Review

Broadly neutralizing antibodies against HIV-1: Templates for a vaccine

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Abstract

The need for an effective vaccine to prevent the global spread of human immunodeficiency virus type 1 (HIV-1) is well recognized. Passive immunization and challenge studies in non-human primates testify that broadly neutralizing antibodies (BrNAbs) can accomplish protection against infection. In recent years, the introduction of new techniques has facilitated the discovery of an unprecedented number of new human BrNAbs that target and delineate diverse conserved epitopes on the envelope glycoprotein spike (Env). The epitopes of these BrNAbs can serve as templates for immunogen design aimed to induce similar antibodies. Here we will review the characteristics of the different classes of BrNAbs and their target epitopes, as well as factors associated with their development and implications for vaccine design.

Introduction

The global successes with many antiviral vaccines raise the question why the development of an HIV-1 vaccine is so challenging. Many of the difficulties lie in the distinct properties of this virus compared with other viruses. Foremost among these is the enormous sequence diversity of HIV-1, which can be as high as...
35% for the envelope glycoproteins (Env) between viruses from different subtypes (Gaschen et al., 2002; Hemelaar et al., 2006; Spira et al., 2003; Taylor et al., 2008). The relative inaccessibility of conserved domains on Env decreases the elicitation of protective antibodies with global coverage (Barouch, 2008; Walker and Burton, 2008). Moreover, the poor understanding of the immune responses that can control HIV-1 replication, for instance in elite controllers and high risk seronegative individuals, makes the development of vaccines that induce such immune responses rather difficult (Miura et al., 2009; Lederman et al., 2010).

It is assumed that a protective vaccine should elicit broadly neutralizing humoral immunity (Pantaleo and Koup, 2004; Walker and Burton, 2008), as passive transfer of broadly neutralizing antibodies (BrNAbs) can completely block infection by chimeric simian-human immunodeficiency virus (SHIV) in non-human primate studies (Baba et al., 2000; Hessell et al., 2009a, 2009b; Mascola et al., 1999, 2000; Parren et al., 2001; Shibata et al., 1999). Furthermore, passive transfer of BrNAbs delays HIV-1 rebound after cessation of antiretroviral therapy in humans (Trikola et al., 2005). Recent studies in humanized mice and non-human primates have further delineated the potential of BrNAbs by showing vaccine-like protection against HIV-1 infection using gene-based antibody delivery (Balazs et al., 2012; Berkhourt and Sanders, 2012). This review will focus on BrNAbs and how their characterization can guide the search for immunogens that elicit such BrNAbs and thus protect against infection.

**Humoral immunity in HIV-1 infection**

In order to better understand the immunogenicity of the HIV-1 Env and the host immune response against it, humoral immunity in the natural course of infection has been studied extensively. The majority of HIV-1-infected individuals mount an HIV-1-specific neutralizing humoral immune response within weeks to months after primary infection (Aasa-Chapman et al., 2004; Tomaras et al., 2008). This response is usually strain-specific as neutralizing activity is generally restricted to the autologous virus variants and mainly directed against the variable domains of Env (Gray et al., 2007; Li et al., 2006; Richman et al., 2003). The emergence of neutralizing antibodies (NAbs) is a burden to the virus and it drives the continuous evolution of HIV-1 Env. Longitudinal studies have shown that HIV-1 rapidly and repeatedly escapes from the NAb response mounted during HIV-1 infection (Bunnik et al., 2008; Deeks et al., 2006; Frost et al., 2005; Gray et al., 2007; Mahalanabis et al., 2009; Moore et al., 2009; Richman et al., 2003; Rong et al., 2009; Wei et al., 2003). As a consequence of this selection, the majority of the virus population in an infected individual is only weakly, if at all, neutralized by the contemporaneous antibody repertoire (Frost et al., 2005; Richman et al., 2003; Wei et al., 2003).

