

Review

A Structural Update of Neutralizing Epitopes on the HIV Envelope, a Moving Target

Emma Parker Miller [†], Maxwell T. Finkelstein [†], Molly C. Erdman, Paul C. Seth and Daniela Fera ^{*†}

Department of Chemistry and Biochemistry, Swarthmore College, Swarthmore, PA 19081, USA; emiller6@swarthmore.edu (E.P.M.); mfinkel1@swarthmore.edu (M.T.F.); merdman1@swarthmore.edu (M.C.E.); pseth1@swarthmore.edu (P.C.S.)

* Correspondence: dfera1@swarthmore.edu; Tel.: +1-610-690-3308

[†] These authors contributed equally to this work.

Abstract: Antibodies that can neutralize diverse HIV-1 strains develop in ~10–20% of HIV-1 infected individuals, and their elicitation is a goal of vaccine design. Such antibodies can also serve as therapeutics for those who have already been infected with the virus. Structural characterizations of broadly reactive antibodies in complex with the HIV-1 spike indicate that there are a limited number of sites of vulnerability on the spike. Analysis of their structures can help reveal commonalities that would be useful in vaccine design and provide insights on combinations of antibodies that can be used to minimize the incidence of viral resistance mutations. In this review, we give an update on recent structures determined of the spike in complex with broadly neutralizing antibodies in the context of all epitopes on the HIV-1 spike identified to date.

Keywords: HIV-1; envelope; spike; neutralizing antibodies; epitope; neutralization; structure



Citation: Parker Miller, E.; Finkelstein, M.T.; Erdman, M.C.; Seth, P.C.; Fera, D. A Structural Update of Neutralizing Epitopes on the HIV Envelope, a Moving Target. *Viruses* **2021**, *13*, 1774. <https://doi.org/10.3390/v13091774>

Academic Editor:
Priyamvada Acharya

Received: 12 August 2021
Accepted: 2 September 2021
Published: 5 September 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

HIV-1 is a rapidly evolving pathogen, which has necessitated the use of antiretroviral therapies that use cocktails of drugs, to treat infected individuals while minimizing the development of resistance. Antibodies, which have longer half-lives in vivo, are increasingly being explored for their potential use both therapeutically and prophylactically [1–6]. Despite the high levels of sequence variation in the HIV-1 spike protein, called the envelope, or “Env”, neutralizing antibodies (nAbs) against semi-conserved regions have been identified. Non-neutralizing HIV-1 antibodies have also been identified and contribute to the in vivo protective immune response, but they are rarely, if ever, structurally characterized [7,8]. Since the majority of available structural information on antibody-based immune responses to HIV is based on neutralizing antibodies, these are the focus of this review. Of particular interest are broadly neutralizing antibodies (bnAbs), which are effective at targeting a wide range of viral strains. BnAbs are the focus of HIV-1 vaccine design programs and have utility in the development of therapeutics.

The first bnAbs were identified in the early 1990s, including b12 against the CD4 binding site (CD4bs), using a phage display library from an asymptomatic infected individual [9,10]. Since then, advances in single B-cell sorting technologies and next-generation sequencing have made possible more efficient identification of bnAbs from the sera of infected individuals [11]. To date, bnAbs have been identified against six major epitopes on HIV-1 Env, essentially covering all the accessible sites. Structural features of many of these sites have been reviewed elsewhere [12–14]. In this review, we focus on bnAbs that have been more recently structurally characterized in complex with Env.

Understanding how these bnAbs interact with their respective epitopes contributes to immunogen design for eliciting bnAbs against HIV-1 by vaccination, and aids the formulation of effective antibody combinations for therapeutic use. The use or elicitation of antibodies with different epitopes or hotspots reduces the chance of viral escape [11,15].

2. HIV-1 Env Structure and Function

HIV-1 Env is a heavily glycosylated class 1 trimeric fusion protein [16–18]. Env is synthesized as a single gp160 molecule and is post-translationally cleaved by furin to form a gp120 and gp41 heterodimer [16,19,20]. The complex is anchored to the membrane via the transmembrane domain of gp41, which interacts with the amino terminus of the gp120 trimer to hold the two subunits together [21]. While much of the Env structure is conserved across different viral strains, there are five loops in the gp120 chain that are highly variable across different strains, called V1V2, V3, V4, and V5 (Figure 1A). The V1V2 loops protrude from the distal end of the inner domain of the gp120 trimer at the trimer apex, and the V3 and V4/V5 loops are on the distal and proximal side of the outer domain, respectively.

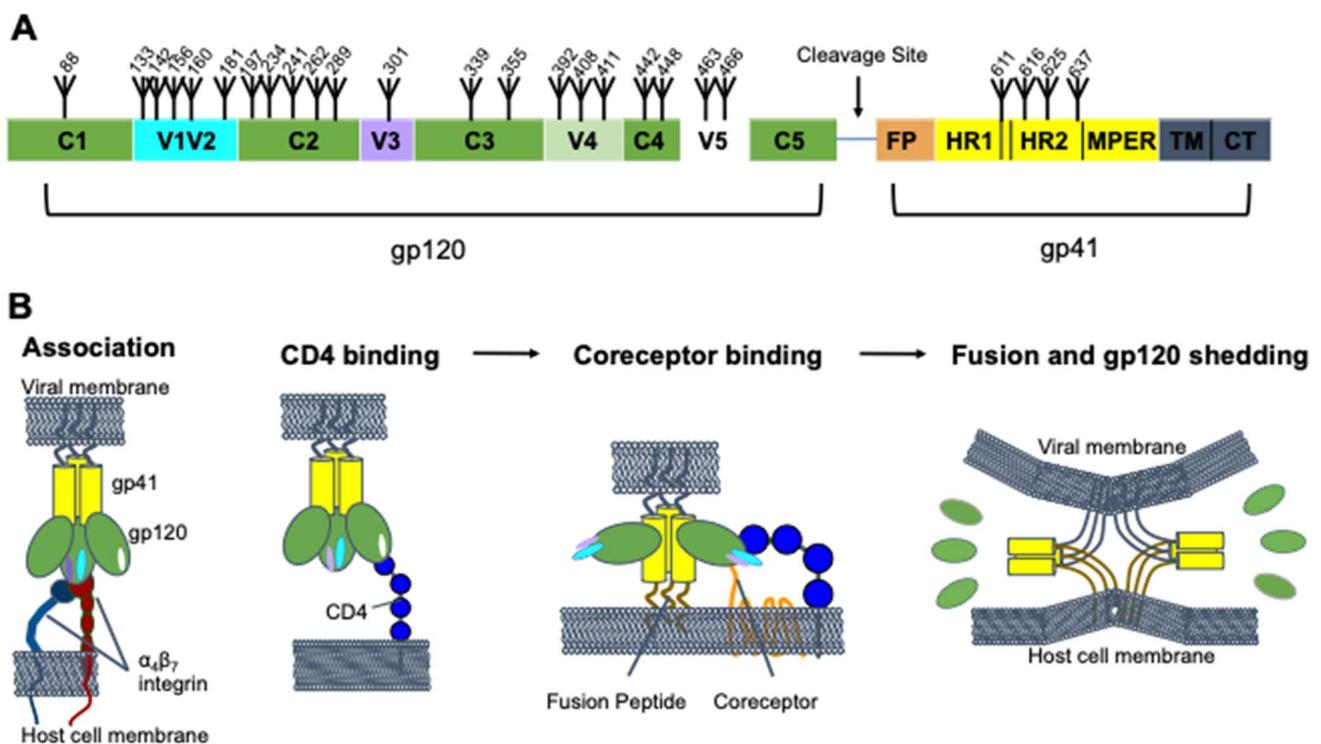


Figure 1. HIV-1 Env structure and dynamics. (A) Domain architecture of HIV-1 Env, with the gp120 and gp41 domains highlighted. The furin cleavage site, fusion peptide (FP), heptad repeats 1 and 2 (HR1 and HR2), membrane proximal external region (MPER), transmembrane domain (TM), and cytoplasmic tail (CT), are shown. Glycosylation sites are numbered (based on the HXB2 strain) and marked with fork symbols. (B) Viral entry. The gp41 (yellow) and gp120 (green) subunits are shown with their variable loops, V1V2 (cyan), V3 (purple), and V5 (white) in different conformations as they go through the fusion process. The fusion peptide (brown) and transmembrane domain (gray) are also shown. V4 is not shown in panel B, and the loops were excluded from the fusion panel for clarity. Binding partners on the host are shown, $\alpha_4\beta_7$ (red, navy), CD4 (blue), and the coreceptor (orange).

Env mediates viral entry by engaging first with the primary cellular receptor, CD4, and then with one of two coreceptors, either CCR5 or CXCR4 [22] (Figure 1B). gp120 binding to CD4 leads to a large conformational change in which the trimer “opens”. Specifically, this involves a rearrangement of the V1V2 and V3 loops and formation of a bridging sheet [22,23]. This conformational change then enables coreceptor binding [22,24]. Coreceptor binding leads to activation of the gp41 fusion peptide, which inserts into the target cell membrane. The gp41 protein then folds into a hairpin structure in which the two heptad repeats, HR1 and HR2, form a six-helix bundle, bringing the viral and host cell membranes into contact to complete fusion [25,26]. The $\alpha_4\beta_7$ integrin has also been suggested to play an important role in HIV entry by interacting with the gp120 V2 loop of

certain viral strains [27,28]. Antibodies can neutralize the virus by interfering with any of these steps.

3. HIV-1 Mechanisms for Evading Antibody Neutralization Responses

Due to the conformational changes Env can undergo, accessibility of epitopes to neutralizing antibodies can be limited, particularly to the CD4bs [29]. This conformational masking can also divert antibody responses and lead to non-neutralizing antibody responses, such as to nonfunctional Env proteins left on the viral surface after viral shedding [30].

In addition to evading immune responses through structural heterogeneity, HIV-1 Env also effectively evades antibody recognition through its high mutation rate. The lack of proofreading mechanisms by its reverse transcriptase allows HIV-1 Env to evolve quickly in response to selective pressure from the host immune response [31,32]. The rapid mutation rate of HIV-1 differentiates it from other viruses in that it generates incredible genetic diversity within a single host throughout the course of infection, in addition to its diversity across different populations worldwide [33,34].

Env also has a “glycan shield” that sterically blocks antibodies from accessing much of its protein surface, with each Env containing 26–30 high-mannose or complex N-linked glycans [17,35] (Figure 2). As viral glycans often resemble “self” within the host, antibodies are less likely to recognize them without peptide sequences, or else they may have some level of autoreactivity [36]. Additionally, mutations in Env can impact glycosylation patterns on its surface, increasing its variability and impacting the neutralization potential of antibodies [37].

