HHS Public Access

Author manuscript

Curr Opin HIV AIDS. Author manuscript; available in PMC 2017 November 01.

Published in final edited form as:

Curr Opin HIV AIDS. 2016 November ; 11(6): 569–575. doi:10.1097/COH.0000000000000317.

Approaches to the induction of HIV broadly neutralizing antibodies

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Abstract

Purpose of Review—A vaccine that elicits antibody responses that can neutralize the diversity of HIV clades has not yet been achieved, and is a major focus of HIV vaccine research. Here we provide an update on the barriers to eliciting such antibodies, and how advances in immunogen design may circumvent these roadblocks, focusing on data published in the last year.

Recent findings—Studies of how broadly neutralizing antibodies (bNAbs) develop in HIV-infected donors continue to produce key insights, suggesting that for some viral targets there are common pathways to developing breadth. Germline-targeting strategies, that aim to recruit rare precursors of bNAbs, have shown promise in immunogenicity studies, and structural biology has led to advances in immunogen design. Mapping of strain-specific Tier-2 vaccine responses has highlighted the challenges that remain in driving antibodies towards breadth.

Summary—Elucidation of the HIV envelope structure, together with an understanding of how bNAbs emerge *in vivo* has guided the design of new immunogens and vaccine strategies that show promise for eliciting protective antibodies.

Keywords

HIV Envelope; Broadly Neutralizing Antibodies; Germline-targeting immunogens; Trimer

INTRODUCTION

HIV-1 is one of the most variable and glycosylated viruses known, making it an especially challenging target for neutralizing antibodies. While almost all infected people develop

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CONFLICTS OF INTEREST

None.

antibodies to the HIV envelope which have some cross-neutralizing activity (1), only about 20-30% of people develop responses that are considered truly broadly neutralizing (2-5). Furthermore, it generally takes years of infection for these broadly neutralizing antibodies (bNAbs) to evolve, and they often have unusual features not favored by the immune system, including extensive somatic hypermutation (SHM), very long or short CDRs, and autoreactivity (6). This suggests difficult pathways to developing bNAbs naturally, even in the context of ongoing viral replication, and poses challenges to their elicitation by vaccination.

Despite this, there is strong rationale for pursuing bNAbs to prevent HIV infection. Passive immunization of bNAbs has long been known to protect non-human primates from infection (reviewed in (6)). Indeed, a recent study shows that a single injection of bNAbs protects against repeated exposure for up to 23 weeks (7). Furthermore, much of the accessible part of the HIV trimer is now known to be vulnerable to bNAbs (8). These conserved epitopes include the V2 site, the N332 glycan supersite, the membrane proximal external region (MPER), the CD4 binding site (CD4bs), and the gp120-gp41 interface, most recently shown to include the fusion peptide (9). Elucidation of the native envelope trimer structure (10-16), and of how bNAbs emerge *in vivo*, has informed the design of new immunogens and vaccine strategies. Some of these immunogens have been tested in non-human primates with promising results, however none have yet broken the barrier to achieving neutralization breadth. This review will cover recent studies that have provided insights into how to overcome these viral and host barriers, and the latest thinking in immunogen design.

VIRUS-ANTIBODY CO-EVOLUTIONARY STUDIES PROVIDE A MODEL FOR BREADTH

The high levels of SHM of many HIV bNAbs suggest a long co-evolutionary trajectory, requiring variation in both the virus and the antibody. Indeed, two recent studies showed that bNAb lineages evolve at least as fast as HIV, particularly at the early stages of their development, though these rates later decline (17, 18). Though selection and mutability account for some of this decrease (18), the role of viral escape in limiting antibody maturation remains understudied. Nonetheless, this incredibly rapid host evolution provides a mechanism for the extraordinary diversity achieved within long-lived bNAb lineages (17).

