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## Passive and active antibody studies in primates to inform HIV vaccines

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### Abstract

**Introduction:** Prevention of infection remains the ultimate goal for HIV vaccination, and there is compelling evidence that antibodies directed to Envelope are necessary to block infection. Generating antibodies that are sufficiently broad, potent, and sustained to block infection by the diverse HIV-1 strains circulating worldwide remains an area of intense study.

**Areas Covered:** In this review, we have summarized progress from publications listed as PubMed citations in 2016–17 in the areas of passive antibody studies using human neutralizing monoclonal antibodies in nonhuman primates, HIV Envelope vaccine development and active vaccination studies to generate potent neutralizing antibodies.

**Expert Commentary:** Passive transfer studies in nonhuman primates using human neutralizing monoclonal antibodies have informed the potency, specificity, and cooperativity of antibodies needed to prevent infection, leading to clinical studies now testing potent antibodies for prevention of HIV. Progress in understanding the structure of Envelope has led to novel vaccine constructs, including mimetics, scaffolds and native-like proteins. As yet, no single approach ensures protection against the circulating global HIV-1 strains, but there is progress in understanding why, and intense research continues in these and other areas for a solution. We offer perspectives on how this knowledge may shape the design of future HIV vaccines.

### Keywords

HIV; vaccines; antibodies; Envelope; passive; monoclonal antibodies; protective efficacy

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Declaration of interest

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## 1. Introduction

In the last three decades, successful vaccines against hepatitis B and human papilloma viruses were developed that induce protective and long-lasting virus-specific antibodies. However, the combined efforts of the global research community to develop a vaccine for HIV-1 (HIV) have fallen short. Although disappointing, the absence of a protective vaccine for HIV is not surprising given the requirement to provide coverage for the antigenic diversity of strains worldwide. Structural features of the trimeric Envelope glycoprotein (Env), the target of protective antibodies, include highly variable regions to which carbohydrate moieties are affixed that disguise and shield critical binding sites. Protective antibodies must overcome these obstacles and recognize conserved determinants on Env that are shared among circulating strains and clades in order to block infection. In the absence of protection, HIV-1 integrates into the genome of the host, leading to a rapid establishment of widely dispersed and latently infected cells that are invisible to the immune system sometimes for many years. Yet, sooner or later, cells within these latent pools can become active continuously producing virus and adding a potentially insurmountable obstruction to viral clearance.

Despite these escape strategies, in most human subjects, HIV-1 infection leads to development of multiple types of antibodies, including neutralizing antibodies (NAbs), with various effector functions, recently reviewed [1]. Studies of NAb development are providing a detailed understanding of the mechanisms of antibody maturation and antigenic escape [2–5]. Technology advancement has led to the rapid discovery and characterization of highly potent human neutralizing monoclonal antibodies (NmAbs) that demonstrate extensive cross reactivity covering multiple HIV-1 clades and virus neutralization capacities [6]. These NmAbs—frequently called bNAbs to include the distinction of breadth—have become indispensable tools in revealing many aspects of the structure of Env. Co-crystals of antibody-bound Env structures have exposed intricate details of epitopes that are sites of vulnerability that may serve as candidates for vaccine targeting, recently reviewed [7] and shown in Fig. 1. At the same time, bNAbs are already in clinical testing as potential therapeutics [8], discussed in more detail below.

In this review, we summarize the progress in passive antibody studies and Env-based vaccines in nonhuman primate (NHP) models of HIV infection and discuss how these studies have influenced vaccine development from the perspective of generating protective antibodies.

It is important, however, to address certain caveats of these models, which use related SIVs or laboratory chimeras of HIV and SIV—SHIVs—due to the lack of a suitable model for HIV-1 itself. In particular, vaccine development or immunotherapy in NHP should be weighed in the context of both the advantages and limitations of these systems in order to interpret experimental outcomes for accurate translation to human studies. Here, we will offer our perspective on considerations critical to the continued and judicious implementation of NHP in HIV research. Some of the questions that will be addressed in the sections below include: (1) How has recent passive antibody research in NHP models enhanced knowledge about the impact of antibodies in blocking or controlling HIV? (2)

What has this research taught us about antibody correlates of protection? (3) What are the most relevant SHIV challenge models for antibody-based vaccine studies? (4) How do we interpret findings from vaccine advances in NHPs to advance clinical studies?

## 2. Progress in developing primate models and lessons from passive antibody studies

### 2.1 Rationale for the development and use of SHIV/maaque models

NHP models for AIDS originally focused on the use of the highly adapted African-origin simian immunodeficiency virus (SIV), which causes disease in Asian macaques, the nonadapted host for SIV. SIV isolates have provided a powerful experimental advantage due to their ability to mirror most aspects of HIV-1 pathogenesis in a compressed time course of disease progression, and models have been developed that utilize various SIV strains in all three Asian macaque species (*M. mulatta*, *M. fascicularis*, and *M. nemestrina*). These virus-host combinations have afforded models to address many important questions in HIV research that cannot be easily addressed in clinical studies. Due to the genetic divergence of SIV and HIV, the Env proteins from these two viruses are not antigenically cross-reactive, making it impossible to study HIV Env-specific antibody responses in SIV infection. The development of chimeric simian/human immunodeficiency viruses (SHIVs) expressing the HIV *env* in an SIV backbone has resulted in tractable models in the same macaque species following passage *in vivo* in order to gain replication fitness. Since the earliest infecting virions access target cells via the coreceptor CCR5, SHIVs bearing Envs that use CCR5 were developed. However, SHIV models have come under criticism for the spontaneous and variable post-acute control of viremia in a subset of infected macaques, as well as reduced replication competence and resulting limited pathogenesis that is likely due to insufficient genetic adaptation. Nonetheless, despite limitations in these models, SHIV expression of HIV Env on the virion surface provided an avenue for human antibody-based passive protection studies in NHP models of infection, as well as a platform for testing vaccine candidates that include Env as an immunogen.

Clinical studies have shown that the development of antibodies commences shortly after infection and recognizes the Env of the infecting transmitted and early founder (T/F) virus, typically one or a few viral variants that persist for at least the first six weeks [9]. T/F strains of HIV have different phenotypic properties than chronic viruses, notably they are more infectious and contain on average more copies of Env per particle but have shorter variable loops and fewer glycan sites. Over time, as variants accrue with mutations in Env, antibodies emerge that bind to these autologous variant Envs, but their detection is delayed relative to the appearance of the antigen, depicted in Figure 2A. Autologous NABs can be measured in a few months after infection and over years, in most subjects, there are increasing levels of heterologous NABs that develop during chronic infection [10], despite only being exposed to variants of the infecting virus. In the figure, these new antibodies are shown to emerge after the variants that precede them in time. Their development occurs as antibodies co-evolve with the autologous virus and undergo somatic hypermutation (SHM), diverging the nucleotide sequences of variable and light chain regions ( $V_{HJ_H}$ ,  $V_{LJ_L}$ ) from away from germline. In a very small subset of people called “elite neutralizers,” this polyclonal

response becomes very “broad” and potent. However, of the broad and potent bNAbs isolated from individuals, the level of SHM is high, ranging from 732%, and varies with bNmAb specificity [11–17]. Other characteristics of potent bNAbs include frequent insertions and deletions (reviewed in [18]) that have been associated with critical protein or glycan contacts [12,16]. In some cases, this exceptional breadth is due to a single clone, as shown by the isolation and characterization of bNAbs from these subjects [19–21].

In a very similar manner, adult and juvenile macaques infected with SHIV show Env divergence from the initial infecting clone or strain. In the last several years, it was shown that SHIV adaptation occurs *in vivo* to better access macaque CD4 (mCD4) thus, increasing virus replication in macaques with only a few and as little as a single but critical point mutation [22,23]. As seen with HIV infection, SHIV-induced antibody responses directed to the HIV Env become more complex and more neutralization-potent over time, as the quasispecies of variants develops. There are strong similarities between SIV and SHIV infection in the dynamics of innate cells, B- and T-cell subsets, cytokine expression, and the development and maturation of T cells and antibodies with various functional activities. Importantly for antibody studies, macaque B cell receptors are capable of recognizing complex epitopes similar to those recognized by certain bNAbs isolated from HIV-1-infected humans, and, importantly, SHIV- infected macaques are able to develop NAbs directed to glycan-specific epitopes recognized by human bNAbs [24].

