Progress toward active or passive HIV-1 vaccination

Amelia Escolano,[1]* Pia Dosenovic,[1]* and Michel C. Nussenzweig[1,2]

[1]Laboratory of Molecular Immunology and [2]Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10065

AIDS is a preventable disease. Nevertheless, according to UNAIDS, 2.1 million individuals were infected with HIV-1 in 2015 worldwide. An effective vaccine is highly desirable. Most vaccines in clinical use today prevent infection because they elicit antibodies that block pathogen entry. Consistent with this general rule, studies in experimental animals have shown that broadly neutralizing antibodies to HIV-1 can prevent infection, suggesting that a vaccine that elicits such antibodies would be protective. However, despite significant efforts over the last 30 years, attempts to elicit broadly HIV-1 neutralizing antibodies by vaccination failed until recent experiments in genetically engineered mice were finally successful. Here, we review the key breakthroughs and remaining obstacles to the development of active and passive HIV-1 vaccines.

The Journal of Experimental Medicine

Introduction

The HIV-1 virus expresses a trimeric envelope glycoprotein (Env) on its surface. Env is a highly variable protein that is shielded from antibody recognition by recessed neutralization-sensitive epitopes and by a large number of host glycans that are attached to Env during its biosynthesis in infected cells (Wyatt and Sodroski, 1998; Wei et al., 2003).

The human immune system has evolved to tolerate and specifically avoid attacking self-glycans, making this form of shielding particularly effective. Nevertheless, a fraction of HIV-1–infected individuals develop antibodies that can neutralize diverse viral isolates by binding to Env. The observation that such antibodies arise during natural infection (Binley et al., 2008; Doria-Rose et al., 2009; Li et al., 2009; Sather et al., 2009; Simek et al., 2009; Gray et al., 2011a; Hraber et al., 2014; Rusert et al., 2016) and that they can block infection in experimental animal models (Eichberg et al., 1992; Emini et al., 1992; Mascola et al., 1999, 2000; Shibata et al., 1999; Baba et al., 2000; Parren et al., 2001; Hessell et al., 2009a,b, 2010; Balazs et al., 2012; Moldt et al., 2012; Pietzsch et al., 2012) suggests that antibody-based vaccines for HIV-1 may be an attainable goal. Indeed, initial efforts to produce HIV-1 vaccines focused on immunization with recombinant Env proteins, but preclinical and clinical observations diminished the enthusiasm for this approach.

Numerous efforts to elicit broadly neutralizing antibodies (bNAbs) in experimental animals including guinea pigs, rabbits and monkeys using Env proteins, or Env-expressing viral vaccine vectors, were unsuccessful (reviewed in McCoy and Weiss [2013] and Sliepen and Sanders [2016]). In most cases, Env-based immunogens elicit antibodies that neutralize laboratory-adapted tier 1 viruses and/or the autologous virus expressing the Env variant used for immunization, but they fail to neutralize heterologous primary isolates. Recently, well-ordered soluble trimers that more faithfully mimic the native HIV-1 spike have also been tested as immunogens in several different animal models (Julien et al., 2013; Lyumkis et al., 2013; Sanders et al., 2013; Pancera et al., 2014; de Taeye et al., 2015; Hu et al., 2015; Sharma et al., 2015; Guenaga et al., 2016; Ingale et al., 2016; Klasse et al., 2016). Although these recently developed native-like trimers produce more consistent neutralizing antibody responses to the corresponding autologous tier 2 viruses, they too failed to elicit bNAbs.

The challenge of eliciting bNAbs by vaccination was also illustrated in human trials (VAX003 and 004) wherein healthy volunteers were immunized with recombinant monomeric gp120 proteins (Flynn et al., 2005; Pitisuttithum et al., 2006). Although these vaccines elicited type-specific neutralizing antibodies, they failed to produce antibodies that neutralized heterologous primary HIV-1 isolates, and there was no protection from infection (Flynn et al., 2005; Pitisuttithum et al., 2006). More recently, the RV144 trial used a prime-boost regimen consisting of four priming injections with a recombinant canarypox vector genetically engineered to express HIV-1 Env, gag, and protease (ALVAC-HIV) and two boosters with a combination of two recombinant gp120s (AIDSVAX B/E). This regimen produced modest levels of protection, but again failed to elicit significant antibody-neutralizing activity against heterologous tier 2 strains of HIV-1 (Rerks-Ngarm et al., 2009; Montefiori et al., 2012). Finally, a vaccine regimen consisting of three DNA prime injections with six plasmids encoding clade B gag, pol, and nef and three gp120 Envs from clade A, B, and C and a boost with a recombinant adenovirus-5 vector expressing the same Envs and a clade B gag-pol fusion protein also failed to elicit bNAbs and did not protect against infection (Hammer et al., 2013).

To better understand bNAb responses to HIV-1 Env, several monoclonal antibodies (MAbs) were isolated from chronically infected individuals, and their epitope specific-

*A Escolano and P. Dosenovic contributed equally to this paper.

Correspondence to Michel C. Nussenzweig: nussen@rockefeller.edu

Abbreviations used: bNAb, broadly neutralizing antibody; CD4bs, CD4-binding site; MAb, monoclonal antibody; SHIV, simian-HIV; SHM, somatic hypermutation.

The Rockefeller University Press $30.00
J. Exp. Med. 2017 Vol. 214 No. 1 3–16
https://doi.org/10.1084/jem.20161765

ities were characterized (Wibmer et al., 2015; Burton and Hangartner, 2016). However, the serum concentration of the first-generation bNAbs required to prevent infection in experimental animal models was so high that achieving this level by immunization seemed unlikely (Mascola and Nabel, 2001; Nishimura et al., 2002).

The failure to elicit bNAbs by immunization by Env immunogens and the relative lack of potency of the best available antibodies shifted the focus of HIV-1 vaccine research away from antibody-based vaccines toward T cell–based protection. Unfortunately, the two clinical trials of T cell–based vaccination conducted to date were ineffective at preventing infection and controlling viral load (HVTN502 [Step study] and HVTN503 [Phambili study]; Buchbinder et al., 2008; Gray et al., 2011b). In fact, there was even evidence of increased infection by HIV-1 in the Step study (Duerr et al., 2012).

In this review, we focus on the recent progress in HIV-1 vaccine development, in particular on the insights gained from cloning anti–HIV-1 antibodies from single memory B cells, how the new concepts are being tested, and what the results may mean for attaining the goal of HIV-1 vaccination.

First-generation monoclonal anti–HIV-1 antibodies

Polyclonal antibodies that neutralize more than just one HIV-1 strain were found in immunized guinea pigs as early as 1990; however, neutralization activity was limited (Javaherian et al., 1990). Subsequent work extended these findings by uncovering antibodies from infected individuals with greater breadth and potency by phage display or by producing immortalized B cell lines. The most potent among the first-generation of human MAbs to HIV-1, b12, 2F5, 4E10, 2G12, and 447-52D show varying degrees of cross-clade neutralizing breadth and potency (Gorny et al., 1992; Buchacher et al., 1994; Burton et al., 1994; Roben et al., 1994; Trkola et al., 1995, 1996; Stiegler et al., 2001; Zwick et al., 2001).

Analysis of first-generation anti–HIV-1 neutralizing antibodies revealed several unusual features, such as long Ig heavy chain complementarity determining regions (CDRH3s), self-reactivity, and high levels of somatic hypermutation (SHM; Haynes et al., 2005; West et al., 2014). However, the number of antibodies analyzed was limited. Therefore, it was difficult to establish the generality of each of these observations, their significance for vaccine development, or which, if any, of these features was required for the development of bNAbs (Burton et al., 2005).

Epitope mapping of the first-generation antibodies identified four major sites of vulnerability on Env: (1) the CD4-binding site (CD4bs; Barbas et al., 1992; Burton et al., 1994); (2) the membrane proximal external region (MPER; Muster et al., 1993; Stiegler et al., 2001; Zwick et al., 2001); (3) a glycan patch on gp120 that includes the N332 glycan (Trkola et al., 1996); and (4) the tip of the V3 loop (Gorny et al., 1992). The availability of information on bNAb epitopes led to the suggestion that immunogens could be designed to mimic these targets and elicit bNAbs (Pantophlet and Burton, 2003; Burton et al., 2005; Sattentau, 2008; Stamatatos et al., 2009). However, as mentioned above, all attempts to do so were disappointing because none of the specifically designed immunogens elicited broad or potent cross-clade neutralizing antibodies.

Why eliciting bNAbs is so difficult remained unclear until the introduction of single B cell antibody cloning (Scheid et al., 2009a,b; Tiller et al., 2009).

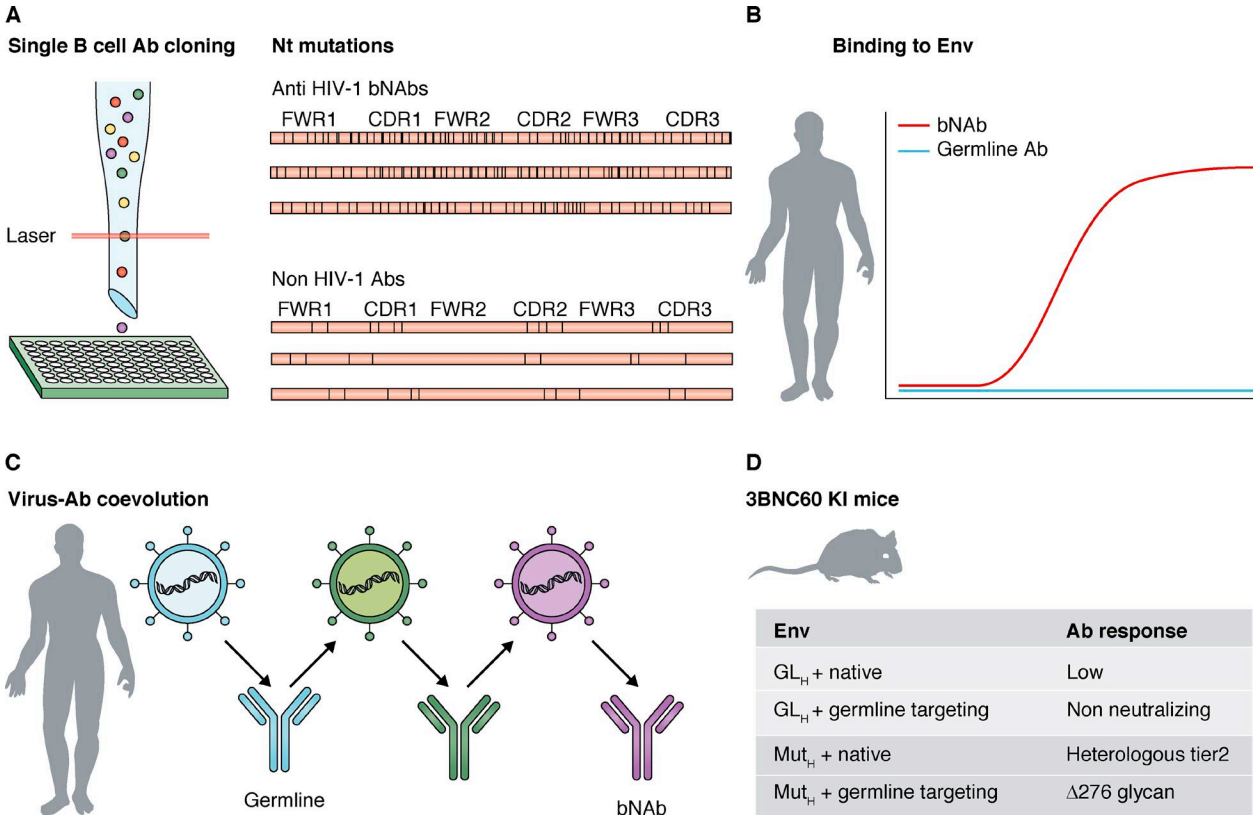
Single-cell antibody cloning

The introduction of single-cell anti–HIV-1 antibody cloning methods in 2008 (Scheid et al., 2009b) and their subsequent use by numerous laboratories led to the discovery of a new generation of potent bNAbs that reinvigorated efforts to produce active and passive HIV-1 vaccines. Moreover, it enabled several observations that helped explain why eliciting anti–HIV-1 bNAbs is so difficult and suggested alternative approaches to vaccine development.

Anti–HIV-1 antibodies were initially cloned from mRNA extracted from individual B cells isolated based on their ability to bind to Env (Scheid et al., 2009a,b). Later, similar methods were adapted to clone antibodies from primary B cell cultures and EBV-transformed B cells starting with PG9 and PG16 (Walker et al., 2009).

