



Review

HIV vaccine development: Challenges and opportunities towards solving the HIV vaccine-neutralizing antibody problem[☆]

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ABSTRACT

Recent advances in HIV vaccine development have created a renaissance in the search for a safe and effective HIV vaccine. These advances include the first demonstration in human clinical trials of a vaccine candidate that provided modest levels of protection from HIV infection; a series of candidates entering into clinical trials with an improved profile of protection against SIV in non-human primate studies, and the identification from HIV infected individuals of new broad and potent monoclonal antibodies against HIV that target conserved, vulnerable regions of the HIV envelope glycoprotein spike. The major challenge for successful HIV vaccine development rests on overcoming the unprecedented hyper-variability of HIV, which likely will require induction of broadly protective neutralizing antibodies to prevent HIV infection, and broad and robust cellular immune responses to control HIV infection. This presentation will review the challenges and opportunities for development of vaccine candidates capable of eliciting broadly neutralizing antibodies against HIV.

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1. Introduction

HIV continues to cause significant morbidity and mortality, particularly in sub-Saharan Africa. Since the identification of HIV as the etiologic agent that causes AIDS [1,2], more than 60 million persons have become HIV infected, with 25 million succumbing to AIDS, over 33 million living with HIV, and over 7000 new infections daily [3]. Despite the remarkable achievements in development of anti-retroviral therapies against HIV, and the recent advances in new prevention technologies, the rate of new HIV infections continue to outpace efforts on HIV prevention and control. Simply put, the world needs an HIV vaccine.

In thirty years since the AIDS virus was first recognized, there have been only three vaccine approaches that have completed human efficacy trials (Table 1). The first was a gp120 monomeric protein in alum, and this candidate failed to prevent or control HIV infection [4]. The second, a recombinant adenovirus type 5 vaccine, containing HIV gag, pol and nef genes, also failed to provide any benefit [5]; in fact, more HIV infections occurred in the vaccinated group when compared with the placebo, for reasons that are still not completely understood. Lastly, in 2009, a prime-boost strategy (RV-144) utilizing a canarypox vector prime + monomeric gp120 boost provided the first signal for prevention of HIV

infection in humans, albeit with a modest 31.2% efficacy [6]. In contrast, this regimen had no effect on control of HIV as measured by viral load in those subjects who subsequently became HIV infected. Currently, there is only one efficacy trial ongoing, a test-of-concept trial assessing the efficacy of a DNA prime + Ad5-HIV vector boost, with data expected from this trial in 2013 [7]. Building on the results from RV-144, plans are underway to gain insights into potential correlates of protection, and to conduct additional efficacy trials with related vector prime plus protein boost-regimens.

Recently, several vaccine concepts assessed in the SIV-rhesus macaque model, have provided greater levels of protection than the Adenovirus type 5-SIV-gag-pol-nef candidate which is analogous to the Merck Ad5-HIV vaccine noted above. These candidates include prime-boost strategies such as DNA + Ad5 [8] and electroporated DNA + IL12 + Ad5 [9], heterologous Adeno vectors [10], and cytomegalovirus (CMV)-based vaccines [11], and we look forward to data from human clinical trials of these and related vaccine concepts in the coming years.

HIV poses multiple scientific challenges to vaccine developers. The first challenge is the lack of an ideal animal model. While the low dose repeated SIV challenge model for T cell based vaccines aimed at controlling HIV infection accurately predicted the failure of the Merck Ad5 based vaccine [12], the current chimeric SHIV models which include HIV-Env have limitations in addressing the hypervariability of HIV. Other challenges include the lack of correlates of protective immunity; the capacity of the virus to target and integrate its genome into cells of the immune system; and most importantly, the unprecedented hyper-variability of HIV [13]. Thus, safe and effective HIV vaccines will likely require induction

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Table 1
HIV vaccine efficacy trials.

	Vaccine candidate	Prevention of HIV infection	Control of HIV infection
2003	VaxGen: gp120	No	No
2007	Merck rAd5: Gag, Pol, Nef	No – more infections in vaccines than placebo	No
2009	RV-144 (Sanofi/VaxGen) Canarypox Gag, Pol, Env/gp120 boost	31% efficacy – first signal in humans for benefit by HIV vaccine	No
2013	Ongoing: NIAID-VRC: DNA + Ad5: gag–pol–nef; Env A, B, C	?	?

of broadly protective neutralizing antibodies to prevent HIV infection, and broad cellular immune responses to control HIV infection. While the product development pipeline includes some approaches for induction of broad cellular immune responses, including the use of conserved epitopes across the genome [14], and mosaic antigens [15], there currently are no candidates in the pipeline that elicit broadly neutralizing antibodies (bnAbs) against HIV.