Important progress in the last few years has led to the development of SHIV strains that encode newly transmitted HIV Env proteins and that incorporate modifications to interact more efficiently with rhesus CD4, enhancing virus entry and replication making them pathogenic *in vivo* [25,26]. These strains can be transmitted by mucosal routes in NHP and are promising for future work. Other SHIV isolates that may be critical for vaccine work include an A/E chimera recently titered *in vivo* that matches strains prevalent in Bangkok, Thailand, identified in the RV144 trial [27]. The T/F viruses are more difficult to neutralize *in vivo*, and their plasma viremia persists longer than many current SHIVs, providing hope that they may be more relevant models for HIV in humans. As with all animal models, a successful clinical trial with clear correlates of protection is necessary to validate one or more of them. If the ongoing human trials of poxvirus plus protein (e.g. HVTN106, [www.hvtn.org](http://www.hvtn.org)) in South Africa are successful, then we may be able to test vaccines or test vaccine-elicited antibodies in NHP models to understand and validate them, as described below. Their use is further discussed as it applies to vaccine development, in Section 3.

## 2.2 Early infection events: viral dissemination and latency

Early innate immune events that occur in response to mucosal HIV infection are not fully characterized, although there has been some significant progress in the last few years. By using fluorescently labeled virus, it has been possible to track migration into vaginal tissues [28] in efforts to visualize the earliest infection stages, where there is evidence that CD4-positive Th17 cells are preferentially targeted by SIV [29]. The HIV-1 viral eclipse phase was described as the period just after virus exposure during which virus replicates in mucosal and proximal lymphoid tissues but is not yet detectable in the circulating plasma [30]. It has been estimated that the eclipse phase in SIV-infected macaques lasts for

approximately 4–7 days, and for HIV-1 infected humans it is about 7–21 days in duration. This predicted eclipse phase either differs in primate models, or may be a result of assay sensitivity, as there are now recent data from several NHP models showing that SIV and SHIV RNA and DNA are detectable in the blood by 3 days after infection in newborn macaques. Moreover, high dose oral inoculation of either SIV or SHIV results in rapid dissemination to lymph nodes and gut within 1–3 days with tissue reservoirs greatly expanded by day 14 [31,32]. The kinetics of SIV distribution to lymphoid tissues following vaginal challenge in adult macaques was observed in 2005 [33], and recent studies with SHIV confirmed these observations [34].

Understanding the kinetics of virus dissemination is important for successful post-exposure prophylaxis by antiretroviral therapy (ART), and this treatment is very effective to prevent infection after needlestick incidents when initiated within 72 hours of exposure. Treatment with ART in individuals with established infection can drive the virus to undetectable levels but it does not eliminate the infected cells, and viral rebound is observed even after extended treatment [35]. Defining the time frame of intervention is important, because once latently infected cells exist, the window of opportunity to eradicate virus appears to be closed, leaving the host vulnerable to breakthrough infections and continued viral replication. In primates, initiating ART during the eclipse phase before detectable viremia, as early as 3 days post inoculation, does not prevent establishment of infection [36]. Elegant clinical studies published in the last year indicate that the source of the continued virus in the blood may arise more commonly from distal sites rather than circulating PBMCs [37]. The application of tissue virus quantification to NHP models has begun to reveal the full impact of SIV and SHIV viral reservoirs in blood and in tissues [32,38]. Likewise, refinements to immunological assays in primates have allowed us to examine the direct effects of the virus on specific subsets of immune cell populations. SIV is found in high levels in lymphoid tissues [39,40], and one major sanctuary is in the B cell follicle in T follicular helper cells [41,42]. This area of research has emerged as the field begins to consider whether it is possible to reduce or eliminate the viral reservoirs [35]. Given these kinetics, how have studies in primates informed the role of antibodies in preventing infection?

### 2.3 Passive antibody for pre-exposure prophylaxis, and modulation of immunity

Combinations of human mAbs (and in some cases humanized murine mAbs) are being used effectively in cancer therapy to ablate certain populations of cells, such as B cells, or more recently to modulate immunity. Their use in infectious diseases has been slower to develop, although a number of influenza-specific mAbs are in clinical testing [43]. Recently, two different combinations of human mAbs were shown to protect nonhuman primates against all signs of Ebola [44,45], and most recently, Zika virus [46]. Despite these successes, antibodies as therapeutics for HIV infection have been dismissed, until very recently, as “too little, too late” for reasons of expense and practicality. During the last decade of HIV-1 antibody discovery, the use of passively transferred antibodies for prevention and cure strategies has gained in relevance because the recent discoveries of extremely potent bNAbs have made a clinically relevant dose more achievable. It is anticipated that the ongoing trials of human bNAbs as PrEP (pre-exposure prophylaxis), if successful, could not only prevent

the establishment of infection but could also provide insight into what may constitute a protective antibody response.

Historically, SHIV/maaque models have shown that even when a single type of bNAb is present before virus exposure, infection by mucosal routes can be blocked and apparent sterilizing immunity can be achieved, summarized in a recent review [1]. This activity is depicted in Figure 2B, where bNAbs can block infection by diverse isolates in NHP models. It is hoped that eventually vaccine-induced pre-existing NAb at the site of entry will either block infection or provide significant viral clearance before subpopulations of CD4+ T cells become productively infected.

## 2.4 Interpreting passive antibody results in NHP models

Data from SHIV challenges consistently shows that the concentration of antibodies required to block infection *in vivo* is dose-dependent and tracks directly with the *in vitro* neutralizing activity of either bNAbs or polyclonal serum IgG preparations, making viral neutralization the primary antiviral function associated with *in vivo* protection [47–49]. Historically, peripheral blood mononuclear cell (PBMC)-based HIV-1 and SHIV neutralization assays with human and/or macaque cells were used to determine the *in vitro* 50% and 90% inhibitory concentrations (IC<sub>50</sub>, IC<sub>90</sub>) of NAb. These assays, and later, neutralization assays using pseudoviruses in TZM-bl cells, were used to determine NAb titers in plasma or serum at the time of challenge during passive transfer studies (plasma dilution resulting in 50% inhibition, [ID<sub>50</sub>]). Importantly, a determination of the potency of a transferred mAb using the concentration measured in plasma at the time of challenge is also essential to understanding the protective outcome of any particular study. This value has been termed the “plasma IC<sub>50</sub> concentration” and is determined based on the *in vitro* neutralization potency of the transferred mAb against the challenge virus as follows: plasma concentration of the transferred mAb divided by the *in vitro* mAb IC<sub>50</sub> measured (reviewed in Pegu et al. [1]). The resulting value incorporates both the amount of mAb in circulation after passive transfer (pg/ml) and the *in vitro* potency of the mAb (pg/ml) and represents the “fold-over the *in vitro* IC<sub>50</sub>.” Both of these values have been used to determine protective efficacy in passive transfer studies, but efficacy in these models has been shown to vary, even among potent and broadly neutralizing mAbs (bNAbs) depending upon the challenge virus, targeted epitope, mAb half-life, and Env glycosylation [50].

Antibody *in vivo* titration studies have shown that complete protection in SHIV models with macaques is seen when serum concentrations constituting neutralizing titers are at least tenfold and more often a few hundred-fold higher than the *in vitro* IC<sub>50</sub> [1,51,52]. However, this range is dependent upon the neutralization assay used to assess the IC<sub>50</sub>, and even considering this caveat, protection efficacy varies among bNAbs and Env targeting specificities. The great potency of the bNAbs discovered since 2010 has effectively translated to requiring much less antibody in macaque serum at the time of challenge for protection. These findings have extremely important implications for prevention and immunotherapy applications, allowing clinically relevant doses to be achieved at reasonable costs. For vaccine research, the potential for a much lower titer of antibody induced by immunization also bolsters the search for immunogen designs and strategies to elicit potent



NAbs, if a reduced risk of infection is expected to be predominately mediated by neutralization. The bar for achieving this goal may still be out-of-reach unless bNAbs of the greatest potency are elicited. To envision how bNAbs used in passive protection studies compare, we have graphed the protective efficacy observed as a function of concentration recorded as the “fold over IC<sub>50</sub>” (Fig. 3). See Table 1 for specifics associated with each passive protection study depicted in this figure. It is important to note that apparent sterilizing immunity provided by even the most potent bNAbs indicates a requirement for serum concentrations many times the *in vitro* IC<sub>50</sub> against the challenge virus, as shown in the 100% efficacy column. However, because the *in vitro* IC<sub>50</sub>s of potent bNAbs are extremely low (usually <0.01 pg/ml), the serum concentrations attributable to protection are also very low, thus making potent bNAbs an attractive therapeutic [1]. The recent report of protection with two broad and potent V2-specific bNAbs, PGDM1400 and CAP256-VRC.25 against a clade C SHIV designed precisely for the study [53], is consistent with the findings of the protection studies shown here because when the bNAbs and challenge virus are carefully matched, the protection results in very low serum concentrations needed for protection. However, even with the most potent bNAbs known today, there is a realization in the field that targeting multiple Env determinants will be needed for expectations of broadening protection coverage either with multi-specific Ab-like constructs or cocktails. This will be especially important for vaccine designers.

