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INVITED REVIEW

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Antibody-virus co-evolution in HIV infection: paths for HIV vaccine development

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Summary

Induction of HIV-1 broadly neutralizing antibodies (bnAbs) to date has only been observed in the setting of HIV-1 infection, and then only years after HIV transmission. Thus, the concept has emerged that one path to induction of bnAbs is to define the viral and immunologic events that occur during HIV-1 infection, and then to mimic those events with a vaccine formulation. This concept has led to efforts to map both virus and antibody events that occur from the time of HIV-1 transmission to development of bnAbs. This work has revealed that a virus-antibody "arms race" occurs in which a HIV-1 transmitted/founder (TF) Env induces autologous neutralizing antibodies that can not only neutralize the TF virus but also can select virus escape mutants that in turn select affinity-matured neutralizing antibodies. From these studies has come a picture of bnAb development that has led to new insights in host–pathogen interactions and, as well, led to insight into immunologic mechanisms of control of bnAb development. Here, we review the progress to date in elucidating bnAb B cell lineages in HIV-1 infection, discuss new research leading to understanding the immunologic mechanisms of bnAb induction, and address issues relevant to the use of this information for the design of new HIV-1 sequential envelope vaccine candidates.

KEYWORDS
co-evolution, HIV neutralization, HIV vaccine

1 | INTRODUCTION

HIV-1 is rapidly evolving with increasing diversity of global strains.[1] In individuals, HIV-1 infection is usually initiated by one or a few transmitted/founder (TF) viruses,[2] and within each infected person, evolves to extraordinary diversity shaped by antibody and T cell responses.[3,4] Moreover, virus integration occurs early on in infection, before a protective antibody or T cell response can occur.[5] Therefore, elicitation of protective antibodies before HIV-1 transmission will be required to achieve protection.[6,7] Antibodies that neutralize HIV-1 bind to the native envelope (Env) trimer, and neutralize by either blocking Env-CD4 receptor or by blocking virion fusion with host cells.[8] A major goal of HIV-1 vaccine development is to induce broadly neutralizing antibodies (bnAbs) that have the properties of binding to Env trimers on virions of difficult-to-neutralize (Tier 2) viruses, and can potently neutralize a substantial percentage of HIV-1 primary isolates.

During the first 30 years of the HIV-1 pandemic, attempts to formulate a protective HIV-1 vaccine using strategies similar to those for licensed vaccines were not successful, nor has immunization with either single HIV-1 envelope glycoproteins (Env), or polyvalent combinations of genetically diverse and unrelated Envs

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succeeded in inducing bnAbs. Recent progress in understanding the immunobiology of bnAbs and founder viruses,[9] and insights from structural studies of bnAbs[10,11] have led to new ways to think about bnAb development, and led to new strategies for bnAb induction.

Hraber et al.[12] demonstrated that 50% of chronically HIV-1 infected individuals develop plasma neutralization breadth for approximately 50% of HIV-1 global strains over several years of infection. Moreover, recent success in establishing and monitoring large cohorts of HIV-infected individuals including those studied from transmission though bnAb development,[5,13,14] and advances in antibody isolation and B cell repertoire analysis have enabled the identification of dozens of new bnAbs from multiple HIV-1 infected adults[14–27] and children.[28,29] In addition, more than one bnAb lineage can occur in the same individual.[16,30] In HIV-1 infected adults, plasma levels of bnAbs usually develop after 2-4 years of infection. However, in children, examples of plasma bnAb breath occurring within the first year of HIV-1 infection have been reported.[28,29] Because many bnAbs have unusual traits of high levels of somatic mutations, long third heavy chain complementarity regions (CDR H3s) and/or poly- or auto-reactivity, it has been postulated that bnAb maturation is disfavored due to control by host tolerance mechanisms.[31,32] Thus, if the key events of virus Env and bnAb lineage evolution could be defined in the setting of HIV-1 infection, then a path to successful induction of bnAbs could be the recapitulation of these events in the setting of vaccination.

Here, we review progress made to date in understanding pathways of bnAb induction in the setting of infection and vaccination, discuss B cell lineage cooperation in bnAb ontogeny and review new strategies of vaccine design.

2 | CHARACTERISTICS OF BROADLY NEUTRALIZING ANTIBODIES

There are six known viral Env regions targeted by bnAbs: the CD4-binding site (CD4bs)[16,17,26,27,33–35]; the gp120 V1V2-glycan region[14,15,19]; the V3-glycan region[20,23–25,28]; the interface between gp120 and gp41 Env glycoproteins,[36] the gp41 fusion domain,[37] and the gp41 Env membrane proximal external region (MPER)[38, 39] (Williams L. D. and Haynes B. F., unpublished) (Figure 1). In this review, we will discuss three bnAb specificities that have been characterized in antibody-virus co-evolution studies: the CD4 binding site, the V3-glycan and the V1V2-glycan regions.

2.1 | CD4-binding site broadly neutralizing antibodies

The functional integrity of the CD4bs is required for virus infectivity and therefore its structure is highly conserved.[40] CD4bs bnAbs have been isolated from multiple HIV-1 infected individuals, suggesting that their induction is relatively common and therefore an achievable goal in the setting of vaccination. Moreover, CD4bs bnAbs isolated

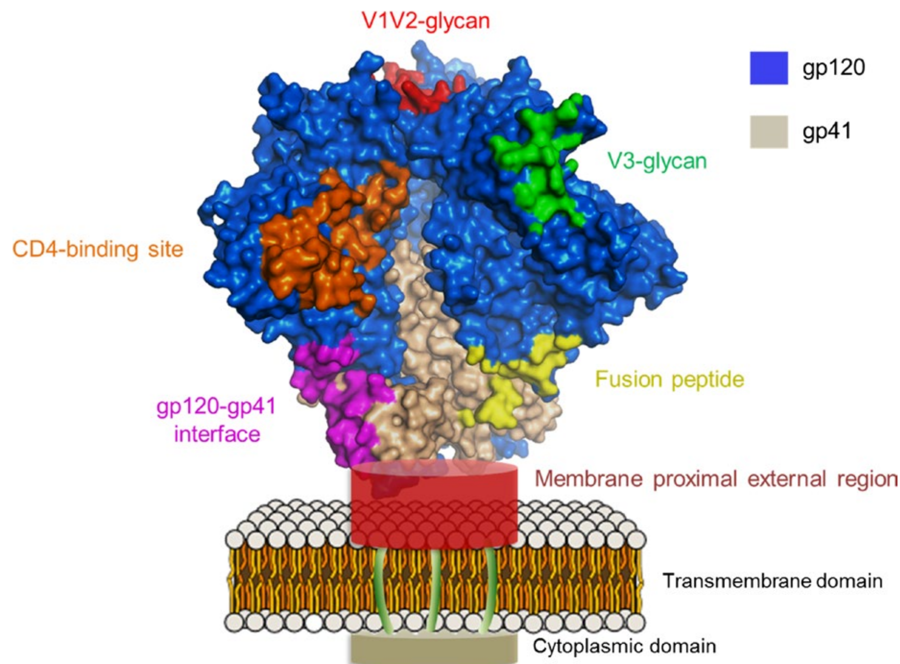

FIGURE 1 Sites of vulnerability on the HIV-1 Env glycoprotein spike. The structure of a HIV-1 prefusion trimer (PDB: 4TVP) is displayed with gp120 and gp41 protomers colored in blue and beige respectively. Epitope mapping of multiple broadly neutralizing antibodies has identified six sites of vulnerability of the HIV-1 Env glycoprotein: the V1V2 loop (red); the base of the V3 loop (green); the CD4-binding site (orange); the interface between gp120 and gp41 proteins (magenta); the fusion peptide region (yellow), and the membrane proximal external region (MPER, brown). Both the V1V2 and the V3 loop bnAb epitopes include direct contact with glycans. The MPER near the base of the Env trimer, the transmembrane and the cytoplasmic domains have only limited structural information and are highlighted for reference (red and sand cylinders and green sticks). Figure derived from structure described by Pancera et al.[59]

from infected individuals share similar V_H and V_L gene characteristics suggesting commonalities in their maturation pathways that could be recapitulated by a B-cell lineage design approach in multiple individuals.[41,42] Finally, CD4bs bnAbs are among the most broad and potent antibodies, surpassed in breadth only by distal MPER gp41 bnAbs such as 10E8 or DH511 (Williams L. D. and Haynes B. F., unpublished).