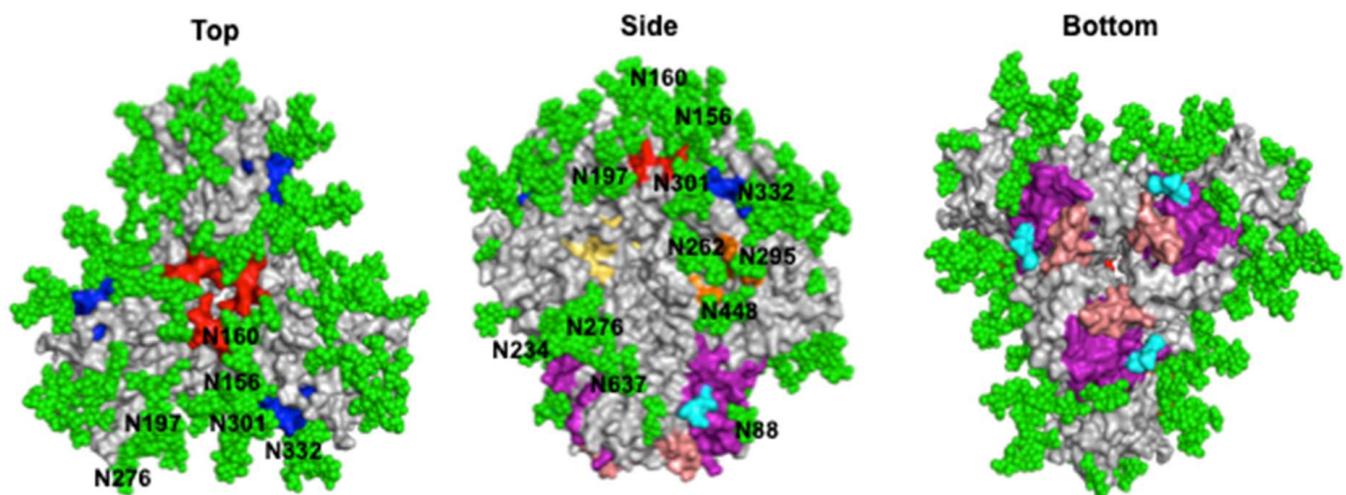


Figure 2. HIV-1 Env glycan shield and broadly neutralizing epitopes. Surface representation of the fully glycosylated BG505 HIV-1 Env (PDB ID: 5T3Z) is shown from different views along with glycans (green spheres labeled with the amino acid that is glycosylated) and broadly neutralizing antibody epitopes colored based on site: CD4bs (yellow), V1V2 (red), V3 glycan supersite (blue), silent face (orange), gp120–gp41 interface (purple), and the gp41 FP (cyan) and a portion of the MPER (salmon).

4. HIV-1 Neutralizing Epitopes

Despite HIV-1 defense mechanisms, bnAbs targeting a significant number of viral variants have been produced by some HIV-1 infected individuals, as well as by mice or other animals. Their identification can be partly attributed to the use of disulfide-stabilized constructs of Env, called “SOSIPs”, which mimic the native ectodomain of the trimer, as a probe [38,39]. SOSIPs have also contributed to antibody–Env complex structure determinations and neutralizing epitope characterizations. To date, bnAbs have been shown to target six epitopes on HIV-1 Env: the CD4bs, the V1V2 loops at the trimer apex, the V3 glycan supersite, the gp120–gp41 bridging region, the silent face, and gp41

(Figure 2). Recently identified bnAbs are listed in Table 1 and their structural features are described below.

Table 1. HIV-1 bnAbs whose high-resolution structures have recently been determined.

Epitope	bnAb	PDB ID	Neutralization Potency (IC ₅₀)	Breadth (%)	Year Published	Reference
CD4bs	PGV19	6B0N	0.34–1.3 µg/mL	70–75	2018	[40]
	1–18	6UDJ	0.048 µg/mL	97	2020	[41]
	1–55	6UDK	0.096 µg/mL	92	2020	[41]
	M1214_N1	6VY2	0.19 µg/mL	65	2020	[42]
	2411a	7JKS	1.49 µg/mL	51	2021	[43]
	2413a	7JKT	3.86 µg/mL	34	2021	[43]
V1V2	CAP256-VRC26.25	6VTT	0.012 µg/mL	59	2020	[44]
	RHA1.V2.01	6XRT	0.35 µg/mL	49	2021	[45]
V3 glycan supersite	DH270.6	6UM6	0.08 µg/mL	55	2019	[46]
	438-B11	6UTK	0.18 µg/mL	67	2020	[47]
	Ab1485	7KDE	0.055 µg/mL	38	2020	[48]
Silent face	SF12	6OKP	0.20 µg/mL	62	2019	[49]
gp120–gp41 interface	1C2	6P65	8.03 µg/mL	85	2019	[50]
gp41	N/A	N/A	N/A	N/A	N/A	N/A

4.1. CD4 Binding Site

The CD4bs is of particular interest because antibodies against this site can prevent interaction with CD4, the primary receptor for viral entry. The CD4bs lies in a cavity formed at the interface of the gp120 inner and outer domains (Figure 2) [22]. Hydrophobic residues deep in the pocket allow for contacts with Phe-43 of CD4 [51]. As a conserved immune target, the epitope is gated by the V1, V2, and V3 loops, as well as multiple N-linked glycans, notably at positions N197, N262, and N276 [52]. Classically, two overarching classes of CD4bs-directed bnAbs have been identified: the first class are CD4 mimics, which contact the CD4bs with heavy chains encoded by either V_H1-2 or V_H1-46, while the second class are “CDRH3-dependent bnAbs”, since their interactions with the CD4bs are dominated by the CDRH3 loop [53].

Recent advances have identified five CD4-mimic bnAbs [40,43] of which three fall within the VRC01 subclass, characterized by a V_H1-2-derived heavy chain and a short, 5-residue CDRL3 used to avoid clashes with the N276 glycan [40,43]. PGV19 is a VRC01-class bnAb that neutralized 70–75% of a 20-pseudovirus panel, and is the first structurally characterized VRC01 class bnAb with a lambda light chain [40,54]. Nonetheless, only subtle differences were found between the binding modes of PGV19 and previously characterized VRC01 class bnAbs. Two other VRC01 subclass bnAbs, 2411a and 2413a, have been identified from humanized mice and are discussed in Section 5.

VRC01 subclass bnAbs are generally broader and more potent than CD4-mimic bnAbs that utilize V_H1-46 to contact the CD4bs. However, Schommers and colleagues isolated two V_H1-46 CD4-mimic bnAbs, called 1–18 and 1–55, with superb neutralization potency, and 1–18 was found to rival or exceed the potency of all previously described CD4bs-directed bnAbs [41]. Like other V_H1-46-encoded CD4 mimics, 1–18 and 1–55 contact the CD4bs loop, the V5 and D loops, and the N197 and N276 glycans on Env (Figure 3). They also overlap with the variable fragment of PGV19 and approach Env from a similar angle. Uniquely, however, 1–18 mimics Phe-43 CD4 contacts via residue F54_{HC} and a V5 loop salt bridge via residue R64_{HC}. 1–18 also contains a six-residue insertion in CDRH1, creating a highly anionic ²⁵DDDPYTDDD³³ motif. This motif is more ionic and makes more interactions with

a conserved cationic patch on the neighboring gp120 protomer than has been previously described for other bnAbs of this class. In accordance with the potency of 1–18, it was found to suppress viral loads in humanized mouse models more effectively than other CD4bs-targeting bnAbs, and it was resistant to VRC01 class escape mutations, indicating promise in potential combination therapies.

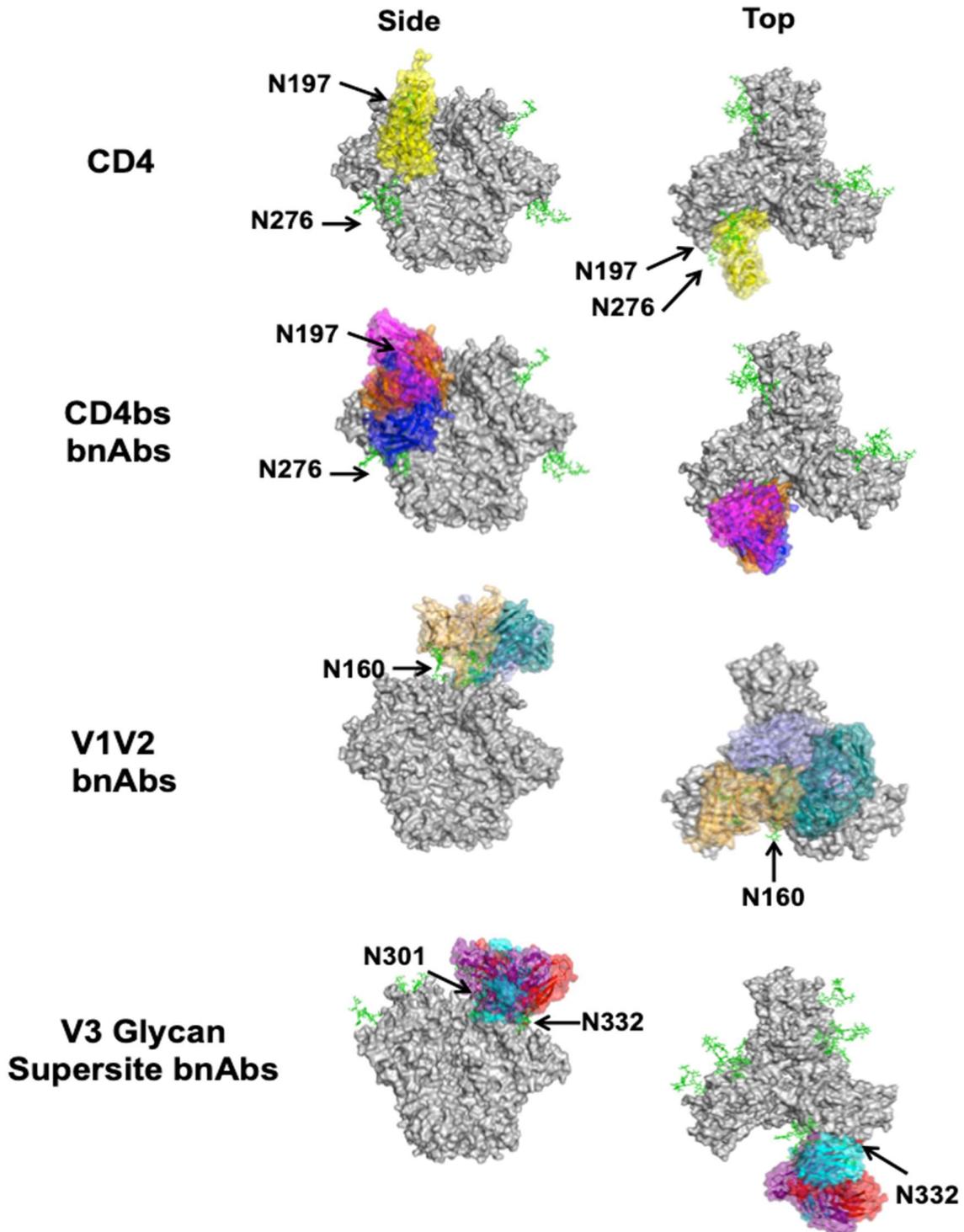


Figure 3. Cont.

