T
his commentary describes a ratio-
nale for the use of breakthrough
viruses from clinical trial participants
to assess neutralizing antibodies as a corre-
late of HIV-1 vaccine efficacy. The ratio-
nale is based on principles of a genetic
sieve analysis, where the 2 analyses may
be cooperative for delineating neutraliz-
ing antibodies as a mechanistic correlate
of protection.

The identification of immunologic
correlates of protection1-3 against HIV-1 is
a major goal that would greatly facilitate
HIV-1 vaccine development. This inform-
ation would provide a basis for rational
immunogen design and could be used to
guide the selection of promising immu-
nogens to advance through preclinical
and clinical testing. It also has value for
qualifying the expected potency of dif-
ferent lots of vaccine preparations and for
predicting vaccine efficacy in populations
where phase 3 trials did not take place.
Multiple cellular and humoral immune
responses are seen in infected individu-
als, and these responses provide a tem-
plate for what should be possible to elicit
with vaccines that aim to either control
virus replication or prevent infection alto-
tgether.4,5 Preventing virus acquisition is a
high priority for a virus like HIV-1 that
integrates genetically and persists despite
robust host immune responses. In this
regard, neutralizing antibodies (nAbs)
are among the most promising responses
to induce with HIV-1 vaccines because
of their well-documented ability to block
infection in nonhuman primate passive
protection studies with simian immu-
nodeficiency virus (SIV) and chimeric
simian-human immunodeficiency virus
(SHIV).6-12 As suggested by results of the
RV144 HIV-1 vaccine efficacy trial and
subsequent correlates studies,13-16 Fc recep-
tor-mediated effector functions might be
another mechanism by which antibodies
can prevent HIV-1 infection.17-19 Indeed,
these findings from RV144 emphasize
the need to consider multiple antiviral
mechanisms when delineating antibody
correlates of protection in HIV-1 vaccine
efficacy trials.

The overall effectiveness of vaccine-
elicted nAbs will depend on the magni-

Keywords: neutralizing antibodies, HIV,
vaccines, correlates, sieve

*Correspondence to: David C Montefiori;
Email: david.montefiori@duke.edu
Submitted: 04/07/2014
Accepted: 04/18/2014
Published Online: 05/01/2014
http://dx.doi.org/10.4161/hv.28950
in preclinical and clinical HIV-1 vaccine trials. \(^{33-36}\) Considerable infrastructure exists to perform these assessments in laboratories that comply with Good Clinical Laboratory Practices (GCLP), which is important for regulatory agency approval. \(^{37,38}\) Current capacity includes laboratories that serve the Collaboration for HIV Vaccine Discovery (CAVD, Bill and Melinda Gates Foundation), the International AIDS Vaccine Initiative (IAVI) and major networks sponsored by the US National Institutes of Health, including the HIV Vaccine Trials Network (HVTN), the Center for HIV/AIDS Vaccine Immunology and Immune Monitoring Discovery (CHAVI-ID), and the Simian Vaccine Evaluation Units (SVEUs).

These laboratory efforts are further strengthened by the availability of well-characterized HIV-1 reference strains that allow standardized neutralization data sets to be compared across vaccine protocols. \(^{36,39-41}\) Notably, the initial panels of reference strains left open key questions about the number and overall composition of strains that will be needed to adequately assess vaccine-elicited responses. Some of these questions are addressed in the design of a recently-described global panel of reference viruses that aims to be applicable to multiple vaccine platforms and clades of HIV-1 in different parts of the world. \(^{42}\) These reference strains are useful for comparing nAb responses among different vaccine immunogens;\(^ {43}\) however, their suitability for delineating nAbs as a correlate of vaccine efficacy remains to be proven. One unanswered question is whether these reference strains, which were selected based on their neutralization profiles with plasma samples from chronic HIV-1 infection, adequately represent the spectrum of epitope variants that need to be targeted by vaccines. This concern is compounded by the inherent limitations of typical case-control analyses that rely on response variability in vaccine recipients, \(^ {44}\) not taking into account differences between vaccine and placebo groups, and where the number of infection cases can be relatively low, especially for more effective vaccines. Thus, the standard practice of assaying the vaccine strain(s) and a small number of heterologous reference strains using case-control serum/plasma samples from vaccine recipients, as was done for RV144, \(^ {14}\) may lack power to detect nAbs as a correlate. Moreover, case-control studies do not distinguish between a measured immune response that is mechanistically responsible for protection vs. a response that is predictive but not a component of the protective mechanism. \(^ {3}\)