Studies in NHP models have shown the dose-dependence of bNAb protection by repeated viral challenge during the decay phase of the passively transferred antibody cocktail [54]. However, the case for neutralizing potency in antibody protection is still strong, even in light of the modest efficacy of RV144 and correlation with high titer binding IgG directed to variable regions [27,55]. When compared for protection against a topical SHIV<sub>SF162P4</sub> (Tier 1) vaginal challenge, no protection was achieved with the weakly-neutralizing mAb, b6, a CD4bs mAb that recognizes a similar and overlapping epitope to bNmAb b12. Surprisingly, a non-neutralizing mAb, F240, that targets the immunodominant region of gp41, protected 2 of 5 macaques [56]. The partial protection by F240 is intriguing and complicates interpreting results of the study. One possibility based on *in vitro* analysis, was the greater ability of F240 compared to b6 and b12 to capture infectious virions although recently this characteristic has not held up as advantageous [57]. The mechanisms behind F240 protection are unknown, but it was hypothesized that even though functionally inactive, the gp41 “stump” epitope recognized by F240 on otherwise infectious virions provided a footing for Fc receptor mediated mechanisms that decreased infectivity of the virus inoculum. This passive study has implications for vaccine-elicited Abs targeting many of the exposed regions of Env during various stages of infection. Thus, unraveling the F240 protection results in the context of RV144 correlates pointing to high titers of binding Abs directed to the V2 region of Env, is extremely important today for parsing the roles of neutralizing, binding, and effector function contributions toward protection.

Polyclonal antibody preparations are also capable of protection against SIV or SHIV infection in NHP models, but only high doses of HIV immune globulin (purified from HIV-positive human or chimpanzee serum) have shown this activity [58]. Similarly, SIVIG and SHIVIG, polyclonal preparations purified from SIV- or SHIV-infected macaques, can protect only at high concentrations (100–200 mg/kg) due to the relatively small proportion

of NAbS in the IgG preparation [59] [60] (earliest work reviewed in [61]). Furthermore, since the development of bNAbs takes years to develop in SHIV-infected NHP, SHIVIG is typically only capable of neutralizing a homologous virus, and heterologous SHIV challenge typically cannot be defended against, as depicted in Figure 2C. Even at high doses, if the potency of SHIVIG against the challenge virus is too low, there is no permanent effect on viremia [62].

More studies are needed, but if cocktails of NmAbs or therapies with bi-modal, or multispecific Ig-based variants (discussed in 2.8 below) are shown to be effective at preventing or limiting viral seeding or preventing viral latency in clinical trials, then logically it follows that if achievable, a ready and present NAb titer of significant breadth will be a critical element of a vaccine-elicited response in order to expect efficacy of a global population. V2-directed autologous Abs were identified as one of two immune correlates of the RV144 clinical vaccine trial [55], but none of the published passive studies to date have fully evaluated the potential of V2-targeted mAb cocktails of the type identified in the vaccine trial analysis for protective efficacy.

## 2.5 Combinations of bNAbs as post-exposure immunotherapy

In primate models, experiments published in the last two years have shown that bNAbs can significantly impact SHIV infection if present early after exposure in blocking infection and/or to modulate disease. As mentioned above, combining different specificities in NmAb cocktails may reduce viral escape and may be a logical choice for effective immunotherapy applications, as these combinations show improvement of neutralization potency *in vitro* [63]. Cocktails that incorporate PGT121 along with VRC01 and its clonal relatives suppressed viral replication and inhibited entry of HIV induced from latent reservoirs into CD4+ T cells [64]. Similar cocktails were given to SHIV-infected macaques on day 10 of infection and resulted in significant reductions in peak viremia and viral replication [65]. The findings in these studies of substantial impact by bNAb cocktails on established viremia spurred our interest in testing bNAb intervention in our infant macaque model developed to study mother-to-child-transmission of HIV-1 (MTCT) at birth. Despite evidence for virus in the blood and tissues by day 1, passive bNAb treatment with the cocktail of PGT121 and VRC07–523 completely protected all ten infants with no evidence of viremia in blood or tissues after six months when given subcutaneously one day after oral SHIV infection [32]. Another recent study also reported the benefits of bNAb therapy applied three days post infection in SHIV-exposed adult macaques infected with SHIV-AD8 [66]. Thus, bNAbs can either attenuate or eradicate different virus strains, as long as they are present near the time of viral acquisition, as shown in Figure 2D.

In addition to the effects seen by bNAbs, polyclonal IgG given early can contribute to viral control and promote beneficial immunomodulatory effects in infected animals, originally shown in SIV infection and more recently with SHIV infection [67,68], depicted in Figure 2E. In this setting, polyclonal IgG is typically not potent enough to block infection, but can control viremia [62] against matched SHIV but not mismatched SHIV, as well as viral seeding in PBMC [69].



We recently reported that polyclonal NAbs given to adult macaques before intrarectal (IR) SHIV infection, but at doses too low to block infection, mitigated B cell dysregulation; this preservation of B cells was correlated with the ability of the animals to develop Env-specific Abs [69].

It is our opinion that, even short of complete eradication of the virus, applications of NmAb immunotherapies during the earliest stages (days) of infection may have an impact in slowing the ravages of HIV-1 on the adaptive immune system. Once deterred, the probable effect would then be the development of strong humoral responses as well as highly functional T cell responses that could contribute to viral control after rebound [66]. Once infection is established and viral reservoirs are present, it is not known whether antibodies could be effective therapeutics, but there has been progress in testing the concept in NHP models. These studies have implications for designing therapeutic strategies to maintain virus remission in infected individuals and remove the indefinite use of ART. Studies in humanized mice and macaque models have shown that several of the known bNAbs can suppress established virus in infected animals, summarized in a recent review [70]. Animal models of viral clearance have begun to test the potential for anti-HIV bNAbs in strategies for curative approaches [8,65,68,71–73].

In summary, attaining viral clearance in macaques using cocktails of bNAbs with different specificities may offer the highest potential for successful therapies to clear infection. Several highly potent bNAbs can neutralize a large portion of known circulating isolates worldwide at extremely low IC<sub>50</sub> concentrations. *In vitro* studies examining mAb combinations suggested that neutralization coverage is improved when different epitopes are targeted and more recently, mathematical models of neutralization and breadth predicted improved protection with combinations of the most potent and broadly neutralizing mAbs [63]. To date only a few *in vivo* studies have been published using combinations of these bNAbs [71,72] and the long term benefit of such therapies is not known.

## 2.6 Recent bNAb discoveries and modifications

Human bNAbs against HIV-1 that were isolated years ago did not have the potency to become a part of an effective immunotherapy in humans. Instead, studies to understand their specific interactions with Env, their neutralization, and their protective efficacies in NHP were their primary contribution to guide vaccine designers as a blueprint for immunogen design, recently reviewed [6]. New B cell technologies leading to the HIV bNAb discovery explosion, heralded in 2009 by the isolation of a panel of very potent bNAbs from an African cohort, headlined with PG9 and PG16 [11], set in motion the subsequent discoveries of many new bNAbs. Large patient cohorts and improved identification of the subjects with the most potent and broad sera continued to expand the collection of super bNAbs [74]. Structural studies of these bNAbs continue to advance understanding of Env vulnerability and requirements for immunogen design [75–78]. The five known antigenic sites on HIV-1 Envelope targeted by bNAbs are shown in Figure 1 along with bNAbs associated with those epitope regions. Since 2010 and the identification of PG9 and PG16 that targeted the Env apex epitopes, other unique determinants have been identified that are associated with discoveries of very potent bNAbs. The high mannose patch is targeted by the PGT families

of bNAbs which show exceptionally potency [15]. PGT121 has shown exceptional protective efficacy at very low doses against SHIV<sub>SSF162P3</sub>, a commonly used Tier 2 virus [49]. As mentioned previously, when using a challenge virus developed to be highly susceptible to neutralization by PDGM1400 and CAP256.VRC26, protection of macaques was also achieved at very low doses and serum concentrations [53]. Together, these studies bolster the idea of targeting the V1V2 apex epitopes for vaccine discovery. Another new epitope discovery is the gp120-gp41 interface harboring unique epitopes from which several bNAbs have been identified [79] [80] [81] and structural studies will no doubt benefit immunogen design concepts. Notably, the bNAb specificities of the apex, the high mannose patch and some of the gp120-gp41 are dependent upon glycans [2]. In 2016, the discovery of the CD4bs bNAb, N6, is the broadest and most potently neutralizing of any so far and covers 98% of known HIV-1 isolates [82]. However, new bNAbs are continuing to be discovered that could surpass the breadth and potency of those known today. The safety and utility of producing antibodies in plants was shown with the Ebola life-saving tri-mAb cocktail. Now, developments with many of the newest and most potent HIV-1 mAbs are also being developed utilizing *Nicotiana species*, for testing in NHP and eventual clinical use [83–85].