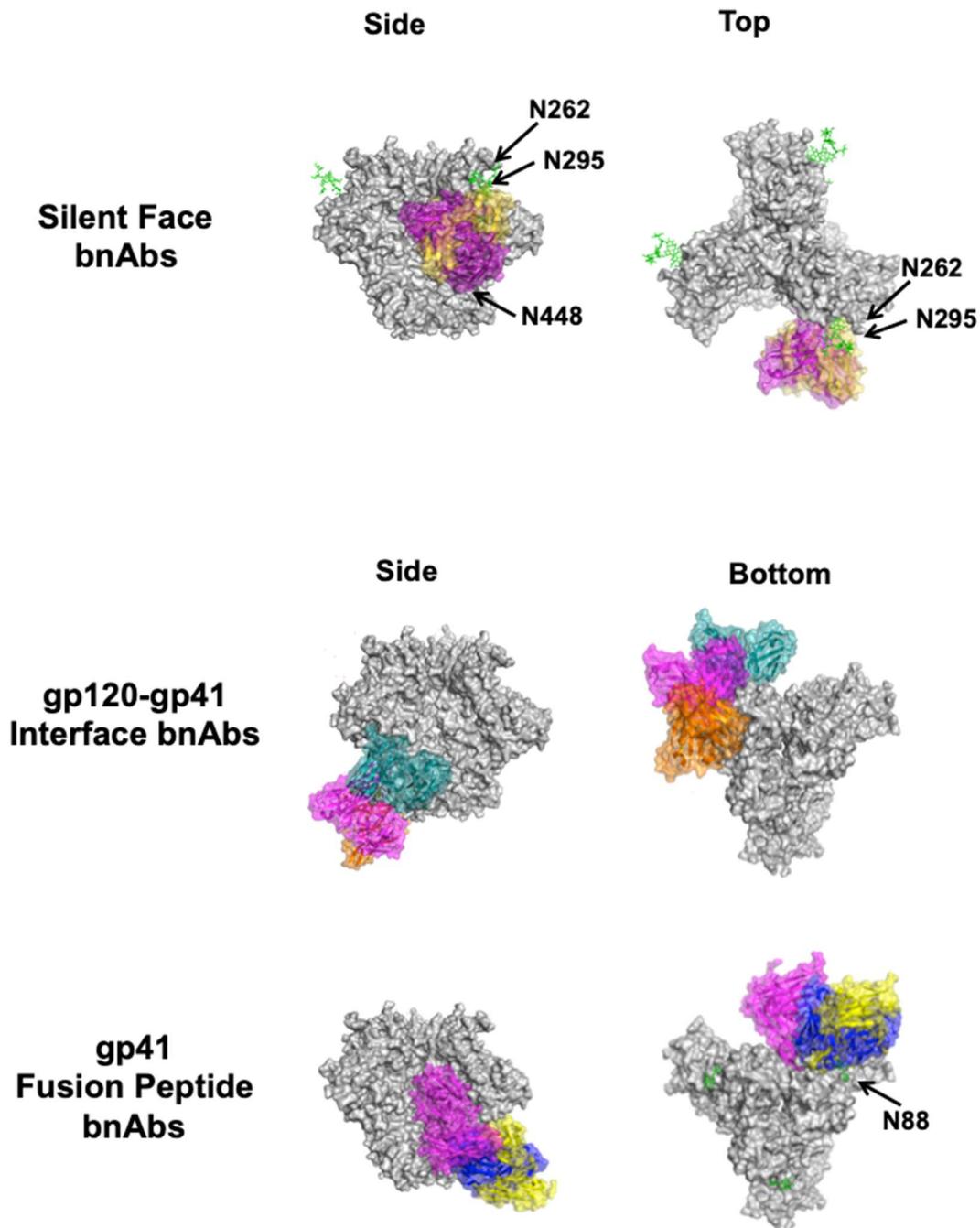


Figure 3. Complexes between bnAbs and HIV-1 Env. Top and side views of CD4 (yellow, PDB ID: 1GC1) in complex with the Env trimer (gray, PDB ID: 5T3Z) are shown for reference along with CD4bs bnAbs VRC01 (blue, PDB ID: 4LST), CH103 (orange, PDB ID: 4JAN), and M1214-N1 (magenta, PDB ID: 6VY2) below. Of the V1V2 apex bnAbs structurally characterized to date, CAP256-VRC26.25 (teal, PDB ID: 6VTT), VRC38 (purple, PDB ID: 5VGJ), and PGT145 (orange, PDB ID: 5V8L) are shown. Of the V3 glycan supersite bnAbs structurally characterized to date, DH270.6 (cyan, PDB ID: 6UM6), 438-B11 (red, PDB ID: 6UTK), and Ab1485 (purple, 7KDE) are shown. The two known silent face bnAbs SF12 (magenta, PDB ID: 6OKP) and VRC-PG05 (yellow, PDB ID: 6BF4) are shown. Of the gp120–gp41 interface bnAbs structurally characterized to date, 8ANC195 (teal, PDB ID: 5CJX), 35O22 (magenta, PDB ID: 4TVP), and 1C2 (orange, PDB ID: 6P65) are shown. Only gp41 FP-directed bnAbs VRC34 (blue, PDB ID: 6NC3), ASC202 (yellow, PDB ID: 6NC2), and PGT151 (magenta, PDB ID: 6DCQ) are shown; MPER-directed bnAbs clash with a closed trimer and are therefore not shown. Relevant glycans are shown as green sticks and labeled according to the amino acid that is glycosylated. For clarity, only the Fv region of the antibodies is shown on a single protomer of the trimer, even if additional copies can bind. Side (left) and bottom or top (right) views are shown.

Unlike CD4-mimic bnAbs, CDRH3-dependent bnAbs have a different approach angle on Env. Jia and colleagues identified M1214_N1, which extends its long CDRH3 into a groove formed by the N197 and N386 glycans to contact the integrin binding site between the V1V2 C and C' strands [55]. In doing so, M1214_N1 approaches the CD4bs closer to the trimer apex than CD4 mimics, similar to CH103 [56] (Figure 3). The epitope is centered on the CD4 binding loop and extends from V2 to V5, dubbed the “V2V5 corridor”. The conservation of this epitope resulted in 65% breadth, but the lower conservation in V2 contacts make M1214_N1 less broad than other CD4bs bnAbs [42]. Additionally, like other CD4bs-directed bnAbs, M1214_N1 had high levels of somatic hypermutation (SHM)—35% SHM of V_H3-66 and 24% SHM of V_L2-11—which may limit its potential for elicitation following immunization. Further studies are required to validate the utility of targeting this neutralizing epitope in other donors.

4.2. V1V2

The V1 and V2 loops are located at the distal end of each of the three gp120 molecules (Figure 2). They form beta strands, stabilized by interstrand disulfide bridges, at the cationic Env trimer apex [55,57,58]. Surrounding these loops is a conserved region covered by glycans, most notably, the N160 glycan. The V1V2 loops undergo large conformational changes after CD4 engagement to expose the otherwise shielded V3 loop and the coreceptor binding site. Even though the V1V2 loops are the most variable on Env and are shielded by glycans, a significant number of bnAbs against this site have been isolated. These bnAbs penetrate the glycan shield via long CDRs, bind almost vertically along the three-fold axis of the trimer, and have several binding modes (Figure 3). They likely neutralize the virus by stabilizing the Env trimer and thereby interfering with structural changes needed for coreceptor binding [59].

One of the most recently structurally characterized V1V2 bnAbs in complex with HIV-1 Env is CAP256-VRC26.25 [44]. CAP256-VRC26.25 is a highly potent bnAb, more so than VRC01, and was found to neutralize 59% of viruses in a 208-strain panel. Its high potency was attributed to the ability of this bnAb to neutralize the virus in a single antibody-to-trimer stoichiometry (unlike CD4bs bnAbs), as well as its extraordinarily long CDRH3 (36 amino acids, the longest of bnAbs of this class). The cryo-electron microscopy (cryo-EM) structure of the CAP256-VRC26.25–Env complex also showed that CAP256-VRC26.25 combines features of different V1V2 bnAbs. For example, it has two sulfated tyrosines that insert into the cationic hole at the three-fold trimer axis, like PGT145 [60]. Moreover, like CH03 in the PG9 class [57], CAP256-VRC26.25 forms intermolecular beta sheet-like hydrogen bonds with the C strand of the V1V2 loop [58]. The interactions between CAP256-VRC26.25 and Env differ from VRC38, which is an outlier in this group in that it has a much shorter, nearly neutral CDRH3 and, unlike the PG9 and PG145 class bnAbs, primarily interacts with the V1V2 complex through side chain interactions [61]. While the N130, N156, and N160 glycans were observed to contact CAP256-VRC26.25, the glycan dependence of this bnAb has yet to be investigated through biochemical studies. Indeed, several bnAbs of this class have been shown to depend on glycans, though the specific glycans can vary from one antibody to the next.

4.3. V3 Glycan Supersite

BnAbs against the V3 glycan supersite have been isolated from numerous infected individuals [62–65]. The V3 glycan supersite is located near the V1V2 region on HIV-1 Env. The base of the V3 loop is located on the opposite side of the gp120 lobe from the CD4bs (Figure 2). It contains a highly conserved ³²⁴GDIR³²⁷ motif, and various high-mannose glycans, most notably the N332 glycan contacted by all known bnAbs targeting this site [47,48,62,63,65]. Conservation at the base of the V3 loop is likely due to its role in CCR5 coreceptor recognition [64,66], so neutralization by bnAbs targeting this epitope is likely mediated by ablating coreceptor binding, or restricting conformational changes

in the V3 loop that would occur following coreceptor binding to enable gp41-mediated membrane fusion [67].

