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Antigenic landscape of the HIV-1 envelope and new immunological concepts defined by HIV-1 broadly neutralizing antibodies

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Abstract

The isolation of HIV-1 broadly neutralizing antibodies (bnAbs) has demonstrated the ability of the human immune system to mount effective antibody responses against the virus. To harness this immune potential to elicit similar antibody responses by vaccination, it is important to understand the immunological processes that produce them. Here we review recent advances in crystal structural determinations of HIV-1 bnAb epitopes that directly portray the antigenic landscape of the HIV-1 envelope glycoprotein. We also summarize new immunological concepts implicated in bnAb sequences and their lineage studies.

Introduction

The isolation and characterization of HIV-1 broadly neutralizing antibodies (bnAbs) [1–24], summarized in Table 1, define the antigenic landscape of the HIV-1 envelope (Env) glycoprotein and provide an opportunity to learn about the immunological processes that produce them. To harness this immune potential by vaccination, current efforts have focused on Env immunogen design and bnAb lineage studies. Structural analyses of HIV-1 bnAb epitopes and their implications for vaccine research have been extensively reviewed [25–30]. Here we focus on recent advances in newly identified bnAb epitopes and immunological concepts implicated in bnAb sequences and their lineage development.

Antigenic landscape of the HIV-1 Env

The native HIV-1 Env trimer has each monomer composed of a surface unit gp120 and a transmembrane unit gp41 non-covalently associated. Antigenically, the Env monomer and trimer are distinct as the trimer packaging sterically shields antigenic sites that are fully exposed on the monomer. Recent generation of the soluble cleaved BG505 SOSIP trimer [31] and its structural determinations (Fig. 1) have greatly advanced our understanding of

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the Env trimer packaging [32–34]. HIV-1 Env is also known to be flexible and undergoes conformational changes from “close, unliganded” to “open, CD4-bound” during viral entry [33–35]. Because the CD4-bound state exposes antibody epitopes that are otherwise shielded in the unliganded state, different conformational states will impact Env antigenicity and immunogenicity.

V1V2 in monomer and trimer context

The disulfide bond-nested first and second variable region (V1V2) of gp120 has generated interest as a target for vaccine development because of data from the RV144 human trial showing V1V2 antibodies inversely correlate with risk of infection [36,37]. Additionally, a panel of bnAbs including PG9, PG16, CH01-04, PGT145, and CAP256-VRC26, all with a long heavy chain CDR3 (H-CDR3), have been identified to target the V1V2 region (Table 1), preferentially in trimer context. Although V1V2 is the most sequence diverse region in gp120 and has large length variability, crystal structural data suggest that it may function as a modular domain with unique structures [38–40]. In stabilized SOSIP trimers [32,33] or a structurally constrained V1V2-scaffold bound to V1V2-specific mAb 830A [40], V1V2 domain is a five-stranded betabarrel structure with strands named A, B, C, C', and D. Crystal structures of PG9 and PG16 in complex with scaffolded V1V2 have shown that the epitopes of these two bnAbs (Fig. 1) consist of Strand C and two glycans at N160 (HXB2 numbering) and N156 or N173 [38,39]. The long H-CDRs of PG9 and PG16 shaped like a hammerhead can reach the backbone of Strand C between the two glycans, forming a strand-strand interaction. Interestingly, Strand C can also adopt helical conformations as shown in the structures of mAbs CH58 and CH59 developed from a RV144 vaccinee [41]. These mAbs were induced by gp120 vaccines, suggesting that Strand C of V1V2 can adopt a helical structure in structurally unconstrained monomeric gp120. Such structural polymorphism may mask this site of vulnerability. Other bnAbs such as PGT145 and its clonal variant PGDM1400 [42] also bind the V1V2 apex of Env trimer, but they are strictly trimer specific. Cryo-EM data suggest that they contact the center of the V1V2 apex on the Env trimer [42].

V3 and its glycan-dense base

The third variable loop (V3), linked by a disulfide bond between Cys296 and Cys331 of gp120, is highly immunogenic, and antibodies targeting its apex crown region are induced in virtually all HIV-1-infected humans. However, because the V3 crown is pointing to the center and packed underneath the V1V2 apex in the unliganded Env trimer [32], antibodies targeting the V3 crown cannot access their epitopes and thus lack neutralizing activity. The V3 base, however, is exposed on the trimer surface, though protected by dense glycans. The bnAb PGT121, PGT128, and PGT135 families (Table 1) recognize discontinuous amino acids of this region and a high-mannose patch near N332, possibly involving other glycans at N137, N301, N332, N386, and N392 [43]. The epitope of PGT128 (Fig. 1) mainly consists of glycans at N301 and N332, and a short stretch of 5 amino acids (residues 323–327) near the V3 C-terminus [16]. This relatively simple composition may render the PGT128 epitope feasible for engraftment onto protein scaffolds [44].