The antibody cloning experiments de-convoluted the serologic activity in individuals that developed broadly neutralizing responses. Although no two individuals were identical, serum-neutralizing activity could be accounted for by a single or a combination of different antibodies targeting different sites of vulnerability on the HIV-1 Env (Scheid et al., 2009a, 2011; Walker et al., 2009, 2011; Wu et al., 2010, 2015; Bonsignori et al., 2011; Huang et al., 2012; Mouquet et al., 2012; Georgiev et al., 2013; Liao et al., 2013; Doria-Rose et al., 2014; Sok et al., 2014; MacLeod et al., 2016; Simonich et al., 2016). Structural and biochemical analysis of the new antibodies enabled a detailed molecular understanding of the new epitopes and revealed novel vaccine targets on Env. Whereas there were only four known neutralizing epitopes in 2009, we now know of additional targets of bNAbs: (1) a trimer-dependent epitope at the apex of the Env spike; (2) the interface of gp120 and gp41; and (3) a recently identified epitope comprising the N88 glycan and the fusion peptide at the N terminus of gp41 (Kwong and Mascola, 2012; West et al., 2014; Burton and Hangartner, 2016; Kong et al., 2016; van Gils et al., 2016). Moreover, some of these epitopes can be recognized by a variety of different mechanisms. For example, there are at least three unique molecular approaches that antibodies use to target the CD4bs of gp120 (Zhou et al., 2015; Gristick et al., 2016).

The antibody cloning experiments uncovered hundreds of new antibodies, several of which were two to three orders of magnitude more potent in virus neutralization than those that were available before 2009 (Burton and Hangartner, 2016). When injected into monkeys, several of these second-generation bNAbs, including 3BNC117, VRC01,



Env	Ab response
GL_H + native	Low
GL_H + germline targeting	Non neutralizing
Mut_H + native	Heterologous tier2
Mut_H + germline targeting	Δ276 glycan

Figure 1. **Requirement for sequential immunization.** (A) Panel illustrates the finding that HIV-1 antibodies are highly somatically mutated. (B) Panel shows that germline-reverted antibodies fail to bind to most HIV-1 antigens. (C) Panel illustrates the observation that HIV-1 and bNAbs develop in sequence over time. (D) Panel shows the results of knock-in mouse immunization experiments that showed that multiple immunogens would be required for bNAb development.

10-1074, and PGT121, protected from simian-HIV (SHIV) infection at concentrations that might be achievable by immunization (Moldt et al., 2012; Shingai et al., 2014). In addition, single injections of antibodies protected macaques from weekly low-dose challenge with SHIV for up to 23 wk (Gautam et al., 2016).

Most important for the vaccine effort, single-cell antibody cloning experiments produced key insights that led to novel approaches to vaccine design. One fundamental observation that emerged was that anti–HIV-1 antibodies, including those that have low or absent levels of neutralizing activity, are generally more somatically mutated than non–HIV-1–specific antibodies cloned from the same B cell pool (Fig. 1; Scheid et al., 2009a). Although high levels of SHM are observed in some chronic infections, they are far lower than in HIV-1–specific antibodies (Breden et al., 2011). bNAbs are a subset of anti–HIV-1 antibodies that display the greatest potency and neutralization breadth and, as such, conform to this more general rule that antibodies to HIV-1 are extensively mutated (Walker et al., 2009; Xiao et al., 2009; Scheid et al., 2011; Klein et al., 2013a; Liao et al., 2013; Doria-Rose et al., 2014; Sok et al., 2014; Burton and Mascola, 2015; Wu et al., 2015).

Somatic mutations are introduced into antibody genes by activation induced cytidine deaminase (AID), an enzyme that converts cytosine to uracil resulting in a DNA mismatch that is repaired by one of a variety of different pathways to produce mutations. The same lesions can also produce deletions, insertions, and even chromosome translocations, but these are far less frequent than somatic mutations (Robbiani and Nussenzweig, 2013; Kepler et al., 2014). AID poses a threat to the genome, and its expression is therefore primarily restricted to dividing B cells in the dark zone of the germinal center (Victora et al., 2010). In addition, its catalytic activity is limited such that it only introduces 1 base pair change per 1,000 nucleotides per cell division (McKean et al., 1984). This relative low rate of mutation limits deleterious off-target effects while permitting efficient selection for individual mutations that increase affinity, accounting in part for the relatively low level of mutation found in most human antibodies. Accumulating up to 100 mutations in the 300 nucleotide IgV_H region (as seen in some bNAbs) would require hundreds of cell division cycles, far more than might be expected from an average round of B cell selection (Victora et al., 2010; Gitlin et al., 2014, 2015; Tas et al., 2016).

One way to account for the high level of mutation in anti–HIV-1 antibodies is by multiple rounds of germinal cen-

ter selection (Scheid et al., 2009a; Victora and Nussenzweig, 2012; Klein et al., 2013b). According to this hypothesis, an antibody that develops to the predominant circulating virus selects against sensitive viral variants, resulting in the emergence of a closely related but antibody-resistant viral variant. The antibody-resistant HIV-1 variant then elicits an additional round of immunoglobulin hypermutation, followed by germinal center–based selection for B cells synthesizing antibodies that recognize the new HIV-1 variant. However, production of the new antibody would lead to selection against the new HIV-1 variant. This cycle of HIV-1 selection and escape from antibody, followed by additional rounds of germinal center B cell selection would be repeated on multiple occasions over time, eventually resulting in the emergence of highly mutated HIV-1–specific antibodies. This scheme is consistent with, and potentially helps explain, the observation that emergence of bNAbs differs from typical humoral immune responses in that they take longer to develop and have more somatic mutations. Moreover, it is in keeping with longstanding ideas about continuing mutation and selection by both the immune system and the virus (Richman et al., 2003; Wei et al., 2003; Burton et al., 2005).

Although prime and boost vaccine regimens with heterologous Env proteins (Klinman et al., 1991; Eda et al., 2006; Mörner et al., 2009; Guenaga et al., 2011) failed to elicit bNAbs, strong support for the idea that bNAbs are elicited sequentially came from prospective studies of several HIV-1–infected individuals who developed bNAbs. In these studies, antibodies and HIV-1 viruses were obtained concurrently from several different time points. The data showed that the initial antibody response was strain specific and targeted the earliest dominant HIV-1 virus in circulation. The first wave of antibodies selected against the concurrently circulating virus, resulting in the emergence of viral variants that were resistant to the initial antibody response. As expected based on the high levels of mutation seen in HIV-1 antibodies, the antibody-resistant virus elicited a secondary antibody response that once again selected for viruses resistant to all antibodies in circulation. Over a period of years, this process was repeated multiple times, giving rise to antibodies to several neutralization-sensitive sites on HIV-1 that eventually accounted for the observed serologic neutralization activity. These elegant prospective studies pioneered by Haynes and colleagues, and confirmed by others (Bonsignori et al., 2011, 2016; Liao et al., 2013; Doria-Rose et al., 2014; Bhiman et al., 2015; Wu et al., 2015; MacLeod et al., 2016), are entirely consistent with the idea that eliciting bNAbs might require a combination of sequential immunogens that target specific precursor B cells and foster prolonged germinal center responses that select for the essential bNAb mutations (Fig. 1; Scheid et al., 2009a; Dimitrov, 2010; Pancera et al., 2010; Zhou et al., 2010; Haynes et al., 2012; Mouquet et al., 2012; Jardine et al., 2013, 2015; Klein et al., 2013b; McGuire et al., 2013; Dosenovic et al., 2015).

Another key finding that came out of the antibody cloning experiments was that somatic mutations are generally required for neutralizing activity of anti–HIV-1 antibodies (Xiao et al., 2009; Mouquet et al., 2010, 2012; Zhou et al., 2010; Bonsignori et al., 2011; Scheid et al., 2011; Hoot et al., 2013; Klein et al., 2013a; Sok et al., 2013; Kepler et al., 2014). Once again, bNAbs, the subset of anti–HIV-1 antibodies displaying the greatest neutralization breadth, conform to this general rule (Xiao et al., 2009; Scheid et al., 2011; Klein et al., 2013a). With the few exceptions obtained from prospective studies where the founder viruses could be identified, the predicted germline precursors of anti–HIV-1 antibodies, including bNAbs, failed to bind to all tested Envs (Fig. 1). Thus, the immunogens that had been used in an attempt to elicit bNAbs in past vaccine trials likely failed to bind to B cells expressing bNAb precursors and were therefore unable to initiate bNAb maturation. This insight led to the proposal that initiation of bNAb maturation would require immunogens that are selected for or specifically designed to bind to B cells expressing bNAb germline precursors (Scheid et al., 2009a; Xiao et al., 2009; Dimitrov, 2010; Klein et al., 2013b).

In conclusion, finding high-level somatic mutation and its critical role in bNAb activity led to the proposal that singular antigens would not be able to elicit bNAbs (Scheid et al., 2009a). It was evident that immunization to achieve this goal would require a novel vaccine scheme modeled on the natural infection in that it would require sequential immunization starting with antigens specifically selected or designed to activate B cells expressing germline precursors of bNAbs (Scheid et al., 2009a; Dimitrov, 2010; Mouquet et al., 2010; Haynes et al., 2012; Klein et al., 2013b).

Germline-targeting antigens

The observation that most recombinant Env proteins are unable to engage the inferred germline version of bNAbs motivated rational immunogen design to engineer Env proteins that do so. These efforts were facilitated by the availability of inferred germline antibodies that were used to screen libraries of Env variants and test the engineered proteins (Xiao et al., 2009; Hoot et al., 2013; Jardine et al., 2013; McGuire et al., 2013). Several different approaches to germline B cell targeting are currently being evaluated.

One approach is to design antigens that specifically target a single bNAb B cell lineage. The potential advantage of this approach is that it focuses the immune response on restricted epitopes on Env that might otherwise be poorly immunogenic. Examples of this approach are the engineered outer domains eOD-GT6 and eOD-GT8 (Jardine et al., 2013, 2015) and the 426c gp140 TM4DV1-3 protein (McGuire et al., 2014). These proteins were selected for binding to germline VRC01 class antibodies and were shown to activate Ca^{2+} flux in vitro by B cell lines expressing inferred VRC01 class precursor antibodies. These new antigens differ from previously available Env antigens in that they make the rather narrow CD4bs more available, in part by removing a glycosylation motif at amino acid position 276–278 that normally interferes with the binding of most antibodies to

this site. Although this first generation of germline-targeting antigens represents a conceptual advance, their spectrum of activity is limited to a specific subset of CD4bs antibodies, and therefore their use as single immunogens may be suboptimal. For example, the affinity of individual germline VRC01 class antibodies, such as VRC01 and 3BNC60 for eOD-GT8, differs, as does the ability of this antigen to elicit responses from B cells expressing the two antibodies (Dosenovic et al., 2015; Jardine et al., 2016; and see section Mice expressing inferred germline antibodies). Whether immunization with this first generation of germline-targeting antigens can in fact expand the limited group of VRC01 precursors in humans remains to be determined (Jardine et al., 2016; Sok et al., 2016; Tian et al., 2016).

Another group of engineered germline-targeting antigens is based on the native-like SOSIP Env trimers (Binley et al., 2000; Sanders et al., 2002). One potential advantage of engineering germline-targeting antigens based on native-like Env trimers is that several independent antibody target sites could be modified to activate germline precursors of more than just one bNAb lineage. An additional advantage of SOS IP Env trimers is that they are stabilized in the prefusion conformation and reduce exposure of nonneutralizing epitopes (Julien et al., 2013; Lyumkis et al., 2013).

Similar to the CD4bs antigens described above (eOD-GT6, eOD-GT8, and 426c gp140 TM4DV1-3), PGT121 germline-targeting SOSIP immunogens are being developed based in part on the molecular characterization of putative intermediates in bNAb development and on selection of candidate antigens using germline-reverted antibodies (Sok et al., 2013; Garces et al., 2014, 2015; Scharf et al., 2016; Steichen et al., 2016). Although these initial SOSIP immunogens were designed to elicit a single bNAb lineage, they could be further engineered to target additional bNAb lineages.

Finally, naturally arising Env variants obtained from transmitted founder viruses that elicited bNAbs represent another approach to germline antibody targeting. This idea was enabled by prospective studies of individuals that develop bNAbs, but it has yet to be tested in experimental animal models (Bonsignori et al., 2011; Liao et al., 2013; Doria-Rose et al., 2014; Wu et al., 2015; MacLeod et al., 2016).

Testing HIV-1 vaccine concepts in genetically engineered mice

Individual B cells carry unique receptors that are randomly assembled by V(D)J recombination leading to a highly diverse repertoire of naive B cells that can potentially respond to nearly any foreign antigen. In the case of HIV-1 Env, this means that humoral immune responses typically involve production of antibodies to several different epitopes (Dosenovic et al., 2009), the vast majority of which, including the immunodominant epitopes, are strain specific or nonneutralizing. Although a diverse response covering many different epitopes would be optimal in a vaccine regimen, the complexity of such a response makes it difficult to analyze.

One way to simplify the problem and focus on B cell responses to a specific epitope is to introduce pre-rearranged VDJ_H and VJ_L into the mouse genome by gene targeting (Goodnow, 1992). Expression of the knock-in antibody transgenes induces allelic exclusion and thereby shuts off V(D)J recombination of endogenous Ig genes (Nussenzweig et al., 1987). Thus, mice that carry pre-rearranged Ig genes express primarily the receptor of interest. In addition to combining the knock-in VDJ_H and VJ_L to produce the fully intact antibody, the two genes can also be studied independently, in which case the introduced transgene pairs with endogenous mouse IgK/L or IgH chains, respectively. There are several advantages to the latter, including a diverse repertoire of B cells with varying specificities that begins to approach the normal repertoire in that it contains a more limited number of bNAb precursors. An important limitation to this approach is that it is restricted to genetically engineered mice. However, a major advantage is that immunology is better studied in the mouse than any other experimental model system, facilitating detailed analysis of immunization experiments.

