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Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine?

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Most existing viral vaccines generate antibodies that either block initial infection or help eradicate the virus before it can cause disease. For HIV-1, obstacles to eliciting protective neutralizing antibodies (NAbs) have often seemed insurmountable. The target of HIV-specific NAbs, the viral envelope glycoprotein (Env), is highly variable in amino acid sequence and glycosylation pattern. Conserved elements of HIV-1 Env seem to be poorly immunogenic, and previous attempts to generate broadly reactive NAbs by vaccination have proven ineffective. However, recent studies show that antibodies in the sera of some HIV-1–infected individuals can neutralize diverse HIV-1 isolates. Detailed analyses of these sera provide new insights into the viral epitopes targeted by broadly reactive NAbs. The findings discussed here suggest that the natural NAb response to HIV-1 can inform future vaccine design. A concerted effort of structure-based vaccine design will help guide the development of improved antibody-based vaccines for HIV-1.

Previous exposure to most viral pathogens, either as the infectious agent or mimicked in a vaccine, results in the generation of antibodies that neutralize the virus and protect against disease[1–3]. Hence, a major goal of HIV-1 researchers is to design a vaccine that elicits protective antibodies *in vivo* that would be present before exposure to the virus[4,5]. The isolation of HIV-1 in 1983 and the appreciation that NAbs would probably target the Env glycoprotein of this retrovirus led to optimism that an effective antibody-based vaccine could be designed within a few years. HIV-1 Env is a trimeric structure consisting of three identical gp160 molecules, each with a surface gp120 noncovalently linked to a membrane-spanning gp41 molecule, and, indeed, recombinant gp160 and gp120 vaccine candidates were rapidly produced and tested in phase 1 clinical trials. To the surprise of most researchers, these studies showed that the vaccine-induced antibodies failed their first *in vitro* test—they were unable to neutralize primary viruses derived from the blood of infected individuals[6]. Ultimately, the results of a phase 3 trial of a gp120 vaccine showed the lack of efficacy of this type of antibody-based vaccine strategy. The vaccine failed to prevent HIV-1 infection and did not lower viral replication or protect against $CD4^+$ T cell decline[7].

Over the ensuing years, numerous attempts to design improved antibody-based vaccine immunogens met with limited success. Vaccine immunogens have elicited NAbs, but only to a minority of circulating HIV-1 strains[4,5]. The lack of progress in this key area of research led to a focus on vaccines that could elicit protective T cell responses[8,9]. Human data suggest that potent $CD8^+$ T cell immunity contributes to the control of HIV-1 replication[10]. In addition, nonhuman primate studies of simian immunodeficiency virus show that the presence of vaccine-induced T cell responses before infection are associated with improved control of viral replication after infection[8]. Unfortunately, the first T cell–based vaccine tested in humans, a recombinant adenovirus encoding several internal HIV-1 proteins, had no effect on viral replication in those who were vaccinated and subsequently infected[11]. Thus, the failure of the first vaccines designed to elicit either protective antibodies or T cells has led to a renewed focus on understanding mechanisms of protective immunity from HIV-1 to better guide the development of improved vaccines. It now seems likely that major improvements in vaccine design will be required for the development of an optimally effective HIV-1 vaccine. In this context, studying the properties of the cross-NAb responses that emerge during natural HIV-1 infection could provide vital clues for the design of immunogens able to elicit broadly neutralizing antibodies that will surely form an important part of an optimal vaccine.

Broadly NAbs in sera—common enough to be good news?

Effective vaccines against viruses such as the hepatitis B, measles and influenza viruses are able to generate an antibody response similar to that which arises during natural infection[1]. However, a chronically replicating retrovirus such as HIV-1 presents some unusual challenges. The extensive genetic variation of HIV-1 is manifested by the numerous genetic subtypes worldwide and by the evolution of multiple viral variants within each infected individual. NAbs against viral Env are generated within weeks after infection, but this early antibody response specifically targets the autologous virus that circulates within each person and is ineffective against heterologous viruses[12]. However, cross-reactive antibodies capable of neutralizing heterologous primary viral isolates can develop during the course of HIV-1 infection[13–20], and it is this NAb response that is of interest to

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vaccine researchers. Passive transfer of potent NAbs into monkeys showed the ability of such antibodies to completely block infection by a chimeric simian-human immunodeficiency virus[21–25]. Hence, an HIV-1 vaccine that can generate preexisting antibodies that neutralize most circulating virus strains has the potential to protect against infection with HIV-1.

