Neutralizing antibodies to HIV-1 induced by immunization

Laura E. McCoy[1,2] and Robin A. Weiss[1,2]

[1]Wohl Virion Centre and [2]Medical Research Council Centre for Medical Molecular Virology, Division of Infection and Immunity, University College London, London WC1E 6BT, England, UK

Most neutralizing antibodies act at the earliest steps of viral infection and block interaction of the virus with cellular receptors to prevent entry into host cells. The inability to induce neutralizing antibodies to HIV has been a major obstacle to HIV vaccine research since the early days of the epidemic. However, in the past three years, the definition of a neutralizing antibody against HIV has been revolutionized by the isolation of extremely broad and potent neutralizing antibodies from HIV-infected individuals. Considerable hurdles remain for inducing neutralizing antibodies to a protective level after immunization. Meanwhile, novel technologies to bypass the induction of antibodies are being explored to provide prophylactic antibody-based interventions. This review addresses the challenge of inducing HIV neutralizing antibodies upon immunization and considers notable recent advances in the field. A greater understanding of the successes and failures for inducing a neutralizing response upon immunization is required to accelerate the development of an effective HIV vaccine.

CORRESPONDENCE
Robin A. Weiss:
r.weiss@ucl.ac.uk

Abbreviations used: MPRE, membrane-proximal region; NHP, nonhuman primate; SIV, simian immunodeficiency virus.

The titer of neutralizing antibodies elicited in plasma or sera correlate closely with protection from infection for almost all human and veterinary viral vaccines where neutralization can be measured (Plotkin, 2008). Not only does this general observation underline the importance of the humoral arm of the immune response in vaccine design but it also highlights the crucial role of those antibodies that block infection at the cellular level. Most neutralizing antibodies act at the earliest steps in the viral replication cycle. They block interaction of the virus with receptors on the cell surface, prevent subsequent conformational changes of viral proteins required for entry into cells, or transition from endocytic vesicles into the cytoplasm (Murphy et al., 2011). The human immunodeficiency viruses types 1 and 2 (HIV-1 and HIV-2) and related simian immunodeficiency viruses (SIVs) are not exceptions. Mutational escape from neutralization in infected individuals shows the relevance of neutralization in the natural history and course of HIV-1 infection (Deeks et al., 2006). Moreover, the passive transfer of neutralizing antibodies can protect against subsequent challenge infection in nonhuman primate (NHP) models (Mascola et al., 1999, 2000; Shibata et al., 1999; Parren et al., 2001; Veazey et al., 2003; Hessell et al., 2009; Watkins et al., 2011). Some protective antibodies can act later in the replication cycle, for example, antibodies involved in ADCC (antibody-dependent cytotoxicity) and ADCVI (antibody-dependent cell-mediated virus inhibition), in addition to or in the absence of neutralizing properties (Forthal and Moog, 2009).

HIV presents special hurdles to generating broad and potent neutralizing antibodies. It was already apparent from the first reports of neutralizing antibodies against HIV-1 (Robert-Guroff et al., 1985; Weiss et al., 1985) that the neutralizing response in infected patients was weak compared with non-neutralizing HIV antibodies. For instance, although anti–envelope glycoprotein (Env) antibody titers were equivalent to those in patients infected with HTLV-1 (human T-lymphotropic virus type 1; measured by binding or by immunofluorescence) neutralizing titers were 100-fold lower (Weiss et al., 1985). Moreover, difficulties in eliciting neutralizing antibodies by vaccination as opposed to infection quickly became apparent with the observation that the neutralizing responses

The Rockefeller University Press $30.00
J. Exp. Med. 2013 Vol. 210 No. 2 209-223
www.jem.org/cgi/doi/10.1084/jem.20121827

The Journal of Experimental Medicine

Table 1. Key characteristics of HIV Env

Characteristic	Description
Neutralization target	Antibodies that can efficiently neutralize HIV do so by interacting with Env. In natural infection, neutralizing antibody elicitation lags behind viral escape within the host.
Evasion of neutralization	Evades effective neutralizing antibodies via a mixture of extensive surface glycosylation, interstrain variability, and conformational masking.
Instability	Comprises three identical gp120/gp41 proteins weakly linked together. Flexibility means epitopes may be sampled at temporally distinct points.
Location	Env is embedded in the viral membrane via the gp41 transmembrane subunit and arrayed at a low density on the virion surface, alongside noninfectious Env variants including gp41 stumps.

elicited by gp120 immunization were more type specific than those produced in natural infection (Weiss et al., 1986).

There are several reasons why HIV is a challenging target for neutralizing antibodies. First, the sheer genetic diversity of concurrent HIV subtypes (clades), circulating recombinant forms, and strains is greater than for any other virus, except possibly hepatitis C virus, and this is reflected in the antigenic diversity of Env which is the target of neutralizing antibodies (Burton et al., 2012; Ndung'u and Weiss, 2012). Second, the neutralizing epitopes are, for the most part, hidden beneath a glycan shield which makes them inaccessible to antibodies, although some epitopes include carbohydrate moieties (Sattentau, 2011). Third, although all strains of HIV bind to the CD4 cellular attachment receptor, the CD4 binding site resides in a pocket to which antibody access is restricted (Kwong et al., 2012). Nevertheless, during the last three years a new generation of mAbs has been identified which offers broad and potent neutralization of diverse HIV strains. Previously, there was concern that a gain in the breadth of neutralization might be accompanied by loss of potency, but we now know that this is not the case. These discoveries have led to increased optimism that vaccines which induce cross-clade neutralizing antibodies will be achieved. The challenge now is to translate the new knowledge of neutralizing epitopes into immunogens that will elicit potent and lasting immunity to HIV infection.

Recently, our understanding of what constitutes a broadly neutralizing antibody against HIV has been revolutionized by the isolation of extremely broad and potent neutralizing mAb from HIV-infected individuals (Walker et al., 2009, 2011; Corti et al., 2010; Wu et al., 2010; Scheid et al., 2011). These mAbs were identified by dissecting the broad neutralization activity seen in specific patient serum samples and by characterizing mAbs from B cells (Beirnaert et al., 2000; Dhillon et al., 2007; Binley et al., 2008; Scheid et al., 2009; Simek et al., 2009; Walker et al., 2009). The application of single B cell cloning techniques (Tiller et al., 2008) enabled the leap forward in neutralizing antibody identification via the use of soluble antigens (Scheid et al., 2009) or baits and recently cell-based antigens (Klein et al., 2012a), alongside the use of direct screening of B cell supernatants for neutralization activity as reviewed extensively in Moir et al. (2011). Although the knowledge garnered from the new generation of HIV neutralizing antibodies can directly inform future immunogen design, as reviewed elsewhere (Pejchal and Wilson, 2010; Walker and Burton, 2010; McMichael and Haynes, 2012), this review aims to provide further insight by considering the efforts made to elicit broadly neutralizing antibodies by vaccination in contrast to their generation during natural HIV infection.

First generation HIV neutralizing mAbs

During the course of chronic HIV infection, neutralizing antibodies are elicited to a variable degree in different individuals (Willey and Aasa-Chapman, 2008; Gray et al., 2011; Bonsignori et al., 2012; Lynch et al., 2012) to Env, the key characteristics of which are summarized in Table 1. For many years, only a limited number of neutralizing mAbs from infected patients were described, but between the available clones the key regions targeted during the neutralization were largely identified and have been thoroughly reviewed elsewhere (Sheppard and Sattentau, 2005; Fig. 1). To summarize, the mAb with the best anti-HIV potency and breadth before 2009 was b12, which was shown to target the CD4-binding site of Env and neutralize up to 40% of strains tested (Burton et al., 1994). Once Env has bound CD4, a conformational change occurs that presents induced epitopes (CD4i epitopes) which can be targeted by neutralizing antibodies (Moulard et al., 2002). The neutralization activity of identified CD4i mAbs, e.g., 17b, is enhanced in the presence of subinhibitory concentrations of sCD4. Moreover, potency and breadth are boosted when this mAb is assayed as a FAB rather than full-length IgG, whereas the reverse is true for the CD4-binding site mAb b12 (Labrijn et al., 2003). These results suggest that antibody access to CD4i epitopes is restricted by steric factors, and thus, although CD4i binding activity is induced in the sera of immunized humans, the sera do not have broad neutralization activity (Vaine et al., 2010).

In addition, an anti-Env glycan-specific neutralizing mAb, 2G12, was identified (Trkola et al., 1996), which has an intriguing domain-swapped architecture (Calarese et al., 2003). Initially N-linked glycans in conserved regions (C) 1, C2, C3, C4 and variable regions (V) 4 were identified with 2G12 binding. More recently, modifications in V1/V2 and V3 have been linked to 2G12 sensitivity (Chaillon et al., 2011). The V3 loop has also been identified as the target of neutralizing antibodies (Durda et al., 1988), notably the mAbs 447-52D (Stanfield et al., 2004; Rosen et al., 2005) and B4E8

(Bell et al., 2008). Gp41 was also identified as a target for neutralizing antibodies isolated from HIV-infected humans; the two most studied mAbs are 2F5 (Barbato et al., 2003) and 4E10 (Stiegler et al., 2001), which both bind within the membrane-proximal region (MPER) and also have a lipid binding ability (Alam et al., 2009). 4E10 exhibits a great breadth of neutralization across subtypes (Mehandru et al., 2004); however, neutralization is only observed at weak to moderate potency (Binley et al., 2004).

Although much was learnt from the first generation of neutralizing mAbs against HIV, there was residual doubt as to whether antibodies with greater breadth and neutralization potency could ever be elicited, even in natural infection in humans, and thus there was speculation as to whether a humoral response induced by vaccination could ever be truly protective. Furthermore, although protection from infection was seen when these mAbs were passively transferred to NHP (Mascola et al., 1999, 2000; Shibata et al., 1999; Parren et al., 2001; Veazey et al., 2003; Hessell et al., 2009; Watkins et al., 2011), viral escape mutants emerged in animal models (Andrus et al., 1998; Poignard et al., 1999) and in patients who had interrupted antiretroviral treatment concurrently with passive transfer of mAbs (Trkola et al., 2005). With the failure of the initial human vaccination trials, based on gp120 protein antigens postulated to induce neutralizing antibodies (Flynn et al., 2005; Pitisuttithum et al., 2006; Gilbert et al., 2010), the prospects for a neutralizing antisera-inducing HIV vaccine appeared bleak and there was a shift within the HIV prevention field toward vaccines designed to induce mainly T cell responses, as previously reviewed (Barouch, 2010; McElrath and Haynes, 2010; Pantaleo et al., 2010; Picker et al., 2012).

