JVI Journals.ASM.org

Role of Human Immunodeficiency Virus Type 1 Envelope Structure in the Induction of Broadly Neutralizing Antibodies

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Very soon after the discovery of neutralizing antibodies (NAbs) toward human immunodeficiency virus type 1 (HIV-1) infection, it became apparent that characterization of these NAbs would be an important step in finding a cure for or a vaccine to eradicate HIV-1. Since the initial description of broadly cross-clade NAbs naturally produced in HIV-1 patients, numerous studies have described new viral targets for these antibodies. More recently, studies concerning new groups of patients able to control their viremia, such as long-term nonprogressors (LTNPs) or elite controllers, have described the generation of numerous envelope-targeted NAbs. Recent studies have marked a new stage in research on NAbs with the description of antibodies obtained from a worldwide screening of HIV-positive patients. These studies have permitted the discovery of NAb families with great potential for both neutralization and neutralization breadth, such as PG, PGT, CH, and highly active agonistic anti-CD4 binding site antibodies (HAADs), of which VRC01 and its variants are members. These antibodies are able to neutralize more than 80% of circulating strains without any autoreactivity and can be rapidly integrated into clinical trials in order to test their protective potential. In this review, we will focus on new insights into HIV-1 envelope structure and their implications for the generation of potent NAbs.

The isolation of new human immunodeficiency virus type 1 (HIV-1) envelope-specific neutralizing antibodies (NAbs) has been a high priority since they were identified as potential targets for vaccine design. Until recently, only a few recombinant NAbs were available for clinical trials. The use of new technologies using single-cell sorting of antigen-specific memory B cells together with PCR amplification of immunoglobulin genes has allowed the production of new antibodies, such as VRC01 (Table 1). Furthermore, high-neutralization screening of short-term memory B cell cultures has yielded PG9 and PG16 monoclonal antibodies (MAbs), which are broadly cross-reactive, and has defined a new target on the gp120 envelope glycoprotein (81, 146). In naturally infected HIV-1 patients, broadly neutralizing Abs (bNAbs) are not commonly produced. Antibodies that are produced are often directed against strain-specific or nonneutralizing determinant sites. Only 10 to 25% of HIV-1-infected individuals generate neutralizing antibody, and a minority of these individuals are able to neutralize several strains with considerable breadth. The development of a highly effective HIV-1 vaccine will likely depend on success in designing immunogens that elicit bNAbs toward naturally circulating strains of HIV-1 (66, 125, 127, 147). Until the last few years, only four NAbs had been defined, but recently, more than a dozen MAbs displaying substantial breadth have been isolated. An understanding of their recognition sites, the structural basis of their interaction with the HIV envelope, and their development pathways provides new opportunities to design vaccine candidates that will elicit bNAbs against this virus. For the majority of licensed vaccines, NAbs have been demonstrated to be one of the best correlates of vaccine efficacy. Their neutralizing activities could be therefore attributed to their capacity to either bind tightly to functional envelope glycoproteins, and thereby block viral entry into the host cells, or initiate antibody-dependent cell-mediated cytotoxicity (ADCC) or antibody-dependent cell-mediated virus inhibition (ADCVI). These mechanisms are mediated via the Fc regions of clustered immunoglobulin G1 (IgG1) or IgG3 recognized by Fc receptors on cells of the innate immune system, leading to the phagocytosis of infected cells upon opsonization, and activation of the classical complement pathway (32, 56). In this review, we will describe the latest insights into the characterization of envelope function during HIV-1 infection. We will then discuss the generation and characterization of new potent broadly cross-clade NAbs.

HIV-1 ENVELOPE GLYCOPROTEIN

The envelope glycoprotein of HIV-1 gp160 (Env) is synthesized in the rough endoplasmic reticulum and subjected to extensive N glycosylation, resulting in high-mannose chains linked to Asn residues at either the Asn-X-Ser or the Asn-X-Thr glycosylation site. gp160 matures by enzymatic cleavage through the host protease furin before anchoring at the membrane surface, in two subunits associated by noncovalent interactions: the surface gp120 (SU) and the transmembrane (TM) gp41 (45, 153). Both gp120 and gp41 are involved in the interaction, recognition, and promotion of the fusion of viral and cellular membranes and are the determinants of viral tropism (16). The Env spikes are thought to be trimeric, and structure-based models forming heterotrimeric complexes have been proposed using tomography by cryo-electron microscopy (9, 119). The envelope proteins are the most immunogenic compounds of HIV-1 particles because they are the only viral protein expressed on the viral membrane. These Env spikes of HIV-1, in their native configuration, elicit partially effective humoral immune responses (107, 149) and represent obvious vaccine targets.