Until recently, we had little information on the prevalence of cross-reactive neutralizing antibody responses during natural HIV-1-infection[17–20], the clinical factors associated with their development or the viral epitopes targeted by such antibodies. Four studies published in the last few months provide new insights into these questions[26–29]. These studies were facilitated by quantitative, high-throughput neutralization assays that use well defined clonal viruses[30] and by improved analytical methodologies that allow researchers to map the viral epitopes targeted by the cross-reactive NAbs found in sera. This new information provides a framework for future studies that can guide the development of novel vaccine immunogens.

In the studies by Doria-Rose *et al.*[27] and Sather *et al.*[29], approximately 25% of the HIV-1–infected subjects who were infected for at least one year, without clinical signs of AIDS and not on antiretroviral drug therapy, were found to have moderate to broadly reactive NAb responses. In another very recent study, the researchers investigated samples from 1,798 mostly non–clade B HIV-1–infected donors with similar selection criteria as the above-mentioned studies and again identified a considerable proportion of donors having modest or broadly reactive NAb responses, with 1% of the donors being designated as "elite neutralizers" with unusually potent activity against a majority of clades[31]. Although there are caveats to these data, such as the use of a single assay to measure serum NAb activities and the lack of knowledge about the precise titer of preexisting NAbs that would be protective, these results demonstrate the ability of the immune system to effectively generate NAbs against HIV-1. The development of a heterologous NAb response correlated with greater time after infection and with a higher level of plasma viremia. Therefore, broadly reactive NAb activities seem to develop over time and are fostered by chronic antigen exposure. These data raise several possibilities. Neutralization breadth may arise by a gradual maturation or focusing of the HIV-1 Env–specific B cell response on a highly conserved epitope, or it may arise as a result of the emergence and accumulation over time of NAbs to several distinct regions of Env. These mechanisms need not be mutually exclusive, although the latter scenario would probably pose greater hurdles for HIV-1 vaccine development. Thus, it becomes important to understand how the immune system is able to generate a cross-reactive NAb response against HIV-1.

Which viral epitopes are vulnerable to broadly NAbs?

As mentioned above, one possibility is that cross-neutralizing antibodies will target regions of Env that are structurally conserved among diverse isolates and that are functionally important for viral attachment and entry into target cells. One such region is the CD4-binding site on gp120, which is required for binding to the CD4 receptor on the surface of specialized cells of the immune system, such as on helper T cells and monocytes. A second structurally conserved Env region is the co-receptor binding site, also on gp120. After engaging CD4, viral Env binds to specific chemokine receptors, primarily CCR5 and CXCR4, on the surface of target cells. Both the CD4-binding site and the co-receptor binding site are immunogenic, and numerous monoclonal antibodies (mAbs) against these regions of Env have been isolated from HIV-1–infected subjects. However, the vast majority of human mAbs to both of these regions are unable to bind the native viral Env trimer and, thus, are unable to neutralize primary strains of HIV-1. This highlights the complex nature of the native structure of HIV-1 Env and the mechanisms used to prevent NAbs from accessing their specific epitope. Over the past 20 years, only a handful of human neutralizing mAbs have been isolated, and only one broadly neutralizing CD4-binding site–specific mAb, b12, has been discovered[32,33]. Last year however, investigators reported that several highly selected neutralizing HIV-1 sera contained NAbs directed to the CD4-binding site[19,20]. These initial reports prompted the more systematic studies mentioned above that shed new light on the epitope specificities of the HIV-specific NAb responses that are generated during HIV infection[26,28,29]. In each study, the NAb epitope specificities were defined by a variety of complementary methodologies such as selective removal of specific antibodies or the use of chimeric viruses presenting specific epitopes. These analyses showed that the complex polyclonal antibody response to the HIV-1 Env can be dissected to understand how sera neutralize diverse HIV-1 viruses. All three studies showed that CD4-binding site–specific antibodies can contribute to the overall cross-neutralizing potential of HIV-1$^+$ sera, and, in rare cases, the broad neutralizing activity of sera is almost exclusively due to CD4-binding site–specific antibodies. Thus, the CD4-binding site of gp120 is a promising target for vaccine design.