Mice expressing inferred germline antibodies

Initially, available knock-in mice carrying first-generation bNAbs were suboptimal for vaccine studies because they expressed the mature antibodies, and not their inferred germline precursors. Moreover, mice carrying antibodies targeting the membrane proximal domain, 2F5 and 4E10, showed severe abnormalities in B cell development because of self-reactivity (Verkoczy et al., 2010, 2011; Doyle-Cooper et al., 2013; Ota et al., 2013).

Knock-in mice expressing the inferred germline version of bNAbs have recently been generated and used to test new vaccine concepts (Dosenovic et al., 2015; Jardine et al., 2015; Briney et al., 2016; Escolano et al., 2016; McGuire et al., 2016; Tian et al., 2016; Zhang et al., 2016). The initial experiments were performed in mice that carry the inferred germline IgH of 3BNC60 or VRC01, both of which belong to the VRC01 class of anti-CD4bs antibodies (Dosenovic et al., 2015; Jardine et al., 2015; McGuire et al., 2016). This group of antibodies shares several features, including the same IgV_H germline gene, V_H1-2*02, which makes critical contacts with Env through CDRH2. They show high levels of somatic mutations, and their heavy chain is always paired with Ig light chains with short (5–amino acid long) CDRL3s (West et al., 2012). The short CDRL3 is rarely found in mouse or human Ig light chains but is an essential feature of these antibodies because it avoids antibody clashes with the V5 region of Env (McGuire et al., 2013).

Immunization of germline 3BNC60 or VRC01 knock-in mice with germline-targeting antigens, eOD-GT8, 426c gp140 TM4ΔV1-3, or eOD-GT6, produced antibody responses of which a fraction were specific for the CD4bs (Dosenovic et al., 2015; Jardine et al., 2015; McGuire et al., 2016; Tian et al., 2016). Single-cell antibody cloning experiments revealed that the germline-targeting immunogens

selectively expanded B cell clones expressing the 3BNC60 or VRC01 heavy chains paired with mouse IgL containing 5–amino acid–long CDRL3s. However, the mice differed in that selection of the 5–amino acid CDRL3 IgL was far more efficient in one of the VRC01 germline knock-in strains than in the other two (Dosenovic et al., 2015; Jardine et al., 2015; Tian et al., 2016). This difference may be caused by a lower affinity of the antigens for germline 3BNC60 and by differences in the way in which the two VRC01 knock-in mice were produced (Jardine et al., 2015; Tian et al., 2016). Irrespective of these differences, and as might be expected, none of the antibodies elicited by immunization with highly modified germline-targeting Env antigens bound to native-like Env antigens or neutralized HIV-1 (Fig. 1).

In contrast to the germline-targeting antigens, native-like antigens failed to induce responses in the VRC01 or 3BNC60 germline IgH knock-in mice (Fig. 1). Thus, germline-targeting antigens were essential to activate naive B cells and select for specific characteristics associated with VRC01 class bNAb development. However, the germline-targeting antigens available to date fail to induce neutralizing responses, and they have a narrow spectrum of activity in that only a very limited number of CD4bs-directed bNAb precursors can be activated by these antigens.

Similar results were obtained with knock-in mice that carry the inferred germline IgH and IgL chain corresponding to PGT121, a bNAb targeting the V3-glycan epitope on the Env spike (Escolano et al., 2016). The germline sequence of PGT121 was based on the CDRH3 sequence of the least mutated clonal relative 10-996 of the same original donor (Mouquet et al., 2012). Native-like SOSIP antigens predictably failed to induce antibody responses in these mice. In contrast, a specifically designed germline-targeting antigen induced B cell responses, although once again immunization with this single immunogen was not sufficient to produce neutralizing antibodies (Escolano et al., 2016; Steichen et al., 2016).

Finally, the VRC01-class germline-targeting eOD-GT8 antigen was also used to immunize transgenic mice carrying human Ig heavy and light chain loci, which gives rise to a diverse repertoire of B cells expressing human antibodies (Sok et al., 2016). Although not measured directly, it was estimated that the precursor frequency for VRC01 class B cells in these mice was on the order of 0.2–1.3 cells per mouse. After immunization, B cells binding to the antigen in a CD4bs-specific manner were obtained by cell sorting. 1% of these antigen-binding memory B cells identified by flow cytometry expressed VRC01-class antibodies, or 28 cells in 17 mice, or 1.6 cells per mouse in the 29% of the mice that responded. Whether this represents a significant expansion that can be further induced to develop into bNAbs remains to be determined.

In conclusion, the initial studies in knock-in mice showed that naive B cells expressing inferred germline versions of bNAbs can be activated by specifically selected or engineered germline-targeting antigens but not by native-like Env trimers. These results, together with the observation that most Envs do not engage germline bNAb precursors, may in part explain why the Env immunogens used in vaccines have failed to elicit neutralizing responses to date.

Sequential immunization in genetically engineered mice

As discussed in the previous section, immunization of germline antibody knock-in mice with germline-targeting immunogens did not lead to the development of neutralizing antibodies. Moreover, it became evident upon uncovering the prevalence and essential nature of anti–HIV-1 antibody mutation (Mouquet et al., 2010, 2012; Zhou et al., 2010; Bonsignori et al., 2011; Scheid et al., 2011; Klein et al., 2013a; Sok et al., 2013) and the natural progression of bNAb development (Richman et al., 2003; Wei et al., 2003; Liao et al., 2013; Doria-Rose et al., 2014; Wu et al., 2015; MacLeod et al., 2016) that immunization to reproduce this phenomenon and elicit bNAbs may require a sequential immunization approach using immunogens that would shepherd the immune response toward bNAbs (Scheid et al., 2009a, 2011; Dimitrov, 2010; Pancera et al., 2010; Zhou et al., 2010; Haynes et al., 2012; Mouquet et al., 2012; Jardine et al., 2013; Klein et al., 2013a,b; McGuire et al., 2013).

This idea was initially tested using a synthetic intermediate 3BNC60 knock-in mouse that carried the mature heavy chain of 3BNC60 paired with random germline mouse light chains (Dosenovic et al., 2015). Whereas germline-targeting antigens elicited antibodies that were only capable of neutralizing HIV-1 viruses lacking the glycan at position 276, immunization with a native-like trimer elicited antibodies with the ability to neutralize heterologous tier 2 HIV-1 strains bearing an intact glycosylation site at position N276 (Dosenovic et al., 2015). Cross-clade neutralizing activity elicited after immunization with the native-like trimer was associated with selection for a highly restricted set of Ig light chains that exclusively expressed the required short CDRL3 of 5 amino acids and showed key mutations that correlated with neutralization breadth. Thus, B cells bearing inferred germline precursors could only be activated by germline-targeting immunogens, but these immunogens did not elicit bNAbs, even when used to immunize mice bearing synthetic intermediates. In contrast, native-like immunogens failed to stimulate B cells bearing inferred germline precursors but were necessary to elicit neutralizing antibodies from B cells expressing the synthetic intermediate antibody (Dosenovic et al., 2015).

These initial experiments established that a combination of different immunogens would be necessary to elicit bNAbs; however, they were limited to a single epitope on Env, they failed to uncover a protocol that elicited bNAbs from germline precursors, and they did not achieve the exceptionally high levels of mutation and neutralizing activity associated with bNAbs. Several of these limitations were subsequently overcome in more recent studies using knock-in mice carrying the inferred germline VRC01 and PGT121 antibodies (Briney et al., 2016; Escolano et al., 2016; Tian et al., 2016).

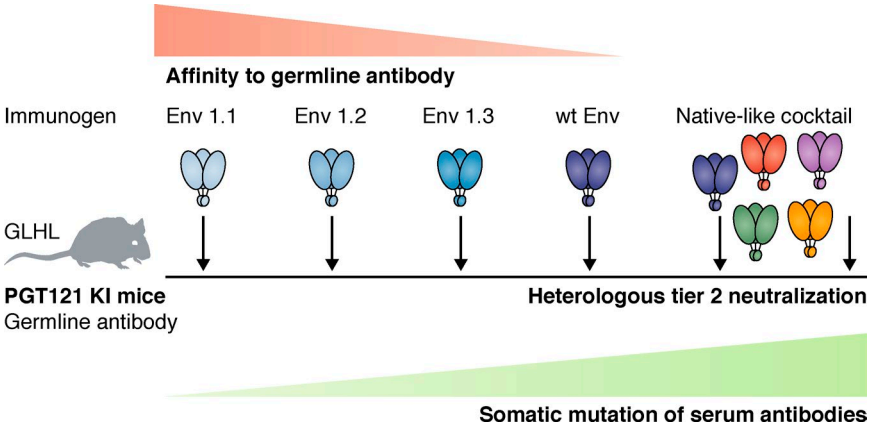


Figure 2. **Linking sequential immunization to somatic mutation and bNAb development.** The figure illustrates the immunization scheme that led to bNAb development in mice that carry the PGT121 germline gene. The initial immunogen had the highest affinity for the germline-reverted antibody, and subsequent immunogens had decreasing affinities. Somatic mutation increased with immunization.

Two studies using mice carrying inferred germline precursors of VRC01 achieved high levels of mutation by sequential immunization starting with germline-targeting Env immunogens followed by boosting with a series of less modified Env immunogens (Briney et al., 2016; Tian et al., 2016). In both cases, the initial immunogen opens up the CD4bs by removing glycans that normally impede antibody access to this epitope. Subsequent immunogens sequentially reincorporate the native sequence. The resulting antibodies neutralized a group of HIV-1 isolates lacking the potential N-linked glycosylation site at position N276. However, the serum of these mice was unable to neutralize HIV-1 viruses that carry an intact potential N-linked glycosylation site at position N276, which includes 95% of all viruses in current databases (Briney et al., 2016; Tian et al., 2016). Two exceptional MAbs neutralized a wild-type virus grown in 293S cells, but these cells failed to incorporate more complex glycans into the virion. Thus, the critical problem of how to elicit CD4bs-directed antibodies that can evade the glycan at position N276 remains to be addressed.

In contrast to the VRC01 mice, sequentially immunized knock-in mice expressing both IgH and IgL of the germline PGT121 antibody developed serologic activity and MAbs that showed significant potency and breadth against heterologous tier 2 isolates (Fig. 2; Escolano et al., 2016). PGT121 germline knock-in mice were immunized with a germline-targeting immunogen, 10MUT, followed by a sequence of modified and native-like Env antigens (Escolano et al., 2016; Steichen et al., 2016). The 10MUT immunogen is based on BG505-SOSIP.664 but differs from it in that the antibody target site is exposed by the introduction of mutations removing glycans at positions N137 and N133, which increase its affinity for germline PGT121 antibody (Garces et al., 2014; Steichen et al., 2016). Each additional immunogen in the sequence partially reverted these mutations, and the final step in the immunization protocol included a combination of different native-like SOSIP Env trimers. The precise sequence was determined by ELISAs on the serum of the immunized mice against a panel of candidate antigens, and the boosting immunogen was selected on the basis of serologic cross-reactivity. Thereby, each step in the immunization protocol introduced antigens with decreasing affinities for germline PGT121. The resulting protocol yielded high levels of SHM that recapitulated several key mutations found in human PGT121-like antibodies.

In conclusion, sequential immunization with antigens designed to shepherd the antibody response toward bNAb development induced high levels of somatic mutation irrespective of the epitope targeted. These are the first results demonstrating that anti–HIV-1 bNAbs can be elicited by immunization. However, there are several caveats to consider—most importantly this was achieved in knock-in mice wherein there is limited B cell diversity. A more diverse immune system may fail to respond in the same way. For example, it was difficult to demonstrate enrichment for memory B cells expressing VRC01-like antibodies in mice that carry a diverse repertoire of human antibody genes (Kymab mice) immunized with eOD-GT8 (Sok et al., 2016), possibly because of limiting naive precursor cells or competition for limiting T cell help by B cells carrying receptors for immunodominant epitopes (Victora and Nussenzweig, 2012). In addition, none of the protocols reported to date elicit antibodies with the neutralization breadth and potency of the most potent bNAbs, nor do they take advantage of the fact that there are multiple neutralizing epitope targets on Env. Finally, although the sequential immunization experiments demonstrate that bNAbs can be elicited by immunization in these engineered systems, complex protocols involving immunization with multiple antigens in a specific sequence may not be practical in parts of the world where HIV-1 vaccines are most needed.

Passive vaccination

The demonstration by Kitasato and von Behring that antibodies can be used to prevent or treat infectious diseases was awarded a Nobel prize in 1901, and serum therapy was used extensively to treat and prevent serious infections in the first part of the 20th century. This form of therapy was largely abandoned with the advent of potent antibiotics and effective vaccines. However, starting 25 years ago, preclinical experiments performed in animal models showed that passively transferred neutralizing sera and first-generation monoclonal

bNAbs are effective in protecting against HIV-1 infection (Prince et al., 1991; Mascola et al., 1999, 2000; Shibata et al., 1999; Baba et al., 2000; Hessell et al., 2010). Nevertheless, passive immunization with first-generation bNAbs was not pursued for HIV-1 prevention because of a combination of poor pharmacokinetic properties and limited overall breadth and potency (Trkola et al., 2005; Joos et al., 2006; Mehandru et al., 2007).