Published ahead of print 26 September 2012

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doi:10.1128/JVI.01110-12

TABLE 1 HIV-1 Env-specific NAbs

Monoclonal antibodies	Isotype	Epitope on:	Epitope core	Epitope type	Clade neutralization		Polyreactivity	Reference(s)
2F5	IgG1	gp41	MPER 663–683, ^{663}ELDKWA^{668}	Linear	IIIB, MN, RF, SF2		Cardiolipin, phospholipid, the centromere B autoantigen	47, 106, 127
4E10	IgG1	gp41	MPER 671–676, ^{671}NWF(D/N)IT^{676}	Linear (discontinous)	A (DJ259, 92RW026, 92RW009), C (94ZW106, DJ258, DU151)	B (JR-FL, HxBc2), E (CM235, 92TH001), F (BZ162, R1), G (G3, RU570)	Cardiolipin, histone, dsDNA, the systemic lupus erythematosus autoantigen SS-A/Ro	47, 161
Z13e1	Fab	gp41	MPER 671–676, ^{671}NWFDIT^{676}	Linear (conformational)	B (JR-FL), C (94ZW109), E (CM235)		Cardiolipin	161
HK20	IgG	gp41 HR1	^{562}QQHLLQLTVWGIKQL^{576}	Linear (conformational)	A 92RW009, B SF1262, C 92BR025	BC CH110.2, CRF01 VI 1888, CRF02 VI 1090	Not available	1, 20, 113
PG9 PG16	IgG	gp120 trimer	V2 and V3 loop	Conformational	A 94UG103, 92RW020 B JRCSF, APV-17, APV-6	C 93IN905, IAVI C22, IAVI C18, IAVI C3, D 92UG024, CRF01_AE CMU02	Not available	146
VRC01	IgG	gp120	CD4 binding site	Conformational	100% clade A, 96% clade B, 87% clade C, 88% clade D	89% CRF01_AE, 81% CRF02_AG, 90% clade G, 100% CRF07_BC	Not available	27, 151
B12	IgG1	gp120	CD4 binding site	Discontinous	B primary JR-FL, JR-CSF, IIIB		Not available	162
2G12	IgG1	gp120	Carbohydrate moieties in C2, C3, and V4	Discontinous	A/B		Not available	57
PGT	IgG	gp120	Man8/9 glycans on gp120	Conformationalgp120 trimer	~70% of globally circulating HIV strains, especially PGT128		Not available	100, 145
VRC PG04	IgG	gp120 SRC	CD4 binding site	Conformational	88% of 162-pseudovirus panel (100% clade A, 90% clade A/E, 100% clade A/G, 94% clade B, 70% clade C)	76% clade D, 93% clade F, 87% clade G, 87% of 97-pseudovirus panel (96% clade A, 88% clade B, 80% clade C)	Not available	31, 151, 157
CH01-04	IgG	gp120	V2/V3	Conformational	36% (CH02), 47% (CH04) of 91 tier-2 pseudoviruses		CH03 (ribonucleoprotein, centromere B), histone antigen CH01-CH03 (HCV E2 protein or gut flora)	6, 7
HJ16	IgG	gp120	CD4bs V3, C1, C2	Conformational	Preferentially neutralized tier-2 isolates, A 92RW009, C isolate VI829	D CI 13, CRF02_AG VI 1090, and CA18, subtype B, subtype A/D	Not available	1, 20
HGN194	IgG	gp120	V3 loop RRSVRIGPGQTF	Conformational	All tier-1 viruses from clades A, B, and C, 11% of tier-2 viruses	C 9B Bal.26, SF162, and SS1196.1 3MW965.26, 96ZM651.2 and 92BR025.9	Not available	1, 20

