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## HIV-1 neutralizing antibodies: understanding nature's pathways

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### Summary

The development of an effective vaccine has been hindered by the enormous diversity of human immunodeficiency virus-1 (HIV-1) and its ability to escape a myriad of host immune responses. In addition, conserved vulnerable regions on the HIV-1 envelope glycoprotein are often poorly immunogenic and elicit broadly neutralizing antibody responses (BNAbs) in a minority of HIV-1-infected individuals and only after several years of infection. All of the known BNAbs demonstrate high levels of somatic mutations and often display other unusual traits, such as a long heavy chain complementarity determining region 3 (CDRH3) and autoreactivity, traits that can be limited by host tolerance controls. Nonetheless, the demonstration that HIV-1-infected individuals can make potent BNAbs is encouraging, and recent progress in isolating such antibodies and mapping their immune pathways of development is providing new strategies for vaccination.

### Keywords

monoclonal antibodies (mAbs); broadly neutralizing antibodies (BNAbs); unmutated ancestor (UA); B-cell lineages; immune tolerance

### Introduction

Protection against viral pathogens is mediated by an intricate interaction of innate and adaptive immune responses that work together to prevent infection or control viral replication. Despite this complexity, a necessary component of most effective viral vaccines is the induction of virus-specific antibodies that inactivate or neutralize the invading pathogen (1). Since human immunodeficiency virus-1 (HIV-1) was first identified in 1983 (2, 3) and shown to be the cause of acquired immunodeficiency syndrome (AIDS) in 1984 (4), there has been a major focus on the development of a vaccine to prevent infection and on the specific viral proteins that would be the target of potentially protective antibodies. Intensive early investigation led to the elucidation of the genomic organization of HIV-1 (5) and the recognition that it was an enveloped lentivirus consisting of a gp120 surface unit and gp41 transmembrane domain (5-7). Further biochemical characterization demonstrated that the HIV-1 envelope glycoprotein (Env) was a heavily glycosylated trimer of gp120 and gp41 heterodimers and that gp120 interacted with the CD4<sup>+</sup> T-cell receptor to initiate viral entry (8, 9). Upon binding CD4, gp120 undergoes conformational changes that result in the formation of a stable intermediate form which binds to a secondary cellular co-receptor, usually the chemokine receptor CCR5 or CXCR4 (10-13). Cellular entry is mediated by

rearrangements of gp41, leading to viral-cell fusion (9, 14). Based on this understanding of viral entry and the requirement for functionally conserved regions of Env, it has long been presumed that antibodies targeting the appropriate regions of gp120 or gp41 could block viral entry and potentially prevent infection (15, 16).

But investigators have also recognized that HIV-1 is different from other viruses for which successful vaccines have been made. HIV-1 infects CD4<sup>+</sup> T-helper lymphocytes that are critical for an effective immune response to the pathogen and upon responding can also become infected (8, 17). HIV-1 also integrates into the genome and rapidly forms a latent pool of resting cells that are largely invisible to the immune system (18, 19). These attributes are obstacles to immune responses that would completely prevent HIV-1 infection. During the phase of chronic infection, the infidelity of the HIV-1 encoded reverse transcriptase (20, 21) and the propensity for *in vivo* recombination result in a continually evolving viral quasispecies that evades the autologous immune response (22, 23). The ability of the HIV-1 Env to shield vulnerable regions from neutralizing antibodies (NAbs) provides yet another obstacle for vaccine design (24-28). Thus, HIV-1 poses a unique set of impediments to the induction of fully protective immune responses.

### Serum neutralizing antibodies: the early days

HIV-1, initially termed lymphadenopathy-associated virus (LAV) (29) and HTLV-III (4), was first isolated by co-cultures of infected patient samples with neoplastic T-cell lines such as H9 or CEM cells (2, 4, 30). By 1985, investigators had demonstrated that sera from infected subjects contained antibodies capable of blocking infection of cells or inhibiting cell to cell spread *in vitro* (31, 32), and later that these antibodies targeted the gp120/gp160 Env proteins (33). These findings, together with the success of the recombinant subunit hepatitis B virus vaccine, provided encouragement for the first wave of HIV-1 candidate vaccines consisting of vaccinia and related pox-virus vectors encoding gp160 (34, 35) and purified recombinant gp120 and gp160 proteins (36-38). Remarkably, these first phase I trials were initiated in the late 1980s within 5-6 years of the discovery of HIV-1 (39). Early phase I trials included pox-virus vectors to prime the immune response and recombinant proteins as a boost or recombinant proteins alone formulated with various adjuvants. Initial results were encouraging, demonstrating the elicitation of high titer anti-gp160 antibodies and robust NAb responses (35-37). However, the enthusiasm for these candidate vaccines was quickly tempered when it was appreciated that viral isolates grown on neoplastic cell lines underwent a process of adaptation that resulted in the selection of a highly neutralization-sensitive subset of viruses (40, 41). The neutralization sensitivity of these T-cell line-adapted (TCLA) viruses was sharply contrasted by relative neutralization resistance of virus isolates isolated by cultures with primary human CD4<sup>+</sup> T cells and thus termed primary HIV-1 isolates (42, 43). It was subsequently shown that vaccine recipients developed antibody responses that were effective against TCLA viral isolates but exhibited little or no neutralization activity against primary HIV-1 isolates (44, 45). Serological mapping indicated that the vaccine-induced antibody response was often preferentially directed against linear epitopes on the viral Env, including the variable region 3 (V3) loop (46-50). Thus, the V3 loop was initially termed the principal neutralizing determinant (PND) (46-48), until it became clear that the V3 regions of Env was usually shielded within the trimeric structure of the viral spike and was relatively inaccessible to NAbs (51-54).

The lack of primary isolate neutralization induced by recombinant gp120 vaccines led to controversy regarding advanced testing of these vaccines, but eventually two phase III efficacy trials of gp120 vaccines were conducted. The VAX004 and VAX003 trials were initiated in 1998 and 1999, respectively. The VAX004 vaccine consisted of two clade B gp120s and was conducted in North America and the Netherlands. The study population was

men who have sex with men and women at high risk for heterosexual transmission. The VAX003 vaccine also contained two gp120s, one from a clade B strain and the other from a clade E (CRF01-AE) viral strain (AIDSVAX® B/E). This trial was conducted in Thailand among intravenous drug users. The results of both studies were reported in 2005 (55-57). Neither vaccine resulted in a reduced risk of HIV-1 infection, nor did either have any impact on the level of plasma viremia upon infection. These results were widely believed to indicate that a different quality of antibody, perhaps neutralizing activity against primary strains of HIV-1, would be required for vaccine protection (39, 58-60).

