Prospects for an HIV vaccine: leading B cells down the right path

Susan Moir, Angela Malaspina & Anthony S Fauci

Until recently, few potent and broadly neutralizing HIV-specific antibodies had been identified, but recent findings have inspired optimism that an effective HIV vaccine can finally be developed. Here we review these studies, which used state-of-the-art high-throughput techniques to collectively describe hundreds of new potent and broad HIV-neutralizing antibodies isolated from HIV-infected individuals.

The quest for an effective preventive HIV vaccine remains an ongoing challenge after over 25 years of sustained, concerted—yet often disappointing—research efforts[1,2]. However, the tide may finally be turning, because several recent advances in the arena of HIV-specific antibodies are giving pause to even the most ardent of vaccine skeptics. Antibodies became the focus of attention in the RV144 vaccine trial conducted in Thailand on low-risk individuals[3], whose results have more recently indicated that non-neutralizing antibodies directed to variable domains of the HIV envelope correlate with a modest degree of protection against the acquisition of HIV infection[4]. In addition, previous landmark studies reported the isolation of a handful of potent and broadly neutralizing HIV-specific antibodies from HIV-infected individuals[5,6]. More recently, exciting research has resulted in the identification of several additional potent and broadly neutralizing antibodies derived from HIV-infected individuals and provided insight into their maturation pathways[7–9]. The hope is that characterization of such antibodies will lead to the identification of precise epitopes in the appropriate conformation on the HIV envelope, which will serve as the basis for vaccine design.

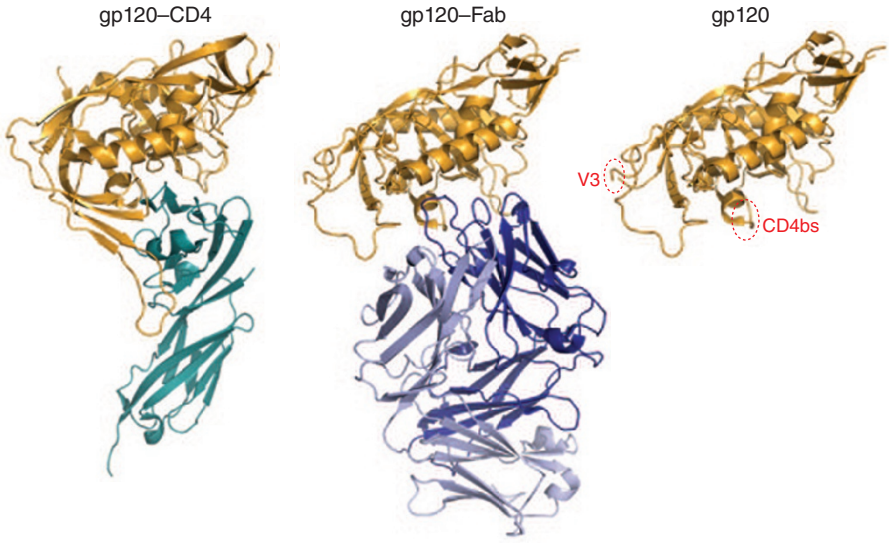

Figure 1 Structural models of gp120 (orange). Left, gp120 in complex with CD4 (PDB 1GC1, teal); middle, gp120 bound to a neutralizing antibody (PDB 3HI1, blue); right, isolated gp120 from the previous complex, with the CD4 binding site (CD4bs) and the position of the V3 loop indicated.

The HIV surface glycoprotein gp120 and its transmembrane counterpart gp41 assemble in trimers to form spikes on the surface of virions. The gp120 envelope glycoprotein contains variable loops V1, V2 and V3, as well as a binding site for its primary receptor CD4 (**Fig. 1**). The high degree of variability in gp120 as well as restricted epitope accessibility due to the glycan shield and other structural features of the HIV envelope glycoproteins are among the reasons cited to explain why potent and broadly neutralizing antibodies to HIV have been difficult to elicit[10,11]. Until recently, only a few naturally occurring broad HIV-neutralizing antibodies were known. However, new approaches developed over the past few years have led to the isolation of several more such antibodies that target gp120. One approach involved targeting the highly conserved CD4 binding site (CD4bs) of gp120 with a probe named RSC3, for resurfaced stabilized core 3. RSC3 was designed, based on crystal structure