Second generation HIV neutralizing mAbs

In 2009, two landmark papers described mAbs isolated from the sera of HIV-infected donors (Scheid et al., 2009; Walker et al., 2009). The use of single B cell cloning, coupled with an antigen-binding-based selection method, revealed that underlying the broad serum neutralization activity of elite neutralizer patients are multiple antibody lineages targeting a range of epitopes on gp120 (Scheid et al., 2009). Concurrently, the adaptation of single B cell cloning to a high-throughput screen of >30,000 B cell clone supernatants for the ability to neutralize HIV led to the isolation of two mAbs, PG9 and PG16 (Walker et al., 2009). These mAbs neutralize between 70 and 80% of >150 strains of HIV tested (Walker et al., 2009) by binding preferentially to trimeric Env via the V1/2 loops of the gp120 subunit (Walker et al., 2009; McLellan et al., 2011). Shortly afterward, a set of mAbs was isolated from

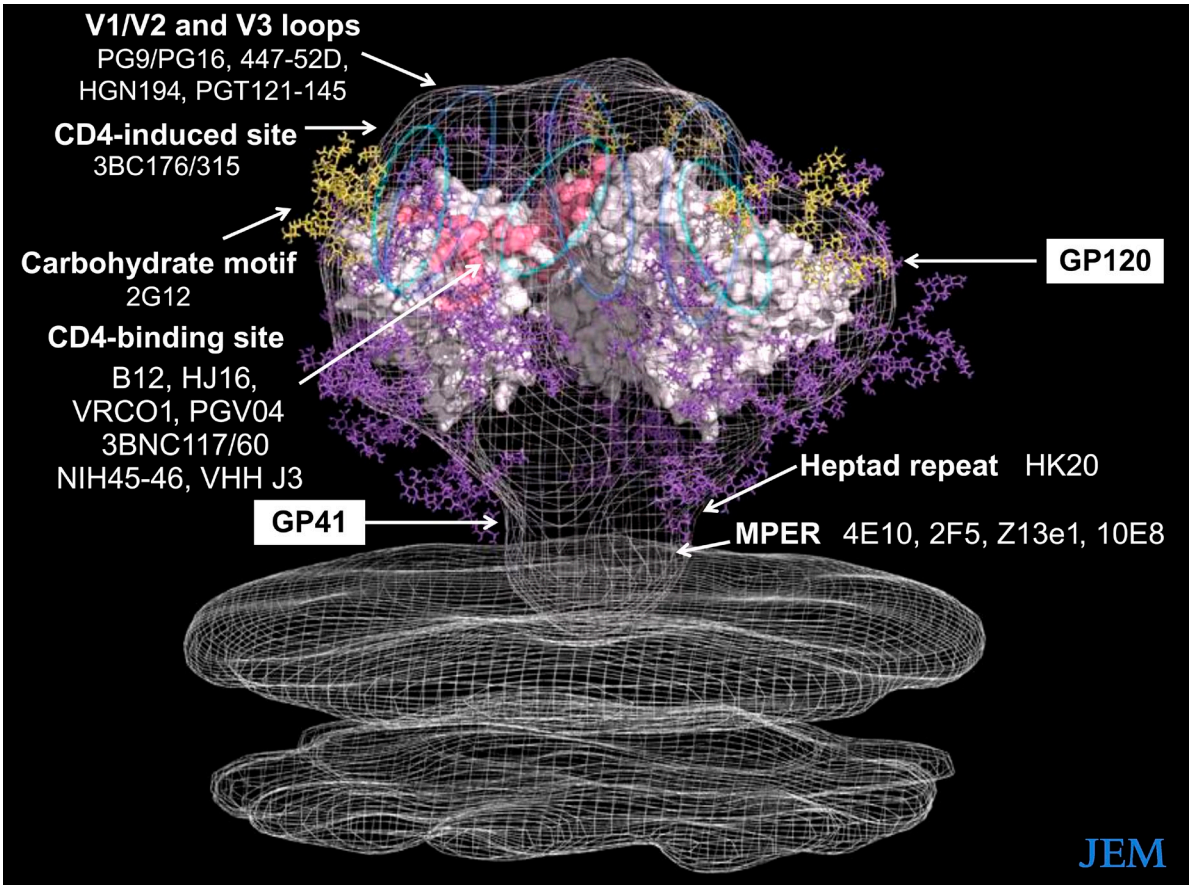

Figure 1. Neutralizing mAbs targeting HIV Env. The diagram was adapted from Burton and Weiss (2010).

multiple patients. Although not as broadly neutralizing as PG9 and PG16, novel epitopes were identified and, in the case of HJ16, showed atypical patterns of virus sensitivity (Corti et al., 2010). Subsequently, a CD4-binding site mAb was isolated, again by a large-scale screening process, but this time using a resurfaced gp120 core protein as bait (Wu et al., 2010). This mAb, VRC01, along with its somatic variant VRC02, neutralizes up to 90% of strains tested (Wu et al., 2010; Zhou et al., 2010; Li et al., 2011). Further improvements to previously used isolation methods (Moir et al., 2011) resulted in the identification of PG9/16-related mAbs designated PGT121-145, which range in breadth from 16 to 80% but exhibit remarkable potency (Walker et al., 2011) and interact with the glycan shield of HIV, with potency potentially arising as a result of Env cross-linking by these mAbs (Pejchal et al., 2011). Substantial advances were also made in terms of the potency of broadly neutralizing CD4-binding site antibodies isolated (Scheid et al., 2011) and differences in mechanism of action of different mAbs targeting the virus at this crucial site (Falkowska et al., 2012). Structural studies on one new anti-CD4 binding site mAb, NIH45-46, revealed that it did not have a large aromatic residue to contact the hydrophobic pocket between the CD4 binding loop and the bridging sheet on Env, as does CD4 itself (Diskin et al., 2011). By mutating a phenylalanine into this position on NIH45-46, an extremely potent CD4bs mAb was generated which could provide protection against ∼75% of circulating strains at levels as low as 0.1 μg/ml (Diskin et al., 2011). Furthermore, the study of CD4bs mAbs from multiple individuals has suggested a common pathway for their development in humans from particular heavy chain germline genes (West et al., 2012). In addition, recent work has shown the potential of combining these broad and potent neutralizing antibodies (Doria-Rose et al., 2012; Klein et al., 2012a,b). These mAbs do not impair one another's function (Doria-Rose et al., 2012), and, given the distinctive neutralization patterns observed, their combined use could provide enhanced breadth and potency. If used either in a multi-antibody therapy/prophylactic or in the ideal case of a vaccine capable of inducing a range of these broad and potent antibodies that between them target multiple epitopes, this could result in serum activity reminiscent of the multispecificity neutralization activity seen in some elite neutralizer patients (Scheid et al., 2009). In addition, combining antibodies that target both gp41 and gp120 subunits has recently been rendered more appealing by the isolation of an anti-MPER 10E8 which effectively neutralizes 98% of viruses tested with a median IC50 of 0.35 μg/ml (Huang et al., 2012).

Now, after 25 years of HIV research, truly cross-reactive human antibodies have been identified which target multiple sites on Env. This demonstrates that improved neutralizing antibody elicitation on vaccination should be possible if the correct immunogens, adjuvants, and immunization schedules can be determined; yet, this is in itself a formidable challenge. Detailed descriptions of these second generation mAb and their mechanisms of action have been reviewed in detail elsewhere (Hessell and Haigwood, 2012) alongside how this information can be used to improve vaccine design (Burton and Weiss, 2010; Walker and Burton, 2010). When used in combination, these second generation mAbs are predicted to provide neutralization coverage approaching 100% breadth (Doria-Rose et al., 2012). In addition, the high potency demonstrated by some mAbs means the titers required for protection are postulated to be within an achievable range for vaccine-induced antibodies (Diskin et al., 2011). Thus, what constitutes an HIV neutralizing antibody has been redefined and the benchmark to aim for is now significantly higher. Despite the extensive study of these mAbs, it is not a straightforward endeavor to elicit them by immunization. They remain rare antibodies in the repertoire of a few patients. A range of attempts has been made with some limited success, as outlined below. However, there remains much uncertainty regarding how such antibodies mature during natural infection let alone after immunization. Thus, there remains a black box in HIV vaccinology which needs to be decoded: how can we elicit broadly neutralizing responses, preferably involving multiple antibody lineages as the predominant response in anti-HIV sera? To begin to unravel this code, it is helpful to reflect on the progress in experimental HIV immunizations to date.

HIV neutralizing responses after animal immunization: successes and failures

Initial immunization studies demonstrated that although high anti-Env titers are inducible, the neutralization phenotype of both sera and isolated mAbs is limited (Robey et al., 1986; Berman et al., 1988; Ho et al., 1988; Nara et al., 1988; Earl et al., 1989; Haigwood et al., 1990). Given the largely unsuccessful immunizations with monomeric gp120 (Berman et al., 1990; Wrin et al., 1995; Mascola et al., 1996; Barnett et al., 1997; Belshe et al., 1998; Connor et al., 1998), attempts have been made to present the immune system with a more accurate representation of the neutralizing epitopes displayed on functional HIV Env spike by using trimeric Env. Although these recombinant immunogens have proved more effective than their monomeric counterparts at inducing neutralizing antisera in side by side comparisons (Kim et al., 2005; McBurney et al., 2007; Beddows et al., 2007; Forsell et al., 2009), particularly in terms of boosting neutralizing titers (VanCott et al., 1997; Barnett et al., 2001; Yang et al., 2002; Bower et al., 2004; Hammonds et al., 2005; Zhang et al., 2007), trimeric Env immunization did not result in the great jump hoped for in the level of neutralization breadth seen in postimmune sera. However, questions have been raised as to the quality of trimer preparations, and recent data suggests homogeneous and stable trimers are advantageous over their monomeric counterparts (Kovacs et al., 2012). Furthermore, it has been demonstrated that sequential immunization of macaques with Env from distinct subtypes results in greater antibody maturation than simultaneous exposure (Malherbe et al., 2011). In addition, some animal immunizations, including trimeric Env immunizations, have led to the production of cross-subtype neutralizing sera or mAb, as detailed in

Table 2 (cross-reactivity is defined as activity against three or more subtypes of HIV). Indeed, neutralization breadth equivalent to that of the new generation of neutralizing mAbs from infected patients has been observed in one study (McCoy et al., 2012). However, this breadth is exhibited by a purified mAb fragment rather than the postimmune sera, which is in fact relatively weakly neutralizing (although cross-reactive). Second, this mAb fragment is derived from a heavy chain–only antibody and so the limits on recognition of recessed epitopes may be less restrictive.

Attempts to focus the immune response on epitopes recognized by neutralizing antibodies and also to increase the breadth of the response have been made by modifying the Env protein immunogen. For example, alterations to the variable loops of Env, including in a study describing a subtype C gp140 immunogen containing a deletion in V2, resulted in rabbit sera that neutralized another subtype C virus and a few heterologous primary isolates (Lian et al., 2005). However, use of the same subunit immunogen in rhesus macaques produced only moderate levels of autologous neutralizing mAb (Lian et al., 2005). Similarly, cynomolgus macaques immunized with a different V2-deleted Env produced sera that neutralized only the homologous HIV strain and a closely related SHIV, although three of the four animals were protected against intrarectal challenge with the same SHIV strain (Ferrantelli et al., 2011). More drastic Env modifications, such as mutation of the CD4-binding site or disruption of the bridging sheet, result in less potent macaque sera neutralization

Table 2. Animal immunizations yielding sera or mAbs that neutralize three or more subtypes

Immunogen	Species	Neutralization	Reference
Oligomeric electrophilic gp120	Mice	mAb which neutralizes 11 strains from subtypes A, B, and C, including tier 2 and 3 isolates, with intermediate potency.	Nishiyama et al., 2009
Multi-subtype (ABC) gp160 DNA immunization + GM-CSF	Mice	Pooled sera from 6 animals neutralized 1 strain from subtype A and C and 3 strains from subtype B.	Rollman et al., 2004
Synthetic peptide derived from C2 residues 218-239 from CRF01_AE Env	Mice	mAb neutralizes 10 strains out of 14 tested, including subtypes A, C, D, and AE but not B	Sreepian et al., 2009
Formaldehyde-stabilized, heat-inactivated virion subtype B	Mice	Sera from 5 animals neutralized 7 strains from subtypes A, B, C, and AE	Poon et al., 2005
cytoplasmic tail Env mutant (more Env on surface)	Rabbits	Sera from 3 animals neutralized 4 strains from subtypes A, B, C, and AE but less potently than murine sera	
VEE virus replicon expressing: gp140/gp160, gp160 lacking cytoplasmic tail	Mice	Sera from 6 animals neutralized 8 nonhomologous subtype B, and pooled sera neutralized subtype C and E viral pseudotypes;	Dong et al., 2003
	Rabbits	Sera from 3 animals neutralized subtype B (SF162) and sera from 2 of these animals also neutralized subtype B (R2)	
Gp140 with novel carbopol-971p and MF59 adjuvant combination	Rabbits	Sera from 6 animals in the combined adjuvant group neutralized 6 strains from subtypes A, B, and C, 5 of which with superior potency to either adjuvants alone.	Lai et al., 2012
2F5 used to select immunogen from combinatorial libraries of recombinant human rhinoviruses displaying ELDKWA	Guinea pigs	Sera from 1 animal neutralized 9 strains from subtypes A, B, C, D, and AE with moderate/weak potency	Arnold et al., 2009
Multiclade (A, B, C) gp140ΔCFI DNA (mutations in the cleavage site, fusion peptide, and interhelical regions) followed by replication defective adenovirus expressing gp140ΔCFI	Guinea pigs	Sera from 4 animals neutralized 13 strains from subtypes A, B, and C with weak potency	Chakrabarti et al., 2005
Gp140 (subtype A or C), verified as homogenous and stable	Guinea pigs	Sera from animals immunized with either gp140 neutralized 1 subtype A, 2 subtype B, and 4 subtype C strains with statistically higher titers for sera resulting from subtype C gp140 rather than gp120 immunization	Kovacs et al., 2012
Gp120 protein from subtype B	Llamas	VHH mAb, neutralizes up to 40% of 59 strains tested from subtypes A, B, C, BC, AG, and AE with variable potency.	Forsman et al., 2008
Gp140 proteins from subtypes A and B/C	Llamas	VHH mAb, neutralizes 70–96% of strains, with 30–100 strains tested from subtypes A, B, C, BC, AG, AE, AC, ACD, D, and G	McCoy et al., 2012; Strokappe et al., 2012
Gp140	Rhesus macaques	Sera from 6 animals potently neutralized 5 strains from tier 1 (subtypes B, C, and A) and weakly neutralized 2 tier 2 strains. Protection: modest, nonsterilizing impact on acquisition of heterologous SHIV	Sundling et al., 2010

