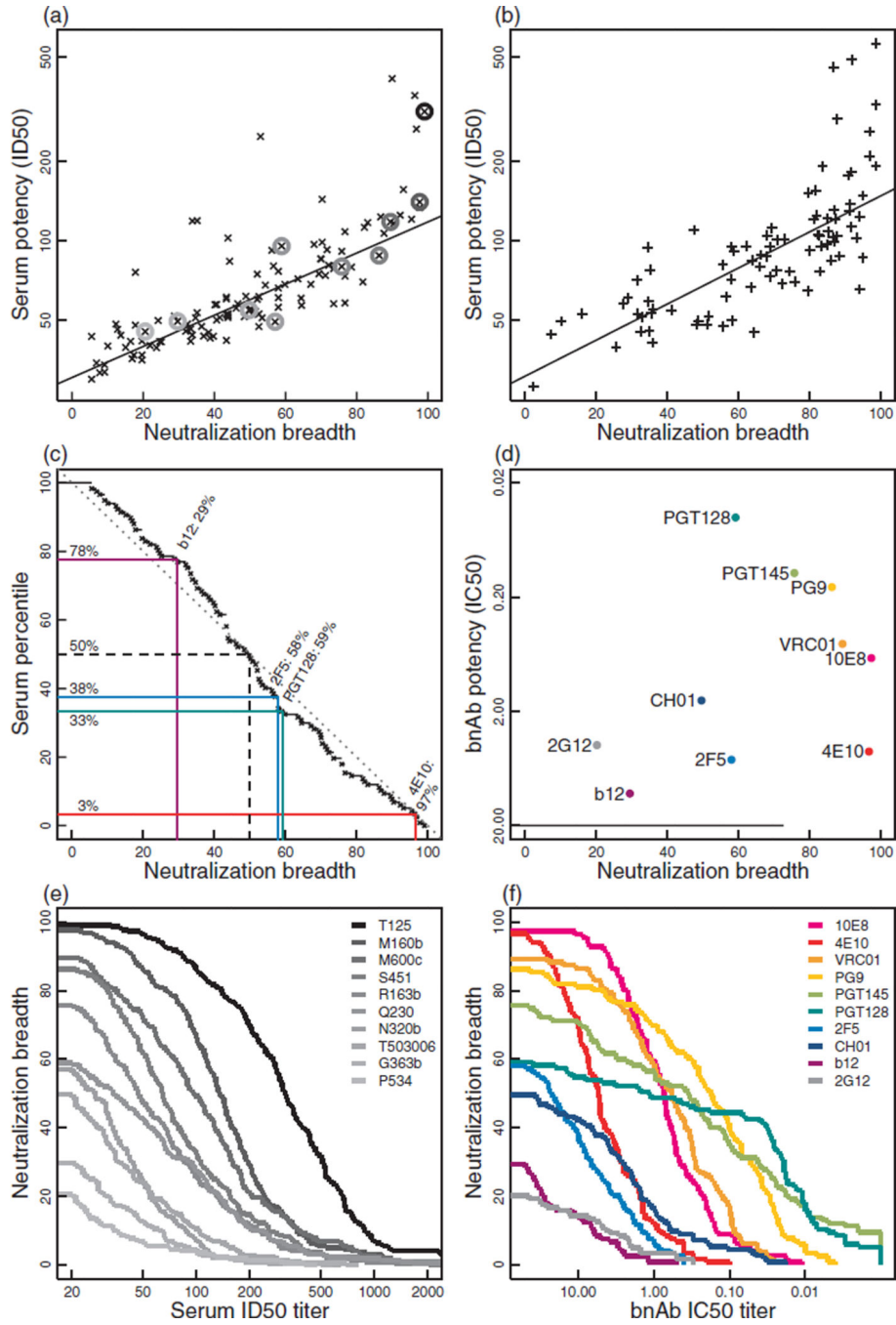


Fig. 2. Serum neutralization breadth and potency continuum

(a) Breadth and potency of neutralization were highly correlated among the 117 nonprescreened sera (Spearman rank correlation, $P = 2.8 \times 10^{-34}$, $\rho = 0.85$), though a few sera had exceptionally high potency. Circles indicate sera profiled in (e), selected for having breadths that match representative human bnAbs. (b) Breadth and potency were highly correlated among 88 sera selected by prescreening (Spearman rank correlation, $P = 4.1 \times 10^{-21}$, $\rho = 0.80$); a few sera again had exceptionally high potency. (c) Serum percentiles on the y-axis showing the proportion of 117 nonprescreened sera with equal or greater breadth

than the breadth shown along the x-axis. Breadths of four representative bnAbs are superimposed for comparison (b12, 2F5, PGT128, 4E10). Serum breadths were consistent with a uniform distribution (Kolmogorov test, $P = 0.31$, approximated due to 27 ties). (d) Breadth and potency of 10 bnAbs. (e) Magnitude-breadth functions showing proportion of viruses neutralized versus ID50 titre for 10 sera having breadths that match representative bnAbs. (f) Magnitude-breadth functions of 10 representative bnAbs.

Neutralization breadth, and proportion of sera with comparable breadth, for 10 human bnAbs tested against a multisubtype panel of 119 tier 2 Env-pseudotyped viruses.

Table 1

	10E8	4E10	VRC01	PG9	PGT145	PGT128	2F5	CH01	b12	2G12
50% neutralization										
Breadth (50) ^a	97.3	96.6	89.2	86.2	75.7	59.1	58.0	49.5	29.4	20.2
Serum %ile ^b	2.6	3.4	9.4	12.0	19.7	33.3	37.6	50.4	77.8	83.8
Breadth (5) ^a	92.8	42.9	82.9	80.2	62.6	53.9	26.1	37.8	7.6	11.8
Serum %ile ^b	6.0	61.5	12.8	14.5	32.5	41.0	78.6	67.5	97.4	94.0
Breadth (1) ^a	59.5	11.8	58.6	69.8	56.5	48.7	5.9	16.2	0.8	3.4
Serum %ile ^b	33.3	94.0	34.2	27.4	39.3	51.3	98.3	90.6	100	100
80% neutralization										
Breadth (50) ^a	97.2	73.9	87.7	80.6	63.0	55.6	42.9	13.5	16.0	12.6
Serum %ile ^b	0	3.4	0.9	1.7	5.1	6.8	12.8	39.3	37.6	41.9
Breadth (5) ^a	65.1	8.4	69.8	70.8	47.2	48.6	5.0	6.7	1.7	3.4
Serum %ile ^b	5.1	51.3	5.1	4.3	11.1	10.3	65.8	61.5	84.6	73.5
Breadth (1) ^a	15.1	0	36.8	55.6	41.7	45.8	0	3.8	0	0
Serum %ile ^b	37.6	94.0	13.7	6.8	13.7	11.1	94.0	71.8	94.0	94.0

bnAbs, broadly neutralizing antibodies.

^aPercentage of bnAb IC50 and IC80 assay results below the concentration (µg/ml) shown in parentheses.

^bPercentage of nonprescreened sera with equal or greater breadth than bnAbs (shown in boldface type).