



Neutralization tiers of HIV-1

David C. Montefiori^a, Mario Roederer^b, Lynn Morris^c,
and Michael S. Seaman^d

Purpose of review

HIV-1 isolates are often classified on the basis of neutralization ‘tier’ phenotype. Tier classification has important implications for the monitoring and interpretation of vaccine-elicited neutralizing antibody responses. The molecular basis that distinguishes the multiple neutralization phenotypes of HIV-1 has been unclear. We present a model based on the dynamic nature of the HIV-1 envelope glycoproteins and its impact on epitope exposure. We also describe a new approach for ranking HIV-1 vaccine-elicited neutralizing antibody responses.

Recent findings

The unliganded trimeric HIV-1 envelope glycoprotein spike spontaneously transitions through at least three conformations. Neutralization tier phenotypes correspond to the frequency by which the trimer exists in a closed (tiers 2 and 3), open (tier 1A), or intermediate (tier 1B) conformation. An increasing number of epitopes become exposed as the trimer opens, making the virus more sensitive to neutralization by certain antibodies. The closed conformation is stabilized by many broadly neutralizing antibodies.

Summary

The tier 2 neutralization phenotype is typical of most circulating strains and is associated with a predominantly closed Env trimer configuration that is a high priority to target with vaccines. Assays with tier 1A viruses should be interpreted with caution and with the understanding that they detect many antibody specificities that do not neutralize tier 2 viruses and do not protect against HIV-1 infection.

Keywords

envelope glycoproteins, epitopes, HIV-1 vaccines, neutralizing antibodies

INTRODUCTION

The identification of HIV-1 vaccines that elicit broadly neutralizing antibodies (bNAbs) will require reliable information on the ability of vaccine-elicited antibodies to neutralize diverse strains of the virus. These antibodies must contend with a high degree of sequence variability and structural plasticity in the trimeric envelope glycoproteins (Env), which mediated virus entry and are the sole targets for neutralization. Much has been done to identify suitable reference strains that represent a spectrum of global Env genetic diversity [1^a,2–5]. These reference strains are used as Env-pseudotyped viruses to assess magnitude and breadth of neutralization in validated reporter gene assays [6–8]. Criteria for reference strain selection places a heavy emphasis on the neutralization phenotype of the Envs as determined with serum samples from chronically infected individuals, with the rationale that the polyclonal nature of the HIV-1-specific antibodies in these sera provides a reasonable sampling of the spectrum of responses that are possible to elicit with vaccines. Large cross-sectional datasets

generated with multiclade panels of Envs and serum samples have shown that HIV-1 isolates exhibit a spectrum of neutralization sensitivity that can be divided into four distinct tiers [9]. Tier 1A is the most sensitive neutralization phenotype and represents a

^aDepartment of Surgery, Duke University Medical Center, Durham, North Carolina, ^bVaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, USA, ^cCenter for the AIDS Program of Research in South Africa (CAPRISA), National Institute for Communicable Diseases and the University of the Witwatersrand, Johannesburg, South Africa and ^dBeth Israel Deaconess Medical Center, Harvard University, Boston, Massachusetts, USA

Correspondence to David C. Montefiori, PhD, Department of Surgery, Division of Surgical Sciences, Duke University Medical Center, Durham, NC 27710, USA. Tel: +1 919 684 5278; E-mail: monte@duke.edu

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KEY POINTS

- Neutralization tier phenotype as currently defined with polyclonal HIV-1 sera corresponds to distinct Env trimer configurations.
- The dynamics of Env trimers on tier 2 HIV-1 isolates selectively detects neutralizing antibodies that are most likely to be protective.
- Tier 1A virus neutralization does not predict an ability to neutralize tier 2 viruses.
- The neutralizing activity of antibodies that only neutralize tier 1A viruses is nonprotective (some of these antibodies may have other antiviral properties, e.g., mediated through Fc receptors).

very minor fraction of circulating strains. Tier 1B is the next most sensitive and represents a larger but still relatively small fraction of circulating strains. Most circulating strains exhibit a moderately sensitive tier 2 phenotype that is considered the most important to target with vaccines; this phenotype comprises the majority of reference strains. Tier 3 is the least sensitive phenotype. Many Env immunogens generate antibodies that neutralize tier 1A and to a lesser extent tier 1B Envs but these antibodies fail to neutralize most tier 2 and 3 Envs. Importantly, an ability to neutralize tier 1A does not predict an ability to neutralize tier 2 Envs [10¹], raising questions about the value of tier 1A Env neutralization as a benchmark for HIV-1 vaccines. Recent structural studies offer molecular insights that help explain the spectrum of neutralization sensitivities the virus exhibits. Other recent work describes a new classification system to differentiate the neutralization potency of serum samples and isolated antibodies. Together this new information serves as a useful guide for monitoring and interpreting neutralizing antibody responses in preclinical and clinical HIV-1 vaccine trials.

EARLY HISTORY: PRIMARY ISOLATES VERSUS LABORATORY STRAINS

Few strains of HIV-1 were available for laboratory investigation and as a source of recombinant vaccine immunogens in the early years of vaccine development. These strains (e.g., IIIB/LAV, MN, SF2) were adapted to replicate in immortalized CD4⁺ human cell lines (e.g., H9, CEM) for ease of production and experimentation. They were classified as laboratory-adapted or more precisely, T cell line-adapted (TCLA) viruses to differentiate them from primary isolates that were passaged only in human peripheral blood mononuclear cells

(PBMCs). TCLA strains were the sole source of vaccine immunogens for many years and were highly sensitive to neutralization by the antibodies they induced [11], generating early enthusiasm in the field. Linear epitopes in the third variable cysteine-cysteine loop (V3) loop of the surface gp120 envelope glycoprotein were responsible for much of this neutralizing activity [12–14], although epitopes in other regions of gp120 may have contributed in part (e.g., V2, CD4 binding site, coreceptor binding site) [15–18]. Indeed, the V3 loop became known as the principal neutralizing domain (PND) and a major focus of early vaccine efforts [19].

