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The Neutralizing Antibody Response to the HIV-1 Env Protein

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Abstract

Objective: Background: A vaccine able to elicit broadly neutralizing antibodies capable of blocking infection by global viruses has not been achieved, and remains a key public health challenge.

Objective: Objective: During infection, a robust strain-specific neutralizing response develops in most people, but only a subset of infected people develop broadly neutralizing antibodies. Understanding how and why these broadly neutralizing antibodies develop has been a focus of the HIV-1 vaccine field for many years, and has generated extraordinary insights into the neutralizing response to HIV-1 infection.

Results: This review describes the features, targets and developmental pathways of early strain-specific antibodies and later broadly neutralizing antibodies, and explores the reasons such broad antibodies are not more commonly elicited during infection.

Conclusion: The insights from these studies have been harnessed for the development of pioneering new vaccine approaches that seek to drive B cell maturation towards breadth. Overall, this review describes how findings from infected donors have impacted on active and passive immunization approaches that seek to prevent HIV-1 infection.

Keywords

HIV-1 envelope glycoprotein; broadly neutralizing antibodies; virus-host co-evolution; viral escape; immunogens; passive immunity

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CONFLICT OF INTEREST

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1. INTRODUCTION

The HIV-1 envelope glycoprotein, which mediates entry into host cells, is the most variable of the viral proteins, and may differ by as much as 30% between different genetic subtypes. Indeed, even within a single infected individual, the level of envelope diversity can be extraordinary. This diversity, enabled by an error-prone reverse transcriptase, is largely a consequence of pressure exerted by the vigorous neutralizing antibody response to HIV-1 infection. The likelihood that a preventative HIV vaccine will need to elicit neutralizing antibodies, and the failure of traditional vaccine approaches, has resulted in extraordinary progress in understanding anti-HIV-1 neutralizing antibody kinetics, targets, and structures. This review will describe what is currently known about the neutralizing antibody response during HIV-1 infection, and how these findings provide both opportunities and hurdles for the design of an effective HIV vaccine.

2. THE EARLY HUMORAL RESPONSE TO HIV-1 INFECTION

2.1. The HIV-1 Envelope Glycoprotein is Recognized by Early Binding Antibodies

The HIV-1 envelope glycoprotein consists of a heterotrimer of three gp120 and three gp41 molecules that are conformationally dynamic and highly sequence variable, particularly in the antibody accessible regions of the envelope. These trimers are relatively sparsely arrayed, with 8–10 spikes per virion [1], in contrast to the greater spike density of viruses such as influenza. An additional defence is the massive levels of glycosylation that characterise the HIV-1 Env. These glycans are so densely packed on the trimer that they occlude much of the underlying protein surface [2]. Immunological decoys also exist in the form of nonfunctional envelope proteins such as gp41 stumps, and monomeric forms of gp120 that expose epitopes occluded within the trimer [3]. The functional envelope spikes therefore present an enormously difficult target for the immune system.

Despite these immunological defences, most infected people mount a robust humoral immune response (Fig. 1). The first B cell response to transmitted HIV-1 consists of binding antibodies that develop within 8 days of detectable viremia, and that initially exist as antigen-antibody complexes [4]. These are followed by circulating anti-gp41 binding antibodies which develop within 2 weeks of infection [5]. The first gp120 responses, binding the V3 region, emerge 2 weeks later [5, 6], however these binding antibodies have no substantial neutralizing (or infection-blocking) capacity for transmitted viruses, and do not exert detectable selective pressure on the envelope.

2.2. Strain-specific Tier-2 Neutralizing Antibodies Develop in All Infected Donors

The first neutralizing antibodies (NAbs) against the infecting virus are substantially delayed compared to those elicited by most viral infections. HIV-1 neutralizing antibodies emerge only 12–20 weeks post-infection [7, 8], perhaps a consequence of the challenging Env properties described above. These initial NAbs are almost invariably strain-specific [7–11], suggesting they target variable regions of the virus. Indeed, studies of early viral evolution, and antibody mapping studies have confirmed that solvent-exposed variable regions such as V1V2, the C3 region in subtype C viruses (which is highly variable despite its designation as

a conserved region) and the V4 region are early targets of these NABs, which often reach titers exceeding 1:4,000 [12–16]. In some cases, these early NABs have been shown to target glycan-deficient patches (or “glycan holes”) on the viral envelope that are normally occluded [17]. The variable nature of these regions results in rapid viral escape by mutations, insertions, deletions and shifting of glycans to change or cover bare patches on the Env [11]. Indeed, titers as low as 1:40 have been shown to be sufficient to drive viral escape, suggesting there is little fitness cost to escape from these early strain-specific antibodies [18]. As viral escape occurs, sequential waves of antibodies develop, some of which target emerging viral escape variants [19], resulting in a constant arms race between host immune responses and viral escape mutants.