In many of the cases, the CD4-binding site was not the sole epitope targeted by serum NAbs. Antibodies to the co-receptor binding region of gp120 also seemed to contribute to the cross-neutralizing activities of some sera[28]. The co-receptor binding site is thought to be only transiently presented to the immune system during virus-cell fusion, after Env binding to its primary receptor, CD4. Thus, access to this co-receptor binding region of Env is limited by the close proximity of the viral Env to the target plasma membrane[34–36]. In fact, known mAbs that bind to the co-receptor binding site on gp120 are unable to neutralize HIV-1, presumably because they cannot effectively access this site on the trimeric viral Env[36]. Hence, the observation that the co-receptor binding site might be the target of cross-neutralizing antibodies that are generated during HIV-1 infection is intriguing and requires more direct confirmation.

In addition to the CD4-binding site and the co-receptor binding site on gp120, the membrane-proximal external region (MPER) of gp41 is highly conserved among diverse HIV-1 strains. The MPER is involved in the process of viral fusion to the target cell and is the known target of the human neutralizing mAbs 2F5, Z13 and 4E10 (ref. 37). Only a minority of the recently studied sera were found to contain NAbs to the MPER. In some cases this was mapped to a specific epitope defined by the human mAb 4E10 (refs. 28,29), and in one case to the epitope recognized by the human mAb 2F5 (ref. 38) In other cases, the epitope of the MPER-directed NAbs was not clearly mapped, suggesting that additional epitopes within the MPER are targets of cross-reactive NAbs generated during natural HIV-1 infection (ref. 26 and E. Gray and L.M., unpublished observations).

The finding that sera can neutralize HIV-1 via antibodies to the MPER is noteworthy because antibodies to this hydrophobic region of gp41 can show cross-reactivity with lipid moieties on human cell membranes. This led to the hypothesis that B cells producing such antibodies would be self reactive and eliminated during B cell differentiation[39]. The fact that cross-reactive MPER-specific NAbs were detected in several HIV-1$^+$ subjects suggests that conserved epitopes within this Env region can be immunogenic, although we have a limited understanding of the viral or host factors that might promote the development of NAb to this region.

An additional observation from these studies is that a substantial fraction of the NAbs in many sera seemed to be directed to

unidentified regions of the viral Env. What could be the nature of these epitopes? One possibility is that they are quaternary epitopes created by the association of three Env molecules that form the functional Env spike on the surface of infectious viral particles. Such complex epitopes would not be identified by most of the reagents used for selection and characterization in these studies, which were based on the monomeric forms of Env or Env-derived peptides. It is also possible that such unidentified epitopes are formed partially or exclusively by carbohydrate molecules that cover large surface areas of the HIV-1 Env. One of the known cross-neutralizing mAbs is 2G12, whose epitope consists exclusively of mannose residues associated with specific N-linked glycosylation sites on the Env[40–42]. Identifying the location and nature of these unknown viral epitopes will probably require the development of improved reagents that can dissect NAb responses to the native viral Env. Furthermore, understanding the structural characteristics of such epitopes has to the potential to inform the design of more relevant vaccine immunogens.