Susan Moir and Anthony S. Fauci are in the Laboratory of Immunoregulation and Angela Malaspina is in the Division of AIDS at the National Institute of Allergy and Infectious Diseases, US National Institutes of Health, Bethesda, Maryland, USA.
e-mail: smoir@niaid.nih.gov or afauci@niaid.nih.gov

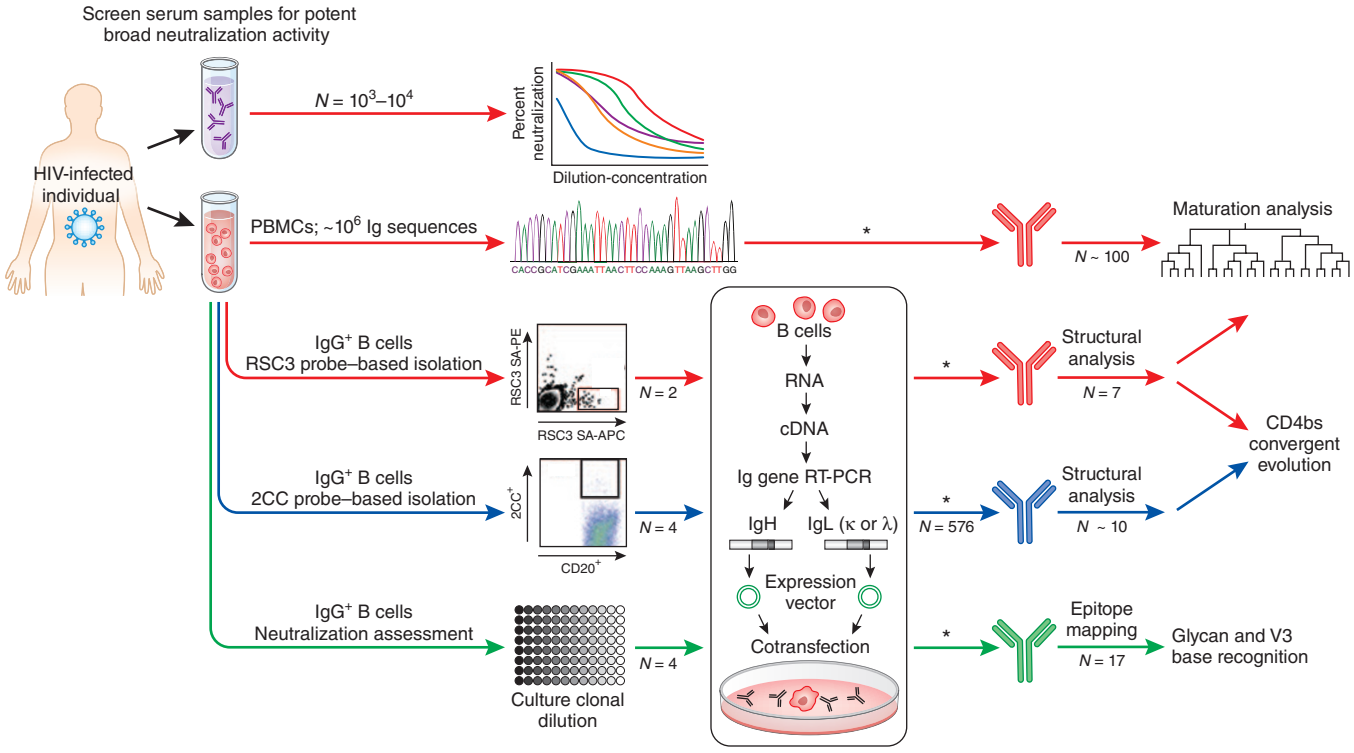

Figure 2 Schematic illustration of strategies used to isolate and characterize HIV-specific antibodies from HIV-infected individuals. Common steps are shown with black arrows or frames and include screening of serum samples and isolation of cells from HIV-infected individuals. Steps specific to each study are indicated by color-coded arrows: red for Wu *et al.*[8], blue for Scheid *et al.*[7] and green for Walker *et al.*[9]. Flow cytometry charts from refs. 6 and 7, with permission. Schematics in the central box were adapted from ref. 31. The variable *N* on the left of the central box refers to numbers of individuals, and on the right it refers to numbers of antibodies or sequences analyzed. Asterisks indicate that cloned or synthesized antibodies were tested for neutralization. Ig, immunoglobin; IgH and IgL, immunoglobin heavy and light chains.

analyses[12], to favor the recognition of the CD4bs while rendering the rest of gp120 unfavorable for recognition by altering or masking immunogenic epitopes. This probe was used to clone the potent and broadly neutralizing CD4bs-specific antibody VRC01 from the B cells of an HIV-infected individual[6]. This study demonstrated that focusing on the CD4bs epitope while masking or deleting the more immunogenic, yet variable, HIV envelope epitopes was crucial to identifying B cells from which potent, highly mutated, broadly neutralizing antibodies could then be isolated[6].

In the three recent studies we review here, each group used a somewhat different approach (**Fig. 2**), but all three took advantage of high-throughput techniques to screen sera from thousands of HIV-infected individuals in order to identify elite neutralizers and clone antibodies from their B cells. It is important to emphasize that the term 'elite neutralizers' refers to the pool of antibodies in the serum of each individual and not necessarily to the clinical status of the person in question. Another common feature in two of the studies was the use of CD4bs probes, similar to the one used to clone the broadly neutralizing antibody VRC01 (ref. 6) in 2010.