To increase the power for detecting nAbs as a correlate of vaccine efficacy, breakthrough viruses from infected vaccine and placebo recipients may be used to seek evidence that the vaccine-elicited nAbs selectively blocked transmission of certain variants. This approach is analogous to a genetic sieve analysis, which looks for features in the sequences of viruses from infected vaccine and placebo recipients that significantly differ relative to the vaccine sequences as evidence of a vaccine effect against certain variants. \(^ {45,46}\) Likewise, a neutralization “sieve” analysis compares the phenotypic properties of viruses from infected vaccine and placebo recipients in terms of their sensitivity to neutralization by pre-infection plasma/serum samples from vaccine recipients at a peak immunogenicity time point. \(^ {25}\) A positive correlation would be indicated if viruses from vaccine recipients are found to be significantly less sensitive to neutralization than viruses from placebo recipients. This outcome would be evidence that the vaccine-elicited nAbs selectively blocked transmission of the more sensitive viruses, implying a direct causal effect in mediating protection. Corroborating evidence would come from a genetic sieve analysis that successfully identifies genetic signatures that correlate with vaccine efficacy and can be shown to be responsible for the neutralization phenotype, as was done in a recent study of vaccine-mediated protection against simian immunodeficiency virus infection in nonhuman primates. \(^ {47}\)

This approach, though simple in principle, is not without challenges. Additional resources would be needed to generate high fidelity molecular clones of functional Env genes from the plasma of infected trial participants to create the virus reagents needed for current assay technologies. \(^ {48}\) In addition, viral diversification during early infection has the potential to compromise the quality of the analysis if the diversification affects vaccine-targeted epitopes prior to sampling. HIV-1 accumulates fixed amino acid changes as the host immune response matures and drives multiple rounds of virus escape from cytotoxic T lymphocyte (CTL) and nAb responses. \(^ {49,50}\) Although this immune pressure starts early in infection, the initial autologous nAb response is delayed and has a very narrow epitope specificity in any single individual, \(^ {49,51}\) possibly explaining why little diversification with potential to affect most antibody epitopes is seen in Env during the first 3–6 mo of infection. \(^ {52}\) Notably, a 6-mo sampling interval did not prevent the identification of a statistically significant genetic sieve effect in RV144. \(^ {45}\) A 6-mo sampling interval also did not prevent the identification of a significant genetic sieve effect in the STEP trial of a HIV-1 Gag, Pol, Nef vaccine that aimed to elicit protective CTL, \(^ {46}\) a finding that was possible under this condition even though the vaccine showed no clinical evidence of efficacy. \(^ {53}\) Although it remains possible that additional and stronger genetic sieve effects would have been detected if samples were obtained more frequently to capture the virus at earlier stages of infection, a sampling interval of no longer than 6 mo seems useful and has proven practical for an acceptable rate of compliance in large clinical trials.

Trial participants who acquire multiple variants at the time of transmission are another possible confounding factor for the neutralization sieve analysis. Current estimates of the rates of multiple variant transmissions are 19% for heterosexually acquired infections, \(^ {54-56}\) 36% for men who have sex with men, \(^ {56}\) and 42% for intravenous drug users (Katie Bar and George Shaw, personal communication). Extra care may be needed to identify these subjects and to include their multiple virus variants in the analysis.

Despite several challenges, neutralization sieve analyses with breakthrough viruses from vaccine and placebo recipients afford important advantages that merit serious consideration for highly variable viruses such as HIV-1. This is likely to be a more powerful method to detect nAb as a correlate of HIV-1 vaccine efficacy than methods that use the
Acknowledgements

The author thanks Celia LaBranche and Peter Gilbert for helpful comments during the writing of this manuscript. The author’s laboratory is funded by grants from US National Institutes of Health and the Bill and Melinda Gates Foundation.

