

# Neutralizing antibodies against HIV-1: can we elicit them with vaccines and how much do we need?

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## Purpose of review

This review describes some of the major obstacles that have impeded progress in the development of an effective neutralizing antibody-based HIV-1 vaccine and explains why it may be possible to overcome these obstacles. A renewed interest in the B-cell response in HIV-1-infected individuals is emphasized.

## Recent findings

New assay technologies and access to large numbers of clinical specimens have permitted a detailed assessment of the neutralizing antibody response in HIV-1-infected individuals. Recent studies have demonstrated that B cells can be stimulated to generate high titers of broadly cross-reactive neutralizing antibodies against multiple genetic subtypes of the virus. Preliminary evidence suggests that some of these antibodies are directed against epitopes in the CD4 binding site on monomeric gp120, whereas many others are directed against epitopes that remain to be identified.

## Summary

The rationale for pursuing an effective neutralizing antibody-based HIV-1 vaccine is strengthened by the recent demonstration of potent neutralizing antibody responses in a subset of HIV-1-infected individuals. Information on how this response develops and what epitopes are targeted could provide the insights that are needed to design improved vaccine strategies.

## Keywords

B-cells, HIV-1 vaccine, neutralizing antibodies

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## Introduction

Despite two decades of empirical and rational vaccine design efforts, including numerous preclinical and clinical trials, the discovery of an HIV-1 vaccine that elicits broadly neutralizing antibodies remains an elusive goal. Neutralizing antibodies are considered important for optimal protection against HIV-1 because they are associated with protection against most viral infections [1–3] and because they provide potent protection against AIDS virus infection in nonhuman primates [4–11]. Unlike other viruses, however, HIV-1 exhibits extensive genetic variability owing to error-prone reverse transcription of the viral genome and a high tolerance for mutations that together afford a substantial advantage for immune evasion and viral persistence. As a consequence of rampant viral evolution under immune pressure, the HIV-1 epidemic is driven by a plethora of genetic variants that comprise a poorly understood level of antigenic diversity [12,13]. Complicating matters further, the envelope glycoproteins gp120 and gp41 that

shielded by *N*-linked glycans and other structurally imposed steric constraints that limit antibody access to potential neutralization epitopes [14–17]. Despite this impressive array of immune-evasion tactics, recent evidence suggests that there are some vulnerabilities in this protective armour of HIV-1 that could provide opportunities for vaccine development. As discussed below, the isolation of several broadly neutralizing monoclonal antibodies (mAbs) and the observation that serum from some HIV-1-infected individuals exhibit strong neutralizing activity against diverse viral isolates, both suggest that it is possible for the humoral immune system to effectively target HIV-1.

## Broadly neutralizing antibodies as an achievable goal for HIV-1 vaccines

Early studies on the neutralization susceptibility of primary HIV-1 patient isolates indicated a high level of resistance [18–20] and that laboratory adaptation in continuous T-cell lines was needed to render the virus

sensitive to neutralization [21–24]. This notion was later challenged by the discovery of a small number of human mAbs from subtype B HIV-1-infected individuals that neutralized a substantial number of primary isolates [25–29]. Discovery of these broadly neutralizing mAbs (e.g., b12, 2G12, 2F5, 4E10) was met with a great deal of interest and optimism as evidence that it should be possible to induce similar antibodies against conserved neutralization epitopes by vaccination. Strangely enough however, these antibodies are infrequently detected among HIV-1-infected individuals [30,31]. Additionally, structural and phenotypic studies demonstrated unusual features of several of these mAbs, suggesting that they arose from atypical B-cell induction pathways. Examples include the apparent self-reactivity of mAbs 2F5 and 4E10 [32,33], an unusually long CDRH3 for mAb IgG1b12 [34] and a rare domain swap that creates a single monovalent antigen-binding surface for mAb 2G12 [35]. Although much has been learned about the epitopes, structure, and protective value of these few antibodies [36], attempts to elicit similar neutralizing antibodies with novel immunogens have met with limited success.

Although it is too early to abandon hope that additional studies with this first generation of broadly neutralizing mAbs will some day yield promising insights, recent years have seen a shift to discover new mAbs and to understand the neutralizing antibody response in a subset of HIV-1-infected individuals whose serum contains high titers of neutralizing antibodies against multiple genetic subtypes of the virus. Only recently has it been appreciated that some chronically infected individuals, perhaps 10–25% depending on the criteria used, possess broadly reactive neutralizing antibodies [37,38\*,39]. In some cases, the magnitude and breadth of neutralizing activity in these serum samples exceeds that of any known mAb. These findings were made, in part, because of new high-throughput neutralizing antibody assay technologies and well characterized clonal Env-pseudotyped viruses that permitted more precise investigations than were previously practical [40].

