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Neutralizing and other antiviral antibodies in HIV-1 infection and vaccination

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Abstract

Purpose of review—New findings continue to support the notion that broadly crossreactive neutralizing antibody induction is a worthwhile and achievable goal for HIV-1 vaccines. Immunogens are needed that can overcome the genetic variability and complex immune evasion tactics of the virus. Other antibodies might bridge innate and acquired immunity for possible beneficial vaccine effects. This review summarizes progress made over the past year that has enhanced our understanding of humoral immunity as it relates to HIV-1 vaccine development.

Recent findings—Although a clear path to designing an effective neutralizing antibody-based HIV-1 vaccine remains elusive, there is new information on how antibodies neutralize HIV-1, the epitopes involved, and clues to the possible nature of protective immunogens that keep this goal alive. Moreover, there is a greater understanding of HIV-1 diversity and its possible limits under immune pressure. Other antibodies might possess antiviral activity by mechanisms involving Fc receptor engagement or complement activation that would be of value for HIV-1 vaccines.

Summary—Recent developments strengthen the rationale for antibody-based HIV-1 vaccine immunogens and provide a stronger foundation for vaccine discovery.

Keywords

adjuvants; AIDS; antibodies; complement; Fc receptors; vaccines

Introduction

Neutralizing antibodies (NAbs) remain a major focus for HIV-1 vaccine development. In addition to a strong association with the success of many approved vaccines [1], NAbs are the only known immune response that has proved capable of completely blocking AIDS virus infection in animal models [2]. New evidence suggests that NAbs might also protect against HIV-1 superinfection in humans [3•], and there is increasing evidence that maternal NAbs can protect against perinatal HIV-1 transmission [4••,5••]. In principle, vaccine-elicited NAbs of sufficient quantity, breadth and duration could confer long-term sterilizing immunity in a large segment of the human population. In cases in which complete protection is not achieved, NAbs might assist in blunting peak viremia and lowering the viral setpoint [6•], thereby making it less likely that an infected individual will develop disease and

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transmit virus, especially if vaccine-elicited antiviral T-cell responses are also present [7]. What has dampened the prospects for an effective NAb-based vaccine is the extraordinary degree of genetic diversity and structural complexity exhibited by the HIV-1 envelope glycoproteins (Env) [8,9]. The results of recent studies are helping to shape the rationale for novel HIV-1 vaccine designs.

Neutralizing antibodies in HIV-1 infection

Previous studies in HIV-1-infected patients who were treated with highly active antiretroviral therapy suggested that a threshold level of viremia is needed to drive NAb production [10,11]. In support of this, low levels of NAbs were seen in a distinct subset of long-term non-progressors ('elite suppressors') who maintained viral loads of less than 50 copies/ml without antiretroviral therapy [12•]. In another recent study [13••], the magnitude of neutralization against heterologous viruses was predicted by the level of viremia in chronically infected individuals. Overall, the NAb response in HIV-1-infected individuals exhibits wide variation in kinetics, magnitude and breadth, but one pattern that is uniform is the substantial delay in NAb production relative to peak viremia after primary infection [14–16]. In general, the earliest NAb response is detected after 3–12 months of infection, and is highly specific for the corresponding early virus variant for up to 2 years of infection. Soon after these NAbs are generated, the virus mutates to evade them, and new serum antibodies slowly emerge that neutralize the escape variant [17–19].

Recent evidence suggests that there are constraints on both the number of escape cycles and the specificity of escape in HIV-1-infected individuals [13••,20•]. Although the virus ultimately wins the battle in most individuals, there is hope that previous vaccination will tip the balance in favor of the host. In this regard, viral evolution through multiple rounds of escape could be a driving force that explains the gradual broadening of serum neutralizing activity over time. Serum from approximately 10% of chronically infected individuals thus contains moderate titers of NAbs against a wide spectrum of viral variants across multiple genetic subtypes [15,21,22]. Of greater interest, serum from approximately 2% of chronically infected individuals contains high titers of broadly crossreactive NAbs (D.C. Montefiori, unpublished). Assuming that NAbs will have greater value either before infection or during acute infection, the neutralizing activity of serum from some HIV-1-positive individuals is evidence that effective NAb induction should be possible by vaccination with the right immunogen. The nature and specificity of the NAb response in these rare HIV-1-infected individuals are thus of considerable interest for vaccine development [23]. In addition, viruses from these individuals are of potential interest as novel vaccine immunogens [24].

The reasons why most HIV-1-infected individuals do not mount a more vigorous NAb response are poorly understood. In some cases, highly conserved and well exposed neutralization epitopes might be autoantigenic and subject to B cell tolerance [25]. In other cases, chronic B-cell activation by gp120 [26•] and, perhaps more importantly, by microbial translocation from the gastrointestinal tract [27••], could be contributing factors. It is also possible that sufficient numbers of cross-neutralizing epitopes on Env, particularly across HIV-1 genetic subtypes, either do not exist or perhaps we lack suitable antibody reagents to identify them. Such differences have previously been highlighted using monoclonal antibodies (MAbs), some of which fail to neutralize non-B subtypes because the epitopes are not present [28,29].

Other potential antiviral antibodies

Despite a delay in NAb production after primary HIV-1 infection, Env-specific binding antibodies are detected very early, and often coincide with the decline in peak plasma

viremia to setpoint levels. These antibodies have the potential to bind exposed epitopes on non-functional Env proteins on the virus surface [30•], where they could mediate biological activities related to Fc receptor (FcR) engagement or complement activation that might either curb or exacerbate disease [31]. HIV-1-positive serum and human MAbs with weak or no detectable neutralizing activity in a conventional peripheral blood mono-nuclear cell (PBMC)-based assay were recently found to possess potent antiviral activity when assayed in immature dendritic cells and macrophages [32•,33••]. This activity was Fcγ receptor-dependent and did not require natural killer cells. The authors proposed that such 'non-neutralizing inhibitory antibodies' might lead to endocytosis and the degradation of opsonized HIV-1 particles as a possible means of protecting against mucosal transmission. In another recent study [34•], the preactivation of monocyte-derived macrophages through the ligation of either FcγRI, FcγRIIa or FcγRIII by an unrelated immune complex led to the inhibition of HIV-1, HIV-2, SIVmac and SIVagm infection at a late stage in the virus replication cycle. The mechanism of virus inhibition in that case could not be identified, but it appeared to be unrelated to cytokine production.