CD4-binding site (CD4bs) and other regions of gp120 core

CD4bs is functionally conserved and located on the gp120 core that consists of an inner domain, an outer domain (OD), and a bridging sheet (formed only in the CD4-bound state) connecting the two [45]. With the CD4bs-targeting bnAb b12 discovered in 1994 [46], the CD4bs has been a known site of vulnerability for many years. The b12 epitope identified the OD region of CD4bs to be structurally stable and antibody accessible [47], a finding that led to the design of RSC3 and isolation of VRC01 (Fig. 1) [1]. To date, the VRC01-class of bnAbs has been isolated from more than 10 donors (Table 1) that share a sequence or genetic signature of the IGHV1–2 gene usage and a short light chain CDR3 (L-CDR3) of 5 amino acids [6,48]. Additionally, the CD4bs is also targeted by 6 other classes of bnAbs exemplified by HJ16, 8ANC131, CH103, VRC13, VRC16, and 179NC75 (Table 1).

Crystal structural analyses of the CD4bs-targeting bnAbs in complex with gp120 core have revealed substantially overlapping epitopes and different modes of gp120 recognition [6,9,49], with the VRC01-class and 8ANC131-class partially mimicking the CD4 interaction with gp120. Although the CD4bs itself is not glycosylated, it is surrounded by glycans, and the bnAbs targeting this site all avoid or accommodate glycans to reach their epitopes. Also, because the CD4bs of gp120 is always available for interaction with the primary cellular receptor CD4, the Env trimer packaging and conformational changes have minimal impact on bnAbs that target this site. However, this is not the case for non-neutralizing monoclonal antibodies (mAbs) that bind only to CD4bs on monomeric gp120 but not on native trimer [50]. Vaccine-induced mAbs to CD4bs to date are not broadly neutralizing against HIV-1 [51]. Other regions of gp120 core, such as the inner domain and the co-receptor-binding site mostly consisting of the bridging sheet, are either buried in Env trimer or subjective to Env conformational changes. Therefore, mAbs directed to these sites cannot effectively neutralize the virus.

The gp120-gp41 interface and fusion peptide (FP)

HIV-1 bnAbs targeting the gp120-gp41 interface and FP are among the newest identified and include 8ANC195, 35O22, PGT151, and VRC34 (Table 1). PGT151 and 35O22 have been used to facilitate structural studies of Env trimer due to their trimer-stabilizing properties [20,21,33]. Co-crystallizing the antigen-binding fragments (Fabs) of 35O22 and PGT122 with BG505 SOSIP led to a 3.1 Å resolution structure of the complex, allowing the assignment of most of gp41 residues [33]. 35O22 was shown to interact with glycans at N88 on gp120 and N618 on gp41, with the N88 glycan accounting for more than half of the buried solvent surface; it also interacts with beta strand 1 of gp120 and helix 9 of gp41 [21,33]. 8ANC195 was initially crystallized in complex with a gp120 core and CD4 (domains 1 and 2) and shown to interact with glycans at N234 and N276 on gp120 and an unidentified protein surface [3]. Later EM and crystallographic data of complexes with Env trimers showed that 8ANC195 also interacts with the N637 glycan, helices 8 and 9 of gp41, and recognize the Env trimer in both closed and partially open conformations [52]. The PGT151 epitope (Fig. 1), revealed by a recent 4.2 Å cryo-EM structure in complex with JR-FL Env CT, consists of multiple glycans at N448 of gp120 and N611 and N637 of the neighboring gp41 [53]. Most interestingly, the FP of gp41 was observed in the PGT151/Env

complex, forming a beta strand interaction with the H3-CDR of PGT151, thus the FP is partially exposed in the Env trimer [53]. The FP is a target for another bnAb VRC34, which also binds to the gp120 N88 glycan [12]. Because the epitopes of these bnAbs do not overlap substantially, the gp120-gp41 interface is likely accessible by antibodies. However, as their epitopes are trimer-dependent and require glycans and amino acid residues from both gp120 and gp41, an immunogen targeting this region will need to be a glycosylated trimer and take into the consideration of trimer conformations and the hydrophobicity of FP.