## The first human broadly neutralizing monoclonal antibodies

In contrast to vaccine sera, several groups of investigators reported that serum from some HIV-1-infected donors was able to neutralize diverse primary strains of HIV-1 (40, 45, 53, 61, 62). Initially, the mechanism of this serum neutralization was poorly understood; in some cases adsorption with gp120 could remove most of the neutralizing activity, while for other sera neutralizing activity was evident in the gp120 flow through fractions (63). Our early knowledge of important viral neutralization epitopes came from the isolation of a handful of neutralizing human monoclonal antibodies (mAbs) that were first reported between 1993 and 1994. These mAbs targeted three distinct regions of the viral Env: the CD4-binding site of gp120 (mAb b12), surface glycans on the outer domain of gp120 (mAb 2G12) and the membrane proximal external region (MPER) of gp41, just prior to the transmembrane spanning sequence (mAbs 2F5, 4E10, and Z13e) (64-66). Numerous other mAbs were also reported, including those to the V1V2 and V3 regions (67), the coreceptor binding region of gp120 and the heptad repeat-1 region of gp41, but these antibodies were generally weakly neutralizing or highly strain specific (24). Importantly, *in vivo* passive infusion studies using chimeric SIV/HIV viral infection of macaque monkeys demonstrated that NABs could completely block viral infection (16, 68-72), thus highlighting the importance of understanding the molecular structure of each of these neutralization epitopes as a means to pursue the design of improved vaccine immunogens. In 1998, the co-crystal structure of the gp120 core protein liganded with soluble CD4 and the coreceptor binding mAb 17b was solved (73, 74). While the gp120 core included N and C-terminal truncations and removal of V1V2 and V3 loops, this structure provided new insights into the mechanism of the gp120-CD4 interaction and set the stage for additional co-crystal structures of NABs. Between 2003 and 2009, investigators solved liganded structures of mAbs 2G12, b12, 2F5, and 4E10 (75-78). These antibody structures not only provided a new level of knowledge about potential vulnerable viral epitopes but also unveiled some rather daunting characteristics of the known BNABs. The 2G12 mAb that binds a cluster of surface glycans was shown to adopt an unusual domain swap configuration in which the two heavy chains interact to form a large monovalent binding surface (75). No prior antibody with a similar configuration had been described, and it was unclear how such an antibody was generated or might be induced via immunization. The CD4bs mAb b12 was derived from a phage display library such that its natural light chain pair was not known. In addition, it demonstrated a rather unusual heavy chain only binding interface (no light chain binding) that was mediated by a moderately long CDRH3 loop (78). Finally, the gp41 membrane-proximal external region (MPER)-directed mAbs 2F5 and 4E10 were both shown to display features of polyreactivity and autoreactivity (79, 80). In addition, both 2F5 and 4E10 have long hydrophobic HCDR3 regions as well as excess somatic mutations, additional traits that can predispose antibodies to tolerance control mechanisms, such as clonal deletion (81).

As additional HIV-1 isolates from geographically diverse regions of the world were studied, it became clear that none of the above BNABs was able to neutralize the majority of diverse HIV-1 strains. The glycan-targeted mAb 2G12 neutralized less than 50% of clade B isolates and was substantially weaker against non-clade B strains. The neutralization pattern of b12

similarly displayed a preference for clade B isolates with an overall breadth of reactivity of less than 50%. Among the two Env gp41 MPER antibodies, 4E10 displayed impressive breadth (~ 90% of viruses tested), but its potency was weak, especially when tested in assays with primary target cells. MAb 2F5 displayed moderate potency, but was generally weak against clade C isolates that were prevalent in many regions of Africa, India and China (53, 82-85).

## Antibodies during natural HIV-1 infection and the next generation of BNABs

The structural and functional analysis of HIV-1 NABs b12, 2F5 and 4E10 led to some pessimism about the potential of the immune system to generate BNABs against HIV-1 (86). This concern was highlighted by the observed lack of natural immunity to HIV-1 (87) and by studies demonstrating the ability of HIV-1 Env to continuously evolve to evade the autologous NAB response (22, 23). Additionally, as the full genetic diversity of HIV-1 was appreciated, we also began to understand the formidable immune evasion mechanisms that are intrinsic to the HIV-1 Env. These include carbohydrates that make up more than half the molecular weight of the HIV-1 Env and variable loops that act in concert with surface glycans to mask important neutralization epitopes (24, 25, 27). In addition, key epitopes such as the receptor binding sites or the MPER of gp41 are either recessed, transiently exposed or have limited spatial accessibility to antibody molecules (24, 27).

This rather bleak view of the HIV-1 humoral immune response began to change with the observation that some HIV-1 infected donors mounted NAB responses that were more potent and cross-reactive than those generated by current vaccine immunogens (40, 45, 53, 61, 62). Several groups of investigators reported that sera obtained after HIV-1-infection could potently neutralize primary isolates of HIV-1, and in some cases, were able to neutralize genetically diverse HIV-1 strains (88-92). Early work assessing serum mediated HIV-1 neutralization was often performed using primary HIV-1 isolates derived from cultures of human peripheral blood mononuclear cells (PBMCs) (40, 41, 93-97). These assays also used PBMCs or CD4<sup>+</sup> T cells as target cells for infection, and infection was often quantified after several rounds of viral replication. While these PBMC-based neutralization assays were critical for our appreciation of the difference between T-cell line adapted and primary HIV-1 isolates, the assays were generally not highly accurate or reproducible. PBMC culture-generated viral stocks contained a quasispecies of viruses, and variation in donor PBMC target cells further contributed to assay variability. Starting in 2005, there was a concerted effort to develop accurate high throughput neutralization assays based on the construction of recombinant Env-pseudoviruses that mediated a single round of viral infection (98-100). The advantage of such assays were that each pseudovirus contained a well-defined clonal sequence and that large panels of diverse HIV-1 Env's could be rapidly constructed, thus better representing the global genetic diversity of HIV-1. These assays allowed the screening of large panels of HIV-1 sera and the identification of individual donors whose sera were able to neutralize the majority of HIV-1 strains. Overall, these data revealed that there was a heterogeneous spectrum of serum neutralization with some sera displaying weak neutralization and others able to neutralize most HIV-1 strains tested (53, 62, 88-92, 101-103). Depending on the cohort tested and the definitions used, these studies revealed that between 10% and 25% of HIV-1 infected subjects generate relatively potent cross-reactive NABs (91, 92, 101, 102, 104-106).

Initially, the difficulty in defining the specific antibodies responsible for mediating broad serum neutralization hindered our ability to understand the nature of this immune response. It was clear that the handful of known neutralizing mAbs did not explain the more potent cross-reactive antibody response found in some donors, but it was unclear if serum neutralization resulted from the activity of polyclonal mixtures of antibodies or from a

limited subset of antibodies targeting conserved epitopes. Although investigators expended substantial effort to isolate additional neutralizing mAbs, several factors limited the discovery of such mAbs. These included the small fraction of B cells that secrete HIV-1 specific neutralizing mAbs (107, 108) and the inefficiency of traditional methods of mAbs isolation. Several methodological advances however, created the framework for the subsequent isolation of numerous new HIV-1 neutralizing mAbs. These methods included the successful large scale culture of memory B-cells at close to limiting dilution concentrations (2-4 B cells per well) under conditions that resulted in sufficient IgG secretion to screen wells directly for HIV-1 neutralization (82, 109, 110). The specific heavy and light chain of individual B-cells were then recovered by PCR amplification and cloned into an IgG expression vector (111, 112). An additional approach was to use fluorescently labeled Env specific protein probes to identify and sort HIV-1 specific B cells and to recover the antibody genes by PCR as described above (84, 107, 108, 113-115). Beginning in 2009, these methodological advances led to the isolation and structural characterization of numerous neutralizing mAbs that have redefined our understanding of the architecture of the HIV-1 Env and vulnerable epitopes that could be targeted by NABs. The major epitopes defined by these new mAbs included regions of the CD4 binding site of gp120 (84, 114, 116-119) and the MPER of gp41 (110, 115, 120), and two new peptide-glycan epitopes on gp120, one in the region of the V1V2 loop (82, 109) and the other in the region of V3 (83). While additional mAbs are likely to be discovered and possibly new viral epitopes defined, the current set of potent and broadly reactive mAbs comes close to recapitulating the potency and breadth of observed serum neutralizing activity (Table 1).