of sensitive viruses compared with sera raised against wild-type trimers (Douagi et al., 2010). Given the extensive glycan shield used by HIV to evade neutralization, modifying the glycosylation of Env immunogens has also been investigated. Several studies have shown increased titers of anti-Env activity in sera but not a concurrent increase in neutralizing activity (Joyce et al., 2008; Ma et al., 2011; Ahmed et al., 2012). However, an increase in neutralization titers was seen in cynomolgus macaques boosted with DNA and Env after a prime comprised a poxvirus expressing an Env mutant lacking an N-linked glycosylation site in the V2 loop, which has been previously characterized as more sensitive to the first generation mAbs b12 and 447-52D (Li et al., 2008). The animals that received the mutant rather than wild-type Env also had lowered viral loads and increased survival upon homologous challenge (Li et al., 2008).

Env immunization has focused primarily but not exclusively on gp120/140, as many immunodominant but non-neutralizing antibodies interact with gp41 (Wilson et al., 1990). However, recently gp41 immunogens have been investigated with a degree of success. An MPER mimotope linked to lipid carriers induced subtype B (SF162.LS) MPER-specific neutralizing sera responses in mice (Zhou et al., 2012). Furthermore, immunization of rabbits with a mimetic of the coiled-coil of gp41 presented by phage display led to the isolation of a single-chain Fv antibody that neutralizes primary HIV of subtypes B and C with modest potency (Nelson et al., 2008). Grafting MPER onto the more highly exposed V1/V2 loop induced neutralizing sera in mice but, unfortunately, not specific for MPER or the V1/V2 loop (Law et al., 2007). Also disappointingly, an NHP study with a gp41 proteoliposome induced MPER binding, but not neutralizing sera (Dennison et al., 2011). Mucosal vaccination with gp41 peptide immunogens has, however, produced sera that can neutralize a limited number of strains (Devito et al., 2004) and block viral transcytosis in vitro (Jain and Rosenthal, 2011). The broadest neutralization induced thus far to gp41 involved immunization of guinea pigs with human rhinoviruses displaying modified versions of the 2F5 epitope which were preselected for binding to this mAb. The postimmune serum from one animal neutralized nine HIV-1 strains from subtypes A, B, C, D, and AE with moderate to weak potency (Table 2; Arnold et al., 2009).

Given the acknowledged role of lipid reactivity in some mAb derived from HIV-positive individuals, and the restrictions placed on the angle of approach of antibodies to the Env spike on a viral particle, strategies for presenting Env in a membrane-bound context have been investigated. A range of viral vectors have been exploited to express Env, including Semliki Forest virus (Forsell et al., 2005), alphavirus replicon particles (Dong et al., 2003; Mörner et al., 2009; Barnett et al., 2010; Table 2), adenoviruses (Lubeck et al., 1997; Zolla-Pazner et al., 1998a; Chakrabarti et al., 2002; Mascola et al., 2005), measles (Lorin et al., 2004), varicella (Traina-Dorge et al., 2010), vesicular stomatitis virus (Schell et al., 2009), and multiple poxviruses (Verrier et al., 2000; Radaelli et al., 2007). A parallel aim of many of these studies was the effective induction of T cell immunity which has been reviewed elsewhere (McElrath and Haynes, 2010). Only two of these studies (Dong et al., 2003; Chakrabarti et al., 2005) resulted in cross-reactive postimmune sera able to neutralize at least three subtypes (Table 2). However, a range of these immunizations resulted in protection from infection (Lubeck et al., 1997; Zolla-Pazner et al., 1998b) or control of viral load in infected animals (Schell et al., 2009; Traina-Dorge et al., 2010). To address concerns about the integrity of recombinant Env immunogens, particularly regarding posttranslation glycosylation, DNA encoding Env has been included in immunization strategies given the preferential uptake of DNA by antigen presenting cells. However, DNA immunization without subsequent viral vector or protein boosts has only produced sera that neutralize three or more subtypes when administered with granulocyte-macrophage colony-stimulating factor (Rollman et al., 2004; Table 2). Overall, the use of viral vectors combined with either Env or DNA has shown some success but, again, has not resulted in a great leap forward in terms of neutralization titers or breadth.

Recent studies have highlighted the importance of including protein components, particularly Env, in immunization protocols to achieve protection against heterologous challenge strains in NHPs (Watkins et al., 2011; Barouch et al., 2012). Although greater understanding of how Env subunit immunogens elicit antibodies has been gained, attempts thus far to focus the immune system on particular epitopes have not resulted in highly specific mAbs. Given that such mAbs have now been identified in multiple infected humans, both mAb structure and mode of action studies should provide clearer insight into how to modify Env to focus the immune response to induce broadly neutralizing sera. Investigations into more selectively focused Env immunizations include several approaches: mutation of immunodominant epitopes; masking of immunodominant epitopes by cross-linking, for example, with aldehydes (Poon et al., 2005; Table 2); and selective alteration of carbohydrate moieties. In addition, when considering the handful of studies (Table 2) that have given rise to cross-reactive responses or mAb (defined as neutralization of three or more HIV subtypes) it is noteworthy that almost half of these studies evaluate individual mAbs rather than serum neutralization, in contrast to most HIV immunization studies. In at least one case, a mAb fragment that neutralizes 96% of strains tested was isolated from weakly neutralizing serum (McCoy et al., 2012). Thus, it may be that if the large-scale techniques applied to searching for neutralizing mAb from infected humans were applied to postimmune sera, it could be clarified whether rare broadly neutralizing antibodies were in fact elicited but at levels too low to dominate the sera neutralization activity. In turn, this knowledge could be used to optimize immunization protocols and improve the stimulation of the appropriate B cell precursors.

Postimmunization protection from HIV and neutralizing responses

Huge efforts have been made to elucidate how humoral responses are induced by Env proteins and to characterize the anti-HIV activity of the resulting sera. However, it is important to ask not only what neutralization activity has been induced upon immunization but also whether the animals are protected from viral challenge. Indeed, protection from both heterologous and homologous challenge in NHP has been observed despite suboptimal serum neutralizing activity and there is residual doubt regarding the role of vaccine-induced neutralizing antibodies in such studies. However, correlation between neutralizing antisera and protection has been noted after Env immunization (Barnett et al., 2008), adenovirus priming followed by Env or alphavirus replicon particles (Bogers et al., 2008), and alphavirus replicon particles with a trimeric Env boost (Barnett et al., 2010). Furthermore, this association between neutralizing antisera and protection is not at the expense of effective cellular immunity. In fact, a separate study of rhesus macaques immunized with alphavirus replicon particles, followed by an Env boost with or without vectors expressing SIV *gag/pol*, showed not only that there is no interference between cellular and humoral immunity but that protection to heterologous challenge was linked to both higher serum neutralization titers and improved cellular immune responses with a few subjects completely protected from infection (Quinnan et al., 2005). Furthermore, peak viral loads after repeated low-dose challenge of rhesus macaques immunized solely with recombinant protein showed a significant inverse correlation with both cross-reactive neutralizing antibodies levels and cellular immunity (Lakhashe et al., 2011). Recently, viral vector combination regimens expressing SIV *gag*, *pol*, and *env* have resulted in 80% or more reduction in infection after repeated challenge with a heterologous, neutralization-resistant SIV, and the inclusion of *env* was shown to be essential to this protection, highlighting a potential role for neutralizing antibodies in the success of this immunization protocol (Barouch et al., 2012). Thus, the challenge is now to identify how this protection is being mediated and to improve immunogens and immunization regimens to promote the protective component of the response. Strategies to improve antibody elicitation have been reviewed elsewhere (Dimitrov et al., 2011; Moir et al., 2011; Verkoczy et al., 2011; Klasse et al., 2012).

Human HIV vaccine studies have in many ways mirrored the studies undertaken in animals. Initial human gp120 immunization resulted in serum neutralization against the strain from which the vaccine was derived (Wrin and Nunberg, 1994; Graham et al., 1996; Mascola et al., 1996; Pincus et al., 1997; Bartlett et al., 1998; Gorse et al., 1998). Subsequently, the first phase III efficacy trial (VAX003/004) involving vaccination with two subtype B gp120 subunits was unable to provide protection from acquisition but did result in serum neutralization of related CXCR4-tropic subtype B strains but not of primary CCR5-tropic isolates (Flynn et al., 2005; Pitisuttithum et al., 2006). The VAX004 trial was also not protective against acquisition but resulted in neutralizing activity against virus expressing the subtype B gp120 immunogen administered to vaccinees. Thus, this weak level of subtype-specific neutralization, which is reminiscent of that shown to protect from homologous challenge in many animal studies, was not protective in humans in a phase III trial (Gilbert et al., 2010).

In 2009, a degree of efficacy was observed in a phase III HIV vaccine trial, with 30% of vaccinees protected relative to placebo for the first time (Rerks-Ngarm et al., 2009). The RV144 trial involved priming with the poxvirus vector ALVAC followed by AIDSVAX B/E gp120 boost, and recently two correlates of risk have been identified. Both focus on the humoral response to vaccination, with an increased risk correlated with high titers of anti-immunogen IgA and decreased risk correlated with high titers of anti-Env IgG that target the V1/V2 loop (Haynes et al., 2012). Interestingly, a previous canarypox-based phase II trial was deemed inadequately effective based on poor cytotoxic T lymphocyte responses despite the elicitation of MN neutralizing sera in 57–94% of subjects (Russell et al., 2007). However, it should be noted that the humoral correlates of risk identified in the analysis of the RV144 trial do not include neutralizing serum responses (Rerks-Ngarm et al., 2009; Haynes et al., 2012; Montefiori et al., 2012).

There is some similarity between the outcomes of many animal immunization experiments and human vaccination trials in that the majority of studies have resulted in serum neutralization which is limited in breadth to highly sensitive viruses. The contrast is that the animal studies often show protection from acquisition in challenge studies, mostly with homologous virus, whereas for human vaccination trials, protection has generally not been evident, presumably as a result of the highly diverse nature of real world exposure to HIV. As outlined in the introduction, until recently the field did not have a clear idea of what levels of breadth and potency could be achieved by human neutralizing antibodies and, as such, the relatively narrow breadth of serum neutralization usually seen upon immunization was not considered suboptimal as it is now judged to be. The knowledge of the caliber of neutralizing antibody that humans can produce during infection provides a high benchmark against which to evaluate immunization studies and the techniques used to isolate these second generation neutralizing mAb can help to assess how close we come to the mark.