With time, as the virus population diversifies and the immune response matures, neutralization can also be detected against heterologous HIV-1 variants (Aasa-Chapman et al., 2004; Gray et al., 2007; Richman et al., 2003; Wei et al., 2003). During the first three years of infection approximately 20–30% of HIV-1 infected individuals develop broadly neutralizing activity with the ability to neutralize viruses from different subtypes (Binley et al., 2004, 2008; Simek et al., 2009; van Gils et al., 2009). In addition, about 1% of HIV-infected individuals, termed elite neutralizers, develop an HIV-1 specific neutralizing activity with remarkable potency and breadth (Euler et al., 2010; Simek et al., 2009). Unfortunately HIV-1-infected individuals do not benefit from broad or elite neutralizing antibody responses (Doria-Rose et al., 2009; Euler et al., 2010; van Gils et al., 2009). The lack of correlation between the presence of broadly neutralizing immunity and disease progression is explained in part by the rapid virus escape, but also by fading humoral responses during disease progression. The waning humoral immunity is exemplified by a decrease in autologous neutralizing antibody responses over time, probably as a result of the depletion of CD4+ T-cell help during chronic infection (Bunnik et al., 2008; Euler et al., 2010; Frost et al., 2005; Richman et al., 2003; Wei et al., 2003), as well as by the reduced responses of HIV-1 infected individuals to vaccination against other pathogens (Madhi et al., 2007; Veit et al., 2009).

**Factors associated with the development of broadly neutralizing antibodies**

To support HIV-1 vaccine development, more insight is needed into the host and viral factors that are associated with the ability of the host to elicit a BrNAb response, and how such a response evolves over time. The development of humoral responses in natural infection can provide clues as to what shapes a BrNAb response against HIV-1.

**High antigenic load**

Several studies have pointed out that the prevalence of broadly neutralizing activity in serum from elite controllers and viremic controllers is much lower as compared to typical progressors (Doria-Rose et al., 2009; Euler et al., 2010; Pereyra et al., 2008; Sather et al., 2009; Scheid et al., 2009). Together with a correlation between the development of broadly neutralizing activity and a high plasma viral RNA load, this suggests that the development of potently neutralizing humoral immunity apparently requires exposure to a sufficiently high amount of Env antigen.

**High viral diversity**

It has also been proposed that a higher degree of viral diversity increases the development of BrNAbs. Dual infection and co-infection can lead to more efficient induction of BrNAb, in particular when the superinfecting strain is from a different subtype (Cortez et al., 2012; Moore et al., 2011; Powell et al., 2010). In addition it has been shown that the viral diversity and divergence in individuals with broadly neutralizing activity is higher compared to individuals without broadly neutralizing activity, with the highest diversity being observed in an elite neutralizer (Euler et al., 2012; Piantadosi et al., 2009). Collectively these data suggest that viral diversity contributes to the formation of BrNAbs.

**Prolonged antigenic stimulation**

The development of a potent broadly neutralizing humoral immune response usually takes at least 2 to 3 years and the breadth of neutralization is correlated with the time since infection (Sather et al., 2009; van Gils et al., 2009), suggesting that prolonged antigenic stimulation may be required for sufficient antibody affinity maturation (McMichael et al., 2010; Stamatos et al., 2009; Walker et al., 2010). Non-HIV-1 antibodies typically accumulate 5–15% changes in the heavy chain during affinity maturation, for example 3–12% in human anti influenza antibodies (Moody et al., 2011; Wrammert et al., 2011), however the known HIV-specific BrNAbs usually have a higher number of somatic mutations, ranging from 13% for b12 (Saphire et al., 2001), to 21% for PG16 (Walker et al., 2009), 32% for VRC01 (Wu et al., 2011), and 36% for NIH45–46 (Scheid et al., 2011) (Table 1). Furthermore, the heavy chain third complementary-determining
region (HCDR3) of HIV-1 specific BrNAbs is often long, up to 30 amino acids for PG9 (Table 1) (Prabakaran et al., 2012), while the average HCDR3 of human antibodies is 14 amino acids (Briney et al., 2012). Interestingly, the predicted unmutated germline ancestors of most BrNAbs have little or no reactivity with standard HIV-1 glycoproteins (Bonsignori et al., 2011; Mouquet et al., 2010; Pancera et al., 2010b; Prabakaran et al., 2012; Xiao et al., 2009), although a recent study revealed that the predicted unmutated germline ancestors of BrNAbs are capable of binding the autologous founder virus (Haynes, 2012).