As might be expected from their greater neutralization breadth and potency against HIV-1 isolates in vitro and good pharmacokinetic properties in vivo, second-generation bNAbs are far more effective than first-generation bNAbs in preventing SHIV infection in macaques (Shingai et al., 2013, 2014; Saunders et al., 2015b; Gautam et al., 2016; Hessell et al., 2016) and HIV-1 infection in humanized mice (Pietzsch et al., 2012; Gruell et al., 2013). A study of 60 macaques treated with one of five different bNAbs found that protection of 50% of the macaques against high-dose intravenous infection requires a relatively modest serum neutralizing titer of 1:100 (Shingai et al., 2014).

In contrast to the high-dose challenge model in macaques, humans are typically exposed to relatively low doses of HIV-1 by a mucosal route, and productive infection usually requires multiple exposures. Gautam et al. (2016) modeled human infection by weekly intrarectal challenge with low doses of $SHIV_{AD8}$ and found that a single passive bNAb infusion protected macaques for up to 23 wk. Protection was directly related to antibody concentration in serum and to antibody half-life. Overall, protection required serum antibody concentrations corresponding to the IC_{80} in TZM-bl assays. For example, VRC01, which is less potent than 3BNC117 but targets the same epitope on HIV-1 Env, protected macaques for an average of 8 wk, whereas 3BNC117 protected for an average of 13 wk. Increasing the half-life of VRC01 by adding a mutation to alter FcRN binding (VRC01-LS) nearly doubled protection from 8 to 14.5 wk (Gautam et al., 2016).

Although passive immunization is protective against SHIV challenge in these animal models, the mechanisms of protection are not fully understood. Fc receptor binding is required (Hessell et al., 2007; Pietzsch et al., 2010; Halper-Stromberg et al., 2014). Two recent studies in rhesus macaques show that passive immunization with protective doses of bNAbs fails to completely sterilize SHIV infection at the site of viral challenge and that small amounts of viral RNA and DNA can be detected in distal tissues (Hessell et al., 2016; Liu et al., 2016). Both studies suggest that in addition to blocking infection, early administration of bNAbs also eliminates small foci of viral replication and thereby limits dissemination and prevents productive infection (Hessell et al., 2016).

Human clinical trials with second-generation bNAbs have shown that VRC01 and 3BNC117 are generally safe and have half-lives of 2 and 2.5 wk, respectively (Caskey et al., 2015; Ledgerwood et al., 2015; Lynch et al., 2015). Addition of the LS mutation that alters FcRN binding will likely increase the half-life by two- to fourfold (Saunders et al., 2015a). Assuming that protection by antibody serum levels corresponds to the IC_{80} in TZM-bl assays, a single subcutaneous injection of the combination of 3BNC117-LS and 10-1074-LS could protect against over 90% of all strains for many months (Gautam et al., 2016). In theory, coverage could be further increased by addition of antibodies that target nonoverlapping sites on the HIV-1 Env, such as PGDM1400 (Sok et al., 2014), or with more potent or broader antibodies (Diskin et al., 2011; Galimidi et al., 2015; Huang et al., 2016).

Conclusions

Significant advances in understanding the structural and immunological determinants of anti–HIV-1 antibody development enabled experiments using engineered mice and immunogens, which finally elicited bNAbs by vaccination. Concurrently, passive immunization studies using potent second-generation bNAbs were highly successful in protecting animals against HIV-1 infection. Antibodies are relatively expensive to manufacture compared with small molecule drugs; however, the low doses required for protection, their long half-life, ease of administration subcutaneously, and decreasing manufacturing costs all make a passive vaccine delivered on a quarterly or biyearly basis an attainable goal. In contrast, vaccination to produce modestly active bNAbs has only been achieved in antibody knock-in mice and by highly complex schemes involving multiple immunogens delivered in a specific sequential manner. Whether this can be achieved in physiologically relevant animal models or humans remains to be determined. Moreover, the potential challenges of developing, manufacturing, and delivering a multicomponent protein vaccine that has to be administered on a specific schedule and likely boosted on a regular basis may make this approach impractical in the near term. Thus, the alternative of a passively administered bNAb vaccine that can be developed rapidly and administered on a quarterly or biannual basis may be a realistic alternative to bridge the gap to a more conventional HIV-1 vaccine.

ACKNOWLEDGMENTS

We thank Yotam Bar On, Marina Caskey, Yehuda Cohen, Lillian Cohn, Ari Halper-Stromberg, Joshua Horwitz, Mila Jankovic, and Gunilla Karlsson Hedestam for valuable input on the manuscript.

M.C. Nussenzweig receives funding from National Institutes of Health (NIH) Center for HIV/AIDS Vaccine Immunology and Immunogen Discovery (CHAVI-ID) UM1 AI100663, Bill and Melinda Gates Foundation grant OPP1033115, NIH grant U19 AI109632, and the Robertson Foundation. A. Escolano is supported by an international postdoctoral fellowship from the Clarin COFUND-Marie Curie program (PCTI-FICYT). P. Dosenovic is supported by an international postdoctoral fellowship from the Swedish Research Council. M.C. Nussenzweig is a Howard Hughes Medical Institute investigator.

The Rockefeller University and M.C. Nussenzweig have patents pending on antibodies 3BNC117 and 10-1074. The authors declare no additional competing financial interests.

Submitted: 20 October 2016
Revised: 30 November 2016
Accepted: 7 December 2016

REFERENCES

Baba, T.W., V. Liska, R. Hofmann-Lehmann, J. Vlasak, W. Xu, S. Ayehunie, L.A. Cavacini, M.R. Posner, H. Katinger, G. Stiegler, et al. 2000. Human neutralizing monoclonal antibodies of the IgG1 subtype protect against mucosal simian-human immunodeficiency virus infection. *Nat. Med.* 6:200–206. http://dx.doi.org/10.1038/72309

Balazs, A.B., J. Chen, C.M. Hong, D.S. Rao, L. Yang, and D. Baltimore. 2012. Antibody-based protection against HIV infection by vectored immunoprophylaxis. *Nature.* 481:81–84. http://dx.doi.org/10.1038/nature10660

Barbas, C.F. III, E. Björling, F. Chiodi, N. Dunlop, D. Cababa, T.M. Jones, S.L. Zebedee, M.A. Persson, P.L. Nara, E. Norrby, et al. 1992. Recombinant human Fab fragments neutralize human type 1 immunodeficiency virus in vitro. *Proc. Natl. Acad. Sci. USA.* 89:9339–9343. http://dx.doi.org/10.1073/pnas.89.19.9339

Bhiman, J.N., C. Anthony, N.A. Doria-Rose, O. Karimanzira, C.A. Schramm, T. Khoza, D. Kitchin, G. Botha, J. Gorman, N.J. Garrett, et al. 2015. Viral variants that initiate and drive maturation of V1V2-directed HIV-1 broadly neutralizing antibodies. *Nat. Med.* 21:1332–1336. http://dx.doi.org/10.1038/nm.3963

Binley, J.M., R.W. Sanders, B. Clas, N. Schuelke, A. Master, Y. Guo, F. Kajumo, D.J. Anselma, P.J. Maddon, W.C. Olson, and J.P. Moore. 2000. A recombinant human immunodeficiency virus type 1 envelope glycoprotein complex stabilized by an intermolecular disulfide bond between the gp120 and gp41 subunits is an antigenic mimic of the trimeric virion-associated structure. *J. Virol.* 74:627–643. http://dx.doi.org/10.1128/JVI.74.2.627-643.2000

Binley, J.M., E.A. Lybarger, E.T. Crooks, M.S. Seaman, E. Gray, K.L. Davis, J.M. Decker, D. Wycuff, L. Harris, N. Hawkins, et al. 2008. Profiling the specificity of neutralizing antibodies in a large panel of plasmas from patients chronically infected with human immunodeficiency virus type 1 subtypes B and C. *J. Virol.* 82:11651–11668. http://dx.doi.org/10.1128/JVI.01762-08

Bonsignori, M., K.K. Hwang, X. Chen, C.Y. Tsao, L. Morris, E. Gray, D.J. Marshall, J.A. Crump, S.H. Kapiga, N.E. Sam, et al. 2011. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. *J. Virol.* 85:9998–10009. http://dx.doi.org/10.1128/JVI.05045-11

Bonsignori, M., T. Zhou, Z. Sheng, L. Chen, F. Gao, M.G. Joyce, G. Ozorowski, G.Y. Chuang, C.A. Schramm, K. Wiehe, et al. NISC Comparative Sequencing Program. 2016. Maturation pathway from germline to broad HIV-1 neutralizer of a CD4-mimic antibody. *Cell.* 165:449–463. http://dx.doi.org/10.1016/j.cell.2016.02.022

Breden, F., C. Lepik, N.S. Longo, M. Montero, P.E. Lipsky, and J.K. Scott. 2011. Comparison of antibody repertoires produced by HIV-1 infection, other chronic and acute infections, and systemic autoimmune disease. *PLoS One.* 6:e16857. http://dx.doi.org/10.1371/journal.pone.0016857

Briney, B., D. Sok, J.G. Jardine, D.W. Kulp, P. Skog, S. Menis, R. Jacak, O. Kalyuzhniy, N. de Val, F. Sesterhenn, et al. 2016. Tailored Immunogens Direct Affinity Maturation toward HIV Neutralizing Antibodies. *Cell.* 166:1459–1470.e11. http://dx.doi.org/10.1016/j.cell.2016.08.005

Buchacher, A., R. Predl, K. Strutzenberger, W. Steinfellner, A. Trkola, M. Purtscher, G. Gruber, C. Tauer, F. Steindl, A. Jungbauer, et al. 1994. Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. *AIDS Res. Hum. Retroviruses.* 10:359–369. http://dx.doi.org/10.1089/aid.1994.10.359

Buchbinder, S.P., D.V. Mehrotra, A. Duerr, D.W. Fitzgerald, R. Mogg, D. Li, P.B. Gilbert, J.R. Lama, M. Marmor, C. Del Rio, et al. Step Study Protocol Team. 2008. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet.* 372:1881–1893. http://dx.doi.org/10.1016/S0140-6736(08)61591-3

Burton, D.R., and L. Hangartner. 2016. Broadly neutralizing antibodies to HIV and their role in vaccine design. *Annu. Rev. Immunol.* 34:635–659. http://dx.doi.org/10.1146/annurev-immunol-041015-055515

Burton, D.R., and J.R. Mascola. 2015. Antibody responses to envelope glycoproteins in HIV-1 infection. *Nat. Immunol.* 16:571–576. http://dx.doi.org/10.1038/ni.3158

Burton, D.R., J. Pyati, R. Koduri, S.J. Sharp, G.B. Thornton, P.W. Parren, L.S. Sawyer, R.M. Hendry, N. Dunlop, P.L. Nara, et al. 1994. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science.* 266:1024–1027. http://dx.doi.org/10.1126/science.7973652

Burton, D.R., R.L. Stanfield, and I.A. Wilson. 2005. Antibody vs. HIV in a clash of evolutionary titans. *Proc. Natl. Acad. Sci. USA.* 102:14943–14948. http://dx.doi.org/10.1073/pnas.0505126102

Caskey, M., F. Klein, J.C. Lorenzi, M.S. Seaman, A.P. West Jr., N. Buckley, G. Kremer, L. Nogueira, M. Braunschweig, J.F. Scheid, et al. 2015. Viraemia suppressed in HIV-1-infected humans by broadly neutralizing antibody 3BNC117. *Nature.* 522:487–491. http://dx.doi.org/10.1038/nature14411

de Taeye, S.W., G. Ozorowski, A. Torrents de la Peña, M. Guttman, J.P. Julien, T.L. van den Kerkhof, J.A. Burger, L.K. Pritchard, P. Pugach, A. Yasmeen, et al. 2015. Immunogenicity of stabilized HIV-1 envelope trimers with reduced exposure of non-neutralizing epitopes. *Cell.* 163:1702–1715. http://dx.doi.org/10.1016/j.cell.2015.11.056

Dimitrov, D.S. 2010. Therapeutic antibodies, vaccines and antibodyomes. *MAbs.* 2:347–356. http://dx.doi.org/10.4161/mabs.2.3.11779

Diskin, R., J.F. Scheid, P.M. Marcovecchio, A.P. West Jr., F. Klein, H. Gao, P.N. Gnanapragasam, A. Abadir, M.S. Seaman, M.C. Nussenzweig, and P.J. Bjorkman. 2011. Increasing the potency and breadth of an HIV antibody by using structure-based rational design. *Science.* 334:1289–1293. http://dx.doi.org/10.1126/science.1213782

