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Development of Broadly Neutralizing Antibodies from Autologous Neutralizing Antibody Responses

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Abstract

Purpose of Review—Detailed genetic and structural characterization has revealed that broadly neutralizing antibodies (bnAbs) against HIV-1 have unusually high levels of somatic hypermutation, long CDRH3 domains, and the ability to target one of four sites of vulnerability on the HIV-1 envelope (Env) glycoproteins. A current priority is to understand how bnAbs are generated during natural infection, and translate this information into immunogens that can elicit bnAb following vaccination.

Recent Findings—Strain-specific neutralizing antibodies (nAb) can acquire broad neutralizing capacity when the transmitted/founder Env or a specific Env variant is recognized by an unmutated rearranged germline that has the capacity to develop bnAb like features. This could be a relatively infrequent event, as only certain germlines appear to possess inherent features needed for bnAb activity. Furthermore, the glycosylation pattern and diversity of circulating HIV-1 Envs, as well as the state of the B cell compartment, may influence the activation and maturation of certain antibody lineages.

Summary—Collectively, studies over the last year suggest that the development of HIV-1 Env immunogens that bind and activate bnAb-like germlines is feasible. However, more information about the features of Env variants and the host factors that lead to breadth during natural infection is needed to elicit bnAbs through immunization.

Keywords

Broadly neutralizing antibodies; affinity maturation; long CDRH3; unmutated common ancestor; HIV-1 envelope evolution

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Introduction

A number of excellent reviews in the last year have highlighted the abundant new knowledge gleaned from studies on HIV-infected individuals with broadly neutralizing antibodies (bnAbs) and speculated on how such information might inform vaccine design (1-7). It is generally accepted that the 4 major sites of vulnerability on the HIV-1 envelope (Env) glycoproteins are the CD4 binding site (CD4bs), glycan dependent epitopes in V1V2 and near the base of V3/C3, and linear epitopes in the membrane proximal external region (MPER) of gp41. However, the presence of unmapped epitopes for some bnAbs suggests that additional sites may exist (1). bnAbs show unusual genetic features including high levels of somatic hypermutation and selective germline gene usage for CD4bs antibodies, long CDRH3s for antibodies that penetrate the glycan shield, and polyreactivity for some MPER antibodies. bnAbs represent subdominant responses, and sera with breadth mostly comprise single specificities, although rarer cases of multiple specificities have been reported (8, 9*, 10, 11) While bnAbs are found only in a subset of people after many years of infection, almost all infected individuals develop strain-specific neutralizing antibodies (nAbs) that can neutralize autologous virus but not heterologous viruses. Previous studies have shown that these mostly target variable regions, unlike bnAbs that target more conserved sites. However, there is some overlap in these epitopes; for example, both bnAb and strain-specific nAb target the CD4bs, V1V2 and the base of the V3/C3 region (12-18). The question of how these bnAbs arise and whether they mature from earlier strain-specific nAbs is only now being revealed.

Ontogeny of broadly neutralizing antibodies

The high degree of somatic mutation in many bnAbs suggests that they undergo multiple rounds of affinity maturation to acquire breadth. It was unknown whether long CDRH3s also develop through the same gradual process or arise through immunoglobulin gene recombination. Results from 2 longitudinal studies have indicated that the pathway to neutralization breadth differs depending on the epitope being targeted (Figure 1). For CD4bs, the initial B cell recognized the infecting virus but neutralization was only achieved after a sufficient level of affinity maturation, resulting in neutralization firstly of the autologous virus and later heterologous viruses (19**). In a second study that followed the development of a V1V2 directed antibody lineage, the unmutated common ancestor (UCA) both bound and neutralized the infecting virus. Breadth required a more modest level of somatic hypermutation than seen for the CD4bs lineage, perhaps explaining how breadth developed early in this donor (20**). This study also revealed that the long CDRH3 characteristic of this class of antibody developed in this case as a result of a recombination event prior to encountering antigen.

The use of deep sequencing has expanded our ability to interrogate the antibody response to HIV-1 even without the benefit of longitudinal sampling. Analysis of the patient from whom the CD4bs bnAb VRC01 was isolated identified clonal relatives that were less somatically mutated (21). Testing of these archived members of the lineage demonstrated the need for extensive affinity maturation for neutralization. Similarly for the V3 glycan PGT121-like antibodies, the degree of somatic mutation was associated with neutralization although

antibodies with half the level of somatic hypermutation still showed significant neutralization (22). bnAbs also show high levels of mutation outside the antigen-binding sites with these mutations in the framework regions required for broad neutralizing activity (23).

