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HIV-1 envelope trimer design and immunization strategies to induce broadly neutralizing antibodies

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Abstract

The identification of multiple broadly neutralizing antibodies (bNAb) against the HIV-1 envelope glycoprotein (Env) trimer has facilitated its structural characterization and guided Env immunogen design. High-resolution structures of a soluble, native-like trimer (BG505 SOSIP.664) enabled the detailed characterization of the multiple bNAb epitopes that cover most of its surface. Several recent studies constitute progress in utilizing this knowledge for the development of an HIV-1 vaccine that induces bNAbs. Native-like Env trimers, including BG505 SOSIP.664, can induce autologous NAb responses against resistant (Tier-2) viruses in several animal models. A major challenge is now to drive those strong but narrowly focused NAb responses towards ones with much greater breadth. Among strategies that merit pursuing are using multiple trimers as sequential or simultaneous immunogens; targeting the germline precursors of bNAbs; delivering sequential lineages of trimers derived from infected individuals who developed bNAbs; and presenting trimers as particulate antigens.

Keywords

HIV-1 vaccine development; Immunogen design; Native-like trimers; Broadly neutralizing antibodies

More than 30 years of research has not yet created a vaccine that provides a useful degree of protection against HIV-1. Most licensed antiviral vaccines work through the induction of neutralizing antibodies (NAbs) [1]. To tackle HIV-1's unprecedented global sequence diversity, a vaccine will most likely have to induce broadly reactive NAbs (bNAbs). The knowledge that ~20–30% of HIV-1 infected individuals do develop bNAbs and observations that passive transfer of bNAbs confers protective immunity in macaques support the feasibility of developing a vaccine to induce such antibodies [2–9]. Here, we will review the strategies that we are pursuing, based around the design and use of native-like, soluble recombinant trimers. Other approaches are beyond the scope of this article. In general, HIV-1 Env vaccine development strategies now parallel, and are often guided by, next-

generation approaches to designing immunogens against other “difficult” pathogens such as Respiratory Syncytial Virus (RSV) and Influenza.

bNAbs target native Env on the HIV-1 surface and impede viral infection of target cells. HIV-1 Env is processed as a gp160 precursor that is proteolytically cleaved by furin into non-covalently linked gp120 and gp41 subunits, which assemble into a trimer of heterodimers. The instability and conformational flexibility of this six-subunit trimer hindered determination of its atomic level structure for 15 years after the core of the HIV-1 gp120 monomer was structurally characterized [10]. The structure of a nearly complete form of the trimer, known as BG505 SOSIP.664, was finally solved in 2013 [11,12]. Rapid progress then provided ever-increasing details of the trimer’s intricacies [11–16]. These cumulative insights have extensively refined our understanding about the metastability of the trimer, and how the gp41 fusion machinery functions to drive HIV-1 entry into cells. Furthermore, the trimer structures have allowed the detailed characterization of bNAb epitopes that is now guiding new structure-based immunogen design programs [16–24].

The trimer’s receptor-binding gp120 subunits mediate the initial attachment of HIV-1 to target cells (most commonly CD4⁺ T-cells), while the gp41 components that anchor the trimer in the virus membrane drive the fusion process. The conformational changes in gp120 driven by CD4 binding, followed by the dissociation of gp120 from gp41 upon co-receptor binding, lead to the step-wise transition of the gp41 sub-units from the pre-fusion structure to the energetically favorable six-helical bundle (6-HB); the energy released by this conformational transition is a critical driver of virus-cell membrane fusion [25].

