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**Virological features associated with the development of broadly neutralizing
antibodies to HIV-1**

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1 The development of a preventative HIV-1 vaccine remains a global public
2 health priority. This will likely require the elicitation of broadly neutralizing
3 antibodies (bNAbs) able to block infection by diverse viral strains from across
4 the world. Understanding the pathway to neutralization breadth in HIV-1
5 infected people will provide insights into how bNAb lineages arise, a process
6 that probably involves a combination of host and viral factors. Here we focus
7 on the role of viral characteristics and evolution in shaping bNAbs during HIV-
8 1 infection, and describe how these findings may be translated into novel
9 vaccine strategies.

11 **Broadly neutralizing antibody responses to HIV-1 infection.**

12 One of the greatest challenges in the HIV-1 vaccine field remains the design
13 of immunogens able to elicit antibodies that can neutralize diverse HIV-1 strains.
14 While almost all HIV-1 infected people develop neutralizing antibodies (NAbs) during
15 the first year of infection, these antibodies are generally strain-specific [1-3]. This is
16 because they target the variable regions of the viral envelope (Env), which differ
17 profoundly between viruses [4-7]. It is only after several years of infection that
18 antibodies develop which can recognize conserved epitopes present on many
19 circulating strains [8-13]. Although a large proportion of infected people develop
20 some degree of neutralization cross-reactivity [12, 14], about 15-30% of individuals
21 develop very broadly neutralizing antibodies (bNAbs). These bNAbs, which are
22 sometimes able to neutralize 80-90% of viruses, are the types of antibodies that an
23 HIV-1 vaccine will ideally need to elicit. However, despite over 20 years of research
24 and many different immunogen designs, bNAbs have not yet been elicited through
25 vaccination, even in the RV144 vaccine trial that showed modest efficacy [15, 16].
26 Understanding the host factors (reviewed in [17-21]) and viral factors that contribute
27 to the development of bNAbs in natural infection may provide valuable insights for
28 vaccine design. Here we will focus specifically on the role of HIV-1 viral properties
29 and evolution in shaping the development of bNAbs during infection.

31 **Isolation of broadly neutralizing antibodies from infected people**

32 Since 2009, the number of broadly neutralizing monoclonal antibodies (bNmAbs)
33 isolated has surged from a handful to more than a 100 [22, 23], largely due to the
34 development of new technologies for human antibody isolation including antigen-
35 specific sorting, B cell culture with micro-neutralization assays for screening of

individual wells, and multiplex RT-PCR for immunoglobulin genes from single cells (reviewed in [22, 24, 25]). Many of these bNmAbs exhibit extraordinary breadth and potency [26-30], and have enabled the identification of five targets on the viral Env. These include the CD4 binding site (CD4bs) of gp120, the membrane proximal external region (MPER) of gp41, the C-sheet of the V1/V2 sub-domain (including the glycan at position 160) and the V3/C3 site on gp120 (including the glycans at residues 301 and 332) [reviewed in [22, 24]. More recently, the identification of a fifth site of vulnerability at the interface between gp120 and gp41 has highlighted the existence of additional epitopes [30-33]. The characterization of large numbers of bNAbs to each of these sites has provided insights into the many modes of recognition that may exist within overlapping regions, leading to their description as supersites rather than epitopes [34].

Many of the bNmAbs have unusual features such as a skewed germ-line usage, high levels of somatic hypermutation associated with extensive affinity maturation (particularly true of CD4bs bNmAbs), polyreactivity and long variable heavy chain third complementarity determining regions (CDR-H3) [27-29, 35-38]. The long CDR-H3 was recently shown to be formed during the initial immunoglobulin recombination event in the CAP256-VRC26 bNmAb lineage, suggesting that elicitation of these bNAbs would require engagement of rare germline B cells [39], although substantial affinity maturation was nonetheless still required for the development of breadth [38]. Together these features suggest a complex pathway towards bNAbs, posing significant challenges for vaccine development.

High viral levels are associated with, but not sufficient for neutralization breadth

Several studies have shown that the development of bNAbs is significantly associated with duration of infection, high viral load, low CD4+ counts, and a higher frequency of T follicular helper (Tfh) cells in the periphery, presumably a surrogate for germinal center Tfh cells [9, 12, 13, 17, 19, 20, 40-42]. Additionally, early preservation of both Tfh cells and B cell function are associated with the later development of breadth [20]. These factors are generally consistent with a requirement for high levels of antigenic stimulation of B cells, and the need for T cell help in driving the maturation of breadth over years of infection. However, although antigenic load is generally necessary for the development of breadth, many infected subjects in the CAPRISA acute infection cohort with high viral loads failed to develop

1 bNAbs over 4 years of infection [12] (Figure 1). In contrast, the occasional
2 development of bNAbs in individuals with low viral loads [43], and perhaps the fact
3 that most HIV-2 infected subjects develop bNAbs regardless of their viral loads [44-
4 46] suggests that high viral load is not the sole determinant of breadth, and that other
5 factors must also play a role.