The membrane proximal external region (MPER) of gp41

With bnAbs such as 2F5, 4E10, and Z13 targeting MPER identified in the mid 1990s and early 2000s [54–57], and the isolation of the potent MPER-targeting bnAb 10E8 in 2012 [17], MPER (Fig. 1) has been a known target for bnAbs for many years (reviewed in [58]). Because this category of bnAbs recognize linear peptides, they have been extensively studied by crystallization [17,59,60], with over 30 crystal structures in the Protein Database (PDB) for 2F5 alone, for example. These structures suggest that their epitopes are helical and relatively straightforward to be engrafted onto protein scaffolds. However, both MPER peptide- and scaffold-based immunogens have been unsuccessful in inducing antibodies similar to the MPER bnAbs. More data suggest that the epitopes of MPER bnAbs are more complex than a helical peptide and may be partially embedded in the lipid membrane, allowing the MPER bnAbs to interact with the membrane lipid to achieve viral neutralization [61,62]. This complexity of the MPER epitopes makes immunogen designs that target them a great challenge.

New immunological concepts defined by HIV-1 bnAbs

Current Env immunogens are immunogenic as supported by high titers of binding antibodies after immunization; however, these antibodies cannot effectively and broadly neutralize the virus, partially due to viral defence mechanisms. Our understanding of the immunological processes of antibody responses can explain the generation of the abundant binding antibodies but not the bnAbs that overcome viral defence. Recent investigations to delineate bnAb development have led to new insights in this immunological process (Fig. 2).

Naïve B cells for HIV-1 bnAbs

As initial responders, the naïve B cells that led to HIV-1 bnAbs are under intensive investigation. Corresponding germline reverted sequences or unmutated common ancestors (UCAs) of bnAbs have been inferred and produced to mimic the reactivity of the naïve B cell receptors (BCRs) with Env immunogens. However, many Env immunogens fail to react with inferred UCAs despite reactivity with mature bnAbs [63–65]. It is unclear whether the lack of reactivity is due to suboptimal design of the immunogen or due to errors in inferred UCAs. Therefore, the search for authentic bnAb precursors has been conducted in human naïve B cells.

With the VRC01 sequence signature [6,48], Jardine et al screened and probed naïve B cells with an improved gp120 OD construct, eOD-GT8, to determine the frequency of naïve B

cells with such signature [66]. They identified a total of 27 naïve B cells displaying the VRC01 sequence signature, with some reacting with eOD-GT8 [66]. The frequency of VRC01-like naïve B cells was estimated to be 1 in 2.4 million, and the affinity K_D with eOD-GT8 ranged from 57 μ M to 125 nM, with a geometric mean of 3.4 μ M. These results indicate that even for rare VRC01-like precursors that are restricted in a specific IGHV gene usage and with an unfavorable L-CDR3 length, the human B cell repertoire is diverse enough to encompass them. This study is the first to characterize the interaction between the antigen and human naïve B cells. Given the importance of B cell priming, there will be additional studies along this line of investigation in the future.

T follicular helper (Tfh) cells for HIV-1 bnAbs

Tfh cells express CD4 and play a central role in T cell-dependent antibody responses. Their potential for vaccine development has been discussed [67]. As the CD4-expressing Tfh cells are also the primary target for HIV-1, one possible solution to this dogma in vaccine strategy is to maintain a low CCR5 expression profile in these cells [67]. Because these cells require lymphoid tissues for differentiation and function, a lack of patient tissue sample has also been problematic. To work around this problem, blood functional PD-1⁺CXCR3⁺CXCR5⁺ Tfh cells have been isolated from HIV-1-infected humans, and their numbers have marginally correlated with bnAb responses [68]. Additionally, CXCL13 has been correlated with bnAb responses and can be used as a plasma biomarker for Tfh and germinal center activities [69]. However, to better understand Tfh cell responses to HIV-1, a relevant animal model such as SHIV-infected rhesus macaques that produce HIV-1 bnAbs will be required [70–73]. Further studies of Tfh cells in tissues, especially their specificity, function, and kinetics in relation to bnAb production [72], will help improve the understanding of this process.