The magnitude and breadth of neutralizing activity that can arise during HIV-1 infection is cause for renewed optimism that the humoral immune response can generate antibodies that target epitopes on a majority of HIV-1 variants. A major goal, therefore, is to understand the vulnerable epitopes targeted and to learn how to exploit them for vaccine development. For example, is broad serum neutralizing activity a result of monospecific or polyspecific antibody responses, and what viral epitopes are targeted? In other words, is broad neutralization of diverse viruses achieved by targeting one or two highly conserved epitopes, or is there a more complex antibody repertoire directed against numerous regions of the viral Env? Data to answer these questions are limited, but new

epitope-mapping techniques have recently allowed a better dissection of the individual neutralizing antibody responses in polyclonal sera, and have provided some initial insights. Several highly selected sera have been shown to contain broadly neutralizing antibodies to the functionally conserved CD4 binding site (CD4bs) of gp120, and this finding has been demonstrated for both clade B and clade C HIV-1-positive sera [31,38\*,41–44]. This situation demonstrates proof-of-concept that neutralizing antibodies to a highly conserved region of HIV-1 can be generated. However, anti-CD4bs antibodies do not appear to constitute the major serum neutralizing fraction in most cases [31,38\*,41–44]. Some sera can neutralize diverse HIV-1 strains by gp120-directed antibodies that are not targeted to the CD4bs, whereas other sera contain neutralizing antibodies not directed to monomeric gp120 at all, and hence may bind to epitopes formed by the native trimeric configuration of the HIV-1 Env. Further investigation and improved epitope-mapping technologies will be required to understand the full spectrum of the neutralizing antibody response against HIV-1.

Might these individuals who have high titers of broadly neutralizing activity in their serum be a rich source of new mAbs? We think the answer to this question is an unqualified yes. Available data strongly suggest that the neutralizing antibody specificities in some serum samples are unlike any available mAbs, or even combinations of known mAbs. In addition, all known broadly neutralizing mAbs have been derived from clade B HIV-1-infected individuals and this limits our knowledge of the full repertoire of HIV-1 neutralization epitopes. For example, mAb b12 neutralized up to 75% of clade B strains of HIV-1, but less than 50% of non-clade B strains [45]. Also, mAb 2G12 has quite limited activity against nonclade B viruses and, like mAb 2F5, is especially weak against clade A and C viruses [45–49]. The isolation of new mAbs may identify new viral epitopes, or perhaps will yield new antibodies to known epitopes [43,50]. Differential serum adsorption analysis has identified anti-CD4bs antibodies in some sera that can neutralize viruses completely resistant to mAb b12, which is the only known CD4bs-directed broadly neutralizing mAb. Current structure-based design efforts would be greatly improved by understanding the contact surface of other CD4bs-neutralizing antibodies and by the discovery of as yet unknown viral epitopes that are targeted by neutralizing antibodies.

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### Role of B-cell regulation

Although the identification and characterization of new epitopes is deemed important, this information alone may not be sufficient to design better vaccines. Additional information may be needed to understand the

interaction of Env with B cells and the role of affinity maturation in the generation of neutralizing antibodies. The unusual characteristics of mAbs b12, 2G12, 2F5, and 4E10 have raised concern that most of the known broadly neutralizing antibodies were the result of atypical B-cell induction pathways that would not commonly occur [51]. It is difficult to address this question with only a handful of available mAbs. For example, we do not know whether there are broadly neutralizing antibodies that arise from a more common B-cell pathway, possibly making them easier to induce with vaccines. We do know that each of the known broadly neutralizing mAbs demonstrates fairly extensive somatic mutation from germ line gene sequence, suggesting a high level of affinity maturation. Hence, the pathway for broadly reactive neutralizing antibodies may require repeated antigen stimulation. This finding seems to be consistent with the observation that most broadly neutralizing HIV-1-positive sera occur in patients who are infected for several years or more. Most of these individuals also appear to have modest levels of viral replication (often less than 50 000 RNA-copies/ml), suggesting that chronic viral replication can stimulate the maturation of the B-cell response as long there is not severe destruction and compromise of the entire immune system. These data provide some hope that in the setting of a normal immune system, appropriate vaccination and B-cell stimulation could lead to the induction of broadly neutralizing antibodies. However, we still know very little about the kinetics of development of effective neutralizing antibody responses, and the concept of having to recapitulate chronic live viral replication with an immunogen is rather daunting. Potential methods to address this issue would be informed by a better understanding of the epitopes we need to target and how B-cell maturation eventually leads to effective antibodies to each epitope.