## 2.7 Bispecific and trispecific antibodies and mimetics

Even with the recent discovery of N6, no single mAb is highly active against all viral strains. Now, innovative research with bispecific and trispecific immunoglobulins (IgGs) composed of antigen-binding fragments from mAbs of different specificities and a common Fc region are being developed as efforts to achieve pan-reactive neutralization coverage [86–89]. These bispecific and trispecific antibodies act against HIV Env with multiple antibody specificities often with improved neutralization coverage over the individual parental antibodies and, importantly, when tested in NHP, were similar in pharmacokinetics comparable to those of the parental bNAbs [86]. When tested for protection, the trispecific mAb engineered to interact with the CD4bs, MPER and the V1V2 glycan site, protected NHP against SHIVs that were resistant to single bNAbs alone [89]. Other biNAbs are being constructed with altered Fab arm flexibility by re-engineering the hinge domain variant of IgG3 [90]. Yet another approach has been to engineer the biNAbs to become positioned at the cell surface with Fab arms designed to link either an anti-CD4 or anti-CCR5 mAb to a gp41-specific bNmAb, creating an antibody-like inhibitor. Although not tested in NHP models, the 10E8v2.0/iMab protected humanized mice from HIV-1 intraperitoneal challenge and reduced virus loads when tested after established infection [82]. The approach is ingenious, providing adaptability and a multi-faceted attack for both prevention and treatment of HIV-1. Other constructs based on multiple targeting include entry inhibitors such as eCD4-Ig, a construct of the immunoadhesin form of CD4, CD4-Ig, fused to a small CCR5-mimetic peptide. Using AAV delivery to macaques, eCD4-Ig remained stable and functional for almost a year and protected macaques from repeated SHIV challenges [91]. This extremely broadly neutralizing molecule was shown to be less immunogenic and could be stably expressed in macaques with AAV for 40+ weeks and protected animals from repeated challenges with SHIV-AD8. Other groups have developed CD4-mimetics to sensitize infected cells to ADCC [92] or to redirect ADCC killing away from uninfected bystander CD4+ T cells [93,94], a surprising new discovery with therapeutic implications. The aim of using entry inhibitors is to sidestep the need for a conventional vaccine. Future

developments and increased efficacy with these tools will be extremely interesting to follow, especially in NHP models of preclinical testing.

## 2.8 Cell-to-cell transmission

Understanding the precise mechanism of HIV-1 passage through virological synapses may become an important consideration for NmAb applications because preventing cell-to-cell transmission will be an important defining characteristic of antibody potency in therapeutic or prophylactic treatments of HIV-1. Although bNAb effectiveness against cell-to-cell transmission by neutralization of transcytosed virus has been shown [95], a recent report raises an alarm regarding the reduced potency of some bNAbs in the face of cell-to-cell infection [96]. The group studied a panel of bNAbs and compared two molecular clones and two transmitted/founder (T/F) clones and compared their sensitivities to each bNAb in cell-free and cell-to-cell infection assays. The *in vitro* findings revealed that bNAbs have reduced neutralization potency in cell-to-cell infections compared to cell-free infections. These results imply that a fraction of virus can escape bNAb neutralization during cell-to-cell transmission, thus compromising full blocking of infection. With new imaging technologies that are being used in animal models it may be possible to visually follow systemic virus spread following infection [39,97] [98]. These technologies applied to studies testing immunotherapy strategies in NHP will be invaluable in assessing the potential for eradication of virus and cure possibilities.

## 2.9 Relevance of effector function in protective efficacy

As summarized in the sections above, there is a strong correlation between the *in vitro* neutralization of the challenge SHIV and *in vivo* protection in passive antibody studies in macaques, while the weight of the contribution by other antiviral activities in the absence of neutralization is still controversial [99]. Nonetheless, there is a growing appreciation for the protective role of antibody effector function in natural infection and vaccine induced responses in NHP [100–103], and reviewed in [104]. Moreover, additional correlates analyses of the RV144 trial suggested that specific qualitative features of the humoral response may be key to protective efficacy, identifying ADCC activity [105], as well as IgG3 antibodies associated with polyfunctional effector profiles [106] as potentially contributing to efficacy.

Antibodies have the ability to modulate the recruitment of innate immune effector cells by cell type and thus the responses induced [104]. ADCC is one of the main effector functions necessary for clinical efficacy of mAbs and is mediated largely through binding of the Fc portion of the Ig molecule to a set of closely related Fcγ receptors capable of activating or inhibitory activities. For HIV infection, the requirement for a particular recruitment may differ during stages of infection or modes of transmission. Also, several antibody characteristics may determine innate immune recruiting capacity including titer, affinity, epitope specificity, and polyclonality, by impacting the geometry and valency of the immune complexes formed. An important study compared bNAb ADCC activity against different scenarios of infection, including reactivation of virus from latent reservoirs. A range of Fc-mediated killing was measured in the panel of bNAbs tested, confirming that a difference in efficacies dependent on the Fab interactions with virions exposed on the surface of infected

cells [107]. Beyond these variable domain features that modulate the potency of the humoral immune response, antibodies provide instructions to the innate immune system on how to clear complexed antigens via their Fc-domain, providing an additional level of regulated and bidirectional control over activity with isotype/subclass, and Fc glycan choice. A recent multi-lab study evaluated a set of 140 blinded IgG samples purified from HIV infected subjects using different FcγR-mediated assays. Surprisingly, ADCC-inducing antibodies were found to be highly enriched in elite controllers (EC) and were shown to coordinate the recruitment of ADCC, monocyte and neutrophil phagocytosis, and complement [108]. Enhanced FcRn function has been shown to not only increase *in vivo* half-life, but also to increase gut mucosal tissue localization, resulting in superior NmAb-mediated protection of macaques against SHIV challenge [109]. Together, the work highlights the critical nature of functionally coordinated antibodies in virus control.

A recent study with non-neutralizing mAbs containing mutations to enhance Fc effector function was performed in NHP [110]. However, even though effector function was enhanced, passive infusion of the non-neutralizing mAbs did not protect animals from infection, but did significantly reduced the number of T/F genomes. This suggests that antibodies that bind HIV-1 Env but fail to neutralize virus in traditional neutralization assays may act against infectious virions by limiting the number of T/F viruses during transmission or, as previously reported, by the ability to capture infectious virions [56].

Despite the growing body of literature that supports the importance of Fc-dependent antibody activities, the impact of FcγR genotype variability in NHP on vaccine efficacy and passive transfer studies of monoclonal or polyclonal therapeutics is still being evaluated. Only recently, studies are beginning to define key similarities and differences between NHP and humans in terms of antibody and effector cell biology. These findings are fundamental to advancing our understanding and correct translation of data from NHP passive studies. A comprehensive analysis of IgG binding characteristics to human and macaque FcγRs has been performed with variants of potent bNmAbs [111]. These data provide us with the expertise and molecular details to customize Fcγ modifications of the human mAbs for optimal interactions with macaque FcγRs *in vivo* [112]. These discoveries should help in identifying the extent to which the NHP model accurately reflects human mechanisms of protective responses, and they are necessary to provide a biological and immunological foundation for animal cohort selection based on FcγR allotypic variations between human and macaques. These studies also underscore the importance of understanding the outcomes of NHP model studies in order to select more promising approaches for clinical trials. As noted above, the first clinical trials with human bNmAbs have begun [71,72].

### **3. Developing vaccines that overcome Env diversity by inducing broad humoral immunity**

#### **3.1 Understanding how HIV Env elicits antibodies to shared regions to enhance vaccine design.**

Inducing immune responses that are broad and potent remains a key objective for vaccines, and the types of antibodies that are elicited will be dependent upon the choice of Env(s) in

the vaccine. One of the major advances in the last few years has been to develop a framework by which we can evaluate potency and breadth of bNAbs and polyclonal responses using a standardized assay. Viral Envs can then be categorized in this system for their relative susceptibility to neutralization by various antibodies, termed “Tiers.” It is expected that a successful vaccine must induce NAbs potent enough to neutralize Tier 2 isolates, which constitute much of inoculum in human transmission events (T/F, discussed in Section 1). Our current thinking is that unless the polyclonal response raised by vaccination induces a majority of targeted and potent antibody responses, it will likely fall short of protection against virus exposure. There are a number of approaches aimed at developing an immunogen effective at eliciting these antibodies. At this writing, the polyclonal responses from vaccinated primates have primarily targeted autologous Tier 2 viral clones [113,114] and have shown limited breadth [115].