The neutralizing activity of early vaccine-elicited antibodies, combined with their ability to protect chimpanzees against experimental challenge with TCLA virus, strengthened the rationale to move two gp120 subunit vaccines into human efficacy trials (Vax003 and Vax004) [20]. Neither vaccine afforded a significant level of protection [21–23] despite inducing strong neutralizing antibody responses against TCLA strains [24–26]. These early efficacy trials were controversial because emerging data indicated that primary isolates were considerably less sensitive to neutralization than TCLA strains when assayed with HIV-1 sera, linear V3-specific antibodies [27–29], and sera from phase 1 trials [30–33]. Indeed, peak immune sera from Vax003 and Vax004 showed only weak neutralizing activity against primary circulating strains assayed as Env-pseudotyped viruses [24,26], offering a possible explanation for why the strong TCLA virus-specific responses in these trials were nonprotective.

With increasing awareness of the importance of strain selection came questions about why TCLA strains and primary isolates were so strikingly different in their neutralization phenotype. Early studies with various combinations of monoclonal antibodies, peptides, Env proteins and mutant viruses indicated that this dichotomy in neutralization sensitivity is due to differences in epitope exposure on native trimeric Env spikes [34–37]. For reasons that are not entirely clear, adaptation to replicate in transformed cell lines selects viral variants that exhibit properties consistent with a more open trimer conformation. This may also be true for primary isolates that are cultured for prolonged periods in the PBMC [38]. Open trimers expose epitopes that are highly immunogenic in HIV-1-infected individuals and in uninfected recipients of Env vaccines. Most circulating strains have evolved under pressure from the host antibody response to conceal these epitopes by adopting a more closed trimer conformation. Paradoxically, HIV-1 variants that have replicated and evolved in the host for many weeks or months prior to the detection of neutralizing

antibodies during acute infection are no more sensitive to heterologous HIV-1 sera than chronic isolates [39,40,41]. Thus, the closed conformation may be more constant *in vivo* than it is *in vitro* in the absence of neutralizing antibodies. Antibodies against epitopes on open trimers are likely induced by nonnative forms of Env, such as unprocessed gp160 and monomeric gp120 that is shed from the virus surface [42,43]. Many vaccine immunogens fall into this nonnative category. Closed native trimers are being sought as immunogens that favor the induction of bNAbs as a more dominant antibody response [44].

Concerns that assays with TCLA strains of virus could yield misleading results led to a growing interest in primary isolates as a higher priority for vaccine immune monitoring. Early efforts suffered from a lack of standardization and the absence of a phenotypic classification system to confirm the primary isolate 'pedigree' based on neutralization-susceptibility. With improved technologies came a recommendation to utilize Env molecular clones that were PCR amplified directly from plasma or PBMC of infected individuals and assayed as Env-pseudotyped viruses [6]. Another recommendation was to create well characterized Env panels that exhibit a neutralization phenotype typical of most circulating strains for use as common reagents across multiple laboratories [6]. As a result, several panels of HIV-1 Env reference strains are now available for standardized assessments of neutralizing antibodies [1,2-5]. These panels have proven valuable for the identification and characterization of a new generation of bNAbs [45,46] and may be an important benchmark for vaccines.

VIRUS PANELS AND THE TIERED CATEGORIZATION OF HIV-1 NEUTRALIZATION PHENOTYPES

Early in the process of creating HIV-1 Env-pseudotyped reference strains it became apparent that a major limitation was the lack of objective data to phenotype and categorize these viruses based on their overall sensitivity or resistance to antibody-mediated neutralization. To address this issue, 109 HIV-1 Env pseudotyped viruses representing a broad range of genetic and geographic diversity were tested against a similarly diverse set of seven clade-specific HIV-1 plasma pools derived from chronically infected individuals [9]. When viruses were rank-ordered according to average ID50 neutralization titers against the plasma pools, a spectrum of sensitivities was observed. Although the majority of viruses exhibited a relatively narrow range of average neutralization sensitivity, subsets

of viruses at either end of the spectrum clearly demonstrated a more sensitive or resistant phenotype. Four subsets of viruses representing distinct tiers of neutralization sensitivity were identified. A small fraction of isolates that demonstrated a highly sensitive phenotype were designated tier 1A (3%, all derived from viruses that were highly passaged *in vitro*), and the remaining viruses that exhibited above average sensitivity were designated tier 1B (19%). The majority of viruses clustered into the tier 2 category (63%), which exhibited average neutralization sensitivity, and tier 3 viruses (15%), which were distinguished as having a more neutralization resistant phenotype. Viruses tended to be more sensitive to neutralization using clade matched plasma pools versus clade mismatched pools; however, no associations were identified between tier categorization and virus characteristics such as clade, stage of infection of the individual from whom the Env gene was cloned (transmitted-founder, acute/early, or chronic), or the source of virus (plasma, cocultured PBMC, or uncultured PBMC).

Subsequent studies expanded on the use of large-scale testing of HIV-1 Env pseudotyped viruses with chronic HIV-1 plasma samples and bNAbs to investigate determinants of neutralization sensitivity and to identify optimal panels of reference viruses. To identify suitable reference strains representative of the global epidemic, deCamp *et al.* [2] utilized an exceptionally large checkerboard-style neutralization data set in which 219 tier 2 and tier 3 Env pseudotyped viruses were assayed with 205 individual chronic HIV-1 plasma samples, where both the Envs and plasma samples were chosen to represent the global diversity of HIV-1 clades and circulating recombinant forms. Using a statistical model selection procedure known as 'lasso', 12 to 9 viruses were selected that accurately captured the spectrum of neutralizing activity that was observed with the larger panel of 219 viruses. An additional 3 viruses were selected to increase the clade representation. All 12 viruses possess a tier 2 neutralization phenotype (while maintaining a spectrum of average sensitivities), and exhibit genetic diversity that includes clades A, B, C and G plus the recombinants CRF01 and CRF07. This Global Reference Panel of HIV-1 Env reference strains allows for practical standardized assessments across various platforms of HIV-1 vaccines currently under development in different regions of the world.