## Eliciting broadly reactive neutralizing antibodies: gaps in our knowledge

### Structural and genetic features of neutralizing mAbs

Remarkably, investigators have recently solved liganded crystal structures of mAbs bound to each of the four major neutralization epitopes, and the structural features of these mAbs has been recently reviewed in detail (28, 121). Here, we focus on some of the key features of these new mAbs and the implications of this knowledge for vaccine design, including key gaps in our knowledge that still limit our ability to elicit HIV-1 specific BNABs. A review of the four major neutralization epitopes on the HIV-1 Env reveals some intriguing structural, genetic, and immunological characteristics of the antibodies to each epitope. The CD4-binding site would seem to be an obvious functionally conserved region of Env, yet most of the initial antibodies isolated to this site were poorly neutralizing. Structural studies of neutralizing and non-neutralizing CD4-binding site mAbs revealed that precise targeting of this region by antibody b12 was necessary to avoid steric and conformational clashes that would hinder binding to the native viral spike (122). Hence, many CD4-binding site mAbs display high affinity binding to recombinant gp120 but are unable to access the CD4-binding site on gp120 (123, 124). The isolation and structural characterization of the broadly reactive VRC01 mAb provided a fascinating solution to this problem: the antibody partially mimics binding by cellular receptor CD4 to gp120 (84, 119). Specifically, a region of the heavy chain of VRC01 is arranged in an antiparallel B sheet configuration that is similar to the interaction of domain 1 of CD4 with gp120. Structures of VRC01-like antibodies from a total of six different donors reveal a highly similar mode of recognition of the CD4-binding site (114, 119, 125, P. Kwong, personal communications). Light chain interactions with somewhat more variable regions of V5 and the D-loop regions of gp120 explain the few cases of viral resistance to this class of mAbs. A rather unique feature of VRC01 class antibodies is that heavy chain binding is mediated largely by CDRH2, a region encoded by the V-gene, rather than CDRH3 which is encoded by V-D-J recombination. Interestingly, all known VRC01 type antibodies derive from VH1-2 or the closely related VH1-46 gene, suggesting these two germline genes are required to generate these antibodies (84, 114, 117, 126). Also, these VRC01 class antibodies are highly somatically mutated (~ 20% – 30% nt



changes in heavy chain compared to germline), and it is difficult to detect any gp120 binding when the amino acid sequence of the heavy chain V-region is reverted to germline (114, 117, 119, 127). This has raised questions about how naive B cells are triggered to generate VRC01 class antibodies (128-130).

Two additional neutralization epitopes on gp120 were identified when antibodies to conserved glycan-containing regions of V1V2 and V3 respectively were described. mAbs PG9 and PG16, and subsequent mAbs in this category, such as CH01-04 and PGT141-145 (82, 83, 109) bind to relatively conserved residues within V2 and require major interactions with surface glycans, most importantly a N-linked glycan at position 160 (131, 132). Similarly, the PGT mAbs described in 2011 bind to residues at the base of V3 and require specific glycan interactions, particularly at position N332 (83, 133). These glycan reactive antibodies are some of the most potent HIV-1 mAbs isolated and they share a common feature of a long CDRH3 loop that interacts with surface glycans and reaches past these glycans to make key contacts with either V2 or V3 residues on gp120. These antibodies are also highly somatically mutated, and hence, a characteristic of HIV-1 glycan reactive antibodies appears to be a long CDRH3 loop together with a high level of affinity maturation to acquire breadth of reactivity. In the case of V1V2 BNABs, the germline reverted versions both CH01 and PG16 have been found to neutralize the same few HIV-1 strains, suggesting a common structural requirement for their induction (109, 134). Finally, a new MPER directed mAb, 10e8, was recently isolated and its co-crystal structure solved (110). This antibody binds to a region of the MPER that overlaps with the 4E10 epitope but includes C-terminal residues close to the transmembrane spanning domain. 10e8 is highly somatically mutated, is 5-10 fold more potent than 4E10 and can neutralize ~ 95% of viruses tested. Interestingly, mAb 10e8 does not possess the polyreactivity that is characteristic of mAbs 2F5 and 4E10, but it does have a long HCDR3 and a high number of somatic mutations (110).

### **Immunological roadblocks to BNAB elicitation**

Despite the detailed knowledge of the major neutralizing mAbs described above and the associated atomic level descriptions of their viral epitopes, we are left with rather formidable barriers to their elicitation. Our best attempts to construct vaccine immunogens that present these key epitopes to the immune system have failed to generate antibodies that neutralize most strains of HIV-1 (28, 39, 44, 45, 58). An example of this phenomenon is observed with monomeric recombinant gp120 used as a vaccine. We have known since 1994 that the neutralizing mAb b12 binds with high affinity to the CD4-binding site on recombinant gp120 (64), yet numerous studies of gp120 immunization have shown that this does not result in the generation of NABs like b12 or newer CD4-binding site antibodies like VRC01 (39, 44, 135, 136). This highlights a long standing dichotomy between antigenicity and immunogenicity that has been described by numerous investigators (24, 27, 63, 137, 138). While additional structural knowledge of new or existing antibodies will likely continue to refine our understanding of these epitopes, we have a wealth of information to provide momentum for our structure-based vaccine design efforts (28, 139, 140). However, a structure-based approach in and of itself will likely not solve the HIV-1 vaccine problem. Rather, the correct antigenic structure must be formulated and delivered in such a way as to overcome the immunological road blocks that appear to limit effective antibody response to the HIV-1 Env (129, 140-142). Thus, the design of improved HIV immunization strategies will likely require a better understanding of epitope dominance and subdominance, immune tolerance and anergy, and the immunogenetics of antibody induction and maturation, including the role of selected germline genes and the process of affinity maturation (Table 2).

Regarding epitope subdominance, we need to understand why certain regions or subregions of the HIV-1 Env are poorly immunogenic and how to engage rare BNAb B-cell precursors and foster their expansion to yield a dominant plasma antibody response (28, 129). As noted previously, the HIV-1 Env is highly immunogenic and induces antibodies to gp41 and gp120 shortly after infection. The hierarchy of the humoral immune response during acute HIV-1 infection has recently been studied in detail (141, 143). The first HIV-1 Env specific antibodies are observed as early as 8 days after plasma viremia is first detected (143). These initial antibodies target immunodominant regions of gp41, followed by the development of non-neutralizing antibodies to gp120, including antibodies to variable loop regions, the CD4-binding site and the CD4-induced co-receptor sites on gp120. Some investigators have speculated that this early antibody response is driven by both surface-exposed immunodominant regions of gp120 and gp41 and by shed gp120 and gp41 stalks that may be a natural part of the viral life cycle (24, 144). Such gp120 and gp41 epitopes may not be accessible on the functional trimeric viral spike, explaining the lack of neutralization by these antibodies.

Over time, virus-neutralizing antibodies develop in most HIV-1 infected individuals, demonstrating that the humoral immune system can target epitopes on the native viral spike. The initial NAb response is directed to the autologous infecting virus and is generally not detected until more than 12 weeks of infection (22, 23, 91, 102, 141). Though these autologous NAb exert immune pressure on the virus, they are highly strain specific; i.e., they can neutralize the autologous virus potently, but display little breadth of activity against heterologous viruses. Initial cross-reactive NAb may arise after several months of infection but the more broadly reactive NAb that target highly conserved epitopes are generally not found until more than two years of infection (91, 102). The kinetics of the HIV-1 Env antibody response raises numerous questions. Why is there a delay of several months or more before viral NAb arise? When these antibodies do arise, what accounts for their initial strain-specificity and what characteristics confer neutralization breadth? Do strain-specific NAb target more variable epitopes in contrast to BNAb, or does a specific NAb lineage mature over time to gain the necessary affinity and precision to target conserved residues within a neutralizing epitope? The answers to these questions have clear implications for vaccine design, as discussed below.