High levels of somatic hypermutation (SHM)

A hallmark of HIV-1 bnAbs is their extreme high levels of SHM. Repeated vaccinations with tetanus toxoid or influenza vaccines induced an average SHM of 5–6%, with the upper limit reaching 10% [74,75]. Prior to the isolation of HIV-1 bnAbs, levels of SHM higher than 10% were thought to be deleterious to the BCR. However, many HIV-1 bnAbs displayed greater than 20% heavy chain SHM, with some reaching 35%. These unprecedented high levels of SHM renewed our understanding of SHM and established a concept that the immune system can accumulate up to 35% heavy chain SHM without impairing the antibody function. Because high levels of SHM are required for the neutralization function of bnAbs, many groups are keen to understand how such high SHMs are accumulated and what are the corresponding immunological mechanisms.

One hypothesis is that bnAb precursors may not be derived from naïve B cells but instead from memory B cells that previously responded to stimuli other than HIV-1 [76–78]. This hypothesis is not supported by longitudinal studies that tracked bnAb lineages to mAbs with only 2–3% SHM [18,23]. Additionally, HIV-1-infected infants also produce bnAbs [79]. Since infants have limited numbers of responded B cells compared to adults [80], infant bnAbs, if highly mutated, do not appear to initiate from previously mutated B cells. Though

not ruled out, a mutated precursor alone cannot account for the elevated SHM in HIV-1 bnAbs. Thus, some mechanisms to promote multiple rounds of B cell selection must be involved. In this case, it probably matters very little whether the precursors initiate from unmutated naïve B cells or from B cells with moderate SHM.

B cell selection and SHM occur in the germinal centers, and mechanisms for high levels of SHM may involve prolonged and/or multiple rounds of germinal center reactions. This is supported by multiple studies tracking bnAb lineages in infected individuals that all found SHMs to increase accumulatively over time [8,18,23,81]. Accordingly, current vaccine strategies often involve multiple immunizations aiming to promote multiple rounds of B cell selection to accumulate SHM, and the Env lineages from bnAb-producing donors are promising candidates to guide the bnAb maturation [11,22,23,82,83]. As visualizing germinal centers requires lymphoid tissue samples, non-human primate models that produce antibody responses similar to HIV-1 bnAbs may be particularly useful for studying the magnitude and duration of germinal center reactions.

Unusual CDR3 lengths

The H-CDR3 and L-CDR3 of an antibody correspond to the VDJ and VJ recombining sites, respectively. Antibodies including HIV-1 bnAbs with long H-CDR3s (> 24 amino acids) have been associated with autoreactivity [84–86]. Consequently, human B cells carrying long H-CDR3s are unfavorably regulated and become infrequent. Structural analysis of HIV-1 bnAbs with long H-CDR3s indicated a critical role of long H-CDR3s in penetrating HIV-1 glycan shields [16,38], demonstrating the contribution of long H-CDR3s to antibody immunity that has been underappreciated. Similar to those in autoreactive antibodies, the long H-CDR3s in HIV-1 bnAbs are established at the original VDJ recombination and involve preferential uses of long D and J genes [87], as well as possible D-D fusion [88].

In contrast to long H-CDR3s, the VRC01-class of bnAbs feature a short L-CDR3 of 5 amino acids with a less than 1% frequency. The average L-CDR3 is 9 and 11 amino acids for kappa and lambda chains, respectively. At the VJ recombining site, the average L-CDR3 only requires minimal nucleotide excisions of the V and J genes; in contrast, an L-CDR3 of 5 amino acids requires extensive excisions of the V and J genes, thus likely to account for its low frequency. Nevertheless, the VRC01-class of bnAbs, all containing a L-CDR3 of 5 amino acids, has been isolated from more than 10 individuals (Table 1), indicating efficient selection and expansion of these rare clones. Structural analyses of the VRC01-class epitopes indicate a short L-CDR3 is required to avoid steric clashes from the V5 loop of gp120 [6]. Taken together, BCRs with long H-CDR3s or short L-CDR3s are rare in the human B cell repertoire but can be effectively activated and clonally selected by the Env antigen.

Unusual mode of antigen recognition

The most common mode of antigen recognition is via the antibody H-CDR3, thus is “conventional” and commonly employed by HIV-1 bnAbs. However, two classes of bnAbs, the VRC01-class and the 8ANC131-class, bind to the CD4bs of gp120 mainly via the

antibody H-CDR2 [9], thus demonstrating an “unconventional” mode of antigen recognition. As the H-CDR2 is part of the IGHV gene, these bnAbs also displayed IGHV genes restricted to IGHV1–2 and IGHV1–46 [9]. Another unconventional bnAb is 2G12 that has exchanged heavy chain variable domains between the two Fabs within a single IgG molecule [89]. Although this unique structure likely renders 2G12 an isolated case and limits its generalizability, the unconventional modes of antigen recognition employed by HIV-1 bnAbs emphasize the diversity and plasticity of the antibody-antigen interaction that should not be overlooked.