CD4bs bnAbs cluster into two classes based on their angle of approach and mode of antigen recognition: *CD4 mimicking* CD4bs bnAbs, such as VRC01, CH31, 8ANC131, and CH235, mimic CD4 in their approach to gp120; within this group, there are VRC01-class bnAbs that use V_H1-2*02 (eg, VRC01 and CH31) and 8ANC131-class of bnAbs that use V_H1-46 (eg, 8ANC131 and CH235).[16,18,26,27,33,42,43] In contrast, the *variable heavy third complementarity determining region (CDR H3)-binder*-class of CD4bs bnAbs, such as CH103, HJ16 and CH98 bnAbs, use multiple V_H genes.[17,21,42,44]

2.2 | V3-glycan broadly neutralizing antibodies

The V3-glycan bnAb class recognizes discontinuous amino acids, including a 4 amino acid linear motif (GDIR) near the HIV-1 V3 loop Env C-terminus, and high-mannose glycans including, among others, N301 and N332.[20, 23, 28, 45–47] While the V3-glycan epitope centers on the glycan at N332, high-mannose glycans at other positions can variably be involved in the bnAb epitope.[46–49] Such diversity is reflected in crystal and electron microscopy structures by different angles of approaches of individual V3-glycan bnAbs.[24,28,45,46,48–50] The V3-glycan bnAb class is of interest for vaccine development because it does not require extensive somatic hypermutation to develop neutralization breadth. Indeed, V3-glycan neutralizing antibodies with limited levels of somatic mutation but neutralization breadth have been recently described in an adult,[20] and as well, in an infant within 1 year from infection.[28] Finally, the composition of the core epitope (4 amino acids and a high mannose glycan at position N332) has facilitated the synthesis of a minimal bnAb epitope construct (Alam S. M., Aussedat B., Vohra Y., Meyerhoff R. R., Cale E. M., Walkowicz W. E., Danishefsky S. J. and Haynes B. F., unpublished).

2.3 | V1V2-glycan broadly neutralizing antibodies

The V1V2-glycan bnAb class was the first of the new generation of bnAbs discovered.[14,15] V1V2-glycan bnAbs, such as the PG9, CH01, PGT145 and CAP256-VRC26 lineages, are characterized by anionic, often tyrosine-sulfated, long and protruding CDR H3s that penetrate HIV envelope glycans and recognize a discontinuous epitope at the apex of the HIV-1 spike.[14,15,19,22, 25,51–54] Crystal structures in complex with a scaffolded V1V2 domain were solved for PG9, PG16 (in the PG9 lineage),[51,52] and CH03, CH04 (in the CH01 lineage).[55] V1V2 glycan bnAbs recognize a discontinuous epitope around an N-linked glycan at position 160, with a preference for short high mannose glycans, eg, $Man_5GlcNac_2$.[52] V1V2 bnAb interactions with various glycans and direct strand-strand contact between the extended CDR H3 and the C strand of the V1V2 domain are common traits among individual V1V2 bnAbs.[51,52,55,56] For immunogen design, despite a preference of V1V2 glycan bnAbs to bind quaternary epitopes, PG9, PG16 and CH01 bnAbs, as well as the CH01 lineage unmutated common ancestor (UCA), can also bind a minor subset of monomeric gp120 Envs[15,57] and minimal Env forms.[58] Crystal structures of the V1V2 glycan Env region in complex with V2 antibodies demonstrated that the V1V2 epitope can assume at least three conformations[52,57]: a β-strand, an α-helix and a 3_{10} helix. V1V2 bnAbs recognize the β-strand conformation, with only slight differences assumed by the scaffolded V1V2 domain in complex with V1V2-glycan bnAbs.[52] Conversely, the α-helix and a 3_{10} helix V1V2 conformations are recognized by ADCC-mediating non- or poorly neutralizing antibodies, such as CH58 and CH59.[57] CH58 and CH59 were isolated from vaccinees from the HIV RV144 vaccine efficacy trial who were immunized with monomeric gp120 Env, suggesting that the α-helix and a 3_{10} helix conformations may be less well represented in native Tier 2 virion Envs.[57] Thus, the plasticity of the gp120 Env V1V2 domain, that allows multiple conformations, represents a hurdle for immunogen design for bnAb induction, since immunogens that bind bnAbs require the retention of the V1V2 domain in the β-strand conformation.[58]

3 | ANTIBODY-VIRUS CO-EVOLUTION

3.1 | CD4-binding site CDR H3-binder bnAb development

We first defined the detailed nature of the arms race between HIV-1 and a bnAb with our studies of the co-evolution of the TF virus and the CH103 CDR H3-binder CD4bs bnAb B cell lineage in the African individual CH505 during the first 3 years of infection.[17]

The UCA of the CH103 bnAb lineage bound the TF HIV-1 envelope glycoprotein as gp140 and gp120 Envs, and early CH103 lineage antibodies only slowly escaped autologous virus variants.[17] Rather, with increasing heterologous breadth of neutralization the CH103 lineage retained the ability to neutralize evolving autologous viruses (Figure 2). This observation gave rise to the discovery of another antibody lineage in the CH505 individual that selected Env variants that were resistant to it, but sensitive to CH103, a phenomenon we termed *B cell lineage cooperation* (see below).[18] The evolution of the CH103 lineage antibody neutralization breadth was preceded by extensive viral diversification in and near the CH103 epitope[17] and viral evolution of the loop D, a binding site for the CH103 lineage, was of particular interest. The loop D was under intense pressure from the very early phases of acute infection: first amino acid substitutions arose as early as 4 weeks post-transmission and by 53 weeks post-transmission no virus was isolated that retained the TF virus amino acid sequence.[18]

3.2 | Cooperating B cell lineages for selecting viruses sensitive to bnAb B cell lineages

In African individual CH505, all viruses with mutated motifs in loop D were more sensitive to CH103 lineage antibody neutralization than the TF virus, implying that those mutations, despite being part of the

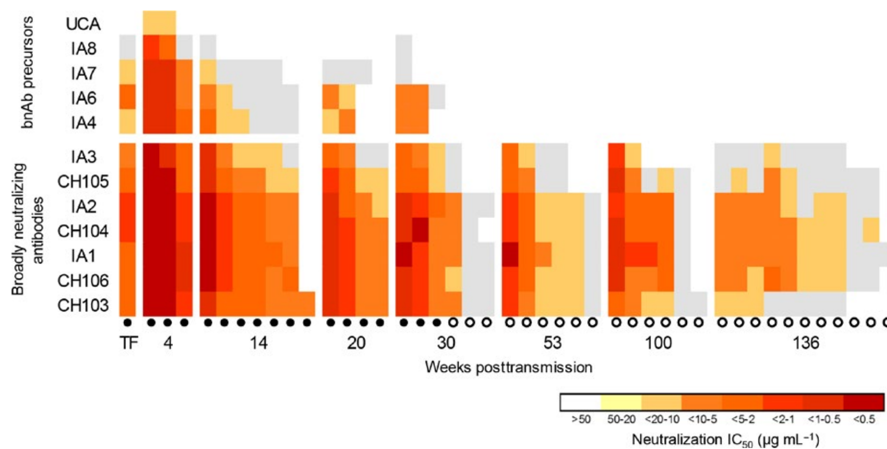

FIGURE 2 Neutralizing activity of CH103 lineage antibodies against longitudinal autologous virus quasi-species variants. Heat map analysis of neutralization data generated from 43 pseudoviruses (X axis) and 12 CH103 lineage mAbs (Y axis). Neutralization potency (IC_{50}) is shown in different shades of color as indicated in the legend, from white (>50 μg/mL) to dark red (<0.5 μg/mL). CH103 lineage mAbs are ordered based on their phylogenic relationship as described in Liao et al.,[17] from the unmutated common ancestor (top) to the most mutated CH103 bnAb (bottom). Less mature CH103 lineage antibodies (UCA-IA4) neutralized only autologous viruses, whereas more somatically mutated mAbs (IA3-CH103) acquired broad neutralization. Autologous neutralization of bnAb precursors was limited to viruses isolated early during the course of infection (●) whereas affinity matured CH103 lineage bnAbs retained the ability to neutralize autologous viruses isolated up to week 136 (○)