References

1. Qin L, Gilbert PB, Corey L, McElrath MJ, Self SG. A framework for assessing immunological correlates of protection in vaccine trials. J Infect Dis 2007; 196:1304-12; PMID:17922394; http://dx.doi.org/10.1086/522428
2. Plotkin SA. Vaccines: correlates of vaccine-induced immunity. Clin Infect Dis 2008; 47:401-9; http://dx.doi.org/10.1086/585862; PMID:18558875
3. Plotkin SA, Gilbert PB. Nomenclature for immune correlates of protection after vaccination. Clin Infect Dis 2012; 54:1615-7; http://dx.doi.org/10.1093/cid/cxs258; PMID:22437257
4. Mascola JR, Montefiori DC. The role of antibodies in HIV vaccines. Annu Rev Immunol 2010; 28:413-44; PMID:20192810; http://dx.doi.org/10.1146/annurev-immunol-030409-101256
5. McMichael AJ. HIV vaccines. Annu Rev Immunol 2006; 24:227-55; PMID:16551249; http://dx.doi.org/10.1146/annurev.immunol.24.021605.090605
6. Shihata R, Igarashi T, Haigwood N, Buckler-White A, Oger R, Ross W, Willey R, Cho MW, Martin MA. Neutralization antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. Nat Med 1999; 5:204-10; http://dx.doi.org/10.1038/5568; PMID:9930869
7. Mascola JR, Stiegler G, VanCott TC, Katinger H, Carpenter CB, Hanson CE, Beary H, Hayes D, Frankel SS, Bix DL, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000; 6:207-10; http://dx.doi.org/10.1038/72318; PMID:10555111
8. Hessel AJ, Poignard P, Hunter M, Hangartner L, Tehrani DM, Bleeker WK, Parren PW, Marx PA, Burton DR. Effective, low-rabbit antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat Med 2009; 15:951-4; http://dx.doi.org/10.1038/nm.1974; PMID:19525960
9. Baha TW, Liska V, Hofmann-Lehmann R, Vlaski J, Xu W, Ayyar KR, Oster MR, Katinger H, Stiegler G, et al. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. Nat Med 2000; 6:200-6; PMID:10565110; http://dx.doi.org/10.1038/73209
10. Nishimura Y, Igarashi T, Haigwood N, Sadjapour R, Plishka RJ, Buckler-White A, Shihata R, Martin MA. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. J Virol 2002; 76:2123-30; PMID:11856389; http://dx.doi.org/10.1128/JVI.76.5.2123-2130.2002
11. Nishimura Y, Igarashi T, Haigwood NL, Sadjapour R, Donau OK, Buckler C, Plishka RJ, Buckler-White A, Martin MA. Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. Proc Natl Acad Sci U S A 2003; 100:1513-6; PMID:12647745; http://dx.doi.org/10.1073/pnas.2436476100
12. Ferrarielli F, Hofmann-Lehmann R, Rasmussen RA, Wang T, Xu W, Li PL, Montefiori DC, Cavacini LA, Katinger H, Stiegler G, et al. Post-exposure prophylaxis with human monoclonal antibodies prevented SHIV/SD120P infection or disease in neonatal monkeys. AIDS 2003; 17:501-9; PMID:12556683; http://dx.doi.org/10.1097/00002030-200302140-00003
13. Reks-Ngarm S, Pirisurithirun P, Nitayaphan S, Kaeuwkungul J, Chiu J, Paris R, Premri N, Namwar C, de Souza M, Adams E, et al.; MOPHTAVIR Investigators. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med 2009; 361:2209-20; http://dx.doi.org/10.1056/NEJMoa0908492; PMID:19843557
14. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Suthenth R, et al. Immune correlates of protection in an HIV-1 vaccine efficacy trial. N Engl J Med 2012; 366:1275-86; http://dx.doi.org/10.1056/NEJMoa1113425; PMID:22475592
15. Gottardo R, Bailer RT, Korber BT, Gnannakar S, Phillips J, Shen X, Tomaras GD, Turk E, Imholte G, Eckler L, et al. Plasma IgG to linear epitopes in the V2 and V3 regions of HIV-1 gp120 correlate with a reduced risk of infection in the RV144 vaccine efficacy trial. Proc Natl Acad Sci U S A 2013; 110:75656; http://dx.doi.org/10.1073/pnas.1307566110; PMID:24086607
16. Zolla-Pazner S, deCamp A, Gilbert PB, Williams C, Yates NL, Williams WT, Howington R, Fong Y, Morris DE, Soderberg KA, et al. Vaccine-induced IgG antibodies to V1V2 regions of multiple HIV-1 subtypes correlate with decreased risk of HIV-1 infection. PLoS One 2014; 9:e87572; http://dx.doi.org/10.1371/journal.pone.0087572; PMID:24904509
17. Bonogirri M, Pollara J, Moody MA, Alpert MD, Chen X, Hwang KK, Gilbert PB, Huang Y, Gurley TC, Koizink DM, et al. Antibody-dependent cellular cytotoxicity-mediated antibodies from an HIV-1 vaccine efficacy trial target multiple epitopes and preferentially use the VH1 gene family. J Virol 2012; 86:11521-S; http://dx.doi.org/10.1128/JVI.01102-S12; PMID:22896626
18. Yates NL, Liao H-X, Fong FY, deCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang Z-Y, Seamon KE, et al. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. Sci Transl Med. 2014; 6(228):228ra39
19. Chung AW, Ghebrehiwet M, Robinson B, Henry E, Choi I, Lane S, Dugast A-S, Schoen MK, Rolland M, Susovich TJ, et al. Polyfunctional Fc-effector profiles mediated by IgG subclass selection distinguish RV144 and VAX0303 vaccines. Sci Transl Med. 2014 Mar 19;6(228):228ra38
20. Hemelaar J, Gouws E, Ghys PD, Osmanov S; WHO-UNAIDS Network for HIV Isolariation and Characterisation. Global trends in molecular epidemiology of HIV-1 during 2000-2007. AIDS 2011; 25:679-89; http://dx.doi.org/10.1097/QAD.0b013e328342f399; PMID:21297425
21. Hemelaar J. Implications of HIV diversity for the HIV-1 pandemic. J Infect 2013; 66:391-400; http://dx.doi.org/10.1016/j.jinf.2012.10.026; PMID:23103289
22. Mascola JR, Snyder SW, Weislow OS, Belay SM, Belshie RB, Schwartz DH, Clements ML, Dolin R, Graham BS, Gorse GJ, et al.; The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. 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; 173:340-8; PMID:5862894; http://dx.doi.org/10.1093/infdis/173.2.340