7 **Do characteristics of the infecting virus determine whether breadth develops?**

8 One possible explanation for why only some infected people develop breadth
9 is that the virus that infects these people has intrinsic features better able to stimulate
10 the precursors of bNAbs. However, identifying specific envelope signatures of acute
11 viruses in individuals who later develop breadth is complicated by the overall
12 sequence diversity among circulating viruses, and by the fact that in many studies,
13 viruses were sequenced at the same point that neutralization breadth was measured,
14 not at the point of infection. Nonetheless, some signatures associated with breadth
15 have been identified. Rademeyer *et al.* showed in subtype C viruses that bNAbs
16 were associated with contemporaneous viruses with shorter variable loops (V1–V4)
17 [41]. However, in contrast, in a much larger study of multiple subtypes, Hraber *et al.*
18 showed that bNAbs were associated with contemporaneous viruses with longer
19 V1V2 regions [47]. In a computational comparison of multi-subtype sequences from
20 subjects with breadth and those without, Gnanakaran *et al.* identified six signatures
21 associated with breadth, mostly in the CD4/coreceptor-binding site of the viral
22 envelope [48]. However all three of these studies were limited in that it is likely that
23 the virus that was sequenced would already have escaped bNAbs, making it
24 impossible to determine if these features are associated with elicitation of breadth or
25 a consequence of breadth. In contrast, in a study of viruses sequenced prior to
26 breadth, Van Kerkhof *et al.* showed that shorter V1 loops, less overall glycosylation,
27 and the absence of a specific glycan at 332 (which forms part of a major bNAb
28 supersite, see above) were associated with increased breadth [49]. Further studies
29 such as this in large diverse cohorts are needed to identify which virological features
30 precede the development of breadth

31 While these examples are suggestive, overall it remains unclear how much of
32 the development of breadth is determined by features of the infecting virus (Box 1).
33 Because neutralization breadth normally emerges years after infection, the infecting
34 virus may be of less relevance than the later viral variant that actually engages the
35 naïve B cell encoding the bNAb precursor (the bNAb-initiating Env, Figure 2). These

1 viral stimuli are often hard to define without detailed longitudinal studies that require
2 the isolation of bNmAbs, followed by deep sequencing at multiple earlier time points
3 to precisely define when the earliest mAb-related transcripts can be detected (Figure
4 2). To date only two such studies have been reported [38, 50], In the study of the
5 CAP256-VRC26 lineage of bNmAbs, the earliest time point at which related
6 transcripts could be detected was 38 weeks after primary infection, and 23 weeks
7 after superinfection. By this time, the superinfecting virus was no longer present,
8 having been replaced by viral recombinants/variants, one of which presumably better
9 reflects the bNAb-initiating Env [38].

10 Identification of the precise Env sequence of the virus that stimulated bNAb
11 lineages through such longitudinal studies may be extremely useful for immunogen
12 design. A notable feature of the unmutated common ancestors (UCA), or germline
13 reverted approximations of many bNAbs, is their general lack of binding to HIV-1 Env
14 [51-54]. While much of this is likely due to the paucity of information used to infer
15 these UCAs, recent studies have defined barriers preventing the binding of UCAs to
16 viral Env. For example, germline-reverted versions of CD4bs bNmAbs such as
17 VRC01, NIH45-46, 3BNC60, and 12A21, all of which use the VH1-2 antibody alleles,
18 generally do not bind viral Env because of obstruction of the light chain [55]. Deletion
19 of a highly conserved glycan at residue 276 in Env results in binding of both heavy
20 and light chains and activation of B cells expressing germline VH1-2 receptors. This
21 finding may account for the inability of previous immunogens to elicit CD4bs
22 antibodies, and as N276-lacking natural Env are rare (5% of HIV-1 strains) may also
23 explain why such specificities are not often elicited in natural infection. Overall,
24 studies that identify bNAb-initiating viral Env (either naturally-occurring or engineered
25 to become germ-line reactive) are important to identify for translation into
26 immunogenicity studies [51-55].

27 28 **Does early viral diversity drive the development of bNAbs?**

29 Another potential contributor to breadth is viral diversity. One of the first
30 observations that suggested that the quasispecies diversity (rather than the founder
31 virus that initiated infection, or the overall viral loads) may contribute to breadth came
32 from the findings that early viral diversity was associated with the subsequent
33 development of bNAbs [40]. The possibility that exposure to diverse envelopes may
34 contribute to breadth suggested that individuals who are superinfected with HIV-1,
35 and thus exposed to multiple variants/recombinants, should also develop better

1 bNAbs, and indeed two studies of superinfection support this. Powell *et al.* showed
2 that dual infection resulted in enhanced breadth, though in this study confounding
3 factors such as the duration of infection could not be excluded [56]. A more recent
4 study reported both increased breadth and potency in superinfected individuals
5 compared with singly infected subjects, with breadth emerging within a year of
6 superinfection, independently of viral load and CD4+ T cell counts [57]. Intriguingly,
7 in this study, both intersubtype superinfection and the persistence of both infecting
8 viruses was linked to increased breadth, again suggesting that exposing the immune
9 system to diverse viruses enhances breadth. Furthermore, the developmental
10 pathway for bNAbs in CAP256, who was superinfected, was recently reported [38].
11 However, it is important to note that in this case, the role of superinfection, beyond
12 simply providing an additional mechanism for viral diversification through
13 recombination, is unclear. If superinfection does indeed drive enhanced breadth, one
14 unanswered question is whether superinfection and increased diversity is simply
15 additive and 'stacks the odds' in favor of encountering an Env able to bind and trigger
16 the appropriate B cell, or whether the presence of multiple genotypes exponentially
17 drives B cell affinity maturation, suggesting a more mechanistic explanation for these
18 findings (Box 1).