HIV-1 trimers have evolved to resist both the binding of NAbs and their induction, and when NAbs *are* induced the mutation rate of the viral genome rapidly drives the emergence of escape mutants. The five highly variable loops (V1–V5) on gp120 shield the more conserved domains associated with receptor binding, a defense mechanism that is dramatically reinforced by the shielding effect of the 25–30 glycan moieties per gp120-gp41 protomer that decorate the trimer surface (fully half the mass of gp120 is carbohydrate). During HIV-1 infection, non-functional Env proteins that expose predominantly immunodominant non-NAb epitopes, such as uncleaved or otherwise defective trimers, dissociated gp120 monomers, the post-fusion, 6-HB, form of gp41, and assorted degradation products, also elicit antibodies [26,27]. Whether non-NAbs impede the NAb response to trimers, or are irrelevant, is under investigation. The induction and binding of NAbs might also be influenced by the conformational flexibility of the trimer, which fluctuates between closed and more-open conformations [28–31].

Despite these viral defenses, the co-evolution between escape variants and NAb affinity maturation drives the development of bNAbs in ~20% of HIV-1 infected individuals [2,5,6]. In general, bNAbs have acquired unusual characteristics that help overcome the trimer’s defenses against antibody binding and neutralization. For example, bNAbs have almost invariably undergone extensive somatic hypermutation (SHM), they have extremely long CDR-H3 loops, they are often polyreactive, and some of them are derived from rare precursor genes [32]. These intrinsic characteristics play a major role in understanding why

it has been, and no doubt will remain, so hard to induce bNAbs by immunization with Env proteins.

In this review, we will describe the currently known bNAb classes and their epitope specificities on the Env trimer. We will then discuss how the design and use of native-like trimers may play a role in bNAb induction.

Broadly neutralizing antibodies

Because bNAbs can neutralize a large proportion of circulating viruses from different clades they are valuable templates for Env immunogen design. For many years, only four bNAbs were known: 2G12, b12, 2F5 and 4E10. A major advance in bNAb isolation and characterization was single antigen-specific B-cell cloning methods that allowed the rapid isolation of monoclonal antibodies (MAbs) [19,20,24,33–40].

Based on their target epitopes, bNAbs can be divided into six different subclasses: the V2 apex; the base of the V3 with associated glycans (V3-glycan); the glycosylated outer domain (OD-glycan); the CD4 binding site (CD4bs); the gp120-gp41 interface; and the gp41 membrane proximal external region (MPER). The way bNAbs recognize these epitope clusters on the HIV-1 Env trimer is shown in Figure 1.

Multiple bNAbs recognize epitopes that are quaternary in nature (i.e., trimer-specific or strongly influenced by trimerization). Several such epitopes are located within the V2 domain at the trimer apex, including PG9, PG16, PGT145, VRC26 and PGDM1400. These epitopes span at least two protomers and hence the bNAbs bind the trimer in a 1:1 stoichiometry; a high mannose glycan at position 160 is critical, as is a long CDHR3 loop that penetrates the glycan shield and recognizes the conserved β -strand C in V2 [21,24,38]. PGDM1400 is one of the most potent bNAbs isolated so far, with cross-clade neutralization coverage of 83% at a median IC₅₀ of 0.003 μ g/mL [20].

The V3-glycan epitopes are part of what is referred to as a “supersite of vulnerability”, the oligomannose glycan patch covering the gp120-OD [22,38]. V3-glycan epitopes for the highly broad and potent PGT121-125 bNAbs comprise the GDIR peptide motif at the base of V3 and elements from one or more of several topologically proximal glycans. Crystal structures of the trimer-PGT122 complex have revealed a detailed understanding of the binding specificities of this bNAb subclass [11,15].

The OD-glycan epitopes are part of the same supersite of vulnerability [22,41]. Thus, bNAbs against the OD-glycan and V3-glycan sub-clusters all depend on the N332 glycan, but approach it from different sides [11,42]. The OD-glycan bNAbs include 2G12 and members of the PGT135-family. One of the first known bNAbs, 2G12, recognizes the high-mannose glycan patch via contacts with only glycans [43,44], while the PGT135 epitope consists of multiple glycans as well as underlying protein segments, including part of the V4-loop [22]. Some bNAbs that interact with the N332-glycan and nearby structures are promiscuous in how they recognize glycans. Thus, some glycan components of OD-glycan epitopes can vary in their precise location without disrupting bNAb binding, an immunological strategy that, here at least, can help counter how viral variation drives neutralization escape [45,46].