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
23. Bures R, Gaitan A, Zhu T, Graziosi C, McGrath KM, Tarraglia J, Caudrelier P, El Habib R, Klein M, Lazzarin A, et al. Immunization with recombinant canarypox vectors expressing membrane-anchored glycoprotein 120 followed by glycoprotein 160 reduces neutralizing antibodies that neutralize R5 primary isolates of human immunodeficiency virus type 1. AIDS Res Hum Retroviruses 2000; 16:2019-35; PMID:11153085; http://dx.doi.org/10.1097/00001635-200010000-00001.

24. Belisle RB, Geese GJ, Mulligan MJ, Evans TG, Keefe MC, Esler JL, Duliege AM, Tarraglia J, Cox WI, McNamara J, et al. NIH AIDS Vaccine Evaluation Group. Induction of immune responses to HIV-1 by canarypox virus (ALVAC-HIV) in HIV-2 seroconvertants in uninfected volunteers. AIDS 1998; 12:2407-15; PMID:9875578; http://dx.doi.org/10.1097/00001635-199810000-00009.

25. Gilbert P, Wang M, Win T, Petropoulos C, Garwitz M, Sinangil F, D’Souza P, Burton DR, Mascola JR.

26. Montefiori DC, Karnasuta C, Huang Y, Ahmed H, Crotty S, Godzik A, Kaufmann DE, McElrath MJ, DeCamp A, Giganti M, et al.; NIAID AIDS Vaccine Evaluation of neutralizing antibodies against HIV-1. J Immunol Methods 2012; 375:57-67; http://dx.doi.org/10.1016/j.jim.2011.09.007; PMID:21968254.

27. Stamatatos L, Morris L, Burton DR, Mascola JR. Broadly neutralizing anti-HIV-1 antibodies: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J Virol 2009; 83:7337-48; PMID:19439467; http://dx.doi.org/10.1128/JVI.01001-09.

28. Montefiori DC, Korber BT. Prevalence of broadly neutralizing antibodies against HIV-1. J Immunol Methods 2009; 350:1-10; PMID:19525964; http://dx.doi.org/10.1016/j.jim.2009.02.013.

29. MacLaren J, et al.; NIAID AIDS Vaccine Evaluation of neutralizing antibodies against HIV-1. J Infect Dis 2012; 206:431-41; http://dx.doi.org/10.1093/infdis/jis367; PMID:22634875.

30. Belfiore M, Ruscetti FW, et al. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. J Infect Dis 2012; 206:411-21; http://dx.doi.org/10.1093/infdis/jis366; PMID:22634875.