2. The HIV vaccine neutralizing antibody problem

Most successful vaccines induce neutralizing antibodies, but HIV will likely require the induction of bnAbs, due to the hyper-variability of the virus. However, HIV has several characteristics that pose formidable challenges to vaccine designers with respect to induction of bnAbs. First, the native HIV envelope glycoprotein (HIV Env) trimer, the target of neutralizing antibodies on the surface of the virus, is unstable, with immunodominant non-native forms of Env on the virion surface capable of inducing non-neutralizing antibodies [16]. Secondly, HIV Env consists of variable and conserved regions, and the variable regions tend to be immunodominant, leading to type-specific neutralizing antibodies with minimal breadth across the global diversity of HIV isolates [17]. Thirdly, there are relatively few native HIV Env trimers on the surface of HIV, which is dominated by non-native forms of HIV Env and host cell proteins captured in the virion membrane in the budding process from infected cells [18]. Lastly, the HIV Env is covered with sugar molecules, making interactions of neutralizing antibodies and virion epitopes of such antibodies difficult [19]. Collectively, these immune evasion mechanisms pose significant obstacles to the induction of bnAbs by vaccines.

3. Identification of broad and potent neutralizing antibodies against HIV

Natural history studies of HIV infected individuals has revealed that between 10% and 30% of HIV+ individuals develop bnAbs, though it usually takes three or more years post infection for such antibodies to develop [20]. However, only 1% of HIV infected individuals termed “elite neutralizers” develop antibodies with outstanding breadth and potency [21]. Mapping of serum reactivities of these individuals reveals only a small number of broadly neutralizing epitopes on HIV Env [22]. Broadly neutralizing monoclonal antibodies (bnMAbs) isolated from these individuals provide more precise clues to the vulnerable regions on HIV Env that can then be targeted for vaccine design.

Prior to 2009, only four bnMAbs (b12, 2G12, 2F5, and 4E10) had been identified. MAb b12 binds to a conformationally conserved surface of HIV Env that overlaps a portion of the CD4 binding site [23]. 2F5 binds the ELDKWA sequence in the heptad repeat 2 region of gp41, and 4E10 to the NWFDT sequence at the point where gp41 inserts into the lipid bi-layer [24,25]. These membrane proximal external region (MPER) antibodies attach to the lipid viral membrane via their hydrophobic CDR H3 loops, which is an important component of their neutralizing activity [26,27]. Lastly, 2G12 targets the high-mannose cluster on the glycan shield of HIV, and has a unique V (H) domain-exchange structure [28–31].

Recently, application of novel technologies to recombinant human monoclonal antibody isolation such as single cell B cell cloning, deep sequencing, and microneutralization assays have led to a large number of new, broader and more potent HIV-1 specific neutralizing monoclonal antibodies than previously described. More importantly, these new tools have catalyzed the HIV-1 vaccine field by providing new targets on HIV-1 Env which may now be exploited for vaccine design. The first of the recent series of such bnMAbs came with the identification of PG9, PG16 and related antibodies, targeting an epitope preferentially expressed on trimeric HIV Env protein which spans conserved regions of variable loops of gp120 [32,33]. Antigenically resurfaced glycoproteins were used as probes to identify sera from donors with neutralizing antibodies to the CD4 binding site which led to the identification of VRC01 and related monoclonal antibodies [34–36]. Moreover, an additional set of broad and potent monoclonal antibodies against HIV was recently identified, many of which target epitopes associated with glycan residues on HIV Env (PGT series of Abs [37]) (Table 2). Collectively, these recent discoveries have now re-focused vaccine design efforts for induction of bnAbs to a limited number of conserved, vulnerable sites on HIV Env. Fig. 1 provides a schematic of the location of the epitopes for the broadly neutralizing antibodies.