V3 glycan supersite bnAbs generally have different binding approaches on Env and are more angled relative to the three-fold axis of the trimer than V1V2-directed bnAbs (Figure 3). While many V3 glycan supersite bnAbs have been reviewed elsewhere [68–70], three more recent high-resolution structures of bnAbs in complex with Env have been determined, two of which are from infected human subjects. DH270.6 was first described in 2017 [62], and found to be a potent neutralizer with 55% neutralization breadth with only 12.9% SHM. Its epitope was later characterized using a glycopeptide mimic of the V3 glycan supersite and recently confirmed in complex with the Env trimer [46,71], showing contacts with both the N332 glycan and GDIR motif. While contacts with the N301 glycan were observed, they were not necessary for binding or neutralization [62]. Like some bnAbs against other epitopes, a single extended CDR loop is necessary for V3 glycan supersite bnAbs to penetrate the high-mannose patch and make contacts with the Env protein surface, but DH270.6 is able to use a shorter CDR (20 amino acids).

Antibody 438-B11 achieved greater breadth than DH270 (up to 67%), with similar neutralization potency but higher SHM (25%) [47]. Despite distinct heavy chain genes (IGHV1-2 for DH270, IGHV1-69*01 for 438-B11) and being rotated roughly 90 degrees relative to DH270.6 in its approach to Env, like DH270, 438-B11 uses its long CDRH3 loop (21 residues) to contact the GDIR motif and N332 glycan, while other parts of the heavy chain interact with the N301 glycan (Figure 3).

4.4. Silent Face of Gp120

The so-called “silent face” of HIV-1 Env is located on a highly glycosylated region of the gp120 outer domain across from the CD4bs, on the same side of the gp120 lobe as V3 glycan supersite but closer to the gp41 region (Figure 2) [36]. The epitope predominantly includes the N262, N295, and N448 glycans on a single gp120 monomer (Figure 3). Silent face antibodies have been suggested to neutralize HIV by inhibiting conformational changes in Env necessary for full receptor binding and cell entry [36]. The neutralization mechanism may also involve the N262 glycan, which was observed to be important for CD4-mediated viral entry [72].

SF12 and VRC-PG05 [36,49] are the only known bnAbs against the center of the silent face epitope that have been structurally characterized to date (Figure 3). VRC-PG05, previously reviewed by Sok and Burton [70], makes extensive contact with the three glycans of this epitope, in addition to some contact with gp120 peptide residues. Until the identification of VRC-PG05, 2G12 was the only known bnAb to target such a glycan-rich region [73]. In contrast to silent face bnAbs, however, 2G12 does not require any Env peptide for its interaction, and it binds closer to the CD4bs. SF12 also recognizes the three glycans of the silent face epitope, but makes more protein contacts than VRC-PG05, which might explain its greater breadth and potency. The binding approach of SF12 on Env is rotated roughly 90 degrees relative to VRC-PG05, but it still interacts with the N448 glycan in a similar way as VRC-PG05. The differences in binding modes for these two bnAbs can be explained in part by their divergent gene usages—SF12 employs $V_{H4-59*01}$ and $V_{K3-20*01}$, whereas VRC-PG05 employs $V_{H3-7*01}$ and $V_{K4-1*01}$.

4.5. Gp120–Gp41 Interface

Located below the gp120 lobe is the gp120–gp41 bridging region, or interface. A diverse set of bnAbs targeting this epitope have been characterized, with different binding approaches on Env and some with entirely non-overlapping epitopes and varying dependency on glycans [50,74–77]. Some bnAbs bind to the gp41 region closer to the viral membrane, whereas others bind closer to the gp120 lobe (Figure 3). Most of these bnAbs are specific for the trimer (rather than binding a single Env protomer) and are dependent on gp120–gp41 cleavage. BnAbs against this site have normal CDR lengths and neutralize Env by several mechanisms. Some bnAbs, such as 8ANC195, can prevent the conforma-

tional changes required for membrane fusion, whereas others, such as 3BC176 and 3BC315, destabilize the trimer [50,74–77]. These latter bnAbs encourage trimer dissociation by disrupting the tryptophan clasp that stabilizes the trimer base [78]. No new bnAbs from human donors against this site have been structurally characterized since the most recent reviews [14,70,79]. One recently identified bnAb against the gp120–gp41 interface, 1C2, was isolated from rabbits and is discussed in Section 5.

4.6. Gp41

The gp41 region of HIV-1 Env consists of multiple immunogenic domains, including the fusion peptide (FP) and membrane-proximal external region (MPER), both of which have known epitopes for bnAbs (Figure 2). Both of these domains serve important roles in host cell entry, in particular through facilitating membrane fusion after recognition of CD4 and coreceptors [80,81].

The FP is largely disordered and hydrophobic due to its interaction with the target cell membrane in both the intermediate and postfusion states [80,82,83]. In the prefusion state, the FP is sequestered in a hydrophobic region of the Env trimer [84]. BnAbs against the FP include VRC34 [85], ACS202 [74], and PGT151 [86]. Notably, in addition to interacting with the FP N-terminal residues, some bnAbs against this site also interact with complex glycans near the gp120–gp41 interface, particularly the N88 glycan. Their binding approaches on Env can vary and are similar to those of gp120–gp41 interface bnAbs (Figure 3).

The MPER is proximal to the viral membrane in the gp41 ectodomain. It is a challenging epitope for immunogen design since bnAbs against this site typically recognize both a lipid component from the viral membrane and a transient Env conformation via long CDR loops [87]. Nevertheless, numerous antibodies against this highly conserved region have been identified: DH511 [88], 4E10 [87,89], 10E8 [90], 2F5 [91], VRC42 [92], LN01 [93], PGZL1 [94], Z13E [95], and CAP206-CH12 [96], and reviewed elsewhere [70,97]. No new bnAb structures against this epitope have been reported since.

5. BnAb Elicitation in Mice and Other Animals

Proper non-human primate models would be helpful for testing prophylactics and therapies before using them in humans. Indeed, several groups have made progress in working with infected rhesus macaque models that have been produced using simian–human immunodeficiency virus (SHIV). Using a SHIV, Roark and colleagues isolated a V1V2 antibody called RHA1.V2.01 that had significant breadth, neutralizing 49% of viruses in a 208-strain panel [45]. Its cryo-EM structure revealed that the single RHA1.V2.01 on the trimer binds at a slightly different angle from PGT145, but is similar to PGT145 in that it has a long linear beta hairpin CDRH3 that is sulfated and reaches into the apex hole [45,60]. Additionally, bnAb Ab1485 was isolated by Wang and colleagues from a SHIV-infected macaque, and binds Env with a similar orientation to the V3 glycan supersite bnAb 438-B11 [48]. Like 438-B11, Ab1485 contacts the GDIR motif using its CDRH3 and CDRH1 loops. While Ab1485 was found to have greater potency than 438-B11 (median $IC_{50} = 0.055 \mu\text{g}/\text{mL}$), it had reduced breadth with 38.1% neutralization of a 42-pseudovirus panel. As these bnAbs resembled binding to Env of human V1V2 and V3 glycan supersite bnAbs, their elicitation in rhesus macaques highlights the utility of using these animals for studying HIV-1 bnAb development.

Elicitation of antibodies with great breadth by vaccination is a major goal of HIV-1 immunogen design. The abundance of structural data of HIV-1 Env alone and in complex with bnAbs has led to the identification of critical features important for their interactions and for immune evasion. Due to the dense glycan shield on Env, one approach researchers have used is to remove glycosylation sites from HIV-1 Env to elicit greater immune responses during initial primes. Boosting regimens would then use Envs in which glycans have been included to help focus the immune response on a desired region. This has produced some promising results. For example, Chen and colleagues isolated VRC01 class bnAbs, 2411a and 2413a, from sequential immunizations of humanized mice expressing

unmutated VRC01 class precursors. These bnAbs achieved moderate breadth and potency (2411a: 51% breadth, median IC₅₀ 1.49 µg/mL; 2413a: 34% breadth, median IC₅₀ 3.86 µg/mL) [43]. As another example, Dubrovskaya and colleagues isolated a gp120–gp41 interface bnAb, called 1C2, from sequential immunization of rabbits [50]. This bnAb had even greater breadth than 3BC315 even though the binding footprint of 1C2 was similar to that of 3BC315. Moreover, 1C2 is similar to gp120–gp41 interface bnAbs 3BC176 and 3BC315 in its ability to destabilize Env [50,76,98]. BnAbs have also been identified in a variety of fusion peptide-vaccinated animal models [99,100], and their structures have been reviewed previously [97]. While it is promising that immunization can produce bnAbs, it remains to be seen whether these responses can be recapitulated in non-human primates and whether they can withstand viral challenge. Furthermore, the protective efficacy of bnAbs against viraemia in vivo in humans also needs to be explored.

6. Conclusions and Future Perspectives

Despite having identified bnAbs over 30 years ago, an HIV-1 vaccine is yet to be developed. Immunogen design has indeed improved over the past decade, largely due to advances in cryo-EM, the development of SOSIP versions of Env, and increases in the available antibody–Env complex structures [38,39]. However, improvements and/or combinations of Envs may still be necessary to mimic the complex bnAb evolution processes observed in HIV-1 infected individuals. HIV-1 mutates rapidly resulting in an “arms race” in infected subjects, whereby the immune response evolves in accordance with viral evolution, and bnAbs typically take at least 2–4 years to develop. Additionally, bnAbs have structural and genetic features that may prevent their elicitation, such as long CDR loops, restricted germline usages, and high rates of somatic hypermutations. Due to the limited number of human subjects who produce bnAbs and that have been studied longitudinally [56,62,101], it becomes imperative to produce other models in which these co-evolutionary trajectories and bnAb development can be studied. The development of SHIVs holds promise in this regard.

With the increasing number of antibody–Env complex structures available, it is expected that there will be advances in antibody therapies. Indeed, antibody combination treatments have shown promise in the clinic [102,103]. Structural information has also led to advances in antigen design, with self-assembling protein nanoparticle immunogens showing promise in directing antibody responses towards broadly reactive epitopes [104–109]. We do note, however, that the structures alone do not indicate the important contacts made between the bnAbs and Env. Neutralization assays will be needed to assess mutations at different contact points to determine the “hot spots” in each complex. This is important because HIV-1 resistance can cause treatments by antibodies to fail. Pinpointing hotspots at antibody–spike interfaces will provide insights on effective antibody combinations to help overcome viral escape mutations and perhaps provide synergistic effects. These studies will also be important for immunogen design.

Author Contributions: D.F. conceptualized the study. D.F., M.T.F., E.P.M., P.C.S., and M.C.E. analyzed structural data and prepared and revised the draft, figures, and table. All authors have read and agreed to the published version of the manuscript.