### Polyreactivity

A remarkable characteristic of a subset of BrNAbs is their polyreactivity (Table 1) (Haynes et al., 2005; Mouquet et al., 2010), which has been suggested to facilitate bivalent heteroligation involving one high-affinity anti-HIV-1 binding site and a second low-affinity binding site for a self-antigen present on HIV-1 virions. Despite the low affinity of the polyreactive second binding site, this characteristic has been proposed to increase the apparent affinity of antibodies to HIV-1. The “raison d’être” of polyspecific NAbs may be the low viral spike density on the viral surface, which may increase the advantage of polyreactive antibodies over nonpolyreactive antibodies (Haynes et al., 2005; Mouquet et al., 2010). Although only 5% of the B cells in the mature naïve B cell compartment are poly- or selfreactive, about 75% of all HIV-1 specific memory B cells in infected individuals were shown to be polyreactive (Mouquet et al., 2010).

### Env characteristics

Env has developed multiple mechanisms to evade the host humoral immune response, including trimeric exclusion, occluded (co)receptor binding sites by conformational masking, (Chen et al., 2005; Decker et al., 2005; Edwards et al., 2001; Kwong et al., 2002; Labrijn et al., 2003; Wei et al., 2003) and the shielding of conserved epitopes by the highly variable flexible loops and a “glycan shield”, which collectively limit the induction of BrNAbs (Wei et al., 2003). It can be hypothesized that a founder virus with more exposed conserved epitopes might elicit NAbs with a greater breadth (Walker and Burton, 2010). For example, variation in the length of the variable loops and the number of glycans might affect the exposure of conserved epitopes on the trimeric Env spike, including the CD4 binding site (CD4BS) (Bunnik et al., 2010; Euler et al., 2011; van Gils et al., 2011). Indeed the development of broadly neutralizing serum activity was found to be associated with a shorter V1 domain and less glycosylation on early Env variants (van den Kerkhof et al., 2012).

### Epitopes targeted by broadly neutralizing antibodies

The BrNAbs present in human sera could provide the templates for vaccines aimed at inducing similar antibodies. In recent years, the introduction of new techniques, such as high throughput single cell BCR-amplification assays, standardized neutralization assays, and sophisticated baits (Scheid et al., 2011; Walker et al., 2011; Wu et al., 2010a), has facilitated the discovery of an unprecedented number of new BrNAbs that target and delineate diverse conserved epitopes on the Env spike. Interestingly, while glycan-dependent BrNAbs, directed to the defensive “glycan shield”, were long considered to be extremely rare—for a long time only the glycan-dependent 2G12 was known, many of the newly identified BrNAbs require glycans. This illustrates that the immunologically “silent face” located on the outer domain of
gp120 (Wyatt et al., 1998), may not be so silent after all and is in fact a valid vaccine target.

The Env spike consists of three gp120 subunits non-covalently associated with three gp41 subunits to form a trimer of heterodimers on the outside of the virion (Land and Braakman, 2001). Gp120 is composed of five conserved domains (C1–C5) that are interspersed with 5 variable domains (V1–V5) (Starchich et al., 1986). The conserved domains form a central core consisting of an inner domain, which interacts with gp41, and an outer domain, which is highly glycosylated. The variable domains can be highly diverse between patients as well as within patients, and form flexible loop structures that protrude from the gp120 core (Zhou et al., 2007). However, substructures within the variable loops can be highly conserved and serve as targets for BrNAbs.

