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Use of broadly neutralizing antibodies for HIV-1 prevention

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Summary

Antibodies have a long history in antiviral therapy, but until recently they have not been actively pursued for HIV-1 due to modest potency and breadth of early human monoclonal antibodies (MAbs) and perceived insurmountable technical, financial, and logistical hurdles. Recent advances in the identification and characterization of MAbs with the ability to potently neutralize diverse HIV-1 isolates has reinvigorated discussion and testing of these products in humans, since new broadly neutralizing MAbs (bnMAbs) are more likely to be effective against worldwide strains of HIV-1. In animal models, there is abundant evidence that bnMAbs can block infection in a dose dependent manner, and the more potent bnMAbs will allow clinical testing at infusion doses that are practically achievable. Moreover, recent advances in antibody engineering are providing further improvements in MAb potency, breadth and half-life. This review summarizes the current state of the field of bnMAb protection in animal models as well as a review of variables that are critical for antiviral activity. Several bnMAbs are currently in clinical testing, and we offer perspectives on their use as pre-exposure prophylaxis (PrEP), potential benefits beyond sterilizing immunity, and a discussion of future approaches to engineer novel molecules.

Keywords

HIV-1 antibodies; pre-exposure prophylaxis; Fc-effector functions; Neutralizing antibodies; SHIV protection

Introduction

During the early part of the 20th century, scientists first began to understand the nature of antibody-based immunity. Paul Ehrlich and Ilya Mechnikov shared the 1908 Nobel Prize in Physiology and Medicine for establishing the concept that antibodies in the blood can neutralize poisons or toxins. Just prior to this, Emil von Behring had been awarded the first Nobel prize (in 1904) for serum therapy, and both Ehrlich and von Behring contributed to the concept and use of serum therapy – the transfer of blood serum to treat diseases such as diphtheria and tetanus (1, 2). After the introduction of antibiotics in the late 1930's, the use

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of serum therapy declined, but subsequent improvements in immunoglobulin purification allowed antibody products to continue to play a major medical role against viral diseases – mainly to prevent infection *via* administration prior to exposure (e.g., hepatitis A, respiratory syncytial virus), or as early post-exposure prophylaxis (e.g., hepatitis B, rabies, varicella-zoster) (3). Importantly, in some cases, an effective vaccine has superseded the need for immunoglobulin prophylaxis (e.g. hepatitis A). Currently, there is only one licensed monoclonal antibody (MAb) product against a viral pathogen (Palivizumab) that is used to prevent respiratory syncytial virus (RSV) disease in high-risk infants. However, the ability to rapidly isolate and produce human antibodies has led to renewed interest in the development of additional MAb products against viral and bacterial pathogens, including Ebola, cytomegalovirus, *Bacillus anthracis*, *Clostridium difficile* and *Staphylococcus aureus* (4–11). The recent demonstration that Ebola MAbs can clear infection in primate models offers hope for this devastating disease and illustrates another example where passive immunotherapy can provide significant benefit against a lethal viral infection (5, 6, 12).

This review will focus on the potential role of HIV-1 specific neutralizing MAbs in preventing HIV-1 infection. The focus on passive immunoprophylaxis for HIV-1 arises, in part, from the difficulty in eliciting cross-reactive neutralizing antibodies (nAbs) by current vaccine immunogens, but also from research over the last 5–6 years demonstrating the isolation and characterization of highly potent and broadly reactive MAbs from B-cells of selected HIV-1 infected individuals. These neutralizing MAbs (nMAbs) provide robust protection in non-human primate challenge models at levels that can likely be readily be achieved by intermittent passive immunization of humans (13–15). In addition, engineering to improve both potency and circulating half-life of MAbs could further enhance their potential to protect (16).

Antibody development in response to HIV-1 infection

HIV-1 establishes a permanent infection by reverse transcribing and integrating its genome into that of the host as the first step toward producing new viral progeny. However, the progression to disease (in the absence of therapy) is highly variable in individual human subjects, even in subjects that are infected from the same virus source. This variation depends upon a number of key variables that will ultimately affect how much virus is produced—including but not limited to host MHC genetics (17) and the number of activated target cells available for virus spread from the site of exposure. When tested for virus in the plasma, subjects who have lower levels of virus experience a slower loss of $CD4^+$ T cells and disease progression compared with subjects who have higher levels of virus production (18). This landmark study, and those that followed, provided the foundational knowledge that led to the use of effective antiretroviral treatment (ART) that target the virally-encoded enzymes to reduce viremia and slow or prevent disease progression.

Virus vs host dynamics

As the virus replicates, both arms of the adaptive immune response are stimulated to generate T cells and antibodies, but these fail to clear HIV-1, since the infected cell population is well established in tissues (19, 20), likely within a few days of exposure (based

on primate model data), and prior to the development of immunity. HIV-1 infection is further aided by the rapid appearance of viral variants over time, termed the quasispecies (21). These quasispecies variants derive from the transmitted founder viruses (22), which are then subjected to immune selection by the developing adaptive response, including nAbs (23, 24). This complex interplay of viral variants and host immunity leads to an evolving situation in which the virus can evade responses but must remain fit enough to infect new cells. As shown in Figure 1, the timing of the development of antibodies (Abs) that can neutralize or directly block infection of HIV-1 *in vitro* depends upon exposure to the Envelope (Env) glycoprotein during infection. The degree of antigen exposure is proportional to the plasma virus levels, with subjects who have moderate to high levels of viremia—Progressors—developing nAbs more rapidly than in subjects who maintain very low viremia for >10 years—Slow Progressors. Individuals who tightly control viremia—Controllers—are quite rare and often have poor Ab responses. The role of viral variants in driving immunity is illustrated by the differences observed in the speed of development of nAbs, as well as how the nAbs change over time to recognize the new variants. HIV-1 evades antibodies through fixing mutations that change or obscure neutralization targets on the Env, for example by shifting or adding sites for glycosylation at or adjacent to one of these sites.

Autologous nAbs and viral control

Despite the production of highly potent nAbs by B cells in some individuals, their virus is not cleared or fully contained, likely due to ongoing viral escape from both nAb and $CD8^+$ T cell responses. This leads to the well-described phenomenon of autologous virus neutralization escape, i.e., an individual's circulating plasma virus is resistant to co-circulating nAbs (23, 25). This has led researchers to suggest that antibodies are of limited importance in HIV-1 infection and control. However, both the T-cell and antibody response induce ongoing selective pressure on the viral quasispecies – and the very nature of a chronic lentiviral infection involves escape from the adaptive immune response. In addition, the virus continues to evolve, even under conditions of prolonged ART (26). Thus, not unlike $CD8^+$ T cells, it is likely that antibodies play a role in limiting the rate of viral replication. Of note, there is at least one clinical case showing that serum nAbs contribute to viral control; Reduction in nAbs and viral rebound occurred concomitantly *in vivo* when CD20 cells were depleted (27). However, taken together, these data suggest that nAbs would be most effective if used as pre-exposure prophylaxis prior to the time of establishment of the viral reservoir. There were early efforts to use purified polyclonal immune globulin from HIV-positive subjects to test whether antibodies could prevent infection or affect established infection. This material, called HIV Immune Globulin or HIVIG, differed in nAb potency from batch to batch due to the source of the antibodies, and early studies in humans resulted in limited and no permanent impact upon viremia in treated adults or upon infection in mother to child transmission (28–30). It was thus incumbent upon researchers in the field to discover human MAbs that could substitute for, and improve upon, the potency of HIVIG.

Broadly neutralizing Ab development in infection

Understanding the potency and targeting of nAbs in infected subjects has been valuable for locating regions in HIV-1 Env that may be an Achilles' heel of the virus, as described in the next section. There is good evidence that antibody responses are robust and that nearly all

subjects neutralize the infecting (autologous) virus (Figure 2). Within 2 to 3 years after infection, the sera from ~50% of infected individuals can neutralize significant numbers of heterologous virus isolates (31), either due to development of an individual antibody lineage with broad reactivity or due to the cumulative effect of many individual nAbs (avidity of the polyclonal response) (32, 33). A small proportion of HIV-positive subjects eventually develop highly potent cross-reactive nAbs that target epitopes shared amongst the diverse clades of HIV-1 worldwide. The individual broadly neutralizing MAbs (bnMAbs) isolated from these subjects have been critical to enhancing our understanding of Env structure and mechanism of HIV-1 neutralization which has led directly to detailed structural information of the neutralization epitopes on the Env trimer and allowed for studies of antibody ontogeny, i.e. how such nAb lineages arise and evolve to achieve broad and potent neutralization (34). The role of bnMAbs in protection is discussed in detail in later sections of this review. Molecular examination of the Env variants in individual subjects, including the transmitted founder (T/F) Env, has provided further insights into the antigenic stimulation of specific nAb lineages (24, 35–37), and this remains an area of intense investigation (38).