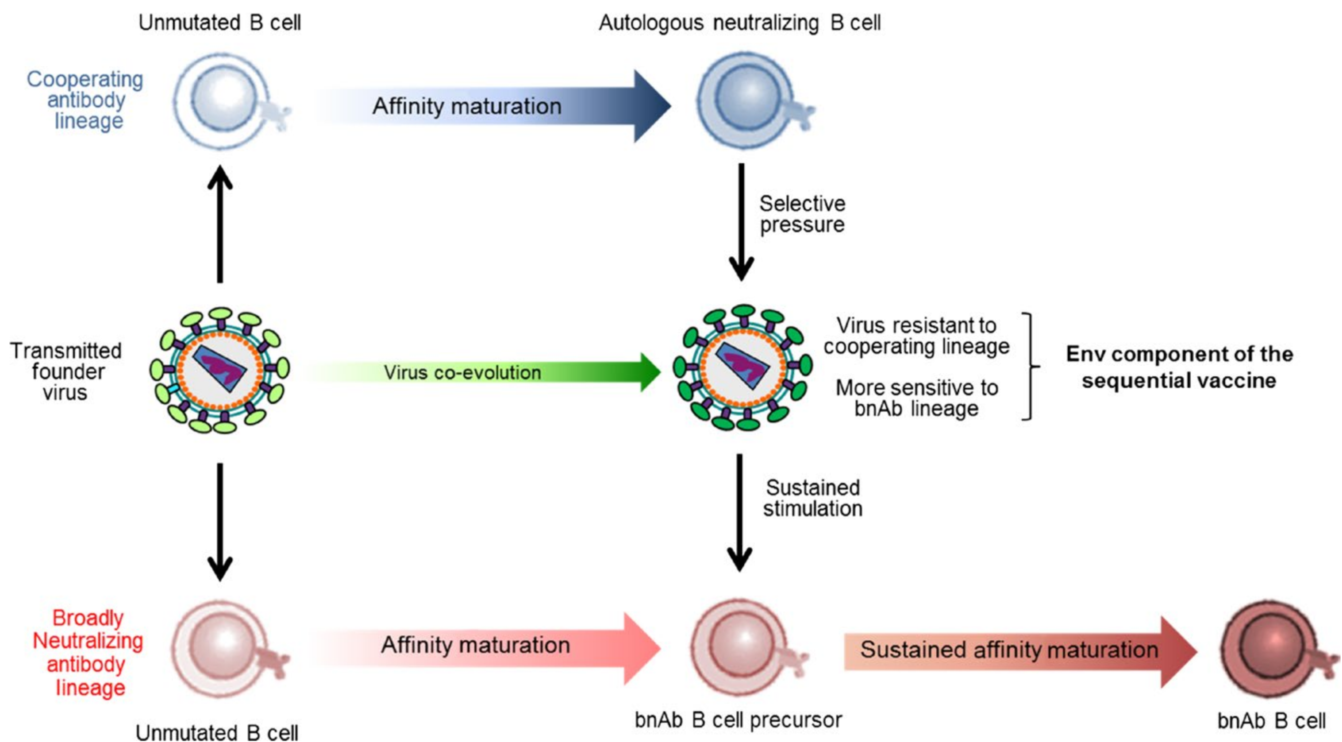

FIGURE 3 Mechanism of cooperation between B cell lineages in inducing HIV-1 broadly neutralizing antibodies. The transmitted/founder virus (green) evolves under pressure of autologous neutralizing antibodies. Among them are lineages that progress to neutralization breadth (red) and cooperating lineages (blue) that target the same epitope. The cooperating lineage selects for virus escape mutants that are more sensitive to neutralization of the evolving broadly neutralizing antibody lineages, thus providing sustained stimulation to bnAb B cell precursors and affinity maturation

binding site of the CH103 bnAb lineage, were selected by antibodies distinct from those in the CH103 B cell lineage.[18] We identified the CH235 autologous neutralizing B cell lineage that cooperated with the CH103 lineage by selecting escape mutants with loop D mutations that made the virus more sensitive to CH103.[18] The presence of cooperating lineages may be a requirement for bnAb development during chronic infection by targeting the same bnAb epitope with a different angle of approach and selecting escape mutants more sensitive to the bnAb lineage, thus supporting the sustained and prolonged maturation of the bnAb lineage (Figure 3). Otherwise, in the absence

of cooperating B cell lineages, once the founder virus fully escaped autologous neutralizing antibodies in B cell lineages with potential to progress to breadth, there would be no additional virus mutants to select affinity matured bnAb lineage B cells.

Identifying cooperating lineages and their effect on evolving autologous virus is also important for vaccine design. Defining cooperating B cell lineages and the virus escape mutants they select informs which virus Envs were involved in bnAb B cell lineage maturation and thus are candidates for inclusion in a vaccine.[16]

3.3 | CD4-binding site CD4 mimicking broadly neutralizing antibody development

As noted above, HIV-1-infected individual CH505 made two types of CD4bs bnAbs, the CH103 CDR H3-binder lineage and the CH235 CD4-mimicking lineage that in its early stage served as a cooperating B cell lineage for the CH103 bnAb. The CH235 lineage progressed over 6 years of infection to extraordinary heterologous neutralization breadth.[16] Like the 8ANC131 CD4 mimic bnAb, CH235 used V_H1-46.[16,26] Co-crystal structural analysis revealed that the angle of approach to gp120 of the CH235 lineage antibodies was the same as for other VRC01-class of antibodies and did not change during bnAb affinity maturation (Figure 4A).[16] As CH235 lineage antibodies progressed to broader and more potent neutralizing activity, they also increased their precision in targeting the CD4 binding supersite of vulnerability with progressively less interactions with the inner domain and the V5 loop (Figure 4B). Targeting precision correlated with neutralization breadth, and somatic hypermutation were necessary to focus antibody recognition of the CD4bs.[16] These findings are relevant because they provide a structural approach to monitor antibody evolution and identify bnAb precursors elicited by vaccines. They also highlight the potential importance of vaccine immunogens that preserve native Env trimer structure for precise targeting of the CD4bs.

Similar to the CH103 UCA, the UCA of the CH235 lineage reacted with the TF virus and with an early mutant Env, M5.[16,18] CH235 lineage V_H transcripts in blood B cells were found as early as 14 weeks post-transmission by next generation sequencing (NGS). The CH235 lineage interaction with HIV-1 Env loop D was mediated by hydrogen bonds between the side chains of Env N280 and CH235 CDR L3.[16] In particular, the N280 loop D mutations observed in the evolving autologous viruses were predicted to disrupt this H-bond network.[16] Acquisition of extraordinary breadth by CH235 lineage bnAbs was associated with the introduction of a compensatory mutation (T30N) in the CDR H1 which formed a new hydrogen bond with R429 in the β20-β21 loop of the HIV-1 gp120 Env C4 region on the opposite face of the CD4bs from loop D and enabled affinity matured CH235 lineage antibodies to neutralize loop D mutants that were initially resistant to neutralization by early members of the CH235 lineage.[16] The

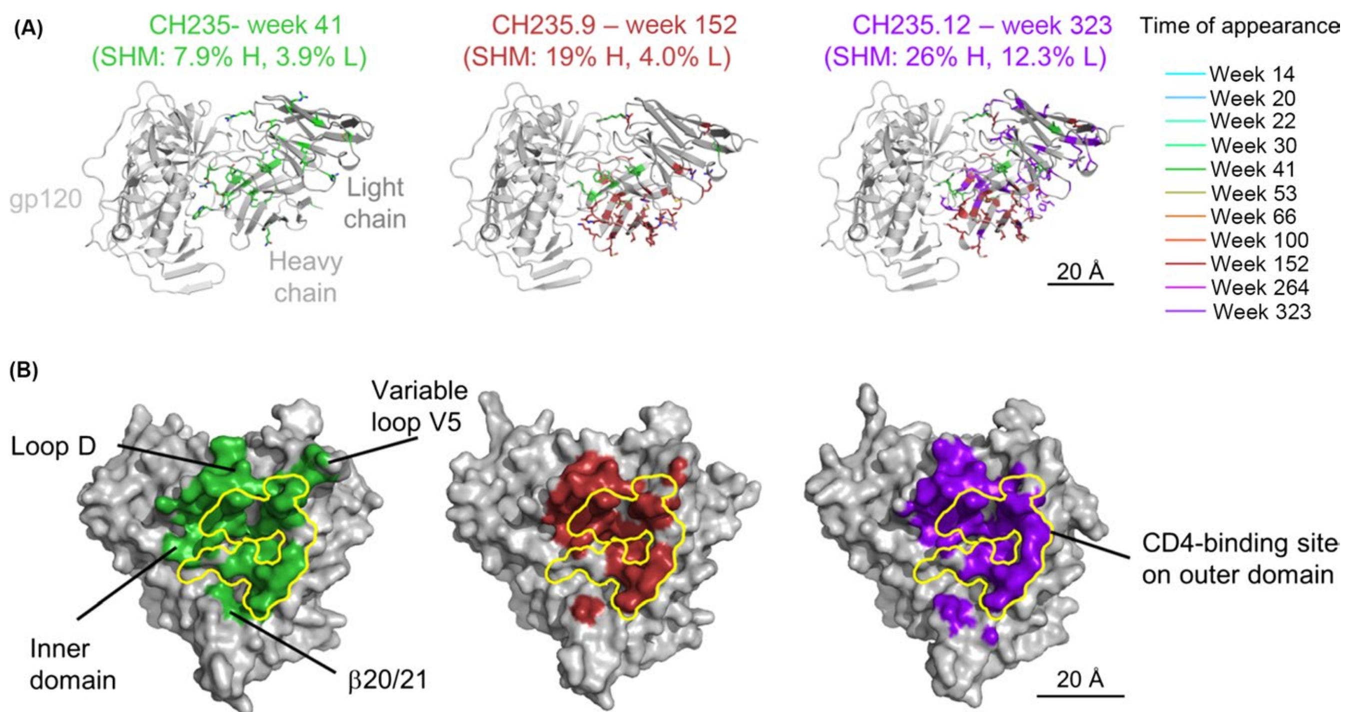

FIGURE 4 Effects of clonal maturation on CH235 VH1-46 bnAb lineage antibody recognition of the CD4-binding site of vulnerability. (A) Co-crystal structures of the antigen-binding fragments (Fabs) of CH235, CH235.9 and CH235.12 antibodies with core gp120. Structures are shown in ribbon diagram, with gp120 in gray and residues altered by somatic hypermutation in stick representation colored by time-of-appearance. The V_H domain mimicked CD4 in Env binding and the gp120 Env-antibody orientation was determined early in bnAb lineage ontogeny and was maintained throughout clonal evolution. (B) The footprints of the CH235, CH235.9 and CH235.12 on gp120 are shown in green, brown, and purple respectively. The footprint of the CD4 supersite of vulnerability is highlighted in yellow. Targeting precision to the CD4 supersite of vulnerability correlated with neutralization breadth. Figure adapted from Bonsignori et al. [16] and used with permission

CH103 CDR H3 binder and the CH235 CD4 mimicking CD4 bnAbs developed in sequence with the CH103 maturing first and the CH235 bnAb maturing 2-3 years later.