Upon gp120 binding to the CD4 receptor, conformational changes occur that create and reveal the co-receptor binding site (Kwong et al., 1998; Liu et al., 2008). Following binding of gp120 to the co-receptor, usually CCR5 or CXCR4 (Bjorndal et al., 1997; Deng et al., 1996; Dragic et al., 1996), gp41 mediates the fusion of the viral and cellular membranes and insertion of viral core and the genomic material into the target cell (Liu et al., 2008; Pancera et al., 2010a). Many Env subdomains can be targets for strain-specific NAbs, however BrNAbs, by virtue of their breadth, target conserved domains of Env (Fig. 1).

**CD4-binding site (CD4BS)**

BrNAbs b12 (Saphire et al., 2001), VRC01 and its relatives VRC03, VRC06 and NIH45–46 (Scheid et al., 2011; Wu et al., 2010a; Zhou et al., 2010), PGV04 (also named VRC-PG04) and 3BNC117 and its relatives (Scheid et al., 2011; Wu et al., 2011) target epitopes that overlap with the CD4BS and block CD4 receptor binding. The CD4BS-directed antibodies mimic the binding of the CD4 receptor, but alanine scan studies showed that different CD4BS BrNAbs recognize the site in subtly different ways (Falkowska et al., 2012; West et al., 2012). In addition, while most CD4BS BrNAbs induce conformational changes in gp120 similar to those induced by CD4, PGV04 does not appear to do so (Falkowska et al., 2012). The breadth and potency of CD4BS BrNAbs has spurred the search for immunogens that effectively mimic the CD4BS to elicit anti-CD4BS antibodies (Azoitei et al., 2011; Wu et al., 2010b).

**Third variable domain (V3)**

Anti-V3 antibodies are among the first antibodies to be elicited in HIV-1 infection, however anti-V3 antibodies were believed to play a minimal role in neutralizing humoral immunity because of the high variability and the occlusion of the V3 loop within the trimeric Env spike of primary viruses (Davis et al., 2009; Krachmarov et al., 2006; Moore et al., 2008). The more recently discovered BrNAbs PGT121–PGT123 and PGT125–PGT131, the most potent ones of each class being PGT121 and PGT128 (Walker et al., 2011), target the conserved base of the V3 domain and unlike other anti-V3 antibodies are very broad and potent. These antibodies are glycan dependent. The crystal structure of PGT128 in complex with a gp120 outer domain shows that it binds to a short β-strand in the V3 domain, including residues 323–327, but also requires glycans attached to residues N301 and N332 (Pejchal et al., 2011).

**First and second variable domains (V1V2)**

Similar to the V3, epitopes in the V1V2 appear to be targeted early in infection. However, this domain is also highly variable and antibodies that target this domain are mostly type-specific. The main task of the V1V2 domain appears to be the shielding of neutralizing epitopes in other parts of Env and escape from neutralizing antibodies is often associated with amino acid substitutions, insertions, deletions, and/or changes in glycosylation in the V1V2 domain (Bontjer et al., 2009, 2010; Moore et al., 2009; Pinter et al., 2004; Rong et al., 2007, 2009; Sagar et al., 2006). However, the recent discovery of the BrNAbs PG9 and PG16 (Walker et al., 2009) together with the discovery of CH01–CH04 (Bonsignori et al., 2011) and PGT145 (Walker et al., 2011), which target a conserved subdomain within the V1V2 and possibly the V3, have highlighted the importance of this subdomain as a BrNAb target. These antibodies are exceptional because they prefer to recognize their epitope in the context of the quaternary structure of the Env trimer, possibly involving multiple V1V2 domains of the Env spike (Walker et al., 2009). The V1V2 domain