It is noteworthy that mAb-mediated protection via passive transfer in NHP is not thought to act solely via the mechanism of neutralization (Hessell et al., 2009; Watkins et al., 2011; Moldt et al., 2012), and there is increasing evidence in support of the importance of antibody effector functions and also trans-cytosis blocking antibodies (Bomsel et al., 1998; Burke and Barnett, 2007; Tudor et al., 2009). Overall, despite the progress in understanding how antibodies can effectively neutralize HIV it is a still the major challenge of the HIV vaccine field to induce a high titer sera response comprising such antibodies. This challenge is even greater when

the predominant mode of HIV transmission is considered, as to induce high concentrations of neutralizing antibody at the mucosal level is even more difficult than on a systemic level (Wright et al., 2004). Thus, it is likely that improving immunization protocols to the level where they can elicit such antibodies will require considerable effort so it is worthwhile to consider how the identified neutralizing mAbs can be used as preventative reagents in the meantime.

Prospects and considerations for the therapeutic use of second generation bnAbs

Given the protection seen in passive immunization studies with the first generation of neutralizing mAbs, which are substantially less potent, it is hypothesized that the second generation of neutralizing mAbs could be used via passive transfer at much lower doses (Diskin et al., 2011), and in combination (Doria-Rose et al., 2012; Klein et al., 2012b), to provide enhanced protection from infection. When could such dosing be useful to prevent transmission in a clinical setting? It seems unlikely that such an intensive intervention would be suitable for prophylactic daily use. Whether passive transfer could be used as postexposure prophylaxis is unclear; data from infusion of polyclonal neutralizing antibodies into NHP 6 h after challenge did provide sterilizing immunity but at 24 h did not (Nishimura et al., 2003). Studies are needed with the second generation mAb, most critically in combination, to assess the time limits and potential advantages of such an approach alongside whether viral escape is feasible in vivo, which in turn will have implications for the combinations of epitopes to be included future vaccination studies.

It cannot be overlooked that there are resource limits which need to be considered when discussing the use of purified mAb to combat HIV, and which may restrict the use of the applications above to a very small proportion of the global population at risk of infection. Efforts are under way, however, to overcome the limits imposed by cost and time intensive production of mAbs so that they could be used prophylactically on a larger scale. The first option is that of mAb-based microbicide. The protection seen with an antiretroviral-based microbicide gel has reinvigorated the HIV microbicide field (Abdool Karim et al., 2010), and although gels containing mAbs have been considered, the cost implications are similar to those for passive transfer of mAbs, with additional problems caused by the need for cold storage of such a microbicide (Gorlani et al., 2012). An alternative prospect is that of a persistent biological microbicide, namely Lactobacilli, genetically modified to express anti-HIV molecules. Vaginal colonization of *Macaca mulatta* with *Lactobacilli jensenii* expressing cyanovirin has already been shown to prevent mucosal SHIV transmission (Lagenaur et al., 2010) and attempts are underway to express mAb fragments in this system. Although *L. jensenii* is a naturally occurring human commensal strain, thorough evaluation of the effects of colonization will be required alongside monitoring of the level of mAb expression. Another attractive approach to using mAbs prophylactically is a gene therapy–based approach. Such an approach was first described 10 years ago (Lewis et al., 2002; Johnson et al., 2009). Last year, this approach made a major advance with the description of a highly effective vector-mediated immunoprophylaxis in SCID mice reconstituted with human PBLs and challenged with a high dose of HIV (Balazs et al., 2012). A range of mAbs were shown to be effective in this system and secreted at 20–250 mg/ml in sera (Balazs et al., 2012), with protection from infection with serum concentrations for b12 and VRC01 of 34 and 8.3 mg/ml, respectively. How well vector-mediated immunoprophylaxis protects from mucosal transmission remains to be established. It will be particularly interesting to see whether combinations of mAbs result in a higher transmission barrier. Other obstacles to this approach are those associated with gene therapy in general. However, it should be noted that the adeno-associated virus vector used is nonintegrating but persistent and thus advantageous over other gene therapy vectors.

Conclusions

Single B cell cloning and systematic high-throughput methods involving the development of novel selection methods have resulted in the identification of the second generation neutralizing mAbs from HIV infected patients, despite such mAbs being relatively rare within the broadly neutralizing B cell repertoires of selected individuals. There is now a need for a similarly systematic approach to identify immunogens that may be capable of eliciting neutralizing antibodies, given that many novel immunogens to date have produced somewhat similar clade-specific weak serum neutralizing responses. In fact, a huge variety of immunization protocols have produced what could almost be termed a consensus humoral immune response comprising homologous neutralization activity in the sera as per initial immunization studies (Weiss et al., 1986), with some exceptions showing an increasing breadth in heterologous activity. A more detailed analysis of the components of the postimmune sera could enable advanced evaluation of the degree to which the immunization was effective, which could in turn inform immunogen and immunization study design. Despite the association observed between neutralizing antisera and a level of protection in some vaccination regimens, neutralization activity in immunized animals and humans is markedly inferior to those in rare HIV-infected individuals who are termed elite neutralizers. Passive immunization studies have shown that if certain levels of neutralizing mAbs are present, complete protection can be achieved (Mascola et al., 1999, 2000; Shibata et al., 1999; Parren et al., 2001; Veazey et al., 2003; Hessell et al., 2009; Watkins et al., 2011). Furthermore, it has been shown that combinations of recently described neutralizing antibodies together could block infection by the vast majority of strains (Doria-Rose et al., 2012; Klein et al., 2012a) and can control infection in humanized mice (Klein et al., 2012b). Therefore, there is a need for a bilateral approach to (1) identify immunogens able to induce neutralization activity (even at low titers) by investigating mAb as well as serum neutralization, and (2) understand how to modulate the immune system to promote the

affinity maturation of these rare clones to predominate over the abundant nonneutralizing antibodies. The desired outcome of such a strategy would be that the required level of neutralizing antibodies are present at the mucosa during the short window between exposure and infection to provide protection from HIV. Although the epidemic continues, we need to find ways to use all the available resources, in this case, the new generation of neutralizing mAb to prevent spread and lower disease burden. Approaches that bypass the difficult elicitation stage to provide broadly neutralizing mAb at the point of need, such as gene therapy and biological microbicides, show much promise. However, many more years of development may be required before these options can be rolled out to the populations most in need of protection.

In conclusion, even though we now have an expanding array of broadly neutralizing antibodies, which block a large proportion of HIV strains by binding to various epitopes on Env, there remains an immense challenge to convert our understanding of how these mAbs bind antigen and block infection into immunogens that will elicit a major humoral response to these targets. Despite the potential applications of HIV neutralizing antibodies detailed above, the challenge of effective immunogen design remains a priority in HIV research as part of the overall goal of developing a prophylactic HIV vaccine. It is to be hoped that the same level of rigor used to identify the new generation of broad and potent neutralizing mAbs will be applied to the challenge of immunogen design, leading to successful vaccine candidates.

Submitted: 13 August 2012
Accepted: 11 December 2012

REFERENCES

Abdool Karim, Q., S.S. Abdool Karim, J.A. Frohlich, A.C. Grobler, C. Baxter, L.E. Mansoor, A.B. Kharsany, S. Sibeko, K.P. Mlisana, Z. Omar, et al; CAPRISA 004 Trial Group. 2010. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. *Science*. 329:1168–1174. http://dx.doi.org/10.1126/science.1193748

Ahmed, F.K., B.E. Clark, D.R. Burton, and R. Pantophlet. 2012. An engineered mutant of HIV-1 gp120 formulated with adjuvant Quil A promotes elicitation of antibody responses overlapping the CD4-binding site. *Vaccine*. 30:922–930. http://dx.doi.org/10.1016/j.vaccine.2011.11.089

Alam, S.M., M. Morelli, S.M. Dennison, H.X. Liao, R. Zhang, S.M. Xia, S. Rits-Volloch, L. Sun, S.C. Harrison, B.F. Haynes, and B. Chen. 2009. Role of HIV membrane in neutralization by two broadly neutralizing antibodies. *Proc. Natl. Acad. Sci. USA*. 106:20234–20239. http://dx.doi.org/10.1073/pnas.0908713106

Andrus, L., A.M. Prince, I. Bernal, P. McCormack, D.H. Lee, M.K. Gorny, and S. Zolla-Pazner. 1998. Passive immunization with a human immunodeficiency virus type 1-neutralizing monoclonal antibody in Hu-PBL-SCID mice: isolation of a neutralization escape variant. *J. Infect. Dis.* 177:889–897. http://dx.doi.org/10.1086/515251

Arnold, G.F., P.K. Velasco, A.K. Holmes, T. Wrin, S.C. Geisler, P. Phung, Y. Tian, D.A. Resnick, X. Ma, T.M. Mariano, et al. 2009. Broad neutralization of human immunodeficiency virus type 1 (HIV-1) elicited from human rhinoviruses that display the HIV-1 gp41 ELDKWA epitope. *J. Virol.* 83:5087–5100. http://dx.doi.org/10.1128/JVI.00184-09

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

Barbato, G., E. Bianchi, P. Ingallinella, W.H. Hurni, M.D. Miller, G. Ciliberto, R. Cortese, R. Bazzo, J.W. Shiver, and A. Pessi. 2003. Structural analysis of the epitope of the anti-HIV antibody 2F5 sheds light into its mechanism of neutralization and HIV fusion. *J. Mol. Biol.* 330:1101–1115. http://dx.doi.org/10.1016/S0022-2836(03)00611-9

Barnett, S.W., S. Rajasekar, H. Legg, B. Doe, D.H. Fuller, J.R. Haynes, C.M. Walker, and K.S. Steimer. 1997. Vaccination with HIV-1 gp120 DNA induces immune responses that are boosted by a recombinant gp120 protein subunit. *Vaccine*. 15:869–873. http://dx.doi.org/10.1016/S0264-410X(96)00264-2

Barnett, S.W., S. Lu, I. Srivastava, S. Cherpelis, A. Gettie, J. Blanchard, S. Wang, I. Mboudjeka, L. Leung, Y. Lian, et al. 2001. The ability of an oligomeric human immunodeficiency virus type 1 (HIV-1) envelope antigen to elicit neutralizing antibodies against primary HIV-1 isolates is improved following partial deletion of the second hypervariable region. *J. Virol.* 75:5526–5540. http://dx.doi.org/10.1128/JVI.75.12.5526-5540.2001

Barnett, S.W., I.K. Srivastava, E. Kan, F. Zhou, A. Goodsell, A.D. Cristillo, M.G. Ferrai, D.E. Weiss, N.L. Letvin, D. Montefiori, et al. 2008. Protection of macaques against vaginal SHIV challenge by systemic or mucosal and systemic vaccinations with HIV-envelope. *AIDS*. 22:339–348. http://dx.doi.org/10.1097/QAD.0b013e3282f3ca57

Barnett, S.W., B. Burke, Y. Sun, E. Kan, H. Legg, Y. Lian, K. Bost, F. Zhou, A. Goodsell, J. Zur Megede, et al. 2010. Antibody-mediated protection against mucosal simian-human immunodeficiency virus challenge of macaques immunized with alphavirus replicon particles and boosted with trimeric envelope glycoprotein in MF59 adjuvant. *J. Virol.* 84:5975–5985. http://dx.doi.org/10.1128/JVI.02533-09

Barouch, D.H. 2010. Novel adenovirus vector-based vaccines for HIV-1. *Curr Opin HIV AIDS*. 5:386–390. http://dx.doi.org/10.1097/COH.0b013e32833cfe4c

Barouch, D.H., J. Liu, H. Li, L.F. Maxfield, P. Abbink, D.M. Lynch, M.J. Iampietro, A. SanMiguel, M.S. Seaman, G. Ferrari, et al. 2012. Vaccine protection against acquisition of neutralization-resistant SIV challenges in rhesus monkeys. *Nature*. 482:89–93. http://dx.doi.org/10.1038/nature10766

Bartlett, J.A., S.S. Wasserman, C.B. Hicks, R.T. Dodge, K.J. Weinhold, C.O. Tacket, N. Ketter, A.E. Wittek, T.J. Palker, and B.F. Haynes; Division of AIDS Treatment Research Initiative. 1998. Safety and immunogenicity of an HLA-based HIV envelope polyvalent synthetic peptide immunogen. DATRI 010 Study Group. *AIDS*. 12:1291–1300. http://dx.doi.org/10.1097/00002030-199811000-00010