Other antibodies might mediate antibody-dependent cellular cytotoxicity (ADCC) as an immune mechanism that results in the clearance of infected cells. Both acquired and innate immunity contribute to ADCC: acquired immunity provides cytophilic antibodies, whereas innate immunity provides the effector cell populations represented by FcγR-bearing granulocytes, natural killer cells, monocytes, macrophages and subsets of γδT cells. The engagement of FcγR on effector cells brings about the release of perforins and granzymes that destroy infected cells [35]. The humoral immune response in HIV-1 infection includes the early production of antibodies that can mediate ADCC [36,37], when the appearance of ADCC-mediating antibodies precedes the NAb response [38].

ADCC has received little attention for HIV-1 in the past, but this is changing as scientists seek to identify a clear correlate of immunity in non-human primate models of candidate AIDS vaccines. Of potential importance, broadly crossreactive ADCC responses can be elicited by current HIV-1 Env immunogens [39•]. Moreover, there was a correlation between ADCC and the partial control of acute viremia in a recent SIVmac vaccine study in which no NAbs against the challenge virus were detected [40]. The authors took this latter observation one step further by purifying high titer ADCC-mediating IgG from the immunized animals, and found that passive infusion of this material into neonatal macaques was not sufficient to protect against oral SIV exposure [41]. This disappointing outcome might be explained by an immature neonatal immune system or by other differences in the design of the two studies. Notably, the passive transfer of non-neutralizing antibodies has provided protection in a similar neonatal model (see below) [42].

The antibody-mediated activation of monocytes, macrophages and natural killer cells through FcγR not only leads to the killing of infected cells, but can also lead to the release of cytokines and chemokines that can affect virus replication. Forthal and colleagues [43•] recently described how human and rhesus PBMCs depleted of CD8 and CD14 cells inhibit SIVmac251 in the presence of non-neutralizing antibodies. This inhibition involved both cytolytic and non-cytolytic mechanisms and was termed antibody-dependent cell-mediated virus inhibition (ADCVI). The study also showed that a non-neutralizing SIV-antibody-positive serum that protected neonatal macaques after oral SIVmac251 challenge contained potent ADCVI activity, suggesting that the activity is relevant *in vivo*. Potent ADCVI activity was also present in a separate non-protective passive IgG study [41].

Interactions between HIV-1 and complement also received attention during the past year. Two studies were published suggesting that HIV-1 is sensitive to complement-mediated lysis and destruction in the presence of antibody. In one study [44], Env-specific antibodies

that were detected before autologous NAbs during peak viral load in primary HIV-1 infection caused viral lysis and inactivation through the classical complement pathway. Complement-mediated inactivation in that case was seen with both autologous and heterologous viruses that were made and assayed in non-lymphoid cells. Notably, no effect was observed when PBMC-grown virus was used. In a second study [45•], non-neutralizing antibodies caused complement-mediated lysis and the inactivation of autologous PBMC-grown virus that correlated with lower plasma viral loads during acute infection.

The results of both studies need to be weighed against strong evidence that HIV-1 evades complement lysis by acquiring complement regulatory proteins as it buds from the host cell membrane [31]. These proteins, which include CD46, CD55 and CD59, are differentially expressed on various cell types and act by blocking activation of the terminal complement pathway that is needed for formation of the membrane attack complex. At the very least, both studies mentioned above indicated that HIV-1-specific complement-activating antibodies are produced very early in infection. The role these antibodies play is uncertain and could involve either virus opsonization or viral lysis by complement [31]. As suggested recently [46••], any role for complement-opsonized HIV-1 is likely to involve interactions with CR2 (CD21)-expressing cells (e.g. B cells, follicular dendritic cells) more so than interactions with CR1 (CD35) on erythrocytes as proposed earlier [47].

Neutralization requirements and mechanisms

Recent progress has been made in understanding the mechanisms of, and requirements for, antibody-mediated HIV-1 neutralization. Crooks *et al.* [48] described a creative set of assays designed to determine the specific stage in virus entry that is targeted by a particular NAb. It was confirmed that all three major stages are potential targets: (1) gp120 binding to its CD4 cell receptor; (2) subsequent binding of gp120 to either its CCR5 or CXCR4 co-receptor; and (3) gp41-mediated membrane fusion. Moreover, relevant NAbs in HIV-1-infected individuals targeted early steps in receptor binding. These assays will be useful in dissecting vaccine-elicited NAb responses and for gaining a better understanding of effective NAb induction. In another recent study [49•], neutralization potency appeared to be influenced by the levels of CCR5 but not CD4 cell expression. Therefore, unusually high levels of CCR5 on genetically engineered cell lines might explain, at least partly, several rare occasions in which a new generation of assays failed to detect neutralization that is readily detected in PBMC assays [50].

Three studies published in 2006 advanced our understanding of the requirements for neutralization. In one study [51•], the introduction of an unrelated epitope into the gp120 V4 region of multiple viral variants was used to show that HIV-1 is not intrinsically resistant to neutralization and that neutralization potency is directly related to the affinity of antibody binding. In a second study [52•], pseudoviruses containing phenotypically mixed Env trimers were used to confirm that antibodies must bind functional trimers in order to neutralize, where binding to a single monomer in each functional trimer on the virus is effective. In a third study [53•], the potency of NAbs against the receptor-binding domain of gp120 was related to the number of different conformational states of monomeric and trimeric gp120 the antibody could bind.