31. Corti D, Lanzavecchia A. Broadly neutralizing anti-HIV-1 antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. Immunity 2012; 37:412-25; PMID:22999947; http://dx.doi.org/10.1016/j.immuni.2012.08.012.

32. Burton DR, Ahmed R, Barouch DH, Butera ST, Crotty S, Godzik A, Kaufmann DE, McElzach MJ, Nussenzwieg MC, Poulidran B, et al. A Blueprint for HIV Vaccine Discovery. Cell Host Microbe 2012; 12:396-407; http://dx.doi.org/10.1016/j.chom.2012.09.008; PMID:22084910.

33. Borsotti M, D’Aquila RT, Burks J, Burton DR, Haigwood NL, Morris L, Sinangil F, D’Souza P, Rodriguez-Chavez IR, Mulenga J, Keele BF, Shaw GM, et al. Inflammatory gene signature on breakthrough HIV-1 sequences from the STEP trial. Nat Med 2011; 17:366-71; http://dx.doi.org/10.1038/nm.2316; PMID:21558627.

34. Coenye T, Cotte M, De Wispelaere J, et al. Early development and therapy. Science 2013; 341:1199-1204; PMID:23845872.

35. Salazar-Gonzalez JF, Bailes E, Pham KV, Keele BF, Lear GH, Turnbull EL, Salazar MG, et al.; CHAVI Clinical Core B. The first T cell response to transmitted/founder virus contributes to the control of acute viremia in HIV-1 infection. J Exp Med 2009; 206:1253-73; PMID:19847823.

36. Buus S, Gray ES, Morris L. Specificity of the autologous neutralizing antibody response. Curr Opin HIV AIDS 2009; 4:358-63; http://dx.doi.org/10.1097/COH.0b013e32832e7a68; PMID:20046808.

37. Rolland M, Edelenbos PT, Larsen BB, DeCamp A, Giganti M, et al. Inflammatory genital infections mitigate a severe genetic bottleneck in heterosexual transmission of subtype A and C HIV-1. PLoS Pathog 2010; 6:e1000274; PMID:20456580; http://dx.doi.org/10.1371/journal.ppat.1000274.

38. Rolland M, Edelenbos PT, Larsen BB, Tovanes DA, Giorgi EE, Seoighe C, Kappes JC, Rountree W, et al.; NIAID AIDS Vaccine Evaluation of neutralizing antibodies against HIV-1. J Immunol Methods 2013; http://dx.doi.org/10.1016/j.jim.2014.02.013; In press; PMID:24293145.

39. Sarzotti-Kelsoe M, Daniell X, Todd CA, Bilka M, Martelli A, Laranche C, Perez LG, Ochsneider C, Kappes JC, Rountree W, et al. Optimization and validation of a neutralizing antibody assay for HIV-1 in A3R5 cells. J Immunol Methods 2014; In press; PMID:24529523; http://dx.doi.org/10.1016/j.jim.2014.02.013.

40. Bartocci MC, Sariola H, et al. Quantifying the multiplication of infection with human immunodeficiency virus type 1 subtype C reveals a non-poison distribution of transmitted variants. J Virol 2009; 83:5566-72; http://dx.doi.org/10.1128/JVI.01322-08; PMID:19593811.
56. Li H, Bar KJ, Wang S, Decker JM, Chen Y, Sun C, Salazar-Gonzalez JF, Salazar MG, Lennar GH, Morgan CJ, et al. High multiplicity infection by HIV-1 in men who have sex with men. PLoS Pathog. 2010 May 13;6(5):e1000890

57. Flynn NM, Forthal DN, Harro CD, Judson FN, Mayer KH, Para MF; rgp120 HIV Vaccine Study Group. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. J Infect Dis 2005; 191:654-65; PMID:15688278; http://dx.doi.org/10.1086/428404

58. Gilbert PB, Peterson ML, Follmann D, Hudgens MG, Francis DP, Gurwich M, Heyward WL, Jobes DV, Popovic V, Self SG, 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; 191:666-77; PMID:15688279; http://dx.doi.org/10.1086/428405

59. Forthal DN, Gilbert PB, Landucci G, Phan T. Recombinant gp120 vaccine-induced antibodies inhibit clinical strains of HIV-1 in the presence of Fc receptor-bearing effector cells and correlate inversely with HIV infection rate. J Immunol 2007; 178:6596-603; PMID:17475891; http://dx.doi.org/10.4049/jimmunol.178.10.6596