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Role of immune mechanisms in induction of HIV-1 broadly neutralizing antibodies

Laurent Verkoczy[1,2,3], **Garnett Kelsoe**[2,4], **M. Anthony Moody**[1,5], and **Barton F. Haynes**[2,3,4]

[1]Duke Human Vaccine Institute, Duke University Medical Center, Durham, North Carolina, USA

[2]Department of Medicine, Duke University Medical Center, Durham, North Carolina, USA

[3]Department of Pathology, Duke University Medical Center, Durham, North Carolina, USA

[4]Department of Immunology, Duke University Medical Center, Durham, North Carolina, USA

[5]Department of Pediatrics, Duke University Medical Center, Durham, North Carolina, USA

Abstract

Although antibodies can be elicited by HIV-1 infection or immunization, those that are broadly neutralizing (bnAbs) are undetectable in most individuals, and when they do arise in HIV-1 infection, only do so years after transmission. Until recently, the reasons for difficulty in inducing such bnAbs have been obscure. Recent technological advances in isolating bnAbs from rare patients have increased our knowledge of their specificities and features, and along with gene-targeting studies, have also begun uncovering evidence of immunoregulatory roadblocks preventing their induction. One critical avenue towards developing an effective HIV-1 vaccine is to harness this emerging information into the rational design of immunogens and formulation of adjuvants, such that structural and immunological hurdles to routinely eliciting bnAbs can be overcome.

Introduction

One key correlate of protection in most effective FDA-approved vaccines is the generation of antibodies that inactivate or neutralize the infectious agent [1]. It is therefore widely held that the rapid elicitation of potent HIV-1 neutralizing antibodies systemically and at mucosal sites would be an important component of a preventive vaccine [2]. Furthermore, because HIV-1 is unlike many pathogens in that it is an integrating retrovirus and rapidly mutates [3,4] a truly efficacious vaccine would require broadly neutralizing antibodies (bnAbs) i.e. those that can protect against a wide array of HIV-1 strains [5,6]. Indeed, passive administration of rare bnAbs (infused as mAbs at physiologically-relevant titers) in non-human primates has demonstrated their ability to completely protect from HIV-1 challenge [7–10]. Thus, defining impediments to their routine induction is critical to successful HIV-1 vaccine development.

Co-contributing Authors:
Barton F. Haynes, Duke Human Vaccine Institute, 2 Genome Court, MSRBII Building, Room 4090, Duke University Medical Center, Durham, NC 27710. Phone: (919) 684-5384; Fax: (919) 684-5230; email: hayne002@mc.duke.edu
Laurent Verkoczy, Duke Human Vaccine Institute, 2 Genome Court, MSRBII Building, Room 3015, Duke University Medical Center, Durham, NC, USA 27710. Phone: (919) 681-3134; Fax: (919) 684-5230; email: laurent.verkoczy@duke.edu

Many reasons for the rarity of bnAbs have been proposed, mostly relating to the unusual features of the HIV-1 Envelope (Env) [11]. These include the Env's predilection for forming an extensive glycan "shield" [3,4,12], rapid neutralizing Ab selection of escape mutants [3,4], transient epitope expression [13], steric hindrance [14,15], and inability to overcome Ab-binding entropic barriers [16]. Another potential reason is that highly immunogenic epitopes present on non-native Env structures may hinder bnAb responses by instead inducing dominant Ab responses that are non-neutralizing or are neutralizing, but strain-specific [11,17]. Recent studies have found that ~20% of infected persons will develop a degree of neutralization breadth, raising hope that induction of these Abs will not be as difficult as originally thought [17,18].

An additional hypothesis for the difficulty in induction of bnAbs has been proposed, based on the observation that certain bnAbs were found to exhibit polyreactivity [19]. This hypothesis postulates that such Abs cannot be made because the B cells from which they originate are sufficiently autoreactive to trigger tolerance mechanisms that eliminate or modify bnAb self-reactivity [20]. In this review, we highlight recent studies that provide evidence for immunoregulation in limiting the expression of certain bnAbs and discuss unusual features common to all bnAbs.