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**Fig. 1. Antigenic map of the Env trimer.** A side view (left) and a top view (right) of the Env trimer with the five BrNAb epitope clusters are shown. A model of glycosylated trimeric gp120 containing the V3 (Bontjer et al., 2010) was fitted into the EM structure of the native spike as previously reported (van Montfort et al., 2011). The gp120 core proteins are represented in ribbon diagrams, the glycans are represented in cyan. The five BrNAb epitope clusters are indicated on one protomer based on the criteria mentioned in the text. The figure was prepared using PyMol (Schrödinger, 2010).
is part of the trimer apex and is involved in oligomeric interactions (Center et al., 2000; Hu et al., 2011). The special ‘hammerhead’ structure of the HCDR3 gives the PG9 and PG16 antibodies the ability to bind both the two glycans attached to residues N156 (or N173 in some strains) and N160, as well as the underlying protein surface (residues 165–171), resulting in high breadth and potency (McLellan et al., 2011). BrNabs PGT145 and CH01–CH04 show similar binding properties as PG9 and PG16, however the exact binding properties of these antibodies need yet to be elucidated.

**Membrane proximal external region (MPER)**

The MPER of gp41 is highly conserved and it is shown to be an essential part of the cell fusion machinery. BrNabs 2F5 and 4E10 (Cardoso et al., 2005; Muster et al., 1993; Ofek et al., 2004; Pejchal et al., 2009; Stiegler et al., 2001; Zwick et al., 2005) have been shown to recognize linear epitopes in the MPER domain. The recently discovered antibody 10E8 is among the broadest and most potent antibodies thus far described and recognizes a structurally conserved site within the gp41 MPER (McLellan et al., 2011, Huang et al., 2012). BrNabs 2F5 and 4E10 are both polyreactive and react with membrane phospholipid cardiolipin, which is believed to increase their affinity to HIV-1, as the MPER domain is close to the viral membrane (Fig. 1) (Haynes et al., 2005). However polyreactivity is not a requirement for MPER-specific antibodies, as BrNab 10E8 is not polyreactive (Huang et al., 2012).

**Antigenic map of the Env trimer**

The five known BrNab epitope clusters allow us to create an approximation of the antigenic map of the Env trimer. The epitope clusters are located at different sites of the Env trimer (Fig. 1), but competition between BrNabs targeting different clusters has been observed, indicating that there is considerable overlap between them (Walker et al., 2009, 2011). These competition experiments have been performed using gp120, not trimeric Env, therefore they may underestimate the overlap because quaternary constraints on the Env trimer may increase the steric interference of BrNabs from different clusters. Since no atomic resolution structure of the Env trimer is available, and since some BrNab epitopes are still ill-defined, this antigenic map can only be an approximation, but it is useful to illustrate a few points.

First, the CD4BS represents the only protein-only BrNab epitope cluster on gp120 (1 in the Fig. 1) and it is recognized through a narrow glycan-free channel that is also used by the CD4 receptor. Apparently, the glycan shield covers the entire conserved protein core except for this site, as well as small protein fragments that can only be recognized in combination with glycans (McLellan et al., 2011; Pejchal et al., 2011). Soluble CD4 can compete for the binding to gp120 with some members of the PGT121–PGT123 and PGT125–PGT131 families, while b12 can compete with PGT135. Therefore, there must be some overlap between the CD4BS epitope cluster and the outer domain (II in the Fig. 1) and V3 base clusters (III in the Fig. 1). Second, the V3 base epitope cluster (III in the Fig. 1) is located near the apex of the trimer and can be competed off by 2G12, PG9 and soluble CD4, suggesting that it overlaps with all other BrNab epitope clusters on gp120. Third, the epitopes for the quaternary structure dependent V1V2 BrNabs (IV in the Fig. 1) are likely located at the apex of the trimer with BrNab PG9 competing strongly with PGT121–PGT123 and weakly with PGT125–PGT131. Yet PG9 cannot be replaced with outer domain or CD4BS directed antibodies, suggesting that it is distant from the outer domain cluster and CD4BS cluster. The outer domain antibodies 2G12 and PGT135 compete strongly with PGT121–PGT131, and b12, suggesting a high degree of overlap with the V3 base cluster, possibly centering on the glycan at N332, as well as with the CD4BS. Finally, one BrNab epitope cluster is situated in gp41. No competition between gp41-directed antibodies and gp120-directed BrNabs has been reported.