Antibody recognition of glycans

Mammalian glycans, especially host-derived glycans displayed on viral antigens, have been recognized as poor immunogens [90,91]. The heavily glycosylated HIV-1 Env is known to protect sites of vulnerability by glycan shields and evade antibody neutralization [92]. For this reason, glycans have been blamed for the poor immunogenicity of the HIV-1 Env. However, there have been at least four categories of glycan-dependent bnAbs (i.e. 2G12, PG9/16, PGT121/128/135, and PGT151) that rely on glycan binding to neutralize the virus. Thus, our view of glycan immunogenicity may need to be adjusted to be more promising, at least in the context of glycoproteins. In current and future immunogen designs, glycans have been and will be an indispensable component, if the immunogen is not constituted purely by glycans [93].

Concluding remarks

Though highly selected and difficult to elicit, HIV-1 bnAbs with 50% breadth are developed in half of HIV-1 chronically infected individuals [94], thus supporting the feasibility of inducing a similar spectrum of antibody responses with vaccines. Because humans and primates have demonstrated the ability to produce HIV-1 bnAbs, studies of human and primate immunology are of utmost importance to fill gaps of knowledge and advance HIV-1 vaccine development.

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Highlights

- HIV-1 bnAbs precisely define sites of vulnerability on the HIV-1 Env.
- HIV-1 Env and its sub-regions each adopt multiple structures.
- Features of HIV-1 bnAbs emphasize the study of human and primate immunology.
- Rare bnAb precursors can be identified and selected from the human repertoire.
- Mechanisms promoting somatic hypermutation are key to HIV-1 bnAb production.

Figure 1.

Representative bnAb epitopes projected onto the Env trimer. The Env trimer is a composition of the high resolution crystal structure of BG505 SOSIP (PDB ID 4TVP) with that of the cryo-EM JR-FL Env CT (PDB ID 5FUU). The MPER region is artificially attached. Each gp120/gp41 monomer is colored with a slightly varying grey.

For HIV-1 bnAb generation:

- i) activate rare naïve B cells: long H-CDR3, short L-CDR3, restricted V-gene use because of H-CDR2-dominated recognition
- ii) better Tfh specificity or greater magnitude and quality?
- iii) extreme SHM → multiple rounds of selection
- iv) glycan or glycopeptide recognition

Figure 2.

Schematic illustration of the key players in B cell responses in germinal centers. The new concepts implicated by HIV-1 bnAbs are listed.