A more recent study focused on developing optimized panels of clade C reference viruses from southern Africa [39], a region of the world highly impacted by the HIV-1 epidemic and where clinical efficacy studies are being conducted to evaluate both active and passive immunization strategies.

A panel of 200 HIV-1 clade C Envs cloned from acute/early infections was assembled, and neutralization tier phenotypes were determined using a panel of 30 chronic clade C HIV-1 serum samples that had been preselected to represent a spectrum of neutralization breadth and potency. K-means clustering analysis of geometric mean ID50 titers demonstrated a similar distribution of tier phenotypes as had been observed with a multiclade panel of Envs [9]. The majority of Envs exhibited a moderately resistant tier 2 phenotype (75%), whereas 1 and 8.5% exhibited sensitive tier 1A and tier 1B phenotypes, respectively, and 15.5% were classified as more resistant tier 3 viruses. A comprehensive analysis of Env traits that impact antibody neutralization showed that increases in the length, glycosylation density, and net negative charge in the V1–V2 and V4 regions of gp120 are associated with lower neutralization sensitivity among tier 2 and 3 Envs, similar to what was seen in other studies [39[¶],40,47,48]. In addition, an amino acid signature was identified in which Arg at position 683 in the membrane-proximal external region was highly enriched in tier 3 viruses, suggesting that this position and region may influence neutralization resistance. The panel of 200 acute/early clade C viruses was utilized to test the breadth and potency of 15 bNAbs targeting four distinct epitopes on Env that are considered promising candidates for HIV-1 prevention and treatment. Mathematical modeling was utilized to predict combinations of bNAbs that would complement one another for the most effective coverage against clade C viruses, taking into account potency, breadth, extent of complete neutralization, and instantaneous inhibitory potential [49[¶]].

While the extended panel of 200 acute/early clade C viruses enabled large-scale studies to robustly interrogate genotypic properties that impact antibody recognition and neutralization sensitivity, smaller panels of more practical size are needed for high-throughput screening of serum samples from clinical vaccine studies or characterizing newly isolated bNAbs. To this end, Hraber *et al.* [1[¶]] used hierarchical clustering of bNAb neutralization titers and magnitude-breadth distributions against the 200 virus panel to down-select optimal panels of either 100 or 50 viruses that exhibit a high degree of overlap with the larger virus panel for all 15 bNAbs tested. These smaller panels allow for more rapid detailed characterization of the breadth and potency of newly isolated bNAbs or potent immune sera, and should reflect the range of virus sensitivities that would be observed with the larger 200 virus panel. To facilitate high-throughput screening of vaccine-sera from large-scale clinical

trials, a smaller, more manageable panel of 12 early/acute clade C viruses was also selected via computational guidance to represent the diverse neutralization sensitivity patterns observed with both polyclonal plasma and monoclonal antibodies. This panel is intended to detect relatively weak or potentially clade-specific tier 2 neutralization responses, which will facilitate the screening of vaccine sera in regions where clade C infections predominate and clade C vaccines are being tested. Together these studies demonstrate how large-scale tier phenotyping data informed the design of optimized and complementary virus panels for the evaluation of candidate HIV-1 vaccines and bnAbs.

MOLECULAR INSIGHTS INTO THE TIERED NEUTRALIZATION PHENOTYPES OF HIV-1 VIRUSES

Native HIV-1 Env on the virus surface consists of a trimolecular complex of gp120-gp41 heterodimers that mediate virus entry through sequential binding of gp120 to CD4 and a coreceptor, most often CCR5, followed by gp41 activation and membrane fusion [50]. Studies of soluble native-like Env trimers (e.g., SOSIPs) using a combination of x-ray crystallography, cryo-electron microscopy/tomography and hydrogen–deuterium exchange have provided detailed structural information on the prefusion form of the closed trimer and the substantial conformational changes that take place upon CD4 binding that lead to outward rotation of gp120 protomers, greater exposure of the V3 loop and formation of the coreceptor binding region [51–58]. A more recent study characterized the antigenic properties of soluble trimers and corresponding gp120 monomers from a tier 3 strain of HIV-1 (CH120.6) and found that many epitopes for non-neutralizing and tier 1 virus-neutralizing antibodies on the gp120 were occluded on the trimers [59[¶]]. Consistent with earlier reports [34–37], these combined observations point to a structure-based explanation for the different neutralization tier phenotypes of HIV-1.

Whereas the above studies used soluble liganded trimers to characterize the open conformation, a separate study used single-molecule fluorescence resonance energy transfer (smFRET) of virion-associated Env to interrogate spontaneous transitions in the configuration of unliganded trimers [60[¶]]. Two strains of trimers were examined, one from a tier 2 virus (JR-FL) and another from a tier 1A virus (NL4-3). Both trimers exhibited spontaneous and reversible conformational changes, with three major conformations identified: closed, open, and intermediate (Fig. 1). Transitions were seen between the closed