Funding: Research for this manuscript was supported by funding from the National Institute of Allergy & Infectious Disease NIH award 1R15AI150484 (DF).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Publicly available datasets were analyzed in this study. The data can be found in the Protein Data Bank (PDB) archives using PDB IDs displayed in Table 1.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Margolis, D.M.; Koup, R.A.; Ferrari, G. HIV Antibodies for Treatment of HIV Infection. *Immunol. Rev.* **2017**, *275*, 313–323. [[CrossRef](#)] [[PubMed](#)]
2. Scheid, J.F.; Horwitz, J.A.; Bar-On, Y.; Kreider, E.F.; Lu, C.-L.; Lorenzi, J.C.C.; Feldmann, A.; Braunschweig, M.; Nogueira, L.; Oliveira, T.; et al. HIV-1 Antibody 3BNC117 Suppresses Viral Rebound in Humans during Treatment Interruption. *Nature* **2016**, *535*, 556–560. [[CrossRef](#)] [[PubMed](#)]
3. Lynch, R.M.; Boritz, E.; Coates, E.E.; DeZure, A.; Madden, P.; Costner, P.; Enama, M.E.; Plummer, S.; Holman, L.; Hendel, C.S.; et al. Virologic Effects of Broadly Neutralizing Antibody VRC01 Administration during Chronic HIV-1 Infection. *Sci. Transl. Med.* **2015**, *7*, 319ra206. [[CrossRef](#)] [[PubMed](#)]
4. Caskey, M.; Klein, F.; Lorenzi, J.C.C.; Seaman, M.S.; West, A.P.; Buckley, N.; Kremer, G.; Nogueira, L.; Braunschweig, M.; Scheid, J.F.; et al. Viraemia Suppressed in HIV-1-Infected Humans by Broadly Neutralizing Antibody 3BNC117. *Nature* **2015**, *522*, 487–491. [[CrossRef](#)] [[PubMed](#)]
5. Bar, K.J.; Sneller, M.C.; Harrison, L.J.; Justement, J.S.; Overton, E.T.; Petrone, M.E.; Salantes, D.B.; Seamon, C.A.; Scheinfeld, B.; Kwan, R.W.; et al. Effect of HIV Antibody VRC01 on Viral Rebound after Treatment Interruption. *N. Engl. J. Med.* **2016**, *375*, 2037–2050. [[CrossRef](#)] [[PubMed](#)]
6. Gruell, H.; Klein, F. Antibody-Mediated Prevention and Treatment of HIV-1 Infection. *Retrovirology* **2018**, *15*, 73. [[CrossRef](#)] [[PubMed](#)]
7. Mayr, L.M.; Su, B.; Moog, C. Non-Neutralizing Antibodies Directed against HIV and Their Functions. *Front. Immunol.* **2017**, *8*, 1590. [[CrossRef](#)] [[PubMed](#)]
8. Mayr, L.M.; Decoville, T.; Schmidt, S.; Laumond, G.; Klingler, J.; Ducloy, C.; Bahram, S.; Zolla-Pazner, S.; Moog, C. Non-Neutralizing Antibodies Targeting the V1V2 Domain of HIV Exhibit Strong Antibody-Dependent Cell-Mediated Cytotoxic Activity. *Sci. Rep.* **2017**, *7*, 12655. [[CrossRef](#)]
9. Burton, D.R.; Pyati, J.; Koduri, R.; Sharp, S.J.; Thornton, G.B.; Parren, P.W.H.I.; Sawyer, L.S.W.; Hendry, R.M.; Dunlop, N.; Nara, P.L.; et al. Efficient Neutralization of Primary Isolates of HIV-1 by a Recombinant Human Monoclonal Antibody. *Science* **1994**, *266*, 1024–1027. [[CrossRef](#)]
10. Zwick, M.B.; Parren, P.W.H.I.; Saphire, E.O.; Church, S.; Wang, M.; Scott, J.K.; Dawson, P.E.; Wilson, I.A.; Burton, D.R. Molecular Features of the Broadly Neutralizing Immunoglobulin G1 B12 Required for Recognition of Human Immunodeficiency Virus Type 1 Gp120. *J. Virol.* **2003**, *77*, 5863–5876. [[CrossRef](#)]
11. Shcherbakov, D.N.; Bakulina, A.Y.; Karpenko, L.I.; Ilyichev, A.A. Broadly Neutralizing Antibodies against HIV-1 As a Novel Aspect of the Immune Response. *Acta Nat.* **2015**, *7*, 11–21. [[CrossRef](#)]
12. Kwong, P.D.; Mascola, J.R. HIV-1 Vaccines Based on Antibody Identification, B Cell Ontogeny, and Epitope Structure. *Immunity* **2018**, *48*, 855–871. [[CrossRef](#)]
13. McCoy, L.E.; Burton, D.R. Identification and Specificity of Broadly Neutralizing Antibodies against HIV. *Immunol. Rev.* **2017**, *275*, 11–20. [[CrossRef](#)]
14. Burton, D.R.; Hangartner, L. Broadly Neutralizing Antibodies to HIV and Their Role in Vaccine Design. *Annu. Rev. Immunol.* **2016**, *34*, 635–659. [[CrossRef](#)] [[PubMed](#)]
15. Starr, T.N.; Greaney, A.J.; Hilton, S.K.; Ellis, D.; Crawford, K.H.D.; Dingens, A.S.; Navarro, M.J.; Bowen, J.E.; Tortorici, M.A.; Walls, A.C.; et al. Deep Mutational Scanning of SARS-CoV-2 Receptor Binding Domain Reveals Constraints on Folding and ACE2 Binding. *Cell* **2020**, *182*, 1295–1310.e20. [[CrossRef](#)]
16. Caillat, C.; Guilligay, D.; Torralba, J.; Friedrich, N.; Nieva, J.L.; Trkola, A.; Chipot, C.J.; Dehez, F.L.; Weissenhorn, W. Structure of HIV-1 Gp41 with Its Membrane Anchors Targeted by Neutralizing Antibodies. *eLife* **2021**, *10*, e65005. [[CrossRef](#)] [[PubMed](#)]
17. Leonard, C.K.; Spellman, M.W.; Riddle, L.; Harris, R.J.; Thomas, J.N.; Gregory, T.J. Assignment of Intrachain Disulfide Bonds and Characterization of Potential Glycosylation Sites of the Type 1 Recombinant Human Immunodeficiency Virus Envelope Glycoprotein (Gp120) Expressed in Chinese Hamster Ovary Cells. *J. Biol. Chem.* **1990**, *265*, 10373–10382. [[CrossRef](#)]
18. Zhu, P.; Chertova, E.; Bess, J.; Lifson, J.D.; Arthur, L.O.; Liu, J.; Taylor, K.A.; Roux, K.H. Electron Tomography Analysis of Envelope Glycoprotein Trimers on HIV and Simian Immunodeficiency Virus Virions. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15812–15817. [[CrossRef](#)]
19. McCune, J.M.; Rabin, L.B.; Feinberg, M.B.; Lieberman, M.; Kosek, J.C.; Reyes, G.R.; Weissman, I.L. Endoproteolytic Cleavage of Gp160 Is Required for the Activation of Human Immunodeficiency Virus. *Cell* **1988**, *53*, 55–67. [[CrossRef](#)]
20. Robey, W.G.; Safai, B.; Oroszlan, S.; Arthur, L.O.; Gonda, M.A.; Gallo, R.C.; Fischinger, P.J. Characterization of Envelope and Core Structural Gene Products of HTLV-III with Sera from AIDS Patients. *Science* **1985**, *228*, 593–595. [[CrossRef](#)]
21. Kowalski, M.; Potz, J.; Basiripour, L.; Dorfman, T.; Goh, W.; Terwilliger, E.; Dayton, A.; Rosen, C.; Haseltine, W.; Sodroski, J. Functional Regions of the Envelope Glycoprotein of Human Immunodeficiency Virus Type 1. *Science* **1987**, *237*, 1351–1355. [[CrossRef](#)]
22. Kwong, P.D.; Wyatt, R.; Robinson, J.; Sweet, R.W.; Sodroski, J.; Hendrickson, W.A. Structure of an HIV Gp120 Envelope Glycoprotein in Complex with the CD4 Receptor and a Neutralizing Human Antibody. *Nature* **1998**, *393*, 648–659. [[CrossRef](#)]
23. Da, L.-T.; Quan, J.-M.; Wu, Y.-D. Understanding of the Bridging Sheet Formation of HIV-1 Glycoprotein Gp120. *J. Phys. Chem. B* **2009**, *113*, 14536–14543. [[CrossRef](#)] [[PubMed](#)]