20 **Viral diversification during the development of bNAbs: chicken or egg?**

21 In addition to the association of viral diversity prior to bNAbs, recent studies
22 have also shown profound viral diversification as bNAbs emerge. Viral neutralization
23 escape is well described, with substitutions, glycan shifts, and indels frequently
24 associated with the development of strain-specific NAbs [2, 58, 59]. Despite the
25 conserved nature of the bNAb epitopes, it has become clear that HIV-1 is also able to
26 escape from bNAbs very effectively [42, 60-64], consistent with the fact that the
27 development of such antibodies has not been shown to confer an obvious clinical
28 benefit [12, 13]. This suggests that, as with strain-specific NAbs, the development of
29 bNAbs would drive increased viral diversification as viral escape occurs. Indeed,
30 Euler *et al.* reported that the peak in envelope sequence diversity coincided with the
31 peak of bNAb titers [65]. However, the two recent studies of the ontogeny of
32 bNmAbs, namely the CH103 CD4bs lineage [50] and the CAP256-VRC26 V2-glycan
33 lineage [38] suggested that in addition to bNAbs driving viral escape, the opposite
34 may also be true, i.e., viral diversification within epitopes through escape may
35 contribute to the maturation of bNAbs. These, and other studies have provided
36 important clues as to the precise mechanisms whereby viral evolution may drive

increased breadth; namely, through the creation or exposure of bNAb epitopes, or through the generation of multiple immunotypes (or epitope variants), described in more detail below.

Immune escape from strain-specific antibodies and the creation of bNAb epitopes

One of the more common bNAb specificities detected in cohorts of infected individuals are those that target the V3/C3 supersite that includes the glycan at position 332 (PNG332) on the viral Env [27, 66, 67]. A longitudinal study of two subjects shown to develop PNG332-dependent bNAbs surprisingly showed that in both cases the infecting virus did not have a glycan at that position (Figure 3A) [42]. Single genome sequencing of viral RNA from longitudinal samples showed the evolution of the 332 glycan and the V3/C3 bNAb epitope by 6 months of infection, prior to bNAb development. Plasma mapping studies showed the presence of strain-specific NAb responses to the V3/C3 region of the envelope [42], and further showed that the insertion of the 332 glycan mediated escape from this early strain-specific antibody. Thus neutralization escape from strain-specific NAbs resulted in viral convergence towards a conserved glycan motif (the 332 glycan is present on 72% of global viruses, see pie chart, Figure 3A) creating the epitope targeted by later bNAbs [68]. This pattern of selection of the 332 glycan was evident at a population level using more than 7,300 single genome derived envelope sequences from 68 acute and 62 chronic HIV-1 infections [42]. However, although the absence of this glycan on infecting viruses has been associated with the subsequent development of bNAbs in a separate study (see above and [49]), this pattern of evolution does not always result in the development of bNAbs, again highlighting the role of other factors in driving this process.

The role of strain-specific NAbs in forming bNAb epitopes was similarly shown in a separate study of CAP257, an infected subject who developed V2-directed bNAbs [64]. Here, an initial strain-specific NAb response was directed to an N167 residue in V2. N167 is very rare among global isolates, occurring in only 5.6% of sequences (pie chart, Figure 3B). Viral escape from this initial response resulted in a N167D mutation by 30 weeks (i.e., a mutation towards the consensus, with D167 present in 87% of global viruses). Viral escape therefore here again resulted in the creation of a V2 epitope that was much more representative of global viruses and presumably became the stimulus for the V2 bNAb response.

While in both these studies, escape from a strain-specific response created a conserved epitope that became the target of later bNAbs [42, 64], a key unanswered question is whether (i) the strain-specific C3- or V2-directed NAb were part of the same lineage as the bNAbs, and matured to target the emerging 332 glycan/D167 residue, or (ii) whether the creation of the bNAb epitope was driven by an unrelated antibody lineage to an overlapping epitope. Although the former scenario may be more amenable to immunogen design, the latter has been directly demonstrated in a follow-up study of the CD4bs bNmAbs described above [69]. In addition to the CH103 bNAb lineage, a second NAb lineage, the CH235 clonal lineage, was isolated from the same donor, CH505. CH235 NAb drove viral escape through several mutations in the D loop of the viral envelope, which forms part of the CD4bs bNAb epitope. These mutations, while effectively mediating escape from CH235-related antibodies, surprisingly resulted in viral variants becoming more sensitive to the developing CH103 bNAb lineage, thereby contributing to the maturation of breadth [69]. This study, through the use of isolated mAbs and viral mutants conclusively demonstrated the role of viral escape from strain-specific NAb in the creation of bNAb lineages, and led to the concept of co-operation between different antibody lineages in the development of neutralization breadth.