Doria-Rose, N.A., R.M. Klein, M.M. Manion, S. O'Dell, A. Phogat, B. Chakrabarti, C.W. Hallahan, S.A. Migueles, J. Wrammert, R. Ahmed, et al. 2009. Frequency and phenotype of human immunodeficiency virus envelope-specific B cells from patients with broadly cross-neutralizing antibodies. *J. Virol.* 83:188–199. http://dx.doi.org/10.1128/JVI.01583-08

Doria-Rose, N.A., C.A. Schramm, J. Gorman, P.L. Moore, J.N. Bhiman, B.J. DeKosky, M.J. Ernandes, I.S. Georgiev, H.J. Kim, M. Pancera, et al. NISC Comparative Sequencing Program. 2014. Developmental pathway for potent V1V2-directed HIV-neutralizing antibodies. *Nature.* 509:55–62. http://dx.doi.org/10.1038/nature13036

Dosenovic, P., B. Chakrabarti, M. Soldemo, I. Douagi, M.N. Forsell, Y. Li, A. Phogat, S. Paulie, J. Hoxie, R.T. Wyatt, and G.B. Karlsson Hedestam. 2009. Selective expansion of HIV-1 envelope glycoprotein-specific B cell subsets recognizing distinct structural elements following immunization. *J. Immunol.* 183:3373–3382. http://dx.doi.org/10.4049/jimmunol.0900407

Dosenovic, P., L. von Boehmer, A. Escolano, J. Jardine, N.T. Freund, A.D. Gitlin, A.T. McGuire, D.W. Kulp, T. Oliveira, L. Scharf, et al. 2015. Immunization for HIV-1 broadly neutralizing antibodies in human Ig knockin mice. *Cell.* 161:1505–1515. http://dx.doi.org/10.1016/j.cell.2015.06.003

Doyle-Cooper, C., K.E. Hudson, A.B. Cooper, T. Ota, P. Skog, P.E. Dawson, M.B. Zwick, W.R. Schief, D.R. Burton, and D. Nemazee. 2013. Immune tolerance negatively regulates B cells in knock-in mice expressing broadly neutralizing HIV antibody 4E10. *J. Immunol.* 191:3186–3191. http://dx.doi.org/10.4049/jimmunol.1301285

Duerr, A., Y. Huang, S. Buchbinder, R.W. Coombs, J. Sanchez, C. del Rio, M. Casapia, S. Santiago, P. Gilbert, L. Corey, and M.N. Robertson. Step/HVTN 504 Study Team. 2012. Extended follow-up confirms early vaccine-enhanced risk of HIV acquisition and demonstrates waning effect over time among participants in a randomized trial of recombinant

adenovirus HIV vaccine (Step Study). *J. Infect. Dis.* 206:258–266. http://dx.doi.org/10.1093/infdis/jis342

Eda, Y., M. Takizawa, T. Murakami, H. Maeda, K. Kimachi, H. Yonemura, S. Koyanagi, K. Shiosaki, H. Higuchi, K. Makizumi, et al. 2006. Sequential immunization with V3 peptides from primary human immunodeficiency virus type 1 produces cross-neutralizing antibodies against primary isolates with a matching narrow-neutralization sequence motif. *J. Virol.* 80:5552–5562. http://dx.doi.org/10.1128/JVI.02094-05

Eichberg, J.W., K.K. Murthy, R.H. Ward, and A.M. Prince. 1992. Prevention of HIV infection by passive immunization with HIVIG or CD4-IgG. *AIDS Res. Hum. Retroviruses.* 8:1515.

Emini, E.A., W.A. Schleif, J.H. Nunberg, A.J. Conley, Y. Eda, S. Tokiyoshi, S.D. Putney, S. Matsushita, K.E. Cobb, C.M. Jett, et al. 1992. Prevention of HIV-1 infection in chimpanzees by gp120 V3 domain-specific monoclonal antibody. *Nature.* 355:728–730. http://dx.doi.org/10.1038/355728a0

Escolano, A., J.M. Steichen, P. Dosenovic, D.W. Kulp, J. Golijanin, D. Sok, N.T. Freund, A.D. Gitlin, T. Oliveira, T. Araki, et al. 2016. Sequential immunization elicits broadly neutralizing anti-HIV-1 antibodies in Ig knockin mice. *Cell.* 166:1445–1458. http://dx.doi.org/10.1016/j.cell.2016.07.030

Flynn, N.M., D.N. Forthal, C.D. Harro, F.N. Judson, K.H. Mayer, and M.F. Para. rgp120 HIV Vaccine Study Group. 2005. Placebo-controlled phase 3 trial of a recombinant glycoprotein 120 vaccine to prevent HIV-1 infection. *J. Infect. Dis.* 191:654–665. http://dx.doi.org/10.1086/428404

Galimidi, R.P., J.S. Klein, M.S. Politzer, S. Bai, M.S. Seaman, M.C. Nussenzweig, A.P. West Jr., and P.J. Bjorkman. 2015. Intra-spike crosslinking overcomes antibody evasion by HIV-1. *Cell.* 160:433–446. http://dx.doi.org/10.1016/j.cell.2015.01.016

Garces, F., D. Sok, L. Kong, R. McBride, H.J. Kim, K.F. Saye-Francisco, J.P. Julien, Y. Hua, A. Cupo, J.P. Moore, et al. 2014. Structural evolution of glycan recognition by a family of potent HIV antibodies. *Cell.* 159:69–79. http://dx.doi.org/10.1016/j.cell.2014.09.009

Garces, F., J.H. Lee, N. de Val, A.T. de la Pena, L. Kong, C. Puchades, Y. Hua, R.L. Stanfield, D.R. Burton, J.P. Moore, et al. 2015. Affinity maturation of a potent family of HIV antibodies is primarily focused on accommodating or avoiding glycans. *Immunity.* 43:1053–1063. http://dx.doi.org/10.1016/j.immuni.2015.11.007

Gautam, R., Y. Nishimura, A. Pegu, M.C. Nason, F. Klein, A. Gazumyan, J. Golijanin, A. Buckler-White, R. Sadjadpour, K. Wang, et al. 2016. A single injection of anti-HIV-1 antibodies protects against repeated SHIV challenges. *Nature.* 533:105–109. http://dx.doi.org/10.1038/nature17677

Georgiev, I.S., N.A. Doria-Rose, T. Zhou, Y.D. Kwon, R.P. Staupe, S. Moquin, G.Y. Chuang, M.K. Louder, S.D. Schmidt, H.R. Altae-Tran, et al. 2013. Delineating antibody recognition in polyclonal sera from patterns of HIV-1 isolate neutralization. *Science.* 340:751–756. http://dx.doi.org/10.1126/science.1233989

Gitlin, A.D., Z. Shulman, and M.C. Nussenzweig. 2014. Clonal selection in the germinal centre by regulated proliferation and hypermutation. *Nature.* 509:637–640. http://dx.doi.org/10.1038/nature13300

Gitlin, A.D., C.T. Mayer, T.Y. Oliveira, Z. Shulman, M.J. Jones, A. Koren, and M.C. Nussenzweig. 2015. Humoral immunity. T cell help controls the speed of the cell cycle in germinal center B cells. *Science.* 349:643–646. http://dx.doi.org/10.1126/science.aac4919

Goodnow, C.C. 1992. Transgenic mice and analysis of B-cell tolerance. *Annu. Rev. Immunol.* 10:489–518. http://dx.doi.org/10.1146/annurev.iy.10.040192.002421

Gorny, M.K., A.J. Conley, S. Karwowska, A. Buchbinder, J.Y. Xu, E.A. Emini, S. Koenig, and S. Zolla-Pazner. 1992. Neutralization of diverse human immunodeficiency virus type 1 variants by an anti-V3 human monoclonal antibody. *J. Virol.* 66:7538–7542.

Gray, E.S., M.C. Madiga, T. Hermanus, P.L. Moore, C.K. Wibmer, N.L. Tumba, L. Werner, K. Mlisana, S. Sibeko, C. Williamson, et al. CAPRISA002 Study Team. 2011a. The neutralization breadth of HIV-1 develops incrementally over four years and is associated with $CD4^+$ T cell decline and high viral load during acute infection. *J. Virol.* 85:4828–4840. http://dx.doi.org/10.1128/JVI.00198-11

Gray, G.E., M. Allen, Z. Moodie, G. Churchyard, L.G. Bekker, M. Nchabeleng, K. Mlisana, B. Metch, G. de Bruyn, M.H. Latka, et al. HVTN 503/Phambili study team. 2011b. Safety and efficacy of the HVTN 503/Phambili study of a clade-B-based HIV-1 vaccine in South Africa: a double-blind, randomised, placebo-controlled test-of-concept phase 2b study. *Lancet Infect. Dis.* 11:507–515. http://dx.doi.org/10.1016/S1473-3099(11)70098-6

Gristick, H.B., L. von Boehmer, A.P. West Jr., M. Schamber, A. Gazumyan, J. Golijanin, M.S. Seaman, G. Fätkenheuer, F. Klein, M.C. Nussenzweig, and P.J. Bjorkman. 2016. Natively glycosylated HIV-1 Env structure reveals new mode for antibody recognition of the CD4-binding site. *Nat. Struct. Mol. Biol.* 23:906–915. http://dx.doi.org/10.1038/nsmb.3291

Gruell, H., S. Bournazos, J.V. Ravetch, A. Ploss, M.C. Nussenzweig, and J. Pietzsch. 2013. Antibody and antiretroviral preexposure prophylaxis prevent cervicovaginal HIV-1 infection in a transgenic mouse model. *J. Virol.* 87:8535–8544. http://dx.doi.org/10.1128/JVI.00868-13

Guenaga, J., P. Dosenovic, G. Ofek, D. Baker, W.R. Schief, P.D. Kwong, G.B. Karlsson Hedestam, and R.T. Wyatt. 2011. Heterologous epitope-scaffold prime:boosting immuno-focuses B cell responses to the HIV-1 gp41 2F5 neutralization determinant. *PLoS One.* 6:e16074. http://dx.doi.org/10.1371/journal.pone.0016074

Guenaga, J., V. Dubrovskaya, N. de Val, S.K. Sharma, B. Carrette, A.B. Ward, and R.T. Wyatt. 2016. Structure-guided redesign increases the propensity of HIV Env to generate highly stable soluble trimers. *J. Virol.* 90:2806–2817. http://dx.doi.org/10.1128/JVI.02652-15

Halper-Stromberg, A., C.L. Lu, F. Klein, J.A. Horwitz, S. Bournazos, L. Nogueira, T.R. Eisenreich, C. Liu, A. Gazumyan, U. Schaefer, et al. 2014. Broadly neutralizing antibodies and viral inducers decrease rebound from HIV-1 latent reservoirs in humanized mice. *Cell.* 158:989–999. http://dx.doi.org/10.1016/j.cell.2014.07.043

Hammer, S.M., M.E. Sobieszczyk, H. Janes, S.T. Karuna, M.J. Mulligan, D. Grove, B.A. Koblin, S.P. Buchbinder, M.C. Keefer, G.D. Tomaras, et al. HVTN 505 Study Team. 2013. Efficacy trial of a DNA/rAd5 HIV-1 preventive vaccine. *N. Engl. J. Med.* 369:2083–2092. http://dx.doi.org/10.1056/NEJMoa1310566

Haynes, B.F., J. Fleming, E.W. St Clair, H. Katinger, G. Stiegler, R. Kunert, J. Robinson, R.M. Scearce, K. Plonk, H.F. Staats, et al. 2005. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. *Science.* 308:1906–1908. http://dx.doi.org/10.1126/science.1111781

Haynes, B.F., G. Kelsoe, S.C. Harrison, and T.B. Kepler. 2012. B-cell-lineage immunogen design in vaccine development with HIV-1 as a case study. *Nat. Biotechnol.* 30:423–433. http://dx.doi.org/10.1038/nbt.2197

Hessell, A.J., L. Hangartner, M. Hunter, C.E. Havenith, F.J. Beurskens, J.M. Bakker, C.M. Lanigan, G. Landucci, D.N. Forthal, P.W. Parren, et al. 2007. Fc receptor but not complement binding is important in antibody protection against HIV. *Nature.* 449:101–104. http://dx.doi.org/10.1038/nature06106

Hessell, A.J., P. Poignard, M. Hunter, L. Hangartner, D.M. Tehrani, W.K. Bleeker, P.W. Parren, P.A. Marx, and D.R. Burton. 2009a. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. *Nat. Med.* 15:951–954. http://dx.doi.org/10.1038/nm.1974

Hessell, A.J., E.G. Rakasz, P. Poignard, L. Hangartner, G. Landucci, D.N. Forthal, W.C. Koff, D.I. Watkins, and D.R. Burton. 2009b. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. *PLoS Pathog.* 5:e1000433. http://dx.doi.org/10.1371/journal.ppat.1000433

Hessell, A.J., E.G. Rakasz, D.M. Tehrani, M. Huber, K.L. Weisgrau, G. Landucci, D.N. Forthal, W.C. Koff, P. Poignard, D.I. Watkins, and D.R. Burton. 2010. Broadly neutralizing monoclonal antibodies 2F5 and 4E10 directed against the human immunodeficiency virus type 1 gp41 membrane-proximal external region protect against mucosal challenge by simian-human immunodeficiency virus SHIVBa-L. *J. Virol.* 84:1302–1313. http://dx.doi.org/10.1128/JVI.01272-09