Implications for vaccine development

The fact that broadly cross-reactive NAb responses arise over a period of years suggests that maturation of the antibody response is required to effectively target specific conserved viral epitopes. Persistent viral replication in the setting of the body's antibody response leads to a continuous evolution of the viral Env to evade NAbs. Such antigenic evolution may gradually focus the NAb response on the less immunogenic but more conserved regions of Env. Therefore, a successful vaccine strategy will probably require a more detailed understanding of the evolution of the viral Env protein and of NAb responses, particularly in those individuals who can eventually mount a cross-reactive NAb response. Of note, plasma virus concentrations in individuals with broad serum NAb responses are generally low to moderate (1×10^3 to 1×10^4 viral RNA copies per ml) despite years of infection in the absence of antiretroviral treatment[29]. We do not currently know whether NAbs have a role in suppressing viral replication during the chronic course of HIV-1 infection, but we do know that once HIV-1 infection is established the virus will eventually find ways to escape from the NAb responses, as it does from T cell–mediated antiviral responses[5,8,9,12]. Therefore, an effective vaccine will need to elicit preexisting NAbs that inactivate the viral inoculum at mucosal sites or during the earliest stages of viral replication, to either completely eliminate the virus or to minimize and delay viral replication such that T cell–mediated antiviral responses can control the virus.

Given that B cells can be stimulated to generate broadly reactive NAbs during the course of HIV-1 infection, it seems reasonable to assume that appropriately designed and delivered vaccine immunogens might also generate such antibody responses. A second consideration is then a quantitative one. How immunogenic are these conserved epitopes during natural HIV-1 infection? Does the concentration of cross-reactive NAbs induced in natural HIV infection correspond to a level that would benefit the host if these responses were induced before infection by vaccination? If the answer to the second question is "yes," then this is indeed good news, as it would suggest that vaccination is targeting a response that has been achieved during natural HIV-1-infection, albeit after prolonged antigen exposure. Current information relevant to NAb levels that provide benefit on exposure to HIV-1 comes largely from studies in macaque models[21–25]. These studies typically show that high titers of NAbs are required to provide sterilizing immunity against high-dose viral challenge. Such titers are greater than those observed in the sera of most HIV-1–infected individuals. However, there are examples where

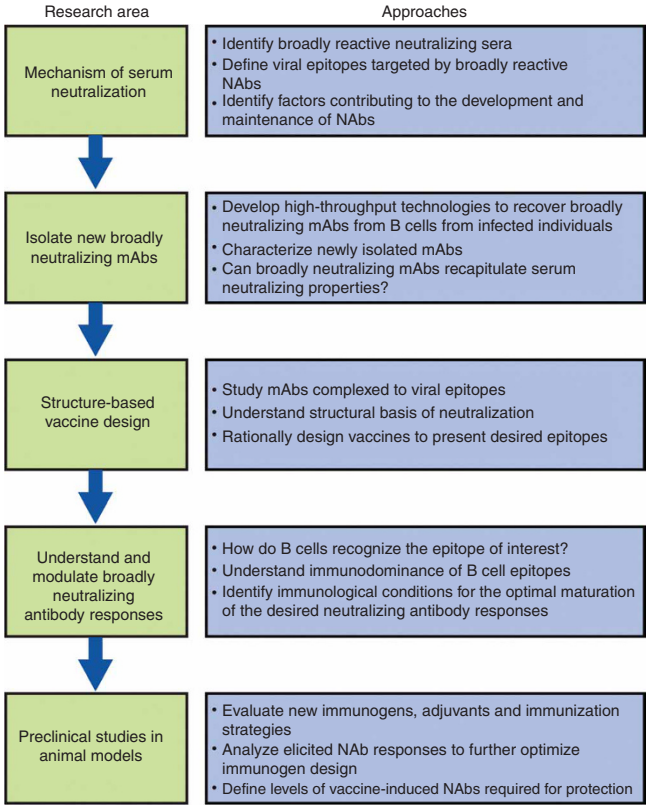

Figure 1 Flow chart of structure-based HIV-1 vaccine design.

antibody-mediated protective immunity is provided at much lower neutralizing titers, particularly when the viral exposure is via the mucosal route[23,43], as is the case for the vast majority of HIV-1 infections worldwide. Furthermore, relatively low NAb titers can provide benefit by increasing the number of challenges to achieve infection in a low-dose repeated challenge macaque model[44], which aims at reproducing the natural setting for HIV-1, in which multiple exposures are believed to be necessary for infection to take place. Therefore, it may well be that the presence of even modest levels of broadly reactive NAbs elicited by immunization could provide a substantial level of protective immunity upon viral exposure. Carefully designed animal studies may provide currently lacking information in this area.