Although these early NAB responses are almost invariably strain-specific, they are nonetheless capable of neutralizing “Tier-2” viruses. Tier-2 viruses, which are defined by their overall relative resistance to neutralization [20] are representative of the majority of transmitted viruses [21]. This is unlike the rarely transmitted Tier-1 viruses which can be neutralized by V3 antibodies, a consequence of their unusually open envelope conformations [21, 22]. Until recently, Tier-1 responses were the only antibodies reliably elicited by HIV vaccination. However, Tier-2 responses are now sporadically elicited in more recent vaccine regimens, highlighting their improvement [23, 24]. Studies of infected subjects provide useful opportunities to understand the biological relevance of vaccine responses, and their potential for maturation towards neutralization breadth (see section below on vaccine implications).

3. BROADLY NEUTRALIZING ANTIBODIES

3.1. Broadly Neutralizing Antibodies Develop in Some Chronically Infected Donors

While early strain-specific Tier-2 neutralizing antibodies exert substantial pressure on the viral population, their lack of breadth limits their value for HIV vaccine design. Thus, the focus in cohort studies in recent years has shifted to the development of broadly neutralizing antibodies (bNABs), able to block infection by diverse viruses from multiple subtypes and geographic locations. Such studies have shown that almost all infected people develop antibodies that have some cross-neutralizing activity [25, 26]. However, only about 20–30% of adults develop responses that are considered truly broadly neutralizing, generally after many years of infection [26–29] (Fig. 1). These are the types of antibodies a vaccine should ideally elicit. Of interest, the proportion of chronically infected children who develop broad and potent neutralizing antibodies is considerably higher, with 70% of children mounting responses that are equivalently broad to the top 20% of adults infected with the same subtype of HIV-1 (Fig. 2) [30]. These bNAB responses in children have been reported to develop earlier than is common in adults, in some cases within the first 2 years of life [31, 32]. Future studies to determine why children mount better responses, and whether their development is generally accelerated compared to adults, will thus be of interest for the vaccine field. Unfortunately, the development of bNABs confers no clinical benefit to those people in whom they develop, a consequence of the ability of the viruses to escape even these antibodies that must target more conserved epitopes [26]. Escape from bNABs has been documented in many studies of infected donors who develop breadth [33–37], and also

in HIV-1 infected donors passively infused with broadly neutralizing monoclonal antibodies [38–41], though few studies have assessed the fitness costs, if any, of these viral mutations.

The development of improved technologies for the isolation of monoclonal antibodies from HIV-1 infected donors has revolutionized studies of the broad neutralizing response to HIV-1. The first generation of bNAbs to HIV-1 (monoclonal antibodies such as b12, 2G12, 2F5, and 4E10) were isolated by phage display or B cell immortalization, and selected for binding to Env peptides or proteins [42]. This was followed by B cell culture and micro-neutralization (functional) screens, and by the use of flow cytometry-based isolation of single B cells using peptide and protein “baits”. Most recently, the design of soluble, recombinant Env trimers and virus-like particles comparable to native Env trimers has provided alternative antigen-specific B cell selection strategies [43–45]. These strategies have enabled the isolation of many dozens of antibodies from elite neutralizers, and some of these have exceptional breadth (approaching 99% of circulating viruses) and potency in the picomolar range [44, 46–48]. As well as providing insights into the host immune response, these remarkable antibodies are now increasingly being tested by passive immunization for either HIV prevention or treatment, with the likelihood of this being successful supported by extensive animal and preclinical human data [38–40, 49–52]. This approach will almost certainly require the use of combinations of bNAbs, both to maximize coverage and (in the context of treatment) to reduce viral escape [53].