24. Chen, B.; Vogan, E.M.; Gong, H.; Skehel, J.J.; Wiley, D.C.; Harrison, S.C. Structure of an Unliganded Simian Immunodeficiency Virus Gp120 Core. *Nature* **2005**, *433*, 834–841. [[CrossRef](#)] [[PubMed](#)]
25. Bartesaghi, A.; Merk, A.; Borgnia, M.J.; Milne, J.L.S.; Subramaniam, S. Prefusion Structure of Trimeric HIV-1 Envelope Glycoprotein Determined by Cryo-Electron Microscopy. *Nat. Struct. Mol. Biol.* **2013**, *20*, 1352–1357. [[CrossRef](#)] [[PubMed](#)]
26. Tamm, L.K.; Lee, J.; Liang, B. Capturing Glimpses of an Elusive HIV Gp41 Prehairpin Fusion Intermediate. *Structure* **2014**, *22*, 1225–1226. [[CrossRef](#)] [[PubMed](#)]
27. Cicala, C.; Arthos, J.; Fauci, A.S. HIV-1 Envelope, Integrins and Co-Receptor Use in Mucosal Transmission of HIV. *J. Transl. Med.* **2011**, *9*, S2. [[CrossRef](#)] [[PubMed](#)]
28. Rao, M.; Peachman, K.K.; Kim, J.; Gao, G.; Alving, C.R.; Michael, N.L.; Rao, V.B. HIV-1 Variable Loop 2 and Its Importance in HIV-1 Infection and Vaccine Development. *Curr. HIV Res.* **2013**, *11*, 427–438. [[CrossRef](#)]
29. Kwong, P.D.; Doyle, M.L.; Casper, D.J.; Cicala, C.; Leavitt, S.A.; Majeed, S.; Steenbeke, T.D.; Venturi, M.; Chaiken, I.; Fung, M.; et al. HIV-1 Evades Antibody-Mediated Neutralization through Conformational Masking of Receptor-Binding Sites. *Nature* **2002**, *420*, 678–682. [[CrossRef](#)]
30. Moore, P.L.; Crooks, E.T.; Porter, L.; Zhu, P.; Cayanan, C.S.; Grise, H.; Corcoran, P.; Zwick, M.B.; Franti, M.; Morris, L.; et al. Nature of Nonfunctional Envelope Proteins on the Surface of Human Immunodeficiency Virus Type 1. *J. Virol.* **2006**, *80*, 2515–2528. [[CrossRef](#)]
31. Richman, D.D.; Wrin, T.; Little, S.J.; Petropoulos, C.J. Rapid Evolution of the Neutralizing Antibody Response to HIV Type 1 Infection. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 4144–4149. [[CrossRef](#)] [[PubMed](#)]
32. Robertson, D.L.; Sharp, P.M.; McCutchan, F.E.; Hahn, B.H. Recombination in HIV-1. *Nature* **1995**, *374*, 124–126. [[CrossRef](#)] [[PubMed](#)]
33. Gaschen, B.; Taylor, J.; Yusim, K.; Foley, B.; Gao, F.; Lang, D.; Novitsky, V.; Haynes, B.; Hahn, B.H.; Bhattacharya, T.; et al. Diversity Considerations in HIV-1 Vaccine Selection. *Science* **2002**, *296*, 2354–2360. [[CrossRef](#)] [[PubMed](#)]
34. McBurney, S.P.; Ross, T.M. Viral Sequence Diversity: Challenges for AIDS Vaccine Designs. *Expert Rev. Vaccines* **2008**, *7*, 1405–1417. [[CrossRef](#)]
35. Cao, L.; Diedrich, J.K.; Kulp, D.W.; Pauthner, M.; He, L.; Park, S.-K.R.; Sok, D.; Su, C.Y.; Delahunty, C.M.; Menis, S.; et al. Global Site-Specific N-Glycosylation Analysis of HIV Envelope Glycoprotein. *Nat. Commun.* **2017**, *8*, 14954. [[CrossRef](#)]
36. Zhou, T.; Zheng, A.; Baxa, U.; Chuang, G.-Y.; Georgiev, I.S.; Kong, R.; O'Dell, S.; Shahzad-Ul-Hussan, S.; Shen, C.-H.; Tsybovsky, Y.; et al. A Neutralizing Antibody Recognizing Primarily N-Linked Glycan Targets the Silent Face of the HIV Envelope. *Immunity* **2018**, *48*, 500–513.e6. [[CrossRef](#)]
37. Wei, X.; Decker, J.M.; Wang, S.; Hui, H.; Kappes, J.C.; Wu, X.; Salazar-Gonzalez, J.F.; Salazar, M.G.; Kilby, J.M.; Saag, M.S.; et al. Antibody Neutralization and Escape by HIV-1. *Nature* **2003**, *422*, 307–312. [[CrossRef](#)]
38. Sanders, R.W.; Vesanen, M.; Schuelke, N.; Master, A.; Schiffner, L.; Kalyanaraman, R.; Paluch, M.; Berkhout, B.; Maddon, P.J.; Olson, W.C.; et al. Stabilization of the Soluble, Cleaved, Trimeric Form of the Envelope Glycoprotein Complex of Human Immunodeficiency Virus Type 1. *J. Virol.* **2002**, *76*, 8875–8889. [[CrossRef](#)]
39. Sanders, R.W.; Derking, R.; Cupo, A.; Julien, J.-P.; Yasmeen, A.; de Val, N.; Kim, H.J.; Blattner, C.; de la Peña, A.T.; Korzun, J.; et al. A Next-Generation Cleaved, Soluble HIV-1 Env Trimer, BG505 SOSIP.664 Gp140, Expresses Multiple Epitopes for Broadly Neutralizing but Not Non-Neutralizing Antibodies. *PLoS Pathog.* **2013**, *9*, e1003618. [[CrossRef](#)]
40. Sarkar, A.; Bale, S.; Behrens, A.-J.; Kumar, S.; Sharma, S.K.; de Val, N.; Pallesen, J.; Irimia, A.; Diwanji, D.C.; Stanfield, R.L.; et al. Structure of a Cleavage-Independent HIV Env Recapitulates the Glycoprotein Architecture of the Native Cleaved Trimer. *Nat. Commun.* **2018**, *9*, 1956. [[CrossRef](#)] [[PubMed](#)]
41. Schommers, P.; Gruell, H.; Abernathy, M.E.; Tran, M.-K.; Dingens, A.S.; Gristick, H.B.; Barnes, C.O.; Schoofs, T.; Schlotz, M.; Vanshylla, K.; et al. Restriction of HIV-1 Escape by a Highly Broad and Potent Neutralizing Antibody. *Cell* **2020**, *180*, 471–489.e22. [[CrossRef](#)]
42. Jia, M.; Liberatore, R.A.; Guo, Y.; Chan, K.-W.; Pan, R.; Lu, H.; Waltari, E.; Mittler, E.; Chandran, K.; Finzi, A.; et al. VSV-Displayed HIV-1 Envelope Identifies Broadly Neutralizing Antibodies Class-Switched to IgG and IgA. *Cell Host Microbe* **2020**, *27*, 963–975.e5. [[CrossRef](#)] [[PubMed](#)]
43. Chen, X.; Zhou, T.; Schmidt, S.D.; Duan, H.; Cheng, C.; Chuang, G.-Y.; Gu, Y.; Louder, M.K.; Lin, B.C.; Shen, C.-H.; et al. Vaccination Induces Maturation in a Mouse Model of Diverse Unmutated VRC01-Class Precursors to HIV-Neutralizing Antibodies with >50% Breadth. *Immunity* **2021**, *54*, 324–339.e8. [[CrossRef](#)]
44. Gorman, J.; Chuang, G.-Y.; Lai, Y.-T.; Shen, C.-H.; Boyington, J.C.; Druz, A.; Geng, H.; Louder, M.K.; McKee, K.; Rawi, R.; et al. Structure of Super-Potent Antibody CAP256-VRC26.25 in Complex with HIV-1 Envelope Reveals a Combined Mode of Trimer-Apex Recognition. *Cell Rep.* **2020**, *31*, 107488. [[CrossRef](#)] [[PubMed](#)]
45. Roark, R.S.; Li, H.; Williams, W.B.; Chug, H.; Mason, R.D.; Gorman, J.; Wang, S.; Lee, F.-H.; Rando, J.; Bonsignori, M.; et al. Recapitulation of HIV-1 Env-Antibody Coevolution in Macaques Leading to Neutralization Breadth. *Science* **2021**, *371*, eabd2638. [[CrossRef](#)]
46. Saunders, K.O.; Wiehe, K.; Tian, M.; Acharya, P.; Bradley, T.; Alam, S.M.; Go, E.P.; Scarce, R.; Sutherland, L.; Henderson, R.; et al. Targeted Selection of HIV-Specific Antibody Mutations by Engineering B Cell Maturation. *Science* **2019**, *366*. [[CrossRef](#)] [[PubMed](#)]