gp120 ENVELOPE GLYCOPROTEIN

gp120 represents the external part of the envelope glycoprotein and is involved in the early steps of viral infection of target cells. It consists of conserved (C) and variable (V) protein domains which, when folded, comprise an inner domain, an outer domain, and a bridging sheet (110). One of the viral defense mechanisms evolved against antibody neutralization is the masking of key regions on SU with glycans. In this respect, 20 to 24 N-glycosylation sites are present on gp120. The V1/V2 and V3 variable regions are extended-loop structures which comprise a significant portion of the gp120 surface features. Hypervariable regions/domains that form exposed "loops" anchored at their bases by disulfide bonds (132) are located near the surface of the molecule. TheV1/V2 loop is subdivided into V1 and V2 by a disulfide bridge and is involved in modulating viral tropism and resistance to neutralization (81, 98, 115). V1/V2 stabilizes proximal gp120-gp120 and/or gp120-gp41 associations either directly or indirectly through stiffening of V1/V2-associated elements (55). V1/V2 consists of 50 to 90 residues that contain two of the most variable portions of the virus, and about 1 in 10 residues are N-glycosylated (111). The gp120 subunit contains functional sites, such as the CD4 binding site (CD4bs). CD4bs consists of a conserved pocket-like structure between the three gp120 units flanked by variable loops and the chemokine coreceptor binding site for CXCR4 and/or CCR5 that is exposed and/or formed after virus attachment to cells. The internal domain is near the point of contact with gp41 and the N and C termini of gp120. Numerous broadly neutralizing human antibodies have been identified that target epitopes on V1/V2 loops or quaternary neutralizing epitopes (QNEs) formed by the interaction of V2 and V3 and expressed on native Env trimers but not soluble Env (37), including PG9 and PG16, which neutralize 70 to 80% of circulating HIV-1 isolates, Abs CH01 to CH04, which neutralize 40 to 50% of isolates, and PGT141 to -145, which neutralize 40 to 80% of strains (6, 145, 146). Most of the QNE-specific Abs show sensitivity for an N-linked glycan at position 160 in V2 (HXB2 numbering) and show preferential binding to the assembled viral spike structure over monomeric gp120 as well as sensitivity to changes in V1/V2 and some V3 residues (65, 146). These quaternary-structure-preferring V1/V2-directed Abs are among the most common broadly neutralizing responses in infected donors (86). Among them we find PG9 and PG16 (which neutralize 70 to 80% of circulating HIV-1 isolates), CH01 to CH04 (which neutralize 40 to 50% of isolates), and PGT141 to -145 (which neutralize 40 to 80%) (6, 145, 146). The third variable loop (V3) of HIV-1 gp120 is comprised of 35 amino acids (aa), which are arranged in a disulfide loop involving two cysteines and play an important role in coreceptor selection (CCR5 or CXCR4), and is a target site for NAbs (105, 157). Indeed, removal of a single N-linked glycosylation site in the V3 loop (position 301) increases the sensitivity of primary isolates to neutralization. Thus, sugars that flank receptor-binding regions on gp120 protect primary HIV-1 isolates from antibody-mediated neutralization (64). N-linked residues are less present in gp120 regions that require structural plasticity and refolding during the infection process, although glycans can also reside on structurally mobile and/or flexible elements, such as the V4 and V5 loops of the outer domain (e.g., from extended loop to a β-strand or α-helix). The hypervariable V4 loop is highly heterogenous due to several insertions and deletions and, as a consequence, the increase of potential N-glycosylation sites (PNG) (2, 41). In contrast, V5 is less glycosylated than V4 (21) and plays a role in CD4 binding and neutralizing antibody responses (51, 71), as shown with VRC01 (2, 41). These host-derived carbohydrates protect the protein surface underlying the gp120 by masking them. The sugar microheterogeneities thereby form a perfect immunological shield that leads to a decrease in the immune response directed against viral particles (19, 97, 116).

gp120 IMMUNOGENICITY

During HIV-1 infection, gp120-specific Abs are naturally generated against conserved sites on the envelope that are crucial to host receptor recognition or viral fusion (159). Immunological and three-dimensional structural studies of Env have shown that these regions were highly flexible during interaction with other surface molecules, thus explaining how Abs specific for some variable regions have neutralizing activity against diverse viruses. However, induction of NAbs by using rationally designed immunogens has, so far, proven unsuccessful (68, 73, 114, 121, 122, 151, 159). Indeed, gp120-specific Abs typically recognize neutralizing epitopes located on variable loops (23, 86) or dominant nonneutralizing conserved epitopes on Env (36, 96). This provides a rational basis for understanding the immunological cross-reactivity of many MAbs targeting the V1/V2 and V3 variable loops of gp120 and the quaternary neutralization epitopes (QNEs) formed by V2 and V3. The V3 loop and the "bridging sheet" contain the binding site for the viral coreceptor (CCR5 or CXCR4) (4, 46, 67, 106). The glycan-rich outer face of gp120 is also the target of the broadly neutralizing MAb (bnMAb) 2G12 (14, 57, 116, 136) (Table 1). In fact, 2G12 together with b12 were the first well-described broadly active NAbs against gp120 (Table 1). 2G12 shows an unusual interlocked VH domain-swapped dimer generating an extended and monovalent binding surface and recognizes clustered $\alpha1\rightarrow2$-linked mannose residues on the distal ends of oligomannose sugars located on the carbohydrate-covered silent face of the gp120 outer domain. 2G12 displays a unique reversible kinetic mechanism of neutralization and inhibits interactions between the V3 loop and the tyrosine sulfate-containing CCR5 amino terminus, and thereby viral entry, by disturbing the formation of fusion complexes (102). The capacity of monomeric or dimeric 2G12 to mediate ADCC or to activate the complement system has been clearly demonstrated *in vitro* (62, 137). B12 was derived from a phage library in which heavy and light chains were randomly reasserted. B12 recognizes an epitope that overlaps the binding site of gp120 to the CD4 receptor and efficiently neutralizes many strains of HIV-1. B12 can also protect macaques from vaginal challenge with simian-human immunodeficiency virus (SHIV) (13, 162).