3.2. Broadly Neutralizing Antibodies Target Much of the HIV-1 Glycoprotein Trimer

The isolation of monoclonal bNAbs from donors with breadth has enabled the identification of viral vulnerabilities to be targeted in vaccine strategies. Currently, five bNAb targets have been identified on the HIV-1 envelope, namely the V2-glycan site, the V3-glycan epitope, the membrane proximal external region (MPER), the CD4 binding site (CD4bs) and the gp120-gp41 interface, including the fusion peptide [54, 55]. Isolation of multiple antibodies to each of these sites from different donors has enabled us to define common features for some of these antibodies, which can thus be classified as “classes” of bNAbs. In some cases, especially a subset of the CD4bs-directed antibodies called the VH-gene restricted or VRC01-class antibodies, the similarities in genetic structure, contact residues and angle of approach between donors are profound [56–58]. Similarly, the prototypical V2-directed bNAbs typically share many features in their structure and mode of recognition [59–61]. Such commonalities are amenable to precise epitope-specific immunogen engineering approaches, described in more detail below.

In contrast, antibodies to other sites exhibit much more variation in their mode of neutralization. For example, antibodies that recognize the V3-glycan epitope, while largely centered on a single glycan moiety at N332, have substantially more variation in their contact sites, and bind a series of overlapping glycan-dependent epitopes in V3, V1 and V4 [47, 62, 63]. This is thus one of the three bNAb sites that have been classified as supersites [55], composed of multiple sub-epitopes that differ subtly, but share the same core contact residues and can be accessed using various immunological solutions and angles of approach.

Already, the definition of distinct bNAb epitopes is blurring as more and more antibodies are isolated, and indeed bNAb sites now span a near-continuum from the apex of the trimer

down to the membrane proximal external region [55]. The continuing isolation of more mAbs, including those from HIV-1 infected children, where the targets have not yet been clearly defined [31], is likely to define both additional bNAb epitopes and supersites in the coming years.

3.3. Factors Associated with the Development of Broadly Neutralizing Antibodies

Many large cohort studies have sought to define host and viral factors that contribute to the development of breadth. These include duration of infection, high viral load, viral diversity and low CD4 T cell counts [26–29, 64]. In children, the high levels of bNAbs are also associated with long-term and high levels of viremia [30]. These associations highlight the substantial antigenic stimulation generally required to drive the high level of somatic hypermutation associated with bNAbs, though there are cases of viral controllers who develop bNAbs [65].

Beyond these gross measures of antigen load, there is evidence for subtler viral contributors to breadth. For example, infection with subtype C viruses is associated with higher levels of bNAbs, and for enrichment of V2-glycan targeted responses compared to subtype B infections which more commonly elicit CD4bs antibodies [27, 64]. Whether viruses that infect donors who later go on to develop bNAbs have unusual characteristics is not yet clear, though there are intriguing hints that this might be the case (Trkola *et al.* Keystone 2017), a finding that would indicate that envelopes from bNAb donors should be harnessed more effectively for immunogen design. This possibility is also being actively pursued in SHIV infection models that aim to recapitulate aspects of viral evolution using viruses from broad neutralizers for studies of bNAbs (Shaw *et al.* Keystone, 2016).

Host factors also play a major role in the development of neutralization breadth, with associations reported between bNAbs and HLA genotype [27] and black ethnicity [64]. Perhaps more important for translation to vaccine design is the role of T follicular helper cells and germinal center function in contributing to the maturation of bNAbs, a finding confirmed in multiple cohorts [66, 67]. These studies rely on circulating “proxies” of cells that are normally only present in the germinal centers, which are the sites of antibody maturation. However, an active area of investigation is the use of fine needle aspirates to directly sample germinal center B cells and T follicular helper cells during immunization [68], and similar studies in donors who develop bNAbs during infection may be informative.

3.4. How bNAbs Mature to Acquire Breadth from their Strain-specific Precursors

The late emergence of bNAbs, generally only years after the initial strain-specific NAb responses, and the overlapping epitopes they targeted suggested that a subset of strain-specific neutralizing antibodies matured to acquire breadth. Understanding this pathway may provide a template for HIV vaccine design, and several elegant and detailed studies of the ontogeny of bNAbs have provided insights into their maturation from these early strain-specific precursors [48, 69–73].

These studies have generally used similar strategies: i) isolation of monoclonal bNAbs, ii) next generation antibody sequencing to infer the evolutionary history and the bNAb

precursor (referred to as the unmutated common ancestor) and iii) parallel virological studies of escape pathways to define the role of viral evolution (Fig. 3).

This approach has enabled extraordinarily deep analyses of the virus-host co-evolutionary processes that lead to breadth.