Env structure and neutralization avoidance

It has long been recognized that HIV-1 rapidly evolves to escape autologous NAbs, explaining why the response fails to contain the virus. Insights into how escape occurs first emerged when variable loop deletions and the removal of certain N-linked glycans on gp120 revealed complex structure-based epitope-masking mechanisms. Additional insights were provided by crystal structures of liganded HIV-1 gp120 core molecules and later by crystal

structures of an unliganded SIV gp120 core molecule, showing how one of the most critical regions for neutralization, the CD4 cell binding site, resides in a recessed pocket that is predicted to be poorly accessible to many antibodies. HIV-1 also imposes entropic barriers to antibodies in the context of a conformationally flexible Env. Excellent reviews on how HIV-1 uses these structural features and entropic barriers to evade NAbs have been published [8,9].

Last year saw the first descriptions of the three-dimensional structure of Env trimer spikes, as visualized on the surface of HIV-1 and SIV by cryoelectron microscopy tomography. Those studies are important because they permit unmodified Env trimers to be examined in their prefusion state as the natural targets for NAbs. One group [54••] reported a deduced structural model of trimeric gp120 that fit well with the crystal structure of an unliganded SIV core gp120 molecule in a profile that resembled a tri-lobed head with an arched apical peak. The most striking finding in that study was that each gp41 monomer in a trimer spike had a ‘leg’ and a ‘foot’ that protruded away from the gp120 head in a tripod fashion and anchored distally in the membrane. Using similar methods, a second group [55••] described a ‘mushroom’ shaped spike with a compact gp41 ‘stem’ in which no separation of gp41 monomers was observed. These opposing models of trimeric gp41 are difficult to explain [56•] and will be important to resolve. For example, a clear model of native gp41 structure might help to explain why some neutralizing antibodies can bind gp41 after virus attachment [48], whereas neutralization epitopes on gp120 are poorly accessible at the virus–cell interface [57].

Genetic and antigenic properties of early transmitted viruses

Viruses transmitted either heterosexually or through homosexual contact, and those transmitted from mother to child via breast-feeding, are primary targets for vaccine-elicited antibody responses. It is possible that the bottleneck that occurs at transmission, when no NAbs are present, selects viruses that show differences in receptor affinities, Env structure, and replication fitness that impact certain neutralization epitopes. Recent studies have thus suggested that early transmitted viruses (ETVs) of subtypes A and C possess unique genetic and antigenic properties, including shorter variable loops and fewer predicted *N*-linked glycans, which might provide less cloaking of neutralization-sensitive targets [58•,59•,60]. Another recent study suggested that these differences might be greater between newly transmitted subtype B and C viruses than between early and late viruses within each subtype [61••]. Although it is not entirely clear that ETVs exhibit greater overall sensitivity to neutralization compared with late viruses, the observation that some ETVs from sexually acquired subtype C infections are more sensitive to neutralization by autologous transmitter plasma compared with the major viral variant in the transmitter [62] suggests that neutralization-escape comes at a cost to transmission fitness. This is not seen in perinatally infected infants, probably because transmission in that case occurs in the presence of maternal NAbs [4••,5••]. It might be that genetic subtype as well as the route of infection play a role in determining the variants that are transmitted. Whether the higher levels of NAbs seen in HIV-1 subtype C-infected patients are caused by an inherent sensitivity of transmitted subtype C viruses or a greater immunogenicity of subtype C Env remains to be determined, as does the impact this will have on vaccine design [58•].

Subtype-specific virus panels for measuring antibody neutralization

Over the past few years a number of virus panels have been compiled in an attempt to standardize the measurement of antibody-mediated neutralization, particularly in vaccine trials. The use of molecularly cloned Env-pseudotyped viruses in luciferase reporter gene assays has facilitated the reproducibility and overall accuracy of measuring antibody-

mediated neutralization, and has provided higher throughput and lower cost. Moreover, in most cases this assay detects the same antibody specificities as the PBMC assay but with slightly greater sensitivity. The use of this assay in a three-tiered approach is now the recommended format for assessing vaccine-elicited NAb responses, with a focus on primary viruses from multiple subtypes that comprise tier 2 and tier 3 reference strains [63]. A subtype B and African subtype C panel of reference strains from acute and early sexually acquired infections have recently been developed and are available through the National Institutes of Health Reference and Reagent Repository [61••,64]. Subtype C is the major global subtype, and therefore additional panels from India, China and possibly other African countries where subtype C dominates are urgently needed, as are panels of subtypes A, D, CRF01_AE and CRF02_AG. Collectively, this covers 90% of all HIV-1 strains worldwide. Viruses chosen for such panels need to be collected within 3 months of infection, transmitted sexually and not be unusually sensitive or resistant to neutralization. A rigorous and regular programme of proficiency testing will ensure adequate competency in laboratories around the world. It is possible that initial panels of reference strains will need to be modified as new information is gained on how to improve their correlative value.

Novel immunogen design and delivery

The failure of soluble monomeric gp120 immunogens to elicit NAbs against most primary HIV-1 isolates and to show efficacy in humans highlights the need for more creative immunogens [65]. In this regard, lead approaches focus on oligomeric Env immunogens that may more effectively present epitopes as they exist on native viral Env. One solution is to introduce a C-terminal trimeric GCN-4 transcription factor motif to form stable gp140 Env trimers. Recent studies have confirmed that these gp140 trimeric proteins elicit higher titers of NAbs than the corresponding monomeric gp120 [66]; however, further design modifications will be required to generate more broadly reactive NAbs. Env trimer construction often requires the elimination of the gp120–gp41 cleavage site to prevent subunit dissociation. A potential problem with this approach is that the uncleaved Env proteins are antigenically distinct from native cleaved Env. To circumvent this problem, Beddows and colleagues [67] constructed a gp140 Env with an intact cleavage site and stabilized the protein by introducing intermolecular disulfide bonds (SOS gp140) between gp120 and gp41. Both clade A and B SOS gp140s are being studied for immunogenicity.