Beddows, S., M. Franti, A.K. Dey, M. Kirschner, S.P. Iyer, D.C. Fisch, T. Ketas, E. Yuste, R.C. Desrosiers, P.J. Klasse, et al. 2007. A comparative immunogenicity study in rabbits of disulfide-stabilized, proteolytically cleaved, soluble trimeric human immunodeficiency virus type 1 gp140, trimeric cleavage-defective gp140 and monomeric gp120. *Virology*. 360:329–340. http://dx.doi.org/10.1016/j.virol.2006.10.032

Beirnaert, E., P. Nyambi, B. Willems, L. Heyndrickx, R. Colebunders, W. Janssens, and G. van der Groen. 2000. Identification and characterization of sera from HIV-infected individuals with broad cross-neutralizing activity against group M (env clade A-H) and group O primary HIV-1 isolates. *J. Med. Virol.* 62:14–24. http://dx.doi.org/10.1002/1096-9071(200009)62:1<14::AID-JMV3>3.0.CO;2-L

Bell, C.H., R. Pantophlet, A. Schiefner, L.A. Cavacini, R.L. Stanfield, D.R. Burton, and I.A. Wilson. 2008. Structure of antibody F425-B4e8 in complex with a V3 peptide reveals a new binding mode for HIV-1 neutralization. *J. Mol. Biol.* 375:969–978. http://dx.doi.org/10.1016/j.jmb.2007.11.013

Belshe, R.B., G.J. Gorse, M.J. Mulligan, T.G. Evans, M.C. Keefer, J.L. Excler, A.M. Duliege, J. Tartaglia, W.I. Cox, J. McNamara, et al; NIAID AIDS Vaccine Evaluation Group. 1998. Induction of immune responses to HIV-1 by canarypox virus (ALVAC) HIV-1 and gp120 SF-2 recombinant vaccines in uninfected volunteers. *AIDS*. 12:2407–2415. http://dx.doi.org/10.1097/00002030-199818000-00009

Berman, P.W., J.E. Groopman, T. Gregory, P.R. Clapham, R.A. Weiss, R. Ferriani, L. Riddle, C. Shimasaki, C. Lucas, L.A. Lasky, et al. 1988. Human immunodeficiency virus type 1 challenge of chimpanzees immunized with recombinant envelope glycoprotein gp120. *Proc. Natl. Acad. Sci. USA*. 85:5200–5204. http://dx.doi.org/10.1073/pnas.85.14.5200

Berman, P.W., T.J. Gregory, L. Riddle, G.R. Nakamura, M.A. Champe, J.P. Porter, F.M. Wurm, R.D. Hershberg, E.K. Cobb, and J.W. Eichberg. 1990. Protection of chimpanzees from infection by HIV-1 after vaccination with recombinant glycoprotein gp120 but not gp160. *Nature*. 345:622–625. http://dx.doi.org/10.1038/345622a0

Binley, J.M., T. Wrin, B. Korber, M.B. Zwick, M. Wang, C. Chappey, G. Stiegler, R. Kunert, S. Zolla-Pazner, H. Katinger, et al. 2004. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. *J. Virol.* 78:13232–13252. http://dx.doi.org/10.1128/JVI.78.23.13232-13252.2004

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

Bogers, W.M., D. Davis, I. Baak, E. Kan, S. Hofman, Y. Sun, D. Mortier, Y. Lian, H. Oostermeijer, Z. Fagrouch, et al. 2008. Systemic neutralizing antibodies induced by long interval mucosally primed systemically boosted immunization correlate with protection from mucosal SHIV challenge. *Virology*. 382:217–225. http://dx.doi.org/10.1016/j.virol.2008.09.016

Bomsel, M., M. Heyman, H. Hocini, S. Lagaye, L. Belec, C. Dupont, and C. Desgranges. 1998. Intracellular neutralization of HIV transcytosis across tight epithelial barriers by anti-HIV envelope protein dIgA or IgM. *Immunity*. 9:277–287. http://dx.doi.org/10.1016/S1074-7613(00)80610-X

Bonsignori, M., D.C. Montefiori, X. Wu, X. Chen, K.K. Hwang, C.Y. Tsao, D.M. Kozink, R.J. Parks, G.D. Tomaras, J.A. Crump, et al. 2012. Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: implications for vaccine design. *J. Virol.* 86:4688–4692. http://dx.doi.org/10.1128/JVI.07163-11

Bower, J.F., X. Yang, J. Sodroski, and T.M. Ross. 2004. Elicitation of neutralizing antibodies with DNA vaccines expressing soluble stabilized human immunodeficiency virus type 1 envelope glycoprotein trimers conjugated to C3d. *J. Virol.* 78:4710–4719. http://dx.doi.org/10.1128/JVI.78.9.4710-4719.2004

Burke, B., and S.W. Barnett. 2007. Broadening our view of protective antibody responses against HIV. *Curr. HIV Res.* 5:625–641. http://dx.doi.org/10.2174/157016207782418533

Burton, D.R., and R.A. Weiss. 2010. AIDS/HIV. A boost for HIV vaccine design. *Science*. 329:770–773. http://dx.doi.org/10.1126/science.1194693

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., P. Poignard, R.L. Stanfield, and I.A. Wilson. 2012. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. *Science*. 337:183–186. http://dx.doi.org/10.1126/science.1225416

Calarese, D.A., C.N. Scanlan, M.B. Zwick, S. Deechongkit, Y. Mimura, R. Kunert, P. Zhu, M.R. Wormald, R.L. Stanfield, K.H. Roux, et al. 2003. Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. *Science*. 300:2065–2071. http://dx.doi.org/10.1126/science.1083182

Chaillon, A., M. Braibant, T. Moreau, S. Thenin, A. Moreau, B. Autran, and F. Barin. 2011. The V1V2 domain and an N-linked glycosylation site in the V3 loop of the HIV-1 envelope glycoprotein modulate neutralization sensitivity to the human broadly neutralizing antibody 2G12. *J. Virol.* 85:3642–3648. http://dx.doi.org/10.1128/JVI.02424-10

Chakrabarti, B.K., W.P. Kong, B.Y. Wu, Z.Y. Yang, J. Friborg, X. Ling, S.R. King, D.C. Montefiori, and G.J. Nabel. 2002. Modifications of the human immunodeficiency virus envelope glycoprotein enhance immunogenicity for genetic immunization. *J. Virol.* 76:5357–5368. http://dx.doi.org/10.1128/JVI.76.11.5357-5368.2002

Chakrabarti, B.K., X. Ling, Z.Y. Yang, D.C. Montefiori, A. Panet, W.P. Kong, B. Welcher, M.K. Louder, J.R. Mascola, and G.J. Nabel. 2005. Expanded breadth of virus neutralization after immunization with a multiclade envelope HIV vaccine candidate. *Vaccine*. 23:3434–3445. http://dx.doi.org/10.1016/j.vaccine.2005.01.099

Connor, R.I., B.T. Korber, B.S. Graham, B.H. Hahn, D.D. Ho, B.D. Walker, A.U. Neumann, S.H. Vermund, J. Mestecky, S. Jackson, et al. 1998. Immunological and virological analyses of persons infected by human immunodeficiency virus type 1 while participating in trials of recombinant gp120 subunit vaccines. *J. Virol.* 72:1552–1576.

Corti, D., J.P. Langedijk, A. Hinz, M.S. Seaman, F. Vanzetta, B.M. Fernandez-Rodriguez, C. Silacci, D. Pinna, D. Jarrossay, S. Balla-Jhagjhoorsingh, et al. 2010. Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. *PLoS ONE*. 5:e8805. http://dx.doi.org/10.1371/journal.pone.0008805

Deeks, S.G., B. Schweighardt, T. Wrin, J. Galovich, R. Hoh, E. Sinclair, P. Hunt, J.M. McCune, J.N. Martin, C.J. Petropoulos, and F.M. Hecht. 2006. Neutralizing antibody responses against autologous and heterologous viruses in acute versus chronic human immunodeficiency virus (HIV) infection: evidence for a constraint on the ability of HIV to completely evade neutralizing antibody responses. *J. Virol.* 80:6155–6164. http://dx.doi.org/10.1128/JVI.00093-06

Dennison, S.M., L.L. Sutherland, F.H. Jaeger, K.M. Anasti, R. Parks, S. Stewart, C. Bowman, S.M. Xia, R. Zhang, X. Shen, et al. 2011. Induction of antibodies in rhesus macaques that recognize a fusion-intermediate conformation of HIV-1 gp41. *PLoS ONE*. 6:e27824. http://dx.doi.org/10.1371/journal.pone.0027824

Devito, C., B. Zuber, U. Schröder, R. Benthin, K. Okuda, K. Broliden, B. Wahren, and J. Hinkula. 2004. Intranasal HIV-1-gp160-DNA/gp41 peptide prime-boost immunization regimen in mice results in long-term HIV-1 neutralizing humoral mucosal and systemic immunity. *J. Immunol.* 173:7078–7089.

Dhillon, A.K., H. Donners, R. Pantophlet, W.E. Johnson, J.M. Decker, G.M. Shaw, F.H. Lee, D.D. Richman, R.W. Doms, G. Vanham, and D.R. Burton. 2007. Dissecting the neutralizing antibody specificities of broadly neutralizing sera from human immunodeficiency virus type 1-infected donors. *J. Virol.* 81:6548–6562. http://dx.doi.org/10.1128/JVI.02749-06

Dimitrov, J.D., M.D. Kazatchkine, S.V. Kaveri, and S. Lacroix-Desmazes. 2011. "Rational vaccine design" for HIV should take into account the adaptive potential of polyreactive antibodies. *PLoS Pathog.* 7:e1002095. http://dx.doi.org/10.1371/journal.ppat.1002095

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

Dong, M., P.F. Zhang, F. Grieder, J. Lee, G. Krishnamurthy, T. VanCott, C. Broder, V.R. Polonis, X.F. Yu, Y. Shao, et al. 2003. Induction of primary virus-cross-reactive human immunodeficiency virus type 1-neutralizing antibodies in small animals by using an alphavirus-derived in vivo expression system. *J. Virol.* 77:3119–3130. http://dx.doi.org/10.1128/JVI.77.5.3119-3130.2003

Doria-Rose, N.A., M.K. Louder, Z. Yang, S. O'Dell, M. Nason, S.D. Schmidt, K. McKee, M.S. Seaman, R.T. Bailer, and J.R. Mascola. 2012. HIV-1 neutralization coverage is improved by combining monoclonal antibodies that target independent epitopes. *J. Virol.* 86:3393–3397. http://dx.doi.org/10.1128/JVI.06745-11

Douagi, I., M.N. Forsell, C. Sundling, S. O'Dell, Y. Feng, P. Dosenovic, Y. Li, R. Seder, K. Loré, J.R. Mascola, et al. 2010. Influence of novel CD4 binding-defective HIV-1 envelope glycoprotein immunogens on neutralizing antibody and T-cell responses in nonhuman primates. *J. Virol.* 84:1683–1695. http://dx.doi.org/10.1128/JVI.01896-09

Durda, P.J., B. Leece, A. Jenoski, H. Rabin, A. Fisher, and F. Wong-Staal. 1988. Characterization of murine monoclonal antibodies to HIV-1 induced by synthetic peptides. *AIDS Res. Hum. Retroviruses*. 4:331–342. http://dx.doi.org/10.1089/aid.1988.4.331

Earl, P.L., M. Robert-Guroff, T.J. Matthews, K. Krohn, W.T. London, and B. Moss. 1989. Isolate- and group-specific immune responses to the envelope protein of human immunodeficiency virus induced by a live recombinant vaccinia virus in macaques. *AIDS Res. Hum. Retroviruses*. 5:23–32. http://dx.doi.org/10.1089/aid.1989.5.23

Falkowska, E., A. Ramos, Y. Feng, T. Zhou, S. Moquin, L.M. Walker, X. Wu, M.S. Seaman, T. Wrin, P.D. Kwong, et al. 2012. PGV04, an HIV-1 gp120 CD4 binding site antibody, is broad and potent in neutralization but does not induce conformational changes characteristic of CD4. *J. Virol.* 86:4394–4403. http://dx.doi.org/10.1128/JVI.06973-11