3.4 | V3-glycan broadly neutralizing antibody development

The development of V3-glycan bnAb B cell lineages over time and the co-evolution with autologous TF virus have been recently elucidated by three independent groups in different individuals: chronically HIV-1 infected adult individuals PC76[20] and CH848 (Bonsignori M, Kreider E. F., Fera D., Meyerhoff R. R., Bradley T., Korber B. T. and Haynes B.F., unpublished), and an infant who became infected during the first 4 months after birth, likely through breastfeeding.[28]

Chronically HIV-1 infected African individual PC76 was monitored for approximately 3.6 years post-transmission. MacLeod et al. isolated a V3-glycan bnAb lineage (the *PCDN lineage*) with moderate neutralization breadth and potency.[20] PCDN neutralization was sensitive to the H330A mutation,[50,59] depended more on the presence of glycans at positions N156, N301, and N332 and less on integrity of the GDIR motif; it also appeared to require interactions with hybrid glycans.[20]

The PCDN V3-glycan bnAb B cell lineage evolved from the very early phases of development into multiple divergent phylogenetic branches that matured in parallel.[20] Approximately 2.3 years post-transmission the autologous virus quasispecies lost the N332 glycosylation site, which likely represented the definitive route of escape from PCDN lineage neutralization and only few PCDN lineage branches kept evolving beyond this time point. While heterologous neutralizing antibodies could be identified in multiple branches, the broadest and most mutated ones arose from the few that survived the virus-induced lineage sieving and kept evolving beyond 2.3 years.[20] The trajectory of somatic mutations that accumulated in the PCDN lineage suggest a more prominent role of AID-dependent intrinsic mutability during the early phases of clonal maturation and a progressively higher prevalence of mutations, including nucleotide reversion mutations at previously mutated sites, controlled by strong selective pressure during the later phases.[20]

Notably, the PCDN B cell lineage UCA did not bind or neutralized any of the isolated autologous viruses.[20] Rather, analysis of the autologous virus quasispecies neutralization suggested that the PCDN lineage was triggered by a virus variant that emerged in-between 5 and 10 months post-transmission after removal of the N-linked glycosylation site at position 335 and a E328K mutation,[20] suggesting the early presence of cooperating lineages.[18]

From an infant who became infected with a clade A virus within 3.8 months of age and acquired plasma Tier 2 neutralizing activity by 1 year of age,[29] Simonich et al.[28] isolated 8 non-clonally related heterologous Tier 2-neutralizing antibodies from memory B cells collected at 15 months of age (11.2 months postinfection). All infant neutralizing antibodies displayed low levels of somatic mutations (2.0%-6.6%). Among them, *BF520.1 bnAb* targeted the V3 glycan epitope and reached 58% neutralization breadth in a 23-virus multi-tier multi-clade panel with potency comparable to that of CD4bs VRC01 bnAb.[28] BF250.1 bnAb was only 6.6% mutated, less than most bnAbs isolated from adults. Interestingly, BF520.1 bnAb as well as the other 7 heterologous neutralizing mAbs neutralized autologous TF quasispecies variants isolated at 6 months of age but did not neutralize earlier variants, albeit they bound to the respective cell surface-expressed trimeric Env and trimeric SOSIP Env. This finding suggests that maturation of the BF520.1 lineage was initiated by binding interactions of the BcR and that subsequent responses to the co-evolving virus led to the selection of B cell expressing neutralizing antibodies.[28]

A V3 glycan bnAb B cell lineage, *the DH270 lineage*, was isolated from African individual CH848 with chronic HIV-1 infection (Bonsignori M, Meyerhoff R. R. and Haynes B. F., unpublished). Sequential sampling of the autologous virus quasispecies was conducted throughout the first 5 years of infection. DH270 bnAb neutralization was restricted to viruses bearing a N332 N-linked glycosylation site; such restriction impacted the overall level of neutralization breadth (55%) measured in global panels that included CRF01AE recombinant forms in which N332 is generally not present. When viruses that did not have N332 were excluded from the same multi-clade panel, the most potent antibody of the DH270 B cell lineage neutralized 77% of isolates (Bonsignori M, Korber B. T. and Haynes B. F., unpublished).

Antibody virus co-evolution studies revealed that V1 loop length of the autologous virus was critical for recognition by DH270 lineage bnAb precursors. The TF virus had a 34-amino acid long V1 loop, which was maintained throughout the first year of infection and DH270 bnAb precursors did not bind these viruses nor bind TF recombinant Envs. One year after transmission, a 10 amino acid deletion in V1 occurred in the TF that rendered the autologous virus sensitive to DH270 lineage antibodies. The V1 loop was a site of intense mutations—including multiple deletions—and autologous virus quasispecies variants with V1 loops ranging from 16 to 43 amino acids co-circulated for 5 years, indicating a complex selective pressure on this site. DH270 lineage antibodies developed neutralization breadth concurrently with the ability to recognize virus Envs with longer V1 loops (Bonsignori M, Korber B. T. and Haynes B. F., unpublished). These observations predicted that immunization strategies will need to use Env variants displaying progressively lengthening of Env V1 loops. Interestingly, the DH270 UCA did not bind the TF Env but rather bound peptides derived from the base of the V3 loop region.

In the CH848 individual we also identified two V3 glycan antibody B cell lineages that cooperated with the DH270 B cell lineage by selecting autologous virus quasispecies with short V1 loops that were sensitive to DH270 neutralization (Bonsignori M and Haynes B. F., unpublished). Thus, these data provided evidence for the hypothesis noted above that cooperating B cell lineages may be a general requirement for bnAb development.

The V3-glycan PCDN, BF520-derived and DH270 bnAb lineages share some common traits: (i) differently from previously described V3-gylcan bnAbs,[23,25] their evolution did not involve insertion/deletion (indel) events, demonstrating that indels are not a universal requirement for the V3-glycan bnAb class to acquire neutralization breadth; (ii) neutralization breadth was acquired with relatively modest levels of somatic hypermutation that can be achieved through vaccination[57,60,61]; and (iii)

the UCA of the V3-glycan lineages did not neutralize or bind autologous TF suggesting the hypothesis that V3-glycan bnAb lineages may arise in response to altered forms of the Env protein.

3.5 | V1V2-glycan broadly neutralizing antibody development

The V2 glycan bnAb lineage VRC26 was isolated from an African individual, CAP256, followed from the time of infection to bnAb development.[19,22,62–64] CAP256 was infected with a clade C TF virus and superinfected with a second clade C TF approximately 15 weeks after primary infection.[63] The CAP256-VRC26 lineage was initiated by the superinfecting virus and the CAP256-VRC26.UCA was likely engaged by modestly mutated viruses.[19,64]

Recombination between primary and superinfecting viruses led to extraordinary diversity in autologous virus evolution with accelerated diversification, including sampling of different amino acids at position 169, and the preferred route of escape of the autologous virus from the CAP256-VRC26 lineage was a rare K169I mutation.[64] Non-bnAbs in the lineage followed two evolutionary pathways: they either displayed limited evolution and did not tolerate diversity at position 169 ("dead-end sublineage") or acquired somatic mutation levels comparable to those of the bnAbs but displayed a more restricted, strain-specific neutralization of autologous quasispecies variants ("off-track antibodies").[64] Conversely, in a mechanism that closely resembled CD4bs CH235 bnAb evolution,[16] CAP256-VRC26 lineage antibody neutralization breadth correlated with the ability to tolerate escape mutations selected by bnAb precursors—for the CH235 lineage it was loop D mutations, for the CAP256-VRC26 lineage is position 169—hence providing a blueprint for vaccine design to elicit V1V2-glycan targeted antibodies.[64]

3.6 | Intrinsic mutability of immunoglobulin genes and accumulation of somatic mutations