From the model it appears that the top of the trimer is mostly delineated by the known four epitope clusters on gp120 and the antigenic map begs the question whether truly novel and unidentified epitope clusters remain to be discovered. Arguably, the most obvious region of the trimer that could harbor unchartered BrNab epitopes could be the gp120-gp41 interface, if accessible at all. There is some evidence that such BrNab epitopes may indeed exist (Zhang et al., 2012).

**Directions for HIV-1 vaccine development**

Monomeric Env proteins such as those used in the AIDSVAX trials, are not able to induce BrNabs (Mascola and Montefiori, 2010; Pitisuttithum et al., 2006). Although most BrNab epitopes are present on gp120, they do not appear to be presented optimally to induce similar BrNabs. A first modest vaccine success was obtained with a pox virus prime, gp120 protein boost vaccine regimen in the RV144 Thai trial. Although no BrNabs were elicited, the vaccine induced some protection (31.2%), and the relative contributions of each vaccine component and the resulting immune responses need to be elucidated. V2-specific antibodies correlated with vaccine protection, but a causal connection has not been established yet (Haynes et al., 2012a; Montefiori et al., 2012; Rolland et al., 2012). Given that no BrNabs were induced, it has been hypothesized that other antibody functions, such as ADCC or ADVCI might have played a role in the achieved protection (Bonsignori et al., 2012; Forthal and Moog, 2009; Haynes et al., 2012a). Although the RV144 trial provides reason for hope, further improvement of Env-based vaccines is necessary to improve vaccine efficacy.

Thus, to date, no immunogen has been able to elicite protective neutralizing immunity. While it is not precisely known what level of BrAbs are required for protection against HIV-1 infection, recent work has shown a correlation between in vitro antibody neutralization capacity and the protective effect in non-human primates (Willey et al., 2010). In addition, examining the efficacy of low antibody titers against low dose repeated pathogenic simian-human immunodeficiency virus challenge in macaques indicates that high concentrations of antibodies may not be needed to provide protective benefit (Hessell et al., 2009a, 2009b).

The neutralizing antibody responses in natural HIV-1 infection may offer new clues for vaccine development and BrNabs
should serve as templates for immunogen design (Mascola and Montefiori, 2010; Pantaleo and Koup, 2004; Stamatatos et al., 2009; Walker and Burton, 2008). The relatively high prevalence of broadly neutralizing activity (20–30%) suggests that the epitopes that are capable of eliciting neutralizing humoral responses are accessible and immunogenic on the native Env spike on HIV-1 particles and that a protective neutralizing antibody-based vaccine against HIV-1 is an achievable goal (Burton et al., 1994; Dhillon et al., 2007; Li et al., 2009; Muster et al., 1993; Sather and Stamatatos, 2010; Steigler et al., 2001; Trkola et al., 1995; Walker et al., 2010). The great sequence diversity of HIV-1, especially in Env (Gaschen et al., 2002; Korber et al., 2001), has always been considered one of the major obstacles in the development of an effective HIV-1 vaccine. However, the fact that many BrNAbs neutralize diverse viruses from different subtypes indicates that HIV-1 Env sequence variation may not be an insurmountable problem. Furthermore, the elicitation of BrNAbs does not appear to be dependent on the subtype from which the Env is derived, as BrNAbs have been isolated from donors infected with diverse HIV-1 subtypes (Table 1).