Animal models for HIV-1 transmission

HIV-1 and SIV models

There are several animal models that have been used as a facsimile of HIV-1 transmission in humans. Nonhuman primate (NHP) hosts exposed to members of the primate lentivirus family are widely used and are the most closely related animal models to human transmission events, summarized in a recent review (39). HIV-1 replicates in chimpanzees, but disease and pathology require 10 years or more, and use of this model for AIDS research was discontinued decades ago. Host restriction factors prevent productive infection by HIV-1 in *Macaca* species (40). Simian immunodeficiency virus (SIV) is endogenous in African macaques but highly pathogenic in Asian macaques. Like HIV-1, transmission of SIV isolates is by mucosal and blood-borne routes and in contrast with HIV-1, results in rapid pathogenesis despite strong antiviral immunity. Cell-mediated immunity plays an important role in the resolution of acute SIV viremia, and post-acute viral control is affected by the MHC Class I haplotype, as with HIV-1 infection. Antibody responses are similarly elicited in both HIV-1 and SIV infection, with binding Abs detected prior to any autologous nAbs and bnAb responses found only after a year or more of infection. Although HIV-1 and SIV have similar receptor and co-receptor usage, the Env proteins are sufficiently different such that nAbs specific for SIV Env are not cross neutralizing for HIV-1, and vice versa.

Introduction of SHIV models

To effectively study HIV-1 bnMAbs in NHP models, it was essential to have a virus bearing the HIV-1 Env protein, and the chimeric SIV-HIV or SHIV viruses were developed for this use (41–45). In these models, chimeric SHIV viruses that express HIV-1 envelope in the backbone of SIV have been used to challenge NHPs *via* various routes that include intravenous (IV), intrarectal (IR), intravaginal (IVAG) and oral routes to model natural HIV-1 transmission. The challenges are carried out using either a single dose of the challenge virus that results in close to 100% infection in all naïve animals or a series of

multiple doses that results in infection of less than a third of the naive animals after each challenge. These models have been used to assess the efficacy of passive transfer of polyclonal IgG (HIVIG and SIVIG) and multiple anti-HIV-1 bnMAbs in providing protection against vaginal, rectal, and oral infection in macaques that range in age from infant to adult (Table 1). In studies of immunoprophylaxis by passive infusion, bnMAbs have been most commonly administered hours to days before challenge and have shown varying degrees of protection, likely impacted by the neutralization sensitivity of the challenge stock to the administered bnMAb as well as the epitope targeted on the HIV-1 Env (14, 15, 46–54).

Limitations and advances in SHIV development

A disadvantage of the SHIV infection model has been the limited number of SHIVs available and the variable sensitivity of these SHIVs to the different bnMAbs (Figure 3). For example, the widely used $SHIV_{SF162P3}$ is sensitive to most CD4 binding site (CD4bs) bnMAbs but is not sensitive to the V2-glycan reactive bnMAbs, e.g., PGDM1400 and PG9. This despite the fact that PGDM1400, targeting the trimer apex, can neutralize >75% of circulating HIV-1 strains at a very high potency. And even amongst CD4bs bnMAbs, $SHIV_{SF162P3}$ is sensitive to VRC01 and VRC07-523 but resistant to 3BNC117, and yet all three bnMAbs neutralize >90% of HIV-1 strains. Also, many current SHIVs are fairly resistant to the MPER-reactive bnMAb 10E8, despite the fact that the 10E8 neutralizes ~98% of HIV-1 strains. Thus, the neutralization phenotype of commonly used SHIVs does not recapitulate the phenotype of most transmitted strains of HIV-1, making it impossible to consistently interpret the results of any given SHIV challenge study to human exposure of diverse strains of HIV-1. Recent advances in the molecular construction of SHIVs have facilitated the construction of several new SHIVs derived from founder HIV-1 strains. These new SHIVs appear to have neutralization phenotypes more similar to HIV-1 strains than to older SHIVs and are sensitive to bnMAbs targeting multiple epitopes, thereby expanding the repertoire of SHIVs and bnMAb combinations that can be tested in passive transfer studies (55–57). Most SHIVs are clonal or oligoclonal in nature, i.e., they are derived from a molecular clone and passaged briefly *in vitro* or *in vivo*. However, some SHIVs have moderate genetic complexity similar to HIV: $SHIV_{SF162P3}$ that was isolated later in infection after variants had developed (58). Other new SHIV stocks may be developed to include characterized heterogeneous variants, as has been recently accomplished for SIVmac251 (59).

Humanized mouse models

In addition to NHP, humanized mouse models are extensively used to study HIV-1 transmission. These models involve reconstitution of severe combined immunodeficiency (SCID) mice with various combinations of human hemato-lymphoid cells, and thus allow for some level of HIV-1 replication. Though there have been recent improvements in the construction of humanized mouse models, the formation of secondary lymphoid tissues is limited and these models are not likely to mimic HIV pathogenesis in humans. Nonetheless, these models appear to be useful for passive immunoprophylaxis studies where the outcome is the presence or absence of HIV-1 infection. Humanized mice were, in fact, used in some of the earliest studies to demonstrate the protective efficacy of bnMAbs (60–62) and have

been used to demonstrate protection by many newer generation bnMAbs. (63–67). Complementing the findings in NHP models, experiments in humanized mice have demonstrated enhanced clearance of infected cells by bnMAbs, and this clearance was attributed to an FcγR-dependent mechanism (68).

Antibody protection studies in animal models

Polyclonal Ig studies

Early studies using polyclonal human immunoglobulin derived from HIV-1 infected donors (HIVIG) demonstrated that potency was critical for protection of chimpanzees from intravenous HIV-1 infection (69, 70). The first neutralizing antibody passive protection studies against HIV-1 primary isolates were conducted in the hu-PBL-SCID mice model (60, 62). One of the earliest studies in the SHIV challenge model used HIVIG derived from HIV-1 infected chimpanzees and a SHIV challenge stock that was based on the HIV-1 strain DH12 (71). Macaques infused with IgG from a HIV-1 DH12 infected chimpanzee, but not an HIV-1 IIIB infected animal, were protected against challenge. Importantly, DH12 IgG, but not the IIIB IgG was able to neutralize the DH12 challenge stock. These were some of the first *in vivo* data suggesting the importance of nAbs in protection against HIV-1.

Protection with first generation bnMAbs

Among the first anti-HIV-1 bnMAbs to show protection in SHIV models were the combination of first generation bnMAbs, 2F5 (gp41 MPER) and 2G12 (gp120 glycan-reactive), that were used together with HIVIG in adult macaques. While individual MAbs displayed partial protection, the combination of 2F5, 2G12 and HIVIG was most effective, providing complete protection against both intravenous and mucosal challenges (46, 52). A similar combination of 2F5 and 2G12 along with the CD4bs-targeting F105 MAb also provided protection against both systemic SHIV challenge as well as oral SHIV challenge in macaque adults and neonates, respectively (47). The use of a single bnMAb to protect against mucosal SHIV challenge was subsequently shown in the case of the CD4bs-targeting bnMAb, b12 (53). In fact, b12 has been used in multiple SHIV protection studies that have helped in understanding both the mechanism as well as durability of protection provided by bnMAbs (48, 49, 72). Other individual bnMAbs, including 2F5, 4E10 and 2G12, were also subsequently shown to protect in mucosal SHIV challenge models (50, 51). Although these studies provided proof of antibody-mediated protective efficacy, relatively high MAb infusion doses (>20 mg/kg) were used, with SHIV challenge performed one day after antibody infusion, when antibody concentrations in plasma were high, often substantially greater than 100 μg/ml. Thus, while these data with first generation anti-HIV-1 MAbs were encouraging proof-of-principle studies for protection with neutralizing antibodies, the amount of antibody needed and lack of data on durability of protection did not support the potential for their use in human populations.