Future areas of investigation

How can the information generated by the recent studies of neutralizing HIV-1$^+$ sera be applied to future vaccine design? Improving our understanding of the structure of HIV-1 Env and of the epitope-targets of NAbs, together with an improved understanding of how to stimulate the appropriate B cell responses, could assist the rational design and preclinical testing of new immunogens and immunization regimens (**Fig. 1**). The observation that more than one pathway leads to the development of cross-reactive NAbs suggests that vaccine immunogens could be designed to mimic several key NAb epitopes. Examples of such epitopes are the CD4-binding site of gp120 and the MPER of gp41. As additional NAb epitopes are discovered, these can also serve as the basis for vaccine immunogens. The isolation of new neutralizing mAbs and the characterization of their epitopes should therefore be a major priority for HIV-1 vaccine development. Novel technologies are already being developed that will facilitate the

isolation of large numbers of mAbs from HIV-1–infected subjects[45,46]. New mAbs may define new neutralization epitopes, and can provide important additional detailed information about known epitopes. High-resolution structural information can reveal the atomic level architecture of the region of Env bound to a NAb, and this information can be used to design better immunogens. As some of these mAbs may recognize epitopes present only on the native trimeric HIV-1 Env, the atomic level crystal structure of this molecule should also be a priority.

An additional roadblock to the generation of NAbs by immunization is our limited knowledge of how B cells recognize key Env epitopes. Many Env immunogens evaluated over the years were designed to include conserved neutralization epitopes, such as those recognized by the existing neutralizing mAb b12, which binds the CD4-binding site of gp120, or mAb 4E10, which binds the MPER of gp41. However, immunization with these constructs did not result in the generation of b12- or 4E10-like NAbs. This dichotomy between the antigenic and immunologic characteristics of a vaccine immunogen is still not understood[4,5]. Also, there are numerous nonneutralizing antibodies whose epitopes overlap those recognized by NAbs. The reasons for the limited immunogenicity of key conserved neutralization epitopes, and the precise structural characteristics that distinguish neutralizing from nonneutralizing antibodies, should be the subject of future studies. As mentioned above, an additional level of complexity is that cross-reactive NAbs may be the result of affinity maturation of B cells that is driven by chronic antigenic exposure. Therefore, we need a better fundamental understanding of how Env antigens are processed and presented to B cells, along with knowledge of the immunological and virologic factors that result in the evolution of cross-reactive NAbs.

It is likely that, in addition to optimizing vaccine antigens, we will have to learn how to better modulate the innate and adaptive immune systems to generate the type of potent and broadly cross-reactive neutralizing antibody response likely to be required to protect against HIV-1 exposure. This may require the use of improved adjuvants and immunization regimens (**Fig. 1**). It is also important to determine whether specific genetic factors are associated with innate and adaptive immune responses required for the optimal elicitation of broad NAb responses through vaccination. Although the majority of HIV-1–infected individuals do not seem to develop broadly reactive NAbs, there is hope that the delivery of suitable immunogens to a healthy immune system could lead to the induction of NAbs in the majority of vaccine recipients.

The encouraging data published in the recent studies discussed here provide evidence that the humoral immune system is capable of generating potent and cross-reactive NAb responses against HIV-1. An understanding of the viral epitopes targeted by serum NAbs, along with detailed structural information of NAbs interacting with their targeted epitopes, will allow a rational approach to the design of improved vaccine immunogens. Even with this approach, there are gaps in our knowledge that may continue to limit the effective design of antibody-based vaccines. Rational immunogen design will need to be coupled to systematic iterative immunization studies to understand how the immune system reacts to specific Env immunogens and vaccine strategies. Progress may occur in small steps, but with an improved understanding of Env structure and of B cell responses to Env, it should be possible to design a vaccine strategy that reproduces the optimal NAb response generated during natural HIV-1 infection.

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