Specificities and traits of bnAbs

Five human bnAbs, 2F5, 4E10, 2G12, b12 and Z13, were initially isolated from HIV-1 infected subjects, and represented the prototypic protective antibodies an HIV-1 vaccine should elicit [21–25]. With the advent of high throughput recombinant Ab technology, many new bnAbs have now been identified, some with higher potency than those of the first generation [26–31]. The rate of bnAb discovery, combined with a body of work over the last decade in characterizing the five original bnAbs, has resulted in two significant advances. First, four distinct Env regions have been identified, each representing a potential Achilles' heel for HIV-1 that could be targeted by an antibody-based vaccine: the gp41 Membrane Proximal External Region (MPER), the gp120 CD4 binding site, quaternary V2/V3 loop epitopes, and Env carbohydrates (reviewed in [11]). Second, these studies have revealed an interesting pattern in which all bnAbs share at least one of the following three unusual characteristics: a) self-/polyreactivity, b) elongated, and highly hydrophobic (and/or charged) heavy chain complementary determining regions (HCDR3), and c) high numbers of Ig somatic mutations ([11];Table 1). These bnAb traits will be discussed in terms of clues they are providing about potential mechanisms controlling bnAb induction, as well as their relevance to bnAb specificity and neutralization potential.

Relationship of bnAb traits to selection checkpoints during normal B cell ontogeny

There is compelling evidence that Abs with ***self-/polyreactivity*** are closely associated with negative selection of the B cells expressing them [32,33]. Early studies in mice clearly demonstrated that removal of self-reactivity occurs via three major mechanisms: deletion, anergy, and receptor editing, at several distinct developmental checkpoints. Studies measuring polyreactivity of Abs sampled from the normal human B cell repertoire have closely mirrored studies of self-reactivity, demonstrating that analogous tolerance mechanisms are associated with reduction of the pre-selected, immature repertoire's enriched polyreactivity from ~55–75% to ~5% in the post-selected, mature B cell repertoire [34].

Another set of bnAb traits, i.e. ***elongated, hydrophobic, and/or charged HCDR3s residues***, appear more prevalent in the immature IgM+IgD- B cell repertoire of both mouse and man

[34–37], and are thus considered potential predictors of negative selection for B cells bearing them. The role of tolerance in removing B cells bearing positively charged residues in HCDRs is best demonstrated *in vivo* in the 3H9 HC series of anti-DNA transgenic mice, where arginine residues qualitatively impact tolerance outcome: an increasing number of arginines in CDRs is accompanied by increased affinity for DNA and triggering of central deletion that is largely independent of LC pairing [38–40]. The mechanisms responsible for counterselecting B cells bearing elongated, hydrophobic HCDR3s are less clear, but deletion or editing of immature IgM+IgD- B cells bearing long HCDR3s has been noted [36]. Of note, B cells bearing elongated and/or unusually charged HCDR3s may be subjected to negative selection even earlier in B cell development. The mechanisms for this remain controversial and may involve clonal deletion by pre-BCR-mediated signals and/or defective pairing of HCs with surrogate light chain (SLC) [41–44]. The latter process, however, is critical in allowing B cells bearing particular V_H genes i.e. V_H81X, to efficiently progress in development [45–47].

Evidence for tolerance mechanisms limiting bnAbs with self-reactive traits

Evidence that B cells expressing bnAbs with self-reactivity are under tolerance controls comes from a series of 2F5 knock-in (KI) mouse lines, made by targeted insertion of the original V_L and/or V_H rearrangements of 2F5, an MPER-specific bnAb with a long, charged, hydrophobic HCDR3, and reactive with HEp-2 cells and several self-antigens including cardiolipin, PS, and histones [19]. In 2F5 V_H KI mice, expression of the 2F5 HC alone is sufficient to trigger profound central tolerance [48], reminiscent of prior KI/tg models expressing HCs with “dominant” self-reactivities, such as those specific for DNA, MHC-I, or red blood cells [49]. While the main developmental blockade in these mice is associated with deletion at the pre-B to immature transition, characteristics of residual 2F5 HC-expressing B cells and serum Ig suggest they are under additional tolerance controls, including anergy [48]. Importantly, in 2F5 $V_H \times V_L$ KI mice, B cells expressing the original 2F5 V_H/V_L pair are even more efficiently deleted, demonstrating that the 2F5 LC contributes to this tolerization [50].

A distinct V_H KI line, made from another MPER-specific bnAb, 4E10, has identified a developmental blockade similar to that seen in 2F5 V_H KI mice, indicating that self-reactivity of 4E10 is also sufficient to induce central deletion of most 4E10 V_H-bearing B cells [51]. Additionally, a study using B-cell tetramers to track the frequencies of developing B cell subsets from the normal C57BL/6 repertoire, also showed a developmentally regulated reduction in B cells specifically recognizing the same nominal epitope recognized by 2F5 [52]. Taken together, these data suggest that many MPER-specific bnAbs are subjected to tolerance controls (Figure 1).