A new generation of highly potent bnMAbs

Due to the foresight of investigators who established large cohorts of HIV-1 infected subjects, and the development of high throughput and accurate virus neutralization assays (73, 74), donors with potent serum neutralization were identified. With the advancement of

technologies to screen cultured memory B cells for neutralization and to isolate antigen-specific B-cells (75, 76), many potent bnMAbs have been isolated in recent years (76–81). The bnMAbs target previously known and newly discovered sites of vulnerability on the native HIV-1 Env trimer, comprised of gp120 and gp41 subunits, and potently neutralize a majority of circulating HIV-1 strains (82). These relatively conserved sites include: (i) the V1V2-glycan site at the apex of the Env trimer targeted by bnMAbs PG9, PGT145, PGDM1400 and CAP256-VRC26.25 (83–85), (ii) the V3-glycan site centered on the glycan at Asn332 targeted by bnMAbs PGT121 and 10-1074 (86, 87), (iii) an exclusively glycan epitope on the outer domain of gp120 targeted by 2G12 (88, 89), (iv) the CD4-binding site of gp120 targeted by the VRC01-class of bnMAbs that partially mimic CD4 receptor binding and include VRC01 and 3BNC117 (75, 76, 90), and additional non-VRC01 class CD4bs bnMAbs that neutralize by other modes of recognition (24, 91, 92), (v) the membrane-proximal external region (MPER) of gp41 targeted by 2F5, 4E10 and 10E8 (81, 89), (vi) an extended region including residues from both gp120 and gp41 between the MPER and gp120 protomers targeted by 35022 and PGT151 (93, 94) and more recently, (vii) the fusion peptide of HIV-1 targeted by VRC34 and PGT151 (95, 96). Importantly however, none of these bnMAbs neutralizes 100% of viral isolates and each bnMAb has a distinct profile of potency and breadth of neutralization (84, 97). This has led to substantial interest in the use of bnMAb combinations that would be complementary (98, 99). Moreover, due to the lack of effective HIV-1 vaccines and the recent discovery of multiple highly potent bnMAbs, significant enthusiasm in the field is growing to investigate the use of these bnMAbs in both prevention and treatment of HIV-1 infection.

Protection with next generation bnMAbs

The identification of newer generation bnMAbs with potencies 10 to 100 fold greater than earlier bnMAbs has renewed interest in their potential clinical use. One of the first studies with a newer generation bnMAb was conducted by Burton and colleagues using the V3-glycan directed antibody PGT121 (14). Using the $SHIV_{SF162P3}$ challenge virus, which is highly neutralization sensitive to bnMAb PGT121, sterilizing immunity was achieved in all animals administered 5 mg/kg and 1 mg/kg and three of five animals administered 0.2 mg/kg PGT121, with corresponding average antibody serum concentrations of 95 μg/mL, 15 μg/mL, and 1.8 μg/mL, respectively (Table 1). This study provided important proof of concept that newer generation potent bnMAbs could provide *in vivo* protection at lower serum concentrations than observed in previous studies (14). Many subsequent studies with next generation potent bnMAbs VRC01, VRC07-523, 3BNC117 (to CD4bs) and 10-1074 (V3-glycan) have also demonstrated protection (13–15, 54, 100–102).

Modifying bnMAbs for improved efficacy

Recent studies have also further delineated the parameters associated with protection including neutralization potency against the challenge virus and persistence of antibody in circulation. A potentially widely applicable approach to extend antibody half-life is to modify antibody binding to the neonatal Fc-receptor (FcRn) to substantially improve the *in vivo* half-life of a bnMAb and increase persistence of bnMAbs at mucosal sites of HIV-1 infection. The FcRn is related to the MHC class of molecules although it is not involved in antigen presentation (103, 104). Instead, it interacts with the Fc region of IgG and is

involved in recycling and transcytosis of IgG within cells. By its capacity to bind to the Fc region with high affinity at low pH found in lysosomal compartments, it prevents degradation of the IgGs when internalized by cells and thereby regulates the serum half-life of IgGs. This interaction is also involved in actively transporting IgGs to sites of pathogen encounter and plays a role in protection against infections. In the case of VRC01, the FcRn enhancing mutation -LS (M428L/N434S (105)) lead to 2–3 fold higher *in vivo* half-life in macaques compared to VRC01 with the wild type Fc region. Compared to VRC01, VRC01-LS also demonstrated increased persistence in the mucosal compartments leading to improved protection against mucosal SHIV challenge (106). This improved half-life also almost doubled the median protection time in a repeated challenge study after a single infusion of the bnMAb (100).

FcRn modulation of IgG in tissues

Hence, when bnMAb VRC01 was engineered to have increased affinity for FcRn (VRC01-LS), increased and prolonged levels of the antibody were detected in mucosal tissue compared to the unmodified VRC01 (106). In fact, VRC01-LS accumulates in vaginal tissues and localizes to the basal and parabasal layers of the vaginal epithelium, similarly to FcRn, as determined by immunohistochemistry. Presumably, FcRn-mediated transport increases the monoclonal antibody levels in this tissue, where it might similarly confer protection against infection.

Since human MAbs are foreign proteins when infused in macaque monkeys, their circulating half-life is much shorter than that of human IgG in humans. This rapid clearance limits the ability to study duration of antibody effect. To overcome this, Saunders et al. modified bnMAb VRC01 to be closer to the sequence of simian antibodies (a process called simianization), without substantially impairing its antigen combining site and neutralization potency (102). After passive administration, simianized VRC01-LS maintained higher concentrations in plasma and rectal tissue than unmodified simianized VRC01. Importantly, both simianized variants of human VRC01 protected animals against SHIV challenge two months after single or multiple antibody infusions. This demonstration that passive immunization with a potent bnMAb could provide sustained protection was an important first step toward the potential clinical use of bnMabs to prevent HIV-1 infection.

Relating bnMAbs to correlates of protection

Importance of neutralization

The studies noted above clearly demonstrate the ability of passively infused bnMAbs to provide complete protection against viral challenge, even when the challenge inoculum results in infecting 100% of control animals – a rate of infection known to be much greater than in the setting of human sexual transmission. A next obvious question is: what antibody function(s) can be directly associated with protection? We discussed above that more potent bnMAbs protect at lower serum concentrations than less potent first generation bnMAbs, and this suggests a key role for virus neutralization. Antibody levels have been determined by directly quantifying the concentration in the plasma just prior to SHIV challenge and these data strongly suggest that the *in vitro* neutralization potency of a bnMAb against a

challenge SHIV is positively associated with the level of protection. For most of the earlier generation of bnMAbs like b12, 2F5, 4E10 and 2G12, plasma concentrations of several hundred micrograms or more appeared to be required for sterilizing protection against high dose mucosal SHIV challenge. The more potent bnMAbs like VRC01, PGT121 and 10E8, provide complete protection at much lower concentrations, depending on their neutralization potency to the challenge SHIV, i.e., these newer bnMAbs neutralize the challenge SHIVs at IC_{50}s 100 to 1000-fold less than the early bnMAbs (14, 15, 48, 50, 51, 53). Thus, protection can be achieved with serum concentrations in the range of 1–10 μg/ml and sometimes lower (13, 14, 100) (Table 1). In protection studies using two SHIVs that have different sensitivities to VRC01, we demonstrated that the more resistant $SHIV_{SF162.P3}$ required a higher VRC01 plasma concentration to block acquisition than the more sensitive $SHIV_{BaL.P4}$ (15) (Figure 4). Hence, the ability of VRC01 to protect was associated with its neutralization potency against the specific SHIV challenge virus used. These data indicate that the neutralization sensitivity to the circulating viral strains should be a key parameter to consider when selecting a bnMAb for use in international prevention trials. The recent identification of exceptionally potent bnMAbs like PGDM1400 (83) and CAP256.VRC26.25 (84) that are 10-fold more potent than any of the previous bnMAbs, suggests that protection at concentrations lower than 1 μg/ml may be possible in these SHIV models, with the caveat that these mAbs do not neutralize all strains of HIV-1. In addition, several recent studies have used repeated low dose SHIV challenge, which may be more reflective of natural HIV-1 exposure in humans. Gautam, Martin and colleagues performed an elegant but simple study, using a single infusion of the potent mAbs VRC01, 3BNC117 and 10-1074, followed by iterative rectal challenge that resulted in infection of all controls at 6–12 weeks. The study also included the longer-half life version of VRC01 (VRC01-LS). This study confirmed the importance of bnMAb potency and half-life in protection and provided additional data demonstrating that relatively low levels of plasma antibody were associated with protection. In total, these studies confirm that relatively low serum concentration of bNAbs can protect and suggest that a single infusion of a bnMAb could provide long lasting (weeks to months) protection (49, 100).