Viral escape and exposure of occluded bNAb epitopes

In CAP257, the same subject who developed bNAbs to the V2 region (described above), a second bNAb response developed later in infection, this time targeting the CD4bs [64]. This enabled us to characterize the relationship between these two evolving specificities [64]. Viral escape from the V2-directed bNAbs drove the deletion of a highly conserved glycan at position 160 in V2 [64]. This deletion resulted in unusual exposure of the CD4bs, which subsequently became the target for the next wave of bNAbs. Thus, viral escape may force the exposure of otherwise occluded conserved epitopes, facilitating the development of breadth. Intriguingly, V2 and CD4bs antibodies were found in three of the four individuals where multiple bNAbs have been described, suggesting this may be a common developmental pathway for consecutive specificities that might be useful for immunogen design [64, 70-72]. These data suggest the possibility that escape from bNAbs, at the expense of potentially deleterious exposure of occluded epitopes, is the result of a trade-off between the need for escape at sites that are normally conserved, and fitness in the presence of bNAbs.

1

2 **Viral escape from bNAbs creates novel immunotypes that drive breadth**

3 Several studies have suggested that bNAbs may mature from earlier binding or
4 strain-specific neutralizing antibodies [38, 50, 64, 73]. This need for ongoing antibody
5 maturation suggests incomplete viral escape from bNAb precursors, which would
6 otherwise not continue to evolve. This is supported by a comparison of the CH103
7 and CH235 mAbs, which showed that the extent and rate of viral escape from the
8 two lineages differed. Complete escape from the CH235 mAb lineage occurred by 53
9 weeks of infection (though it is not known exactly when the lineage was initiated). In
10 contrast, members of the more broadly neutralizing CH103 lineage continued to
11 neutralize viral variants as far as 100 weeks post-infection, which is at least 86 weeks
12 after the antibody lineage was first detected by deep sequencing. These findings
13 suggest that the continued ability to neutralize autologous viral variants might be an
14 important aspect of bNAb maturation [69]. There is evidence to support this notion for
15 both strain-specific nAbs and bNAbs. Studies of strain-specific plasma responses to
16 autologous viruses have shown differential sensitivity of viral variants at a given time
17 point to strain-specific NAbs [69, 74]. Similarly, Murphy *et al.* showed that two
18 antibody light chain variants paired with a single heavy chain displayed differential
19 neutralization of autologous viruses containing escape mutations at the base of
20 V3/C3 region [75]. These findings suggested that viral evolution drives strain-specific
21 NAbs to recognize amino acid variants within a given epitope [75]. The subject of this
22 study did not go on to develop bNAbs, however Wibmer *et al.* documented similar
23 findings in CAP257 who did develop bNAbs to the CD4bs [64]. This study combined
24 detailed mapping of evolving plasma specificities with longitudinal analyses of viral
25 escape mutations, to show that the CD4bs bNAbs acquired increased breadth in
26 response to the serial emergence of escape mutations within the epitope [64]. The
27 early CD4bs bNAb response in CAP257 was only able to neutralize heterologous
28 viruses with an N279 residue in the D loop, part of the bNAb epitope. Global viruses
29 generally contain either a N279 (50%) or a D279 (47%). Thus an antibody with
30 complete dependence on N279 would only be able to neutralize half of global
31 viruses, whereas the ability of a bNAb to tolerate either a N279 or a D279 would
32 theoretically confer double the potential neutralization breadth. Interestingly, early
33 escape from the initial CD4bs response was mediated by a N279D mutation. The
34 maturation of the plasma response to tolerate this escape mutation in circulating viral
35 variants corresponded with the increased breadth of this plasma, and with the ability
36 to neutralize heterologous viruses with either an N or D at this position. The similar

1 maturation of this CD4bs bNAbs response to neutralize later autologous escape
2 variants containing mutations at additional sites within the epitope (at residues 276
3 and 456) prior to complete neutralization escape only 70 weeks later supports a
4 model where escape mutants result in evolution of the bNAbs response to tolerate
5 variation at some sites within an epitope, with a 'footprint' becoming increasingly
6 focused on core conserved motifs (Figure 4). Thus the evolving antibody swarm in an
7 infected individual may be driven towards breadth through exposure to multiple
8 epitope variants generated as the viruses 'toggles' between early escape pathways,
9 before complete viral escape. This ability to recognize multiple immunotypes,
10 emerging as a result of neutralization escape has also been associated with
11 increased breadth in other studies [38, 61, 76]. These data suggest this may be a
12 common feature of subjects with breadth, with the potential to be exploited for
13 immunogen design.