Improving the breadth of the NAb response may also be possible by the inclusion of polyvalent Envs. In preclinical studies using DNA priming and oligomeric protein boosting, a combined clade B and C Env immunogen generated high titers of antibodies to both vaccine strains of virus [68]. Similarly, Wang and colleagues [69] used the DNA/protein platform to immunize rabbits with Envs from clades A, B, C, D, E, F and G. In both studies, NAbs against some non-clade B viruses were elicited but the total breadth of the NAb response remained limited. An alternative approach is to construct immunogens based on centralized (ancestral or consensus) Env sequences to minimize the genetic distance between vaccine strains. Such immunogens might elicit NAbs to conserved antibody determinants on Env. Initial centralized Env constructs based on group M and subtype C HIV-1 have been found to elicit both T-cell and antibody responses in small animals; further studies are ongoing [70]. Liao *et al.* [71•] compared a group M consensus Env immunogen to wild-type Envs from clades A, B and C, and found the former to elicit NAb responses at least as good as any individual wild-type Env.

Although much of the existing effort towards improving NAb responses is based on novel immunogen design, there has been some progress in improving NAb responses by using prime-boost delivery platforms and through the use of adjuvants. Several recent studies have confirmed that either plasmid DNA or recombinant adenovirus immunization effectively

primes for soluble protein boosting and results in high titers of NAbs [68,69,72]. In addition, the co-delivery of granulocyte macrophage colony-stimulating factor DNA with Env DNA in monkeys led to a more rapid and higher avidity antibody response than Env DNA without adjuvant [73]. For soluble trimeric protein, use of the GSK series of adjuvants (AS01B, AS02A and AS03; Glaxo-SmithKline Biologicals, Rixensart, Belgium) generated higher titer NAb responses than a commercially available Ribi adjuvant [66].

Neutralization epitope mapping

Improved Env designs could be guided by assays that not only measure virus neutralization but, more specifically, what epitopes those NAbs target. Several groups of investigators have begun to analyze vaccine sera to understand better the antibody specificities generated. Three papers published last year reported that vaccine-elicited autologous virus neutralization was mediated by NAbs against the V1 region of gp120 [66,74,75]. Interestingly, all three groups identified the same hypervariable region at the tip of the V1 loop. Whether or not some common and partly conserved motifs exist in V1 that could serve as a useful vaccine antigen remains to be determined.

Antibodies against the V3 region of gp120 are also readily generated by many Env immunogens. Anti-V3 antibodies have inherent limitations because the V3 region of most HIV-1 strains is not accessible. Nonetheless, a minority of HIV-1 isolates, probably 10–15%, are sensitive to V3 MAbs. Efforts to optimize the NAb response against V3 include protein scaffolds that present the known V3 MAb 447–52D epitope [76] and specific design modifications to stabilize and increase the immunogenicity of V3 [74,75]. A potentially interesting observation is that anti-V3 antibodies derived from non-clade B-infected patients, or non-clade B immunization, might be more crossreactive than anti-V3 antibodies from clade B HIV-1-infected subjects [74,77].

As new assays to map neutralization epitopes become more refined, they may be applied to sera from HIV-1-infected patients, particularly those who possess broadly NAbs. Such studies might provide insight about how neutralization breadth can be achieved. For example, it is not known whether broadly neutralizing sera target conserved Env regions or contain polyclonal antibodies that target several variable neutralization epitopes simultaneously. One study of 92 HIV-1-positive plasma samples found little or no reactivity to the membrane proximal external region of HIV-1 gp41 engrafted into SIV [78•], whereas another study found that cloned viral Envs from two patients with broadly neutralizing sera developed mAb 2F5 and 4E10 escape mutations, suggesting that NAbs to the membrane proximal external region may play a role in driving virus evolution [24]. Decker *et al.* [79] used non-conventional strains of HIV-2 to show that sera from HIV-1-infected individuals contain high titers of antibodies against highly conserved neutralization epitopes in the co-receptor binding domain of gp120. Unfortunately, these epitopes are poorly exposed on most strains of the virus [57,61••]. Further studies of broadly neutralizing sera will be required to understand how neutralization breadth is achieved in natural HIV-1 infection.

Conclusion

Two decades of intensive research have failed to yield an effective antibody-based HIV-1 vaccine. Despite the obstacles towards effective Env vaccine design, new evidence has emerged to support the notion that NAbs and possibly other types of antiviral antibodies are indeed worth pursuing. The field continues to make progress in understanding viral Env structure and to engage in a combination of empirical and rational approaches to immunogen design. The interface of these approaches may yet yield an effective vaccine. Future prospects would benefit from progress in a number of key areas: (1) detailed studies of

ETVs from the major genetic subtypes derived from multiple routes of infection, including breast-milk transmissions; (2) the crystal structure of native unliganded trimeric Env as well as gp120 structures from non-B subtypes; (3) broadly neutralizing MAbs with novel specificities, including specificities that are both immunogenic and antigenic; (4) additional assays to scrutinize the epitope specificities of NAbs generated by infection and vaccination; (5) better adjuvants; (6) closer inspection of non-neutralizing antibodies; and (7) a greater understanding of the ontogeny of the B-cell response to HIV-1 Env, including host genetic factors.

Abbreviations

ADCC	antibody-dependent cellular cytotoxicity
ADCVI	antibody-dependent cell-mediated virus inhibition
ETV	early transmitted virus
MAb	monoclonal antibody
NAb	neutralizing antibody
PBMC	peripheral blood mononuclear cell

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

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 241–242).