There is also evidence that the BNAb response is limited by self-tolerance and clonal deletion. Structural studies of mAbs 2F5 and 4E10 with respective gp41 peptides show that only a small part of their CDRH3s bind the gp41 epitope (76, 77). Additional studies suggested that remaining hydrophobic CDRH3 regions are important for antibody interactions with the viral membrane, to allow the antibody to lie in close proximity to the membrane. This appears to position the mAbs for high-affinity binding to the gp41 MPER (145-148). Interestingly, both 2F5 and 4E10 react with anionic lipids such as cardiolipin, consistent with the observations that the antibodies bind both to gp41 and to the nearby virion membrane (79, 80, 149). Alam and colleagues (80, 148) demonstrated that both 2F5 and 4E10 encounter the viral membrane followed by induction of a gp41 conformation change that leads to high affinity mAb docking to the MPER epitope on gp41. In this process the 'hinge' region of the MPER is extracted from contact with the viral membrane (145, 150). Importantly, *in vitro* polyreactivity of HIV-1 mAbs may be of clinical significance, as passive infusion of mAbs 4E10, 2F5, and 2G12 resulted in a prolonged partial thromboplastin time (PTT coagulation test) in subjects to whom they were administered (151, 152), although no clinical adverse events were noted. This prolonged PTT test, due to lipid-reactive antibodies that mediate lupus anticoagulant activity, can be detected *in vitro* for 4E10, but not for 2F5, 2G12, or other mAbs such as b12 or VRC01 (79, 153), suggesting that the prolonged PTT effect was the result of infusion of mAb 4E10.

The polyreactivity of some HIV-1 BNABs raised the important concept of tolerance and immunoregulatory host controls as one set of factors limiting the frequent elicitation of these antibodies (142). To test this hypothesis, Verkoczy and colleagues (142, 154, 155) made homologous recombinant knock-in mice that expressed the mature and germline versions of the 2F5 antibody heavy and light chains. The polyreactivity of both the germline and mature 2F5 was sufficient to result in deletion of 95% of the antibody precursors in bone marrow at the first tolerance checkpoint. Importantly for vaccine development, not all 2F5 B cells were deleted but could be found in peripheral LN and spleen in an anergic state (155). These anergic BNAB B cells could be awakened by repetitive immunization with the gp41 MPER peptide epitope embedded in lipids (L. Verkoczy and B. Haynes, unpublished data). A similar deletion at the first tolerance check point was found in 4E10 knock-in mice (L. Verkoczy L and B Haynes, unpublished data). Thus, overcoming tolerance controls that limit BNAB induction has emerged as a key concept in HIV-1 vaccine development. Nussenzweig and colleagues (108, 117, 156, 157) evaluated hundreds of HIV-1 Env specific mAbs from chronically HIV-1 infected donors and showed that these mAb were often highly affinity matured and polyreactive. Interestingly, the plasma cell repertoire of patients acutely infected with HIV-1 also contains autoreactive antibodies. Liao and colleagues found that initial gp41 directed antibodies were often highly mutated and polyreactive, and some were shown to react with gut flora antigens (158). These data raised the hypothesis that B cells were initially activated by gut flora prior and that cross-reactivity with gp41 may further shape the memory B-cell repertoire after HIV-1 infection.

A similar tolerance obstacle may affect BNABs that interact with peptide-glycan epitopes in the V1V2 and V3 regions. These antibodies have extended CDRH3 structures that are required to reach peptide residues obstructed by glycans (Table 1). Both mouse and human studies have demonstrated a selection against long CDRH3 loops, presumably due to their increased auto or polyreactivity. Mouse studies suggest that many B cells encoding long CDRH3 regions are eliminated before reaching the periphery (159, 160), and human studies show a decreased prevalence of CDRH3 sequences in IgG memory compared to naive B-cell subsets (161) and a selection bias against long CDRH3 loops, particularly those with hydrophobic patches (162). Thus, a key question for eliciting these glycan-reactive BNAB is whether their long CDRH3 loops are encoded during germline recombination or whether an iterative affinity maturation process could result in short insertion events that gradually lengthen the CDRH3. This latter process would require immunization to drive an unprecedented level of affinity maturation. Since we know that a relatively small fraction of B-cell receptors (BCRs) encode for long CDRH3 lengths (161-163), perhaps the more likely scenario is that these long CDRH3 BCRs are selected by specific circulating Env immunogens with high enough affinity to drive their expansion and to overcome their potential autoreactive properties.

Another potential immunological roadblock arises when considering antibodies such as VRC01 that potently neutralize via binding to a conserved region of the CD4-binding site. As noted above, VRC01-type antibodies from several different donors display a high level of structural similarity and predominant gp120 contacts are made via the heavy chain CDR2 which is encoded by the VH1-2 gene (114, 119, 125). Hence, it appears that this potent class of antibodies derive from a highly restricted set of VH genes and subsequently undergo extensive affinity maturation to achieve effective broad and potent neutralization (121). Investigators have made the rather disconcerting observation that reversion of V-gene mutations to germline VH1-2, even with an intact mature CDRH3, abolished detectable binding to gp120 (117, 119). This appears true even when testing large panels of diverse gp120s (127) and has raised the question about the nature of the antigen that stimulated the naive VH1-2 BCR, e.g. was it an HIV-1 Env antigen or some unrelated antigen (128, 129)? Several groups of investigators have begun to use structural information to design variants



of HIV Env immunogens that would bind to germline (or V-gene reverted) versions of VRC01. Finally and has already been noted, BNABs appear to take months to years to evolve and have high levels of somatic mutation. This suggests a long and potentially tortuous pathway of evolution. Recent studies have shown that vaccination with HIV-1 gp120 induces anti-gp120 antibodies with ~3-5% somatic mutations and an upper range of 8% (164-166). In contrast, the mean level of somatic mutations among BNABs is 16% with some BNABs accumulating 32% mutations compared to germline nucleotide sequence (121, 129) (Table 1). Fortunately, newer BNABs have recently been described that have normal CDRH3 lengths, fewer somatic mutations, and arise much earlier in HIV-1 infection (118). Thus, studies of BNAB lineages and their evolution from early infection through the process of maturation to potent neutralization may provide insights for vaccine immunogen design and immunization strategies.

## Nature as a guide: the study of B-cell lineages to guide vaccine design

As noted above, immunization with antigenic HIV-1 Envs has not resulted in induction of subdominant BNAB B-cell responses similar to those that arise in some HIV-1-infected individuals. Rather, new approaches will likely be required to induce otherwise disfavored antibody lineages. We know that naive B cells compete for survival in germinal centers, and those with the highest affinity occupy the germinal center and expand to manifest plasma antibody levels (167-169). However, we know little about the early events that trigger the development of BNABs responses in HIV-1 infection. Thus, several groups of investigators have begun to study these events in detail to gain insights for HIV vaccine design.

### B-cell lineages from natural infection

The recent isolation and sequencing of numerous BNABs provides opportunities to study how these antibodies develop and evolve. When more than one BNAB is isolated from an HIV-1 infected donor, either by antigen-specific B-cell sorting or by memory B-cell cultures, the isolated antibodies are often part of the same clonal lineage, suggesting that one lineage can account for a major fraction of the serum NAb response (82-84, 109, 114, 170). Once the genetic sequence of a BNAB is known, next generation sequencing provides a way to dramatically expand the view of an antibody clonal family (114, 162, 171-173). Bioinformatics based analyses allows the identification of intermediate antibody (IA) sequences and the potential to infer the unmutated ancestor (UA) of the BNAB lineage, i.e. the naive BCR sequence. Wu and colleagues (114) used 454 pyrosequencing of antibody gene transcripts to study the heavy and light chain antibody lineages of mAbs VRC01 and VRC-PG04 in their respective donors. Novel bioinformatics analysis allowed the identification of thousands of sequences that formed a specific BNAB lineage. While these studies included a single time point from a chronically infected donor, the investigators were able to identify heavy and light chain clonal relatives with far fewer somatic mutations than the mature isolated antibody. This allowed the expression and testing of antibody intermediates and the demonstration that high levels of affinity maturation were required for the full potency and breadth of the antibody. In spite of the success of elucidating BNAB clonal lineages from chronically infected subjects, these initial studies were limited by lack of samples from the time of transmission and therefore lack of the inciting transmitted-founder virus Env that was involved in stimulating the antibody lineage.