Ferrantelli, F., M.T. Maggiorella, I. Schiavoni, L. Sernicola, E. Olivieri, S. Farcomeni, M.R. Pavone-Cossut, S. Moretti, R. Belli, B. Collacchi, et al. 2011. A combination HIV vaccine based on Tat and Env proteins was immunogenic and protected macaques from mucosal SHIV challenge in a pilot study. *Vaccine*. 29:2918–2932. http://dx.doi.org/10.1016/j.vaccine.2011.02.006

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

Forsell, M.N., Y. Li, M. Sundbäck, K. Svehla, P. Liljeström, J.R. Mascola, R. Wyatt, and G.B. Karlsson Hedestam. 2005. Biochemical and immunogenic characterization of soluble human immunodeficiency virus type 1 envelope glycoprotein trimers expressed by semliki forest virus. *J. Virol.* 79:10902–10914. http://dx.doi.org/10.1128/JVI.79.17.10902-10914.2005

Forsell, M.N., W.R. Schief, and R.T. Wyatt. 2009. Immunogenicity of HIV-1 envelope glycoprotein oligomers. *Curr Opin HIV AIDS*. 4:380–387. http://dx.doi.org/10.1097/COH.0b013e32832edc19

Forsman, A., E. Beirnaert, M.M. Aasa-Chapman, B. Hoorelbeke, K. Hijazi, W. Koh, V. Tack, A. Szynol, C. Kelly, A. McKnight, et al. 2008. Llama antibody fragments with cross-subtype human immunodeficiency virus type 1 (HIV-1)-neutralizing properties and high affinity for HIV-1 gp120. *J. Virol.* 82:12069–12081. http://dx.doi.org/10.1128/JVI.01379-08

Forthal, D.N., and C. Moog. 2009. Fc receptor-mediated antiviral antibodies. *Curr Opin HIV AIDS*. 4:388–393. http://dx.doi.org/10.1097/COH.0b013e32832f0a89

Gilbert, P., M. Wang, T. Wrin, C. Petropoulos, M. Gurwith, F. Sinangil, P. D'Souza, I.R. Rodriguez-Chavez, A. DeCamp, M. Giganti, et al. 2010. Magnitude and breadth of a nonprotective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine. *J. Infect. Dis.* 202:595–605. http://dx.doi.org/10.1086/654816

Gorlani, A., J. Brouwers, C. McConville, P. van der Bijl, K. Malcolm, P. Augustijns, A.F. Quigley, R. Weiss, H. De Haard, and T. Verrips. 2012. Llama antibody fragments have good potential for application as HIV type 1 topical microbicides. *AIDS Res. Hum. Retroviruses*. 28:198–205. http://dx.doi.org/10.1089/aid.2011.0133

Gorse, G.J., M.J. McElrath, T.J. Matthews, R.H. Hsieh, R.B. Belshe, L. Corey, S.E. Frey, D.J. Kennedy, M.C. Walker, and M.M. Eibl; National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. 1998. Modulation of immunologic responses to HIV-1MN recombinant gp160 vaccine by dose and schedule of administration. *Vaccine*. 16:493–506. http://dx.doi.org/10.1016/S0264-410X(97)80003-5

Graham, B.S., M.C. Keefer, M.J. McElrath, G.J. Gorse, D.H. Schwartz, K. Weinhold, T.J. Matthews, J.R. Esterlitz, F. Sinangil, and P.E. Fast; NIAID AIDS Vaccine Evaluation Group. 1996. Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120. A randomized, double-blind trial. *Ann. Intern. Med.* 125:270–279.

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; and the CAPRISA002 Study Team. 2011. 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

Haigwood, N.L., J.R. Shuster, G.K. Moore, H. Lee, P.V. Skiles, K.W. Higgins, P.J. Barr, C. George-Nascimento, and K.S. Steimer. 1990. Importance of hypervariable regions of HIV-1 gp120 in the generation of virus neutralizing antibodies. *AIDS Res. Hum. Retroviruses*. 6:855–869. http://dx.doi.org/10.1089/aid.1990.6.855

Hammonds, J., X. Chen, T. Fouts, A. DeVico, D. Montefiori, and P. Spearman. 2005. Induction of neutralizing antibodies against human immunodeficiency virus type 1 primary isolates by Gag-Env pseudovirion immunization. *J. Virol.* 79:14804–14814. http://dx.doi.org/10.1128/JVI.79.23.14804-14814.2005

Haynes, B.F., P.B. Gilbert, M.J. McElrath, S. Zolla-Pazner, G.D. Tomaras, S.M. Alam, D.T. Evans, D.C. Montefiori, C. Karnasuta, R. Sutthent, et al. 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. *N. Engl. J. Med.* 366:1275–1286. http://dx.doi.org/10.1056/NEJMoa1113425

Hessell, A.J., and N.L. Haigwood. 2012. Neutralizing antibodies and control of HIV: moves and countermoves. *Curr. HIV/AIDS Rep.* 9:64–72. http://dx.doi.org/10.1007/s11904-011-0105-5

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. 2009. 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

Ho, D.D., J.C. Kaplan, I.E. Rackauskas, and M.E. Gurney. 1988. Second conserved domain of gp120 is important for HIV infectivity and antibody neutralization. *Science*. 239:1021–1023. http://dx.doi.org/10.1126/science.2830667

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

Jain, S., and K.L. Rosenthal. 2011. The gp41 epitope, QARVLAVERY, is highly conserved and a potent inducer of IgA that neutralizes HIV-1 and inhibits viral transcytosis. *Mucosal Immunol.* 4:539–553. http://dx.doi.org/10.1038/mi.2011.21

Johnson, P.R., B.C. Schnepp, J. Zhang, M.J. Connell, S.M. Greene, E. Yuste, R.C. Desrosiers, and K.R. Clark. 2009. Vector-mediated gene transfer engenders long-lived neutralizing activity and protection against SIV infection in monkeys. *Nat. Med.* 15:901–906. http://dx.doi.org/10.1038/nm.1967

Joyce, J.G., I.J. Krauss, H.C. Song, D.W. Opalka, K.M. Grimm, D.D. Nahas, M.T. Esser, R. Hrin, M. Feng, V.Y. Dudkin, et al. 2008. An oligosaccharide-based HIV-1 2G12 mimotope vaccine induces carbohydrate-specific antibodies that fail to neutralize HIV-1 virions. *Proc. Natl. Acad. Sci. USA*. 105:15684–15689. http://dx.doi.org/10.1073/pnas.0807837105

Kim, M., Z.S. Qiao, D.C. Montefiori, B.F. Haynes, E.L. Reinherz, and H.X. Liao. 2005. Comparison of HIV Type 1 ADA gp120 monomers versus gp140 trimers as immunogens for the induction of neutralizing antibodies. *AIDS Res. Hum. Retroviruses*. 21:58–67. http://dx.doi.org/10.1089/aid.2005.21.58

Klasse, P.J., R.W. Sanders, A. Cerutti, and J.P. Moore. 2012. How can HIV-type-1-Env immunogenicity be improved to facilitate antibody-based vaccine development? *AIDS Res. Hum. Retroviruses*. 28:1–15. http://dx.doi.org/10.1089/aid.2011.0053

Klein, F., C. Gaebler, H. Mouquet, D.N. Sather, C. Lehmann, J.F. Scheid, Z. Kraft, Y. Liu, J. Pietzsch, A. Hurley, et al. 2012a. Broad neutralization by a combination of antibodies recognizing the CD4 binding site and a new conformational epitope on the HIV-1 envelope protein. *J. Exp. Med.* 209:1469–1479. http://dx.doi.org/10.1084/jem.20120423

Klein, F., A. Halper-Stromberg, J.A. Horwitz, H. Gruell, J.F. Scheid, S. Bournazos, H. Mouquet, L.A. Spatz, R. Diskin, A. Abadir, et al. 2012b. HIV therapy by a combination of broadly neutralizing antibodies in humanized mice. *Nature*. 492:118–122.

Kovacs, J.M., J.P. Nkolola, H. Peng, A. Cheung, J. Perry, C.A. Miller, M.S. Seaman, D.H. Barouch, and B. Chen. 2012. HIV-1 envelope trimer elicits more potent neutralizing antibody responses than monomeric gp120. *Proc. Natl. Acad. Sci. USA*. 109:12111–12116. http://dx.doi.org/10.1073/pnas.1204533109

Kwong, P.D., J.R. Mascola, and G.J. Nabel. 2012. The changing face of HIV vaccine research. *J. Int. AIDS Soc.* 15:17407.

Labrijn, A.F., P. Poignard, A. Raja, M.B. Zwick, K. Delgado, M. Franti, J. Binley, V. Vivona, C. Grundner, C.C. Huang, et al. 2003. Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1. *J. Virol.* 77:10557–10565. http://dx.doi.org/10.1128/JVI.77.19.10557-10565.2003

Lagenaur, L.A., V.A. Villarroel, V. Bundoc, B. Dey, and E.A. Berger. 2010. sCD4-17b bifunctional protein: extremely broad and potent neutralization of HIV-1 Env pseudotyped viruses from genetically diverse primary isolates. *Retrovirology*. 7:11. http://dx.doi.org/10.1186/1742-4690-7-11

Lai, R.P., M.S. Seaman, P. Tonks, F. Wegmann, D.J. Seilly, S.D. Frost, C.C. LaBranche, D.C. Montefiori, A.K. Dey, I.K. Srivastava, et al. 2012. Mixed adjuvant formulations reveal a new combination that elicit antibody response comparable to Freund's adjuvants. *PLoS ONE*. 7:e35083. http://dx.doi.org/10.1371/journal.pone.0035083

Lakhashe, S.K., V. Velu, G. Sciaranghella, N.B. Siddappa, J.M. Dipasquale, G. Hemashettar, J.K. Yoon, R.A. Rasmussen, F. Yang, S.J. Lee, et al. 2011. Prime-boost vaccination with heterologous live vectors encoding SIV gag and multimeric HIV-1 gp160 protein: efficacy against repeated mucosal R5 clade C SHIV challenges. *Vaccine*. 29:5611–5622. http://dx.doi.org/10.1016/j.vaccine.2011.06.017

Law, M., R.M. Cardoso, I.A. Wilson, and D.R. Burton. 2007. Antigenic and immunogenic study of membrane-proximal external region-grafted gp120 antigens by a DNA prime-protein boost immunization strategy. *J. Virol*. 81:4272–4285. http://dx.doi.org/10.1128/JVI.02536-06

Lewis, A.D., R. Chen, D.C. Montefiori, P.R. Johnson, and K.R. Clark. 2002. Generation of neutralizing activity against human immunodeficiency virus type 1 in serum by antibody gene transfer. *J. Virol*. 76:8769–8775. http://dx.doi.org/10.1128/JVI.76.17.8769-8775.2002

Li, Y., B. Cleveland, I. Klots, B. Travis, B.A. Richardson, D. Anderson, D. Montefiori, P. Polacino, and S.L. Hu. 2008. Removal of a single N-linked glycan in human immunodeficiency virus type 1 gp120 results in an enhanced ability to induce neutralizing antibody responses. *J. Virol*. 82:638–651. http://dx.doi.org/10.1128/JVI.01691-07

Li, Y., S. O'Dell, L.M. Walker, X. Wu, J. Guenaga, Y. Feng, S.D. Schmidt, K. McKee, M.K. Louder, J.E. Ledgerwood, et al. 2011. Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. *J. Virol*. 85:8954–8967. http://dx.doi.org/10.1128/JVI.00754-11

Lian, Y., I. Srivastava, V.R. Gómez-Román, J. Zur Megede, Y. Sun, E. Kan, S. Hilt, S. Engelbrecht, S. Himathongkham, P.A. Luciw, et al. 2005. Evaluation of envelope vaccines derived from the South African subtype C human immunodeficiency virus type 1 TV1 strain. *J. Virol*. 79:13338–13349. http://dx.doi.org/10.1128/JVI.79.21.13338-13349.2005