Vaccine design considerations based on BrNAb epitopes

Based on the knowledge of the failed gp120 vaccines and the new knowledge on BrNAb epitopes, two major approaches can be taken to improve the induction of BrNAbs. The first approach, termed reverse vaccinology, uses the structural information of a BrNAb and its epitope to graft this epitope on a protein scaffold. Scaffold approaches are currently being pursued for the CD4BS, V1V2 and MPER domains (Azoitei et al., 2011; Wahome et al., 2010). The choice of scaffold needs careful selection and optimization in order to allow for optimal presentation and stability of the epitope. Env fragments, for example gp120 itself or gp120 outer domain fragments, can also be used as scaffolds to facilitate the presentation of the epitope of choice in its natural environment. By using glycans to cover other potentially immunogenic regions on the Env protein or scaffold, the response can be focused on the desired epitope.

While scaffolded epitope approaches focus on one particular epitope, the opposite approach is to create an Env protein that will expose all or most BrNAb epitopes in the right quaternary context. A stable mimic of the native Env spike is such an ideal protein and considerable efforts are aimed at creating soluble gp140 mimics of the native Env spike (Forsell et al., 2009; Sanders, 2011). However, while it has been shown repeatedly that trimeric soluble gp140s of various designs are more efficient at inducing NAbs against neutralization-sensitive tier 1 viruses compared to monomeric gp120 (Beddows et al., 2005; Earl et al., 2001; Kang et al., 2009; Kim et al., 2005; Kovacs et al., 2012; Melchers et al., 2012; Yang et al., 2001), none of them induce BrNAbs that neutralize neutralization-resistant tier 2 and tier 3 viruses (Seaman et al., 2010). Most of the current trimeric soluble gp140s do not mimic the native spike perfectly, as they do not interact very efficiently with BrNAbs that are dependent on the proper quaternary structure of the Env spike such as PG16 and PGT145. Moreover, most gp140s interact efficiently with a number of non-NAbs which is further testimony for their imperfect structure. Furthermore, most gp140 trimers are not processed in their gp120 and gp41 components because the cleavage site is usually deliberately mutated in order to enhance trimer stability. Uncleaved trimers are not adequate mimics of the native spike by virtue of them being uncleaved while the native spike is cleaved, and cleaved and uncleaved trimers have different antigenic properties (Binley et al., 2000; Dey et al., 2009; Herrera et al., 2005; Pancera and Wyatt, 2005; Si et al., 2003). We note however, that current generation cleaved gp140 trimers have not been better at inducing tier 2 neutralization than uncleaved gp140 trimers (Beddows et al., 2005, 2007; Kang et al., 2009). The new BrNAbs as well as non-NAbs should be used as quality checks to verify whether newly designed gp140 trimers have the desired structure. Whether a perfect mimic of the native spike will be a good immunogen remains to be seen, as such a construct will still have many of the immunosuppressive and immunoevasive defense strategies of the native Env spike.

Another approach is the use of a B-cell mosaic vaccine (Corey and McElrath, 2010; Kong et al., 2009; Korber and GnanaKaran, 2009). The principle of mosaic vaccine design has worked for the induction of cross-reactive cellular responses by T-cell mosaic vaccines (Fischer et al., 2007; Santra et al., 2010, 2012). Obviously, the generation of a B-cell mosaic Env involving conformational epitopes is less straightforward than generating mosaic proteins for linear T cell epitopes, yet here again the new knowledge on BrNAbs may facilitate such designs. The different vaccine approaches aimed at inducing BrNAbs may be complementary. For example, one can envisage priming with a scaffold-based vaccine to focus the immune response on one particular BrNAb epitope, then boosting with a gp140 trimer to select and boost specificities that recognize that epitope in the right quaternary context.

Vaccine design considerations based on BrNAb epitopes

The development of BrNAbs during natural infection in humans can further guide vaccine design. Many BrNAbs have one or more unusual characteristics, including polyreactivity for host antigens, extensive somatic hypermutation and long variable HCDR3 domains. Furthermore, the predicted germline ancestors of BrNAbs usually do not bind Env immunogens very well. These factors may increase the difficulty to elicit such antibodies by one single immunogen, yet there may be strategies to overcome these issues. In addition, not all BrNAbs show all the unusual characteristics, and therefore immunogen design does not have to overcome all these obstacles.