From 2005 to 2012, the Center for HIV/AIDS Vaccine Immunology (CHAVI) enrolled acute HIV-1-infected subjects and followed them for 3-5 years. A subset of these subjects developed cross-reactive Nabs, and their samples are now being studied for NAb and virus co-evolution. Numerous studies have shown that the initial NAb response is directed primarily to the autologous virus and that there is an ongoing pattern of viral escape and further maturation of the NAb response (22, 23, 93, 94, 102, 174, 175). The co-evolution of

HIV-1 and the polyclonal antibody response can be thought of as an ‘arms race’, wherein the infecting virus induces an autologous NAb response that is initially limited in breadth. Viral escape mutants appear to drive the antibody response and ~20% of HIV-1 infected subjects develop cross-reactive NAb (91, 102, 175). In a recent study, Moore and colleagues (175) reported two examples of HIV-1-infected donors whose transmitted-founder viruses did not have an N-linked glycosylation site at position 332 (a critical glycan for binding by the PGT group of mAbs). The transmitted viruses in each donor were initially neutralized by their autologous plasma, but later escaped by adding a glycan at position 332 (175). The development of N332-glycan containing viruses preceded the development of more broadly NAb that targeted this N332 site. Thus, a BNAb response arose in response to viral escape from earlier autologous NAb.

Liao and colleagues (118) recently isolated several closely related CD4-binding site NAb from a CHAVI cohort seroconverter who had been HIV-1 infected for approximately 2.5 years, and solved the structure of one of these mAbs (CH103) bound to the outer domain of gp120. Using samples derived within several weeks of infection and sequentially thereafter, they were able to establish the initial transmitted-founder viral Env sequence and use antibody gene sequencing to infer the full antibody clonal lineage, including the UA and IA sequences of the CH103 lineage. Notably, the UA of CH103 avidly bound the transmitted-founder Env gp140, and early intermediate antibodies predominantly neutralized the early autologous virus strains. Importantly, the evolution of neutralization breadth was preceded by extensive viral diversification in and near the defined CH103 binding epitope. This was the first demonstration that a specific clonal HIV-1 antibody lineage (in this case to the CD4-binding site) evolves from restricted autologous virus neutralization to broad cross reactive neutralization (Fig. 1). Further studies of the co-evolution of the infecting Env sequences in relation to specific BNAb lineages are likely to provide additional key information on the maturation pathways of BNAb.

### **Strategies to elicit BNAb: guiding the immune response**

We have described several major obstacles to the elicitation of broadly reactive NAb against HIV-1. These include epitope subdominance, tolerance and clonal deletion, gene-restricted antibody lineages, and high levels of affinity maturation. While each of these obstacles is formidable, our recent structural understanding of antibody mode of recognition together with genetic studies of antibody evolution are beginning to elucidate the pathways for BNAb development. This knowledge has raised additional questions to investigate but also provides new approaches to BNAb elicitation (Table 2).

There are at least two related approaches to address epitope subdominance: one is to remove or mask dominant epitopes (28, 176-178), and the other involves the structure-based design of immunogens that present specific conserved epitopes to the immune system (139, 179, 180). Numerous studies with variable region deleted Envs (181-183) or with glycan-masked immunogens (184) suggest that these approaches alone do not solve the problem, though they may have utility as part of a more complex approach. For example, native deglycosylation of gp140 Env exposes gp41 epitopes and enables the UA of some gp41 mAb to bind HIV-1 Env and has the potential to improve Env immunogenicity (185). It may be possible to focus the immune response by initially immunizing with a restricted epitope and boosting with a native form of gp120 or gp140 (28, 179, 186). This has the potential to stimulate the correct naïve BCR and to affinity mature the response against the same epitope as it exists in its native form on the Env trimer. In this regard, there has been recent progress in the design of protein scaffolds on which specific HIV-1 epitopes can be transplanted and retain their native configuration (187-190), and several studies have shown proof-of-concept that such protein scaffolds can induce an epitope specific antibody response (187, 188). There has also been progress in the design of cleaved gp140 molecules that

retain antigenic reactivity to BNABs (191-193). Investigators are just beginning to take advantage of these new immunogens and immunization strategies.

We have already discussed the obstacle of self-tolerance and clonal deletion in some detail. Antibodies with long hydrophobic CDRH3 regions may be disfavored during the process of affinity maturation and this may impact elicitation of glycan reactive BNABs to the V1V2 and V3 regions (e.g. PG9, PGT128). Further studies should inform our understanding of how these V1V2 and V3 glycan BNABs arise, if the long CDRH3 is selected at the germline level, and if glycan reactivity results from affinity maturation. This information would inform the design of vaccines to elicit these glycan-reactive antibodies. For example, the PG9 (V1V2-glycan) and PGT128 (V3-glycan) groups of BNABs make both protein and glycan contacts on gp120. If the early forms of these antibodies make mainly protein contacts (i.e. glycan reactivity evolves over time with affinity maturation), the approach to elicitation might favor presentation of the appropriate protein contacts surface first. However, if the early antibodies bind both protein and glycan, elicitation may require initial immunization with an appropriately glycosylated native trimer. Self-reactivity may be a particular problem for elicitation of antibodies to the MPER of gp41. BNABs 2F5 and 4E10 are thought to have hydrophobic CDRH3 regions to close approximation to viral membrane, which favors antibody interaction with the gp41 MPER epitope. However, the recent discovery of broadly neutralizing 10e8 antibody demonstrates that the immune system can target the MPER without engendering substantial lipid reactivity. The isolation and characterization of additional antibodies such as the non-polyreactive 10e8 may offer insights into common features of this immune response. Strategies to overcome these disfavored antibody lineages include structure-based design of immunogens for high affinity binding to the BCR, high avidity multivalent antigen presentation, potent adjuvants and specific modulators (e.g. Toll-like receptor ligands) to enhance the innate and adaptive immune response (28, 129, 139, 140).