Assessing protective in vivo titers

Regarding virus neutralization as a key component of protection, it is instructive to assess not only the plasma MAb level at the time of challenge, but also the plasma neutralization IC_{50} value. However, there are various formats used to measure i*n vitro* neutralization, which may complicate cross-study comparisons. One common and highly standardized assay format involves the use of lentiviral vectors pseudotyped with the HIV-1 or SHIV Env. However, Env-pseudotyped viruses are clonal (one Env sequence) and may not represent the quasispecies of Env in the challenge SHIV stock. Thus, it is preferable to assay the actual replication competent SHIV challenge stock for neutralization to provide a direct link between *in vitro* neutralization assessment and *in vivo* protection (i.e. using the very same SHIV stock for challenge and *in vitro* assays). Given the variation in neutralization assay formats, including virus stock not directly linked to the challenge, and different target cells used, it is not surprising that a wide range of reciprocal serum ID_{50} titers ranging from <100 to >2000 has been reported to be protective. In some cases, the levels of bnMAbs at the sites of mucosal challenge were also determined (14, 50, 53). Generally much lower

concentrations of bnMAbs were detected at these sites compared to the plasma, which might have implications for the mucosal titers needed to be achieved by a successful protective HIV-1 vaccine. Understanding how the levels of antibodies in plasma correlate with local concentrations necessary to prevent infection by mucosal exposure is difficult. IgG levels in mucosal samples can vary depending on the collection method used and even over time in the animal. However, recent studies using improved methods to quantify IgG in mucosal tissues have allowed a clearer understanding of the role of mucosal IgG in protection, as discussed below (106–111). Mechanisms that contribute to protection by bNmAbs are not limited to virus neutralization (48, 112), as discussed further below. Also, by engineering bnMAbs to increase affinity for FcγRs that are expressed on immune effector cells, additional details of the specific antibody-mediated effector functions that impact protection may become clear (113).

Contribution of antibody effector functions to protection

Fc receptor binding in NHPs

IgG contains two antigen combining sites (Fabs) and an Fc region that interacts with various Fc receptors (FcRs) on immune cells. The first NHP experiments to show the importance of Fc receptor binding in protection was performed with two Fc variants of IgG1 b12 in SHIV-challenged macaques. Complement activation was ablated with b12 variant KA (K322A) and both complement and Fcγ receptor binding were abolished with a double mutation variant LALA (L234A/L235A) (48). The finding was reinforced in a follow-up study regimen that more closely mimicked human transmission events using a repeated but lowered virus inoculation dose and an ongoing low-dose weekly administration of either b12 or the effector function knock-out version, LALA. Importantly, this regimen maintained a low but fairly constant and systemic presence of neutralizing antibody at the time of each mucosal challenge. When compared, b12 was more than twice as effective as LALA in reducing the infection risk at each repeated challenge (49). Hence, this bnMAb provided optimal protection when Fc-mediated effector functions were intact. Unexpectedly, protection by the KA variant, that should only be deficient in complement activation, was equal in protection to parental b12, suggesting a negligible role by complement in protection against HIV-1. Recognizing the dual functionality of all antibodies, these results were not especially surprising, but a direct confirmation of effector function involvement with protection against mucosal SHIV challenge in macaques was novel. It is important to emphasize that both of the b12 Fc variants had identical neutralization capacities as the parental b12. Thus, the findings only imply that - at least for HIV-1 protection - antibody Fc receptor binding and resulting anti-viral activities may augment neutralization and that it is most likely that full protection requires multifunctional antiviral activities of neutralizing antibodies.

Effector functions correlate with better outcomes

In HIV-infected individuals, serum titers of HIV-1 specific ADCC antibodies correlate with $CD4^+$ T cell counts (114), suggesting a benefit of the recruitment of anti-viral immune functions. In fact, many studies have shown strong correlations between ADCC titers and improved clinical outcomes (115–118). Likewise, NHP studies have shown correlative data

indicating a beneficial role for Fc-mediated effector function in controlling post-infection viremia (115, 119–121). However, no evidence has been reported in animal models that antibody effector functions alone, in the absence of neutralization, can prevent infection. For example, non-neutralizing polyclonal antibodies that showed strongly functional ADCC did not protect neonatal macaques from oral SIVmac251 challenge (122). Likewise, no protection was seen with passive transfer of non-neutralizing antibodies from HIV^+ individuals that were highly enriched for potent ADCC activity (123). Thus, despite the association with reduced risk of infection by binding antibodies in RV144 vaccinees and animal models showing post-infection viral control, antibody Fc-mediated antiviral activity alone has not been shown to be adequate for blocking infection after either SIV or SHIV challenge. Furthermore, simply enhancing Fcγ receptor binding efficiency, by using a nonfucosylated variant of bnMAb b12 did not improve protection against SHIV challenge (72).

Why can't antibody effector functions alone prevent infection?

The protection seen in animal models with neutralizing bnMAbs could be due to the theory of HIV-1 Env heterogeneity that suggests non-neutralizing antibodies do not bind forms of HIV-1 Env that can mediate viral entry into host cells. The model proposes that infectious virions also express accessible nonfunctional (i.e. not entry competent) forms of envelope, and antibody binding to these forms is sufficient to permit virion capture and even ADCC, but does not lead to neutralization (82, 124). The model also helps to reconcile the paradox of non-neutralizing mAbs with known affinity for the CD4 binding site and other epitopes associated with protection, yet do not prevent viral infectivity.

Compromised protection without neutralization

Figure 5 depicts the scenarios of how non-neutralizing or weakly neutralizing mAbs may effectively mediate FcγR anti-viral activities that result in viremia blunting and possible reduction in the risk of acquisition, but do not prevent infection. In the first panel, non-neutralizing or weakly neutralizing Abs at the mucosal surface in the presence of an HIV-1 inoculum are more likely to permit penetration of infectious virus into the epithelium through abrasions or tearing that can occur during sexual transmission or exist as a consequence of sexually-transmitted diseases (STD). Without a mechanism to neutralize the transmitted/founder (T/F) viruses, host cells within the epithelium can become infected and migrate through the mucosae and into draining lymphatics or enter the blood circulation. This occurs because Env binding by non-neutralizing mAbs leaves host cells vulnerable to infectious Env spikes not bound by mAbs that can block entry. Some non-neutralizing mAbs are capable, however, of virus capture and coating virions and signaling *via* FcγR binding that lead to capture and clearance of virus and killing infected cells. Based on the data to date, these processes, although important in protection, are not sufficient to completely prevent infection because infectious Env spikes are left unbound and $CD4^+$ host cells are then vulnerable to infection. Eventually, infection spins out of control as more host cells are infected even though Ab effector functions are ongoing. In contrast, and illustrated in the second panel, is the setting of bnMAbs intercepting a virus inoculum before tissue penetration. Neutralizing-capable Abs effectively bind to Env spikes that will lead to infection, blocking viral entry while also carrying out FcγR binding and effector functions

against any productively infected cells. Potentially, the right bnMAb in sufficient concentration or combinations of bnMAbs may effectively clear most or even all of the inoculum exposure. Thus, animal passive protection studies have successfully modeled sterile protection with neutralizing antibodies when a sufficient concentration of bnMAb is present at the time of challenge or shortly thereafter. These results are also in agreement with the observation that the sensitivity of the challenge virus to the bnMAb generally determines the efficacy of protection against the virus stock as discussed earlier.

Evidence in mouse models

In very early passive protection studies, bnMAb IgG1 b12, Fab b12 and the non-neutralizing Fab b13 were compared for protection against HIV-1_{SF2} in hu-PBL-SCID mice. Briefly, the results showed that the pattern of the *in vivo* protection results followed the rank order of the neutralizing potency of IgG1 b12 > Fab12 > Fab 13, i.e. IgG1 b12 gave strong protection (5/5), Fab b12 gave some protection (1/5), and Fab b13 gave no protection (61). The compromised protection with Fab b12 suggested that neutralization measured *in vitro* may not be the only anti-viral functions associated with protection *in vivo*. This finding has been reported again using humanized mice (125) and reiterates the substantial role of *in vivo* FcγR-mediated mechanisms in the protective activity of anti-HIV-1 bnMAbs (112).

Not all bnMAbs are equal

Recent studies suggest that not all bnMAbs are equal in the capacity to kill HIV-infected cells by Fc-mediated effector functions, and variation in this activity can occur with, e.g., lower bnMAb affinity that may cause instability of Env-bnMAb complexes on the cell surface or less activity due to poorly exposed Env epitopes on T/F viruses (126). Bruel, et al. visualized the disappearance of Gag^+ target cells in the presence of NK cells in time-lapse microscopy as a readout for ADCC activity and found that even some of the most potent bnMAbs did not induce FcγRIIIa stimulation and killing of HIV-infected cells. These findings may be important for designing optimal bnMAb cocktails for immunotherapy regimens, and provides a rationale for re-engineering Fc regions of some bnMAbs to optimize therapeutics for cure research. Moreover, the differential affinity by bnMAbs for FcγRs, as well as the availability of innate immune cells, is a critical determinant of where and which type of antibody effector function could be optimized for bnMAb HIV protective therapeutics. For example, Sips, et al. found that phagocytic cells, macrophages and neutrophils, were the predominant FcγR-bearing effector cells in mucosal tissue (127). Therefore, as the authors predict, due to the landscape of effector cells available in mucosal tissue at sites of HIV-1 transmission, phagocytosis may be more frequent and relevant to HIV-1 protection and therapy than ADCC. These recent findings cause reflection on the results in macaques with the IgG1 b12 KA variant that suggested complement binding did not contribute to the protection efficacy (48). It is reasonable to hypothesize that within the specific parameters of the experimental design, including the required interactions with macaque Fcγ receptors, other effector functions such as ADCC or phagocytosis or combinations thereof were better suited for protection by b12.