The evolution of the CH235 CD4 mimic CD4bs antibody lineage proceeded in a manner that depended less on antigen selection and more on intrinsic mutability of the V_H1 gene, since mutations accumulated at positions that also mutate frequently in non-HIV-1 antibodies.[16] Similarly, amino acid substitutions in CH235 at these positions were also frequently seen in non-HIV-1 antibodies.[16] These commonalities extended to other V_H1-2*02 and V_H1-46 CD4 mimic bnAbs[16] and V_H1-2*02 V3-glycan bnAbs (Bonsignori M and Haynes B. F., unpublished), implying that intrinsic mutability at specific nucleotide sites is a more general biological phenomenon that plays a role in dictating the degree of somatic mutations.[16,32,65,66]

We dissected the role of intrinsic mutability and activation-induced cytidine deaminase (AID) hotspots and cold spots in guiding the early phases of maturation of the DH270 V3-gylcan bNAb lineage (Bonsignori M and Haynes B. F., unpublished). The heavy chain rearrangement of intermediate antibody (IA) DH270.IA4, the least mutated IA in the DH270 lineage, differed from the DH270.UCA by 4 non-synonymous nucleotide mutations in the V_H1-2*02 gene segment. All four mutations involved sites of intrinsic mutability. While three mutations occurred at AID hotspots and resulted in both positional and identity conformity to non-HIV-1-reactive antibodies, the fourth mutation occurred at an AID cold spot (Bonsignori M and Haynes B. F., unpublished). This improbable Env mutation had two effects: (i) it disrupted an overlapping AID hotspot within the same codon and (ii) it introduced a new AID cold spot at the same position, thus fixing the improbable mutation throughout lineage maturation (Bonsignori M and Haynes B. F., unpublished). All subsequent mutations at sites of intrinsic mutability throughout the DH270 lineage maturation occurred at AID hotspots. The functional relevance of the improbable mutation in the DH270 bnAb lineage is that it was sufficient and necessary for neutralizing activity (Bonsignori M and Haynes B. F., unpublished).

Thus, the analysis of bnAb lineage evolution in the context of AID-induced intrinsic mutability is a powerful tool to identify which mutations are both critical to maintain lineage evolution on its path toward neutralization breadth and less likely to occur, so that immunogens can be designed to specifically target these rare mutations and ensure their selection through lineage maturation[16] (Bonsignori M and Haynes B. F., unpublished).

4 | NEUTRALIZATION ASSESSMENT OF BNAB LINEAGE DEVELOPMENT

Since 2009, several technologic advances have helped pave the way to the discovery of a new generation of bnAbs. Very few bnAbs were known prior to this time (eg, b12, 2G12, 2F5, 4E10), and the breadth and potency of these early bnAbs pale in comparison to the newer bnAbs.[12] High throughput assay technologies with molecularly cloned Env-pseudotyped viruses,[67–69] combined with a heightened awareness of the importance of the "Tier 2" neutralization phenotype of most circulating strains[70,71] were major contributors. New reporter gene assays in either TZM-bl or U87.CD4.CCR5.CXCR4 became available that are more rapid, sensitive and less costly than other assays, and are amenable to high standards of optimization and validation.[72] These refinements permitted robust large-scale testing to identify HIV-1-infected individuals who were promising sources of bnAbs that possess extraordinary potency and breadth.[73] They also permitted accurate, high throughput screening of thousands and sometimes tens of thousands of culture supernatants from activated memory B cells to rapidly identify viable neutralizing antibody-secreting B cell clones for immediate IgG gene amplification, sequencing and cloning. Equally importantly, researchers began to use Tier 2 rather than Tier 1 viruses in screening assays, which improved the selective identification of bnAbs among a plethora of other, less relevant antibodies that neutralize rare Tier 1 strains but do not neutralize common Tier 2 circulating strains. Large multi-clade panels of genetically diverse HIV-1 Env-pseudotyped viruses are now being used to assess and compare bnAb activity and to predict the optimal bnAb combinations that are likely to provide maximum clinical benefit.[74] Algorithms based on common patterns of neutralizing activity against panels of diverse Env strains are available, that can rapidly estimate whether a new bnAb targets

a known epitope.[75–79] Often times the epitope can be confirmed by testing neutralizing activity against mutant strains that contain known bnAb-specific diagnostic escape mutations.[80,81] Moreover, computational analyses of Env sequences and corresponding patterns of bnAb activity have been used to identify genetic signatures within and outside bnAb epitopes that compliment crystal structure information in guiding novel immunogen designs.[82,83]

These tools also play important roles in delineating bnAb lineage development. High fidelity functional Env clones of the TF virus and later variants obtained by single genome amplification (SGA) have been used to demonstrate multiple rounds of autologous neutralization and escape over the course of infection.[67] In most cases contemporaneous serum at each stage of escape is capable of neutralizing earlier but not later autologous Env variants.[67,84] Finally, as mentioned above, it is noteworthy that the UCA of most bnAb lineages exhibited little or no neutralizing activity against the autologous Tier 2 TF virus. This observation highlights the challenges of identifying suitable immunogens that will stimulate appropriate germline B cells as an essential early event in bnAb development.

5 | B CELL LINEAGE IMMUNOGEN DESIGN

The HIV-1 vaccine development field has realized that immunization with a single HIV envelope protein will not be successful at inducing bnAbs.[41] Moreover, with evidence for a role of host immune tolerance control mechanisms in limiting the induction of bnAbs,[32,41] the biology of bnAbs has begun to be elucidated. The role of the structure of the Env immunogen is undoubtedly important, as the Env must contain sufficiently native bnAb epitopes to bind in optimal affinities and correct orientation to the UCA, ie, the naïve B cell receptor (BcR), of bnAb lineages and maturation intermediate bnAb precursors avoiding, at the same time, the selection of maturing B cells with BcR that are drifting off-track from acquisition of neutralization breadth.[85,86] Thus, the concept of B cell lineage immunogen design has arisen, whereby the phylogeny of bnAbs from HIV-1 infected individuals is defined, and Envs are chosen from the co-evolving autologous virus quasispecies for sequential immunizations based on optimal affinity of Env immunogens for maturing on their path toward neutralization breadth[86] (Figure 5).

The B cell lineage immunogen design strategy is based on mapping the co-evolution of autologous virus and neutralizing lineages over time to select Env variants of the autologous virus quasispecies that participated in the selection of bnAb precursors by progressively engaging bnAb precursors, starting with the UCA and selection of immunogens with characteristics of Envs involved in natural induction of bnAbs in vivo.[16–19, 64]

While Envs have been designed for reacting with UCAs of heterologous bnAb lineages,[85, 87, 88] we have taken the approach of identifying, in selected HIV-1-infected individuals who make bnAbs, the natural sequence of Envs that were implicated in bnAb lineage maturation in order to select sequential immunogens. While such immunogens are designed for the UCA and intermediate antibodies of one particular bnAb lineage, they hold promise for inducing bnAb lineages in multiple individuals because of the remarkable conserved usage of V_H and V_L genes of bnAbs and the restricted nature of antibody motifs for many bnAb types, particularly for the gp41 membrane proximal region,[89] the CD4 binding site[42] and the V1V2-glycan site.[15,41,55,56] To mimic the

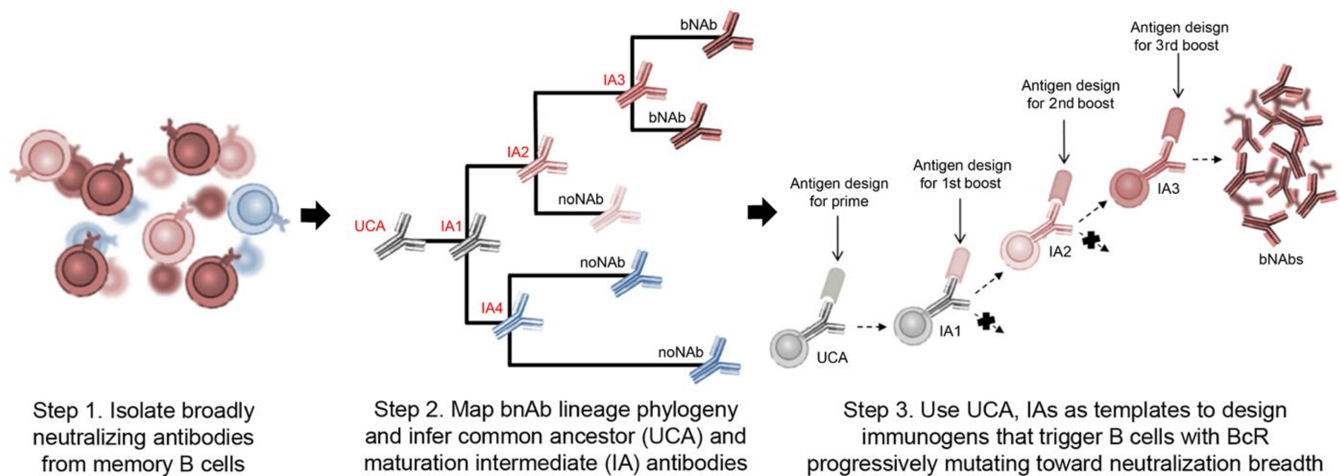

FIGURE 5 B-cell lineage-based approach to vaccine design. Affinity matured broadly neutralizing antibodies (bnAbs) and bnAb precursors are isolated from HIV-1 infected donors using methods such as memory B cell cultures or antigen-specific B cell sorting (step 1). Based on known bnAb sequences, next-generation sequencing can be used to retrieve numerous V_HDJ_H and V_LJ_L clonally related rearrangements. If appropriate longitudinal samples are available, it is possible to define the full lineage phylogeny and infer the unmutated common ancestor (UCA) and early maturation intermediate antibodies (IA) (step 2). Recombinant monoclonal antibodies expressing the bnAb precursor V_HDJ_H and V_LJ_L rearrangements from UCA and through IAs can then be used to design HIV-1 immunogens that will engage and select for B cells with BcRs evolving to neutralization breadth. Studying the co-evolution of autologous virus and bnAb lineages and the selection operated by cooperating lineages on autologous virus has in many cases identified Env immunogens that can engage the bnAb germline UCA antibody, and defined which HIV-1 Envs participated in bnAb lineage development, thus enabling the design of sequential immunogens (step 3)