A corollary to the hypothesis that poly-/self-reactive bnAbs are not routinely made due to tolerization of their B cells, is that they can be made in situations where tolerization is either mitigated or absent. Indeed, a possible clinical correlate supporting this is a lower-than-expected reporting rate of coincident HIV-1 and SLE disease [53–55]. Also, studies of the 2F5 bnAb and its specific nominal MPER epitope support the notion that self-reactive bnAb-specific B cells are enriched when tolerance controls are removed. This is seen in the case of autoimmune-prone mice [56], and in mice reconstituted with B cells developed *in vitro* under conditions permissive for self-reactivity, wherein an enrichment for MPER-specific B cells is accompanied by robust anti-MPER responses to immunization with MPER peptides [52,57]. Finally, B cells from 2F5 $V_H \times V_L$ KI bone marrow that are normally deleted *in vivo*, can be rescued by *in vitro* culture and immortalized as hybridomas which display the same reactivity and neutralizing properties of 2F5 [51].

Functional relevance of bnAb traits to HIV-1 binding and neutralization

Polyreactivity in bnAbs appears to be functionally relevant in at least two regards. First, a bnAbs's given spectrum of polyreactivity may be required for its functional specificity, for example the lipid polyreactivity of the MPER-specific bnAbs 2F5 and 4E10 is critical for their mechanism of neutralization [58–60]. Second, general polyreactivity has been proposed to increase the probability of bivalent binding by anti-Env antibodies to HIV virions [61]. Extensive HIV-induced polyreactivity (70% of mAbs isolated) was shown to enhance the avidity of anti-Env antibodies by facilitating heteroligation, *i.e.*, bivalent antibody binding involving distinct Env-specific and non-Env (virion/host) components [62]. An analogous mechanism was reported for a polyreactive CD4-inducible antibody, 21c, in which an adjacent bound CD4 receptor is used as part of the CD4i epitope [63].

With respect to the unusual length, hydrophobicity, and charge of bnAb HCDR3s, hydrophobic HCDR3 residues in MPER-specific bnAbs are thought to be critical for lipid reactivity that permits subsequent binding with the exposed MPER epitope and both events are critical for neutralization [58,64]. Positively charged HCDR3 residues are critical for 2F5's interactions with the MPER epitope [65,66], but not necessarily 4E10's [67].

Finally, it is not known why most bnAbs have an unusually high frequency of somatic mutations, although two related possibilities in the context of HIV-1 hyperactivation/GC dysregulation is either aberrant regulation of the somatic mutation machinery or defective selection of B cells undergoing affinity maturation. Understanding these mechanisms during the course of HIV-1 infection, as well as performing Env immunization studies in KI models of reverted (unmutated) bnAbs, will be critical in ascertaining how affinity maturation impacts acquisition of broad neutralizing function.

Challenges faced and lessons to be learned

In designing strategies to elicit self-reactive bnAbs, three questions emerge from the above studies: 1) are all bnAbs with self-/polyreactive traits (including those that don't target the MPER), also subjected to tolerance controls? 2) what percentage of bnAbs have or had such traits at some point of their maturational pathway; and, 3) in terms of bnAb structure-function correlates, does bnAb tolerizing self-reactivity overlap with binding required for neutralization?

The existence of non-autoreactive bnAbs such as VRC01, with an unremarkable HCDR3, but with an extremely high rate of somatic mutations suggests that a valid vaccination strategy would need to focus drive a prolonged maturation process to elicit these kinds of bnAbs. Certainly, not all polyreactive B cells are subjected to tolerance controls and not all B-cell clones normally subjected to tolerance controls produce pathogenic antibodies. In the case of the gp41 MPER-specific bnAbs 2F5 and 4E10, their passive infusion in humans has not been associated with pathogenicity [68], although in neonatal infant macaques, a possible bleeding event was seen [69]. Assuming pathogenicity is not a general problem of MPER-specific bnAbs, M66 and 4E10 could be two attractive self-/polyreactive bnAb candidates to elicit, because they have relatively low frequencies of Ig somatic mutations (Table 1) and may recognize less complex and restrictive Env epitopes [70]. Related to this issue, bnAbs like 4E10 may have the additional advantage of using functionally conserved V_H genes *i.e.* used in multiple viral responses and well represented in the nascent B cell repertoire; indeed, the V_H1–69 gene used by 4E10 is common to many Abs against hepatitis C and influenza [71].