The correlation between the development of broadly neutralizing activity and a high plasma viral RNA load indicates that a certain level of antigen is required to drive the humoral immune response. In the AIDSVAX and RV144 trials, individuals were vaccinated with 600 μg gp120, which is unusually high compared with the doses of traditional subunit vaccines (e.g., 20 μg for HBsAg, 40 or 120 μg for HPV L1), yet such high doses may indeed be required for efficient humoral immunity against HIV-1.

An ideal vaccine would elicit protective immunity with one immunization. However, many vaccines require booster immunizations, often at 1 or 2 months after the first immunization and again around 6 months after the first immunization (McElrath and Haynes, 2010). It may be naïve to expect induction of protective immune responses with a few vaccinations in a limited time frame, while the development of a BrNAbs response in HIV-1 infected individuals takes several years, suggesting that the affinity maturation of HIV-1 BrNAbs requires a longer time frame and multiple or continuous antigen exposures. Future clinical trials may need to follow the example of the AIDSVAX trials where 7 immunizations of 600 μg gp120 were given over a time period of 3 years (Flynn et al., 2005; Pittisutthitham et al., 2006). Furthermore, to mimic an evolving viral quasispecies to stimulate late NAb affinity maturation, it may be necessary to immunize with multivalent vaccines, administered simultaneously and/or sequentially. Indeed Malherbe et al. studied the sequential immunization with a collection of Env variants representing the
viral quasispecies in an infected individual over time. They observed that sequential exposure to different Env variants led to better antibody maturation and increased neutralization breadth (Malherbe et al., 2011).

The crucial issue might not be whether a vaccine immunogen binds to known BrNAbs, but whether it can engage the germline precursors of these BrNAbs and start the affinity maturation process. B-cell-lineage vaccine strategies use computationally derived clonal lineages of known BrNAbs as templates for immunogens that are able to bind to the naïve B-cells and the consecutive somatically mutated memory B-cells (Haynes et al., 2012b). Many Env immunogens appear not to bind to the predicted germline ancestors of BrNAbs (although this may be different for transmitted/founder viruses) (Haynes, 2012), therefore it may be necessary to specifically modify or design Env immunogens that do bind these germline precursors.

The specific Env characteristics of vaccine antigens should in part be guided by the properties of BrNAb epitopes and/or by creating an optimal and stable mimic of the native Env spike (see above). Nevertheless characteristics of Env that are associated with the development of BrNAbs during natural infection can further “polish” such Env immunogens. For example the finding that short V1 domains are associated with induction of BrNAbs (van den Kerkhof et al., 2012), suggests that we should use Env immunogens with short V1 domains. BrNAbs epitopes on Env immunogens with shorter V1 domains may be more accessible and have a higher propensity to induce BrNAbs.

T help may be crucial to supply B cells with the required activation signals to develop high affinity BrNAbs to HIV-1. Protein subunit vaccines are usually not very efficient in inducing T cell responses so it remains to be established in what formulation the immunogen should be delivered. Many possibilities are being developed, from soluble proteins to DNA plasmids and viral vectors, which can all be used in multiple prime-boost combinations (Girard et al., 2006; McElrath and Haynes, 2010). Fusing Env immunogens to costimulatory molecules may be an alternative approach for aiding B cell targeting and activation (Melchers et al., 2011, 2012). Efforts to design novel immunogens, should be conducted in parallel to studies on the optimization of the immunogens and immunization schedule regimens.

Conclusions

The fact that many recently identified BrNAbs have neutralizing activity combined against the majority of primary HIV-1 variants combined with the relatively high prevalence of broadly neutralizing activity in HIV-1 infected individuals, implies that the epitopes of these BrNAbs are, at least under some circumstances, accessible and immunogenic, thereby predicting that a vaccine capable of eliciting these types of antibodies is an achievable goal. The known BrNAb epitopes can serve as templates for Env- and epitope-based vaccine strategies.

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