47. Kumar, S.; Ju, B.; Shaperro, B.; Lin, X.; Ren, L.; Zhang, L.; Li, D.; Zhou, Z.; Feng, Y.; Sou, C.; et al. A V(H)1-69 Antibody Lineage from an Infected Chinese Donor Potently Neutralizes HIV-1 by Targeting the V3 Glycan Supersite. *Sci. Adv.* **2020**, *6*. [[CrossRef](#)] [[PubMed](#)]
48. Wang, Z.; Barnes, C.O.; Gautam, R.; Cetrulo Lorenzi, J.C.; Mayer, C.T.; Oliveira, T.Y.; Ramos, V.; Cipolla, M.; Gordon, K.M.; Gristick, H.B.; et al. A Broadly Neutralizing Macaque Monoclonal Antibody against the HIV-1 V3-Glycan Patch. *eLife* **2020**, *9*. [[CrossRef](#)] [[PubMed](#)]
49. Schoofs, T.; Barnes, C.O.; Suh-Toma, N.; Golijanin, J.; Schommers, P.; Gruell, H.; West, A.P.J.; Bach, F.; Lee, Y.E.; Nogueira, L.; et al. Broad and Potent Neutralizing Antibodies Recognize the Silent Face of the HIV Envelope. *Immunity* **2019**, *50*, 1513–1529.e9. [[CrossRef](#)]
50. Dubrovskaya, V.; Tran, K.; Ozorowski, G.; Guenaga, J.; Wilson, R.; Bale, S.; Cottrell, C.A.; Turner, H.L.; Seabright, G.; O'Dell, S.; et al. Vaccination with Glycan-Modified HIV NFL Envelope Trimer-Liposomes Elicits Broadly Neutralizing Antibodies to Multiple Sites of Vulnerability. *Immunity* **2019**, *51*, 915–929.e7. [[CrossRef](#)] [[PubMed](#)]
51. Zhou, T.; Xu, L.; Dey, B.; Hessell, A.J.; Van Ryk, D.; Xiang, S.-H.; Yang, X.; Zhang, M.-Y.; Zwick, M.B.; Arthos, J.; et al. Structural Definition of a Conserved Neutralization Epitope on HIV-1 Gp120. *Nature* **2007**, *445*, 732–737. [[CrossRef](#)]
52. Stewart-Jones, G.B.E.; Soto, C.; Lemmin, T.; Chuang, G.-Y.; Druz, A.; Kong, R.; Thomas, P.V.; Wagh, K.; Zhou, T.; Behrens, A.-J.; et al. Trimeric HIV-1-Env Structures Define Glycan Shields from Clades A, B and G. *Cell* **2016**, *165*, 813–826. [[CrossRef](#)]
53. Zhou, T.; Lynch, R.M.; Chen, L.; Acharya, P.; Wu, X.; Doria-Rose, N.A.; Joyce, M.G.; Lingwood, D.; Soto, C.; Bailer, R.T.; et al. Structural Repertoire of HIV-1-Neutralizing Antibodies Targeting the CD4 Supersite in 14 Donors. *Cell* **2015**, *161*, 1280–1292. [[CrossRef](#)]
54. Zhou, T.; Zhu, J.; Wu, X.; Moquin, S.; Zhang, B.; Acharya, P.; Georgiev, I.S.; Altae-Tran, H.R.; Chuang, G.-Y.; Joyce, M.G.; et al. Multi-Donor Analysis Reveals Structural Elements, Genetic Determinants, and Maturation Pathway for Effective HIV-1 Neutralization by VRC01-Class Antibodies. *Immunity* **2013**, *39*, 245–258. [[CrossRef](#)]
55. Pan, R.; Gorny, M.K.; Zolla-Pazner, S.; Kong, X.-P. The V1V2 Region of HIV-1 Gp120 Forms a Five-Stranded Beta Barrel. *J. Virol.* **2015**, *89*, 8003–8010. [[CrossRef](#)]
56. Liao, H.-X.; Lynch, R.; Zhou, T.; Gao, F.; Alam, S.M.; Boyd, S.D.; Fire, A.Z.; Roskin, K.M.; Schramm, C.A.; Zhang, Z.; et al. Co-Evolution of a Broadly Neutralizing HIV-1 Antibody and Founder Virus. *Nature* **2013**, *496*, 469–476. [[CrossRef](#)] [[PubMed](#)]
57. Gorman, J.; Soto, C.; Yang, M.M.; Davenport, T.M.; Guttman, M.; Bailer, R.T.; Chambers, M.; Chuang, G.-Y.; DeKosky, B.J.; Doria-Rose, N.A.; et al. Structures of HIV-1 Env V1V2 with Broadly Neutralizing Antibodies Reveal Commonalities That Enable Vaccine Design. *Nat. Struct. Mol. Biol.* **2016**, *23*, 81–90. [[CrossRef](#)] [[PubMed](#)]
58. McLellan, J.S.; Pancera, M.; Carrico, C.; Gorman, J.; Julien, J.-P.; Khayat, R.; Louder, R.; Pejchal, R.; Sastry, M.; Dai, K.; et al. Structure of HIV-1 Gp120 V1/V2 Domain with Broadly Neutralizing Antibody PG9. *Nature* **2011**, *480*, 336–343. [[CrossRef](#)] [[PubMed](#)]
59. Löving, R.; Sjöberg, M.; Wu, S.-R.; Binley, J.M.; Garoff, H. Inhibition of the HIV-1 Spike by Single-PG9/16-Antibody Binding Suggests a Coordinated-Activation Model for Its Three Protomeric Units. *J. Virol.* **2013**, *87*, 7000–7007. [[CrossRef](#)] [[PubMed](#)]
60. Lee, J.H.; Andrabi, R.; Su, C.-Y.; Yasmeen, A.; Julien, J.-P.; Kong, L.; Wu, N.C.; McBride, R.; Sok, D.; Pauthner, M.; et al. A Broadly Neutralizing Antibody Targets the Dynamic HIV Envelope Trimer Apex via a Long, Rigidified, and Anionic β -Hairpin Structure. *Immunity* **2017**, *46*, 690–702. [[CrossRef](#)]
61. Cale, E.M.; Gorman, J.; Radakovich, N.A.; Crooks, E.T.; Osawa, K.; Tong, T.; Li, J.; Nagarajan, R.; Ozorowski, G.; Ambrozak, D.R.; et al. Virus-like Particles Identify an HIV V1V2 Apex-Binding Neutralizing Antibody That Lacks a Protruding Loop. *Immunity* **2017**, *46*, 777–791.e10. [[CrossRef](#)] [[PubMed](#)]
62. Bonsignori, M.; Kreider, E.F.; Fera, D.; Meyerhoff, R.R.; Bradley, T.; Wiehe, K.; Alam, S.M.; Aussedat, B.; Walkowicz, W.E.; Hwang, K.-K.; et al. Staged Induction of HIV-1 Glycan-Dependent Broadly Neutralizing Antibodies. *Sci. Transl. Med.* **2017**, *9*, eaai7514. [[CrossRef](#)] [[PubMed](#)]
63. Simonich, C.A.; Williams, K.L.; Verkerke, H.P.; Williams, J.A.; Nduati, R.; Lee, K.K.; Overbaugh, J. HIV-1 Neutralizing Antibodies with Limited Hypermutation from an Infant. *Cell* **2016**, *166*, 77–87. [[CrossRef](#)]
64. Sok, D.; Pauthner, M.; Briney, B.; Lee, J.H.; Saye-Francisco, K.L.; Hsueh, J.; Ramos, A.; Le, K.M.; Jones, M.; Jardine, J.G.; et al. A Prominent Site of Antibody Vulnerability on HIV Envelope Incorporates a Motif Associated with CCR5 Binding and Its Camouflaging Glycans. *Immunity* **2016**, *45*, 31–45. [[CrossRef](#)]
65. Walker, L.M.; Huber, M.; Doores, K.J.; Falkowska, E.; Pejchal, R.; Julien, J.-P.; Wang, S.-K.; Ramos, A.; Chan-Hui, P.-Y.; Moyle, M.; et al. Broad Neutralization Coverage of HIV by Multiple Highly Potent Antibodies. *Nature* **2011**, *477*, 466–470. [[CrossRef](#)]
66. Shaik, M.M.; Peng, H.; Lu, J.; Rits-Volloch, S.; Xu, C.; Liao, M.; Chen, B. Structural Basis of Coreceptor Recognition by HIV-1 Envelope Spike. *Nature* **2019**, *565*, 318–323. [[CrossRef](#)] [[PubMed](#)]
67. Wilen, C.B.; Tilton, J.C.; Doms, R.W. HIV: Cell Binding and Entry. *Cold Spring Harb. Perspect. Med.* **2012**, *2*, a006866. [[CrossRef](#)] [[PubMed](#)]
68. Daniels, C.N.; Saunders, K.O. Antibody Responses to the HIV-1 Envelope High Mannose Patch. *Adv. Immunol.* **2019**, *143*, 11–73. [[CrossRef](#)]
69. Moyo, T.; Kitchin, D.; Moore, P.L. Targeting the N332-Supersite of the HIV-1 Envelope for Vaccine Design. *Expert Opin. Ther. Targets* **2020**, *24*, 499–509. [[CrossRef](#)]
70. Sok, D.; Burton, D.R. Recent Progress in Broadly Neutralizing Antibodies to HIV. *Nat. Immunol.* **2018**, *19*, 1179–1188. [[CrossRef](#)]

71. Fera, D.; Lee, M.S.; Wiehe, K.; Meyerhoff, R.R.; Piai, A.; Bonsignori, M.; Aussedat, B.; Walkowicz, W.E.; Ton, T.; Zhou, J.O.; et al. HIV Envelope V3 Region Mimic Embodies Key Features of a Broadly Neutralizing Antibody Lineage Epitope. *Nat. Commun.* **2018**, *9*, 1111. [[CrossRef](#)]
72. Moore, J.P.; Willey, R.L.; Lewis, G.K.; Robinson, J.; Sodroski, J. Immunological Evidence for Interactions between the First, Second, and Fifth Conserved Domains of the Gp120 Surface Glycoprotein of Human Immunodeficiency Virus Type 1. *J. Virol.* **1994**, *68*, 6836–6847. [[CrossRef](#)]
73. Seabright, G.E.; Cottrell, C.A.; van Gils, M.J.; D’addabbo, A.; Harvey, D.J.; Behrens, A.-J.; Allen, J.D.; Watanabe, Y.; Scaringi, N.; Polveroni, T.M.; et al. Networks of HIV-1 Envelope Glycans Maintain Antibody Epitopes in the Face of Glycan Additions and Deletions. *Structure* **2020**, *28*, 897–909.e6. [[CrossRef](#)]
74. Van Gils, M.J.; van den Kerkhof, T.L.G.M.; Ozorowski, G.; Cottrell, C.A.; Sok, D.; Pauthner, M.; Pallesen, J.; de Val, N.; Yasmeen, A.; de Taeye, S.W.; et al. An HIV-1 Antibody from an Elite Neutralizer Implicates the Fusion Peptide as a Site of Vulnerability. *Nat. Microbiol.* **2016**, *2*, 16199. [[CrossRef](#)]
75. Blattner, C.; Lee, J.H.; Sliopen, K.; Derking, R.; Falkowska, E.; de la Peña, A.T.; Cupo, A.; Julien, J.-P.; van Gils, M.; Lee, P.S.; et al. Structural Delineation of a Quaternary, Cleavage-Dependent Epitope at the Gp41–Gp120 Interface on Intact HIV-1 Env Trimers. *Immunity* **2014**, *40*, 669–680. [[CrossRef](#)] [[PubMed](#)]
76. Lee, J.H.; Leaman, D.P.; Kim, A.S.; Torrents de la Peña, A.; Sliopen, K.; Yasmeen, A.; Derking, R.; Ramos, A.; de Taeye, S.W.; Ozorowski, G.; et al. Antibodies to a Conformational Epitope on Gp41 Neutralize HIV-1 by Destabilizing the Env Spike. *Nat. Commun.* **2015**, *6*, 8167. [[CrossRef](#)] [[PubMed](#)]
77. Scharf, L.; Scheid, J.F.; Lee, J.H.; West, A.P.; Chen, C.; Gao, H.; Gnanapragasam, P.N.P.; Mares, R.; Seaman, M.S.; Ward, A.B.; et al. Antibody 8ANC195 Reveals a Site of Broad Vulnerability on the HIV-1 Envelope Spike. *Cell Rep.* **2014**, *7*, 785–795. [[CrossRef](#)]
78. Pancera, M.; Zhou, T.; Druz, A.; Georgiev, I.S.; Soto, C.; Gorman, J.; Huang, J.; Acharya, P.; Chuang, G.-Y.; Ofek, G.; et al. Structure and Immune Recognition of Trimeric Prefusion HIV-1 Env. *Nature* **2014**, *514*, 455–461. [[CrossRef](#)]
79. Chuang, G.-Y.; Zhou, J.; Acharya, P.; Rawi, R.; Shen, C.-H.; Sheng, Z.; Zhang, B.; Zhou, T.; Bailer, R.T.; Dandey, V.P.; et al. Structural Survey of Broadly Neutralizing Antibodies Targeting the HIV-1 Env Trimer Delineates Epitope Categories and Characteristics of Recognition. *Structure* **2019**, *27*, 196–206. [[CrossRef](#)]
80. Eckert, D.M.; Kim, P.S. Mechanisms of Viral Membrane Fusion and Its Inhibition. *Annu. Rev. Biochem.* **2001**, *70*, 777–810. [[CrossRef](#)]
81. Kong, R.; Xu, K.; Zhou, T.; Acharya, P.; Lemmin, T.; Liu, K.; Ozorowski, G.; Soto, C.; Taft, J.D.; Bailer, R.T.; et al. Fusion Peptide of HIV-1 as a Site of Vulnerability to Neutralizing Antibody. *Science* **2016**, *352*, 828–833. [[CrossRef](#)]
82. Harrison, S.C. Viral Membrane Fusion. *Nat. Struct. Mol. Biol.* **2008**, *15*, 690. [[CrossRef](#)]
83. Colman, P.M.; Lawrence, M.C. The Structural Biology of Type I Viral Membrane Fusion. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 309–319. [[CrossRef](#)] [[PubMed](#)]
84. Ananthaswamy, N.; Fang, Q.; AlSalmi, W.; Jain, S.; Chen, Z.; Klose, T.; Sun, Y.; Liu, Y.; Mahalingam, M.; Chand, S.; et al. A Sequestered Fusion Peptide in the Structure of an HIV-1 Transmitted Founder Envelope Trimer. *Nat. Commun.* **2019**, *10*, 873. [[CrossRef](#)]
85. Kong, R.; Louder, M.K.; Wagh, K.; Bailer, R.T.; de Camp, A.; Greene, K.; Gao, H.; Taft, J.D.; Gazumyan, A.; Liu, C.; et al. Improving Neutralization Potency and Breadth by Combining Broadly Reactive HIV-1 Antibodies Targeting Major Neutralization Epitopes. *J. Virol.* **2015**, *89*, 2659–2671. [[CrossRef](#)]
86. De la Peña, A.T.; Rantalainen, K.; Cottrell, C.A.; Allen, J.D.; van Gils, M.J.; Torres, J.L.; Crispin, M.; Sanders, R.W.; Ward, A.B. Similarities and Differences between Native HIV-1 Envelope Glycoprotein Trimers and Stabilized Soluble Trimer Mimetics. *PLoS Pathog.* **2019**, *15*, e1007920. [[CrossRef](#)]
87. Irimia, A.; Sarkar, A.; Stanfield, R.L.; Wilson, I.A. Crystallographic Identification of Lipid as an Integral Component of the Epitope of HIV Broadly Neutralizing Antibody 4E10. *Immunity* **2016**, *44*, 21–31. [[CrossRef](#)] [[PubMed](#)]
88. Williams, L.D.; Ofek, G.; Schätzle, S.; McDaniel, J.R.; Lu, X.; Nicely, N.I.; Wu, L.; Lougheed, C.S.; Bradley, T.; Louder, M.K.; et al. Potent and Broad HIV-Neutralizing Antibodies in Memory B Cells and Plasma. *Sci. Immunol.* **2017**, *2*, eaal2200. [[CrossRef](#)]
89. Cardoso, R.M.F.; Zwick, M.B.; Stanfield, R.L.; Kunert, R.; Binley, J.M.; Katinger, H.; Burton, D.R.; Wilson, I.A. Broadly Neutralizing Anti-HIV Antibody 4E10 Recognizes a Helical Conformation of a Highly Conserved Fusion-Associated Motif in Gp41. *Immunity* **2005**, *22*, 163–173. [[CrossRef](#)]
90. Huang, J.; Ofek, G.; Laub, L.; Louder, M.K.; Doria-Rose, N.A.; Longo, N.S.; Imamichi, H.; Bailer, R.T.; Chakrabarti, B.; Sharma, S.K.; et al. Broad and Potent Neutralization of HIV-1 by a Gp41-Specific Human Antibody. *Nature* **2012**, *491*, 406–412. [[CrossRef](#)] [[PubMed](#)]
91. Ofek, G.; Tang, M.; Sambor, A.; Katinger, H.; Mascola, J.R.; Wyatt, R.; Kwong, P.D. Structure and Mechanistic Analysis of the Anti-Human Immunodeficiency Virus Type 1 Antibody 2F5 in Complex with Its Gp41 Epitope. *J. Virol.* **2004**, *78*, 10724–10737. [[CrossRef](#)]
92. Krebs, S.J.; Kwon, Y.D.; Schramm, C.A.; Law, W.H.; Donofrio, G.; Zhou, K.H.; Gift, S.; Dussupt, V.; Georgiev, I.S.; Schätzle, S.; et al. Longitudinal Analysis Reveals Early Development of Three MPER-Directed Neutralizing Antibody Lineages from an HIV-1-Infected Individual. *Immunity* **2019**, *50*, 677–691.e13. [[CrossRef](#)] [[PubMed](#)]