14 15 **Concluding remarks: implications for HIV vaccine design**

16 Studies of the viral drivers of breadth are based on the premise that, unlike host
17 factors, defining the virological factors associated with breadth may provide
18 opportunities for translation into immunogen design. One unanswered question is
19 whether viruses infecting people who later develop bNAbs are intrinsically better
20 immunogens. This possibility is supported by the observation that BG505, an Env
21 that forms unusually stable trimers, and as such is a major current focus for
22 immunogen design, was derived from an HIV-1-infected infant who developed
23 bNAbs. Whether this trimeric recombinant Env protein is able to induce bNAbs in
24 vaccinated individuals remains to be determined [77-79]. Regardless, it is unlikely to
25 be this simple, and the unusual features of the bNAbs together with the fact that a
26 minority of individuals develop very broad responses, suggest that the development
27 of breadth is a complex interaction of multiple factors. However the data described
28 here suggest the evolving virological features are likely to play a key role.
29 Comparison of bNAbs Env with those from subjects who fail to develop significant
30 breadth is likely to continue to provide insights into viral signatures of breadth.
31 However immunogenicity studies would be the 'gold standard' in testing these
32 signatures to define whether their incorporation into vaccine strategies may be
33 valuable.

34 The increasing appreciation that viral evolution may be important in shaping
35 bNAbs (through at least three mechanisms, summarized in Figure 5) has provided

support for the notion that exposure of the immune system to epitope variants through vaccination may drive the development of enhanced breadth [22, 23, 25, 38, 42, 50, 64, 69, 80-82]. Thus, a vaccine strategy may need to begin with a (natural or engineered) Env immunogen able to engage the appropriate B cell precursor, followed by the sequential use of immunogens incorporating key epitope variants associated with driving breadth. Indeed, such studies, using sequentially isolated envelopes from infected subjects who rapidly developed breadth have shown moderate improvements in NAb responses [80, 81, 83]. Emerging data that better defines key immunotypes and synergies between specificities (such as that between the V2 and CD4bs bNAb epitopes) will provide exciting new opportunities for testing these concepts in immunization strategies.

Box 1. Outstanding questions

- Are envelopes from subjects with breadth intrinsically better immunogens?
- Does superinfection and increased diversity simply 'stack the odds' in favour of encountering an Env able to bind and trigger the appropriate B cell, or is there a more mechanistic explanation for how diversification drives breadth?
- Can the immune system be 'coaxed' along the pathway to breadth through exposure to serial immunogens?

Figure Legends

Figure 1. Viral load is necessary but not sufficient for neutralization breadth. Infected subjects from the CAPRISA acute infection cohort stratified by viral load and neutralization breadth (defined as neutralizing >40% of a panel of 42 heterologous viruses at three years post-infection [12]). Subjects with bNAbs and high viral load are shown in red, no bNAbs despite high viral load in green, and those with no bNAb and low viral load in blue. X-axis, weeks post-infection, Y-axis, viral load (RNA copies/ml).

Figure 2. Longitudinal bNAb and virus co-evolution studies will enable the identification of viral envelopes that engaged the B cell expressing the unmutated common ancestor (UCA) of a bNAb lineage (the bNAb-initiating Env). **(A)** bNmAb

isolation from infected subjects followed by retrospective deep sequencing at multiple time points to identify the time point at which the unmutated common ancestor emerged. **(B)** Parallel virological studies, also at multiple time points, will enable the identification of the bNAb-initiating Env.

Figure 3. Strain-specific antibodies result in the creation of bNAb epitopes through viral escape. **(A)** Strain-specific antibodies to the C3 region result in viral escape through the addition of a glycan at position 332 [42]. This glycan, conserved in 72% of global viruses (pie chart, right) became the target of maturing/newly developing NABs, resulting in the development of bNABs. **(B)** Similarly, a strain-specific V2 response targets the rare N167 residue (present in only 5.6% of global viruses). Viral escape occurs through a N167D mutation, with D167 representing the consensus at the position, present in 87% of viruses (pie chart, right), resulting in the development of bNABs.

Figure 4. Model for the maturation of strain-specific plasma responses to acquire breadth through exposure to developing immunotypes (epitope variants). **(A)** Strain-specific antibodies (black) recognize defined epitopes (shown in gray, in a 4-domain lock-and-key schematic). **(B)** Early NABs drive initial viral escape mutations (represented as a yellow shape at the fourth domain) within epitopes. Affinity maturation enables the maturing NABs to tolerate both the gray and the yellow variants, through a more focused footprint. **(C)** Further viral escape occurs (orange shape, first domain), resulting in maturation of NABs to tolerate epitope variation at domains 1 and 4. **(D)** The emergence of further (red) mutations at domain 1, 3, and 4 results in maturation of NABs to focus on a conserved epitope core (or 'footprint', shaded in the gray box) present on heterologous viruses, enabling the maturation of bNABs. Note: an earlier iteration of this model was published in [17].

Figure 5. Summary of mechanisms whereby viral evolution shapes the development of HIV-1 neutralizing breadth. This can occur through **(A)** creation of bNAb epitopes through viral escape from strain-specific antibodies, **(B)** exposure of bNAb epitopes through viral escape mutations, and **(C)** affinity maturation of NAb lineages by exposure to different immunotypes.