While the focus has been on subjects with significant breadth, analysis of large cohorts reveals that most infected individuals develop some level of cross-neutralizing activity suggesting breadth occurs as a continuum rather than an extreme phenotype (17, 24-26). The immune system is capable of making potent neutralizing antibodies, but why all strain-specific nAbs do not eventually mature to acquire breadth remains an important and unanswered question. This may be related to unsuitable germline gene usage, auto-reactivity, suboptimal angle of approach due to glycan interference or insufficient long-term antigenic stimulation.

Role of viral evolution in shaping broadly neutralizing antibody responses

Several studies have suggested that viral factors contribute to the development of breadth, beyond simply providing sufficient antigenic stimulation in the form of high viral loads. During the last year, there has been progress in defining the precise viral mechanisms driving improved breadth, through detailed longitudinal studies. Moore *et al*. described the evolution of a N332 glycan-dependent bnAb epitope in the V3/C3 region, absent on the infecting virus, through immune escape from earlier strain-specific nAb responses that targeted the same region of the envelope (27). This study showed neutralization escape from strain-specific nAbs resulted in viral convergence towards conserved glycan motifs, creating the epitope for later broad nAbs (28). In a subsequent study breadth was similarly associated with a reduced probability of the N332 residue being glycosylated on the infecting virus (29).

A second mechanism was identified in an infected subject who developed sequential broad neutralizing antibodies to both V2 and the CD4bs. Here, viral escape drove the deletion of a highly conserved glycan at N160 in V2 (9*) resulting in exposure of the CD4bs, which immediately became the target for the next wave of bnAbs. Thus viral escape may force the exposure of otherwise occluded conserved epitopes, facilitating the development of breadth. Intriguingly, in three of four studies where multiple bnAbs emerge, the antibodies target V2 and the CD4bs, suggesting a common developmental pathway for consecutive specificities (8, 9*, 10, 30).

Neutralization breadth is known to be associated with increased viral diversity (25, 31, 32). Recent studies have extended these analyses to examine the contribution of dual or superinfection to breadth. Powell *et al*. showed that dual infection resulted in enhanced breadth, though in this study confounding factors such as duration of infection could not be excluded (33). A more recent study reported both increased breadth and potency in superinfected individuals, with breadth emerging within a year of superinfection, independently of viral load and CD4+ T cell counts (34*). Interestingly, in this study, both intersubtype superinfection and persistence of both infecting viruses was associated with breadth, suggesting that a more divergent circulating viral population was a major

contributing factor. Finally, in two studies describing the maturation of bnAbs from binding/strain-specific antibodies (see ontogeny section above) (19**, 20**), extensive viral diversification preceded the development of breadth, further supporting the notion that multiple circulating versions of epitopes may drive breadth.

In the first study to propose a mechanism for these observations, Murphy *et al*. showed that two antibody light chain variants paired with a single heavy chain displayed differential neutralization of autologous viruses containing escape mutations in the base of V3/C3 region. This suggested that viral evolution drives strain-specific nAbs to recognize amino acid variants within a given epitope, implying that escape variants increase breadth (15*). However in that study, bnAbs did not develop against this early epitope. Wibmer *et al.* subsequently detailed the development of both broadly neutralizing V2 and CD4bs nAbs in response to the sequential accumulation of escape mutations, with later plasma responses able to tolerate new epitope variants (9*). This ability to recognize multiple immunotypes, emerging as a result of neutralization escape was also associated with increased breadth in other studies (20**, 35, 36), providing a model for the maturation of bnAbs that is consistent with the viral diversification observed prior to breadth (Figure 2) (19**, 20**). The increasing appreciation of how such antibodies mature and acquire breadth provides a template for the design of novel sequential immunization strategies (1, 9*).