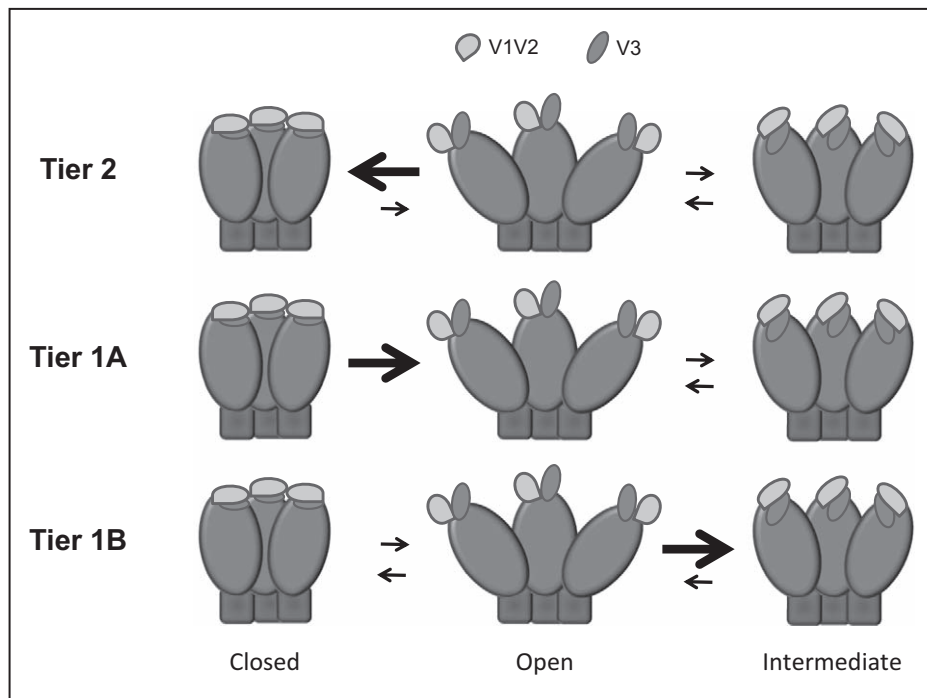


FIGURE 1. Balance of conformational states in the HIV-1 Env trimer. Shown is a model based on the findings of Munroe *et al.* [60**] and also proposed by Cai *et al.* [59**]. Native Env trimers on the virus surface are structurally dynamic and constantly transitioning between at least three conformations that differentially expose a variety of epitopes for nonbNAbs. Env trimers on tier 2 viruses predominantly occupy a closed conformation that masks nonbNAb epitopes. Env trimers on tier 1A viruses frequently transition to an open conformation that exposes these epitopes, making the viruses highly sensitive to neutralization by polyclonal HIV-1 sera and many current vaccine-elicited antibodies. Tier 1B viruses may spend relatively more time in an intermediate conformation that partially exposes these epitopes.

and open states and between the intermediate and open states but rarely between closed and intermediate states. Soluble CD4 stabilized the open conformation of NL4-3 whereas CD4 + 17b (a coreceptor mimic) and 17b alone stabilized the intermediate conformation of this trimer. CD4 and CD4+ 17b stabilized both the open and intermediate conformation of JR-FL equally well but here 17b alone had no effect. Together these observations indicate that the open conformation corresponds to the CD4-bound state, whereas the intermediate conformation corresponds to the CD4/coreceptor-bound state. Also, the coreceptor binding site is more occluded on JR-FL (tier 2) than NL4-3 (tier 1A). Open trimers that are stabilized by ligation would only exist at the virus-cell interface, where they are relatively inaccessible to antibodies. Many bNAbs stabilize the closed state [54,57,60**,61], which may contribute to entry-inhibiting activity.

Equilibrium in the structural dynamics of HIV-1 Env trimers provides the necessary balance between entry fitness and immune evasion but also appears to give rise to the neutralization tier phenotypes. As illustrated in Fig. 1, Env trimers on tier 2 viruses spend more time in a closed conformation, which is

a target for bNAbs and autologous NAbs but few other Env-specific antibodies. Trimers on tier 1A viruses spend more time in an open conformation, providing access to many epitopes that are occluded on tier 2 Envs. By analogy, trimers on tier 1B viruses may spend more time in an intermediate conformation that partially exposes epitopes. Thus, the degree of openness distinguishes the tiers and is quantifiable on the basis of the neutralization potency of antibodies to epitopes that are occluded on the closed trimer.

RECOMMENDATIONS ON THE USE OF TIERED REFERENCE STRAINS FOR VACCINE EVALUATION

A major goal of HIV-1 vaccine development is the induction of antibodies able to broadly neutralize tier 2 viral strains. Most experimental HIV-1 vaccines to date have only been able to elicit antibodies that neutralize tier 1A and a subset of tier 1B viruses, which represent a minor fraction of circulating strains. Though some success has been made at generating antibodies that neutralize vaccine-matched tier 2 strains through the use of native-like

trimeric Envs, these antibodies have very little activity against heterologous tier 2 strains and thus are not broadly neutralizing [62–64].

As mentioned earlier, the Vax003 and Vax004 trials demonstrated that potent tier 1A virus neutralizing antibodies were unable to provide protection from HIV-1 infection [25,26]. Although the moderate efficacy in another phase 3 trial, RV144, was not linked to neutralizing activity, tier 1A virus neutralization was identified as a secondary correlate when the IgA fraction was removed [65]. In chronic HIV-1 infection, antibodies able to neutralize tier 1A viruses have been shown to exert some selection pressure against autologous viruses, and mAb isolation confirmed that they target epitopes in V3 and the CD4 binding site (CD4bs) [66]. Furthermore, in the setting of mother-to-child infection where viruses are shared, antibodies that target the V3 were shown to correlate with a reduced risk of HIV-1 transmission [67,68[¶]]. Thus, while there is evidence that tier 1A virus neutralizing activity has some potential for protection, the impact is likely to be limited. The prevention of sexual HIV-1 transmission will likely require an ability to neutralize tier 2 viruses.