progression of maturation of bnAb lineages, each Env should engage a bnAb precursor with affinity sufficient to trigger the B cell but with low binding affinity to allow for affinity maturation to the next stage of bnAb development. By selecting sequential Envs that progressively lost reactivity with the earliest, less mutated members of the bnAb lineage and either acquired or maintained high affinity for more mature antibodies on the observed pathway toward breadth, this strategy aims at providing an evolutionary advantage to antibodies accumulating mutations that will lead to the development of bnAbs in vivo (Figure 5).

This strategy aims at recapitulating the key events and interactions with the evolving autologous Env that shaped the development of a bnAb lineage toward breadth during chronic infection. Examples are the need for selection of improbable mutations and antibody exposure to progressively longer V1 loops for the V3-glycan *DH270* lineage maturation (Bonsignori M., Korber B. T. and Haynes B. F., unpublished), and specific mutations in Env loop D for the CD4bs CH103 bnAb lineage.[18] Hence, the goal is to favor a maturation pathway of naïve B cells in the repertoire of a vaccine recipient that can pass through specific stages that are instrumental for acquisition of neutralization breadth.

During HIV-1 infection, the TF virus can initiate HIV-1 B cell lineages in two ways. First, the TF can engage the BcR of a naïve, unmutated B cell and initiate B cell proliferation and accumulation of somatic mutations in V(D)J rearrangements as shown in Figure 3: for example,

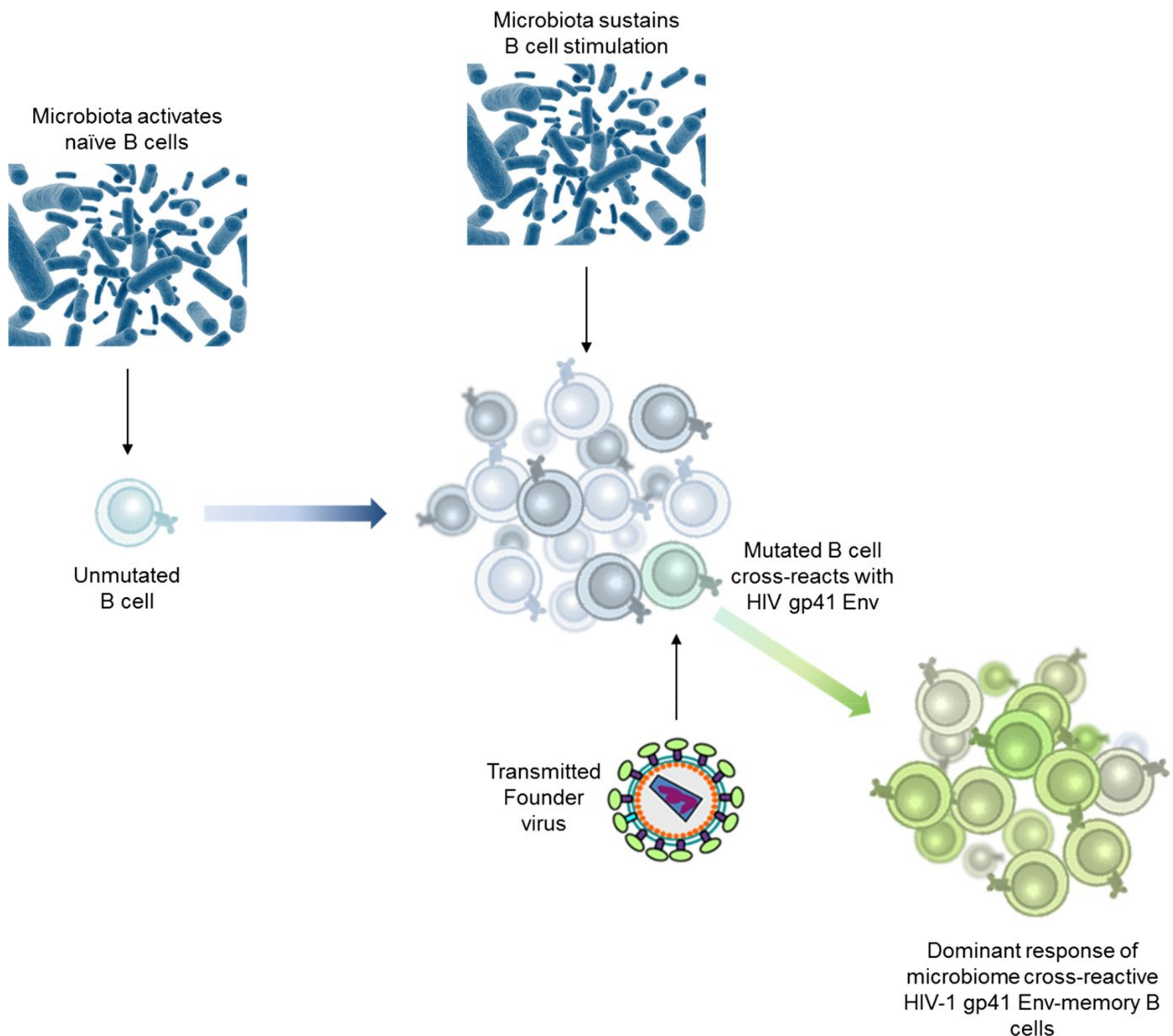

FIGURE 6 Mechanism of diversion of cross-reactive memory B cells by transmitted founder virus. Naïve B cells can be triggered by antigens derived from the microbiota or other environmental antigens and give rise to a somatically mutated clonal pool of memory B cells that undergoes affinity maturation against the cognate antigen (blue pathway). Among the microbiota-induced memory B cells, we have demonstrated that some cells can cross-react with HIV gp41 Env.[90–92] Transmitted/founder virus Env can engage these mutated cells, even if the unmutated ancestor naïve B could not, and diverge clonal evolution to affinity maturation against Env (green pathway). This mechanism resulted in a dominant expansion of non-neutralizing gp41-microbiota cross-reactive memory B cells

CD4bs bnAbs CH103[17] and CH235[18] derived from an African clade C HIV-1-infected individual bound to the autologous TF Env. Second, the TF Env may not engage the HIV-1 antibody UCA but rather engages latter members of the antibody lineage (Figure 6). We have found this to be the case with commonly made non-broadly neutralizing gp41 Env antibodies that are the first to arise in HIV-1 infection, for which the gp41 lineage was initiated by antigens derived from the microbiome before HIV-1 infection and, upon HIV-1 infection, gp41 Env engaged cross-reactive affinity matured B cells[90–92] (Figure 6). Diversion of pre-existing B cell responses to non-HIV antigens may be at play for some bnAb UCAs as well. Whereas the UCA of some bnAbs interact with Env antigens,[15, 17, 18, 58, 93] others may not.[87, 94–97] Thus, an alternative strategy in which non-HIV-1 antigens need be included as immunogens may be necessary to drive the initial maturation of non-HIV-reactive UCAs to maturation intermediates amenable to diversion toward HIV-1 specificity.

6 | ENV REGIMENS THAT DERIVE FROM ANTIBODY-VIRUS CO-EVOLUTION STUDIES

We have now reconstructed bnAb lineages from a number of HIV-1 infected individuals that made either gp41 membrane proximal lineage bnAbs[89,98] (Williams L. D. and Haynes B. F., unpublished), CD4bs bnAbs,[16–18] V1V2-glycan bnAbs[15] or V3-glycan bnAbs (Bonsignori M. and Haynes B. F., unpublished). Here, we discuss the use of antibody and virus co-evolution mapping data to design a first generation of immunogens for induction of two types of CD4bs bnAb B cell lineages, CDR H3-binder and V_H1-46 CD4 mimicking CD4bs bnAbs, and for V3-glycan bnAbs.