A key event in the development of breadth is the viral diversification that precedes bNAb emergence (19-21). A detailed study of the V2-targeting CAP256-VRC26 lineage provided a mechanism for how this diversification contributed to breadth, showing that as the antibody lineage matured, some members learned to tolerate diversity that had been created by viral mutations at key sites in the epitope (22). In contrast, “dead-end” antibodies that were unable to tolerate diversity failed to mature, while other “off-track” antibodies matured, though not towards breadth (22). In that study, virus mutations occurred through escape from earlier members of the same lineage, but another explanation for increased viral diversity has come from studies of “co-operating antibody lineages” within single individuals (23, 24). This was first shown in donor CH505, where the CH235 CD4bs lineage drove escape mutations that enhanced neutralization by a second CD4bs lineage, CH103, resulting in

breadth. A fascinating follow-up study showed that ultimately the CH235 lineage acquired even greater breadth, highlighting the role of two distinct antibody lineages in curtailing the ability of the virus to completely escape either, and providing continued stimulation of both bNAbs. Intriguingly, a recent observation of enhanced breadth following passive administration of another CD4bs bNAb, 3BNC117 might suggest a passively administered antibody serving the same role as a co-operating lineage (25), though this needs further study.

These studies of infection provide mechanistic insights into how breadth develops and are the basis of sequential, B-cell lineage based, immunization strategies that have gained wide support (reviewed in (26)). This approach seeks to mimic aspects of the viral evolutionary processes using immunogens that gradually incorporate mutations to drive antibody tolerance of diversity and promote increased breadth. This is supported by modeling (27) and by recent data showing maturation towards breadth in knock-in mice expressing germline reverted N332-dependent PGT121 antibodies (Escolano *et al*, Keystone, 2016). This important proof-of-concept study suggests that if the right antibody precursor is activated, breadth can be nurtured.

PREDICTABLE PATHWAYS OF BNAB DEVELOPMENT

Studies of bNAb evolution suggest that there are similar B cell ontogenies and antibody structures between donors, which is encouraging for vaccine design. This is particularly true of CD4bs antibodies, and includes both CDR H3-restricted and VH-gene restricted (commonly referred to as VRC01-like) CD4bs antibodies (24, 28-30). A study of 14 donors showed that the 2 classes of antibodies had distinct ontogenies, but both could neutralize highly effectively (with using one of two optimal angles of approach) despite recognizing very different paratopes. For both types of CD4bs bNAbs, the precision of the angle of approach correlated with breadth. However, a longitudinal study of the CH235 CD4bs VH-restricted lineage found that although early antibodies had a nearly perfect angle of approach, significant affinity maturation was required for breadth. Structural studies of VRC01-like antibodies showed that affinity maturation correlated with increased flexibility in an induced-fit mechanism of binding (31), perhaps to accommodate variable HIV epitopes and glycans. Studies of the antibody genes during maturation showed that for VH-restricted bNAbs, common mutations were acquired during the course of SHM, suggesting a shared evolutionary pathway (24). The observation that these mutations were also seen in HIV-uninfected individuals suggests that the SHM required for breadth is largely a consequence of “intrinsic mutability” at specific sites within the VH1-2 and VH1-46 genes commonly used by CD4bs antibodies. Similar observations from influenza studies may suggest that this is a broad contributor to affinity maturation (32).

V2-targeting bNAbs also share structural and developmental commonalities. These antibodies are characterized by a long CDRH3 that is highly anionic and tyrosine sulphated (20). Comparison of the atomic level interactions of antibodies from 4 donors showed similarities in strand-strand protein recognition and quaternary interactions with glycans suggesting a common mode of interaction, though subtle differences in precise targeting were noted. An important D-gene encoded YYD motif in the long CDRH3 that characterizes

this class of bNAb has a variable role in V2 binding (33, 34), which may suggest that the conservation of this motif can be attributed more to the preferred anionic nature of the paratope, and the enrichment of this motif among germline genes with long CDRH3s (33). Comparison of the ontogenies of V2 antibodies also suggests some common features, with the long CDRH3 of such antibodies established at the recombination stage, and showing partial glycan recognition early in the developmental pathway (33). Intriguingly, V2 antibodies PG9 and CAP256-VRC26 from unrelated donors were derived from similar germline genes (99% identity) (34). Furthermore, as with the CD4bs antibodies certain mutations were shared between lineages, suggesting common pathways of maturation. Along with the association of longer CDRH3s with certain germlines (35), this suggests that some antibody genes may be more amenable to development of V2 specificities than others. Overall, in both CD4bs and V2 bNAbs, common developmental pathways may provide roadmaps for vaccination, discussed below.