A long-known bNAb target is the CD4bs; NAbs against this conserved gp120 structure neutralize the virus by blocking trimer binding to the CD4 receptor. The first CD4bs bNAb was b12, although by modern standards its breadth and potency are quite limited [47]. The much later identification of VRC01, which has far greater breadth and potency, was followed by the discovery of many others that also target the CD4bs [37,48–50]. However, depending on the angle at which they approach the trimer, various glycans can impede the access of bNAbs to CD4bs-associated epitopes. As a result, bNAbs targeting these sites have evolved ways to avoid clashes with one or more glycans. How they do so defines two CD4bs bNAb sub-families [51]. The first is restricted by the engagement of a specific bNAb germline precursor B-cell from either the VH1-2 or VH1-46 lineage, while the second sub-family includes a CDRH3-dominated cluster of Abs that has a diverse B-cell ontogeny. Each sub-family has an optimal angle of approach to the CD4bs that maximizes neutralization potency and breadth [51]. Structure-guided awareness of the importance of the angle of approach of a bNAb to its target, particularly the CD4bs, is now a critical issue in immunogen design. Thus, many antibodies interact strongly with the CD4bs on gp120 monomers but bind the cognate trimers poorly and hence have limited neutralization activity; the reason is that several approach angles that allow access to the CD4bs on gp120 monomers are blocked off in the sterically constrained environment of the trimer. As a result, inducing CD4bs bNAbs that approach the trimer at an appropriate angle is problematic for gp120 monomers or non-native gp140 proteins [52].

A recently discovered bNAb epitope cluster involves the gp120-gp41 interface. bNAbs in this family usually interact with both gp120 and gp41 subunits as well as glycans, and hence are trimer-specific or, at least, strongly influenced by trimerization. PGT151, the first member of this family, is exquisitely specific for native-like trimers, which it binds with an unusual 2:1 stoichiometry [19,40]. Two others, 35O22 and 8ANC195, target separate areas of the gp120-gp41 interface [16,18]. The 35O22 and 8ANC195 epitopes are partially overlapping but both are separated from the PGT151 site; 8ANC195 and PGT151 do, however, cross-compete for trimer binding via an indirect, steric hindrance mechanism [42]. The gp120-gp41 interface antibodies probably neutralize HIV-1 infectivity by stabilizing the pre-fusion state and/or by impeding conformational changes necessary for fusion. Thus, 8ANC195 can trap the Env glycoprotein in a partially open state, preventing further downstream conformational changes that initiate fusion [16]. Two related bNAbs, 3BC315 and 3BC376, were first described as targeting an epitope similar to that seen by V3 or CD4i antibodies [33]. The availability of new tools for epitope mapping reveals that both bNAbs bind a distinctive epitope overlapping the 35O22 site, although with a greater proportional involvement of the gp41 subunit and hence a location closer to the viral membrane [17]. 3BC315 and 3BC376 neutralize via a unique mechanism, in which antibody binding increases the rate of trimer decay into inactive forms, including the shedding of gp120.

A similar mechanism that results in trimer disintegration also applies to two other gp41 bNAbs, 2F5 and 4E10, that target the MPER, which is even closer to the viral membrane [53–55]. The 10E8 epitope also involves the MPER, but this bNAb also interacts with viral membrane lipids via its CDR-H3 region [35,56]. 10E8 may impede the fusion process by binding to a fusion-intermediate conformation of gp41 [56,57].

An overview antigenic map of the trimer and its bNAb epitopes has been assembled [42]. Strikingly, despite the extensive glycosylation and the sequence variation within the variable loops, bNAbs are now known to recognize almost every part of the trimer surface (Fig. 1). As a result, old views that there are only a few sites of vulnerability on HIV-1 Env are now changing; there are, in fact, many. The key question now is whether we can exploit these multiple targets by designing and using immunogens that elicit bNAbs.