Numerous Abs, including NAbs 17b, X5, m18, and m14, have been described for their capacity to bind to the CD4-induced site. All of these contain long H3s regions that play a major role in their mechanism of binding. The H3s regions of X5, m6, and m9 appear to be very flexible and highly potent at neutralizing HIV (88, 105, 150, 158). Recent works from Corti et al. have described new and potent bNAbs HGN194 and HJ16, directed against gp120 (20) (Table 1). HJ16 was obtained from a donor infected with a clade C virus. This antibody binds to CD4bs, thus preventing interaction of HIV-1 with CD4. HJ16 recognizes a discontinuous epitope on a surface proximal to the CD4bs on gp120. This epitope is completely distinct and nonoverlapping with that recognized by

CD4bs-specific antibody b12. HJ16 exhibits a broad neutralizing activity comparable in breadth to that of b12. HJ16 and b12 present complementarity in their neutralizing properties toward a group of viruses, referred to as tier-2 isolates, which have moderate sensitivity to antibody-mediated neutralization (20, 120). HGN194 was isolated from memory B cells of a long-term nonprogressor (LTNP) infected with an HIV-1 clade AG circulating recombinant form (CRF). This Ab recognizes a conformational epitope in the V3 loop and neutralizes all tier-1 viruses (those with a low sensitivity to antibody-mediated neutralization) across clades but only 11% of tier-2 viruses tested by Wu et al. (148). *In vivo*, administration of the IgG1 HGN194 in rhesus monkeys significantly decreases virus titers after HIV-1 challenge and seems to drastically affect viral reservoirs (148) (Table 1).

Numerous broadly gp120-specific NAbs were discovered using a multistep strategy (146). A new approach of NAb screening from a clade A-infected patient has been described; this approach combines binding of Abs to recombinant gp120 and gp41 with a high-throughput microneutralization assay, resulting in the improvement of the quality of selected candidates. B cells from infected donors obtained in the selection step were isolated (146). Pools of the VH- or VL-region clones were subcloned into a human IgG1 constant domain context. The first described potent bNAbs were PG9 and PG16. They are somatically related and appear to be derived from the same recombination of heavy and light chains. These antibodies have been identified by a large screening of 1,800 serum samples from HIV-positive donors. PG9 and PG16 both target discontinuous epitopes involving the conserved regions of V1, V2, and V3 loops and overlapping the CD4bs present in the assembled viral spike. They are sensitive to a loss of the N332 glycan and a N160/K168 substitution in the V2 loop or an isoleucine at position 215 in the C2 region (133) but are clearly distinct from 2G12 (97, 146). Despite the vaunted diversity of the HIV-1 gp120 envelope and the high sequence variability in the V2 and V3 regions (146), neutralization assays indicate that the recognized epitope is conserved in 70 to 80% of circulating viral isolates. Recently, McLellan et al. (80) solved the atomic structure of PG9, in complex with V1/V2, which allowed the identification of conserved features that enable recognition of gp120 (80). PG9 overcomes variability of the V1/V2 protein sequence by binding to the atomic backbone of the protein rather than to the variable amino acid side chains. Associated with the strong evolutionary conservation of N332 glycans in gp120, PG9 is able to recognize 80% of HIV-1 circulating strains. Interaction between PG9 and V1/V2 occurs mainly with N-linked glycan asPG9 binding the glycan at position 160 on gp120. The third complementarity-determining region (CDR) of the heavy chain (CDRH3) reaches through the glycan shield to contact the protein-proximal *N*-acetylglucosamine area. In addition to glycan recognition, a strand in the CDRH3 of PG9 forms intermolecular parallel β-sheet-like hydrogen bonds to V1/V2 strand C. Strand C is the most variableV1/V2 strand, and this sequence-independent means of recognition probably allows for an increased breadth of recognition (81). The net charge in β-strand C influences the interaction with PG9 and PG16. The length and number of glycosylation sites in V2 confer resistance to PG9 and PG16 by creating a hindrance in antibody binding or hiding crucial residues of PG9 and PG16 epitopes (108). The CDRH3 of PG9 comprises a hammerhead that occupies a slot between two glycans on gp120, contacting the flanking glycan surfaces and binding to the protein surface at its base (115). Cryo-electron microscopy results concerning the binding of PG9 with glycan 160 (Man5GlcNAc2) suggest this glycan to be of an appropriate size such that it does not clash with the antibody light chain and does not stretch between the tip and base of the PG9 CDRH3 (81). Thus, PG9 is an example of the immune system actually exploiting the viral glycan defenses by binding directly to them. The changes in the heavy and light chains that allow for glycan recognition occur during affinity maturation and provide a possible explanation for the observed increase in PG9 (and PG16) breadth and affinity. The overall neutralization sensitivity is the consequence of characteristic molecular features of the V2 loop, and neutralization by PG9/16 is balanced by the glycans, the net positive charge and, possibly, the length of the V2 loop. Neutralization by PG9 correlates strongly with that by PG16, indicating that these Abs recognize a common HIV-1 epitope. This suggests that a common surface of the paratope on PG9 and PG16 might be involved in HIV-1 recognition. Substantial differences in sequence are found between PG9 and PG16 (28, 97). An analysis of the antibody variable genes revealed two pairs of somatic variants, each one containing a long heavy-chain complementarity determining region 3 (CDRH3). Long CDRH3 loops have been previously associated with polyreactivity of MAbs, such as 2F5, 4E10, or 1b12, which also bind different autoantigens, such as phospholipids (cardiolipin, phosphatidylserin) or histone antigen (50, 141) (Table 1).