Hessell, A.J., J.P. Jaworski, E. Epson, K. Matsuda, S. Pandey, C. Kahl, J. Reed, W.F. Sutton, K.B. Hammond, T.A. Cheever, et al. 2016. Early short-term treatment with neutralizing human monoclonal antibodies halts SHIV infection in infant macaques. *Nat. Med.* 22:362–368. http://dx.doi.org/10.1038/nm.4063

Hoot, S., A.T. McGuire, K.W. Cohen, R.K. Strong, L. Hangartner, F. Klein, R. Diskin, J.F. Scheid, D.N. Sather, D.R. Burton, and L. Stamatatos. 2013. Recombinant HIV envelope proteins fail to engage germline versions of anti-CD4bs bNAbs. *PLoS Pathog.* 9:e1003106. (published erratum appears in *PLoS Pathog.* 2013. http://dx.doi.org/10.1371/annotation/ed7c0148-97eb-4416-824d-6e6d1aaeceef) http://dx.doi.org/10.1371/journal.ppat.1003106

Hraber, P., M.S. Seaman, R.T. Bailer, J.R. Mascola, D.C. Montefiori, and B.T. Korber. 2014. Prevalence of broadly neutralizing antibody responses during chronic HIV-1 infection. *AIDS.* 28:163–169. http://dx.doi.org/10.1097/QAD.0000000000000106

Hu, J.K., J.C. Crampton, A. Cupo, T. Ketas, M.J. van Gils, K. Sliepen, S.W. de Taeye, D. Sok, G. Ozorowski, I. Deresa, et al. 2015. Murine antibody responses to cleaved soluble HIV-1 envelope trimers are highly restricted in specificity. *J. Virol.* 89:10383–10398. http://dx.doi.org/10.1128/JVI.01653-15

Huang, J., G. Ofek, L. Laub, M.K. Louder, N.A. Doria-Rose, N.S. Longo, H. Imamichi, R.T. Bailer, B. Chakrabarti, S.K. Sharma, et al. 2012. Broad and potent neutralization of HIV-1 by a gp41-specific human antibody. *Nature.* 491:406–412. http://dx.doi.org/10.1038/nature11544

Huang, J., B.H. Kang, E. Ishida, T. Zhou, T. Griesman, Z. Sheng, F. Wu, N.A. Doria-Rose, B. Zhang, K. McKee, et al. 2016. Identification of a CD4-binding-site antibody to HIV that evolved near-pan neutralization breadth. *Immunity.* 45:1108–1121. http://dx.doi.org/10.1016/j.immuni.2016.10.027

Ingale, J., A. Stano, J. Guenaga, S.K. Sharma, D. Nemazee, M.B. Zwick, and R.T. Wyatt. 2016. High-density array of well-ordered HIV-1 spikes on synthetic liposomal nanoparticles efficiently activate B cells. *Cell Reports.* 15:1986–1999. http://dx.doi.org/10.1016/j.celrep.2016.04.078

Jardine, J., J.P. Julien, S. Menis, T. Ota, O. Kalyuzhniy, A. McGuire, D. Sok, P.S. Huang, S. MacPherson, M. Jones, et al. 2013. Rational HIV immunogen design to target specific germline B cell receptors. *Science.* 340:711–716. http://dx.doi.org/10.1126/science.1234150

Jardine, J.G., T. Ota, D. Sok, M. Pauthner, D.W. Kulp, O. Kalyuzhniy, P.D. Skog, T.C. Thinnes, D. Bhullar, B. Briney, et al. 2015. HIV-1 vaccines. Priming a broadly neutralizing antibody response to HIV-1 using a germline-targeting immunogen. *Science.* 349:156–161. http://dx.doi.org/10.1126/science.aac5894

Jardine, J.G., D.W. Kulp, C. Havenar-Daughton, A. Sarkar, B. Briney, D. Sok, F. Sesterhenn, J. Ereño-Orbea, O. Kalyuzhniy, I. Deresa, et al. 2016. HIV-1 broadly neutralizing antibody precursor B cells revealed by germline-targeting immunogen. *Science.* 351:1458–1463. http://dx.doi.org/10.1126/science.aad9195

Javaherian, K., A.J. Langlois, G.J. LaRosa, A.T. Profy, D.P. Bolognesi, W.C. Herlihy, S.D. Putney, and T.J. Matthews. 1990. Broadly neutralizing antibodies elicited by the hypervariable neutralizing determinant of HIV-1. *Science.* 250:1590–1593. http://dx.doi.org/10.1126/science.1703322

Joos, B., A. Trkola, H. Kuster, L. Aceto, M. Fischer, G. Stiegler, C. Armbruster, B. Vcelar, H. Katinger, and H.F. Günthard. 2006. Long-term multiple-dose pharmacokinetics of human monoclonal antibodies (MAbs) against human immunodeficiency virus type 1 envelope gp120 (MAb 2G12) and gp41 (MAbs 4E10 and 2F5). *Antimicrob. Agents Chemother.* 50:1773–1779. http://dx.doi.org/10.1128/AAC.50.5.1773-1779.2006

Julien, J.P., A. Cupo, D. Sok, R.L. Stanfield, D. Lyumkis, M.C. Deller, P.J. Klasse, D.R. Burton, R.W. Sanders, J.P. Moore, et al. 2013. Crystal structure of a soluble cleaved HIV-1 envelope trimer. *Science.* 342:1477–1483. http://dx.doi.org/10.1126/science.1245625

Kepler, T.B., H.X. Liao, S.M. Alam, R. Bhaskarabhatla, R. Zhang, C. Yandava, S. Stewart, K. Anasti, G. Kelsoe, R. Parks, et al. 2014. Immunoglobulin gene insertions and deletions in the affinity maturation of HIV-1 broadly reactive neutralizing antibodies. *Cell Host Microbe.* 16:304–313. http://dx.doi.org/10.1016/j.chom.2014.08.006

Klasse, P.J., C.C. LaBranche, T.J. Ketas, G. Ozorowski, A. Cupo, P. Pugach, R.P. Ringe, M. Golabek, M.J. van Gils, M. Guttman, et al. 2016. Sequential and simultaneous immunization of rabbits with HIV-1 envelope glycoprotein SOSIP.664 trimers from clades A, B and C. *PLoS Pathog.* 12:e1005864. http://dx.doi.org/10.1371/journal.ppat.1005864

Klein, F., R. Diskin, J.F. Scheid, C. Gaebler, H. Mouquet, I.S. Georgiev, M. Pancera, T. Zhou, R.B. Incesu, B.Z. Fu, et al. 2013a. Somatic mutations of the immunoglobulin framework are generally required for broad and potent HIV-1 neutralization. *Cell.* 153:126–138. http://dx.doi.org/10.1016/j.cell.2013.03.018

Klein, F., H. Mouquet, P. Dosenovic, J.F. Scheid, L. Scharf, and M.C. Nussenzweig. 2013b. Antibodies in HIV-1 vaccine development and therapy. *Science.* 341:1199–1204. http://dx.doi.org/10.1126/science.1241144

Klinman, D.M., K.W. Higgins, and J. Conover. 1991. Sequential immunizations with rgp120s from independent isolates of human immunodeficiency virus type 1 induce the preferential expansion of broadly crossreactive B cells. *J. Exp. Med.* 173:881–887. http://dx.doi.org/10.1084/jem.173.4.881

Kong, R., K. Xu, T. Zhou, P. Acharya, T. Lemmin, K. Liu, G. Ozorowski, C. Soto, J.D. Taft, R.T. Bailer, et al. 2016. Fusion peptide of HIV-1 as a site of vulnerability to neutralizing antibody. *Science.* 352:828–833. http://dx.doi.org/10.1126/science.aae0474

Kwong, P.D., and J.R. Mascola. 2012. Human antibodies that neutralize HIV-1: identification, structures, and B cell ontogenies. *Immunity.* 37:412–425. http://dx.doi.org/10.1016/j.immuni.2012.08.012

Ledgerwood, J.E., E.E. Coates, G. Yamshchikov, J.G. Saunders, L. Holman, M.E. Enama, A. DeZure, R.M. Lynch, I. Gordon, S. Plummer, et al. VRC 602 Study Team. 2015. Safety, pharmacokinetics and neutralization of the broadly neutralizing HIV-1 human monoclonal antibody VRC01 in healthy adults. *Clin. Exp. Immunol.* 182:289–301. http://dx.doi.org/10.1111/cei.12692

Li, Y., K. Svehla, M.K. Louder, D. Wycuff, S. Phogat, M. Tang, S.A. Migueles, X. Wu, A. Phogat, G.M. Shaw, et al. 2009. Analysis of neutralization specificities in polyclonal sera derived from human immunodeficiency virus type 1-infected individuals. *J. Virol.* 83:1045–1059. http://dx.doi.org/10.1128/JVI.01992-08

Liao, H.X., R. Lynch, T. Zhou, F. Gao, S.M. Alam, S.D. Boyd, A.Z. Fire, K.M. Roskin, C.A. Schramm, Z. Zhang, et al. NISC Comparative Sequencing Program. 2013. Co-evolution of a broadly neutralizing HIV-1 antibody and founder virus. *Nature.* 496:469–476. http://dx.doi.org/10.1038/nature12053

Liu, J., K. Ghneim, D. Sok, W.J. Bosche, Y. Li, E. Chipriano, B. Berkemeier, K. Oswald, E. Borducchi, C. Cabral, et al. 2016. Antibody-mediated protection against SHIV challenge includes systemic clearance of distal virus. *Science.* 353:1045–1049. http://dx.doi.org/10.1126/science.aag0491

Lynch, R.M., E. Boritz, E.E. Coates, A. DeZure, P. Madden, P. Costner, M.E. Enama, S. Plummer, L. Holman, C.S. Hendel, et al. VRC 601 Study Team. 2015. Virologic effects of broadly neutralizing antibody VRC01 administration during chronic HIV-1 infection. *Sci. Transl. Med.* 7:319ra206. http://dx.doi.org/10.1126/scitranslmed.aad5752

Lyumkis, D., J.P. Julien, N. de Val, A. Cupo, C.S. Potter, P.J. Klasse, D.R. Burton, R.W. Sanders, J.P. Moore, B. Carragher, et al. 2013. Cryo-EM structure of a fully glycosylated soluble cleaved HIV-1 envelope trimer. *Science.* 342:1484–1490. http://dx.doi.org/10.1126/science.1245627

MacLeod, D.T., N.M. Choi, B. Briney, F. Garces, L.S. Ver, E. Landais, B. Murrell, T. Wrin, W. Kilembe, C.H. Liang, et al. IAVI Protocol C Investigators & The IAVI African HIV Research Network. 2016. Early antibody lineage diversification and independent limb maturation lead to broad HIV-1 neutralization targeting the Env high-mannose patch. *Immunity.* 44:1215–1226. http://dx.doi.org/10.1016/j.immuni.2016.04.016

Mascola, J.R., and G.J. Nabel. 2001. Vaccines for the prevention of HIV-1 disease. *Curr. Opin. Immunol.* 13:489–494. http://dx.doi.org/10.1016/S0952-7915(00)00246-6

Mascola, J.R., M.G. Lewis, G. Stiegler, D. Harris, T.C. VanCott, D. Hayes, M.K. Louder, C.R. Brown, C.V. Sapan, S.S. Frankel, et al. 1999. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. *J. Virol.* 73:4009–4018.