Design of Env trimer immunogens

There are multiple ways to design immunogens intended to induce bNAbs, including, but not limited to, gp120 monomer-lineages, non-native gp140 proteins, gp120-core and eOD proteins, epitope-specific scaffolds and epitope-based peptides. All these approaches are beyond the scope of this article, but have been reviewed elsewhere (Sliepen & Sanders 2015). Here, we will focus on the design and use of immunogens based on recombinant, soluble native-like trimers that closely mimic the native Env complex on the HIV-1 virus. As most of the native-like trimers described to date bind most bNAbs, their potential for eliciting relevant immune responses is clear.

The trimer's functionally essential metastability, particularly the non-covalent interactions between the gp120 and gp41 subunits, were major obstacles for creating soluble recombinant versions that mimicked the membrane-associated spikes on the virus surface. To make soluble gp140 proteins, gp41 must be truncated prior to the transmembrane domain. However, without further modifications, the trimers disintegrate because the individual sub-units dissociate. As a result, for many years, the standard HIV-1 Env immunogen design involved monomeric gp120 subunits, as their production is relatively straightforward (although not without problems, due to proteolytic damage to V3, inappropriate dimerization via intermolecular disulfide bonds and the related formation of aberrantly scrambled disulfide bonds) [58–61]. The gp120 monomers did not induce NABs against primary (i.e., neutralization-resistant) isolates and failed to provide protection in two efficacy trials [62–64]. These outcomes are probably explained by one or more defects, including the presentation of immunodominant non-NAB epitopes; the lack of NAB epitopes that depend on quaternary structure and/or the presence of gp41; and the absence of steric constraints on the CD4bs that allow the generation of “off-target” non-NABs that approach the trimer from an inappropriate angle (see above).

Various stabilization strategies were used to generate soluble gp140 proteins that contain both gp120 and the gp41 ectodomain (gp41_{ECTO}). The engineered inactivation of the furin cleavage site between gp120 and gp41_{ECTO}, followed later by the introduction of a trimerization domain (most commonly Foldon) to the gp41_{ECTO} C-terminus allowed the production of soluble “uncleaved” gp140 glycoproteins that were nominally “trimers” (although often contaminated with higher m.wt. aggregates that formed via intermolecular disulfide bonds). The advent of negative-stain EM, combined with a range of other analytical techniques, revealed that the uncleaved gp140s rarely if ever adopted a native-like conformation that resembled the Env spike on viruses. Instead, the gp120 subunits were separated, splayed out around a central gp41_{ECTO} core that has a configuration akin to the post-fusion form of these subunits [52,58,65–68]. Moreover, the gp120 subunits of

uncleaved gp140s are damaged by the formation of aberrant intermolecular disulfide bonds, and contain multiple, highly processed glycan structures that differ from the high-mannose forms that are hallmarks of native-like trimers [52,58–60,65,66,68]. These multiple structural defects account for why, in animal immunization studies, multiple uncleaved gp140s of a range of genotypes have failed to induce consistent NAb responses against neutralization-resistant (Tier-2) viruses, including the autologous virus [69–75].

An alternative strategy for making soluble trimers evolved over many years, leading to the BG505 SOSIP.664 trimer that is now widely used as a platform for structural studies and immunogen design. The SOSIP design embraced the need for the gp120 and gp41_{ECTO} subunits to be proteolytically cleaved and indeed included mutations to make cleavage more efficient [76]. The resulting and inevitable instability was overcome by introducing an intermolecular disulfide bond (501C-605C; SOS) to strengthen the gp120-gp41_{ECTO} interaction and a mutation (I559P) in gp41 to prevent these subunits transiting from their pre-fusion (i.e., native) structure [77,78]. A later design improvement involved truncating gp41_{ECTO} at position 664, removing the hydrophobic MPER and its associated bNAb epitopes to improve solubility [79,80].