Lorin, C., L. Mollet, F. Delebecque, C. Combredet, B. Hurtrel, P. Charneau, M. Brahic, and F. Tangy. 2004. A single injection of recombinant measles virus vaccines expressing human immunodeficiency virus (HIV) type 1 clade B envelope glycoproteins induces neutralizing antibodies and cellular immune responses to HIV. *J. Virol*. 78:146–157. http://dx.doi.org/10.1128/JVI.78.1.146-157.2004

Lubeck, M.D., R. Natuk, M. Myagkikh, N. Kalyan, K. Aldrich, F. Sinangil, S. Alipanah, S.C. Murthy, P.K. Chanda, S.M. Nigida Jr., et al. 1997. Long-term protection of chimpanzees against high-dose HIV-1 challenge induced by immunization. *Nat. Med*. 3:651–658. http://dx.doi.org/10.1038/nm0697-651

Lynch, R.M., L. Tran, M.K. Louder, S.D. Schmidt, M. Cohen, R. Dersimonian, Z. Euler, E.S. Gray, S. Abdool Karim, J. Kirchherr, et al; CHAVI 001 Clinical Team Members. 2012. The development of CD4 binding site antibodies during HIV-1 infection. *J. Virol*. 86:7588–7595. http://dx.doi.org/10.1128/JVI.00734-12

Ma, B.J., S.M. Alam, E.P. Go, X. Lu, H. Desaire, G.D. Tomaras, C. Bowman, L.L. Sutherland, R.M. Scearce, S. Santra, et al. 2011. Envelope deglycosylation enhances antigenicity of HIV-1 gp41 epitopes for both broad neutralizing antibodies and their unmutated ancestor antibodies. *PLoS Pathog*. 7:e1002200. http://dx.doi.org/10.1371/journal.ppat.1002200

Malherbe, D.C., N.A. Doria-Rose, L. Misher, T. Beckett, W.B. Puryear, J.T. Schuman, Z. Kraft, J. O'Malley, M. Mori, I. Srivastava, et al. 2011. Sequential immunization with a subtype B HIV-1 envelope quasispecies partially mimics the in vivo development of neutralizing antibodies. *J. Virol*. 85:5262–5274. http://dx.doi.org/10.1128/JVI.02419-10

Mascola, J.R., S.W. Snyder, O.S. Weislow, S.M. Belay, R.B. Belshe, D.H. Schwartz, M.L. Clements, R. Dolin, B.S. Graham, G.J. Gorse, et al; The National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. 1996. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1. *J. Infect. Dis*. 173:340–348. http://dx.doi.org/10.1093/infdis/173.2.340

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

Mascola, J.R., A. Sambor, K. Beaudry, S. Santra, B. Welcher, M.K. Louder, T.C. Vancott, Y. Huang, B.K. Chakrabarti, W.P. Kong, et al. 2005. Neutralizing antibodies elicited by immunization of monkeys with DNA plasmids and recombinant adenoviral vectors expressing human immunodeficiency virus type 1 proteins. *J. Virol*. 79:771–779. http://dx.doi.org/10.1128/JVI.79.2.771-779.2005

McBurney, S.P., K.R. Young, and T.M. Ross. 2007. Membrane embedded HIV-1 envelope on the surface of a virus-like particle elicits broader immune responses than soluble envelopes. *Virology*. 358:334–346. http://dx.doi.org/10.1016/j.virol.2006.08.032

McCoy, L.E., A.F. Quigley, N.M. Strokappe, B. Bulmer-Thomas, M.S. Seaman, D. Mortier, L. Rutten, N. Chander, C.J. Edwards, R. Ketteler, et al. 2012. Potent and broad neutralization of HIV-1 by a llama antibody elicited by immunization. *J. Exp. Med*. 209:1091–1103. http://dx.doi.org/10.1084/jem.20112655

McElrath, M.J., and B.F. Haynes. 2010. Induction of immunity to human immunodeficiency virus type-1 by vaccination. *Immunity*. 33:542–554. http://dx.doi.org/10.1016/j.immuni.2010.09.011

McLellan, J.S., M. Pancera, C. Carrico, J. Gorman, J.P. Julien, R. Khayat, R. Louder, R. Pejchal, M. Sastry, K. Dai, et al. 2011. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. *Nature*. 480:336–343. http://dx.doi.org/10.1038/nature10696

McMichael, A.J., and B.F. Haynes. 2012. Lessons learned from HIV-1 vaccine trials: new priorities and directions. *Nat. Immunol*. 13:423–427. http://dx.doi.org/10.1038/ni.2264

Mehandru, S., T. Wrin, J. Galovich, G. Stiegler, B. Vcelar, A. Hurley, C. Hogan, S. Vasan, H. Katinger, C.J. Petropoulos, and M. Markowitz. 2004. Neutralization profiles of newly transmitted human immunodeficiency virus type 1 by monoclonal antibodies 2G12, 2F5, and 4E10. *J. Virol*. 78:14039–14042. http://dx.doi.org/10.1128/JVI.78.24.14039-14042.2004

Moir, S., A. Malaspina, and A.S. Fauci. 2011. Prospects for an HIV vaccine: leading B cells down the right path. *Nat. Struct. Mol. Biol*. 18:1317–1321. http://dx.doi.org/10.1038/nsmb.2194

Moldt, B., M. Shibata-Koyama, E.G. Rakasz, N. Schultz, Y. Kanda, D.C. Dunlop, S.L. Finstad, C. Jin, G. Landucci, M.D. Alpert, et al. 2012. A nonfucosylated variant of the anti-HIV-1 monoclonal antibody b12 has enhanced FcγRIIIa-mediated antiviral activity in vitro but does not improve protection against mucosal SHIV challenge in macaques. *J. Virol*. 86:6189–6196. http://dx.doi.org/10.1128/JVI.00491-12

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

Moulard, M., S.K. Phogat, Y. Shu, A.F. Labrijn, X. Xiao, J.M. Binley, M.Y. Zhang, I.A. Sidorov, C.C. Broder, J. Robinson, et al. 2002. Broadly cross-reactive HIV-1-neutralizing human monoclonal Fab selected for binding to gp120-CD4-CCR5 complexes. *Proc. Natl. Acad. Sci. USA*. 99:6913–6918. http://dx.doi.org/10.1073/pnas.102562599

Murphy, K. 2011. The distribution and functions of immunoglobulin isotypes. *In* Janeway's Immunobiology. 8th edition. Garland Science, New York. 408–417.

Nara, P.L., W.G. Robey, S.W. Pyle, W.C. Hatch, N.M. Dunlop, J.W. Bess Jr., J.C. Kelliher, L.O. Arthur, and P.J. Fischinger. 1988. Purified envelope glycoproteins from human immunodeficiency virus type 1 variants induce individual, type-specific neutralizing antibodies. *J. Virol*. 62:2622–2628.

Ndung'u, T., and R.A. Weiss. 2012. On HIV diversity. *AIDS*. 26:1255–1260. http://dx.doi.org/10.1097/QAD.0b013e32835461b5

Nelson, J.D., H. Kinkead, F.M. Brunel, D. Leaman, R. Jensen, J.M. Louis, T. Maruyama, C.A. Bewley, K. Bowdish, G.M. Clore, et al. 2008. Antibody elicited against the gp41 N-heptad repeat (NHR) coiled-coil can neutralize HIV-1 with modest potency but non-neutralizing antibodies also bind to NHR mimetics. *Virology*. 377:170–183. http://dx.doi.org/10.1016/j.virol.2008.04.005

Nishimura, Y., T. Igarashi, N.L. Haigwood, R. Sadjadpour, O.K. Donau, C. Buckler, R.J. Plishka, A. Buckler-White, and M.A. Martin. 2003. Transfer of neutralizing IgG to macaques 6 h but not 24 h after SHIV infection confers sterilizing protection: implications for HIV-1 vaccine development. *Proc. Natl. Acad. Sci. USA*. 100:15131–15136. http://dx.doi.org/10.1073/pnas.2436476100

Nishiyama, Y., S. Planque, Y. Mitsuda, G. Nitti, H. Taguchi, L. Jin, J. Symersky, S. Boivin, M. Sienczyk, M. Salas, et al. 2009. Toward effective HIV vaccination: induction of binary epitope reactive antibodies with broad HIV neutralizing activity. *J. Biol. Chem*. 284:30627–30642. http://dx.doi.org/10.1074/jbc.M109.032185

Pantaleo, G., M. Esteban, B. Jacobs, and J. Tartaglia. 2010. Poxvirus vector-based HIV vaccines. *Curr Opin HIV AIDS*. 5:391–396. http://dx.doi.org/10.1097/COH.0b013e32833d1e87

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

Pejchal, R., and I.A. Wilson. 2010. Structure-based vaccine design in HIV: blind men and the elephant? *Curr. Pharm. Des*. 16:3744–3753. http://dx.doi.org/10.2174/138161210794079173

Pejchal, R., K.J. Doores, L.M. Walker, R. Khayat, P.S. Huang, S.K. Wang, R.L. Stanfield, J.P. Julien, A. Ramos, M. Crispin, et al. 2011. A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. *Science*. 334:1097–1103. http://dx.doi.org/10.1126/science.1213256

Picker, L.J., S.G. Hansen, and J.D. Lifson. 2012. New paradigms for HIV/AIDS vaccine development. *Annu. Rev. Med*. 63:95–111. http://dx.doi.org/10.1146/annurev-med-042010-085643

Pincus, S.H., K.G. Messer, R. Cole, R. Ireland, T.C. VanCott, A. Pinter, D.H. Schwartz, B.S. Graham, and G.J. Gorse. 1997. Vaccine-specific antibody responses induced by HIV-1 envelope subunit vaccines. *J. Immunol*. 158:3511–3520.

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

Plotkin, S.A. 2008. Vaccines: correlates of vaccine-induced immunity. *Clin. Infect. Dis*. 47:401–409. http://dx.doi.org/10.1086/589862

Poignard, P., R. Sabbe, G.R. Picchio, M. Wang, R.J. Gulizia, H. Katinger, P.W. Parren, D.E. Mosier, and D.R. Burton. 1999. Neutralizing antibodies have limited effects on the control of established HIV-1 infection in vivo. *Immunity*. 10:431–438. http://dx.doi.org/10.1016/S1074-7613(00)80043-6

Poon, B., J.F. Hsu, V. Gudeman, I.S. Chen, and K. Grovit-Ferbas. 2005. Formaldehyde-treated, heat-inactivated virions with increased human immunodeficiency virus type 1 env can be used to induce high-titer neutralizing antibody responses. *J. Virol*. 79:10210–10217. http://dx.doi.org/10.1128/JVI.79.16.10210-10217.2005

Quinnan, G.V. Jr., X.F. Yu, M.G. Lewis, P.F. Zhang, G. Sutter, P. Silvera, M. Dong, A. Choudhary, P.T. Sarkis, P. Bouma, et al. 2005. Protection of rhesus monkeys against infection with minimally pathogenic simian-human immunodeficiency virus: correlations with neutralizing antibodies and cytotoxic T cells. *J. Virol*. 79:3358–3369. http://dx.doi.org/10.1128/JVI.79.6.3358-3369.2005

Radaelli, A., O. Bonduelle, P. Beggio, B. Mahe, E. Pozzi, V. Elli, M. Paganini, C. Zanotto, C. De Giuli Morghen, and B. Combadière. 2007. Prime-boost immunization with DNA, recombinant fowlpox virus and VLP(SHIV) elicit both neutralizing antibodies and IFNgamma-producing T cells against the HIV-envelope protein in mice that control env-bearing tumour cells. *Vaccine*. 25:2128–2138. http://dx.doi.org/10.1016/j.vaccine.2006.11.009

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

Robert-Guroff, M., M. Brown, and R.C. Gallo. 1985. HTLV-III-neutralizing antibodies in patients with AIDS and AIDS-related complex. *Nature*. 316:72–74. http://dx.doi.org/10.1038/316072a0

Robey, W.G., L.O. Arthur, T.J. Matthews, A. Langlois, T.D. Copeland, N.W. Lerche, S. Oroszlan, D.P. Bolognesi, R.V. Gilden, and P.J. Fischinger. 1986. Prospect for prevention of human immunodeficiency virus infection: purified 120-kDa envelope glycoprotein induces neutralizing antibody. *Proc. Natl. Acad. Sci. USA*. 83:7023–7027. http://dx.doi.org/10.1073/pnas.83.18.7023