6.1 | Immunogen design for CD4-binding site CDR H3-binder bnAb immunogens

To choose Envs that may initiate CH103-like bnAb lineages, we assayed approximately 30 CH505 gp140 Envs over time (Figure 7) and selected four that bound well at each stage of the CH103 bnAb lineage starting with the TF envelope. With these immunogens, we elected to test two strategies: (i) to use an initial priming immunogen with a lower affinity for the UCA and lineage intermediates in order to select for progressively higher affinity antibodies with each successive boost, as conceptualized in the B cell lineage design approach, and (ii) to use an immunogen with higher affinity for the UCA (and each selected maturation IA) to expand a subdominant bnAb precursor pool. Thus, a 4-valent CH505 Env immunogen has been made as gp120s, as gp140 oligomers and as stabilized gp140 SOSIP trimers, each with progressively greater affinity for the CH103 UCA (Saunders K. O. and Haynes B. F., unpublished). When gp41-containing Env immunogens are administered to humans the phenomenon described above in Figure 6 occurs whereby the vaccine-induced Env response to gp41 is a dominant microbiome cross-reactive non-neutralizing antibody response.[91] Members of gp41 antibody lineages were present in prevaccination blood samples of vaccinees, demonstrating the existence of pre-existing gp41 cross-reactive clonal lineage members.[91] Thus, the use of gp120 sequential Env immunogens to induce CD4bs CDR H3-binder type of bnAbs will test the hypothesis that gp120s that are antigenic for Env lineages can initiate and select antibody lineage members with progressive affinity maturation and neutralization capacity while bypassing gp41 diversion of gp120 antibody responses. Interestingly, this phenomenon of gp41 diversion appears to be human-specific in that we have been unable to document this phenomenon in Rhesus macaques that have been immunized with gp41-containing Env vaccines (Han V., Saunders K. O., Permar S., Von Rompay, K. and Haynes B. F., unpublished).

6.2 | Immunogen design for CD4 mimicking CD4 binding site bnAb immunogens

The second bnAb lineage that occurred in CH505 was a V_H1-46, CD4-mimicking CD4bs bnAb lineage that over 6 years developed extraordinary potency and breadth (Figure 8A). Here, an extensive set of approximately 100 CH505 evolved autologous Envs were produced as both gp120s and/or gp140s and tested for binding to both CH103

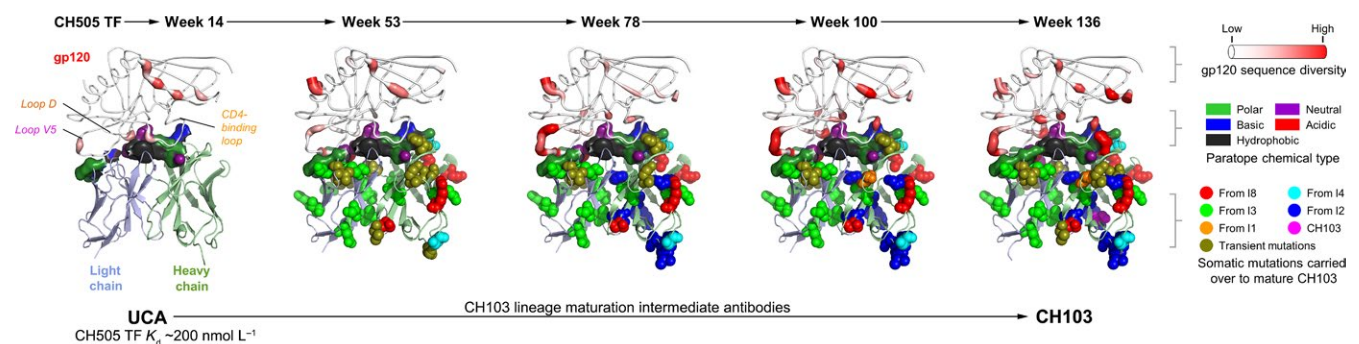

FIGURE 7 Immunogen design for CDR H3-binding CD4bs bnAbs. Interactions between evolving virus and the developing CH103 clonal lineage mapped onto models of CH103 developmental variants and contemporaneous virus as indicated. The outer domain of HIV gp120 is shown in worm representation, with thickness and color (white to red) mapping the degree of per-site sequence diversity at each time point. Models of antibody intermediates are shown in cartoon diagram, with somatic mutations at each time-point highlighted in spheres and colored according to first appearance of each mutation in IAs and CH103 bnAb as indicated. Paratope residues are shown in surface representation and colored by their chemical types as indicated. Figure adapted from Liao et al.[17] and used with permission

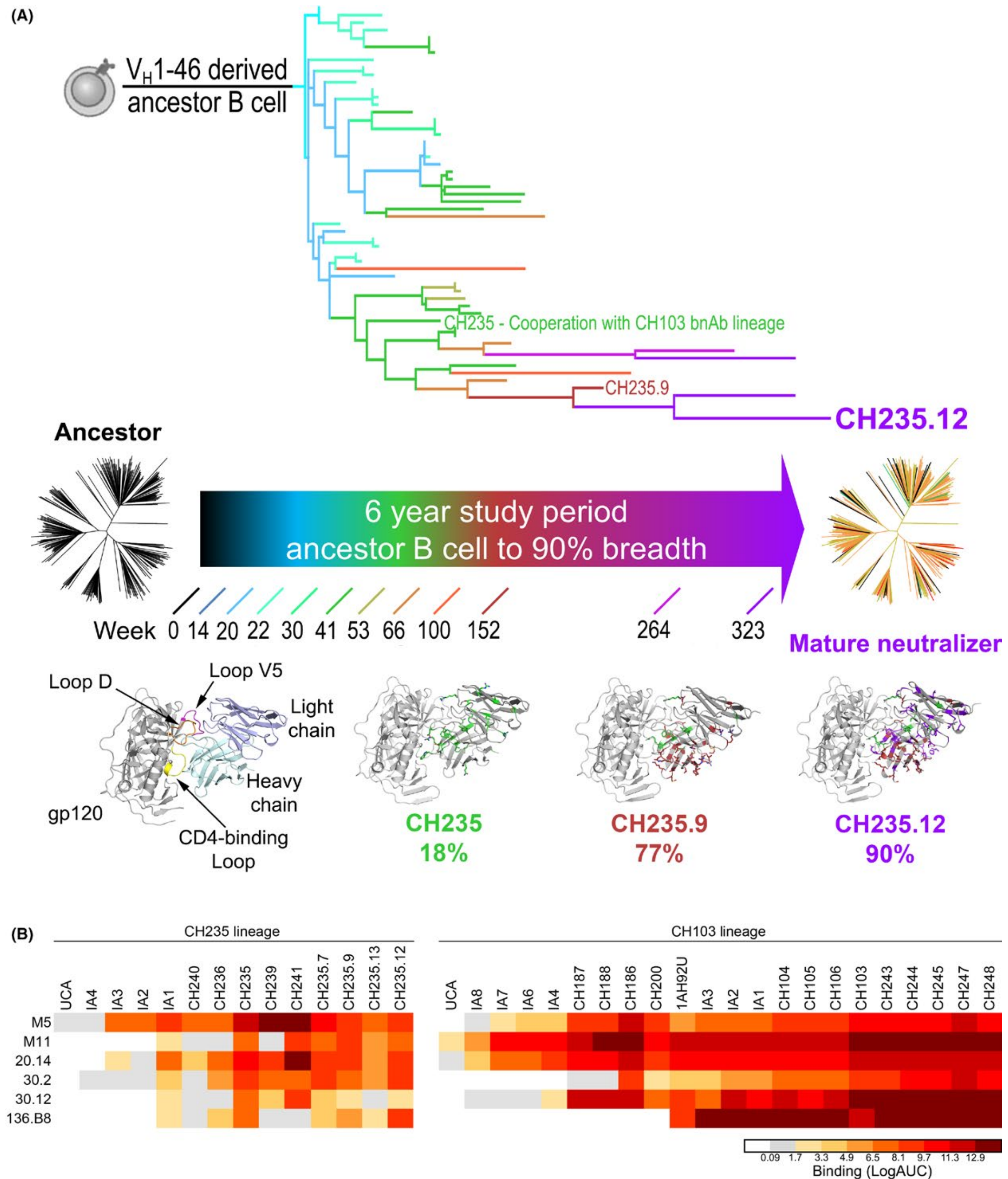

FIGURE 8 Immunogen design for concurrent elicitation of CD4 mimic and CDR H3-binder bnAbs. (A) Phylogenetic tree of the CH235 lineage, colored by first time (weeks postinfection) from which sequences were obtained. Cooperation with the CH103 lineage was exerted by bnAb precursors, such as the CH235 mAb, which displayed limited breadth. The structures of CH235, CH235.9 and CH235.12 Fabs in complex with gp120 (gray) show the residues altered by somatic hypermutation colored by time of appearance. As maturation progressed, CH235 lineage antibodies broaden their spectrum of neutralization to 90% for CH235.12. Neutralization dendrograms display single mAb neutralization of a genetically diverse panel of 199 HIV-1 isolates. Coloration is by IC_{50}. (B) Heat map analysis of selected autologous gp120 Env quasi-species variants binding to CH235 and CH103 lineage antibodies. Strength of binding (LogAUC) is shown in different shades of color as indicated, from white (<0.09) to dark red (>12.9). The gp120 Envs is a selection of immunogens optimized to induce both CH235- and CH103-like bnAbs based on their ability to progressively engage members of both antibody lineages with increasing binding strength. The M5 and M11 gp120 Envs are CH505 TF loop D mutants that best bound to the UCA of the two lineages.[16] Figures adapted from Bonsignori et al.[16] with permission