Many Env sequences do not yield fully native-like SOSIP.664 trimers, for reasons that are still not fully understood [81]. However, a trimer based on an clade A founder virus isolated from infant-BG505 by the Overbaugh group and its Kenyan collaborators turned out to have exceptional properties [82,83]. Thus, the BG505 SOSIP.664 trimer adopts a stable native-like conformation and displays almost all known bNAb epitopes while binding a subset of non-NAbs only weakly [83]; its glycan composition is enriched for native-like high mannose structures (Pritchard et al., 2015); and it is free of aberrantly scrambled disulfide bonds [58]; Go et al., submitted). The reproducible homogeneity and stability of the BG505 SOSIP.664 trimer have enabled its structure to be solved at increasing, and now atomic-level, resolution by three different groups [11,12,14–16,84]. The same trimer has been used to characterize bNAbs to unknown epitopes, to refine our understanding of existing bNAb epitopes [16–19,40,42], and to isolate new bNAbs [20,24]. In immunization studies in rabbits, the BG505 SOSIP.664 trimers induced NAbs against the autologous Tier-2 virus, a stepping stone in the path towards bNAbs [75]. Macaques also responded, although the autologous NAb response was weaker in these animals [75]. Whether changes in the adjuvant and/or dosing regimen will improve the response in macaques is under active investigation, as these animals are immunologically closer to humans than rabbits are. The NAb specificities induced in rabbits targeted various epitopes that, in some cases, resembled those of glycan-dependent bNAbs. A second native-like trimer, B41 SOSIP.664, also induced a strong autologous NAb response against the autologous Tier-2 virus in rabbits [30,75].

Although, as noted, many Env sequences do not yield fully native-like SOSIP.664 trimers, various techniques have now allowed the production of ones based on the clade A isolate 92UG037.8, the clade B isolates B41, JR-FL and AMC008, and the clade C strains DU422, ZM197M, 16055 and CZA97.012 [30,58,81,85,86]. The existence of these reagents, and others as yet unpublished, provides opportunities to explore whether the simultaneous or sequential use of multiple, genetically diverse native-like trimers will be useful for broadening the NAb response at the Tier-2 level (Fig. 2). While BG505 and B41 SOSIP.664

proteins form exclusively native-like trimers that be purified by the non-selective 2G12 bNAb followed by size exclusion chromatography, some other SOSIP.664 proteins yield mixtures of native-like and aberrant trimers that can only be separated by the appropriate use of antibody-affinity columns. Thus, a native-like sub-fraction of SOSIP.664 trimers based on the JR-FL or 16O55 sequences can be isolated by using a CD4bs non-NAb negative-selection column to deplete the predominant non-native trimer population [85]. Conversely, positive-selection affinity columns based on quaternary epitope-specific bNAbs, PGT145 or PGT151, successfully purify native-like trimers from several genotypes [30,86]. A comparative study using SOSIP.664 trimers based on the CZA97.012 and 92UG037.8 genotypes shows why positive selection columns can be a powerful tool, compared to less selective purification strategies such as Ni-NTA (His-tagged trimers) or lectin columns [58].

Flexibly linked (NFL) or single-chain (SC) trimers are alternative approaches yielding trimers based on BG505 and JR-FL (NFL) or BG505 (SC) that appear to be native-like when viewed by EM and assessed antigenically; in both cases a flexible Gly-Ser linker strand replaces the Furin cleavage site (REKR) between gp120 and gp41_{ECTO} [87,88]. A 10-residue (GGGSGGGGS) linker was used in one study [87]; in the other, a range of linker lengths (1–20 residues) was evaluated and best found to be (GGSGGGSGGGSGG, i.e., 15 residues) [88]. The flexible linker allows gp120 to associate properly with gp41_{ECTO}, a necessity for forming native-like trimers, without the need for proteolytic cleavage. However, both flexible linker trimer designs rely on other elements of the SOSIP.664 blueprint: the truncation at position 664; the I559P or a related substitution; and, in the case of the SC-trimer, also the 501C-605C disulfide bond [87,88]. The adverse consequences of not including the disulfide bond and/or the I559P change to flexible linker trimers are clear [66,89]. Whether uncleaved NFL and/or SC trimers are precisely equivalent to cleaved SOSIP.664 trimers remains to be determined, as does their immunogenicity. One epitope disrupted by the flexible linker in the SC-trimers is PGT151 at the gp120-gp41_{ECTO} interface. From the production perspective, the transient transfection yields and purification strategies seem comparable for all three native-like trimer design variants.