Host factors

In addition to viral evolution, host factors have been implicated in the development of breadth. A genome-wide association study (GWAS) revealed a decreased prevalence of the protective HLA allele B*57 in individuals with neutralization breadth, while the unfavorable HLA allele B*07 was enriched in this population (37). CSF-1R, the receptor for colony stimulating factor-1 and a regulator of macrophages, also showed a potential association with neutralization breadth. While no B cell-specific genetic markers were identified in this GWAS study, the functional state of the B cell compartment is likely to influence the development of breadth. One study reported that more peripheral naïve B cells, but less tissue-like and activated memory B cells (a phenotype more like healthy individuals) favored neutralization breadth (38). In contrast, Boliar *et al*. found that neutralization breadth was present despite marked dysregulation of peripheral B cell subsets, and that hypergammaglobulinemia, a measure of B cell dysfunction, correlated directly with neutralization breadth (39). Additionally, *in vitro* binding of gp120 to peripheral B cells via the $\alpha 4\beta 7$ integrin may contribute to suppression of B cell activation and proliferation *in vivo* through TGF-$\beta 1$ production and other mechanisms that could interfere with the development of robust neutralizing activity (40). Current approaches to enhance B cell/antibody responses during immunization include incorporation of adjuvants and stimulatory cytokines such as GM-CSF, APRIL, and IL-21 directly into gp120 (41-43). Adjuvants will undoubtedly also be incorporated into human vaccine trials to enhance antibody responses (44, 45) and see the review on “Modulation of HIV-1 Immunity by Adjuvants” in this same issue. There is also evidence that a higher frequency of a functional memory subset of T follicular helper cells in the periphery in early infection, and maintenance of this population over time, may contribute to the development of bnAb activity (46*). Taken together, these studies

illuminate avenues to enhance T and B cell responses that could promote the development of bnAbs.

Activation of germline B cells

A significant obstacle to generating bnAbs during natural infection or by immunization could be that Env is poorly recognized by the germline-like (GL) versions of the B cell receptors (BCRs) for these antibodies. To investigate this, various methods to infer the reverted GL version of mature bnAbs (or UCA) have been employed in the absence of longitudinal data. Despite the ability of bnAbs to neutralize diverse HIV-1 Envs, the corresponding GL antibodies, soluble or expressed on the surface of a B cell, often do not recognize those same Envs produced as recombinant proteins (47, 48). However as pointed out above, in cases where bnAb lineages have been investigated from early infection, reverted GL versions of the mature antibodies do recognize an early autologous Env variant (19**, 20**).

Targeted modification of Env proteins can increase recognition by GL versions of bnAbs. Removal of conserved glycans known to modulate sensitivity to CD4bs bnAbs facilitated activation of B cells expressing the GL BCRs of CD4bs bnAbs (49*). Removal of glycan N276, which is conserved in 95% of HIV-1 strains, eliminated the steric constraints of the GL antibody versions. Computational design has been used to develop an optimized Env immunogen that is recognized by CD4bs bnAbs and their GL precursors (50**). The final construct had high affinity for the mature and GL derived CD4bs bnAbs. In a parallel study, a chimeric CD4bs antibody containing the GL VH and mature VL showed a similar angle of approach and outer domain contacts as the mature antibody, but lacked critical inner domain and bridging sheet contacts (51). Together these studies suggest that it is difficult to identify naturally occurring Envs that will elicit bnAbs, but that immunogens can indeed be rationally designed to potentially elicit CD4bs bnAbs.

Given the potential to develop and test Env immunogens that are recognized by GL-bnAbs, it will be important to understand the pathways to bnAb in nonhuman primates. To this end, Sundling *et al*. showed that VH gene family structure is similar between humans and rhesus macaques (RM), with high homology (average of 92%) between VH genes (52*). These investigators also recovered CD4bs mAbs from an immunized RM that had high affinity for gp120 and tier 1 neutralizing capacity, modest somatic hypermutation levels (~5% from germline), and relatively longer CDRH3 domains. However, these CD4bs mAbs were derived from RM VH3 and VH4 families, as opposed to the human VH1 germlines that give rise to most CD4bs bnAbs. Env immunogens designed to interact with GL-versions of human bnAbs may therefore not activate the expected germline in RMs (50**). Thus, using nonhuman primates to model the development of bnAbs will require a thorough understanding of the biological similarities and limitations.

Vaccine induced responses in human subjects

The ultimate goal of eliciting bnAbs following immunization is still elusive. The RV144 human vaccine trial elicited cross-reactive but weakly neutralizing antibodies directed against linear and conformational epitopes in V2 that were inversely correlated with the rate

of HIV-1 infection (53). Liao *et al*. found that mAbs from a single vaccinated RV144 subject targeted residue 169 in the V2 loop, had tier 1 neutralizing activity, and mediated killing of HIV-1 infected CD4 T cells *in vitro* (54). Further studies suggest that the vaccine elicited V2 mAbs recognize a conformation of V1V2 that is distinct from that recognized by V1V2 bnAbs (54, 55). This has led to the development of V1V2 glycopeptide immunogens that bind with high affinity to GL and mature V1V2 bnAbs, but with much lower affinity to the vaccine elicited, strain-specific V2 mAbs (56*). It remains unclear whether these vaccine-induced antibodies are the precursors of bnAbs, or whether they can be coaxed along the pathway to breadth with novel immunization strategies.