1. Robbins JB, Schneerson R, Szu SC. Perspective: serum IgG antibody is sufficient to confer protection against infectious diseases by inactivating the inoculum. J Infect Dis. 1995; 171:1387–1398. [PubMed: 7769272]

2. Mascola JR. Defining the protective antibody response for HIV-1. Curr Mol Med. 2003; 3:211–218.

3•. Smith DM, Strain MC, Frost SDW, et al. Lack of neutralizing antibody response to HIV-1 predisposes to superinfection. Virology. 2006; 355:1–5. This study reports the first anecdotal evidence that NAbs might protect against superinfection. [PubMed: 16962152]

4••. Dickover R, Garratty E, Yusim K, et al. Role of maternal autologous neutralizing antibody in selective perinatal transmission of human immunodeficiency virus type 1 escape variants. J Virol. 2006; 80:6525–6533. This is the largest study of perinatally infected mother–infant pairs reported to date and includes a refined genetic analysis in addition to autologous NAb assays. The results strongly suggest that in-utero transmission of clade B HIV-1 involves neutralization-escape variants from the mother. They also imply that maternal autologous NAbs are associated with non-transmission. [PubMed: 16775339]

5••. Wu X, Parast AB, Richardson BA, et al. Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. J Virol. 2006; 80:835–844. This study shows that the perinatal transmission of non-clade B HIV-1 is associated with neutralization-escape variants in the transmitting mothers. This finding is in contrast with previous reports that sexual transmission in adults favors autologous neutralization-sensitive variants, suggesting that different antigenic variants are selected depending on the route of transmission. [PubMed: 16378985]

6•. Ferrantelli F, Buckley KA, Rasmussen RA, et al. Time-dependence of protective postexposure prophylaxis with human monoclonal antibodies against pathogenic SHIV challenge in newborn

macaques. Virology. 2007; 358:69–78. This study confirmed the protective role of pre-existing NAbs and demonstrates the protective role of NAbs in the absence of complete ‘sterilizing’ immunity. [PubMed: 16996554]

7. Letvin NL, Barouch DH, Montefiori DC. Prospects for vaccine protection against HIV-1 infection and AIDS. Annu Rev Immunol. 2002; 20:73–99. [PubMed: 11861598]
8. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science. 1998; 280:1884–1888. [PubMed: 9632381]
9. Pantophlet R, Burton DR. GP120: target for neutralizing HIV-1 antibodies. Annu Rev Immunol. 2006; 24:739–769. [PubMed: 16551265]
10. Binley JM, Trkola A, Ketas T, et al. The effect of highly active antiretroviral therapy on binding and neutralizing antibody responses to human immuno-deficiency virus type 1 infection. J Infect Dis. 2000; 182:945–949. [PubMed: 10950795]
11. Montefiori DC, Hill TS, Vo HTT, et al. Neutralizing antibodies associated with viremia control in a subset of individuals after treatment of acute human immunodeficiency virus type 1 infection. J Virol. 2001; 75:10200–10207. [PubMed: 11581388]

12•. Bailey JR, Lassen KG, Yang H-C, et al. Neutralizing antibodies do not mediate suppression of human immunodeficiency virus type 1 in elite suppressors or selection of plasma virus variants in patients on highly active antiretroviral therapy. J Virol. 2006; 80:4758–4770. An excellent study showing that potent viremia control can be achieved in some HIV-1-infected individuals without the need for NAbs, and also suggesting that a threshold level of viremia is needed to drive NAb production. [PubMed: 16641269]

13••. Deeks SG, Schweighardt B, Wrin T, et al. Neutralizing antibody responses against autologous and heterologous viruses in acute versus chronic human immunodeficiency virus (HIV) infection: evidence for a constraint on the ability of HIV to completely evade neutralizing antibody responses. J Virol. 2006; 80:6155–6164. This study shows that HIV-1 might lose its ability to evade the autologous NAb response completely during chronic infection, suggesting a limited number and type of escape mutations the virus can tolerate. Above a certain threshold, individuals with low viremia were more likely to have NAbs against their contemporaneous autologous virus, suggesting a protective role for NAbs in some cases. Chronically infected individuals who had higher plasma virus loads were more likely to possess cross-reactive NAbs. [PubMed: 16731954]

14. Pellegrin I, Legrand E, Neau D, et al. Kinetics of appearance of neutralizing antibodies in 12 patients with primary or recent HIV-1 infection and relationship with plasma and cellular viral loads. J Acquir Immune Defic Syndr. 1996; 11:438–447.
15. Pilgrim AK, Pantaleo G, Cohen OJ, et al. Neutralizing antibody responses to human immunodeficiency virus type 1 in primary infection and long-term nonprogressive infection. J Infect Dis. 1997; 176:924–932. [PubMed: 9333150]
16. Moog C, Fleury HJA, Pellegrin I, et al. Autologous and heterologous neutralizing antibody responses following initial seroconversion in human immunodeficiency virus type 1-infected individuals. J Virol. 1997; 71:3734–3741. [PubMed: 9094648]
17. Kelly HR, Urbanski M, Burda S, et al. Neutralizing antibody patterns and viral escape in HIV-1 non-B subtype chronically infected treatment-naive individuals. Human Antibodies. 2005; 14:89–99. [PubMed: 16720979]
18. Wei X, Decker JM, Wang S, et al. Antibody neutralization and escape by HIV-1. Nature. 2003; 422:307–312. [PubMed: 12646921]
19. 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. [PubMed: 12644702]

20•. Draenert R, Allen TM, Liu Y, et al. Constraints on HIV-1 evolution and immunodominance revealed in monozygotic adult twins infected with the same virus. J Exp Med. 2006; 203:529–539. Similar clinical, virological and immunological profiles were seen in monozygotic twins who were simultaneously infected with the same HIV-1 variant through needle sharing, included strikingly similar NAb responses. [PubMed: 16533886]

21. Donners H, Willems B, Beinaert E, et al. Cross-neutralizing antibodies against primary isolates in African women infected with HIV-1. AIDS. 2002; 16:501–503. [PubMed: 11834970]

22. Braibant M, Brunet S, Costagliola D, et al. Antibodies to conserved epitopes of the HIV-1 envelope in sera from long-term nonprogressors: prevalence and association with neutralizing activity. AIDS. 2006; 20:1923–1930. [PubMed: 16988513]

23. Zhang M-Y, Choudhry V, Sidorov IA, et al. Selection of a novel gp41-specific HIV-1 neutralizing human antibody by competitive antigen panning. J Immun Meth. 2006; 317:21–30.