An obvious challenge for bnMAb immunotherapy (i.e. treatment of those that are already infected) are escape mechanisms inherent to HIV-1 Env. Thus, continued animal modeling

will be critical pre-clinical steps for determining the most likely bnMAb combinations for optimal protection efficacy and outwitting Env diversity. Regarding immunoprophylaxis, a combination of empirical data and mathematical modeling have been used to understand optimal bnMAb combinations that would cover the large majority of diverse HIV-1 isolates, as we mentioned earlier (98, 99, 128).

Implications of bnMAb biodistribution in protection

Until recently, it was assumed that passively infused IgG entered the mucosa *via* passive mechanisms (e.g. transudation). However, as we detailed above, the recent appreciation of the role of the FcRn in antibody tissue distribution suggests that IgG in mucosal tissues is actively modulated by interaction with FcRn, which is expressed in mucosal tissues (102, 106). Mucosal secretions collected in early passive transfer studies provided evidence that passively transferred bnMAbs are transduced systemically and through the mucosae (14, 49, 52, 53). These and other studies have documented the kinetics in blood of passively transferred polyclonal and monoclonal antibodies.

New evidence of bnMAb activities in tissues

Clear evidence of IgG tissue distribution after passive infusion, although assumed to be widespread, has only recently been defined. In 2012, the concentrations and kinetics of b12 and its LALA effector function deficient variant were assessed in adult female macaques (129). The findings demonstrated that the rapid and broad distribution to lymphoid and mucosal tissues by both b12 and LALA were equivalent. Thus, the reduced protective capacity of LALA compared to b12 cannot be attributed to a differential effect of biodistribution. One prior macaque study, using polyclonal neutralizing IgG, demonstrated protection against SHIV challenge when the IgG was administered 6 hours, but not 24 hours after SHIV challenge (130). This year, we used an infant macaque oral SHIV challenge model to examine the effectiveness of early post-exposure bnMAb therapy. Short-term treatment with bnMAb cocktails started one day after virus exposure and administered three more times over 10 days prevented infection in all 10 infants, despite evidence of infection within the first few days in multiple tissues (107). These data provided direct evidence that bnMAb could impact and eradicate infection even 24 hours after viral challenge. Using serial sacrifice, the biodistribution of polyclonal SIVIG and bnMAb cocktails of PGT121 and VRC07-523 was measured in multiple lymphoid, gut and organs of infant macaques. A visualization of bnMAb spread 24 hours after subcutaneous (s.c.) delivery confirmed the rapid and extensive biodistribution of the antibodies (Figure 6). A subsequent study in adult macaques has confirmed the initial finding of bnMAb clearance of infection in diverse tissues; in this case delivered pre-exposure (131). It is important to note that (a) there was no difference in the distribution of the bnMAbs in the infants, whether or not a virus inoculum was present one day before bnMAb infusion, and (b) the kinetics and spread of virus closely matched the distribution of the bNmAbs. The targeted distribution of bnMAbs in lymphoid, gut and mucosal tissues, especially in early stages of infection and virus spread, allows for co-localization and the formation of immune complexes (ICs) that are required for a full complement of antibody-mediated anti-viral activities. For HIV-1 transmission, mucosal vulnerability in the rectum and vagina are particularly relevant for rapid delivery or vaccine

induction of protective immunity, and notably, bnMAbs can be detected at both sites by one day after s.c. delivery. Thus, is seems likely that the bnMAb cocktail stopped the development of SHIV-infection by killing and clearing newly infected cells in blood and tissues (107). This study emphasizes the multi-functionality of antibodies while demonstrating that bnMAbs can be protective when administered during the earliest stages of infection. Infected cell killing requires recruitment of effector cells *via* antibody Fc, a reminder of the dual roles of bnMAbs in protection – neutralization and effector mechanisms – already shown to be powerful in the current cache of bnMAbs.

Anti-viral mechanisms of bnMAbs vs ART

An important distinction between bnMAbs and small molecule antiretroviral drugs that target protease, reverse transcriptase or integrase is that the viral reservoir includes Env variants that are selected to escape from the natural adaptive antibody response, whereas drug resistance mutations more commonly arise only after initiation of ART. This may mean that HIV-1 therapy with bnMAbs will be challenging. Initial clinical studies with single bnMAbs have shown substantial virological effects, but with evidence of fairly rapid selection of escape variants (132, 133). In animal models, combinations of bnMAbs demonstrate more sustained viral suppression than single bnMAbs (134–137). Ongoing animal model and human studies are addressing the potential role of HIV bnMAbs, in combination and with other models of ART. Our challenge may be in optimizing these weapons and designing treatment regimens, establishing a new reinforcement to curb the pandemic while vaccine efforts continue.

Effects of bnMAbs on virus control and immune modulation

Timing of bnMAb effectiveness

Early virological events are critical, and thus the timing of bnMAbs is critical to their success in preventing infection. As discussed above, widespread lymphoid, gut, and organ infection with SHIV can occur within one day of exposure (107). These results mirrored earlier studies using SIV in infants (138). A study in adult macaques infected with SHIV suggest that passive treatment beginning at ten days post-exposure is as effective as drug treatment (ART) at viral suppression, and both of these treatments result in reducing viral reservoirs (139). However, as discussed earlier, bnMAb may only be able to eradicate the very early nidus of infection, i.e., within 24 hours after exposure, and this would not be feasible for most human HIV-1 exposure events. However, in the setting of MTCT, some infants are exposed to HIV-1 during passage through the birth canal, where they are exposed to HIV-1. In this setting, it is feasible to envision passive transfer as a means to protect from intrapartum infection and additional, as immunoprophylaxis during breastfeeding.

Immune modulation by bnMAbs

In addition to blocking and blunting viral replication after passive infusion, other therapeutic effects of neutralizing mAbs independent of virus neutralization have been seen. The modulation of endogenous immune responses was reported in a murine model after a short immunotherapy using neutralizing mAbs. The mice receiving the mAbs a week following infection with FrCas(E) murine retrovirus survived healthy and mounted a long-lasting

protective antiviral immunity with strong humoral and cellular immune responses (140). The enhancement of endogenous neutralizing antibody responses has been reported in three of our studies using neutralizing polyclonal IgG. The first of these was post-exposure SIVIG treatment in juvenile SIV-infected macaques (141) and the second and third were with pre-exposure prophylaxis using SHIVIG with sub-sterilizing levels of bnMAbs in newborn SHIV-infected macaques (142, 143). In these studies, an immunomodulatory effect was seen following antibody passive transfer that manifested as reduced pathogenesis and decreased progression to disease in animals receiving neutralizing antibody treatment compared to untreated animals. Importantly, this effect was not seen using SHIVIG with "mismatched" neutralizing activity specific to a different SHIV (142). One explanation for how this may work is through the process known as "antibody feedback," a theory associated with how germinal center B cells are signaled for triggering earlier onset of somatic mutations (144). Michaud, et al. (140), posit that immune complexes formed during antibody passive immunotherapy could affect the magnitude and quality of an anti-viral humoral response. Recently, immunotherapy with 3BNC117 in HIV-1 infected individuals was reported to enhance host humoral immunity against HIV-1 providing support for this theory (145). As more evidence is gathered regarding the power of bnMAbs against HIV-1 infection, these theories will become important as a basis to understand the scope of effective mechanisms *in vivo*. Taken together, bnMAbs are promising therapeutics regardless of whether neutralization, effector function, or some combination of both, are the keys to their protective mechanism.

Contribution of IgA in HIV-1 protection

To date, the antibodies that have been investigated intensively have all been IgGs, in part because these are most commonly isolated by current technologies, and due to the ease of constructing and expressing antibodies in this format. Thus other isotypes have not been widely evaluated as MAbs for prophylaxis. IgA in the form of secretory IgA (SIgA) is a predominant antibody isotype in mucosal secretions and plays an essential role in mucosal immunity. For HIV-1, the role of IgA is not well defined; serum IgA levels were correlated with increased risk of infection in the setting of the RV144 vaccine trial (146). Despite its less dominant role compared to IgG, a correlation between mucosal SIgA and enhanced protection against infection has been demonstrated for vaginally transmitted viruses such as HPV and HIV-1 (147–149). HIV-1 specific IgA from highly exposed persistently seronegative (HEPS) individuals has been shown to neutralize and inhibit transcytosis of primary HIV-1 isolates across a human epithelial lining (150) and is thought to contribute to the apparent resistance of these individuals to HIV infection (151).