The findings of these co-evolutionary analyses, reviewed elsewhere in detail [73, 74], show that bNAbs are derived from precursors that bind (and sometimes neutralize) the transmitted/founder virus, or another early viral variant [69, 70]. These early strain-specific responses result in viral mutations within the epitope, as the virus attempts to escape this initial response. Alternatively, early epitope variants are created by unrelated “helper” neutralizing antibodies that target an overlapping epitope and similarly drive viral mutations [71]. These emerging viral mutants in turn are recognized by maturing descendants of the precursor antibodies, generated during affinity maturation. In this way, the (now swarms) of viral variants select diverse swarms of related “sister” antibodies, some of which have considerable neutralization breadth. Thus, constant exposure of the immune system to diverse viral variants in an ongoing “arms race” selects antibody lineages that are able to tolerate viral diversity [69], and thus indirectly drives the maturation of bNAbs.

3.5. Features of Broadly Neutralizing Antibodies

Many bNAbs have unusual features that provide a variety of challenges both to their natural development and for vaccine approaches. A key feature of many bNAbs is their high levels of somatic hypermutation, indicative of the prolonged maturation pathway described above. These mutations are present both in the complementarity determining regions of the antibodies, but also in the usually conserved framework regions of the antibody structures [75]. Although some of these mutations may be redundant for breadth, as illustrated for the CD4bs bNAbs VRC01 and for membrane proximal external region bNAbs 10E8 [76, 77], the precursors of bNAbs are strain-specific [48, 70, 72] highlighting the importance of acquiring some degree of somatic hypermutation in developing breadth.

Furthermore, many bNAbs display some degree of autoreactivity or polyreactivity [78] which present an additional challenge for their development. Interestingly, efforts to engineer enhanced breadth and potency into isolated bNAbs monoclonal antibodies sometimes results in enhanced polyreactivity [79], suggesting that there is a very fine balance between useful affinity maturation and polyreactivity in the maturation of these lineages [80].

Lastly, the highly constrained accessibility to conserved Env epitopes sometimes necessitates the selection of precursors that have unusually long or short antigen binding loops. For example, many CD4 binding site bNAbs have extremely rare, short antigen binding loops that avoid steric clashes with hypervariable regions and glycans [81]. In contrast, V1V2-directed bNAbs isolated thus far have unusually long, anionic antigen binding loops that are associated with autoreactivity, and thus their precursors are frequently deleted through tolerance mechanisms [48, 82–86]. The pool of potential precursors for such bNAbs is extremely limited.

3.6. Why don't all Chronically Infected Donors Make Broadly Neutralizing Antibodies?

As discussed above, many people develop some degree of cross-reactivity in their plasma, however elite neutralizers are rare. Although bNAbs are enriched in individuals with higher viral loads, many people who have equivalently high viral loads over many years do not develop breadth [74]. Indeed, some donors fail to develop any cross-reactivity after years of infection [26]. Most studies thus far have focused on elite neutralizers, however less is known about why this is not a more common outcome of chronic infection.

A potential bottleneck for the development of bNAbs is the activation of the rare B cell precursors, which are in some cases present at very low levels. A second roadblock is the engagement of these precursors by a cognate viral antigen which is a stochastic event. While some viral envelopes have been shown to better engage these precursors [59, 60, 87], overall the necessary specific virus-B cell interaction is likely to be a low probability event, enhanced in the context of high viral loads.

Another aspect that may curtail the maturation of breadth is the rate of viral escape, a consequence of antibody targeting. Rapid viral escape, possible for antibodies that target highly variable regions, will likely deprive B cells of the necessary antigenic stimulation required for their continued maturation and such lineages will not acquire high levels of somatic hypermutation. In contrast, studies of donors who develop bNAbs are characterized by slow viral escape from bNAb lineages, sometimes over a period of years [48, 70]. Of note, escape from passively administered bNAbs is not necessarily as slow as escape during infection. Indeed, in a recent clinical trial of the V3-glycan bNAb 10-1074, over 90% of viruses had escaped within four weeks of infusion [40]. Thus, the intrinsic “escapability” from bNAbs targetting conserved epitopes may also be impacted by a more dynamic interplay of viral fitness and escape in the context of polyclonal plasma [71, 97].

A final impediment for bNAb development is that not all antibody maturation leads to breadth. Within a bNAb line-age, we have highlighted “off-track” antibodies that acquire the same level of somatic hypermutation as bNAbs, but which have little to no breadth [69]. Favorable antibody maturation is in response to autologous viral variants within that donor, with the acquisition of breadth being a side-effect of that evolution (Sacks, Moore *et al.*, unpublished). Overall, defining the reasons for the lack of maturation towards breadth will require additional studies, both of donors who remain strain-specific for many years despite high levels of antigenic stimulation, and of antibodies within bNAb lineages that fail to acquire breadth.