24. Cham F, Zhang PF, Heyndrickx L, et al. Neutralization and infectivity characteristics of envelope glycoproteins from human immunodeficiency virus type 1 infected donors whose sera exhibit broadly cross-reactive neutralizing activity. Virology. 2006; 347:36–51. [PubMed: 16378633]

25. Haynes B, Fleming J, St Clair E, et al. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science. 2005; 308:1906–1908. [PubMed: 15860590]

26•. He B, Qiao X, Kalsse PJ, et al. HIV-1 envelope triggers polyclonal Ig class switch recombination through CD4-independent mechanism involving BAFF and C-type lectin receptors. J Immunol. 2006; 176:3931–3941. This study showed that polyclonal B-cell activation and class switch recombination can be induced by gp120 in a CD40-independent pathway that might lead to B-cell exhaustion in HIV-1-infected individuals. [PubMed: 16547227]

27••. Brenchley JM, Price DA, Schacker TW, et al. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med. 2006; 12:1365–1371. This study used plasma lipopolysaccharide as a well-established indicator of microbial translocation and a potent immunostimulating product of Gram-negative bacterial cell walls to provide compelling evidence that the breakdown of the gut mucosal barrier is a defining feature that leads to chronic immune activation in HIV-1-infected individuals. [PubMed: 17115046]

28. Binley JM, Wrin T, Korber B, et al. Comprehensive cross-clade neutralization analysis of a panel of antihuman immunodeficiency virus type 1 monoclonal antibodies. J Virol. 2004; 78:13232–13252. [PubMed: 15542675]

29. Gray ES, Meyers T, Gray G, et al. Insensitivity of paediatric HIV-1 subtype C viruses to broadly neutralising monoclonal antibodies raised against subtype B. PLoS Medicine. 2006; 3:e255. [PubMed: 16834457]

30•. Moore PL, Crooks ET, Porter L, et al. The nature of nonfunctional envelope proteins on the surface of human immunodeficiency virus type 1. J Virol. 2006; 80:2515–2528. This study provides key evidence that non-functional Env proteins on the virus surface can be bound by non-NAbs. [PubMed: 16474158]

31. Montefiori DC. Role of complement and Fc receptors in the pathogenesis of HIV-1 infection. Springer Semin Immunopathol. 1997; 18:371–390. [PubMed: 9089955]

32•. Holl V, Peressin M, Schmidt S, et al. Efficient inhibition of HIV-1 replication in human immature monocyte-derived dendritic cells by purified anti-HIV-1 IgG without induction of maturation. Blood. 2006; 107:4466–4474. This is the first study to demonstrate the antibody-mediated inhibition of HIV-1 infection in immature dendritic cells by an FcR-mediated mechanism. [PubMed: 16469871]

33••. Holl V, Peressin M, Decoville T, et al. Nonneutralizing antibodies are able to inhibit human immunodeficiency virus type l replication in macrophages and immature dendritic cells. J Virol. 2006; 80:6177–6181. This study is further confirmation that antibodies can inhibit HIV-1 infection in immature dendritic cells and in macrophages by an Fc receptor-mediated mechanism. Assays with a large panel of human MAbs showed that epitopes in the V3 loop of gp120 and in the immunodominant region of gp41 can mediate this activity. Activity was also present with several MAbs that are broadly neutralizing in conventional PBMC-based assays (e.g. IgG1b12, 2G12, 2F5, 4E10). [PubMed: 16731957]

34•. David A, Sáez-Cirión A, Versmisse P, et al. The engagement of activating FcγRs inhibits primate lentivirus replication in human macrophages. J Immunol. 2006; 177:6291–6300. This study shows a common Fc receptor-mediated mechanism for the inhibition of HIV-1, HIV-2 and SIV in monocyte-derived macrophages. [PubMed: 17056559]

35. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001; 22:633–640. [PubMed: 11698225]

36. Lyerly HK, Reed DL, Matthews TJ, et al. Anti-GP 120 antibodies from HIV seropositive individuals mediate broadly reactive anti-HIV ADCC. AIDS Res Hum Retroviruses. 1987; 3:409–422. [PubMed: 2833917]

37. Koup RA, Sullivan JL, Levine PH, et al. Antigenic specificity of antibody-dependent cell-mediated cytotoxicity directed against human immunodeficiency virus in antibody-positive sera. J Virol. 1989; 63:584–590. [PubMed: 2536094]

38. Sawyer LA, Katzenstein DA, Hendry RM, et al. Possible beneficial effects of neutralizing antibodies and antibody-dependent, cell-mediated cytotoxicity in human immunodeficiency virus infection. AIDS Res Hum Retroviruses. 1990; 6:341–356. [PubMed: 1971182]

39•. Gomez-Roman VR, Florese RH, Peng B, et al. An adenovirus-based HIV subtype B prime/boost vaccine regimen elicits antibodies mediating broad antibody-dependent cellular cytotoxicity against non-subtype B HIV strains. J Acquir Immune Defic Syndr. 2007; 43:270–277. Evidence that current HIV-1 vaccine immunogens are capable of eliciting broadly cross-reactive ADCC responses. [PubMed: 16940858]

40. Gomez-Roman VR, Patterson LJ, Venson D, et al. Vaccine-elicited antibodies mediate antibody-dependent cellular cytotoxicity correlated with significantly reduced acute viremia in rhesus macaques challenged with SIV. J Immun. 2005; 174:2185–2189. [PubMed: 15699150]

41. Florese RH, Van Rompay KK, Aldrich K, et al. Evaluation of passively transferred, nonneutralizing antibody-dependent cellular cytotoxicity-mediating IgG in protection of neonatal rhesus macaques against oral SIVmac251 challenge. J Immunol. 2006; 177:4028–4036. [PubMed: 16951366]