Other very recent studies have given rise to a number of Abs with different properties of potency and breadth of neutralization (100, 145, 146). A new family of NAbs was isolated by screening antibody-containing memory B cell supernatants for broad neutralizing activity from the top four elite neutralizers from approximately 1,800 HIV-infected donors. The eliciting is based on a score incorporating both breadth and potency. Antibody variable genes were rescued from B cell clones showing cross-clade neutralizing activity and expressing full-length IgGs. It was revealed that all of the monoclonal Abs isolated from each individual donor belong to a distant, but clonally related, cluster of Abs. Two of six bNAbs (PGT125 to -128 and -130 to -131) that bind specifically to the Man8/9 glycans on gp120 have also been described recently and are potently neutralizing across clades (100, 145) (Table 1). A model of the Fab PGT128 complex with a fully glycosylated gp120 outer domain reveals that the antibody penetrates the glycan shield and recognizes two conserved glycans N332 and N301 as well as a short β-strand segment of the gp120 V3 loop (100). It was also observed that their neutralization of HIV may be mediated by cross-linking Env trimers on the viral surface. PGT128, the broadest of these Abs, neutralizes over 70% of globally circulating viruses and is, on average, an order of magnitude more potent than the recently described PG9, PG16, VRC01, and VRCPG04 bnMAbs and two orders of magnitude more potent than the prototype bnMAbs described earlier (4, 146, 151, 152). PGT Abs neutralize the cell surface trimer with more potency than their Fab and also exhibit the interesting property of being able to reduce the half-life of the virus, while 2G12 had no effect on the half-life of the virus. By electronic microscopy, it has been demonstrated that PGT127 and -128 are able to cross-link with the spikes on the surfaces of virus particles, thereby increasing their affinity through avidity effects, but not on the surfaces of envelope-expressing cells (100). These Abs target a sensitive conserved and highly exposed epitope, including two glycans (N301/N332), and V3 loop backbone components that make this region an at-

tractive immunogen for vaccine design (145). In the same family of bNAbs, PGT141 to -145 present a strong preference for quaternary epitopes similar to those described for PG9 and PG16. These Abs neutralize around 40 to 80% of circulating HIV-1 isolates and present the same properties of CDRH3 as PG9 or PG16, with an extended CDRH3 loop and a β-hairpin tip able to penetrate the N-linked glycan barrier and reach the protein surface beneath.