Table 1

Summary of unique HIV-1 bnAbs isolated during the past 6 years

#	mAb ID	Donor (viral clade)	Env target, B-cell probe	V-genes (hypermutation)	CDR3 length (amino acids)	Isolation year, reference
Isolated by HIV-1 Env probes						
1	VRC01	NIH45 (B)	CD4bs [*] , RSC3	VH1-2 (32%), VK3-20 (18%)	H3: 12, L3: 5	2010, [1]
2	3BNC117	RU3 (B)	CD4bs, 2cc core	VH1-2 (26%), VK1-33 (16%)	H3: 10, L3: 5	2011, [2]
3	12A12	IAVI57	CD4bs, 2cc core	VH1-2 (23%), VK1-33 (19%)	H3: 13, L3: 5	2011, [2]
4	1B2530	RU1 (B)	CD4bs, 2cc core	VH1-46 (28%), VL1-47 (18%)	H3: 16, L3: 11	2011, [2]
5	8ANCI131	RU8 (B)	CD4bs, 2cc core	VH1-46 (26%), VK3-20 (19%)	H3: 16, L3: 9	2011, [2]
6	8ANCI195	RU8 (B)	gp120-gp41, 2cc core	VH1-3 (28%), VK1-5 (16%)	H3: 20, L3: 9	2011, [2,3]
7	VRC-PG04	IAVI74 (AD)	CD4bs, RSC3	VH1-2 (30%), VK3-20 (19%)	H3: 14, L3: 5	2011, [4]
8	VRC-CH31	CH0219 (A)	CD4bs, RSC3	VH1-2 (24%), VK1-33 (15%)	H3: 13, L3: 5	2011, [4]
9	3BC176	RU3 (B)	trimer, cell BaL gp140	VH1-2 (24%), VL2-23 (15%)	H3: 19, L3: 10	2012, [5]
10	VRC-PG19	IAVI23	CD4bs, RSC3	VH1-2 (23%), VL2-14 (14%)	H3: 11, L3: 5	2013, [6]
11	VRC23	NIH-127/C (B)	CD4bs, RSC3	VH1-2 (22%), VK3-15 (15%)	H3: 12, L3: 5	2013, [7]
12	CH103	CH505 (C)	CD4bs, RSC3	VH4-61 (17%), VL3-1 (11%)	H3: 13, L3: 10	2013, [8]
13	VRC13	NIH44 (B)	CD4bs, RSC3	VH1-69 (34%), VL2-14 (24%)	H3: 21, L3: 6	2015, [9]
14	VRC16	NIH-C38 (B)	CD4bs, RSC3	VH3-23 (18%), VK1-39 (19%)	H3: 20, L3: 9	2015, [9]
15	VRC18	NIH-C38 (B)	CD4bs, RSC3	VH1-2 (27%), VK3-20 (18%)	H3: 10, L3: 5	2015, [9]
16	VRC27	NIH-Z258 (B)	CD4bs, RSC3	VH1-2 (30%), VK1-33 (27%)	H3: 13, L3: 5	2015, [9]
17	179NC75	EBI79 (B)	CD4bs, 2cc core	VH3-21 (28%), VL3-1 (22%)	H3: 24, L3: 10	2015, [10]
18	DRVIA7	DRVI01	CD4bs, RSC3	VH1-2 (19%), VK1-5 (17%)	H3: 11, L3: 5	2016, [11]
19	N123-VRC34	N123	gp120-gp41, FP [*] , SOSIP	VH1-2 (15%), VK1-9 (10%)	H3: 13, L3: 9	2016, [12]
Isolated by B-cell culture and micro-neutralization screening						
20	PG9	IAVI24 (A)	V1V2 quaternary	VH3-33 (13%), VL2-14 (6%)	H3: 28, L3: 11	2009, [13]
21	CH01	CH0219 (A)	V1V2 quaternary	VH3-20 (13%), VK3-20 (10%)	H3: 24, L3: 9	2011, [14]
22	PGT121	IAVI17 (A)	N332 supsite	VH4-59 (17%), VL3-21 (18%)	H3: 24, L3: 12	2011, [15]
23	PGT128	IAVI36 (AG)	N332 supsite	VH4-39 (19%), VL2-8 (9%)	H3: 19, L3: 10	2011, [15,16]
24	PGT135	IAVI39 (C)	N332 supsite	VH4-39 (17%), VK3-15 (16%)	H3: 18, L3: 9	2011, [15]
25	PGT145	IAVI84 (A or D)	V1V2 quaternary	VH1-8 (18%), VK2-28 (16%)	H3: 31, L3: 9	2011, [15]

#	mAb ID	Donor (viral clade)	Env target, B-cell probe	V-genes (hypermutation)	CDR3 length (amino acids)	Isolation year, reference
26	10E8	NIH-N152 (B)	MPER *	VH3-15 (21%), VL3-19 (14%)	H3: 20, L3: 12	2012, [17]
27	VRC24	NIH-N27 (B)	N332 supersite	VH4-4 (23%), VL1-15 (18%)	H3: 24, L3: 9	2013, [7]
28	CAP256-VRC26	CAP256 (C)	V1V2 quaternary	VH3-30 (14%), VL1-51 (10%)	H3: 37, L3: 12	2014, [18]
29	PGT151	IAVI31 (C)	gp120-gp41, FP	VH3-30 (20%), VK2-29 (12%)	H3: 26, L3: 9	2014, [19,20]
30	35O22	NIH-N152 (B)	gp120-gp41	VH1-28 (35%), VL2-14 (24%)	H3: 14, L3: 10	2014, [21]
31	CH235	CH505 (C)	CD4bs	VH1-46 (8%), VK3-15 (5%)	H3: 13, L3: 8	2014, [22,23]
Isolated by other methods						
32	HJ16	242315 (B)	CD4bs	VH3-30 (29%), VK4-1 (20%)	H3: 19, L3: 8	2010, [24]

* CD4bs, CD4-binding site; FP, fusion peptide; MPER, membrane proximal external region.