In the first study[7], Scheid *et al.* cloned and characterized several new highly active agonistic CD4bs-directed antibodies, or HAADs, from B cells of four unrelated HIV-infected individuals having elite neutralizer sera. The authors used a modified single-cell sorting strategy, originally described in 2003, to evaluate autoreactivity among human B-cell precursors[13]. This strategy has been at the center of important advances in basic B-cell immunology[14,15] and autoimmune and primary immunodeficiency diseases (reviewed in ref. 16), and it has more recently been applied to infectious diseases, including HIV and influenza[17–19]. The selection procedure, shown schematically in **Figure 2**, involved the identification of HIV-specific B cells by flow cytometry, sorting of antigen-specific B cells into single wells of a multiwell plate and amplification of the corresponding variable heavy and light chain immunoglobulin gene regions, followed by cloning and expression in cells designed to secrete high levels of antibodies.

In addition, Scheid *et al.* used PCR primers to target more conserved upstream regions of the immunoglobin genes. This approach was based on the observation that antibodies to the CD4bs (VRC01–VRC03)[6] are products of highly mutated immunoglobin genes. Thus, primers used previously to target more downstream regions may not recognize the genes of the highly mutated antibodies being sought. This new primer set was used to more efficiently recover IgG from a subset of HIV-specific B cells that recognized the CD4 binding site probe 2CC core, which consists of a gp120 core glycoprotein stabilized in the CD4-bound conformation and stripped of variable loops V1 and V3 (ref. 20).

With this approach (**Fig. 2**, blue lines), Scheid *et al.* cloned 576 new antibodies from the four individuals and carried out mapping and neutralization assays to identify several HAADs from each individual. These HAADs potently neutralized a wide spectrum of HIV isolates, including isolates classified as transmitted founder viruses, and those antibodies with the highest breadth and potency were also highly mutated. As with the previously described VRC01–VRC03 antibodies[6], these HAADs were cloned from IgG$^+$ memory B cells, which—in contrast to plasma cells—do not actively secrete antibodies, raising the question of whether such antibodies actually

circulate in the blood of the individuals studied. To address this issue, the authors isolated plasma cells from the bone marrow of two of the individuals and identified immunoglobin gene variants with sequences very similar to those of several HAADs from the corresponding donors. They also used mass spectrometry to demonstrate that the antibodies cloned from memory B cells could be found in the sera of these individuals.