Tier 1A viruses continue to be used for immune monitoring in preclinical and human clinical trials despite a paucity of evidence that they are indicators of a desirable response. Moreover, their continued use has potential to create confusion over the goals for HIV-1 vaccines. In 2016, the Global HIV Vaccine Enterprise convened a workshop to discuss the merits of tier 1A viruses and whether it was time to ‘break an old habit’ by discontinuing their use altogether. It was noted that assessments with tier 1A viruses can provide an indication of the overall immunogenicity of a vaccine, for example, by showing increased antibody titers following boosting with protein vaccines [69]. These titers also enable general comparison between different vaccines and different regimens as well as the durability of antibody responses, much the same as binding antibody assays. It was recommended that tier 1A viruses could be used on a limited basis for these types of observations to be made, with the understanding that an ability to neutralize only these viruses is unlikely to protect against infection, or to be an early indicator of progress toward tier 2 virus neutralization.

Questions were raised at the workshop concerning the possibility that tier 1B virus neutralization might be an indicator of early progress toward tier 2 virus neutralization. Although these viruses are more sensitive to neutralization compared to tier 2 viruses, most are resistant to V3 and weak mAbs, confirming they are antigenically distinct from tier

1A viruses [9]. Studies that have used tier 1B viruses to monitor vaccine responses have shown titers to be at least 10-fold lower compared with those against tier 1A viruses [70,71]. The kinetics of tier 1B responses also differed from those against tier 1A viruses and in some cases, tier 1B virus neutralization was not inhibited by V3 peptides [72,73]. Furthermore, tier 1B virus neutralizing titers track with tier 2 rather than tier 1A virus neutralizing titers [74] suggesting they share an Env trimer configuration that more closely resembles tier 2. Immunization experiments using a SOSIP.664 trimer made from a tier 1B isolate, AMC008 showed that it induced autologous virus neutralizing antibodies and minimal tier 1A virus neutralizing antibodies, similar to other SOSIP proteins [64]. This suggested that once stabilized, Env from tier 1B resemble those made using tier 2 Envs. As mentioned above, the increased sensitivity of tier 1B viruses to certain antibodies is likely explained by the more frequent sampling of an open conformation compared to tier 2 viruses. Because tier 1B viruses are diverse, spanning a range of neutralization sensitivities, it may be possible to identify a panel of tier 1B viruses that have greater value than tier 1A for immune monitoring.

NEUTRALIZATION POTENCY SCORES FOR SERUM AND MONOCLONAL ANTIBODIES

Decisions on which vaccine (or prophylactic passive immunization) strategies should be prioritized for development and large-scale testing are made on a variety of criteria. One of the most important is based on a comparison of the breadth of neutralization that can be elicited. However, there is no simple way to do this. For example, is a mAb that neutralizes a minority of tier 3 virus and a large majority of tier 2 viruses preferable to one that neutralizes half of each category? Trying to compare the distributions of neutralization breadths across two arms of a clinical trial (e.g. phase 1 comparing two different vaccines) is even more difficult.

Graber *et al.* [75^{¶¶}] devised a metric by which antisera or antibodies can be compared in order to inform these decisions in an objective manner. This metric can be calculated using an on-line tool (<http://hiv.lanl.gov/content/sequence/NI/ni.html>). In essence, the output of the calculation is a ‘neutralization potency’ that is scaled to be similar to the tier values of viruses. For example, a neutralization potency value of 1 indicates that the antisera neutralizes only tier 1-like viruses; a neutralization potency value of 1 indicates an antiserum that can neutralize at least some tier 3 viruses. Because the neutralization potency is a continuous metric, it

can be used to compare elicited antisera and rank potential vaccine candidates accordingly.

The metric is based on a logistic regression of neutralization (yes/no) at a single dilution of antisera, against a set of viruses. Given the current standard of performing the neutralization assay at eight dilutions, this makes the proposed use of neutralization potency more suitable to high-throughput measurements. Given the logistical and economic constraints of requiring eight dilutions per virus, it has been proposed to use a subset of ~12 viruses from the large panel of more than 200 that can provide a reasonable estimate of breadth [1[■],2]. The same has been done for the neutralization potency metric; alternatively, a much larger set of viruses can be tested in the single-dilution format to achieve a more precise breadth score.

Finally, the logistic regression of the neutralization data provides not only the neutralization potency value (related to breadth), but also a 'slope' value. The slope indicates how much the neutralization potency of the antisera depends on the neutralization resistance (tier) of tested viruses. In the case of monoclonal antibodies, this dependence is typically very low, i.e., the neutralization of a bNAb depends primarily on the presence or absence of the epitope (which is itself not usually correlated to tier category). Hraber *et al.* [75[■]] suggest that the slope value of antisera may indicate how polyepitopic the dominant neutralization response is, and that antisera that are primarily directed against a single epitope can be distinguished from antisera that neutralize through multiple epitopes. Thus, the neutralization potency analysis might provide another discriminatory metric by which vaccine strategies can be objectively and quantitatively compared.

CONCLUSION

The neutralization tier phenotypes of HIV-1 isolates can be understood in the context of the dynamic nature of Env trimers on the virus surface. These trimers spontaneously transition between closed, open, and at least one intermediate conformation, where open trimers expose more epitopes than closed trimers. Natural variation in transition time influences the neutralization phenotype of the virus, where Env trimers that spend more time in a closed conformation display a tier 2 phenotype, while those that spend more time in the open conformation display a tier 1 phenotype. Other structural features of Env contribute to the spectrum in neutralization-sensitivity seen within the tier 2 category of viruses. A new neutralization potency score utilizes this variation to quantitatively compare serum antibody responses and monoclonal antibodies.