The inefficiency in isolating broadly reactive CD4bs-directed NAbs from human B cells is probably due to the use of immunogens that are reactive with many HIV-1-specific antibodies, including nonneutralizing antibodies. Recent observations on envelope structure have been combined with computer-assisted protein design to define recombinant forms of HIV-1 Env that specifically interact with NAbs directed against the CD4 binding site (70, 71, 151, 156). A resurfaced stabilized core (RSC) HIV-1 gp120 molecule, mimicking the site of initial CD4 receptor binding, has allowed the isolation of the CD4bs-specific MAb VRC with extensive neutralization breadth. To isolate these MAbs, antigen-specific memory B cell sorting was used, together with single-cell PCR, to amplify IgG H- and L-chain genes from individual B cell cDNA (117). Memory B cells able to bind to the RSC were selected. The matched H- and L-chain genes were amplified and cloned into IgG1 expression vectors that reconstitute the constant regions allowing the expression of full IgG. Three Abs (VRC01, VRC02, and VRC03) were elicited by this strategy. Comparisons of the nucleotide sequence of heavy and light chains revealed that VRC01 and VRC02 are somatic variants of the same IgG1 clone. The heavy-chain CDR3 of the two MAbs was composed of the same 14 aa, while the heavy chain CDR3 of VRC03 contained 16 aa. Both MAbs are highly somatically mutated, with 32% of the heavy chain variable gene (VH) and 17 to 19% of the kappa light chain variable gene (VK) nucleotides divergent from putative germ line gene sequences. VRC03 was potentially derived from a different IgG1 clone, but its heavy chain was derived from the same alleles as VRC01 and VRC02. VRC03 also presents a hypersomatic mutation with an unusual amino acid insertion in heavy-chain framework 3 and with 30% of VH and 20% of VK nucleotides derived from putative germ line gene sequences. Overall, the three MAbs share common sequence motifs in heavy chain CDR1, CDR2, and CDR3. A cryo-electron microscopic analysis of binding characteristics revealed that VRC01 and VRC02 act as partial CD4 agonists in their binding with gp120 and partially mimic the interaction of CD4 with gp120 (135). This characteristic may explain their breadth, as interaction with CD4 is crucial for infection. In these studies, it was observed that among circulating HIV-1 isolates tested for sensitivity to VRC01, 173 of 190 were neutralized and only 17 of 190 were resistant. Data for VRC02 are very similar. VRC03 is less broad than VRC01 and VRC02, neutralizing only 57% of viruses (151). A structural analysis of these resistant isolates showed important variations in the V5 region (Table 1). However, the low VRC01 resistance frequency suggests that VRC01 uses a recognition mechanism that allows binding despite V5 variations. Studying the interaction of VRC01 with V5 shows that VRC01 recognition of the V5 domain in gp120 is different than that seen with CD4. The V5 loop fits into the gap between heavy and light chains. A model of the interaction between VRC01 and the Env functional trimer has recently been proposed. In this model, VRC01 locks the Env functional trimer in a low-energy conformation and disables further rearrangements that occur during virus entry (71). Thus, interaction with the more conserved residues at the loop base is sufficient for VRC01 activity independently of variation in the top of the V5 loop (156). Finally, the observation that VRC01 does not display substantial self-reactive or polyreactive properties could facilitate the use of passive-protection studies in nonhuman primates and then in humans.

Recently, deep-sequencing technology combined with bioinformatics has been used for the first time to identify bNAb sequences from millions of variable heavy-chain sequences in HIV-infected individuals (27, 152). This deep screening has allowed the isolation of many antibodies, referred to as VRC and VRC-like antibodies, including the bNAb VRCPG04 isolated from one donor (with a virus subtyped as clade A1/D) and VRC-CH31 from another donor. Those Abs are similar to VRC01 in their breadth and potency. They also bind to the CD4Bs, are highly affinity matured, and are derived from the IgVH1-2 germ line gene. PGV04 was sorted from single memory B cells of an elite neutralizer by using the RSC3 protein and a CD4bs-defective version for selective isolation of potent CD4bs MAbs (152). VRC-PG04 has a neutralizing profile distinct from those of CD4, b12, and VRC01. In the first neutralizing assays against a multiclade 162-pseudovirus panel, PGV04 neutralized 88% of the pseudoviruses, while PG9 neutralized only 75% (31). Therefore, VRC-PG04 exhibited enhanced breadth compared to PG9 with a similar potency. In a second neutralization assay with a 97-pseudovirus panel, PG9, PGV04, and VRC01 neutralized 82%, 87%, and 93% of viruses, respectively. Therefore, VRC01 and PGV04 have nearly the same breadth and the same profile of neutralization. In addition, VRC-PG04 showed no evidence of polyreactivity (31). Several alanine substitutions affect CD4bs MAb neutralization and binding differently; D279A substitution decreases neutralization by VRC01 and CD4-IgG but does not affect neutralization by b12, while the I420A and I423A substitutions decrease VRC01 neutralization but increase both CD4-IgG and b12 neutralization. However, each of these three substitutions (D279A, I420A, and I423A) abrogates PGV04 neutralization. The highly conserved nature of the residues that are important for VRC-PG04 recognition likely explains how PGV04 is able to achieve broad neutralization.

In contrast to VRC01, VRC-PG04 did not enhance 17b or X5 binding to its epitope (the CD4-induced [CD4i] site) in the coreceptor region on the gp120 monomer (31). VRC-PG04 displayed both a heavy-chain variable gene (VH) mutation frequency and a level of affinity maturation similar to those observed with VRC01-03. The VRC-PG04 Fab was crystallized in complex with a gp120 core from the recombinant clade A/E 93TH057 that was previously crystallized with VRC01 (156). The crystallized complex showed strong similarity with the one obtained with VRC01, despite different donor origins and only 50% amino acid identity in the HC variable region. An overlay of the heavy chains of VPG04 and VRC01 revealed small differences, but critical interactions in the Asp^{368} gp120 salt bridge to Arg^{71} VRC01 were maintained in VRC-PG04. The remarkable convergence in recognition observed with VRC01, VRC03, and VRC-PG04 suggests a similar mechanism of HIV-1 gp120 recognition, conserved between infected donors. The precision required for this mode of recognition likely arises as a consequence of the multiple mechanisms of immune evasion that protect the site of CD4 attachment on HIV-1 gp120 (19). Thus, precise H-binding is required for the convergence in structure that enhances regions with hydrophobic interactions and focuses precisely on the initial site of CD4 receptor attach-