Conclusion

The last year has seen significant progress in defining the pathway to breadth and identifying important viral and host factors. However, additional longitudinal studies on infected individuals that develop bnAbs are needed to determine whether there is a limited number of pathways to breadth, and whether these are epitope-specific. In particular, given the frequency of V3/C3 as a target, and the ability of these bnAbs to use multiple germline genes, this class of antibodies should be a focus (11, 24, 57). It may also be important to examine individuals with limited or no breadth, to determine if subdominant bnAb responses (i.e. aborted lineages) can be detected by deep sequencing. Existing studies have highlighted the difficulties in eliciting bnAbs. These roadblocks would need to be overcome in order to stimulate effective and broad neutralizing antibody responses by vaccination. For example, how do we stimulate particular germline alleles? How do we stimulate low frequency B cells with long CDRH3? Will it be possible to achieve the required level of somatic hypermutation by vaccination? Given the rapid pace of new discoveries in this field, we anticipate that the coming year will make inroads into answering many of these questions.

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Key Points

1. The pathway to neutralization breadth differs depending on the epitope targeted, but all require significant somatic hypermutation.
2. Long CDRH3 regions, formed through germline immunoglobulin gene recombination, may be particularly well suited for developing bnAbs that penetrate the glycan shield.
3. Viral diversity plays a key role in the development of neutralization breadth, regardless of the epitope targeted, with specific glycan changes near the base of the V3/C3 region and in V1V2 playing key roles.
4. Functional memory T follicular helper cells may favor the development of breadth, but more studies are needed to define the role of additional host factors.
5. Env immunogens can be designed to better engage germline-like B cell receptors for bnAbs derived from humans, but testing them in nonhuman primates may be complicated by germline differences.