Evidence from SHIV/macaque *in vivo* studies suggest that mucosally applied IgA correlates with a higher level of protection against rectally transmitted HIV (152, 153). Nonetheless, an important experiment for evaluating the impact of neutralizing SIgA at the mucosal surface of human transmission sites will be the passive transfer of dimeric IgA (dIgA) in macaques. If shown (or engineered) to effectively interact with macaque polymeric Ig Receptor (pIgR) in plasma, a more natural deposition of SIgA at the mucosal surface could be accomplished following transcytosis through the mucosal epithelium, possibly shedding light on the impact of SIgA against a virus inoculum. More studies are warranted in this area, especially

to parse the weight of neutralization vs Fcα mediated effector functions at the mucosal surface.

Passive transfer studies in humans – where are we now?

There have been a limited number of studies using anti-HIV-1 Abs in humans. Most of the earlier studies had been focused on Ab treatment of HIV-1 infected individuals with some limited success (154–157). In more recent studies, both 3BNC117 and VRC01 have been shown to have an anti-viral effect when administered to HIV-1 infected patients (132, 133, 158). In the case of prevention, no studies have been completed in humans to test the protective efficacy of bnMAbs against HIV-1 infection.

Opportunities and challenges

Recently, two large multi-center phase IIb clinical trials to test the efficacy of VRC01 in preventing HIV-1 transmission in high-risk populations have been initiated. These trials, collectively called the Antibody-Mediated Prevention (AMP) studies, are the first to test the ability of a bnMAb to prevent HIV-1 infection. Importantly, if significant protection against infection is observed, these studies are designed to also assess the level of antibody in plasma associated with protection. Thus, the results from this study could help in setting protective benchmarks for development of newer bnMAbs as well as defining the target antibody or neutralization levels needed to be induced by preventive HIV-1 vaccines.

Despite the encouraging developments in the field of HIV-1 bnMAbs and their potential use in prevention, there still lie many challenges before they can be widely used for prevention. The foremost challenge is the viral diversity observed in circulating HIV-1 strains. Although a few bnMAbs can cover more than 90% of the circulating strains, no single bnMAb is able to potently neutralize all HIV-1 strains. Therefore a combination of two or more bnMAbs would be needed to potently cover the entire range of viral strains of HIV-1 (98, 99). In this regard, use of the latest antibody technologies to make bi-specific antibodies targeting multiple HIV-1 Env epitopes to increase breadth and potency of bnMAbs shows promise (159, 160). Another challenge to development of bnMAbs for clinical use is the cost related to clinical grade production. This can be tackled in several ways. First, the use of more potent bnMAbs will lead to lower dosage at each injection. In this regard, newly discovered extremely potent bnMAbs like PGMD1400 and CAP256.VRC26.25 represent suitable candidates for development (83, 84). Second, engineering of antibodies to have longer half lives can lead to reduction in the number of doses needed to be administered every year (105, 106). Finally, ongoing improvements in efficient and cost-effective options to manufacture bnMAbs will significantly impact the overall cost of the final products and make them more feasible for global use (161).

Future advances

Alternative approaches for future clinical testing include advances in bi-specific antibodies (159, 160), engineered immunomodulatory proteins (162–164), and vector-delivered antibody molecules (165), which have the potential to mediate improved antibody effects compared to naturally-occurring antibodies. Newer visualization techniques may help to

inform the mechanisms by which protection and infected cell killing occurs at mucosal sites. Although beyond the scope of this review, antibody-based treatments, including bnMAb combinations, are in testing to determine their impact on viremia and on latent viral reservoir in established infection (134, 135). Potent bnMAb treatment, combined with inducing or latency-reversing agents, may contribute to partial or functional clearance of the viral reservoir.

Concluding remarks

For more than thirty years, the HIV-1 field has cast a wide net of vaccine approaches and anti-retroviral drugs in global Herculean efforts to address the continuing threat of human transmission of this fatal virus infection. The advances in ART drugs and regimens have undoubtedly prolonged the lives of millions. Educational and humanitarian programs, especially in resource-limited areas of the world have also contributed to a slowing of the worldwide spread of HIV-1/AIDS. Vaccine designers are making progress, but at best, only 30% protection has been seen to date in the clinic and possibly 50% based on recent animal models (166, 167). The field now has a new arsenal of powerful human monoclonal antibodies with the potential to prevent HIV-1 infection, and if used early enough - to modulate or ablate viremia and potentially enhance immune responses. Antibodies are dual functioning molecules that act as operative communicators with effector immune cells. As such, HIV-1 antibodies that bring together virus neutralization and Fc-effector functions, can be effective contributors to improving the lives of infected individuals and in some demographics, preventing infection altogether.

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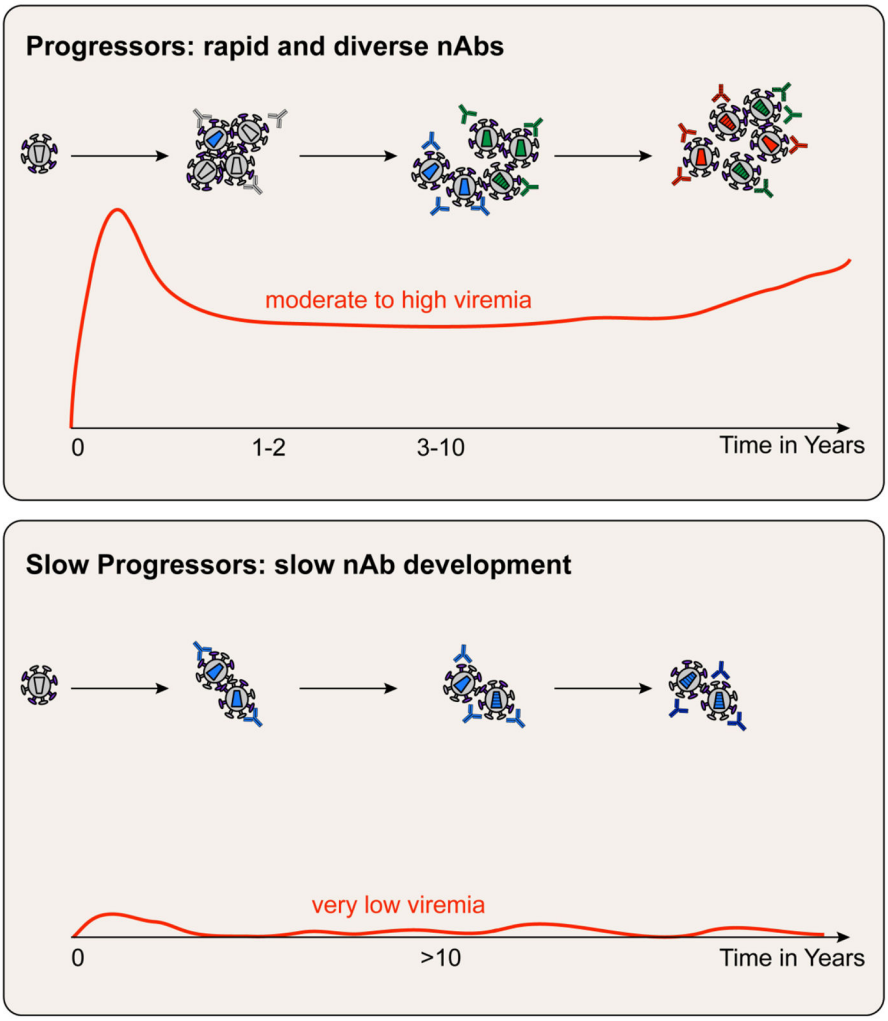

Figure 1. Timing and complexity of neutralizing antibodies in human subjects with different virus levels

HIV-1 accumulates at least one mutation every time it replicates and thus, develops into a swarm of closely related viruses called the quasispecies that differs in each individual. The number of variants in the quasispecies depends upon the interplay between the virus and the adaptive immune response. Progressors have moderate to high viremia that is accompanied by the development of variants and antibodies that become diverse within a few years. Slow Progressors have low or undetectable viremia for 10 years or more, and they develop nAbs slowly and at low titers as a result of exposure to less antigen, and less diverse antigen.