Mascola, J.R., G. Stiegler, T.C. VanCott, H. Katinger, C.B. Carpenter, C.E. Hanson, H. Beary, D. Hayes, S.S. Frankel, D.L. Birx, and M.G. Lewis. 2000. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. *Nat. Med.* 6:207–210. http://dx.doi.org/10.1038/72318

McCoy, L.E., and R.A. Weiss. 2013. Neutralizing antibodies to HIV-1 induced by immunization. *J. Exp. Med.* 210:209–223. http://dx.doi.org/10.1084/jem.20121827

McGuire, A.T., S. Hoot, A.M. Dreyer, A. Lippy, A. Stuart, K.W. Cohen, J. Jardine, S. Menis, J.F. Scheid, A.P. West, et al. 2013. Engineering HIV envelope protein to activate germline B cell receptors of broadly neutralizing anti-CD4 binding site antibodies. *J. Exp. Med.* 210:655–663. http://dx.doi.org/10.1084/jem.20122824

McGuire, A.T., A.M. Dreyer, S. Carbonetti, A. Lippy, J. Glenn, J.F. Scheid, H. Mouquet, and L. Stamatatos. 2014. HIV antibodies. Antigen modification regulates competition of broad and narrow neutralizing HIV antibodies. *Science.* 346:1380–1383. http://dx.doi.org/10.1126/science.1259206

McGuire, A.T., M.D. Gray, P. Dosenovic, A.D. Gitlin, N.T. Freund, J. Petersen, C. Correnti, W. Johnsen, R. Kegel, A.B. Stuart, et al. 2016. Specifically modified Env immunogens activate B-cell precursors of broadly neutralizing HIV-1 antibodies in transgenic mice. *Nat. Commun.* 7:10618. http://dx.doi.org/10.1038/ncomms10618

McKean, D., K. Huppi, M. Bell, L. Staudt, W. Gerhard, and M. Weigert. 1984. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA.* 81:3180–3184. http://dx.doi.org/10.1073/pnas.81.10.3180

Mehandru, S., B. Vcelar, T. Wrin, G. Stiegler, B. Joos, H. Mohri, D. Boden, J. Galovich, K. Tenner-Racz, P. Racz, et al. 2007. Adjunctive passive immunotherapy in human immunodeficiency virus type 1-infected individuals treated with antiviral therapy during acute and early infection. *J. Virol.* 81:11016–11031. http://dx.doi.org/10.1128/JVI.01340-07

Moldt, B., E.G. Rakasz, N. Schultz, P.Y. Chan-Hui, K. Swiderek, K.L. Weisgrau, S.M. Piaskowski, Z. Bergman, D.I. Watkins, P. Poignard, and D.R. Burton. 2012. Highly potent HIV-specific antibody neutralization in vitro translates into effective protection against mucosal SHIV challenge in vivo. *Proc. Natl. Acad. Sci. USA.* 109:18921–18925. http://dx.doi.org/10.1073/pnas.1214785109

Montefiori, D.C., C. Karnasuta, Y. Huang, H. Ahmed, P. Gilbert, M.S. de Souza, R. McLinden, S. Tovanabutra, A. Laurence-Chenine, E. Sanders-Buell, et al. 2012. Magnitude and breadth of the neutralizing antibody response in the RV144 and Vax003 HIV-1 vaccine efficacy trials. *J. Infect. Dis.* 206:431–441. http://dx.doi.org/10.1093/infdis/jis367

Mörner, A., I. Douagi, M.N. Forsell, C. Sundling, P. Dosenovic, S. O'Dell, B. Dey, P.D. Kwong, G. Voss, R. Thorstensson, et al. 2009. Human immunodeficiency virus type 1 env trimer immunization of macaques and impact of priming with viral vector or stabilized core protein. *J. Virol.* 83:540–551. http://dx.doi.org/10.1128/JVI.01102-08

Mouquet, H., J.F. Scheid, M.J. Zoller, M. Krogsgaard, R.G. Ott, S. Shukair, M.N. Artyomov, J. Pietzsch, M. Connors, F. Pereyra, et al. 2010. Polyreactivity increases the apparent affinity of anti-HIV antibodies by heteroligation. *Nature.* 467:591–595. http://dx.doi.org/10.1038/nature09385

Mouquet, H., L. Scharf, Z. Euler, Y. Liu, C. Eden, J.F. Scheid, A. Halper-Stromberg, P.N. Gnanapragasam, D.I. Spencer, M.S. Seaman, et al. 2012. Complex-type N-glycan recognition by potent broadly neutralizing HIV antibodies. *Proc. Natl. Acad. Sci. USA.* 109:E3268–E3277. http://dx.doi.org/10.1073/pnas.1217207109

Muster, T., F. Steindl, M. Purtscher, A. Trkola, A. Klima, G. Himmler, F. Rüker, and H. Katinger. 1993. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. *J. Virol.* 67:6642–6647.

Nishimura, Y., T. Igarashi, N. Haigwood, R. Sadjadpour, R.J. Plishka, A. Buckler-White, R. Shibata, and M.A. Martin. 2002. Determination of a statistically valid neutralization titer in plasma that confers protection against simian-human immunodeficiency virus challenge following passive transfer of high-titered neutralizing antibodies. *J. Virol.* 76:2123–2130. http://dx.doi.org/10.1128/jvi.76.5.2123-2130.2002

Nussenzweig, M.C., A.C. Shaw, E. Sinn, D.B. Danner, K.L. Holmes, H.C. Morse III, and P. Leder. 1987. Allelic exclusion in transgenic mice that express the membrane form of immunoglobulin mu. *Science.* 236:816–819. http://dx.doi.org/10.1126/science.3107126

Ota, T., C. Doyle-Cooper, A.B. Cooper, K.J. Doores, M. Aoki-Ota, K. Le, W.R. Schief, R.T. Wyatt, D.R. Burton, and D. Nemazee. 2013. B cells from knock-in mice expressing broadly neutralizing HIV antibody b12 carry an innocuous B cell receptor responsive to HIV vaccine candidates. *J. Immunol.* 191:3179–3185. http://dx.doi.org/10.4049/jimmunol.1301283

Pancera, M., J.S. McLellan, X. Wu, J. Zhu, A. Changela, S.D. Schmidt, Y. Yang, T. Zhou, S. Phogat, J.R. Mascola, and P.D. Kwong. 2010. Crystal structure of PG16 and chimeric dissection with somatically related PG9: structure-function analysis of two quaternary-specific antibodies that effectively neutralize HIV-1. *J. Virol.* 84:8098–8110. http://dx.doi.org/10.1128/JVI.00966-10

Pancera, M., T. Zhou, A. Druz, I.S. Georgiev, C. Soto, J. Gorman, J. Huang, P. Acharya, G.Y. Chuang, G. Ofek, et al. 2014. Structure and immune recognition of trimeric pre-fusion HIV-1 Env. *Nature.* 514:455–461. http://dx.doi.org/10.1038/nature13808

Pantophlet, R., and D.R. Burton. 2003. Immunofocusing: antigen engineering to promote the induction of HIV-neutralizing antibodies. *Trends Mol. Med.* 9:468–473. http://dx.doi.org/10.1016/j.molmed.2003.09.001

Parren, P.W., P.A. Marx, A.J. Hessell, A. Luckay, J. Harouse, C. Cheng-Mayer, J.P. Moore, and D.R. Burton. 2001. Antibody protects macaques against vaginal challenge with a pathogenic R5 simian/human immunodeficiency virus at serum levels giving complete neutralization in vitro. *J. Virol.* 75:8340–8347. http://dx.doi.org/10.1128/JVI.75.17.8340-8347.2001

Pietzsch, J., J.F. Scheid, H. Mouquet, M.S. Seaman, C.C. Broder, and M.C. Nussenzweig. 2010. Anti-gp41 antibodies cloned from HIV-infected patients with broadly neutralizing serologic activity. *J. Virol.* 84:5032–5042. http://dx.doi.org/10.1128/JVI.00154-10

Pietzsch, J., H. Gruell, S. Bournazos, B.M. Donovan, F. Klein, R. Diskin, M.S. Seaman, P.J. Bjorkman, J.V. Ravetch, A. Ploss, and M.C. Nussenzweig. 2012. A mouse model for HIV-1 entry. *Proc. Natl. Acad. Sci. USA.* 109:15859–15864. http://dx.doi.org/10.1073/pnas.1213409109

Pitisuttithum, P., P. Gilbert, M. Gurwith, W. Heyward, M. Martin, F. van Griensven, D. Hu, J.W. Tappero, and K. Choopanya. Bangkok Vaccine Evaluation Group. 2006. Randomized, double-blind, placebo-controlled efficacy trial of a bivalent recombinant glycoprotein 120 HIV-1

vaccine among injection drug users in Bangkok, Thailand. *J. Infect. Dis.* 194:1661–1671. http://dx.doi.org/10.1086/508748

Prince, A.M., H. Reesink, D. Pascual, B. Horowitz, I. Hewlett, K.K. Murthy, K.E. Cobb, and J.W. Eichberg. 1991. Prevention of HIV infection by passive immunization with HIV immunoglobulin. *AIDS Res. Hum. Retroviruses.* 7:971–973. http://dx.doi.org/10.1089/aid.1991.7.971

Rerks-Ngarm, S., P. Pitisuttithum, S. Nitayaphan, J. Kaewkungwal, J. Chiu, R. Paris, N. Premsri, C. Namwat, M. de Souza, E. Adams, et al. MOPH-TAVEG Investigators. 2009. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N. Engl. J. Med.* 361:2209–2220. http://dx.doi.org/10.1056/NEJMoa0908492

Richman, D.D., T. Wrin, S.J. Little, and C.J. Petropoulos. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. USA.* 100:4144–4149. http://dx.doi.org/10.1073/pnas.0630530100

Robbiani, D.F., and M.C. Nussenzweig. 2013. Chromosome translocation, B cell lymphoma, and activation-induced cytidine deaminase. *Annu. Rev. Pathol.* 8:79–103. http://dx.doi.org/10.1146/annurev-pathol-020712-164004

Roben, P., J.P. Moore, M. Thali, J. Sodroski, C.F. Barbas III, and D.R. Burton. 1994. Recognition properties of a panel of human recombinant Fab fragments to the CD4 binding site of gp120 that show differing abilities to neutralize human immunodeficiency virus type 1. *J. Virol.* 68:4821–4828.

Rusert, P., R.D. Kouyos, C. Kadelka, H. Ebner, M. Schanz, M. Huber, D.L. Braun, N. Hozé, A. Scherrer, C. Magnus, et al. Swiss HIV Cohort Study. 2016. Determinants of HIV-1 broadly neutralizing antibody induction. *Nat. Med.* 22:1260–1267. http://dx.doi.org/10.1038/nm.4187

Sanders, R.W., M. Vesanen, N. Schuelke, A. Master, L. Schiffner, R. Kalyanaraman, M. Paluch, B. Berkhout, P.J. Maddon, W.C. Olson, et al. 2002. Stabilization of the soluble, cleaved, trimeric form of the envelope glycoprotein complex of human immunodeficiency virus type 1. *J. Virol.* 76:8875–8889. http://dx.doi.org/10.1128/JVI.76.17.8875-8889.2002

Sanders, R.W., R. Derking, A. Cupo, J.P. Julien, A. Yasmeen, N. de Val, H.J. Kim, C. Blattner, A.T. de la Peña, J. Korzun, et al. 2013. A next-generation cleaved, soluble HIV-1 Env trimer, BG505 SOSIP.664 gp140, expresses multiple epitopes for broadly neutralizing but not non-neutralizing antibodies. *PLoS Pathog.* 9:e1003618. http://dx.doi.org/10.1371/journal.ppat.1003618

Sather, D.N., J. Armann, L.K. Ching, A. Mavrantoni, G. Sellhorn, Z. Caldwell, X. Yu, B. Wood, S. Self, S. Kalams, and L. Stamatatos. 2009. Factors associated with the development of cross-reactive neutralizing antibodies during human immunodeficiency virus type 1 infection. *J. Virol.* 83:757–769. http://dx.doi.org/10.1128/JVI.02036-08

Sattentau, Q. 2008. Correlates of antibody-mediated protection against HIV infection. *Curr. Opin. HIV AIDS.* 3:368–374. http://dx.doi.org/10.1097/COH.0b013e3282f9ae79

Saunders, K.O., A. Pegu, I.S. Georgiev, M. Zeng, M.G. Joyce, Z.Y. Yang, S.Y. Ko, X. Chen, S.D. Schmidt, A.T. Haase, et al. 2015a. Sustained delivery of a broadly neutralizing antibody in nonhuman primates confers long-term protection against simian/human immunodeficiency virus infection. *J. Virol.* 89:5895–5903. http://dx.doi.org/10.1128/JVI.00210-15

Saunders, K.O., L. Wang, M.G. Joyce, Z.Y. Yang, A.B. Balazs, C. Cheng, S.Y. Ko, W.P. Kong, R.S. Rudicell, I.S. Georgiev, et al. 2015b. Broadly neutralizing human immunodeficiency virus type 1 antibody gene transfer protects nonhuman primates from mucosal simian-human immunodeficiency virus infection. *J. Virol.* 89:8334–8345. http://dx.doi.org/10.1128/JVI.00908-15

Scharf, L., A.P. West, S.A. Sievers, C. Chen, S. Jiang, H. Gao, M.D. Gray, A.T. McGuire, J.F. Scheid, M.C. Nussenzweig, et al. 2016. Structural basis for germline antibody recognition of HIV-1 immunogens. *eLife.* 5:e13783. http://dx.doi.org/10.7554/eLife.13783

Scheid, J.F., H. Mouquet, N. Feldhahn, M.S. Seaman, K. Velinzon, J. Pietzsch, R.G. Ott, R.M. Anthony, H. Zebroski, A. Hurley, et al. 2009a. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. *Nature.* 458:636–640. http://dx.doi.org/10.1038/nature07930

Scheid, J.F., H. Mouquet, N. Feldhahn, B.D. Walker, F. Pereyra, E. Cutrell, M.S. Seaman, J.R. Mascola, R.T. Wyatt, H. Wardemann, and M.C. Nussenzweig. 2009b. A method for identification of HIV gp140 binding memory B cells in human blood. *J. Immunol. Methods.* 343:65–67. http://dx.doi.org/10.1016/j.jim.2008.11.012

Scheid, J.F., H. Mouquet, B. Ueberheide, R. Diskin, F. Klein, T.Y. Oliveira, J. Pietzsch, D. Fenyo, A. Abadir, K. Velinzon, et al. 2011. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. *Science.* 333:1633–1637. http://dx.doi.org/10.1126/science.1207227