4. WHAT VACCINE LESSONS HAVE WE LEARNED FROM STUDIES OF INFECTION?

One of the main reasons for the focus on the neutralizing response of HIV-1 infected donors who develop bNAbs is the hope that these studies will inform the design of an HIV vaccine. These studies of bNAb evolution have identified several hurdles that require the use of innovative strategies. The first of these, recruiting the correct precursor B cells with features amenable to maturation into bNAbs has spawned an array of germline-targeting approaches.

These include scaffolded proteins with features designed to enrich for B cell precursors, an approach that has been pioneered for the CD4 binding site antibodies [81, 88–90], but is now being expanded to other epitopes through engineering or identification of ‘bNAb-initiating envelopes’ [59, 60, 91]. An alternative approach to enriching the pool of precursors is the transient relaxation of immune tolerance mechanisms through checkpoint inhibitors, now being tested in non-human primates (Bradley *et al.*, HIV Vaccines Keystone, 2017).

The second major hurdle is the high levels of somatic hypermutation (as high as 30%) that appear to be required for breadth. This level of maturation is considerably higher than has been achieved through protein vaccination thus far. This, and the detailed understanding of the co-evolution between virus and host have led to the notion of sequential immunization or B cell lineage design, first proposed by Barton Haynes and colleagues [92], and now being widely pursued, with the first experimental confirmations of the validity of this approach emerging in the literature [93]. This approach has the potential to drive higher levels of somatic hypermutation, but is also based on the premise that clever use of immunogens may lower the threshold required for breadth by enriching for the “right” mutations [76]. Studies of lineages that exhibit breadth with more moderate levels of somatic hypermutation, such as pediatric bNAbs [32] and the recently described CD4bs PC63 lineage (Landais *et al.*, HIV Vaccines Keystone 2017), will prove useful in the selection of tailored immunogens

Another approach (which both complements and extends the germline-targeting approaches above) is the use of native-like Env trimers. This has been an area of enormous growth over the past 5 years, reviewed in more detail in [94]. The design of stabilized soluble trimers, and advances in their expression and purification have provided intriguing immunogenicity data with periodic Tier-2 neutralization, rarely achieved in previous vaccine regimens. These Tier-2 NABs generally target rare glycan holes [95], similar to the strain-specific NABs seen frequently in early infection (described above). However, during infection, the development of breadth is not determined by the overall Tier-2 autologous neutralizing responses, which do not differ between donors who develop breadth and those who remain strain-specific over many years [7, 26]. Whether these glycan-hole directed antibodies can be matured towards breadth is likely to be a major question in the coming years. The lack of correlation of Tier-2 NABs with breadth during infection highlights the remaining challenges in achieving breadth, despite these promising data.

CONCLUSION

The neutralizing response to HIV-1 infection has been explored with enormous depth over the last decade. Much is now known about how bNAbs, which many consider necessary for an effective preventative HIV vaccine, develop during infection. These studies have generated novel immunological and virological insights into HIV-1 infection, that are generally applicable to other highly mutable pathogens. These studies are the basis of innovative vaccine strategies that seek to recruit appropriate B cells, and drive them towards breadth. Structural biology and engineering has been used to design an extraordinary range of immunogens, and human immunogenicity trials of some of these are likely to begin within the next year, and will be crucial as the lessons from infection described in this review are translated into vaccine design. Lastly, the identification of bNAbs with

exceptional breadth and potency has led to their use for passive vaccination, which bypasses the considerable immunological hurdles described above. Ongoing and planned clinical trials will provide important insights into protective correlates which will further inform active vaccine approaches as well as directly providing tools for HIV prevention and treatment.

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LIST OF ABBREVIATIONS

bNAbs	Broadly neutralizing antibodies
CDbs	CD4 binding site
Env	Envelope
HLA	Human leukocyte antigen
NAbs	Neutralizing antibodies

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Fig. (1).
Schematic depicting the timing of the development of binding antibodies, strain-specific Tier-2 neutralizing antibodies and broadly neutralizing antibodies.

Neutralization breadth at ~5 years p.i.

Fig. (2).

Comparison of the development of broadly neutralizing antibodies in adults and children at approximately 5 years post-infection. Adapted from (26) and (30).

Fig. (3). Co-evolutionary studies rely on i) bNAb isolation from HIV-1 infected donors, ii) next generation antibody sequencing to infer the evolutionary history of antibody lineages and iii) virological studies of escape pathways to define the role of viral evolution in shaping breadth. Adapted from [48, 69, 96].