Single molecule fluorescence, electron microscopy and hydrogen-deuterium exchange experiments have shown that both native (virion-associated) and soluble SOSIP.664 trimers “breathe”, by alternating between closed and more open conformations [16,28,31,67,90]. Moreover, multiple potent bNAbs preferentially recognize the closed, pre-fusion form of the trimer and some non-NAb epitopes become accessible when the trimer opens up [28,29]. These findings underpin the desirability of modifying SOSIP.664 trimers to further stabilize them in the closed pre-fusion state. Thus, by reducing the antigenicity/exposure of immunodominant non-NAb epitopes (such as but not limited to V3) it may be possible to reduce their immunogenicity and thereby focus the response on bNAb epitopes. Here, it is also relevant that various Env immunogens are more easily engaged by the germline precursors of non-NAbs than of bNAbs [91]. Overall, reducing the immunodominance of non-NAb epitopes may benefit various vaccine strategies aimed at inducing bNAbs (Fig. 2).

One way to stabilize BG505 SOSIP.664 trimers in the ground state and prevent spontaneous sampling of the CD4-induced state is to introduce an intrasubunit disulfide bond (201C-433C) within gp120 [13]. Our own approach has involved adding several stabilizing

point substitutions, E64K or H66R and A316W, into clade A (BG505), clade B (B41 and AMC008) and clade C (ZM197M) SOSIP.664 constructs. In combination, the twin substitutions stabilize the closed conformation of the trimer and reduce the exposure of several non-NAb epitopes, including in V3. These stabilized trimer variants are designated SOSIP version 4 (SOSIP.v4) [86]. In immunization studies in rabbits or mice, the BG505, B41 and AMC008 SOSIP.v4 trimers induced lower titers of anti-V3 antibodies and Tier-1 NAb, without impairing the autologous NAb responses [86].

Presenting Env proteins as particulate antigens can enhance B-cell stimulation via B-cell receptor crosslinking and hence increase their immunogenicity. In a pilot experiment, ferritin-based protein nanoparticles displaying multiple copies of BG505 SOSIP.664 trimers were more immunogenic in rabbits than the corresponding soluble trimers [92]. Virus-like particles displaying multiple copies of predominantly native-like, full-length JR-FL Env induced Tier-2 autologous NAb that targeted a hole in the glycan shield created by the absence of the N197-glycan [93]. Various similar and different approaches to presenting native-like trimers in particulate form are no doubt being pursued.

Steering bNAb development pathways with lineage vaccines

Although the use of combinations of genetically diverse trimers, delivered sequentially or simultaneously, is an approach that is being pursued in animal studies, it should be recognized that bNAbs are not produced in the earliest stages of HIV-1 infection but emerge through co-evolutionary processes. Thus, viral escape from a rapid, autologous NAb response drives renewed cycles of B cell activation and affinity maturation in a process that is unlikely to be mimicked by the use of a few “standard trimers” delivered together. As an alternative approach, “lineage vaccine” strategies are being pursued to mimic the co-evolution process that takes place during infection. One lineage vaccine approach is based on *env* gene sequences derived over time from infected people who develop bNAb responses (“natural lineages”) [24,49,94,95]. Alternatively, immunogens are engineered to steer the Ab response to a specific bNAb epitope (“designed lineages”) [96–100]. The two strategies are not necessarily mutually exclusive. A detailed summary of all aspects of the designed lineage approach is beyond the scope of this review.