Sequence and structural analyses conducted on the new HAADs indicated highly similar affinity maturation pathways for these antibodies. Remarkably, despite extensive somatic hypermutation, the heavy chains of HAADs within and among different individuals had many similarities, including the fact that they originated from two closely related immunoglobin variable heavy-chain gene families and showed conserved contact points with the HIV envelope. In addition, similarly to VRC01, the HAADs closely mimicked CD4 binding and caused conformational changes in the native trimeric form of the HIV envelope that led to virus fusion, suggesting that these antibodies could interfere with viral entry. Overall, Scheid *et al.* make a strong argument that broadly neutralizing antibodies specific to the CD4 binding site arise in HIV-infected individuals by mutational convergence toward similar epitope recognition and may account for the neutralization observed in certain individuals.

In the second study, Wu *et al.* used 454 pyrosequencing and functional genomics to dissect the process of affinity maturation of VRC01-like antibodies[8]. The rationale of this approach was that analyzing the developmental pathway of these highly mutated and potent antibodies from germline in the context of natural infection and demonstrating the predictability and reproducibility of these results could lead to more effective vaccine strategies. This strategy (see **Fig. 2**, red lines) used the same CD4bs-directed RSC3 probe that had previously led to the identification of VRC01-like antibodies among B cells of an HIV-infected individual whose serum was broadly neutralizing[6].

In the most recent study, Wu *et al.*[8] cloned seven new VRC01-like antibodies from 2 of 12 elite neutralizers whose sera showed high levels of RSC3 reactivity. Collectively, the VRC01-like antibodies showed high levels of mutation in the immunoglobin heavy chain (50% divergence), yet the gene family *IGHV1-2* was common to all seven antibodies, and even more remarkably, it was identical to one of the two related heavy chain families isolated by the Nussenzweig group[7]. Genes for the light chains and J-chain segments varied somewhat among the seven VRC-like antibodies. However, despite amino acid sequence variations, structural analyses of VRC01-like antibodies in complex with gp120 revealed striking similarities. Such structural convergence in epitope recognition has rarely been observed, and it led Wu *et al.* to consider using analysis of immunoglobin gene transcripts (cDNA) to evaluate the affinity maturation process of VRC01-like antibodies. Whereas cloning antibodies from a pool of antigen-specific B cells is a laborious process that yields information on a minute fraction of the B-cell repertoire, sequencing cDNA derived from peripheral blood mononuclear cells (PBMCs) of HIV-infected individuals could potentially lead to information on millions of predicted antibodies. The data could also be filtered to identify sequences with characteristics similar to those of VRC01-like antibodies. An important caveat to interrogating the B-cell antibody repertoire solely on the basis of sequence analysis is that immunoglobin genes for heavy and light chains are expressed independently, so one can only speculate about the mechanism for the actual pairing of heavy and light chains to form a functional antibody. In this regard, Wu *et al.* have considerably narrowed the degree of speculation required. First, they showed that heavy chains from the VRC01-like antibodies of three donors could be paired with several different light chains and still maintain neutralization capacity, suggesting that heavy and light chain sequence data from PBMCs could be informative even if the precise pairing was not known. The authors obtained deep sequencing data on the two donors from whom VRC03 and VRC-PG04 had been cloned and applied the criteria of high divergence from the inferred germline genes and high sequence similarity to the actual VRC01-like antibody to synthesize new genome-derived VRC01-like antibodies. This analysis was conducted on both heavy and light chains, and in each case, the new antibodies showed potent HIV-neutralization capacity.

Armed with hundreds of new sequences from genome-derived VRC01-like antibodies, Mascola and colleagues addressed how these sequences evolved[8]. The authors observed structural convergence in gp120 epitope binding and functional complementation between heavy and light chains of VRC01-like antibodies. They thus postulated that such antibodies should mature in similar, hence predictable, ways. To this end, they used various phylogenetic methods to demonstrate that inter- and intradonor sequences of neutralizing antibodies clustered together and that similarities existed between VRC01-like antibody maturation pathways, including common maturation intermediates. Furthermore, cross-donor phylogenetic analyses of sequences within the immunoglobin heavy chain demonstrated an incremental accumulation of mutations that corresponded with increasing neutralization capacity. Thus, evidence for focused evolution—from putative unmutated germline ancestors through maturation intermediates to the final highly mutated mature VRC01-like antibodies—was established on the basis of thousands of sequences and close to 100 actual and genomic-derived antibodies.