42. Van Rompay KK, Berardi CJ, Dillard-Telm S, et al. Passive immunization of newborn rhesus macaques prevents oral simian immunodeficiency virus infection. J Infect Dis. 1998; 177:1247–1259. [PubMed: 9593009]

43•. Forthal DN, Landucci G, Stefano-Cole K, et al. Rhesus macaque polyclonal and monoclonal antibodies inhibit simian immunodeficiency virus in the presence of human or autologous rhesus effector cells. J Virol. 2006; 80:9217–9225. This paper describes the utility of the ADCVI assay for studies of humoral immunity in non-human primate models of AIDS virus infection and vaccination. [PubMed: 16940533]

44. Aasa-Chapman MMI, Holuigue S, Aubin K, et al. Detection of antibody-dependent complement-mediated inactivation of both autologous and heterologous virus in primary human immunodeficiency virus type 1 infection. J Virol. 2005; 79:2823–2830. [PubMed: 15709001]

45•. Huber M, Fischer M, Misselwitz B, et al. Complement lysis activity in autologous plasma is associated with lower viral loads during the acute phase of HIV-1 infection. PLoS Med. 2006; 3:e441. This study found a correlation between complement-activating antibodies and viremia control in primary HIV-1 infection that might be related to complement-mediated virus lysis and inactivation. [PubMed: 17121450]

46••. Bánki Z, Wilflingseder D, Ammann CG, et al. Factor I-mediated processing of complement fragments on HIV immune complexes targets HIV to CR2-expressing B cells and facilitates B cell-mediated transmission of opsonized HIV to T cells. J Immunol. 2006; 177:3469–3476. This study shows for the first time that iC3b and C3d are probably the major complement fragments to deposit on HIV-1 particles, implying that opsonized virions are more likely to bind CD21-positive cells than cells bearing other complement receptors. This finding helps to clarify the many possible consequences of the virus's interaction with complement. [PubMed: 16920989]

47. Zhou JT, Montefiori DC. Complement-activating antibodies that target human immunodeficiency virus type 1 to complement receptor type 1 (CR1/CD35). Virology. 1996; 226:13–21. [PubMed: 8941318]

48. Crooks ET, Moore PL, Richman D, et al. Characterizing anti-HIV monoclonal antibodies and immune sera by defining the mechanism of neutralization. Human Antibodies. 2005; 14:101–113. [PubMed: 16720980]

49•. Choudhry V, Zhang M-Y, Harris I, et al. Increased efficacy of HIV-1 neutralization by antibodies at low CCR5 surface concentration. Biochem Biophys Res Commun. 2006; 348:1107–1115. The importance of CCR5 surface expression levels shown in this study has important implications for the latest NAb assay technologies that utilize genetically engineered cell lines with very high levels of CCR5 on their surface. [PubMed: 16904645]

50. Brown BK, Karasavvas N, Beck Z, et al. Monoclonal antibodies to phospha-tidylinositol phosphate neutralize human immunodeficiency virus type 1: role of phosphate-binding substrates. J Virol. 2007; 81 (in press).

51•. Yang X, Lipchina I, Cocklin S, et al. Antibody binding is a dominant determinant of the efficiency of human immunodeficiency virus type 1 neutralization. J Virol. 2006; 80:11404–11408. This study provides the most direct evidence to date that the affinity of antibody binding to native Env trimers determines neutralization efficiency and that primary isolates are not intrinsically resistant to antibody-mediated neutralization. [PubMed: 16956933]

52•. Herrera C, Klasse PJ, Kibler CW, et al. Dominant-negative effect of hetero-oligomerization on the function of the human immunodeficiency virus type1 envelope glycoprotein complex. Virology. 2006; 351:121–132. Pseudoviruses containing phenotypically mixed Env trimers were used to confirm that neutralization requires antibodies that bind functional trimers. Also, preliminary results suggested that antibody binding to one spike does not affect adjacent trimers. [PubMed: 16616288]

53•. Yuan W, Bazick J, Sodroski J. Characterization of the multiple conformational states of free monomeric and trimeric human immunodeficiency virus envelope glycoproteins after fixation by cross-linker. J Virol. 2006; 80:6725–6737. This study provides additional insights into the nature of the thermodynamic and kinetic barriers that restrict access of NAbs to native Env. [PubMed: 16809278]

54••. Zhu P, Liu J, Bess J Jr, et al. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature. 2006; 441:847–852. One of two studies published in 2006 showing Env trimer spikes in their native form on the virus as revealed by cryoelectron tomography. This model aims to bridge the gap between partial crystal structures and complete topology to explain key functional and antigenic properties of the virus. In this study, trimeric gp41 was viewed and modeled as having a tripod structure. [PubMed: 16728975]

55••. Zanetti G, Briggs JAG, Grunewald K, et al. Cryo-electron tomographic structure of an immunodeficiency virus envelope complex *in situ*. PLoS Path. 2006; 2:e83. This study appeared soon after the paper by Zhu et al. [54••] and shows the gp41 trimer to have a compact stalk-like structure rather than resembling a tripod on the surface of SIV.

56•. Subramaniam S. The SIV surface spike imaged by electron tomography: one leg or three? PLoS Path. 2006; 2:e91. This is an excellent opinion paper that explains how cryoelectron tomography is used to model protein structure. It also describes several variables and limitations in methodology that might account for the different structural models of gp41 trimers reported recently.