The high level of affinity maturation of most BNABs presents a major potential obstacle to their elicitation by traditional immunization methods. It is clear from over 20 years of study of HIV-1 Env immunogenicity that immunization with a current Env vaccines will not induce broad NABs (39, 194). In addition, only modest levels of affinity maturation have been reported with subunit protein immunization in humans (164-166). To overcome these limitations, we may have to employ new approaches to stimulate suitable B-cell lineages and guide affinity maturation. Studies such as those discussed above, have begun to trace the co-evolution of virus and BNAB lineages to carefully define the Envs immunogen associated with BNAB development (118) (Fig. 1). For vaccine design, the expression and study of these unmutated and early ancestor antibodies may be critical for defining Env immunogens with binding affinity for the appropriate naive B-cell receptor. This approach of B-cell lineage immunogen design has three steps (Fig. 2). The first step is the isolation of one or more clonally related broadly neutralizing mAbs from an HIV-1 infected donor. Second, using the sequences of the known mAb and next generation sequencing to expand the clonal family, one reconstructs the antibody lineage back to the UA. Third, using recombinant antibody technology, the members of the BNAB lineage are expressed and Env constructs are selected that optimally bind to each clonal lineage member at each stage of the lineage. These specific Env immunogens can be used in a prime and boost fashion to engage the naive BCR and to further stimulate the evolution of the NAB response. (Fig. 2). Recently, Env antigens that bind to the unmutated and early intermediate antibodies of a number of B-cell lineages have been defined (109, 195), and in one case, those Envs with higher binding to the 2F5 UA have proved to induce the highest MPER antibodies (185). Importantly, detailed mapping of the co-evolution of a BNAB lineage and Env escape provides key information for informed design of either sequential or polyvalent mixtures of Envs or Env subunits. Thus, in addition to the recently defined CH103 BNAB lineages, it will be important

to define additional antibody lineages, including those directed to other conserved neutralization epitopes such as the MPER, V1V2-glycan, and V3-glycan sites. In addition to a highly lineage directed approach, it is also likely that the most effective vaccine-induced antibody response will be polyclonal and be directed to multiple neutralizing epitopes, thus future vaccine strategies may also include polyvalent immunogens as a way to stimulate multiple B-cell lineages or as a means to focus the response on common conserved regions (196-198).

The observation that some HIV-1 BNABs are restricted to BCRs that derive from specific VH genes has raised the additional question of how to stimulate these specific B cells. VRC01 type antibodies engage the CD4-binding site mainly via CDRH2 interactions, which is encoded by the VH gene. Thus, it is interesting that the UA (or even just V-gene reverted) version of VRC01 antibodies do not bind to heterologous Envs (117, 119, 127). This has led to the hypothesis that stimulation of such antibodies will require the rational design of modified Env immunogens to engage the appropriate BCR (121, 128-130, 139, 199). Several groups of investigators are pursuing variants of the core of gp120 (78, 179, 186, 200) or the more restricted outer domain of gp120 to stimulate CD4-binding site antibodies (201-203). Schief and colleagues (204) have recently reported on the design of an outer domain construct that can bind the V-gene reverted versions of VRC01 and related antibodies. These highly specific germline binding immunogens can be tested empirically for elicitation of VRC01 like antibodies. However, mice and presumably most other small animal species do not have a close homologue to the human VH1-2 gene. Even rhesus macaques that share a similar IgG locus and an average 93% genetic homology to humans (136) may not be the ideal model to test such immunogens, since small changes in the germline sequence may alter key contact sites (114, 119, 126). It may be possible to test such immunogens in mice engineered to have a human IgG locus, though we do not know if these mice can generate the level of affinity maturation required to achieve detectable HIV-1 neutralization (205). In contrast to the VRC01 class of antibodies, HIV-1 Env constructs have been found to bind the germline reverted versions of V1V2 (109, 134) and gp41 membrane proximal external region BNABs (195). Likewise, the UA of the recently isolated CD4-binding site CH103 mAb can bind its donor autologous transmitted-founder Env with high affinity (118). Thus, lack of reported Env binding by germline reverted HIV-1 specific NAb appears to be a particular characteristic of the highly somatically mutated VRC01 class of CD4-binding site antibodies that bind predominantly via the V-gene encoded CDRH2. Even for these antibodies, investigators have not yet studied their development in enough detail to know if the germline antibodies can bind to the transmitted-founder Env.

## Human vaccine studies: lessons from clinical efficacy studies

Early HIV-1 vaccine efficacy trials using gp120 envelope proteins demonstrated that these vaccines were not protective (56, 57). Similarly, a recombinant adenovirus type 5 vector based vaccine that expressed HIV-1 Gag, but not Env, induced antigen-specific CD8<sup>+</sup> T cells but provided no protection (206). Taken together these data suggested a better quality of antibody responses would be needed to achieve some level of protection in a HIV-1 efficacy trial. In 2009, the results of the fourth HIV-1 vaccine efficacy trial, the RV144 trial in Thailand, was published (207). This trial used a canarypox vector (ALVAC) expressing HIV-1 clade B Gag, protease and CRF01\_AE Env gp120 as a prime, and used both the ALVAC plus the same AIDSVAX® B/E gp120s used in the failed VAX003 trial as boosts. Unlike the prior two gp120 trials, this RV144 study demonstrated an estimated vaccine efficacy for prevention of transmission of 31%. While this level of efficacy was not sufficient to deploy this vaccine, it gave vaccine developers hope that a preventive vaccine is possible and that improved vaccines can be designed.

An extensive immune correlates analysis was performed on samples of RV144 vaccinees that received the vaccine. This involved a comparison of vaccine elicited immune responses in those who did not get infected versus those that did get infected (208). These analyses revealed two correlates of HIV-1 infection. First, it was shown that higher plasma levels of vaccine-elicited antibodies to the V1V2 loop was associated with a reduced risk of transmission, suggesting that these antibodies may have played a role in preventing transmission. Surprisingly, the second correlate was that high envelope plasma IgA levels correlated with infection risk, such that the higher the plasma IgA Env antibody level, the higher the transmission risk. Since there was no enhancement of overall infection risk due to the vaccine, these data suggested that high IgA levels might mitigate the effect of other potentially protective antibodies. In a separate study, investigators found a selection, or sieve effect, in the V2 region of viruses that infected RV144 vaccine recipients (209). Specifically, viruses in vaccine recipients were more likely to have an amino acid alteration at position 169 or 181 in the Env V2 region. These data complimented the finding of an association between high V1V2-binding antibodies and reduced risk of HIV-1 acquisition and further suggested that antibodies to the V2 region may have played a role in the vaccine mediated protection. To further understand the potential and mechanism of vaccine-induced V1V2 antibodies, Liao, Haynes, and colleagues (166) used an unbiased B-cell culture method to isolate mAbs from RV144 vaccine recipients. Among the 25 mAbs isolated, four were antibodies to the V2 region. Co-crystal structures and binding analysis demonstrated that these V2 mAbs recognized residue K169 within the V2 region, the same residue implicated in the sieve analysis mentioned above. Additional studies demonstrated that V1V2 antibodies that were induced by the RV144 vaccine could capture virions and neutralize infection of neutralization sensitive (tier 1 or TCLA) viruses but could not capture virions and neutralize the more-difficult-to neutralize (tier 2 or primary) viruses. However, these V1V2 antibodies could bind to tier 2 primary virus Envs on the surface of virus infected CD4 T cells and mediate their killing by natural killer cells through the process of antibody-dependent cellular cytotoxicity (ADCC). Interestingly, it has been demonstrated that RV144-induced IgA antibodies against the same epitopes as are bound by IgG, can indeed block the mediation of ADCC by IgG Env antibodies (210). A recent repeat of the immune correlates of protection in RV144 confirmed the correlation of antibody against the V1V2 region as a significant correlate of decreased transmission risk (211). Thus, these data suggest that protection in the RV144 study was mediated by a vaccine elicited antibody response, likely including antibodies to the V1V2 region of gp120. The specific *in vivo* mechanism of protection is not clear but could involve neutralization of a subset of sensitive viral strains, virion capture at the mucosal surface (212), and Fc receptor-mediated ADCC or related mechanisms by which antibody could kill cells expressing HIV-1 Env on the cell surface. Additional information regarding these possible mechanisms may come from passive antibody protection studies of the RV144 vaccine elicited V1V2 mAbs using in the rhesus chimeric simian-human immunodeficiency viruses (SHIVs) infection model. These immune correlates will also serve as markers for study in future vaccine efficacy trials. Efforts to improve on the RV144 immunogen by designing formulations of Env and adjuvants that can improve the quantity of potentially protective antibodies are underway. Obviously, these efforts will proceed in parallel with continued efforts to design vaccines that can elicit more robust and cross-reactive NAbs.