4. HIV bnMAbs protect monkeys from SHIV infection

HIV vaccines assessed to date have not elicited bnAbs. The VaxGen monomeric gp120 vaccine conferred high levels of non-neutralizing binding antibodies, and type-specific neutralizing antibodies primarily against Tier 1 sensitive isolates of HIV, but failed to prevent HIV infection. Similarly, the ALVAC prime + VaxGen gp120 boost regimen assessed in the RV-144 trial did not confer bnAbs. The correlates of the modest protection observed in RV-144 is currently the subject of intensive investigation, and to date, no immune correlates have been conclusively identified.

Experiments in the rhesus macaque monkey model have demonstrated that SIV or SIV/HIV hybrid virus (SHIV) infections can be prevented by passive infusion of bnAbs [29,38–43] or by vector-mediated delivery of genes expressing bnAbs [44]. Complete protection against SHIV infection in macaques was obtained by passively administering HIV neutralizing monoclonal antibodies 2G12 and 2F5 and anti-HIV immunoglobulin's [42,45] or by infusing monoclonal antibodies 2F5 or 4E10 [46]. Similarly, b12 conferred complete protection in a dose-dependent manner [47], and 2G12 conferred protection at relatively low doses of antibody [29]. Under

Table 2
Broadly neutralizing monoclonal antibodies against HIV.

Target on HIV Env	Antibodies	References
CD4 binding site	b12; VRC01; PGV04; HJ16	[23,34–36]
MPER	2F5 and 4E10	[24–27]
Conformational epitope in conserved regions of variable loops	PG9; PG16; CH01–CH04	[32,33]
Glycan related epitopes	2G12; PGT series	[28–31,37]

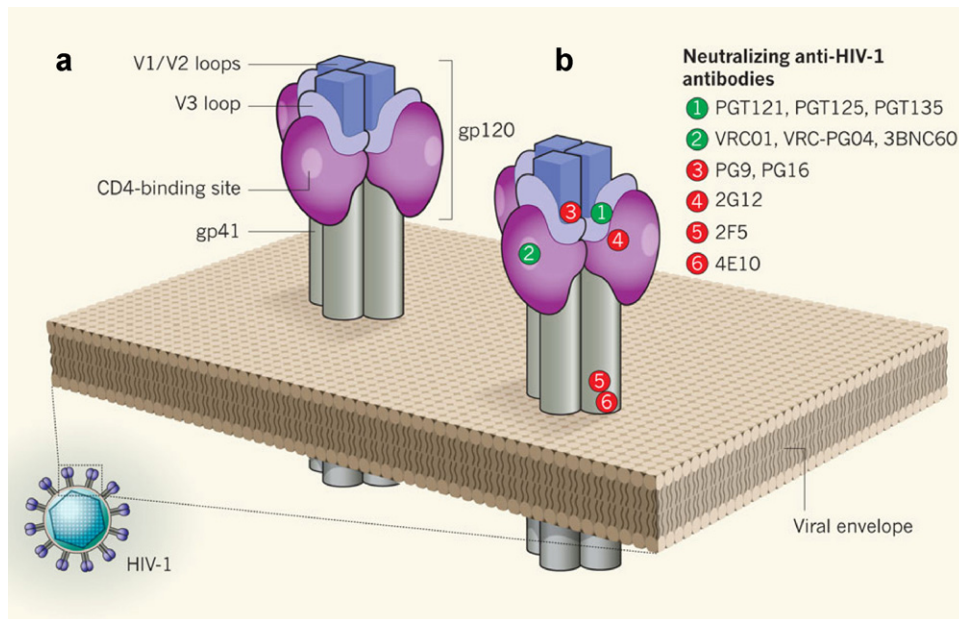


Fig. 1. The envelope of HIV-1 carries spikes. (a) Each spike is made of three molecules of the surface glycoprotein gp120 and three molecules of the transmembrane glycoprotein gp41. Glycoprotein gp120 contains variable V1/V2 and V3 loops, as well as the binding site for CD4. (b) The binding sites of broadly acting and potent HIV-1-specific neutralizing antibodies are shown as colored circles.

similar conditions, non-neutralizing antibodies have been reported to not be protective [43,45]. Recently, b12 was found to protect macaques from SHIV infection while b6, a weak-neutralizing antibody which targets the CD4 binding site, was not protective [48]. In this same study, however, a non-neutralizing antibody targeting gp41 gave inconclusive partial protection when topically administered. Newborn macaques could be also protected from oral SHIV challenge by passive immunization with bnMAbs, b12, 2G12 and 2F5 [38,49]. These studies suggest that induction of bnAbs by vaccination could prevent HIV infection, and thus immunogen design efforts aimed at eliciting such antibodies should continue to be a priority in HIV vaccine discovery.