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Conflicts of interest

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REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Hraber P, Rademeyer C, Williamson C, *et al.* Panels of HIV-1 subtype C Env reference strains for standardized neutralization assessments. *J Virol* 2017; 91: pii: e00991-17. doi: 10.1128/JVI.00991-17. [Epub ahead of print]. Panels of 100, 50 and 12 well characterized tier 2 clade C Envs were established from an original panel of 200 Envs as standard reference reagents for neutralization assays.
 2. deCamp A, Hraber P, Bailer RT, *et al.* Global panel of HIV-1 Env reference strains for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 2014; 88:2489–2507.
 3. Simek MD, Rida W, Priddy FH, *et al.* 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. *J Virol* 2009; 83:7337–7348.
 4. Li M, Salazar-Gonzalez JF, Derdeyn CA, *et al.* Genetic and neutralization properties of subtype C human immunodeficiency virus type 1 molecular env clones from acute and early heterosexually acquired infections in southern Africa. *J Virol* 2006; 80:11776–11790.
 5. Li M, Gao F, Mascola JR, *et al.* Human immunodeficiency virus type 1 env clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J Virol* 2005; 79:10108–10125.
 6. Mascola JR, D'Souza P, Gilbert P, *et al.* Recommendations for the design and use of standard virus panels to assess the neutralizing antibody response elicited by candidate human immunodeficiency virus type 1 vaccines. *J Virol* 2005; 79:10103–10107.
 7. Sarzotti-Kelsoe M, Bailer RT, Turk E, *et al.* Optimization and validation of the TZM-bl assay for standardized assessments of neutralizing antibodies against HIV-1. *J Immunol Methods* 2014; 409:131–146.
 8. Richman DD, Wrin T, Little SJ, Petropoulos CJ. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc Natl Acad Sci U S A* 2003; 100:4144–4149.
 9. Seaman MS, Janes H, Hawkins N, *et al.* Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessments of neutralizing antibodies. *J Virol* 2010; 84:1439–1452.
 10. Klasse PJ, LaBranche CC, Ketas TJ, *et al.* Sequential and simultaneous immunization of rabbits with HIV-1 envelope glycoprotein SOSIP.664 trimers from clades A, B and C. *PLoS Pathog* 2016; 12:e1005864.
- A recent example of the lack of correlation between the ability of vaccine-elicited antibodies to neutralize tier 1 and tier 2 viruses. In this case the tier 2 virus was autologous to the vaccine.

11. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. *Annu Rev Immunol* 2010; 28:413–444.
 12. Montefiori DC, Graham BS, Zhou JT, *et al.* V3-specific neutralizing antibodies in sera from HIV-1 gp160-immunized volunteers block virus fusion and act synergistically with human monoclonal antibody to the conformation-dependent CD4 binding region of gp120. *J Clin Invest* 1993; 92:840–847.
 13. Davis KL, Gray ES, Moore PL, *et al.* High titer HIV-1 V3-specific antibodies with broad reactivity but low neutralizing potency in acute infection and following vaccination. *Virology* 2009; 387:414–426.
 14. Bures R, Gaitan A, Zhu T, *et al.* Immunization with recombinant canarypox vectors expressing membrane-anchored gp120 followed by gp160 protein boosting fails to generate antibodies that neutralize R5 primary isolates of human immunodeficiency virus type 1. *AIDS Res Hum Retroviruses* 2000; 16:2019–2035.
 15. Decker JM, Bibollet-Ruche F, Wei X, *et al.* Antigenic conservation and immunogenicity of the HIV coreceptor binding site. *J Exp Med* 2005; 201:1407–1419.
 16. Nakamura GR, Byrn R, Wilkes DM, *et al.* Strain specificity and binding affinity requirements of neutralizing monoclonal antibodies to the C4 domain of gp120 from human immunodeficiency virus type 1. *J Virol* 1993; 67:6179–6191.
 17. Thali M, Furman D, Ho D, *et al.* Discontinuous, conserved neutralization epitopes overlapping the CD4-binding region of human immunodeficiency virus type 1 gp120 envelope glycoprotein. *J Virol* 1992; 66:5635–5641.
 18. Fung MSC, Sun CRY, Gordon WL, *et al.* Identification and characterization of a neutralization site within the second variable region of human immunodeficiency virus type 1 gp120. *J Virol* 1992; 66:848–856.
 19. Javaherian K, Langlois AJ, McDanal C, *et al.* Principal neutralizing domain of the human immunodeficiency virus type 1 envelope glycoprotein. *Proc Natl Acad Sci U S A* 1989; 86:6768–6772.
 20. Francis DP, Gregory T, McElrath MJ, *et al.* Advancing AIDSVAX to phase 3. Safety, immunogenicity, and plans for phase 3. *AIDS Res Hum Retroviruses* 1998; 14(Suppl 3):S325–S331.
 21. Flynn NM, Forthal DN, Harro CD, *et al.* Placebo-controlled phase 3 trial of recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J Infect Dis* 2005; 191:654–665.
 22. Gilbert PB, Ackers ML, Berman PW, *et al.* HIV-1 virologic and immunologic progression and initiation of antiretroviral therapy among HIV-1-infected subjects in a trial of the efficacy of recombinant glycoprotein 120 vaccine. *J Infect Dis* 2005; 192:974–983.
 23. Pitisuttithum P, Gilbert PB, Gurwith M, *et al.* Randomized, placebo controlled efficacy trial of a bivalent rgp120 HIV-1 vaccine among injecting drug users in Bangkok, Thailand. *J Infect Dis* 2006; 194:1661–1671.
 24. Montefiori DC, Karnasuta C, Huang Y, *et al.* Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. *J Infect Dis* 2012; 206:431–441.
 25. Gilbert PB, Peterson ML, Follmann D, *et al.* Correlation between immunologic responses to a recombinant glycoprotein 120 vaccine and incidence of HIV-1 infection in a phase 3 HIV-1 preventive vaccine trial. *J Infect Dis* 2005; 191:666–677.
 26. Gilbert P, Wang M, Wrin T, *et al.* Magnitude and breadth of a nonprotective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine. *J Infect Dis* 2010; 202:595–605.
 27. Moore JP, Cao Y, Qing L, *et al.* Primary isolates of human immunodeficiency virus type 1 are relatively resistant to neutralization by monoclonal antibodies to gp120, and their neutralization is not predicted by studies with monomeric gp120. *J Virol* 1995; 69:101–109.
 28. Matthews TJ. Dilemma of neutralization resistance of HIV-1 field isolates and vaccine development. *AIDS Res Hum Retroviruses* 1994; 10:631–632.
 29. Cohen J. Jitters jeopardize AIDS vaccine trials. *Science* 1993; 262:980–981.
 30. Belshe RB, Gorse GJ, Mulligan MJ, *et al.* Induction of immune responses to HIV-1 canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. *AIDS* 1998; 12:2407–2415.
 31. Spenlehauer C, Saragosti S, Fleury HJA, *et al.* Study of the V3 loop as a target epitope for antibodies involved in the neutralization of primary isolates versus T-cell-line-adapted strains of human immunodeficiency virus type 1. *J Virol* 1998; 72:9855–9864.
 32. Mascola JR, Snyder SW, Weislow OS, *et al.* Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. *J Infect Dis* 1996; 173:340–348.
 33. Vancott TC, Polonis VR, Loomis LD, *et al.* Differential role of V3-specific antibodies in neutralization assays involving primary and laboratory-adapted isolates of HIV type 1. *AIDS Res Hum Retroviruses* 1995; 11:1379–1391.
 34. Moore JP, Sattentau QJ, Wyatt R, Sodroski J. Probing the structure of the human immunodeficiency virus surface glycoprotein gp120 with a panel of monoclonal antibodies. *J Virol* 1994; 68:469–484.
 35. Bou-Habib DC, Roderiquez G, Oravec T, *et al.* Cryptic nature of envelope V3 region epitopes protects primary monocytotropic human immunodeficiency virus type 1 from antibody neutralization. *J Virol* 1994; 68:6006–6013.
 36. Sullivan N, Sun Y, Li J, *et al.* Replicative function and neutralization sensitivity of envelope glycoproteins from primary and T-cell line-passaged human immunodeficiency virus type 1 isolates. *J Virol* 1995; 69:4413–4422.
 37. Sattentau QJ, Moore JP. Human immunodeficiency virus type 1 neutralization is determined by epitope exposure on the gp120 oligomer. *J Exp Med* 1995; 182:185–196.
 38. Pugach P, Kuhmann SE, Taylor J, *et al.* The prolonged culture of human immunodeficiency virus type 1 in primary lymphocytes increases its sensitivity to neutralization by soluble CD4. *Virology* 2004; 321:8–22.
 39. Rademeyer C, Korber B, Seaman MS, *et al.* Features of recently transmitted HIV-1 clade C viruses that impact antibody recognition: implications for active and passive immunization. *PLoS Pathog* 2016; 12:e1005742.
- Genotypic and neutralization properties of 200 recently transmitted clade C viruses showed evidence of clade effects, antigenic drift and important distinctions among the epidemics in southern Africa, Thailand, and other parts of the world that may impact antibody efficacy.
40. Hraber P, Korber BT, Lapedes AS, *et al.* Impact of clade, geography and age of the epidemic on HIV-1 neutralization by antibodies. *J Virol* 2014; 88:12623–12643.
 41. Ping L-H, Joseph SB, Anderson J, *et al.* Comparison of viral Env proteins from acute and chronic infections with subtype C human immunodeficiency virus type 1 identifies differences in glycosylation and CCR5 utilization and suggests a new strategy for immunogen design. *J Virol* 2013; 87:7218–7233.
 42. Moore PL, Crooks ET, Porter L, *et al.* Nature of nonfunctional envelope proteins on the surface of human immunodeficiency virus type 1. *J Virol* 2006; 80:2515–2528.
 43. Parren PWHI, Burton DR. HIV-1 antibody – debris or virion? *Nat Med* 1997; 3:366–367.
 44. de Taaey SW, Moore JP, Sanders RW. HIV-1 envelope trimer design and immunization strategies to induce broadly neutralizing antibodies. *Cell Press* 2016; 37:221–232.
 45. Burton DR, Hangartner L. Broadly neutralizing antibodies to HIV and their role in vaccine design. *Annu Rev Immunol* 2016; 34:635–659.
 46. Wibmer CK, Moore PL, Morris L. HIV broadly neutralizing antibody targets. *Curr Opin HIV AIDS* 2015; 10:135–143.
 47. van Gils MJ, Bunnik EM, Boeser-Nunnink BD, *et al.* Longer V1V2 region with increased number of potential N-linked glycosylation sites in the HIV-1 envelope glycoprotein protects against HIV-specific neutralizing antibodies. *J Virol* 2011; 85:6986–6995.
 48. Sagar M, Wu X, Lee S, Overbaugh J. Human immunodeficiency virus type 1 V1-V2 envelope loop sequences expand and add glycosylation sites over the course of infection, and these modifications affect antibody neutralization sensitivity. *J Virol* 2006; 80:9586–9598.
 49. Wagh K, Bhattacharya T, Williamson C, *et al.* Optimal combinations of broadly neutralizing antibodies for prevention and treatment of HIV-1 clade C infection. *PLoS Pathog* 2016; 12:e1005520.
- Neutralization assays with large panels of tier 2 Env-pseudotyped viruses provided a wealth of information for predicting bNAbs efficacy.
50. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science* 1998; 280:1884–1888.
 51. Julien JP, Cupo A, Sok D, *et al.* Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science* 2013; 342:1477–1483.
 52. Lyumkis D, Julien JP, de Val N, *et al.* Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. *Science* 2013; 342:1484–1490.
 53. Pancera M, Zhou T, Druz A, *et al.* Structure and immune recognition of trimeric prefusion HIV-1 Env. *Nature* 2014; 514:455–461.
 54. Tran EE, Borgnia MJ, Kuybeda O, *et al.* Structural mechanism of trimeric HIV-1 envelope glycoprotein activation. *PLoS Pathog* 2012; 8:e1002797.
 55. Harris A, Borgnia MJ, Shi D, *et al.* Trimeric HIV-1 glycoprotein gp140 immunogens and native HIV-1 envelope glycoproteins display the same closed and open quaternary molecular architectures. *Proc Natl Acad Sci U S A* 2011; 108:11440–11445.
 56. Guttman M, Garcia NK, Cupo A, *et al.* CD4-induced activation in a soluble HIV-1 Env trimer. *Structure* 2014; 22:974–984.
 57. Guttman M, Cupo A, Julien J-P, *et al.* Antibody potency relates to the ability to recognize the closed, prefusion form of HIV Env. *Nat Commun* 2015; 6:6144.
 58. Liu J, Bartesaghi A, Borgnia MJ, *et al.* Molecular architecture of native HIV-1 gp120 trimers. *Nature* 2008; 455:109–113.
 59. Cai Y, Karaca-Griffin S, Chen J, *et al.* Antigenicity-defined conformations of an extremely neutralization-resistant HIV-1 envelope spike. *Proc Natl Acad Sci U S A* 2017; 114:4477–4482.
- One of the best recent reports describing the differential antigenicity of an Env trimer and its corresponding gp120 monomers, highlighting how trimer organization is important for occluding epitopes and conferring neutralization resistance.
60. Munro JB, Gorman J, Ma X, *et al.* Conformational dynamics of single HIV-1 envelope trimers on the surface of native virions. *Science* 2014; 346:759–763.
- This study used smFRET to identify three spontaneous conformational states of Env trimers on virus particles and to characterize and compare the transition rates between these states on tier 1 and tier 2 viruses. These three conformational states are primary determinants of neutralization tier phenotype.
61. Guttman M, Cupo A, Julien J-P, *et al.* Antibody potency relates to the ability to recognize the closed, prefusion form of HIV Env. *Nature* 2015; 6:6144.