93. Pinto, D.; Fenwick, C.; Caillat, C.; Silacci, C.; Guseva, S.; Dehez, F.; Chipot, C.; Barbieri, S.; Minola, A.; Jarrossay, D.; et al. Structural Basis for Broad HIV-1 Neutralization by the MPER-Specific Human Broadly Neutralizing Antibody LN01. *Cell Host Microbe* **2019**, *26*, 623–637.e8. [[CrossRef](#)]
94. Zhang, L.; Irimia, A.; He, L.; Landais, E.; Rantalainen, K.; Leaman, D.P.; Vollbrecht, T.; Stano, A.; Sands, D.I.; Kim, A.S.; et al. An MPER Antibody Neutralizes HIV-1 Using Germline Features Shared among Donors. *Nat. Commun.* **2019**, *10*, 5389. [[CrossRef](#)]
95. Pejchal, R.; Gach, J.S.; Brunel, F.M.; Cardoso, R.M.; Stanfield, R.L.; Dawson, P.E.; Burton, D.R.; Zwick, M.B.; Wilson, I.A. A Conformational Switch in Human Immunodeficiency Virus Gp41 Revealed by the Structures of Overlapping Epitopes Recognized by Neutralizing Antibodies. *J. Virol.* **2009**, *83*, 8451–8462. [[CrossRef](#)] [[PubMed](#)]
96. Morris, L.; Chen, X.; Alam, M.; Tomaras, G.; Zhang, R.; Marshall, D.J.; Chen, B.; Parks, R.; Foulger, A.; Jaeger, F.; et al. Isolation of a Human Anti-HIV Gp41 Membrane Proximal Region Neutralizing Antibody by Antigen-Specific Single B Cell Sorting. *PLoS ONE* **2011**, *6*, e23532. [[CrossRef](#)]
97. Caillat, C.; Guilligay, D.; Sulbaran, G.; Weissenhorn, W. Neutralizing Antibodies Targeting HIV-1 Gp41. *Viruses* **2020**, *12*, 1210. [[CrossRef](#)] [[PubMed](#)]
98. Turner, H.L.; Andrabi, R.; Cottrell, C.A.; Richey, S.T.; Song, G.; Callaghan, S.; Anzanello, F.; Moyer, T.J.; Abraham, W.; Melo, M.; et al. Disassembly of HIV Envelope Glycoprotein Trimer Immunogens Is Driven by Antibodies Elicited via Immunization. *Sci. Adv.* **2021**, *7*, eabh2791. [[CrossRef](#)]
99. Xu, K.; Acharya, P.; Kong, R.; Cheng, C.; Chuang, G.-Y.; Liu, K.; Louder, M.K.; O'Dell, S.; Rawi, R.; Sastry, M.; et al. Epitope-Based Vaccine Design Yields Fusion Peptide-Directed Antibodies That Neutralize Diverse Strains of HIV-1. *Nat. Med.* **2018**, *24*, 857–867. [[CrossRef](#)]
100. Dingens, A.S.; Acharya, P.; Haddox, H.K.; Rawi, R.; Xu, K.; Chuang, G.-Y.; Wei, H.; Zhang, B.; Mascola, J.R.; Carragher, B.; et al. Complete Functional Mapping of Infection- and Vaccine-Elicited Antibodies against the Fusion Peptide of HIV. *PLoS Pathog.* **2018**, *14*, e1007159. [[CrossRef](#)]
101. Doria-Rose, N.A.; Schramm, C.A.; Gorman, J.; Moore, P.L.; Bhiman, J.N.; DeKosky, B.J.; Ernandes, M.J.; Georgiev, I.S.; Kim, H.J.; Pancera, M.; et al. Developmental Pathway for Potent V1V2-Directed HIV-Neutralizing Antibodies. *Nature* **2014**, *509*, 55–62. [[CrossRef](#)]
102. Mendoza, P.; Gruell, H.; Nogueira, L.; Pai, J.A.; Butler, A.L.; Millard, K.; Lehmann, C.; Suárez, I.; Oliveira, T.Y.; Lorenzi, J.C.C.; et al. Combination Therapy with Anti-HIV-1 Antibodies Maintains Viral Suppression. *Nature* **2018**, *561*, 479–484. [[CrossRef](#)]
103. Bar-On, Y.; Gruell, H.; Schoofs, T.; Pai, J.A.; Nogueira, L.; Butler, A.L.; Millard, K.; Lehmann, C.; Suárez, I.; Oliveira, T.Y.; et al. Safety and Antiviral Activity of Combination HIV-1 Broadly Neutralizing Antibodies in Viremic Individuals. *Nat. Med.* **2018**, *24*, 1701–1707. [[CrossRef](#)]
104. Brouwer, P.J.M.; Antanasijevic, A.; de Gast, M.; Allen, J.D.; Bijl, T.P.L.; Yasmineen, A.; Ravichandran, R.; Burger, J.A.; Ozorowski, G.; Torres, J.L.; et al. Immunofocusing and Enhancing Autologous Tier-2 HIV-1 Neutralization by Displaying Env Trimers on Two-Component Protein Nanoparticles. *NPJ Vaccines* **2021**, *6*, 1–14. [[CrossRef](#)] [[PubMed](#)]
105. Karch, C.P.; Bai, H.; Torres, O.B.; Tucker, C.A.; Michael, N.L.; Matyas, G.R.; Rolland, M.; Burkhard, P.; Beck, Z. Design and Characterization of a Self-Assembling Protein Nanoparticle Displaying HIV-1 Env V1V2 Loop in a Native-like Trimeric Conformation as Vaccine Antigen. *Nanomed. Nanotechnol. Biol. Med.* **2019**, *16*, 206–216. [[CrossRef](#)]
106. Sok, D.; Briney, B.; Jardine, J.G.; Kulp, D.W.; Menis, S.; Pauthner, M.; Wood, A.; Lee, E.-C.; Le, K.M.; Jones, M.; et al. Priming HIV-1 Broadly Neutralizing Antibody Precursors in Human Ig Loci Transgenic Mice. *Science* **2016**, *353*, 1557–1560. [[CrossRef](#)]
107. He, L.; de Val, N.; Morris, C.D.; Vora, N.; Thinnis, T.C.; Kong, L.; Azadnia, P.; Sok, D.; Zhou, B.; Burton, D.R.; et al. Presenting Native-like Trimeric HIV-1 Antigens with Self-Assembling Nanoparticles. *Nat. Commun.* **2016**, *7*, 12041. [[CrossRef](#)]
108. Jardine, J.G.; Ota, T.; Sok, D.; Pauthner, M.; Kulp, D.W.; Kalyuzhniy, O.; Skog, P.D.; Thinnis, T.C.; Bhullar, D.; Briney, B.; et al. Priming a Broadly Neutralizing Antibody Response to HIV-1 Using a Germline-Targeting Immunogen. *Science* **2015**, *349*, 156–161. [[CrossRef](#)]
109. Jardine, J.; Julien, J.-P.; Menis, S.; Ota, T.; Kalyuzhniy, O.; McGuire, A.; Sok, D.; Huang, P.-S.; MacPherson, S.; Jones, M.; et al. Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors. *Science* **2013**, *340*, 711–716. [[CrossRef](#)]