5. IAVI's Neutralizing Antibody Consortium (NAC)

IAVI has established an international consortium of laboratories that is working to understand the interplay of bnAbs and HIV Env, and translating this information to the design of vaccines ideally capable of eliciting such potent HIV-specific neutralizing antibodies [50]. The NAC has two complementary strategies for vaccine design. The first, termed reverse engineering [51] focuses on identification of the epitopes targeted by bnMAbs, understanding the molecular structure of these epitopes and designing immunogens to mimic the epitopes. The second strategy uses the native HIV-1 Env trimer as the starting point for vaccine design, with the goal of modifying the trimer to target the immune responses to the desired epitopes.

6. Reverse engineering of HIV vaccines

For many viruses, where natural infection gives effective neutralizing antibody responses, the favored strategy for vaccine development would be mimicry of natural infection, via the development of live-attenuated vaccines. However, due to the mutability of HIV and the potential for an attenuated mutant to revert to wild-type, there currently is no active program aimed at developing a live-attenuated HIV vaccine. Similarly, whole inactivated vaccines which have been successfully developed against other viral infections such as polio have not been effective in eliciting bnAbs against

HIV. This is due, in large part, to instability of the HIV Env spike protein and immunodominant epitopes which focus the immune response on the development of non-neutralizing or type-specific neutralizing antibody responses.

As a result, one strategy being considered for HIV vaccine development, and for other variable viral pathogens such as influenza and hepatitis C, is the reverse engineering of vaccines [51]. Rather than attempting to mimic the pathogen, this approach begins with the identification of bnAbs, mapping the targets of these antibodies on the viral protein (for HIV, this would be Env), elucidating the structure of the antibody in complex with its epitope, and then mimicking the structure with candidate immunogens. This strategy is currently focusing on four primary targets on HIV Env, where bnAbs have been identified.

6.1. CD4 binding site

The CD4 binding site is the major site of attachment of HIV Env gp120 to its primary host cell receptor (CD4), which initiates the infection process, and thus is a primary target for HIV vaccine design. The recent identification of several broad and potent neutralizing antibodies to this site has re-invigorated efforts in immunogen design. Strategies being assessed include presentation of the outer domain of HIV Env and mimics of the CD4 binding sites on gp120 in prime-boost regimens, on virus-like particle platforms, and in computational designed protein scaffolds. In addition, recent studies identifying the putative germ line of bnAbs targeting the CD4 binding site as VH1-02*-02 [52] has led to the search for immunogens that effectively bind the germ line.

6.2. Membrane proximal external region (MPER)

The MPER on gp41 has two linear epitopes for which broadly neutralizing, but relatively less potent neutralizing antibodies have been identified (2F5 and 4E10). As linear epitopes, significant efforts in peptide vaccine design have focused on this region of HIV Env. However, more recently, the recognition that these epitopes are juxtaposed to the lipid membrane of the virus, presents more challenges in the induction of such antibodies via immunization.

Strategies for immunogen design are thus focused on presentation of the epitopes in the context of lipid membranes, either as proteoliposomes, heterologous epitope scaffolds, and other configurations.

6.3. Epitopes defined by PG9 and PG16

The recently identified broad and potent monoclonal antibodies, PG9 and PG16, bind HIV Env trimer preferentially and spans conserved regions of variable loops V1/V2/V3 of HIV gp120. Four recently identified bNAbs, CH01–CH04 are also V2/V3 conformationally epitope specific [32]. Thus, these epitopes on HIV Env have become an important vaccine target. The crystal structure of the antigen-binding fragment (Fab) of PG16 at 2.5Å resolution revealed its unusually long, 28-residue, complementarity determining region (CDR) H3 forms a unique, stable sub-domain that sits above the antibody surface [53]. Strategies to design immunogens to this site have included determination of the clonal lineage of such antibodies, and identification of immunogens that bind the common reverted un-mutated ancestor, and on computationally designed protein scaffolds to mimic the site.

6.4. Glycan dependent epitopes defined by the PGT series of bnMAbs

The recent identification of the PGT series of bnMAbs [37], and the glycan dependency of their epitopes, has focused attention on these targets. Strategies for immunogen design to these targets are currently being developed, and include presentation of glycans on virus-like particles.