Rollman, E., J. Hinkula, J. Arteaga, B. Zuber, A. Kjerrström, M. Liu, B. Wahren, and K. Ljungberg. 2004. Multi-subtype gp160 DNA immunization induces broadly neutralizing anti-HIV antibodies. *Gene Ther*. 11:1146–1154. http://dx.doi.org/10.1038/sj.gt.3302275

Rosen, O., J. Chill, M. Sharon, N. Kessler, B. Mester, S. Zolla-Pazner, and J. Anglister. 2005. Induced fit in HIV-neutralizing antibody complexes: evidence for alternative conformations of the gp120 V3 loop and the molecular basis for broad neutralization. *Biochemistry*. 44:7250–7258. http://dx.doi.org/10.1021/bi047387t

Russell, N.D., B.S. Graham, M.C. Keefer, M.J. McElrath, S.G. Self, K.J. Weinhold, D.C. Montefiori, G. Ferrari, H. Horton, G.D. Tomaras, et al; National Institute of Allergy and Infectious Diseases HIV Vaccine Trials Network. 2007. Phase 2 study of an HIV-1 canarypox vaccine (vCP1452) alone and in combination with rgp120: negative results fail to trigger a phase 3 correlates trial. *J. Acquir. Immune Defic. Syndr.* 44:203–212. http://dx.doi.org/10.1097/01.qai.0000248356.48501.ff

Sattentau, Q.J. 2011. Vaccinology: A sweet cleft in HIV's armour. *Nature*. 480:324–325. http://dx.doi.org/10.1038/480324a

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. 2009. 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, 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

Schell, J., N.F. Rose, N. Fazo, P.A. Marx, M. Hunter, E. Ramsburg, D. Montefiori, P. Earl, B. Moss, and J.K. Rose. 2009. Long-term vaccine protection from AIDS and clearance of viral DNA following SHIV89.6P challenge. *Vaccine*. 27:979–986. http://dx.doi.org/10.1016/j.vaccine.2008.12.017

Sheppard, N., and Q. Sattentau. 2005. The prospects for vaccines against HIV-1: more than a field of long-term nonprogression? *Expert Rev. Mol. Med*. 7:1–21. http://dx.doi.org/10.1017/S1462399405008859

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

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

Sreepian, A., J. Permmongkol, W. Kantakamalakul, S. Siritantikorn, N. Tanlieng, and R. Sutthent. 2009. HIV-1 neutralization by monoclonal antibody against conserved region 2 and patterns of epitope exposure on

the surface of native viruses. *J. Immune Based Ther. Vaccines*. 7:5. http://dx.doi.org/10.1186/1476-8518-7-5

Stanfield, R.L., M.K. Gorny, C. Williams, S. Zolla-Pazner, and I.A. Wilson. 2004. Structural rationale for the broad neutralization of HIV-1 by human monoclonal antibody 447-52D. *Structure*. 12:193–204.

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

Strokappe, N., A. Szynol, M. Aasa-Chapman, A. Gorlani, A. Forsman Quigley, D.L. Hulsik, L. Chen, R. Weiss, H. de Haard, and T. Verrips. 2012. Llama antibody fragments recognizing various epitopes of the CD4bs neutralize a broad range of HIV-1 subtypes A, B and C. *PLoS ONE*. 7:e33298. http://dx.doi.org/10.1371/journal.pone.0033298

Sundling, C., M.N. Forsell, S. O'Dell, Y. Feng, B. Chakrabarti, S.S. Rao, K. Loré, J.R. Mascola, R.T. Wyatt, I. Douagi, and G.B. Karlsson Hedestam. 2010. Soluble HIV-1 Env trimers in adjuvant elicit potent and diverse functional B cell responses in primates. *J. Exp. Med.* 207:2003–2017. http://dx.doi.org/10.1084/jem.20100025

Tiller, T., E. Meffre, S. Yurasov, M. Tsuiji, M.C. Nussenzweig, and H. Wardemann. 2008. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J. Immunol. Methods*. 329:112–124. http://dx.doi.org/10.1016/j.jim.2007.09.017

Traina-Dorge, V., B. Pahar, P. Marx, P. Kissinger, D. Montefiori, Y. Ou, and W.L. Gray. 2010. Recombinant varicella vaccines induce neutralizing antibodies and cellular immune responses to SIV and reduce viral loads in immunized rhesus macaques. *Vaccine*. 28:6483–6490. http://dx.doi.org/10.1016/j.vaccine.2010.07.018

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

Tudor, D., M. Derrien, L. Diomede, A.S. Drillet, M. Houimel, C. Moog, J.M. Reynes, L. Lopalco, and M. Bomsel. 2009. HIV-1 gp41-specific monoclonal mucosal IgAs derived from highly exposed but IgG-seronegative individuals block HIV-1 epithelial transcytosis and neutralize CD4(+) cell infection: an IgA gene and functional analysis. *Mucosal Immunol.* 2:412–426. http://dx.doi.org/10.1038/mi.2009.89

Vaine, M., S. Wang, Q. Liu, J. Arthos, D. Montefiori, P. Goepfert, M.J. McElrath, and S. Lu. 2010. Profiles of human serum antibody responses elicited by three leading HIV vaccines focusing on the induction of Env-specific antibodies. *PLoS ONE*. 5:e13916. http://dx.doi.org/10.1371/journal.pone.0013916

VanCott, T.C., J.R. Mascola, R.W. Kaminski, V. Kalyanaraman, P.L. Hallberg, P.R. Burnett, J.T. Ulrich, D.J. Rechtman, and D.L. Birx. 1997. Antibodies with specificity to native gp120 and neutralization activity against primary human immunodeficiency virus type 1 isolates elicited by immunization with oligomeric gp160. *J. Virol.* 71:4319–4330.

Veazey, R.S., R.J. Shattock, M. Pope, J.C. Kirijan, J. Jones, Q. Hu, T. Ketas, P.A. Marx, P.J. Klasse, D.R. Burton, and J.P. Moore. 2003. Prevention of virus transmission to macaque monkeys by a vaginally applied monoclonal antibody to HIV-1 gp120. *Nat. Med.* 9:343–346. http://dx.doi.org/10.1038/nm833

Verkoczy, L., G. Kelsoe, M.A. Moody, and B.F. Haynes. 2011. Role of immune mechanisms in induction of HIV-1 broadly neutralizing antibodies. *Curr. Opin. Immunol.* 23:383–390. http://dx.doi.org/10.1016/j.coi.2011.04.003

Verrier, F., S. Burda, R. Belshe, A.M. Duliege, J.L. Excler, M. Klein, and S. Zolla-Pazner. 2000. A human immunodeficiency virus prime-boost immunization regimen in humans induces antibodies that show interclade cross-reactivity and neutralize several X4-, R5-, and dualtropic clade B and C primary isolates. *J. Virol.* 74:10025–10033. http://dx.doi.org/10.1128/JVI.74.21.10025-10033.2000

Walker, L.M., and D.R. Burton. 2010. Rational antibody-based HIV-1 vaccine design: current approaches and future directions. *Curr. Opin. Immunol.* 22:358–366. http://dx.doi.org/10.1016/j.coi.2010.02.012

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

Watkins, J.D., N.B. Siddappa, S.K. Lakhashe, M. Humbert, A. Sholukh, G. Hemashettar, Y.L. Wong, J.K. Yoon, W. Wang, F.J. Novembre, et al. 2011. An anti-HIV-1 V3 loop antibody fully protects cross-clade and elicits T-cell immunity in macaques mucosally challenged with an R5 clade C SHIV. *PLoS ONE*. 6:e18207. http://dx.doi.org/10.1371/journal.pone.0018207

Weiss, R.A., P.R. Clapham, R. Cheingsong-Popov, A.G. Dalgleish, C.A. Carne, I.V. Weller, and R.S. Tedder. 1985. Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. *Nature*. 316:69–72. http://dx.doi.org/10.1038/316069a0

Weiss, R.A., P.R. Clapham, J.N. Weber, A.G. Dalgleish, L.A. Lasky, and P.W. Berman. 1986. Variable and conserved neutralization antigens of human immunodeficiency virus. *Nature*. 324:572–575. http://dx.doi.org/10.1038/324572a0

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

Willey, S., and M.M. Aasa-Chapman. 2008. Humoral immunity to HIV-1: neutralisation and antibody effector functions. *Trends Microbiol.* 16:596–604. http://dx.doi.org/10.1016/j.tim.2008.08.008

Wilson, C., M.S. Reitz Jr., K. Aldrich, P.J. Klasse, J. Blomberg, R.C. Gallo, and M. Robert-Guroff. 1990. The site of an immune-selected point mutation in the transmembrane protein of human immunodeficiency virus type 1 does not constitute the neutralization epitope. *J. Virol.* 64:3240–3248.

Wright, P.F., J. Mestecky, M.J. McElrath, M.C. Keefer, G.J. Gorse, P.A. Goepfert, Z. Moldoveanu, D. Schwartz, P.W. Spearman, R. El Habib, et al; National Institutes of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. 2004. Comparison of systemic and mucosal delivery of 2 canarypox virus vaccines expressing either HIV-1 genes or the gene for rabies virus G protein. *J. Infect. Dis.* 189:1221–1231. http://dx.doi.org/10.1086/382088

Wrin, T., and J.H. Nunberg. 1994. HIV-1MN recombinant gp120 vaccine serum, which fails to neutralize primary isolates of HIV-1, does not antagonize neutralization by antibodies from infected individuals. *AIDS*. 8:1622–1623. http://dx.doi.org/10.1097/00002030-199411000-00017

Wrin, T., T.P. Loh, J.C. Vennari, H. Schuitemaker, and J.H. Nunberg. 1995. Adaptation to persistent growth in the H9 cell line renders a primary isolate of human immunodeficiency virus type 1 sensitive to neutralization by vaccine sera. *J. Virol.* 69:39–48.

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

Yang, X., J. Lee, E.M. Mahony, P.D. Kwong, R. Wyatt, and J. Sodroski. 2002. Highly stable trimers formed by human immunodeficiency virus type 1 envelope glycoproteins fused with the trimeric motif of T4 bacteriophage fibritin. *J. Virol.* 76:4634–4642. http://dx.doi.org/10.1128/JVI.76.9.4634-4642.2002

Zhang, P.F., F. Cham, M. Dong, A. Choudhary, P. Bouma, Z. Zhang, Y. Shao, Y.R. Feng, L. Wang, N. Mathy, et al. 2007. Extensively cross-reactive anti-HIV-1 neutralizing antibodies induced by gp140 immunization. *Proc. Natl. Acad. Sci. USA*. 104:10193–10198. http://dx.doi.org/10.1073/pnas.0608635104

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, M., I. Kostoula, B. Brill, E. Panou, M. Sakarellos-Daitsiotis, and U. Dietrich. 2012. Prime boost vaccination approaches with different conjugates of a new HIV-1 gp41 epitope encompassing the membrane proximal external region induce neutralizing antibodies in mice. *Vaccine*. 30:1911–1916. http://dx.doi.org/10.1016/j.vaccine.2012.01.026

Zolla-Pazner, S., M. Lubeck, S. Xu, S. Burda, R.J. Natuk, F. Sinangil, K. Steimer, R.C. Gallo, J.W. Eichberg, T. Matthews, and M. Robert-Guroff. 1998a. Induction of neutralizing antibodies to T-cell line-adapted and primary human immunodeficiency virus type 1 isolates with a prime-boost vaccine regimen in chimpanzees. *J. Virol*. 72:1052–1059.

Zolla-Pazner, S., S. Xu, S. Burda, A.M. Duliege, J.L. Excler, and M.L. Clements-Mann. 1998b. Neutralization of syncytium-inducing primary isolates by sera from human immunodeficiency virus (HIV)-uninfected recipients of candidate HIV vaccines. *J. Infect. Dis*. 178:1502–1506. http://dx.doi.org/10.1086/314452