ment (152, 156, 157). Surprisingly, this convergence in epitope recognition is not matched in antibody sequence identity. Indeed, 10 antibodies that bind RSC3 use the IGHV1-2*02 germ line and accrue 70 to 90 nucleotide changes. Even if these mature Abs share the same epitope recognition, only two residues from the germ line IGHV1-2*02 allele mature to give the same amino acids (152). Both of these changes occur at a hydrophobic contact in the critical region 2 (CDRH2). Despite this divergence in maturation, a comparison of the VRC01, VRC03, and VRC-PG04 paratopes shows that many of these changes are of a conserved chemical character. Overall, these observations suggest that affinity maturation does converge with epitope recognition even if there is divergence in specific residue maturation and that VRC-PG04, VRC01, b12, and CD4-IgG recognize the CD4bs in somewhat different ways.

Isolation of single memory B cells from the blood of HIV-infected individuals using parts of the HIV Env protein has been also described recently (117, 118). Variable regions of the light and heavy chains of the antibody genes expressed by B memory cells were amplified by a new strategy of PCR and then used to produce new MAbs (118). Different primers were designed to amplify even highly mutated antibody genes from B cells by binding to less-mutated parts of the antibody sequences (117). As is the case for other recently described HIV-specific bNAbs, the most potent new bNAbs identified show an unusually high degree of somatic hypermutation (152). Regarding their capacity to efficiently neutralize HIV-1 strains, several antibodies were selected, including the VRC01 variant NIH45-46 and 3BNC60. These Abs showed binding patterns similar to that of VRC01 on gp120. New classes of highly active agonistic anti-CD4 binding site antibodies (HAADs) that expose the coreceptor binding site on gp120 and mimic binding of the host receptor CD4 have also been described (118). This antibody class shares IgVH and IgVL consensus sequences, including the contact residues between VRC01 and the HIV spike. In five different donors, these antibodies originate from only two closely related IgVH genes and three IgVL genes. The variable portions of the heavy chain of the most potent new bNAbs and VRC01 have about 68% of their amino acid sequence in common and also share a origin in that they are derived from two related germ line genes, IgVH1-2 and IgVH1-46. In addition, the crystal structure of the Fab region of one of the new bNAbs, 3BNC60, was similar to VRC01, suggesting that it binds gp120 in a similar way and by mimicking CD4 binding and disrupting the envelope trimers. More recently, the structure of the most potent clonal variant of VRC01, NIH45-46, has been described in more detail (27). This antibody resembles VRC01 but includes a four-residue insertion (residues 99a to 99d) within CDRH3, a property acquired by somatic hypermutation. Three of these residues are important in the binding to gp120 and neutralization activities. In comparison with VRC01 neutralization data, NIH45-46 was more potent than VRC01 on 62 of the viruses tested from a panel of 82 tier-2 and -3 viruses representing all known clades but still less potent than 3BNC117 (118, 151). In the same study (27), the ability of VCR03 to bind gp120 and to increase neutralizing activity by modifying the contact zones with gp120 was attempted by constructing a new variant of NIH45-46 by tryptophan substitution. The mutant obtained showed enhanced potency of neutralization since it neutralizes six NIH45-46-resistant strains, making this antibody, NIH45-46^{G54W}, one of the rare MAbs that could be a candidate in the design of a new vaccine.

In order to elicit NAbs from the sera of an African donor infected with a clade A strain of HIV-1 (125), Bonsignori et al. used a combination of different methods of memory B cell isolation, clonal or oligoclonal culture systems, single-cell flow sorting, Epstein-Barr virus (EBV) transformation, and recombinant antibody cells (6). A family of Abs from the same clonal lineage of bNAbs was identified, such as quaternary bNAbs (CH01 to CH04), CD4 binding site (VRC01-like) bNAbs (CH30, CH31, and CH32), V3 NAb (CH19), and a novel gp41 NAb against the 3-glycan gp41 site. CH11.CH01 to -04 antibodies recognize a conformational epitope on the V2/V3 region. Similarly to MAbs PG9 and PG16, CH01 to CH04 recognize an epitope on a single protomer that is usually, but not exclusively, conferred to the gp120 envelope glycoprotein by trimer formation (6, 22, 144). MAbs CH01 to CH04 bind a PG9/PG16-like conformational epitope but present differences in their breadths of neutralization and residue sensitivities compared to those of PG9/PG16. They neutralize 36% (CH02) to 47% (CH04) of 91 tier-2 pseudoviruses. However, when CH01 and VRC-CH31 are combined, they neutralized 86 of 91 of HIV-1 strains (95%) (7). The CH01 to -04 antibodies share similar V(D)J rearrangements, the same length of CDRH3, and high degrees of similarity to CDRH3 and CDRL3, thus indicating that they belong to the same clonal family. Somatic mutations of the VDJ nucleotide sequences of CH01 to CH04 (11.5 to 14.3%) are similar to those of MAbs PG9 (11.9%) and PG16 (13.2%). In the same way as for anti-gp41 MPER NAbs, MAb CH03 is autoreactive for ribonucleoprotein, centromere B, and histone antigens, and MAbs CH01 to CH03 are polyreactive with the hepatitis C virus E2 protein or gut flora antigens. This autoreactivity could potentially and indeed does probably induce tolerance mechanisms, such as anergy or deletion (50, 141). Like PG9/PG16, CH01/CH04 NAbs present a long region 3 (HCDR3). This suggests a common mechanism of generation or selection of Abs with a long CDHR3.