ENGAGING GERMLINES - HOW TO KICK START BNABS THROUGH VACCINATION

A major barrier for vaccine design is the fact that the many germline-reverted bNAbs fail to bind recombinant envelope (29, 36-40). These findings, along with the rare nature of the B cells that need to be targeted (35, 41), and the ontogenic similarities described above, have led to the design of immunogens specifically engineered to stimulate precursors by vaccination. This is most advanced for CD4bs antibodies, described below. However, for V2 bNAbs, potential “bNAb-initiating envelopes” have recently been identified through longitudinal studies of infected subjects (22), or by large scale screening of germline-reverted bNAbs against diverse viruses (33, 34). Viral strains with enhanced reactivity for V2-bNAb precursors have been incorporated into novel immunogens (33), and provide an opportunity to extend germline-targeting to V2 antibodies.

Antigens designed to bind to the germline precursors of VRC01 class antibodies have recently been tested in knock-in mouse models, providing convincing data in support of this concept. Immunization of mice expressing germline reverted VRC01 or 3BNC60 heavy chains with germline-targeting immunogens (called eOD8-GT8) selected for B cells with 5 amino acid long CDRL3s characteristic of VRC01 class antibodies (42, 43). In contrast native Env failed to activate these naïve B cells, even in this model where precursors are highly enriched, supporting the germline-targeting model (42). Furthermore, direct probing of the human immune repertoire for eOD8-GT8 reactive B cells confirmed that despite their generally rare nature, in the context of $>10^{11}$ B cells per person, such VRC01-class precursors are likely to be present at sufficient frequency to be reliably elicited in most people (41).

Recruitment of these rare B cells, while alleviating a key roadblock, is however not sufficient if they do not acquire enough productive mutations to become reactive to immunogens that better represent circulating viruses (Figure 1). Fortunately, although these vaccine elicited germline-derived antibodies did not neutralize HIV-1 (42, 43), some acquired mutations shared with the mature VRC01, enabling them to bind a second more

native-like boosting immunogen (43). However, this boosting immunogen, like eOD-GT8, lacked the N276 glycan, conserved in ~95% of viruses. Inclusion of this glycan in the following boosting immunogens will be crucial for neutralization (see below). Indeed, in knock-in mice expressing the mature 3BNC60 heavy chains, immunization with eOD-GT8 and BG505-SOSIP both elicited antigen specific B cells but only the trimer elicited neutralizing antibodies (42). These elegant studies have therefore provided the first stage in an immunization strategy, but clearly highlight the need for boosting with more native-like immunogens.

ENGINEERING AND TESTING NEXT-GENERATION TRIMERIC ENV IMMUNOGENS

The structural characterization of the envelope trimer (10, 11), and the development of native-like trimer immunogens has been a major step forward. The best described structure is the BG505 SOSIP.664 Env which is stabilized using disulphide bonds (SOS) to link gp120 and gp41 (which is truncated at position 664 to delete the transmembrane domain), and a I558P mutation that stabilizes the gp41 ectodomain. This protein is a good representation of the envelope trimer, displaying bNAb epitopes (except the deleted MPER region), while largely shielding the normally occluded epitopes recognized by non-neutralizing antibodies. Structural studies have also defined the glycan shield in the trimeric context, providing our first insights into glycan conservation, processing and the glycan-glycan interactions that shield the Env from many antibodies (44-46). Notably, uncleaved and monomeric gp120 contains more complex glycans than trimer, perhaps a consequence of reduced steric constraints on glycan processing enzymes, further highlighting the importance of trimeric immunogens (45-47).