To design natural lineage immunogens it is necessary to understand how bNAbs emerged in the relevant individual, and to identify the Env characteristics that drove the response [24,49,101]. Deep-sequencing is a valuable tool for identifying what Env sequences initiated the bNAb development pathway, and the resulting escape mutations that then drove the broadening process [94]. Key sequences can then be used for Env-immunogen design, for example monomeric gp120s based on the CH505 lineage [49,102]. As one test of this approach, we are using SOSIP trimers based on sequences that evolved in the BG505 virus-infected infant during the period of bNAb development [103]. Basing natural lineage trimers on sequences from infected people who developed bNAbs unusually rapidly may be particularly useful [103,104].

The designed lineage approach involves making immunogens that initiate a specific bNAb development pathway. The first stage of a bNAb response is the engagement of naïve B-cells

that express the unmutated germline-bNAb precursor. However, recombinant Env glycoproteins of “standard designs” usually recognize these precursors rather poorly [98–100,105]. To try to engage the correct germline-bNAb precursor B-cell, various gp120 monomer-based constructs, such as engineered outer domain (eOD) or gp120 core proteins, have been made. These proteins generally involve deleting variable loops and/or glycan sites, and are narrowly focused on the CD4bs family of bNAbs [98–100,105]. Our own approach to the designed lineage concept is based around the use of structural and other insights to successfully engineer various SOSIP trimers (designated SOSIP-GL) to engage multiple germline-bNAbs *in vitro*. Ongoing immunogenicity studies are testing the concept of using the SOSIP-GL trimer to initiate bNAb lineages, followed by boosting with one or more “mature” SOSIP trimers to drive the pathway towards bNAbs (Fig. 2). The outcome of studies with BG505 SOSIP.664 trimers and other immunogens in 3BNC60 knock-in mice supports this strategy [96]. In this context, it is also relevant that some “mature” SOSIP.664 trimers themselves bind various human bNAb precursor antibodies *in vitro* [106,107]. Whether they can do so *in vivo* is being evaluated in mouse models.

One reason why we prefer a trimer-based approach to engaging bNAb precursors is the greater steric constraints that apply to various bNAb epitopes on trimers compared to monomeric gp120-based constructs. Thus, as noted above, the angle at which a bNAb approaches the virus (i.e., native trimer) can be critical, particularly in respect of the CD4bs-associated epitopes. If the immunogen does not impose the appropriate restriction on the approach angles, the likelihood is that “off-target” non-NABs will be induced instead of, or as well as, the more desirable bNAbs. This scenario may account for why the eOD-GT6 construct, which is based on gp120-OD monomers, induced only non-NABs when tested in a VRC01 knock-in mouse model [97]. When two germline-targeting immunogens (eOD-GT6 and 426c.TM₄ V1-3) were tested in knock-in mice bearing the mature or germline versions of the 3BNC60 bNAb heavy chain, they were able to engage germline heavy chain B-cells and drive the appropriate selection and subsequent affinity maturation of the mouse light chain [96]. Under the same conditions, BG505 SOSIP.664 trimers did not engage germline heavy chain B-cells, probably because of the obstructive effect of the N276 glycan in the native-like trimer context [96,97]. However, when the mature heavy chain knock-in mice were immunized with the same SOSIP.664 trimers a highly restricted set of mouse light chains was selected, which resulted in the induction of 3BNC60-like bNAbs. In contrast, the germline-targeting eOD-GT6 immunogen triggered much weaker NAb responses, probably because the comparatively unconstrained angles of approach to the CD4bs recruited a more diverse set of light chains that favored the production of non-NABs.

While these knock-in mouse models are useful to test the ability of Env immunogens to activate very specific human germline bNAb precursors, they do not take into account how well the same proteins would perform in a mixed germline repertoire where mechanisms such as epitope immunodominance and subdominance are relevant [91]. Thus, while a series of natural lineage immunogens might induce bNAbs potentially in specific knock-in mice, this desirable response could be thwarted by the additional induction of immunodominant non-NABs when the immunogens tested in the complete human germline repertoire.