7. Trimer-based immunogen designs

The native HIV Env trimer is the major target for broadly neutralizing antibodies, and it appears that binding to the trimer is required for neutralization [54,55]. Thus, a recombinant native trimer would represent an optimal starting point for immunogen design. However, the instability of the native trimer on HIV has significantly impeded efforts for development of native recombinant trimers. In the absence of an atomic resolution structure of the native HIV Env trimer, two primary approaches have been advanced. The first aims to rationally design HIV Env with modifications that stabilize recombinant Env trimers in a native conformation.

This approach includes trimeric gp140 molecules (gp120 + the ectodomain of gp41) stabilized by the addition of heterologous trimerization motifs at the C-terminus of the gp41 sequence [56,57] and similar trimers with a deletion of the hypervariable V2 loop have been designed, with the aim of better exposing the neutralization epitopes that overlap the CD4-binding site [58–60]. Similarly, trimeric gp140 molecules internally stabilized by a disulfide bond between gp120 and gp41 (SOS proteins) have been designed and screened preclinically [61,62]. Immunogenicity studies with these trimer mimics, and other related trimer mimics, have generally thus far showed very modest improvements in breadth and potency when compared with monomeric gp120, likely due to the imperfect nature of mimicking the trimer in the absence of its atomic structure. Screening of trimer mimics with the latest set of broad and potent monoclonal antibodies directed against HIV may provide improvements in antigenic profiling of trimer mimics, and thus potentially better candidate immunogens. This hypothesis is currently under test.

The second strategy for trimer-based immunogens has utilized viral vectors expressing HIV Env which have been shown to fuse CD4+ CCR5+ target cells as evidence for the functionality of the

trimer, as the starting point for immunogen design efforts. This strategy, using negative strand RNA viruses to present the trimer in native form, is currently entering preclinical studies, and such immunogens will then be modified to mask immunodominant variable regions of the trimer in efforts to focus immune responses on the conserved, broadly neutralizing targets.

8. Other challenges and opportunities in design of vaccines to elicit bnAbs against HIV

While the two principal strategies discussed above, reverse engineering of epitope mimics and trimer-specific immunogens are being pursued vigorously with the discovery of the latest set of broad and potent monoclonal antibodies against HIV, there remain other challenges and opportunities, which are briefly described below.

1. Modification of native trimer based immunogens: while the native HIV Env trimer is an excellent starting point for immunogen design, such immunogens may be impeded by immunodominant epitopes. Thus, immunization strategies aimed at focusing the immune response to those epitopes representing the binding sites of broadly neutralizing antibodies remains a challenge.
2. B cell pathways that regulate broadly neutralizing antibody production: potent and broadly neutralizing monoclonal antibodies often display unusual characteristics such as extensive somatic hypermutation, and long HCDR3 regions which may pose challenges to recapitulate via host immunoregulatory responses to HIV vaccines. While deep sequencing efforts have now yielded important insights into the evolution of such broadly neutralizing antibodies [52], novel immunogen design strategies may be required using computationally derived clonal lineages as templates [32], in order to drive B cells along the appropriate maturation pathways. Similarly, novel immunization strategies may also be required, for example using heterologous prime and boost immunogens based on binding to the inferred unmutated ancestral B cell receptor and intermediates along the maturation pathway. Here, the rules of human immunogenicity in terms of driving specific somatic hypermutation remain in their infancy, and thus ripe for future investigation.

9. Summary and future directions

Significant efforts are ongoing to design and screen immunogens with the goal of inducing broad and potent neutralizing antibodies capable of preventing HIV infection. However, to date, this goal has not been achieved, in large part due to the remaining scientific challenges posed by HIV Env. For example, there currently remains a lack of understanding of the structure of the native HIV trimer, the target of bNAbs, a lack of understanding of all the bNAb epitopes on HIV Env, and a lack of understanding of why only a small percentage of HIV+ subjects elicit broad and potent neutralizing antibodies and why it takes 3+ years to elicit such antibodies. Our approach to the problem has been to attempt to merge the talents of creative academic scientists focused on understanding the interaction of bNAbs and HIV Env, together with dedicated industrial scientists and vaccine discovery platforms to create a global consortium to advance HIV vaccine development. The current renaissance in neutralizing antibody-based HIV vaccine development, in large part due to the recent advances in identification and characterization of bNAbs against HIV, offers significant hope to the future development of safe and globally effective HIV vaccines.

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For more information, see www.iavi.org.

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