There has been progress in identifying the ideal properties of Envs to include in vaccines, in part, through understanding the development of bNmAbs in HIV infection of elite neutralizers. One advance has been to explore in detail the co-evolution of Env on the surface of virions and the antibody response, as diagrammed in Figure 1A and described in a recent review [116]. Developing Env immunogens that engage germline B cell receptors (BCR) of potent bNmAbs has recently emerged as an attractive strategy to drive antibody maturation, and understanding the development of antibody lineages leading to neutralization breadth of the kind seen in elite neutralizers. Elegant recent work demonstrated that T/F Envs expressed on the surface of the virion can bind to germline BCR [75]. However, the mechanisms at play to drive lineages toward specific epitopes may be more complex and varied and apparently do not necessarily include T/F viruses [117].

Important advances in understanding how the glycan shield limits access to the Env protein has come from structural studies of antibodies co-crystallized with recombinant trimers, and last year was no exception with several new revealing reports [76–79,81]. From a structural perspective, it has been tempting to speculate that we can design vaccines that will present desired epitopes and avoid those that are less desirable, as described in a series of recent reviews [7,118]. One key measure is to examine the immunogenicity of these Env proteins in NHPs, compared with recombinant scaffolds that expose regions of interest to the B cells, partially or fully.

### 3.2 Understanding Env delivery methods, constructs and adjuvants

Further advances have been made in vector and adjuvant development that can directly influence the cell tropism, magnitude, location, and type of antibodies induced. Some of the remaining obstacles to address include the following question: Which immunogen is the most effective for generating breadth - an Env that mimics nature with relatively disordered components, or an engineered Env that presents epitopes in an ordered structure? Even if we knew which Envs were most effective in eliciting breadth, there are many other factors that influence the magnitude and longevity of the antibody response. These include: the molecular form of Env, e.g. monomer, trimer, modified trimer, or protein embedded in liposome; the Env delivery method, e.g. protein, viral vector, DNA; the amount of Env used; the regimen and the adjuvant. Each of these may contribute in different ways and may affect

the ability to stimulate protective B cell memory. The following sections address these areas further.

### 3.2 Mimicking nature: natural Envelopes to mimic the pathway of bNAbs

A concept put forward as a strategy to “educate” the immune system of vaccinated hosts into eliciting bNAbs is to sequentially immunize with natural quasispecies sequences that have evolved during the development of breadth. The hypothesis behind this approach is that B cells target conserved, functional regions on Env by sequential exposure to viral variants and that this process can be recapitulated by chronological immunizations using specific circulating Envs isolated from these subjects. In recent years several groups, including our own, have tested this hypothesis using natural Env immunogens isolated from timepoints contemporaneous with or preceding the development of neutralization breadth. In the last year, we showed that Envs cloned from two unrelated HIV-infected subjects who developed moderate NAbs during infection could be used in co-immunization with gp160 DNA and gp140 trimeric Env protein. This Env- based vaccine using uncleaved trimeric gp140 proteins induced NAbs in vaccinated macaques that had modest breadth and the capacity to neutralize autologous Tier 2 virus. T<sub>FH</sub> activation and increasing avidity developed concomitantly during the 20 weeks of vaccination [113], which appears to be critical for optimal humoral responses [119] and was also observed for constrained Env immunogens, discussed below [120]. The commitment to sequential boosting by other groups has become increasingly innovative and has been combined with germline targeting strategies with some success in manipulating antibody evolution in a controlled manner [121–123]. A further refinement in using natural quasispecies Envs is the B cell lineage approach based on mapping the co-evolution of autologous virus and neutralizing lineages over time [116]. Using this process, it has been possible to select the rare autologous Env variants that participated in the selection of bnAb precursors by aligning their sequential engagements (including the unmutated common ancestor). Env immunogens can then be chosen with features shown to be involved in natural induction of bnAbs *in vivo*. Such mapping analyses also revealed the existence of cooperative lineages for the development of bnAb responses [124]. Therefore, these studies reveal how the earliest development of NAbs may inform how breadth can intensify and target conserved determinants to generate heterologous NAbs in vaccinees. Very recently this concept was tested in macaques immunized with CH505 lineage proteins delivered sequentially as gp140 trimers, where some modest neutralization of heterologous tier 2 viruses was observed in a single macaque [125].

### 3.3 Engineered Env immunogens that expose key epitopes

An important limitation of the HIV vaccine field is the difficulty in generating Env immunogens that can recapitulate the native Env conformation as seen on the functional viral spike. Natural Env trimers have been described as too flexible or metastable, and it has been proposed that Env on virions “breathes” by oscillating between the closed (pre-fusion), and open (post-fusion, CD4 receptor bound) states. The more open conformation allows greater access to the trimer surface by weaker, less potent NAbs and the closed conformation restricts access only to bNAbs. Therefore, several groups began to design Envs that could address this problem [126–128]. More recently, further improvements have led to modified native-like Envs that favor this closed state by increasing trimer stability and constraining its



conformation to induce NABs that exclusively interact with epitopes targeted only by bNABs [129,130]. Thus in theory, these native-like Env immunogens will elicit NABs that can neutralize Tier 2 virus, and would have the greatest chance for protection efficacy when tested against challenge with a heterologous Tier 2 virus.

The first generation clade B, YU-2 and JRFL gp140-Foldons were soluble trimers in which the transmembrane gp41 domain was replaced by the foldon motif (derived from T4 bacteriophage Fibrin) in order to improve trimer stability. Native flexibly linked (NFL) trimers were then developed to contain a flexible peptide covalent linkage of Env subdomains to produce soluble, homogeneous, and cleavage-independent Env mimics that elicit higher immune responses [131]. Vaccine-elicited mAbs in NHP vaccinated with the NFLs and reverted to their most likely inferred germline state could bind these gp140 immunogens, indicating progress with this strategy [132]. Env gp140 foldons were characterized as immunogens by cloning antibodies from immunized macaques using single B cell sorting [133]. Characterization of these mAbs showed that, the antibody response in NHPs is highly polyclonal and different from the bNABs in infected human elite neutralizers due to its lower level of SHM and distinct binding specificities compared to bNABs. This powerful technology is very important as a tool to understand how well vaccines function in primates, as the resulting mAbs can be compared with mAbs cloned from infected or vaccinated humans [134].

Another spike mimetic design is the cleaved recombinant BG505 SOSIP.664 trimer based on a T/F clade A Env sequence containing a covalent disulfide bridge between gp120 and gp41 and an I/P mutation in gp41 to improve trimerization and stability, summarized in a recent review [129]. Compared to uncleaved gp140 trimers, BG505 SOSIP best recapitulates the native Env spike on the viral surface as demonstrated by extensive structural and antigenic characterization. This SOSIP strategy has been applied to Env trimers from multiple clades (A, B, C) and significantly increased constraint was reached in the SOSIP.v4 constructs to reduce presentation of V3 and other non-neutralizing epitopes [135,136]. In immunized rabbits, these modifications significantly increased neutralization of autologous Tier 2 viral isolates but their effect on heterologous neutralization was modest [125,136]. In addition, the autologous NAb response against BG505 SOSIP.664 targets a glycan shield hole (residue 241) rarely present in Env populations, thus raising the concern of skewing the immune response towards rare epitopes [137]. NAb development best correlated with booster immunization GC B cell magnitude and Tfh characteristics of the Env-specific CD4 T cells [120]. Encouraging evidence for improvement in inducing Tier 2 NABs was observed in macaques immunized with BG505 SOSIP.664 immunogens delivered by the subcutaneous route in an extended regimen [115].

Other engineering approaches have been developed including immunogen modifications to increase interaction with BCRs. In knock-in mouse models, the BG505 trimers were engineered to gain the ability to engage the germline BCR of PGT121 NmAb *in vitro* and *in vivo* [123]. A reengineered version of this trimer, BG505 SOSIP.v4.1-GT1, was shown to bind to multiple bNAB germline precursors and to activate B cells in two different knock-in mouse strains [138]. Iterative rounds of computational design and *in vitro* antigenic testing resulted in the engineering of eOD-GT6, a gp120 outer domain construct able to interact

with germline and mature CD4 binding site BCRs, capable of priming VRC01-class responses in knock-in mice [139].

Additional approaches that have shown some promise include the use of synthetic liposomes for native-like trimers [130], the use of chemical cross-linking stabilized native-like trimers [140]. Other groups have made scaffolds based on natural self-folding proteins that have a multimeric or trimeric structure and can theoretically direct immune responses to the displayed regions MPER [141] or V1V2 [142] and have tested these in rabbits, but few of these have advanced to testing in NHP models.