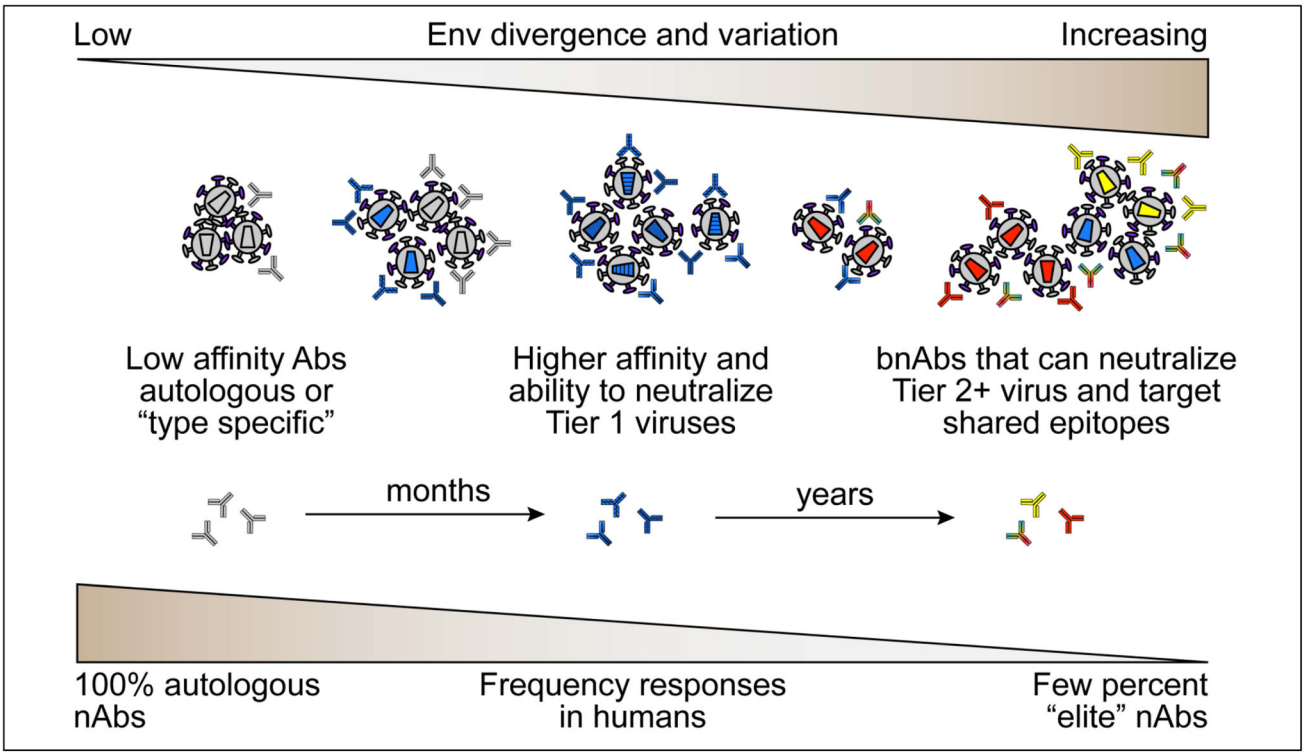

Figure 2. HIV-1 Env divergence and variation during infection

Following infection, the autologous or "type specific" response develops first, with low affinity Abs against the T/F viruses (gray) and early variants (light blue). This occurs in essentially all individuals and affinity and neutralization against the autologous virus increase over time. As the virus responds to autologous NAbs, escape variants emerge that are divergent from the T/F virus, creating more Env sequence diversity, which in turn becomes subject to the host humoral responses. As the quasispecies diverges, the nAb responses become higher affinity and broadens to accommodate resistant variants. This results in the ability of an individual sera to neutralize viruses from other individuals. After a number of years, some sera are able to cross-react with a range of heterologous strains by targeting one or more shared epitopes. This results and "broad" nAbs (bnAbs) that can neutralize many diverse Tier 2 viruses. A small percentage of individuals are "elite" neutralizers or have antibodies that can potently neutralize the large majority of virus isolates.

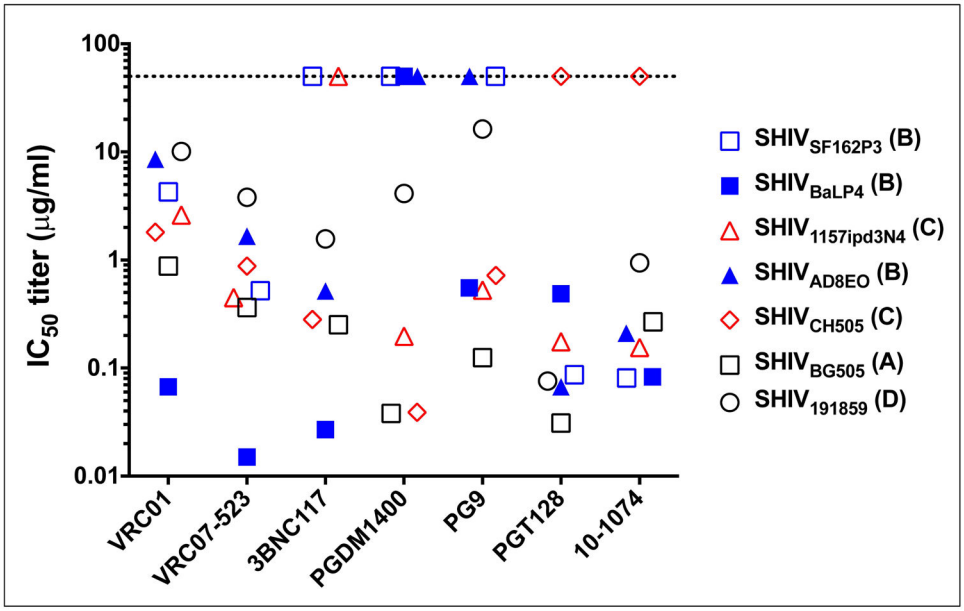

Figure 3. Neutralization sensitivity of SHIVs to anti-HIV-1 bnMAbs

The IC_{50} titers of the bnMAbs shown were determined against a panel of replication competent SHIVs in a single-round of infection assay with TZM-bl target cells. The SHIV stocks were made in rhesus PBMCs. The clade of each SHIV is indicated in parenthesis.

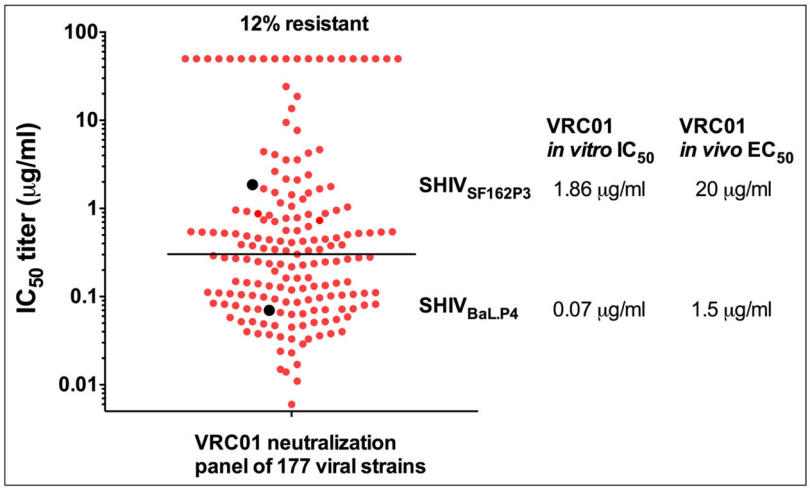

Figure 4. VRC01 serum concentrations needed to protect against SHIV infection

The neutralization sensitivity (IC_{50}) of a panel of 177 HIV-1 strains to VRC01 is shown (red) along with the sensitivity of two SHIVs ($SHIV_{SF162P3}$ and $SHIV_{BaL.P4}$) (black). The plasma concentration of VRC01 needed to protect 50% of animals (EC_{50}) after mucosal challenge is indicated for each SHIV.