In understanding the relative importance for immunization approaches to induce epitope specificity and polyreactivity for the neutralizing function of MPER-targeted Abs, recent

studies have provided interesting, initial clues. Dennison et al. have shown that many gp41-specific mAbs are lipid reactive but do not neutralize HIV-1 because they bind to non-neutralizing gp41 epitopes [72]. Thus, for these types of Abs, lipid reactivity is necessary but not sufficient for neutralization. Moreover, Guenaga et al. [73] and Dennison and colleagues [74] have used immunization strategies with scaffolds as well as heterologous prime-boosts to focus the Ab response on the nominal gp41 MPER neutralizing epitope ($_{664}$DKW) that is recognized by 2F5 [59,65]. However, in both instances, Abs did not neutralize HIV-1, and in the Dennison *et al.* study [74], the induced $_{664}$DKW Abs were not polyreactive with lipids. Thus, for eliciting 2F5-like bnAbs, i.e. directed at the nominal 2F5 neutralizing epitope, it appears that induction of polyreactive Abs that bind this epitope *and* virion lipids is critical for efficient HIV-1 neutralization, and it is these types of Abs that are regulated most stringently.

Conclusions

The pace at which novel HIV bnAbs have been identified and characterized has provided new insight into the traits of HIV-1 bnAbs, and has now positioned the field to determine the critical requirements for broad and potent HIV-1 antibody induction. Ongoing studies will establish what bnAb traits are relevant for bnAb regulation and function, and secondly, will provide a much deeper understanding of the basic B cell biology regarding how bnAbs are generated, the B cell subsets they originated from, the relevant checkpoints during their ontogeny, and how somatic hypermutation and affinity maturation impact Ab function. This information should provide guidance for immunogen/adjuvant based strategies aimed at targeting relevant B cell populations and stimulating B cell diversification processes, thus optimizing the possibility that relevant bnAb functional specificities can be safely induced.

The main hurdle in developing a protective Ab-based HIV-1 vaccine continues to be the inability of current vaccination strategies to induce effective bnAb responses (i.e. those that are high titered and long lasting). The key remaining gaps in knowledge that future studies need to address include comprehensively establishing which traits in bnAbs are most relevant for their functions and *in vivo* regulation, developing a much deeper understanding of the ontogeny of bnAbs, including defining the precursor B cell subsets from which bnAbs originate, defining the relevant checkpoints bNabs are under, and understanding how somatic hypermutation and affinity maturation impact acquisition of bnAb functional properties. Another perhaps equally important consideration is defining the mechanisms that drive existing/current Env immunogens to elicit dominant non-neutralizing responses that may hinder the induction of bnAb responses. Thus, future studies should be aimed at defining the specific Env structures and/or residues required for naïve B cell activation, the host genetic factors that regulate bnAb induction, and the B cell maturation pathways that lead to bnAb responses.