62. Sanders RW, Derking R, Cupo A, *et al.* A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not nonneutralizing antibodies. *PLoS Pathog* 2013; 9:e1003618.
63. Sanders RW, van Gils MJ, Derking R, *et al.* HIV-1 VACCINES. HIV-1 neutralizing antibodies induced by native-like envelope trimers. *Science* 2015; 349:aac4223.
64. de Taeye SW, Ozorowski G, Torrents de la Pena A, *et al.* Immunogenicity of stabilized HIV-1 envelope trimers with reduced exposure of nonneutralizing epitopes. *Cell* 2015; 163:1702–1715.
65. Haynes BF, Gilbert PB, McElrath MJ, *et al.* Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N Engl J Med* 2012; 366:1275–1286.
66. Moody MA, Gao F, Gurley TC, *et al.* Strain specific V3 and CD4 binding site autologous HIV-1 neutralizing antibodies select neutralization-resistant viruses. *Cell Host Microbe* 2015; 18:354–362.
67. Permar SR, Fong Y, Vandergrift N, *et al.* Maternal HIV-1 envelope-specific antibody responses and reduced risk of perinatal transmission. *J Clin Invest* 2015; 125:2702–2706.
68. Martinez DR, Vandergrift N, Douglas AO, *et al.* Maternal binding and neutralizing IgG responses targeting the C-terminal region of the V3 loop are predictive of reduced peripartum HIV-1 transmission risk. *J Virol* 2017; 91: pii: e02422-16. doi: 10.1128/JVI.02422-16.
- Confirmed an earlier association between high levels of maternal plasma V3-specific tier 1A virus-neutralizing responses and lower mother-to-child transmission risk.
69. Gray GE, Mayer KH, Elizaga ML, *et al.* Subtype C gp140 vaccine boosts immune responses primed by the South African AIDS Vaccine Initiative DNA-C2 and MVA-C HIV vaccines after more than a 2-year gap. *Clin Vaccine Immunol* 2016; 23:496–506.
70. Zolla-Pazner S, Kong XP, Jiang X, *et al.* Cross-clade HIV-1 neutralizing antibodies induced with V3-scaffold protein immunogens following priming with gp120 DNA. *J Virol* 2011; 85:9887–9898.
71. Bricault CA, Kovacs JM, Nkolola JP, *et al.* A multivalent clade C HIV-1 Env trimer cocktail elicits a higher magnitude of neutralizing antibodies than any individual component. *J Virol* 2015; 89:2507–2519.
72. Shen X, Basu R, Sawant S, *et al.* HIV-1 gp120 protein and MVAgp140 boost immunogens increase immunogenicity of a DNA/MVA HIV-1 vaccine. *J Virol* 2017; 91: pii: e01077-17. doi: 10.1128/JVI.01077-17.
73. Townsley S, Mohamed Z, Guo W, *et al.* Induction of heterologous tier 2 HIV-1-neutralizing and cross-reactive V1/V2-specific antibodies in rabbits by prime-boost immunization. *J Virol* 2016; 90:8644–8660.
74. Slieden K, Ozorowski G, Burger JA, *et al.* Presenting native-like HIV-1 envelope trimers on ferritin nanoparticles improves their immunogenicity. *Retrovirology* 2015; 12:82.
75. Hrabec P, Korber B, Wagh K, *et al.* A single, continuous metric to define tiered serum neutralization potency against HIV. *eLife* 2017; in press.
- This article describes a metric called the neutralization potency score that is a tier-like approach for comparing serum antibody responses and monoclonal antibodies. This new metric offers an alternative to the more conventional magnitude-breadth curves.