Sharma, S.K., N. de Val, S. Bale, J. Guenaga, K. Tran, Y. Feng, V. Dubrovskaya, A.B. Ward, and R.T. Wyatt. 2015. Cleavage-independent HIV-1 Env trimers engineered as soluble native spike mimetics for vaccine design. *Cell Reports.* 11:539–550. http://dx.doi.org/10.1016/j.celrep.2015.03.047

Shibata, R., T. Igarashi, N. Haigwood, A. Buckler-White, R. Ogert, W. Ross, R. Willey, M.W. Cho, and M.A. Martin. 1999. Neutralizing antibody directed against the HIV-1 envelope glycoprotein can completely block HIV-1/SIV chimeric virus infections of macaque monkeys. *Nat. Med.* 5:204–210. http://dx.doi.org/10.1038/5568

Shingai, M., Y. Nishimura, F. Klein, H. Mouquet, O.K. Donau, R. Plishka, A. Buckler-White, M. Seaman, M. Piatak Jr., J.D. Lifson, et al. 2013. Antibody-mediated immunotherapy of macaques chronically infected with SHIV suppresses viraemia. *Nature.* 503:277–280. http://dx.doi.org/10.1038/nature12746

Shingai, M., O.K. Donau, R.J. Plishka, A. Buckler-White, J.R. Mascola, G.J. Nabel, M.C. Nason, D. Montefiori, B. Moldt, P. Poignard, et al. 2014. Passive transfer of modest titers of potent and broadly neutralizing anti-HIV monoclonal antibodies block SHIV infection in macaques. *J. Exp. Med.* 211:2061–2074. http://dx.doi.org/10.1084/jem.20132494

Simek, M.D., W. Rida, F.H. Priddy, P. Pung, E. Carrow, D.S. Laufer, J.K. Lehrman, M. Boaz, T. Tarragona-Fiol, G. Miiro, et al. 2009. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. *J. Virol.* 83:7337–7348. http://dx.doi.org/10.1128/JVI.00110-09

Simonich, C.A., K.L. Williams, H.P. Verkerke, J.A. Williams, R. Nduati, K.K. Lee, and J. Overbaugh. 2016. HIV-1 neutralizing antibodies with limited hypermutation from an infant. *Cell.* 166:77–87. http://dx.doi.org/10.1016/j.cell.2016.05.055

Sliepen, K., and R.W. Sanders. 2016. HIV-1 envelope glycoprotein immunogens to induce broadly neutralizing antibodies. *Expert Rev. Vaccines.* 15:349–365. http://dx.doi.org/10.1586/14760584.2016.1129905

Sok, D., U. Laserson, J. Laserson, Y. Liu, F. Vigneault, J.P. Julien, B. Briney, A. Ramos, K.F. Saye, K. Le, et al. 2013. The effects of somatic hypermutation on neutralization and binding in the PGT121 family of broadly neutralizing HIV antibodies. *PLoS Pathog.* 9:e1003754. (published erratum appears in *PLoS Pathog.* 2013. http://dx.doi.org/10.1371/annotation/f1f8c791-61e9-45c6-a455-92c6dadf9f06) http://dx.doi.org/10.1371/journal.ppat.1003754

Sok, D., M.J. van Gils, M. Pauthner, J.P. Julien, K.L. Saye-Francisco, J. Hsueh, B. Briney, J.H. Lee, K.M. Le, P.S. Lee, et al. 2014. Recombinant HIV envelope trimer selects for quaternary-dependent antibodies targeting the trimer apex. *Proc. Natl. Acad. Sci. USA.* 111:17624–17629. http://dx.doi.org/10.1073/pnas.1415789111

Sok, D., B. Briney, J.G. Jardine, D.W. Kulp, S. Menis, M. Pauthner, A. Wood, E.C. Lee, K.M. Le, M. Jones, et al. 2016. Priming HIV-1 broadly

neutralizing antibody precursors in human Ig loci transgenic mice. *Science.* 353:1557–1560. http://dx.doi.org/10.1126/science.aah3945

Stamatatos, L., L. Morris, D.R. Burton, and J.R. Mascola. 2009. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine?. *Nat. Med.* 15:866–870. http://dx.doi.org/10.1038/nm.1949

Steichen, J.M., D.W. Kulp, T. Tokatlian, A. Escolano, P. Dosenovic, R.L. Stanfield, L.E. McCoy, G. Ozorowski, X. Hu, O. Kalyuzhniy, et al. 2016. HIV vaccine design to target germline precursors of glycan-dependent broadly neutralizing antibodies. *Immunity.* 45:483–496. http://dx.doi.org/10.1016/j.immuni.2016.08.016

Stiegler, G., R. Kunert, M. Purtscher, S. Wolbank, R. Voglauer, F. Steindl, and H. Katinger. 2001. A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1. *AIDS Res. Hum. Retroviruses.* 17:1757–1765. http://dx.doi.org/10.1089/08892220152741450

Tas, J.M., L. Mesin, G. Pasqual, S. Targ, J.T. Jacobsen, Y.M. Mano, C.S. Chen, J.C. Weill, C.A. Reynaud, E.P. Browne, et al. 2016. Visualizing antibody affinity maturation in germinal centers. *Science.* 351:1048–1054. http://dx.doi.org/10.1126/science.aad3439

Tian, M., C. Cheng, X. Chen, H. Duan, H.L. Cheng, M. Dao, Z. Sheng, M. Kimble, L. Wang, S. Lin, et al. 2016. Induction of HIV neutralizing antibody lineages in mice with diverse precursor repertoires. *Cell.* 166:1471–1484.e18. http://dx.doi.org/10.1016/j.cell.2016.07.029

Tiller, T., C.E. Busse, and H. Wardemann. 2009. Cloning and expression of murine Ig genes from single B cells. *J. Immunol. Methods.* 350:183–193. http://dx.doi.org/10.1016/j.jim.2009.08.009

Trkola, A., A.B. Pomales, H. Yuan, B. Korber, P.J. Maddon, G.P. Allaway, H. Katinger, C.F. Barbas III, D.R. Burton, D.D. Ho, et al. 1995. Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IgG. *J. Virol.* 69:6609–6617.

Trkola, A., M. Purtscher, T. Muster, C. Ballaun, A. Buchacher, N. Sullivan, K. Srinivasan, J. Sodroski, J.P. Moore, and H. Katinger. 1996. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. *J. Virol.* 70:1100–1108.

Trkola, A., H. Kuster, P. Rusert, B. Joos, M. Fischer, C. Leemann, A. Manrique, M. Huber, M. Rehr, A. Oxenius, et al. 2005. Delay of HIV-1 rebound after cessation of antiretroviral therapy through passive transfer of human neutralizing antibodies. *Nat. Med.* 11:615–622. http://dx.doi.org/10.1038/nm1244

van Gils, M.J., T.L. van den Kerkhof, G. Ozorowski, C.A. Cottrell, D. Sok, M. Pauthner, J. Pallesen, N. de Val, A. Yasmeen, S.W. de Taeye, et al. 2016. An HIV-1 antibody from an elite neutralizer implicates the fusion peptide as a site of vulnerability. *Nat. Microbiol.* 2:16199. http://dx.doi.org/10.1038/nmicrobiol.2016.199

Verkoczy, L., M. Diaz, T.M. Holl, Y.B. Ouyang, H. Bouton-Verville, S.M. Alam, H.X. Liao, G. Kelsoe, and B.F. Haynes. 2010. Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. *Proc. Natl. Acad. Sci. USA.* 107:181–186. http://dx.doi.org/10.1073/pnas.0912914107

Verkoczy, L., Y. Chen, H. Bouton-Verville, J. Zhang, M. Diaz, J. Hutchinson, Y.B. Ouyang, S.M. Alam, T.M. Holl, K.K. Hwang, et al. 2011. Rescue of HIV-1 broad neutralizing antibody-expressing B cells in 2F5 VH x VL knockin mice reveals multiple tolerance controls. *J. Immunol.* 187:3785–3797. http://dx.doi.org/10.4049/jimmunol.1101633

Victora, G.D., and M.C. Nussenzweig. 2012. Germinal centers. *Annu. Rev. Immunol.* 30:429–457. http://dx.doi.org/10.1146/annurev-immunol-020711-075032

Victora, G.D., T.A. Schwickert, D.R. Fooksman, A.O. Kamphorst, M. Meyer-Hermann, M.L. Dustin, and M.C. Nussenzweig. 2010. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell.* 143:592–605. http://dx.doi.org/10.1016/j.cell.2010.10.032

Walker, L.M., S.K. Phogat, P.Y. Chan-Hui, D. Wagner, P. Phung, J.L. Goss, T. Wrin, M.D. Simek, S. Fling, J.L. Mitcham, et al. Protocol G Principal Investigators. 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. *Science.* 326:285–289. http://dx.doi.org/10.1126/science.1178746

Walker, L.M., M. Huber, K.J. Doores, E. Falkowska, R. Pejchal, J.P. Julien, S.K. Wang, A. Ramos, P.Y. Chan-Hui, M. Moyle, et al. Protocol G Principal Investigators. 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. *Nature.* 477:466–470. http://dx.doi.org/10.1038/nature10373

Wei, X., J.M. Decker, S. Wang, H. Hui, J.C. Kappes, X. Wu, J.F. Salazar-Gonzalez, M.G. Salazar, J.M. Kilby, M.S. Saag, et al. 2003. Antibody neutralization and escape by HIV-1. *Nature.* 422:307–312. http://dx.doi.org/10.1038/nature01470

West, A.P. Jr., R. Diskin, M.C. Nussenzweig, and P.J. Bjorkman. 2012. Structural basis for germ-line gene usage of a potent class of antibodies targeting the CD4-binding site of HIV-1 gp120. *Proc. Natl. Acad. Sci. USA.* 109:E2083–E2090. http://dx.doi.org/10.1073/pnas.1208984109

West, A.P. Jr., L. Scharf, J.F. Scheid, F. Klein, P.J. Bjorkman, and M.C. Nussenzweig. 2014. Structural insights on the role of antibodies in HIV-1 vaccine and therapy. *Cell.* 156:633–648. http://dx.doi.org/10.1016/j.cell.2014.01.052

Wibmer, C.K., P.L. Moore, and L. Morris. 2015. HIV broadly neutralizing antibody targets. *Curr. Opin. HIV AIDS.* 10:135–143. http://dx.doi.org/10.1097/COH.0000000000000153

Wu, X., Z.Y. Yang, Y. Li, C.M. Hogerkorp, W.R. Schief, M.S. Seaman, T. Zhou, S.D. Schmidt, L. Wu, L. Xu, et al. 2010. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. *Science.* 329:856–861. http://dx.doi.org/10.1126/science.1187659

Wu, X., Z. Zhang, C.A. Schramm, M.G. Joyce, Y.D. Kwon, T. Zhou, Z. Sheng, B. Zhang, S. O'Dell, K. McKee, et al. NISC Comparative Sequencing Program. 2015. Maturation and diversity of the VRC01-antibody lineage over 15 years of chronic HIV-1 infection. *Cell.* 161:470–485. http://dx.doi.org/10.1016/j.cell.2015.03.004

Wyatt, R., and J. Sodroski. 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. *Science.* 280:1884–1888. http://dx.doi.org/10.1126/science.280.5371.1884

Xiao, X., W. Chen, Y. Feng, Z. Zhu, P. Prabakaran, Y. Wang, M.Y. Zhang, N.S. Longo, and D.S. Dimitrov. 2009. Germline-like predecessors of broadly neutralizing antibodies lack measurable binding to HIV-1 envelope glycoproteins: implications for evasion of immune responses and design of vaccine immunogens. *Biochem. Biophys. Res. Commun.* 390:404–409. http://dx.doi.org/10.1016/j.bbrc.2009.09.029

Zhang, R., L. Verkoczy, K. Wiehe, S. Munir Alam, N.I. Nicely, S. Santra, T. Bradley, C.W. Pemble IV, J. Zhang, F. Gao, et al. 2016. Initiation of immune tolerance-controlled HIV gp41 neutralizing B cell lineages. *Sci. Transl. Med.* 8:336ra62. http://dx.doi.org/10.1126/scitranslmed.aaf0618

Zhou, T., I. Georgiev, X. Wu, Z.Y. Yang, K. Dai, A. Finzi, Y.D. Kwon, J.F. Scheid, W. Shi, L. Xu, et al. 2010. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. *Science.* 329:811–817. http://dx.doi.org/10.1126/science.1192819

Zhou, T., R.M. Lynch, L. Chen, P. Acharya, X. Wu, N.A. Doria-Rose, M.G. Joyce, D. Lingwood, C. Soto, R.T. Bailer, et al. NISC Comparative Sequencing Program. 2015. Structural repertoire of HIV-1-neutralizing antibodies targeting the CD4 supersite in 14 donors. *Cell.* 161:1280–1292. http://dx.doi.org/10.1016/j.cell.2015.05.007

Zwick, M.B., A.F. Labrijn, M. Wang, C. Spenlehauer, E.O. Saphire, J.M. Binley, J.P. Moore, G. Stiegler, H. Katinger, D.R. Burton, and P.W. Parren. 2001. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. *J. Virol.* 75:10892–10905. http://dx.doi.org/10.1128/JVI.75.22.10892-10905.2001