### 3.4 Advances in adjuvants and vectors to enhance antibody responses

Research efforts have also focused on the development of new adjuvants to add to the very limited number licensed currently for human use. Recently, different adjuvants were compared head-to-head for their effect on Env protein vaccination in rhesus macaques. Binding and Tier 1 neutralizing antibody titers were increased by alum/TLR7 and by MF59 alone or with TLR4 while MF59, poly IC:LC, and ISCOMs (immune stimulating complexes) transiently improved Env-specific memory B cells. However, there were no changes in CDRH3 length or SHM, two antibody features associated with bNAb development [143]. MF59 was further evaluated for efficacy in NHPs vaccinated with the components of the RV144 clinical trial (ALVAC canarypox vector prime and Env gp120 modified prime-boost), since this adjuvant is included in the HVTN 100 clinical trial underway in South Africa to test the RV144 approach with a clade C Env immunogen. Surprisingly, vaccine efficacy against SIV intrarectal challenge was not associated with the MF59 adjuvanted responses despite being higher than alum-adjuvanted responses, the adjuvant used in the RV144 trial [144]. V2 specific antibodies appeared important but there were other important signals of protection, including induction of Env-dependent intestinal lymphoid cells. However, antibodies from MF59-adjuvanted ALVAC/gp120 vaccinated NHPs captured more infectious virus than the alum group, resulting in a higher number of T/F strains [57]. Thus understanding the relative importance of neutralizing and capture antibodies in mucosal acquisition models remains a key goal of adjuvant and antigen design for HIV vaccines.

The partial efficacy of the RV144 clinical trial [27] also increased interest in the development of viral vector vaccine delivery systems. In particular, the double-stranded DNA poxvirus vectors (MVA, NYVAC and ALVAC), which have an extensive safety record, have been used as priming agents to elicit T cell responses. In a recent head-to-head comparison between NYVAC and ALVAC, the vaccine regimen with NYVAC was shown to induce stronger cellular and humoral immune responses [145]. Recently, a DNA-MVA-protein vaccine was shown to elicit mucosal immunity in LNs [110]. Another type of viral vectors actively pursued is adenovirus (Ad) since it is able to target mucosal sites, an important feature for HIV vaccines. Adenoviruses can infect dividing and nondividing cells and generate humoral and cellular immune responses while also accommodating large transgenes. However, the main drawback is the anti-vector immunity in particular to highly prevalent serotypes such as human Ad5, which was included in the failed STEP trial. This issue was partially resolved by using serotypes with lower prevalence such as human Ad4,

Ad26, Ad35 and A48. Ad26, Ad35 and Ad48 are non-replication competent whereas Ad4 is replication-competent which has the potential to better mimic natural HIV infection and to act as adjuvant. Ad26 used in an Ad prime/protein boost approach recently showed 50% protection against SIV intrarectal challenge [111]. Another solution was to develop Ad vectors based on Ads from other species (chimpanzees and rhesus macaques), but these vectors do not replicate in humans and may not confer immunity that is as potent. There was evidence for significantly better viral control in a recent study that combined either human Ad4 or simian Ad7 expressing Gag and Env with recombinant Env protein [146].

#### 4. Expert commentary

In 1984, vaccine researchers had little reason to doubt the proclamation by Health and Human Services Director Margaret Heckler and NIH researcher Dr. Robert Gallo that an HIV vaccine would be forthcoming in two years, heady from the development of the first recombinant hepatitis B vaccine. Within a few years, however, they came the realization that the road to a successful vaccine would be much longer. HIV has proven to be a formidable nemesis to vaccinology, much more challenging than predicted. Disagreements within the field developed among researchers about whether the vaccine should be designed to elicit cellular or humoral immunity delayed the testing of combination approaches. The importance of generating both types of immunity is now well appreciated, and a great deal of progress has been made in vector development as well as DNA and RNA vaccines, some of which has led to rapid clinical vaccine development for emerging viral outbreaks such as Zika [147,148]. Adjuvant development is focused on enhancing innate responses [149] and understanding how different adjuvants affect antibody specificities [150,151]. Much of the progress using Env-based approaches stems from continued refinements to NHP models that led to a better understanding of the potency and breadth of antibodies needed for protection from infection, which cannot be achieved by T cell-directed vaccines. By using the current collection of human bNAbs, it is now possible to predict which combinations of mAbs targeting different epitopes are most effective in preventing infection against strains from many different clades. This information is guiding the development of vaccines that will optimally expose these conserved regions to maximize protective immunity. There is firm evidence from NHP models supporting the inclusion of Env in HIV vaccines to elicit protective antibodies as well as to enhance the potential for viral control. Furthermore, these models have provided insights into other benefits of antibodies through analysis of passive transfer studies with antibodies that did not confer sterilizing immunity. Testing vaccine sera/IgG for protection in primates may be a next step forward as a strategy for understanding potency and breadth. Beyond measuring titers of NAbs in sera of vaccinated macaques, the HIV antibody field has moved forward with new ways to evaluate induced immunity e.g., focusing on the development of mucosal immunity, quantifying the activation of  $T_{\text{fh}}$  cells in germinal centers, and defining the kinetics and distribution of viremia in blood and tissues of vaccinated NHP after viral challenges. These types of studies, along with new imaging technologies [98] and advances in immunogen design and delivery are moving the field to a more comprehensive understanding of vaccinology and may lead to contributions to HIV cure research. The contributions of NHP models to HIV vaccine pursuits are both direct and indirect and argue for more investment into discovery research to understand

mechanisms critical to HIV vaccine and immunotherapy success, as part of model validation, so that the current pandemic can be more rapidly contained and eliminated.

## 5. Five year view

The lessons learned from passive antibody studies in primate models and prophylactic vaccines in nonhuman primates and humans have provided a large body of data to serve as guidelines to inform the potency of immunity necessary for protective versus controlling HIV vaccines. However, until there is a highly successful trial that shows more than 50% protection, primate challenge models and *in vitro* assays for antibody activity cannot be validated. In the next five years, the outcomes from both the VRC01 PrEP trial, as well as the poxvirus plus protein HIV vaccine trial in South Africa will be available, and antibody correlates for protection from infection should be more clearly defined. It is highly likely that antibody cocktails or specifically engineered antibody-based molecules with multiple specificities will be developed that can be used as relatively infrequent PrEP, much as the long-lasting drugs in development today. We propose that antibody-based therapy may well supplant drug therapy in certain instances, where the subject requires a drug holiday for treatment of another disease, or due to disabling side effects. In the setting of mother-to-child-transmission, long lasting antibodies may provide a welcome opportunity to breastfeeding babies born to HIV-positive mothers without fear of transmission.

At a minimum, a protective vaccine-elicited polyclonal antibody response can be expected to be potently neutralizing and broadly cross-reactive, and this goal will be achieved only after immunogens are designed to engage the appropriate B-cell receptors to induce antibodies of the desired specificity. Anamnestic responses will arise too slowly requiring titers of pre-existing NAb ready for action, preferably at mucosal sites. Neutralizing IgG or potentially IgA should be present in the gastrointestinal and genitourinary mucosal tissue, which will require establishment of long-lived plasma cells and mucosal homing factors. Avidity and persistence of the polyclonal response must be maximized. Affinity maturation of the polyclonal response in vaccinees promoted by the immunization regimen will be necessary in order to develop high affinity antibodies and autoreactive B cells must be marginalized or deleted. Many of the NAb isolated from HIV+ subjects after years of infection have extensive somatic mutations which may be impossible to replicate by immunizations. We also note for thoroughness in discussion of a protective vaccine response, that both arms of the adaptive immune response, cellular and humoral, will be needed to provide durable immunity. Cytotoxic T cell responses are also needed to eliminate infected cells that have slipped through a NAb blockade. Memory T cell responses are also essential, and the generation of T follicular helper (T<sub>FH</sub>) cells is clearly important for NAb development. The best hope for vaccine designers may be to aim to elicit less extensively 'matured' antibodies that bind to a few critical epitopes on Env and augment with potent mucosal T cell anti-HIV responses. If it is possible to generate a protective antibody response to HIV-1 by vaccination, we predict that it will be comprised of multiple epitope targeting, possibly necessitating multiple immunizations. Nonetheless, in the same time frame, we propose that Env immunogens effective at generating antibodies directed at conserved regions will be discovered, and the pathways for generating very broad antibodies *in vivo* will be delineated.