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### How much antibody is needed?

Now that there is growing recognition that the humoral immune system, even in the setting of the insult associated with CD4 T-cell destruction, can generate potent neutralizing antibody responses against HIV-1, a critical question is whether the serum level of these antibodies would be sufficient to prevent HIV-1 infection if they existed prior to virus exposure in a vaccine setting. Several recent studies in the nonhuman primate model have provided new quantitative information on the level of pre-existing neutralizing antibody needed for protection. Early viral challenge studies were done with either intravenous challenge, or relatively high dose mucosal challenge, enough to infect all control animals with one exposure. Recent experiments using a low dose mucosal challenge models show that the level of antibody required to protect against infection may be as much as 10-fold lower than previously thought [52,53]. In

addition, the serum level of antibody required for protection seems to vary depending on the antibody itself, suggesting that the mechanism of action of in-vivo protection may depend on the epitope targeted [52]. In total, the nonhuman primate data suggest a strong correlation between neutralizing antibodies and protection, though Fc-mediated effector functions of antibodies also likely play a role in protection [54]. The serum neutralization level required for protection may vary depending on the antibody and the viral challenge used, but the preponderance of the data suggests that when serum neutralization levels, even undiluted, are sufficient to mediate 90% neutralization in common in-vitro assays, protective effects *in vivo* are observed. Until more is known from human vaccine efficacy trials, this level of 90% neutralization at low serum dilutions is a reasonable benchmark for new antibody-based vaccine candidates. The gp120 vaccines previously tested in a phase III efficacy trial did not achieve this level of serum-neutralizing antibodies against circulating strains of HIV-1 (Montefiori *et al.*, unpublished data).

It is also important to distinguish between the potential protective benefit of pre-existing neutralizing antibodies that may be able to act on low viral inocula near the site of viral entry, and the apparent lack of clinical benefit of neutralizing antibodies during the chronic phase of HIV-1 infection. In principle, antibodies could have a greater advantage if present prior to virus exposure or shortly after infection acquisition, when they would not face the enormous challenge of overcoming viral evolution and escape in a setting of ongoing virus replication. In the absence of vaccination, autologous neutralizing antibodies to HIV-1 arise only after several months of infection. The virus rapidly mutates to escape these initial antibodies and, in a vicious cycle, the virus continues to adapt as new neutralizing antibodies are made against escape variants. Although antibodies do exert some pressure on the virus during chronic infection, the end result is continued high level viremia and progression to disease. Hence, efforts to design vaccines that elicit the same broadly neutralizing antibodies seen in infected individuals are based on the premise that these antibodies have the potential to prevent acquisition of infection and perhaps to control early virus replication and dissemination.

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### Conclusion

The design of an immunogen capable of inducing anti-HIV-1 neutralizing antibodies remains a critical goal for HIV-1 vaccine researchers. Despite substantial advances in our understanding of Env structure and of the atomic level contact surface of several neutralizing mAbs, the translation to improved vaccine immunogens has proven to be a major scientific challenge. The complex level of

antigenic diversity of HIV-1, the shielding of key epitopes within the three-dimensional structure of the native Env trimer, and the failure of newer versions of Env proteins to elicit broadly reactive antibodies, have led to some pessimism regarding the potential to ever elicit neutralizing antibodies against diverse strains of HIV-1. But nature tells us that B cells can be stimulated to secrete a potent and cross-reactive antibody response against HIV-1. These antibodies exist in the sera of some HIV-1-infected patients, and this challenges us to understand at a more fundamental level just how neutralizing antibodies arise. Many questions remain about the innate and adaptive immune pathways that result in the generation of neutralizing antibodies, the kinetics of their development and the viral epitopes targeted. It is essential to bring together the optimal clinical specimens, and expertise in B-cell biology and structural virology, to address these key questions, and to begin to translate this knowledge into tangible vaccine development.

## References and recommended reading

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- 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 (p. 453).

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