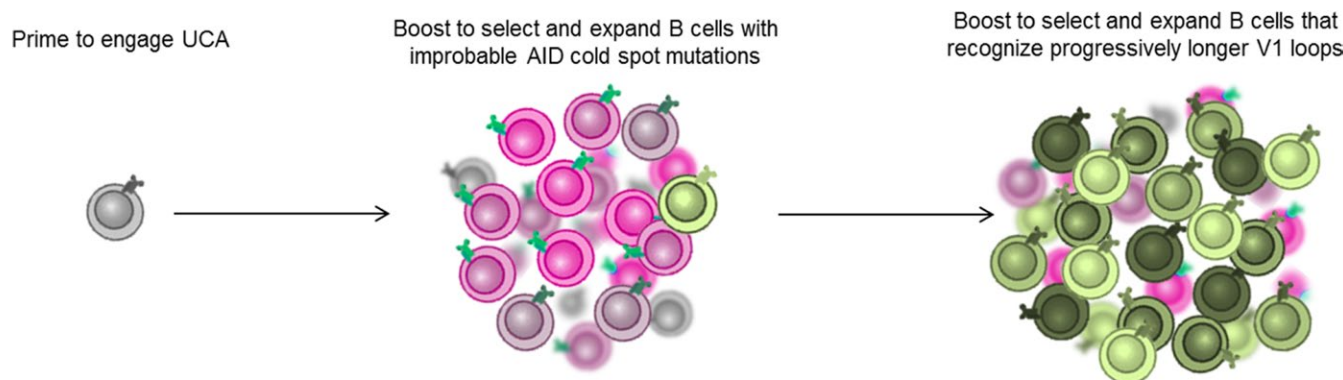

FIGURE 9 Immunogen design for V3-glycan broadly neutralizing antibodies. An immunization strategy to elicit DH270-like V3 glycan bnAbs is composed of three steps. First, prime with an immunogen that binds to the UCA. We have identified a synthetic Man_9 V3 glycopeptide as a candidate immunogen (Bonsignori M., Alam S. M. and Haynes B. F., unpublished). Clonal differentiation subsequent to priming will lead to the introduction of somatic mutations, including improbable mutations critical for clonal maturation toward neutralization breadth (green). Second, boosting with an immunogen that engages of DH270.IA4-like antibodies and select for the these improbable mutations, providing an evolutionary advantage to a subdominant response. Third, boost with Env immunogens with longer V1 loops, as the ability to neutralize viruses with longer V1 loops was correlated with broader neutralization in the DH270 lineage (Bonsignori M, Korber B. T. and Haynes B.F., unpublished)

and CH235 bnAb lineage members. From this analysis a 6-valent set of immunogens was chosen predicted to have optimal affinity to induce both CH103-like and CH235-like CD4bs lineages (Figure 8B). The best binder to the CH235 UCA was the M5 Env with a mutation in loop D that was found early on (approximately 4 weeks) after CH505 TF transmission.[18] At this time point, the TF sequence represented 88% of viral Envs while the M5 Env (which differed from the TF by one mutation in loop D, N279K), represented 10% of viral Envs.[18] The second Env chosen was the CH505 M11 Env isolated at week 30 after transmission, that had become partially resistant to the CH235 cooperating lineage but bound better to the CH103 UCA than the TF.[16, 18] Thus, in this regimen, a combination of M5 and M11 Env will be used as a priming immunization, followed by the 20.14 Env (from 20 weeks post-transmission), the 30.20 Env and 30.12 Env (from 30 weeks post-transmission) administered in sequence as boost and ending with a final boost of the week 136 Env, 136.B18.[16] Studies in CH235 and CH103 UCA V_H+V_L knock-in mice will facilitate choosing the Env forms (gp120s, gp140s, stabilized SOSIP trimers, multimers) of these Envs for optimal bnAb lineage initiation and selection of affinity matured bnAb precursors.

6.3 | Immunogen design for V3-glycan bnAb site immunogens

As noted above, neither we (Bonsignori M. and Haynes B. F., unpublished) nor others[20,99,100] have found binding of a TF Env to the V3-glycan UCA. Rather, peptides from the base of the gp120 V3 loop bound to the UCA suggesting Env fragments may initiate V3-glycan bnAb lineages (Bonsignori M., Alam S. M. and Haynes B. F., unpublished). Similar to the strategy for the design of the CH103 and CH235 CD4bs bnAb immunogens, we have expressed approximately 100 autologous Envs and then chosen them on the basis of (i) binding affinity to the expressed Env; (ii) autologous neutralization Tier; and (iii) the length of the Env V1 loop length (Figure 9).

An Env was chosen as one of the few Envs with ability to bind to the early DH270.IA4 IA with an improbable mutation, which represented a checkpoint for acquisition of neutralizing activity by the DH270 lineage. Subsequent Envs with short V1 loops were chosen for binding to intermediates of the DH270 lineage. The remaining Envs have progressively longer V1 loops and progressively weaker bnAb IA binding to provide selection of lineage members with affinity maturation at the later stages of bnAb maturation (Bonsignori M, Korber B. T. and Haynes B. F., unpublished).

7 | CONCLUSIONS

Work over the past 10 years has demonstrated that bnAb B cell lineages are disfavored, are in many cases controlled by tolerance or other improbable events, and will require targeting of specific B cell lineages with sequential immunogens to achieve induction of bnAbs. It has become apparent that to induce bnAbs several conditions must be optimized.

First, the immunogen will need optimization. The form of the immunogen may vary with different stages of the bnAb lineage, with a high affinity gp120[85,87,88] or minimal immunogen necessary for UCA BcR activation, and stabilized trimers may be necessary either to prime or for boosting at the mid- and later stages of lineage selection. A key aspect of the immunogen is the affinity of binding of Env by the bnAb lineage antibody. If each immunogen is selected for high levels of lineage BcR binding, then affinity maturation will likely be stifled with antigen binding at near maximal levels.[101] Rather, affinity of BcR binding will likely need to be progressively less as the lineage matures to be able to select bnAb lineage B cell BcRs with accumulations of somatic mutations needed for higher affinity virion Env binding and neutralization breadth.

Second, the sequence of Envs used will need to be optimized. Here, we have described some of the considerations that are used from

antibody-virus co-evolution studies. New bioinformatics tools have been developed to more precisely select Envs for immunization.[102] New considerations are the identification of the improbable AID cold-spot mutations that are bottleneck mutations required for lineage development (Bonsignori M. and Haynes B. F., unpublished) and Envs of viruses selected by cooperating B cell lineages that are highly sensitive to bnAb lineages.[18]

Third, the adjuvant to be used is critical, and will need to selectively drive high levels of T follicular helper cells (T_{fh}) and not activate or induce low levels of T regulatory cells (T_{reg}) in germinal centers.[103,104]

Finally, it has become clear that host controls of full bnAb lineage maturation are preventing the full development of bnAbs in the setting of vaccination. Some bnAb types such as gp41 MPER bnAbs (2F5, 4E10, DH511 and 10E8) must have hydrophobic CDR H3s for binding to the virion membrane, and bnAb precursors with these characteristics are either deleted in bone marrow or became anergic in the periphery.[98, 105–109] Other bnAb types such as CD4bs antibodies are either not deleted in bone marrow or less so than gp41 antibodies, but rather antibody poly-reactivity or auto-reactivity is acquired later in bnAb B cell lineage maturation.[17] Thus, for many types of bnAbs, the concept has arisen that a component of a successful vaccine for induction of bnAb B cell lineages to full neutralization breadth will be the formulation of Env vaccines in adjuvants that promote a profile of the immune system in bnAb development with high T_{fh} and low T_{reg}, and promote repeated rounds of affinity maturation in germinal centers.[103,104] In addition, HIV-1 infection induces autoimmune manifestations in approximately 50% of individuals, and those HIV-1 infected individuals that make bnAbs have higher frequencies of autoantibodies than those that do not make bnAbs, indicating that bnAbs arise in the setting of HIV-1-induced loosening of immune tolerance controls.[103] Thus, depending on the bnAb lineage, adjuvants or other inhibitors of immune tolerance controls may need to be utilized to achieve full bnAb maturation.

The path to bnAb induction remains difficult, and more than one bnAb type will need to be induced by a successful vaccine to prevent TF escape following transmission. Nonetheless, the recognition that induction of bnAbs is dependent on complex virus Env-antibody interactions and is under host controls has paved the way for design of new sequential immunogens that have the potential to overcome the hurdles standing in the way of bnAb induction.

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