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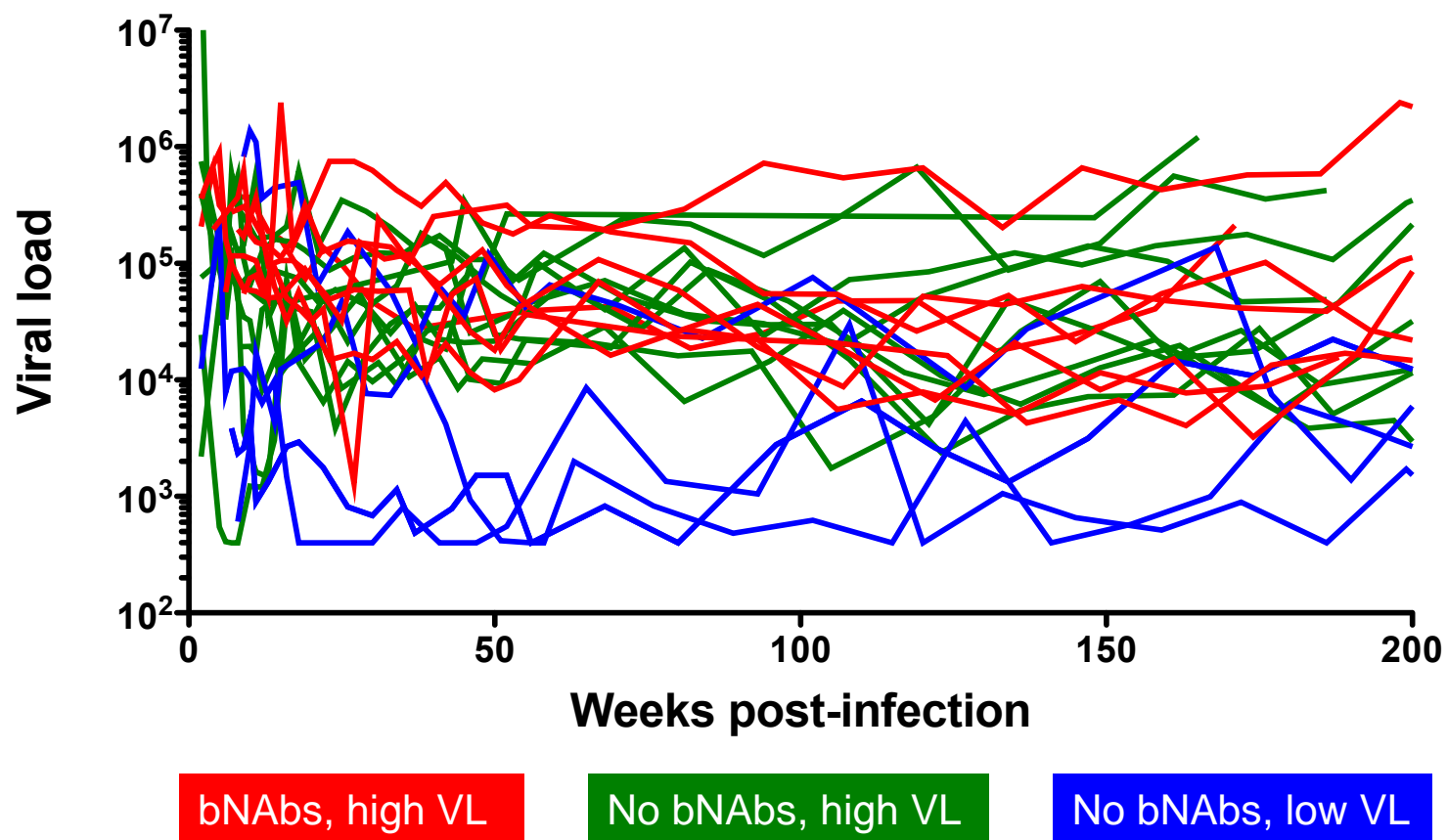


Figure 1

A.

First detection of mAb
related transcripts using
deep sequencing

Isolation of mAbs



Strain-specific plasma
antibodies

Plasma bNAbs

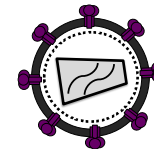
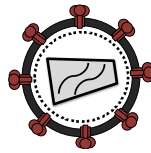
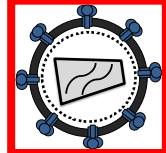
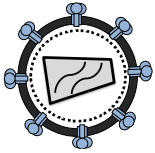
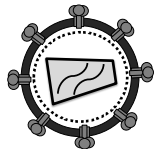
B.