These observations raise two interesting questions: how common is it for an antibody response to be driven by focused evolution, and can the VRC01-like antibody maturation pathway be predictably emulated in the setting of an antibody response to vaccination? Although the analyses of the evolution of VRC01-like antibodies described above were conducted on a cross-section of HIV-infected individuals, future longitudinal analyses will help evaluate the actual evolution of the neutralizing antibody response over time and help determine which elements are unique to natural infection and which can be reproduced from an HIV vaccine designed to induce neutralizing antibodies. In this regard, recent evidence from an animal model shows that sequential immunization with envelopes corresponding to viral quasipecies that evolved over the course of infection leads to a broadening of the HIV-neutralizing B-cell response[21].

In the third study, Walker *et al.* identified a diverse set of broad and potent neutralizing antibodies, directed mainly to the base of variable loop V3 of gp120 and collectively referred to as PGT monoclonal antibodies[9]. Although the starting point of all three studies was the same (that is, the identification of donors based on high serum titers of broad and potent HIV-neutralizing antibodies), Walker *et al.* followed a different strategy to clone antibodies from B cells. Instead of using a specific probe to CD4bs, they used a nonselective high-throughput memory B-cell culture system[5], originally designed to screen culture supernatants for secreted neutralizing IgG and recover corresponding genes by near clonal dilution of the input B cells (**Fig. 2**, green lines). With this approach, 17 new monoclonal antibodies were reconstituted from four elite neutralizers. Some of these antibodies had neutralization profiles that

were superior in breadth and potency to PG9, PG16 and VRC01 (refs. 5,6). Several of the new PGT monoclonal antibodies individually showed neutralization breadth and potency similar to that measured in the serum, suggesting that the serologic activity in the elite neutralizers could be accounted for by a relatively restricted number of B-cell clones. The new PGT monoclonal antibodies were all glycan dependent, with some showing preferential recognition of a quaternary epitope found only on the native trimeric form of the HIV envelope, similarly to PG9 and PG16 (ref. 5). The others bound equally well to monomeric gp120, with most of them recognizing an epitope in the vicinity of the V3 loop.

Sequence analysis revealed that many of the PGT antibodies isolated from each donor were related to one another, with differing degrees of neutralization potency and breadth among related clones, suggesting that successive selection of antibody variants had occurred, possibly driven by escaping virus variants. Given that the majority of PGT antibodies were directed to the base of the V3 loop, it is not surprising that Walker *et al.* found corresponding mutations among related antibodies that recognize a variable region of the HIV envelope known to be highly immunogenic[22]. Variable regions of the HIV envelope have long been considered undesirable targets for vaccines, based mainly on reasonable assumptions that these regions are unlikely to provide the broad coverage required in an effective vaccine and that variants can quickly arise and escape neutralization. However, more recent evidence of conserved structural features in the V2-V3 loops and delineation of the residues that are highly variable[22] have led to the design of new V3- and V2-scaffold protein immunogens that could be used in prime-boost multiepitope vaccine therapies[23]. Thus, although concern regarding escape from antibodies specific to V2-V3 loops remains, several of the new PGT antibodies showed surprisingly broad cross-clade neutralization that may be explained by structural conservation in this region. Supporting the concept of conserved epitope recognition, recent structural analyses on two of these PGT antibodies showed that they recognize a conserved glycan-protein epitope exposed on gp120 and induce potent neutralization partly by cross-linking trimers on the viral surface[24]. Furthermore, at low concentrations, a few of these PGT antibodies were superior in breadth of HIV neutralization compared to other broadly neutralizing antibodies, and coverage from combinations of antibodies at low concentration was highest with PGT antibodies. As proposed by Poignard and colleagues[9], the concept of coverage at low concentrations of a combination of antibodies, especially those with complementary profiles, may be important in strategies for vaccine development, given that high serum HIV-antibody titers have been difficult to achieve[25].