The immunogenicity of these stabilized trimers has been assessed in rabbits, and elicited antibodies able to neutralize the autologous Tier-2 (neutralization resistant) virus, though strong V3 responses able to neutralize Tier-1 viruses (i.e. viruses with unusual neutralization sensitivity, due to a more open conformation) were also elicited (48). Similar, though weaker, responses were observed in macaques. As the elicitation of non-neutralizing responses may decoy the immune response away from the bNAb epitopes, a further stabilized trimer (SOSIP.v4 trimer) was engineered to skew the intrinsic “breathing” of the Env towards a more closed conformation, as in Tier-2 viruses. This next generation trimer had reduced exposure and therefore immunogenicity of the V3 and CD4i epitopes (49). These stabilized trimers are being extended to include additional envelopes from multiple subtypes, purification approaches and platforms such as liposomal vehicles to block the unglycosylated underside of the trimer that is only exposed, and immunogenic in soluble trimers (49-54). The consistent, albeit low neutralizing responses elicited by these trimers is a step forward as inducing Tier-2 responses by vaccination is rare, and while this is not a “home run”, it forms a base from which to build vaccine strategies that elicit bNAbs.

Promising alternative approaches to the design of stabilized Env trimers include cleavage independent native flexibly linked (NFL) or single-chain (SC) trimers (containing flexible glycine-serine linkers that replace the furin-dependent cleavage site) (55, 56) and the use of

trimer-enriched virus-like particles (which have the advantage of presenting trimers within a native-like lipid membrane) (57). Indeed the latter approach has shown potential in small animal immunogenicity experiments, also eliciting Tier-2 neutralizing responses.

ELICITING TIER-2 NEUTRALIZATION IS LIKELY NECESSARY BUT NOT SUFFICIENT FOR BNAB INDUCTION

Most immunogens elicit antibodies able to neutralize Tier-1 viruses, but these do not correlate with more relevant Tier-2 responses (48), and the targets are generally different. It is thus unlikely that a Tier-1 response can be matured to a Tier-2 response, and indeed the vaccine field is re-evaluating the utility of measuring Tier-1 responses. Strain-specific Tier-2 neutralizing responses, which develop in all infected people, may have greater potential to be driven towards breadth, but in the context of infection only a minority do so (58). Pushing strain-specific Tier-2 responses towards breadth is the next challenge in the vaccine field, and requires understanding the targets of these responses compared to bNAbs, and incorporating diversity into immunogens, to shape maturation of breadth.

Mapping the targets of vaccine-elicited Tier-2 antibodies is providing useful insights. In a study using a cocktail of gp140 trimers from donor CAP206, a subject with anti-MPER bNAbs, Tier-2 antibodies were elicited in some macaques (though not to the MPER) and interestingly overlapped with the Tier-2 neutralizing responses seen in the human donor (suggesting common viral determinants of these specificities). However these bound an unusual “glycan hole” in V5 and loop E, and structural analyses suggested they were unlikely to mature towards breadth (59). Targeting of these “glycan holes” (regions of the underlying Env protein exposed through deletion of normally conserved glycans) occurs often in infection (58, 60, 61), and is an emerging theme in vaccination. Trimer-enriched virus-like particles also elicited Tier-2 neutralizing antibodies that targeted a hole in the “glycan fence” that normally surrounds the CD4bs, through a deleted N197 glycan (57). Similarly, rabbits immunized with the BG505 SOSIP, which lacks the glycan at position N241 targeted this glycan hole (Burton *et al*, Keystone, 2016). This has been interpreted as meaning that immunogen design might benefit from glycan deletion to expose underlying vulnerabilities (57, 62), in the same way that the CD4bs germline-targeting immunogens lack the conserved N267 glycan. However as these missing glycans are often present on circulating viruses, its not clear how (or whether) these Tier-2 neutralizing antibodies can be “educated” into neutralizing viruses that do contain these normally conserved glycans, perhaps by sequential addition of more native “elements” into boosting immunogens. This is likely to be a major focus in the near future.