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### Reference annotations

\* Of interest

\*\* Of considerable interest

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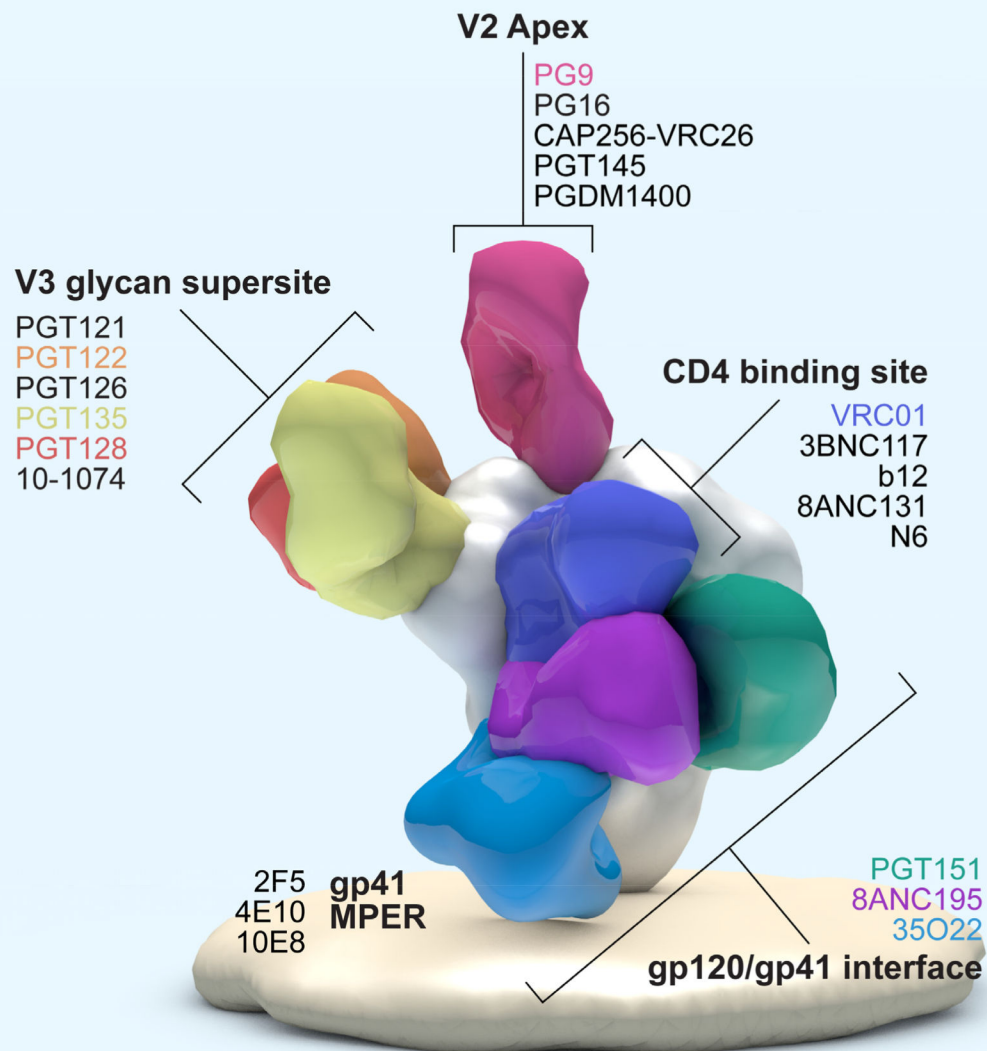
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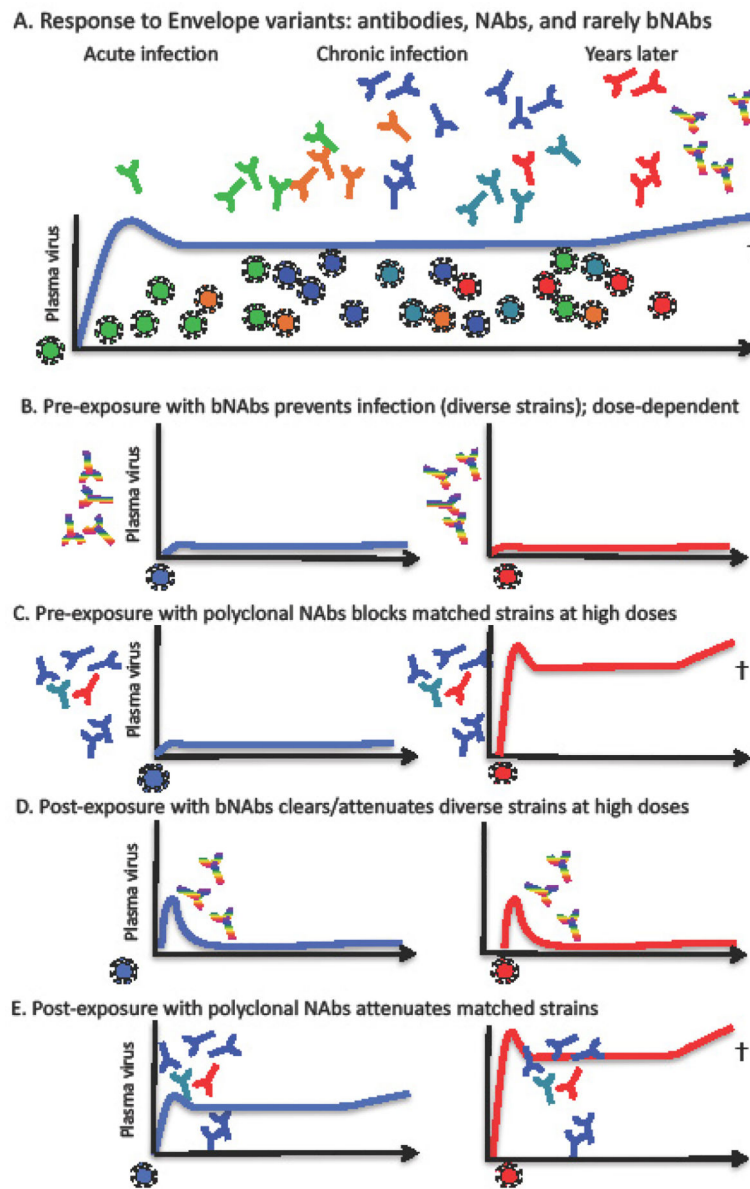
## 6. Key issues:

- Despite imperfections, primate models that use SIV and SHIV have been instructive in understanding the necessity for HIV antibodies to prevent infection in models of HIV infection in macaques.
- New SHIV isolates are available based on transmitted/founder viruses that are designed to interact more efficiently with rhesus CD4, thus enhancing virus entry and replication.
- Neutralizing antibodies (NAbs) have multiple effects on infection in NHP models: blocking, modulating immunity, and killing infected cells; non-neutralizing antibodies have yet to demonstrate protection from infection.
- Polyfunctionality of antibodies may contribute to viral control through cell killing mechanisms via the Fc portion of the molecule.
- The window for antibody treatment leading to virus clearance appears to be only a few days after virus exposure in NHP models; new technologies are helping to define viral kinetics.
- Achieving durable reductions in viral load in blood and tissues with passive bNAbs during established, chronic infection appears to be challenging.
- Bispecific and trispecific antibodies and mimetics are promising therapeutic approaches.
- Several novel strategies for Env presentation to obscure non-neutralizing regions while exposing neutralization epitopes are in testing.
- Iterative testing of vaccines in NHP models, followed by cloning of mAbs from the macaques, is yielding valuable information about antigen presentation and the pathways to broadening of the immune response.
- Prime-boost vaccines modeled after RV144 have provided similar levels of protection, adding confidence to the NHP models.
- In NHP models, no vaccines tested to date have been shown to elicit NAbs that are capable of blocking Tier 2 virus strains, and more research is needed to break this barrier.



**Figure 1. Antigenic sites on HIV-1 Envelope targeted by bNAbs**

Shown are binding locations for bNAbs to the CD4-binding site; to a high-mannose-patch referred to as the V3 glycan supersite; to the V2 apex; and to the gp41 membrane proximal external region (MPER) of HIV-1 Envelope. bNAbs associated with each of these determinants are listed. This figure is adapted from Burton and Mascola, 2015 [157].