## Closing remarks

The discovery HIV-1 broadly neutralizing mAbs and their specific modes of recognition on the viral Env provide an initial framework for improved vaccine design. Building on this knowledge, our recent understanding of immune pathways for BNAb development has provided insights for both vaccine design and immunization strategies. Together with the proof of concept that a vaccine can prevent HIV-1 infection in humans, this new knowledge



has invigorated the field of HIV-1 vaccine research. Nonetheless, the way forward is formidable with the realization that a successful vaccine may have to overcome multiple immunoregulatory controls of BNABs, and focus the immune response on subdominant epitopes. Our ultimate success will likely require an in depth knowledge of host-pathogen immunobiology unlike that required for any prior vaccine. Taking nature's lead, the development of a vaccine that elicits potent and cross-reactive NABs is possible and such a vaccine has the prospect of effective prevention of HIV-1 infection.

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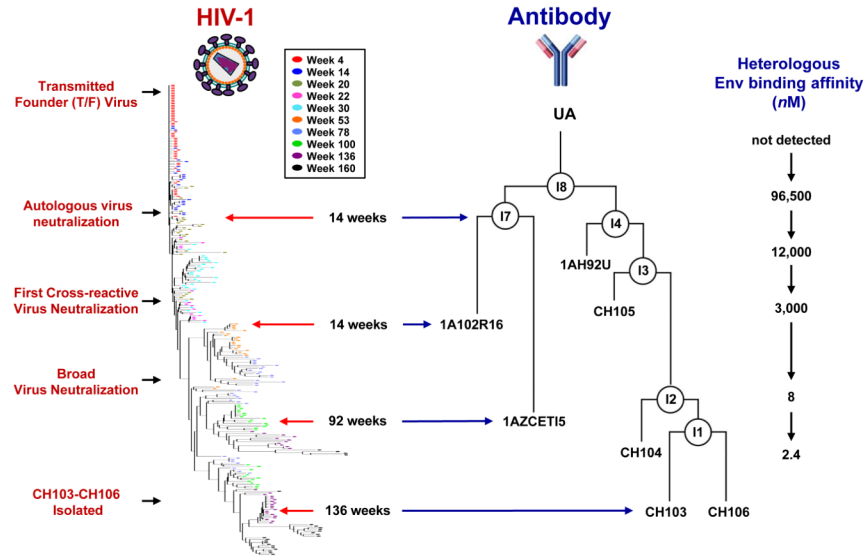
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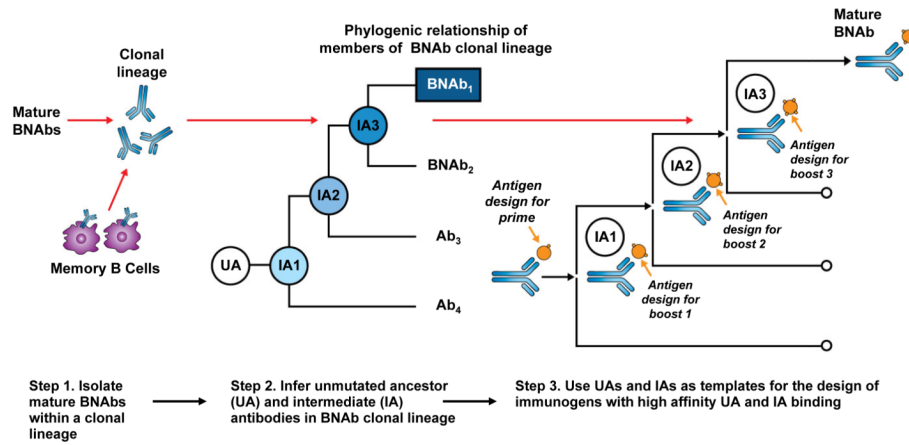
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**Fig. 1. Coevolution of virus and a single antibody lineage in an HIV-1 seroconverter** Mature CD4-binding site antibodies CH103-106 were isolated from circulating memory B-cells at week 136. Longitudinal sampling allowed inference and reconstruction of the evolution of the infecting viral sequence and of the specific neutralizing antibody lineage. The heavy chain antibody lineage was augmented with sequences derived from B-cell gene sequencing and bioinformatics analyses were used to infer early intermediates (IA) and the unmutated ancestor (UA). The UA and IA heavy chain lineages are shown alongside viral evolution. This antibody lineage evolved to gain high affinity Env binding, and virus neutralization evolved from strain-specific autologous virus activity to cross-reactive neutralization of heterologous viruses.



### Fig. 2. B-cell lineage-based approach to vaccine design

Mature BNABs can be isolated from HIV-1 infected donors using modern methods such as memory B-cell culture or sorting of antigen-specific B-cells. Based on the known BNAB sequence, next generation sequencing can be used to find numerous clonal relatives of the mature BNAB. If appropriate longitudinal samples are available, it is possible to infer the full antibody lineage, including the UA and IA. The expressed UA and IA sequences can then be used as templates for the design of HIV-1 immunogens with high affinity binding. Since the antibody lineage is known to evolve in response to viral evolution, it may be possible to design sequential immunogens with high affinity binding for the UA and IA, thus guiding the antibody response toward the mature antibody with broad neutralizing activity.

Table 1

Genetic characteristics of HIV-1 broadly neutralizing monoclonal antibodies

Viral Epitope	Antibody binding characteristics <sup>a</sup>	Antibody clonal family	Year Published	Isotype and subclass <sup>b</sup>	Heavy chain V-gene	Light chain V-gene (K or L)	CDRH3 length (Kabat AA) <sup>c</sup>	VH mutation (nt %) <sup>d</sup>	VH mutation (AA %) <sup>d</sup>	Neutralization Breadth <sup>e</sup>	Neutralization Potency <sup>f</sup>	Polyreactive <sup>g</sup>	References
MPER of gp41	contiguous sequence	2F5	1993	IgG3	2-5	K1-13	22	14	15	++	++	yes	53, 65, 66, 77, 85
	contiguous sequence	4E10	1994	IgG3	1-69	K3-20	18	14	20	++++	++	yes	53, 65, 66, 76, 85
	contiguous sequence	M66.6	2011	NR	5-51	K1-39	21	4.3	10	+	++	yes	120
V1V2-glycan	contiguous sequence	CAP206-CH12	2011	IgG1	1-69	K3-20	15	12	19	+	++	yes	115
	contiguous sequence	10e8*	2012	IgG3	3-15	L3-19	20	21	26	++++	+++	no	110
	peptido-glycan	PG9, PG16	2009	NR	3-33	L2-14	28	12-13	17-20	+++	+++	no	82
Outer domain glycan	peptido-glycan	CH01 - 04	2011	IgG1	3-20	K3-20	24	14-17	23-29	+	++	no <sup>g1</sup>	109, 170
	peptido-glycan	PGT 141-145	2011	NR	1-8	K2-28	31-32	12-18	21-29	+++	+++	NR	83
	glycan only	2G12	1994	IgG1	3-21	K1-5	14	21	32	+	++	NR	53, 65, 75
V3-glycan	peptido-glycan	PGT121-123	2011	NR	4-59	L3-21	24	17-21	21-27	++	++++	NR	83
	peptido-glycan	PGT125-131	2011	NR	4-39	L2-8	19	15-23	23-33	++	++++	NR	83
	peptido-glycan	PGT135-137	2011	NR	4-39	K3-15	18	17-20	25-29	+	++	NR	83
CD4 binding site	CDRH3 loop	b12	1994	NR	1-3	K3-20	18	13	20	+	++	no <sup>g2</sup>	53, 64, 78
	no liganded structure	HJ16	2010	NR	3-30	K4-1	19	15	37	+	++	NR	116
	CDRH3 loop	CH103 - 106	2013	IgG1	4-59	L3-1	13	14-16	22	++	++	yes <sup>g3</sup>	118
mimics CD4 via CDRH2	mimics CD4 via CDRH2	VRC01 - 03	2010	IgG1	1-2	K3-20 <sup>#</sup>	12-14	30-32	40-48	++++	+++	no	84, 119
	mimics CD4 via CDRH2	VRC-PG04, 04b	2011	IgG1	1-2	K3-20	14	30	38-39	+++	+++	no	114
	mimics CD4 via CDRH2	VRC-CH30 - 34	2011	IgG1	1-2	K1-33	13	23-24	36-40	+++	+++	no	114, 170
mimics CD4 via CDRH2	no liganded structure	3BNC117, 3BNC60*	2011	NR	1-2	K1-33	10	27	37-40	+++	+++	yes <sup>g4</sup>	117
	mimics CD4 via CDRH2	NIH45-46*	2011	IgG1	1-2	K3-20	16	30	41	++++	+++	yes	117, 125
	no liganded structure	12A12, 12A21*	2011	NR	1-2	K1-33	13	23-25	38-40	++++	+++	yes	117
no liganded structure	no liganded structure	8ANC131, 134*	2011	NR	1-46	K3-20	16	27	37-38	+++	++	yes	117
	no liganded structure	INC9, 1B2530*	2011	NR	1-46	L1-47	16-19	24-26	36-38	+	++	yes	117