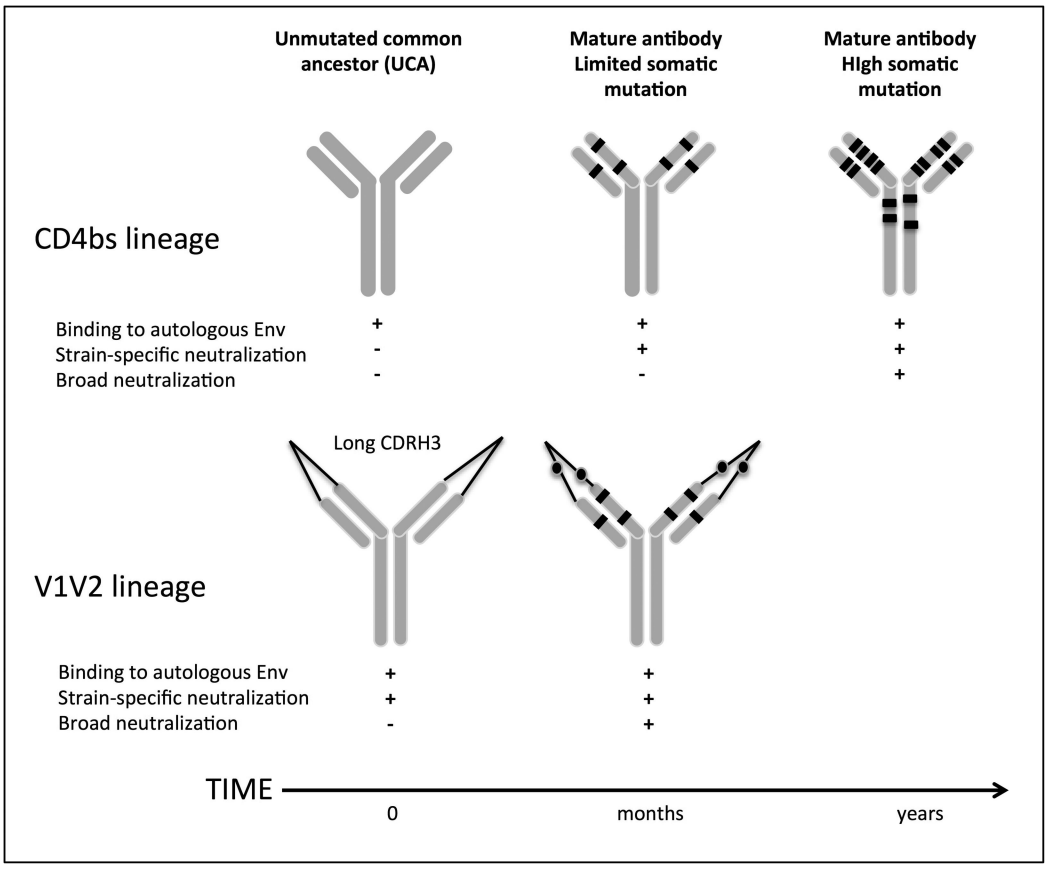

Figure 1. The pathway to neutralization breadth

Maturation of a CD4bs lineage required somatic hypermutation for both strain-specific and broad neutralizing capacity (19). This is consistent with high levels of somatic hypermutation seen in other CD4bs bnAbs e.g. VRC01. In contrast, V1V2 directed bnAbs selected a germline with preexisting long CDRH3s and had the inherent ability to bind and neutralize the founder virus. Additional levels of somatic hypermutation were needed to mediate broad neutralizing activity (20).

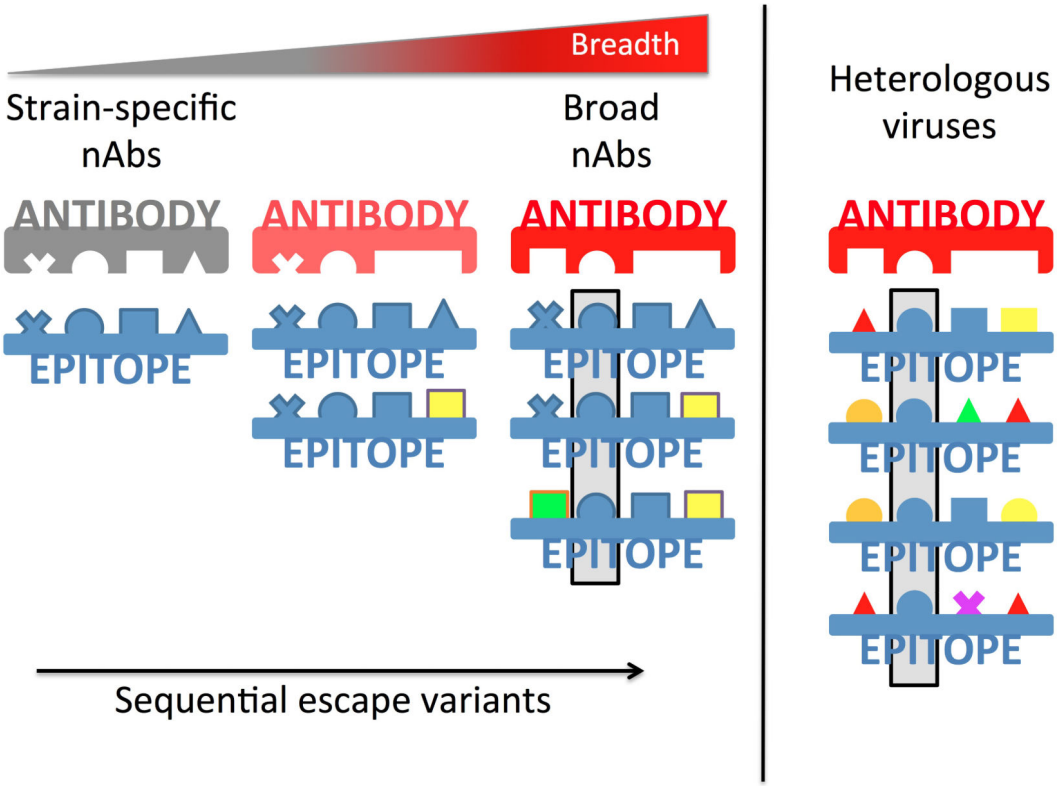

Figure 2. Model for the maturation of strain-specific plasma responses to acquire breadth

Strain-specific antibodies (gray) recognize defined epitopes (shown in blue, in a lock-and-key schematic). These antibodies drive viral escape mutations (represented as multi-colored shapes) within epitopes. The emergence of sequential escape mutations creates multiple immunotypes (or epitope variants) within a single infected subject. Maturation of nAbs to tolerate multiple residues at a given position (e.g. either a triangle or a square) or to recognize a smaller core epitope (e.g. the invariant sphere at the second position within the epitope, highlighted in the gray box) thereby better encompasses the global epitope variants in heterologous viruses (shown on the right), and enables the development of broad neutralization antibodies (red).