**Figure 2. Roles of antibodies at various stages of HIV infection**

Schematic representation of the development of neutralizing antibodies (NAbs) and broadly neutralizing antibodies (bNAbs) during HIV-1 infection. Shown in panel (2A) is the depiction of the co-evolution of autologous antibodies and viral variants over time during infection. The first autologous NABs are shown in green, the same as the infecting virus. As Envelope variants develop during years of chronic infection, antibodies that match those viruses appear, lagging behind the appearance of the variants as depicted by the colors of the NABs. Antibodies emerge following each iteration of quasispecies development causing a delay in effective response to autologous variant Envs. In (2B), potent bNAbs (shown as rainbow colors to represent breadth against multiple clades and tiers of virus) have been shown to block infection when given before SHIV exposure to macaques. A comparison with polyclonal NABs is shown in (2C) where it is critical that the majority of the



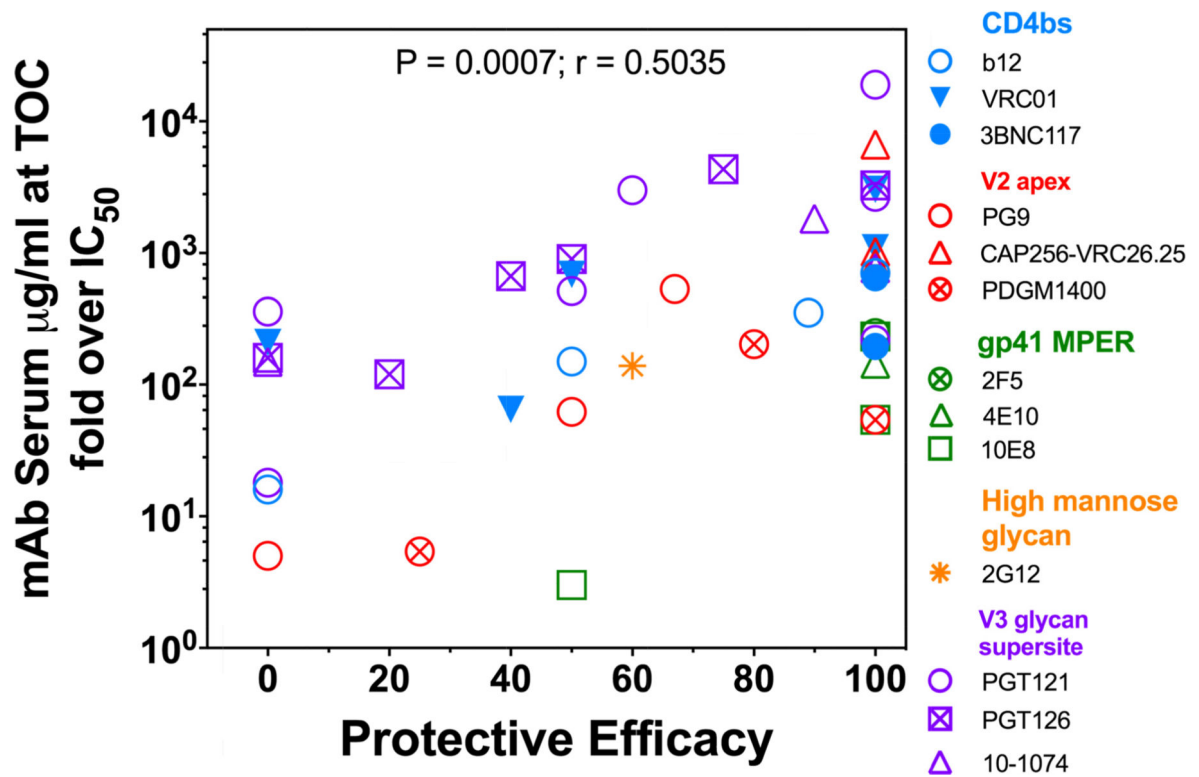
neutralizing activity is matched (or derived from) the challenge SHIV. In **(2D)**, bNAb cocktails, if given very soon after virus exposure can ablate early sites of infection. In contrast **(2E)**, even if closely matched, polyclonal Nabs can, at best, attenuate infection.

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**Figure 3. Correlation of neutralization potency and protection from SHIV challenge in macaque passive studies**

Data from multiple published passive antibody protection studies in macaques have been graphed showing the strong association between the potency of the antibody present in the sera at the time of challenge and the resulting protective efficacy. The fold over  $IC_{50}$  was calculated as the passive antibody serum concentration at time of challenge (TOC) divided by the antibody neutralization potency (50% inhibitory concentration,  $[ID_{50}]$  *in vitro*), in pg/ml. Details of each study depicted here are detailed in Table 1.

Table 1.

Summary of SHIV bNAb Passive Protection Studies in Macaques

Region of Env	Antibody	SHIV(Route) Tier(Clade)	Challenge dose (TCID <sub>50</sub> )	mAb dose (mg/kg)	IC50 (Mg/ml)/ Neutralization Assay	TOC serum concentration (Mg/ml)	Fold >IC50	No. Protected /Total	Protective Efficacy (percent)	Ref.
CD4bs	b12	SF162P4(IVAG) Tier 1(B)	300	25	1.0/rhPBMC	705	705	4/4	100	[51]
		5		149		2/4	50			
		1		16		0/4	0			
	VRC01	SF162P3(IVAG) Tier 2(B)	300	25	1.6/rhPBMC	563	352	8/9	89	[152]
				20		65	4/4	100		
				20		79	4/4	100		
		BAL(IR) Tier 1(B)	12,800	20	0.02	61	3,050	6/6	100	[153]
				5		22	6/6	100		
				0.3		1.3	4/10	40		
		AD8EQ(IR) Tier 2(B)	1,000	50	0.94/TZM-bl	649	690	1/2	50	[153]
				20		197.3	0/2	0		
				30		350.9	1/2	50		
3BNC117	AD8EQ(IR) Tier 2(B)	1,000	5	0.14/TZM-bl	91	650	2/2	100	[153]	
			1		27.35	195	2/2	100		
			20		283.8	2,027	2/2	100		
	DH12-V3AD8 Tier 2(B)	1,000	5	0.14/TZM-bl	123.85	885	1/2	50		
			1		26.45	650	1/2	50		
			0.2		4.8	195	0/2	0		
CAP256-VRC26.25	325c(IVAG) Tier 2(C)	500	2	0.003/TZM-bl	20	6,667	4/4	100	[53]	
			0.4		2.5	833	4/4	100		
			0.08		0.75	250	4/5	80		
PGDM1400	BAL-P4(IR) Tier 1(B)	12,800	2	0.037/TZM-bl	7.5	203	1/4	25		
			0.4		2	54	5/5	100		
			0.08		0.2	5.4	4/6	67		
VIV2	PG9	BAL-P4(IR) Tier 1(B)	20	0.06/TZM-bl	32	533	3/6	50	[50]	
			5		3.7	62	3/6	50		

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Region of Env	Antibody	SHIV(Route) Tier(Clade)	Challenge dose (TCID50)	mAb dose (mg/kg)	IC50 (Mg/ml)/ Neutralization Assay	TOC serum concentration (Mg/ml)	Fold >IC50	No. Protected /Total	Protective Efficacy (percent)	Ref.
V3 glycan	PGT121	SF162P3(IVAG) Tier 2(B)	300	0.3	0.005/TZM-bl	0.28	5	0/6	0	[49]
				5		95	19,000	5/5	100	
	[153]		AD8EO(IR) Tier 2(B)	1,000	1	0.1/TZM-bl	15	3,000	3/5	60
					0.2		1.8	360	0/5	0
					20		260.75	2,608	2/2	100
					5		51.45	515	1/2	50
					1		21.85	219	2/2	100
					0.2		1.8	18	0/2	0
					20		207.9	20,790	4/4	100
					1		22.2	2,220	1/2	50
					0.2		2.35	235	2/2	100
					0.05		1.15	115	0/2	0
MPER	PGT126	SF162P3(IVAG) Tier 2(B)	300	10	0.03/TZM-bl	98	3,267	5/5	100	[154]
				2		20	667	2/5	40	
				0.4		3.6	120	1/5	20	
				10		130	4,333	3/4	75	
				2		27	900	2/4	50	
				0.4		4.8	160	0/4	0	
	10-1074	AD8EO(IR) Tier 2(B)	1,000	20	0.15/TZM-bl	273.75	1,825	2/2	100	[153]
				5		768	768	2/2	100	
				1		149	149	0/2	0	
				20		231.8	3,863	2/2	100	
				5		95.95	1,599	2/2	100	
				1		23.5	392	1/2	50	
Man-9glycans	2G12	SF162P3(IR) Tier 2(B)	500	40	7.6/TZM-bl 200/rhPBMC	1053	139	3/5	60	[155]
				5		326	0/2	0		
MPER	10E8	BAL.P4(IR) Tier 1(B)	12,800	20	0.57/TZM-bl	133	233	6/6	100	[50]
				5		31	54	6/6	100	

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Region of Env	Antibody	SHIV(Route) Tier(Clade)	Challenge dose (TCID50)	mAb dose (mg/kg)	IC50 (Mg/ml)/ Neutralization Assay	TOC serum concentration (Mg/ml)	Fold >IC50	No. Protected /Total	Protective Efficacy (percent)	Ref.
				0.3		1.8	3	3/6	50	
	4E10	BAL(IR) Tier 1(B)	2.000	50	0.8/TZM-bl 6.0/rhPBMC	866	1,125 144	6/6	100	[156]
	2F5	BAL(IR) Tier 1(B)	2.000	50	0.4/TZM-bl 3.0/rhPBMC	742	1,855 247	6/6	100	