<sup>a</sup>Major binding characteristics indicated by co-crystal structural analysis. mAb 2G12 utilizes unusual domain swap structure - see indicated references. mAb b12 was isolated from phase display library and natural light chain pair was not retained; b12 displays heavy chain only binding in co-crystal structure with gp120.

<sup>b</sup>Isotype indicates the natural isotype and subclass of the isolated antibody. In some cases, this was not reported. In many cases, the PCR amplified heavy and light chain genes are expressed in an IgG1 expression vector even though the natural antibody was a different subclass.

<sup>c</sup>Reported CDRH3 lengths are often based on IMGT or Kabat nomenclature. The Kabat definition is generally used for structural studies and is often 2 AA residues shorter than the IMGT definition. For explanation of the differences in nomenclature, see [www.imgt.org](http://www.imgt.org).

- <sup>d</sup>The percent mutations (nt) of an antibody heavy chain are based on comparison to the inferred germline gene. Percent mutations are sometime also reported based on translated AA sequences (AA).
- <sup>e</sup>Neutralization breadth is indicated for antibodies tested on large panels of > 100 tier 2 Env-pseudoviruses and the values shown are the percent of viruses neutralized at an IC50 of < 50 ug/ml. +++++ = > 90%; +++ = 75 - 90%; ++ = 50 - 74%; + = < 50%. If there are several members of a clonal family, the broadest clonal member was used.
- <sup>f</sup>Neutralization potency indicated by median or geometric mean IC50 (ug/ml) of neutralized viruses (i.e. excluding non-neutralized viruses); +++++, IC50 < 0.05; ++++, IC50 = 0.05 - 0.49; ++, IC50 = 0.5 - 4.9; +, IC50 = 5.0 - 50.
- <sup>g</sup>Poly or self reactivity as assessed by a combination of common autoimmune assays, including cardiolipin ELISA, Hep2 cell IFA and Athena assay for antinuclear antibodies.
- <sup>g1</sup>One antibody in this clonal lineage, CH103 is polyreactive.
- <sup>g2</sup>Some have found b12 polyreactive, but studies in b12 VHDJH + VLJL knock-in mice have demonstrated the degree of polyreactivity for this antibody is not sufficient to predispose these antibodies to tolerance deletion (D. Nemazee, personal communication).
- <sup>g3</sup>CH103, CH104, CH106 are autoreactive, CH105 is not autoreactive.
- <sup>g4</sup>3BNC117 is reported to 3y polyreactive, but not 3BNC60.
- <sup>\*</sup> Additional members of the clonal family also reported in the primary publication. NIH45-45 is same donor and clonal family as VRC01. For mAbs INC9 and 1B2530, neutralization data only available for 1B2530
- <sup>#</sup>VRC01 and 02 were initially reported to derive from inferred VK3-11, but additional analysis of the antibody lineages strongly suggests VK3-20. Also, VRC03 was initially inferred to be from different clonal family than VRC01, but more detailed analysis suggests that VRC01, 02 and 03 are clonal relatives (J. Mascola, P. Kwong, unpublished data)

Notes: NR, not reported.



**Table 2**

## Obstacles to the elicitation of HIV-1 broadly neutralizing antibodies

Obstacles and Questions	Approach
<p><b>Epitope dominance/subdominance</b></p> <ul style="list-style-type: none"> <li>• Do dominant variable region epitopes compete out subdominant conserved epitopes</li> <li>• Do specific epitopes provide weak B cell receptor (BCR) stimuli</li> <li>• Are some neutralization epitopes only recognized by rare germline precursor BCRs</li> <li>• What are structural and genetic characteristics of BNABs to subdominant epitopes; e.g., antibodies to the CD4 binding site</li> </ul>	<ul style="list-style-type: none"> <li>• Dampen dominant epitopes; e.g. add glycans, remove variable loops</li> <li>• Minimal epitope constructs; e.g., structure based designs of gp120 core, transplanted epitopes on to protein scaffolds</li> <li>• Native Env trimer to focus immune response on accessible viral spike epitopes</li> </ul>
<p><b>Tolerance: deletion/energy</b></p> <ul style="list-style-type: none"> <li>• Are self-reactive BCRs limiting responses to some epitopes; e.g. MPER, glycan epitopes</li> <li>• Does requirement for poly (lipid)-reactivity for MPER BNABs lead to clonal deletion</li> <li>• How does a non-lipid reactive BNAB effectively target the MPER</li> <li>• Are long CDRH3 in BNABs encoded at germline or result from affinity maturation and insertions</li> <li>• How do we overcome selection against long CDRH3 antibodies</li> </ul>	<ul style="list-style-type: none"> <li>• Antigen design for strong BCR signals to overcome energy;</li> <li>• High affinity epitope specific antigens; e.g., scaffolds to present optimal MPER epitope</li> <li>• Antigen presentation; e.g. multivalency and adjuvants</li> <li>• Understand prevalence of long CDRH3 BCRs and how to engage these BCRs</li> </ul>
<p><b>Selected BCRs and Affinity Maturation</b></p> <ul style="list-style-type: none"> <li>• If some germline antibodies (e.g. VRC01 class) do not bind gp120, how is the BCR stimulated</li> <li>• What are defining structural and genetic characteristics of VH1-2 restricted antibodies</li> <li>• How much somatic mutation is required to achieve virus neutralization</li> <li>• How to promote affinity maturation via immunization</li> <li>• Does virus evolution specifically drive antibody maturation toward broad neutralization</li> <li>• What is optimal CD4 T-help to promote affinity maturation</li> </ul>	<ul style="list-style-type: none"> <li>• Rational immunogen design to engage appropriate BCRs (e.g. VH1-2 gene for VRC01 class antibodies)</li> <li>• Genetic analysis of BNAB antibody lineages to understand germline and antibody evolution, and interaction with virus evolution</li> <li>• Structural studies of mature and germline antibody binding</li> <li>• B-cell lineage-based vaccine design (Fig. 2). Design immunogens based on unmutated and intermediate ancestors of known BNAB lineages. Guide B-cell maturation</li> </ul>