It has been proposed that an obstacle to bNAb development is created by the self-mimicry of critical epitopes on Env proteins. As a result, B-cells with the potential to evolve into bNAb producers are depleted at tolerance checkpoints [108,109]. Native-like trimer variants from which self-mimicking epitopes have been deleted without compromising bNAb epitopes constitute one way to explore this hypothesis.

The development of native-like trimers during the past few years has opened several areas of active investigation that are aimed at developing a bNAb-based vaccine. Progress in this area melds well with the ever-increasing knowledge base on the very many bNAbs that have been isolated and characterized over approximately the same period; their collective existence strongly underpins the concept of a bNAb vaccine. The determination of what are now atomic-level structures of the BG505 SOSIP.664 trimer has provided insights not only into the design of new Env immunogens, but also into the many bNAb epitopes and neutralization mechanisms that constitute vulnerable targets ripe for the exploitation. Very few immunogenicity studies have yet been completed and reported, but it is clear that SOSIP trimers can induce the Tier-2 autologous NAb responses that are widely seen as a necessary step in various more complex pathways towards neutralization breadth. Multiple different immunization strategies based on native-like trimers, including next-generation, structure-guided variants, can now be tested in various animal models. The cumulative outcome of such experiments will generate yet more knowledge that will help our collective understanding of how to further refine the design and delivery of this new family of Env immunogens. Knowledge of how advances are made in the RSV and Influenza vaccine areas, among others, will also be harnessed to guide the design of HIV-1 Env vaccines, and perhaps *vice versa*.

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Trends Box

The structure of the HIV-1 Env trimer has been solved by both X-ray crystallography and cryo-EM, guiding immunogen design improvements.

Recombinant native-like HIV-1 Env trimers, based on the SOSIP.664 design, induce autologous Tier-2 neutralizing antibodies in rabbits and macaques.

Stabilizing HIV-1 Env trimers in the closed pre-fusion state reduces their propensity to undergo reversible conformational transitions (“breathing”), which may be important for reducing their induction of immunodominant non-NAb responses.

Knock-in mice that express specific inferred germline B-cell receptors (BCRs) or the complete human germline BCR repertoire are valuable for evaluating Env immunogens.

Outstanding questions

How can we increase the breadth and potency of autologous NAb responses?

Do non-NAb responses actively interfere with the elicitation of bNAbs, or they are merely irrelevant?

How do autologous NAbs elicited in animals after immunization with native-like trimers compare to autologous responses raised during HIV-1 infection?

What is/are the epitope(s) of autologous Tier-2 NAbs induced by native-like Env trimers? Are similar or different epitopes immunogenic when native-like trimers from different isolates are used?

Can native-like trimers based on Env sequences that evolve in infected people that develop bNAbs be used to mimic the Env-antibody co-evolution processes that take place during the course of infection?

Are native-like trimers based on all sequences equivalently immunogenic? If not, what structural or antigenic properties most influence their immunogenicity?

Figure 1. bNAb epitopes mapped onto the 3D structure of the BG505 SOSIP.664 trimer
 The bNAbs labeled in different colors are modeled onto an EM density map of the BG505 SOSIP.664 trimer (colored in grey). The figure includes bNAbs recognizing five different epitope clusters: PG9 (V2apex), PGT122 and PGT128 (V3-glycan); PGT135 and 2G12 (OD-glycan); VRC01 (CD4bs); and PGT151, 35O22, 3BC315 and 8ANC195 (gp120-gp41 interface). Only one Fab fragment per trimer is shown for clarity. Thus, the model does not indicate the stoichiometry of bNAb binding, only the location of the epitope. This figure is an updated version of Fig.4 from Derking et al., 2015. We thank Gabe Ozorowski and Andrew Ward for preparing it.

Figure 2. Immunization schedules
An overview of various immunization schemes involving native-like trimers and aimed at inducing bNAbs. Displaying the trimers in particulate form (e.g., nanoparticles), and reducing their exposure of immunodominant, non-neutralizing epitopes would benefit all of these regimens.