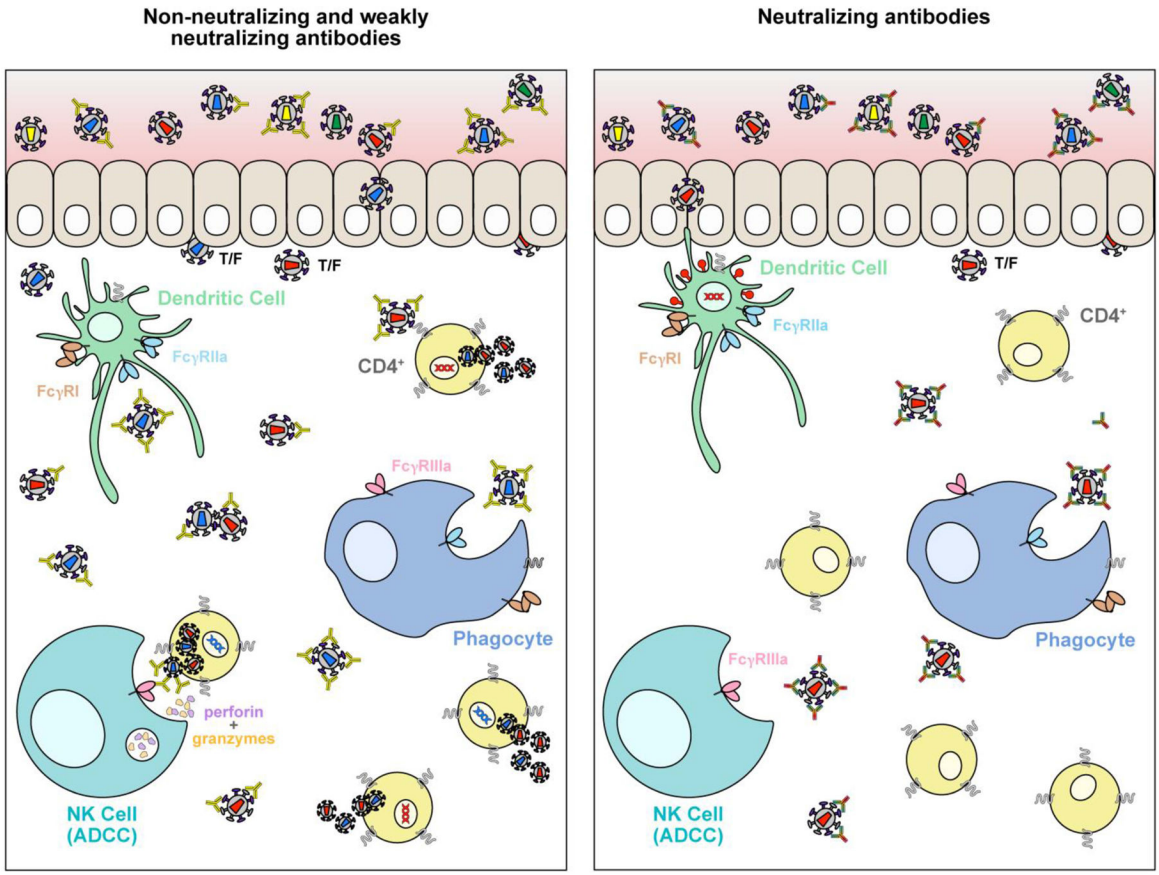

Figure 5. Antiviral activities of antibodies against HIV

The two panels depict scenarios of the protection outcome of HIV-1 exposure at mucosal sites when non-neutralizing or weakly neutralizing antibodies are present, compared to exposure in the presence of potently neutralizing antibodies. The former may be unable to block virus entry into cells, but can kill infected cells *via* Fc-mediated functions. These anti-viral activities are important for blunting viremia and slowing viral replication, but may not be sufficient to prevent eventual infection that overwhelms the antibody response. Conversely, neutralizing antibodies block entry into cells and also mediate Fc effector functions to kill and clear any infected cells, thus preventing establishment of infection.

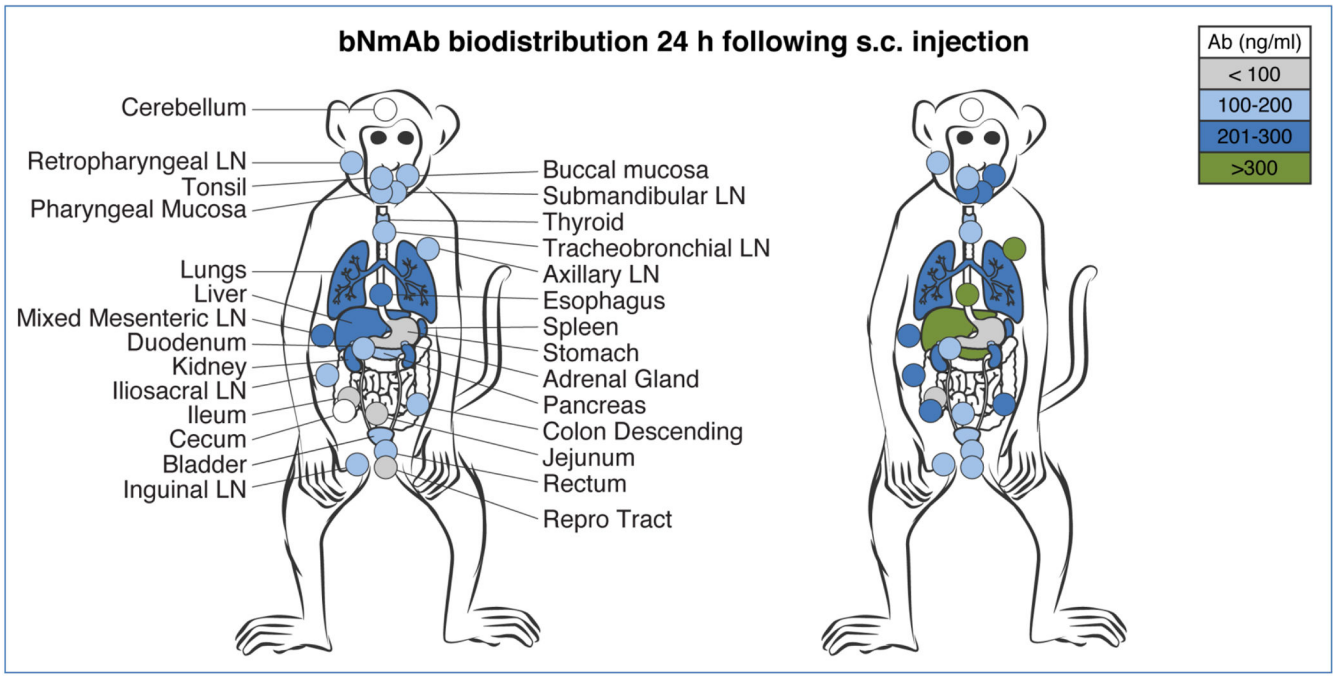

Figure 6. Biodistribution of bnMAb cocktail following subcutaneous (s.c.) injection

A cocktail containing 5 mg/kg of each PGT121 and VRC07-523 was delivered s.c. in the upper back to infant (~1 month old) rhesus macaques. Multiple tissues were collected one day later, homogenized, and assayed for bnMAb cocktail concentration. Two representative animals are shown from experiments with no SHIV inoculation.

Table 1

SHIV protection studies

HIV-1 Env epitope	Antibody	Challenge virus (route)	Rate of Protection	Conc.[a] (μg/ml)	Reference
CD4 binding site	b12	$SHIV_{SF162P4}$ (vaginal)	4/4	705	53
		$SHIV_{SF162P4}$ (vaginal)	2/4	149	53
		$SHIV_{SF162P4}$ (vaginal)	0/4	16	53
		$SHIV_{SF162P3}$ (vaginal)	8/9	563	48
	VRC01	$SHIV_{SF162P3}$ (vaginal)	4/4	65	15
		$SHIV_{SF162P3}$ (rectal)	4/4	79	15
		$SHIV_{SF162P3}$ (oral)	2/2	287	107
		$SHIV_{SF162P3}$ (oral)	4/5	64	107
		$SHIV_{BaL.P4}$ (rectal)	4/4	4	Unpublished
		$SHIV_{BaL.P4}$ (rectal)	4/10	1.3	15
	VRC07-523LS[b]	$SHIV_{SF162P3}$ (rectal)	6/6	25	Unpublished
	3BNC117	$SHIV_{AD8EO}$ (rectal)	2/2	91	54
V1V2	PG9	$SHIV_{BaL.P4}$ (rectal)	4/6	32	15
V3-glycan	PGT121	$SHIV_{SF162P3}$ (vaginal)	5/5	15	14
		$SHIV_{SF162P3}$ (vaginal)	3/5	1.8	14
		$SHIV_{AD8EO}$ (rectal)	2/2	22	54
	PGT126	$SHIV_{SF162P3}$ (vaginal)	5/5	98	13
		$SHIV_{SF162P3}$ (vaginal)	2/5	20	13
		$SHIV_{SF162P3}$ (rectal)	3/4	130	13
		$SHIV_{SF162P3}$ (rectal)	2/4	27	13
	10-1074	$SHIV_{AD8EO}$ (rectal)	2/2	115	54
High mannose glycan	2G12	$SHIV_{SF162P3}$ (vaginal)	3/5	1053	50

HIV-1 Env epitope	Antibody	Challenge virus (route)	Rate of Protection	Conc.[a] (μg/ml)	Reference
MPER	4E10	$SHIV_{Ba-L}$ (rectal)	6/6	866	51
	2F5	$SHIV_{Ba-L}$ (rectal)	6/6	742	51
	10E8	$SHIV_{BaL.P4}$ (rectal)	6/6	31	15
		$SHIV_{BaL.P4}$ (rectal)	3/6	1.8	15

[a] Average concentration of antibody in serum at the time of challenge is listed.

[b] VRC07-523 contained the FcRn enhancing mutation (LS) to extend *in vivo* half life.