CONCLUSION

We now have a roadmap defining the steps needed to recruit rare bNAb precursors, and achieve neutralization breadth using immunogens that can drive antibody tolerance of diversity and of glycans (Table 1). However, the long-lived evolution seen in naturally occurring bNAb lineages (17, 20, 24) suggests that retention of mutating B cells within germinal centers might need equally long-lived antigenic stimulation, perhaps in the form of replicating vectors. In addition, supporting a permissive environment for long-term bNAb

development in germinal centers will be crucial, as it is during infection (3, 63-65). The frequent deletion of autoreactive bNAb precursors (recapitulated experimentally in knock–in mice (66)) may suggest an unprecedented need to transiently lower immune tolerance controls during vaccination (26). Human immunogenicity studies using immunogens described above are likely to start in the next year, and will provide key data for the field. Overall, the major advances made over the last year have taken the HIV vaccine field closer than ever before to an HIV vaccine able to elicit broadly neutralizing antibodies.

ACKNOWLEDGEMENTS

We thank Drs Lynn Morris, Jinal Bhiman and Kurt Wibmer for critical comments on the manuscript and useful discussions.

FINANCIAL SUPPORT

PLM is supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa and a U01 grant (AI116086-01). CW receives support from the Collaboration for AIDS Vaccine Discovery (CAVD). PLM and CW receive funding from the Centre for the AIDS Programme of Research (CAPRISA) and the SA Medical Research Council SHIP program.

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KEY POINTS

- Studies of antibody virus co-evolution continue to provide important insights into how neutralization breadth develops
- Common developmental pathways exist for some classes of broadly neutralizing antibodies
- Proof-of-concept studies provide strong support for germline-targeting immunogens having the potential to activate rare precursors of broadly neutralizing antibodies
- Advances in our understanding of the HIV envelope structure continue to improve trimeric immunogen design
- Elicitation of Tier-2 neutralizing antibodies is likely necessary but not sufficient for breadth, with mapping studies highlighting challenges in eliciting breadth

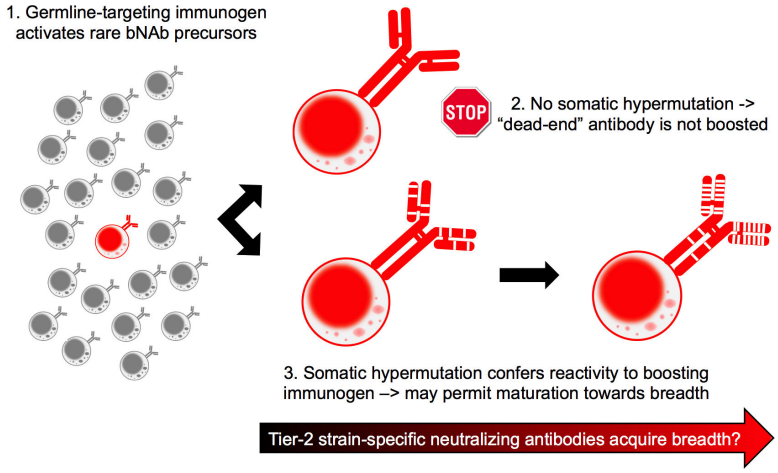

Figure 1. Maturation of breadth requires selection of rare B cell precursors, and their retention and maturation towards breadth

Germline-targeting approaches enable selection and activation of rare B cells, but if these do not acquire mutations that enable them to bind to more native-like boosting immunogens, they will not be boosted. Acquisition of sufficient productive mutations to enable binding to boosting antigens will be required for maturation of Tier-2 strain-specific neutralizing antibodies.

Table 1

Pathway to eliciting broadly neutralizing antibodies through vaccination: recent developments

	Challenges	Proposed solutions
Kickstart bNAb precursors	Rare, frequently autoreactive, and may not bind HV Env	Engineer germline-reactive immunogens
		Screen for germline-reactive natural Env
		Measure early indicators of success (e.g. antibody allele, angle of approach)
Elicit Tier-2 nAbs and drive them towards breadth	Native-like HIV Env immunogens needed, off-target responses to glycan holes are common, breadth may require extensive SHM and tolerance of epitope diversity/ glycans	SOSIP and other trimer platforms (e.g. NFL-trimers and trimer-enriched VLPs)
		Immunogen redesign to reduce off-target neutralization
		B cell-lineage based immunogens
		Cocktails
		Replicating vectors for persisting antigen

Curr Opin HIV AIDS. Author manuscript; available in PMC 2017 November 01.