0

100

200

Weeks
post-
infection



Transmitted/
founder
virus

Longitudinal viral variants



bNAb-initiating Env

Figure 2

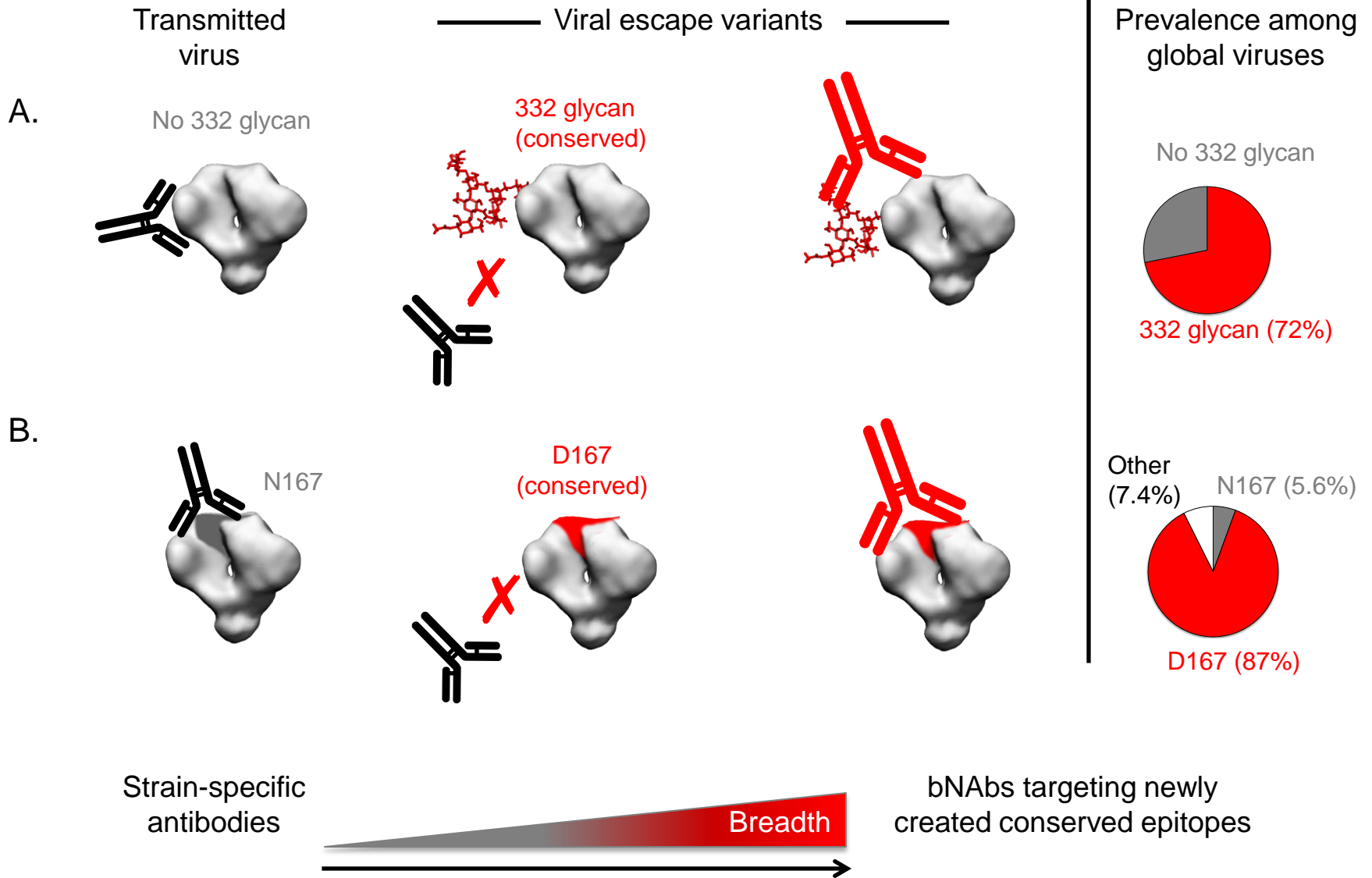


Figure 3

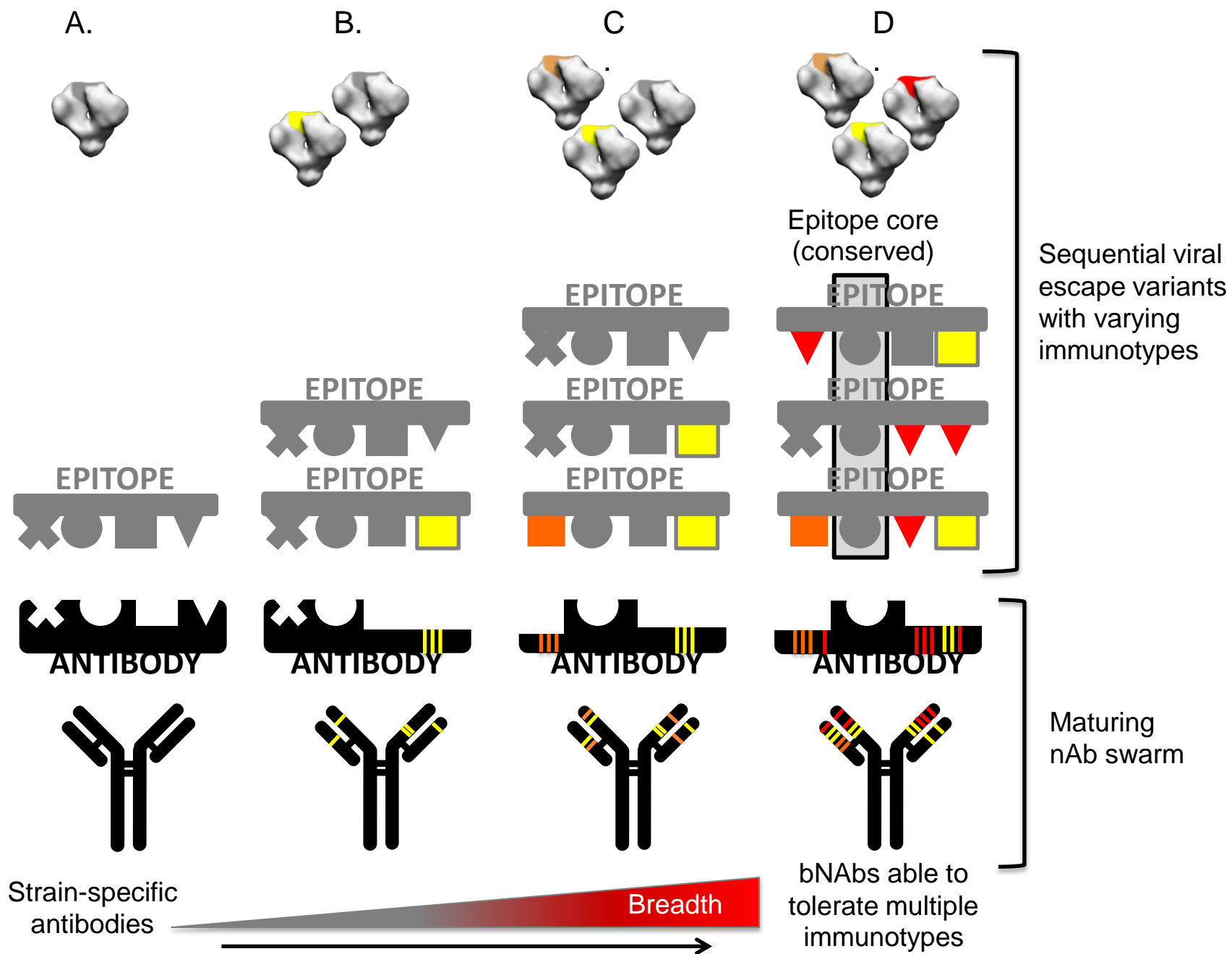


Figure 4

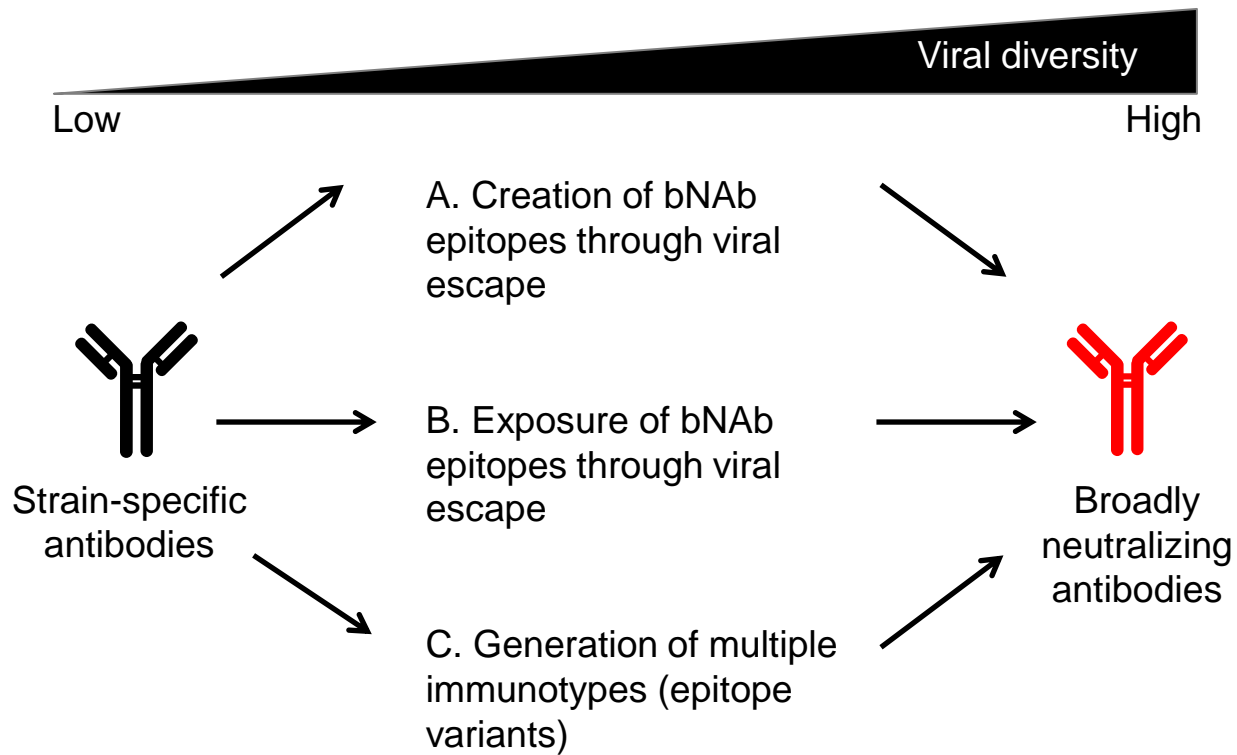


Figure 5