Collectively, these three studies[7–9], along with two earlier ones[5,6], clearly demonstrate that broad and potent HIV-neutralizing antibodies do indeed arise in HIV-infected individuals. Estimates based on serologic analyses indicate that 10–30% of HIV-infected individuals develop such protective antibodies[26], and these B cell–based studies now suggest that, at least for some individuals, a few B-cell clones expressing highly mutated immunoglobulin genes may account for all the neutralizing activity measured in their serum[7–9].

The two studies that targeted the highly conserved CD4bs of gp120 (refs. 7,8) showed that despite being highly mutated, the CD4bs antibodies were similar in structure, providing evidence for mutational convergence toward similar epitope recognition. It is unclear at present whether an HIV immunogen containing the CD4bs epitope can be successfully designed to elicit a similarly predictable and reproducible antibody maturation pathway following vaccination; however, it is certainly worth exploring this possibility, given the remarkable breadth and potency of the matured CD4bs antibodies that were isolated in these studies. In this regard, structure-based efforts are being made to further increase the potency of CD4bs-directed antibodies and improve immunogen design[27]. In addition, the study by Walker *et al.* provides evidence that other epitopes on the HIV envelope can elicit potent HIV antibodies that are broadly neutralizing even at low doses[9], suggesting that a combined antibody response might confer protection at titers that can realistically be elicited by a vaccine.

What is less clear from all three studies is whether such antibodies actually control viremia in the individuals studied. In fact, for HIV-infected individuals identified as elite neutralizers and whose B cells either have the potential to make[8,9] or are shown to make[7] these broad and potent HIV-neutralizing antibodies, there is no concrete evidence that such antibodies slow disease progression. The paucity of evidence indicating that antibodies—or for that matter cellular immunity—can clear HIV or prevent superinfection underscores the need for vaccine strategies that prevent infection entirely. However, a number of studies have shown that replicating viruses are more susceptible to neutralization in the early phase of HIV infection than in the chronic phase, which is when most of the analyses have been conducted. As such, restricting early virus replication may be achievable with antibodies that are not quite so potent and broadly neutralizing (reviewed in ref. 28). Thus, the use of these potent antibodies in passive immunization could prove helpful in determining how capable they are of preventing infection in certain well-defined circumstances or of blunting the progression of disease in already infected individuals.

These studies also raise other important questions, including whether a long affinity maturation process, driven by years of antigen exposure, is required to elicit these newly described HIV antibodies and, if so, whether a vaccination scheme can be devised to induce such highly mutated antibodies in uninfected individuals. In this regard, additional antibody properties that may be difficult to elicit in uninfected individuals could also be required. For example, considerable attention has been given to polyreactivity, a property usually associated with unmutated natural IgM antibodies that are the first line of host defense[29]; however, polyreactive B-cell clones are also deleted or tolerized during B-cell development in order to minimize pathological self-reactivity[16]. The first polyreactive HIV-specific antibodies described were 2F5 and 4E10, both of which recognize a membrane-proximal epitope in gp41 and host-derived phospholipids[30]. A recent study by Nussenzweig and colleagues has provided evidence that polyreactivity is common for HIV-specific antibodies isolated from HIV-infected individuals[18] and that this may be a desirable property, given that the envelope spikes on an HIV virion are farther apart than the distance between the two epitope-binding sites of most antibodies. Thus, this study suggests that heteroligation enhances neutralization, allowing polyreactive HIV-specific antibodies to simultaneously ligate one epitope on the viral envelope with one arm and a host membrane component with the other[18]. Several of the HAADs, but not VRC01-like and PG antibodies, showed polyreactivity, however it remains to be seen whether this property is essential for optimal neutralization of HIV and, if so, whether such antibodies can be elicited by vaccination.

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The authors declare no competing financial interests.

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