57. Labrijn A, Poignard P, Raja A, et al. Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1. J Virol. 2003; 77:10557–10565. [PubMed: 12970440]

58•. Li B, Decker JM, Johnson RW, et al. Evidence for potent autologous neutralizing antibody titers and compact envelopes in early infection with subtype C human immunodeficiency virus type 1. J Virol. 2006; 80:5211–5218. The results of this study suggest differences in the epitopes that are targeted by the autologous NAb response in clade C compared with clade B HIV-1 infection. It will be important to explore these differences in greater detail as they relate to vaccine development. [PubMed: 16699001]

59•. 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. An elegant study using chimeric subtype A envelopes showing that early viruses have shorter V1–V2 loops and fewer glycosylation sites and are more sensitive to antibody neutralization than envelopes from the same patient later in infection. [PubMed: 16973562]

60. Chohan B, Lang D, Sagar M, et al. Selection for human immunodeficiency virus type 1 envelope glycosylation variants with shorter V1–V2 loop sequences occurs during transmission of certain genetic subtypes and may impact viral RNA levels. J Virol. 2005; 79:6528–6531. [PubMed: 15858037]

61••. 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. This paper describes the first set of HIV-1 subtype C reference strains that may be used as Env-pseudotyped viruses for the standardized assessments of NAb responses. It also compares the

genetic and antigenic differences between early and late variants of subtypes B and C. [PubMed: 16971434]

62. Derdeyn CA, Decker JM, Bibollet-Ruche F, et al. Envelope-constrained neutralization-sensitive HIV-1 after heterosexual transmission. Science. 2004; 303:2019–2022. [PubMed: 15044802]
63. Mascola JR, D'Souza P, Gilbert P, et al. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate human immunodeficiency virus type 1 vaccines. J Virol. 2005; 79:10103–10107. [PubMed: 16051803]
64. 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. [PubMed: 16051804]
65. Haynes BF, Montefiori DC. Aiming to induce broadly reactive neutralizing antibody responses with HIV-1 vaccine candidates. Exp Rev Vaccines. 2006; 5:347–363.
66. Li Y, Svehla K, Mathy NL, et al. Characterization of antibody responses elicited by human immunodeficiency virus type 1 primary isolate trimeric and monomeric envelope glycoproteins in selected adjuvants. J Virol. 2006; 80:1414–1426. [PubMed: 16415019]
67. Beddows S, Kirschner M, Campbell-Gardener L, et al. Construction and characterization of soluble cleaved and stabilized trimeric env proteins based on HIV type 1 env subtype A. AIDS Res Hum Retroviruses. 2006; 22:569–579. [PubMed: 16796532]
68. Lian Y, Srivastava I, Gomez-Roman VR, et al. Evaluation of envelope vaccines derived from the south African subtype C human immunodeficiency virus type 1 TV1 strain. J Virol. 2005; 79:13338–13349. [PubMed: 16227256]
69. Wang S, Pal R, Mascola JR, et al. Polyvalent HIV-1 env vaccine formulations delivered by the DNA priming plus protein boosting approach are effective in generating neutralizing antibodies against primary human immunodeficiency virus type 1 isolates from subtypes A B C D and E. Virology. 2006; 350:34–47. [PubMed: 16616287]
70. Kothe DL, Li Y, Decker JM, et al. Ancestral and consensus envelope immunogens for HIV-1 subtype C. Virology. 2006; 352:438–449. [PubMed: 16780913]
71•. Liao H-X, Sutherland LL, Xia S-M, et al. A group M consensus envelope glycoprotein induces antibodies that neutralize subsets of subtype B and C HIV-1 primary viruses. Virology. 2006; 353:268–282. This study provides initial proof that an artificial Env immunogen based on a consensus sequence is immunogenic and generates readily detectable NAbs in small animals. [PubMed: 17039602]
72. Shu Y, Winfrey S, Yang Z-Y, et al. Efficient protein boosting after plasmid DNA or recombinant adenovirus immunization with HIV-1 vaccine constructs. Vaccine. 2007; 25:1398–1408. [PubMed: 17113201]
73. Robinson HL, Montefiori DC, Villinger F, et al. Studies on GM-CSF DNA as an adjuvant for neutralizing antibody elicited by a DNA/MVA immunodeficiency virus vaccine. Virology. 2006; 352:285–294. [PubMed: 16740288]
74. Wu L, Yang Z-Y, Xu L, et al. Cross-clade recognition and neutralization by the V3 region from clade C human immunodeficiency virus-1 envelope. Vaccine. 2006; 24:4995–5002. [PubMed: 16690178]
75. Derby NR, Kraft Z, Kan E, et al. Antibody responses elicited in macaques immunized with human immunodeficiency virus type 1 (HIV-1) SF162-derived gp140 envelope immunogens: comparison with those elicited during homologous simian/human immunodeficiency virus SHIV (SF162P4) and heterologous HIV-1 infection. J Virol. 2006; 80:8745–8762. [PubMed: 16912322]
76. Chakraborty K, Durani V, Miranda ER, et al. Design of immunogens that present the crown of the HIV-1 V3 loop in a conformation competent to generate 447-52D-like antibodies. Biochem J. 2006; 399:483–491. [PubMed: 16827663]
77. Gorny MK, Williams C, Volsky B, et al. Cross-clade neutralizing activity of human anti-V3 monoclonal antibodies derived from the cells of individuals infected with non-B clades of human immunodeficiency virus type 1. J Virol. 2006; 80:6865–6872. [PubMed: 16809292]
78•. Yuste E, Sanford HB, Carmody J, et al. Simian immunodeficiency virus engrafted with human immunodeficiency virus type 1 (HIV-1)-specific epitopes: replication, neutralization, and survey of HIV-1-positive sera. J Virol. 2006; 80:3030–3041. Anti-HIV-1 neutralizing MAb epitopes

were introduced into the corresponding regions of SIVmac239 by site-directed mutagenesis and used in neutralization assays. The authors show that antibodies with 2F5 and 4E10 specificities are rare among HIV-1-positive individuals. [PubMed: 16501112]

79. Decker J, Bibollet-Ruche F, Wei X, et al. Antigenic conservation and immunogenicity of the HIV coreceptor binding site. J Exp Med. 2005; 201:1407–1419. [PubMed: 15867093]