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•• of outstanding interest

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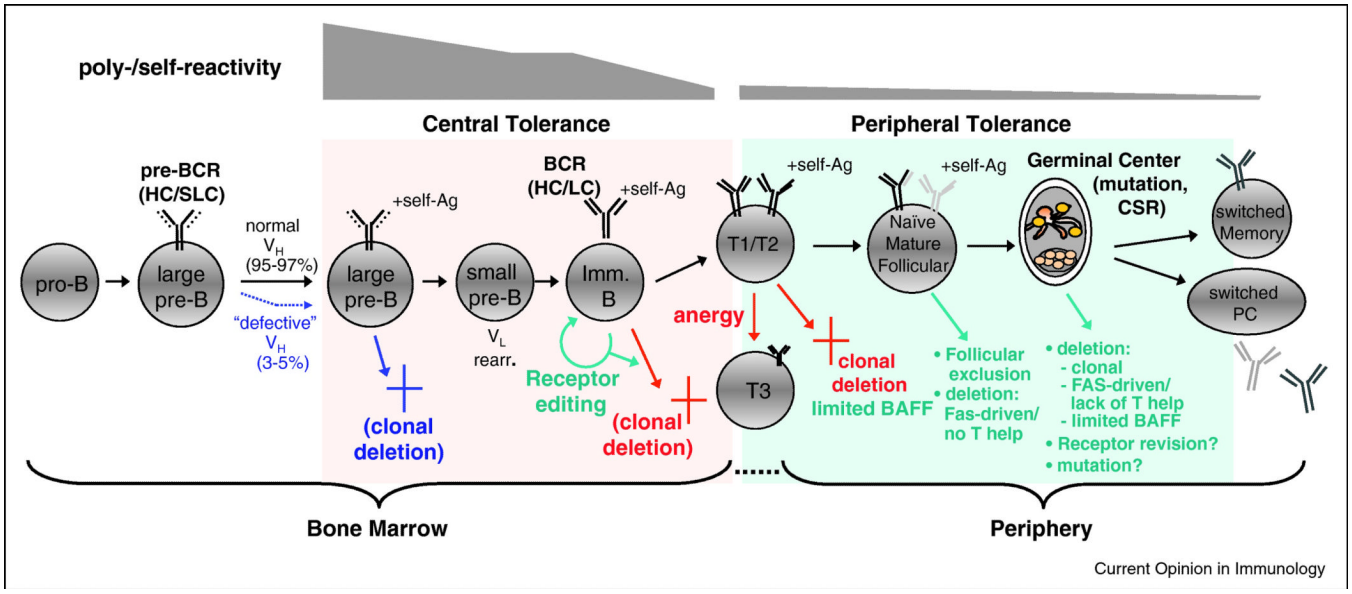

Figure 1.

Table 1

Immunogenetic and functional characteristics of representative bnAbs

bnAb[a]	Env epitope specificity	Neutralization[b]		Isotype	V family usage		% V_H mutations[d]	HCDR3 features[e]			Polyreactivity	Original ref.
		Breadth	Potency		V_H	Vκ/Vλ		length	#pos. charges	avg. hydrop.		
2F5	gp41 MPER	60	2.3	IgG3	2–5	κ1–13	15.2	24	3	−0.04	Yes	[22]
4E10	gp41 MPER	98	3.2	IgG3	1–69	κ3–20	15.6	20	3	−0.27	Yes	[23]
M66.6	gp41 MPER	25	18.0	IgG1[c]	5–51	κ1–39	9.3	23	2	−0.89	Yes	[31]
2G12	gp120 glycan shield	32	2.4	IgG1	3–21	κ1–5	31.7	16	3	−1.5	Yes	[24]
1b12	gp120 CD4 binding site	41	1.8	IgG1[c]	1–3	κ3–20	13.1	20	1	−1.2	Yes	[21]
VRC01,VRC02	gp120 CD4 binding site	91	0.34,0.32	IgG1[c]	1–2	κ3–11	32.1	14	3	−1.9	No	[29••]
VRC03	gp120 CD4 binding site	57	0.45	IgG1[c]	1–2	κ3–20	30.2	16	2	−0.89	No	[29••]
HJ16	gp120 CD4 DMR core	36	8.0	IgG1[c]	3–3	κ4–1	14.6	21	ND[f]	ND	ND	[27]
PG9,PG16	gp120 quaternary V2,V3	79,73	0.22,0.15	IgG1[c]	3–33	λ2–14	16.7, 20.5	30	2,3	−1.4,−1.1	No	[28••]
CH01–CH04	gp120 quaternary V2,V3	35–45	0.46–1.1	IgG1[c]	3–20	κ3–20	11.5–14.3	24	1,1,1,0	−0.4,.8,.4,.7	Yes, except CH04	[26]
CH05	gp120 quaternary V2,V3	44	0.79	IgG1[c]	3–20	κ1–6	11.5	24	0	−0.7	No	[26]

[a] bnAbs listed in the same row are somatic variants of the same clone, those shaded represent a likely clonally-related lineage.

[b] breadth is defined as the percent of primary isolates neutralized with an IC50<50 μg/ml; potency is defined as the mean IC50 value (μg/ml) within the group of viruses neutralized with IC50 values <50 μg/ml.

[c] IgG1 vector construct; original isotype not reported/not determined.

[d] estimate based on aa sequence comparisons of original (mutated) and inferred reverted (unmutated) V(D)J rearrangements.

[e] average hydrophobicity (GRAVY) values of HCDR3 aa were determined using the Duke University Laboratory for Computational Immunology's SoDA program; for reference, mean HCDR3 aa length and number of positive charges in the normal mature naïve human B cell repertoire are 13.5, and 0.67, respectively [34].

[f] ND=not determined