Recently, the human mAb2909, isolated by immortalization of peripheral blood mononuclear cells (PBMC) from asymptomatic and drug-naïve HIV-1 patients (38), has been shown to bind to a quaternary structure on virions expressing only V2, V3, and the CD4 binding site but not to soluble monomeric gp120 (17, 37). This antibody demonstrates a high neutralizing activity for primary isolates such as SF162. Its neutralizing activity against SF162 is 750- to 100,000-fold more potent than those of other well-characterized NAbs. HIV-1 SF162 contains a rare polymorphism consisting of a single-amino-acid substitution in the V2 domain (K^{160} nonpermissive for N glycosylation). This substitution leads to the loss of the conserved N-linked glycosylation site that is essential for the epitope of PG9 and PG16 (146). The occurrence of MAb 2909 suggests the possible existence of additional oligomer-specific Abs that may possess broader neutralizing activity.

gp41 ENVELOPE GLYCOPROTEIN

The binding of gp120 to CD4 and the viral coreceptors results in conformational changes and triggers the establishment of a "fusion complex" composed of gp41 trimers (29, 91, 113). gp41 is the transmembrane (TM) domain for the entire envelope glycoprotein and thus anchors the viral spikes in the bilayer lipid membrane of viral particles and, as stated above, plays an important role in membrane fusion and cell entry. It presents an extracellular domain, a TM domain, and an intracytoplasmic domain. gp41 sequences are more conserved than those of gp120 and contain

only four N-glycosylation sites on their ectodomain. These glycosylation sites are highly conserved and appear to contribute to optimal viral replication efficiency (58). The N-terminal hydrophobic region consists of the fusion peptide (aa 512 to 527), a polar region (PR, aa 525 to 543) that is also called the fusion peptide proximal region (FPPR), the N-terminal heptad repeat (NHR or HR1) (546 to 581), and the C-terminal heptad repeat (CHR or HR2). These regions are folded as α-helices and linked by a loop, generally referred to as the immunodominant loop (598 to 604), containing a disulfide bridge. A highly conserved tryptophan-rich membrane-proximal ectodomain/external region (MPER; 660 to 683) is also present (15). Finally, the membrane-spanning domain or transmembrane (MSD/TM) and the intracytoplasmic tail or C-tail are two other hydrophobic regions present at the C-terminal portion. A complete structural description of whole-molecule gp41 is not yet clearly defined. A crystallized intact trimer is currently unavailable, because it seems that large portions appear to be in constant motion, probably as part of the conformational masking defense of potential epitopes from NAbs (30, 80).

gp41 IMMUNOGENICITY

gp41 is largely occluded by quaternary interactions within native Env (18, 126, 153, 155, 158). During the fusion process, conformational changes occurring after interaction with gp120 expose different gp41 regions accessible for NAbs. Some of these exposed regions are essential for the infection process, and blocking them can inhibit infection and neutralize the virus (Table 1). The NAbs 2F5, Z13e1, and 4E10 can specifically bind Env and block a late stage of the fusion process (26, 92, 93, 130, 160). Despite great efforts, high NAb titers against any of the conserved sites on Env have not yet been elicited (61, 68, 101). Recently, the HK20 gp41-specific antibody was obtained from immortalized memory B cells from an HIV-1-infected individual (20) (Table 1). This Ab targets the conserved hydrophobic pocket in HR1. The crystal structure of HR1-specific human MAb HK20 in complex with the 5-helix domain of gp41 shows that HK20 binds to the same region as that recognized by D5, a human antibody directed against the N-heptad region (83) but differs significantly from D5 in terms of contact sites and shows a role for somatic mutations in affinity maturation (77). These aspects influence the potency and breadth of neutralization, which are higher for HK20 than for D5 and depend on somatically mutated residues. In addition, it was shown that in the case of HK20, the scFv is at least 15-fold more potent in neutralization than IgG, which is consistent with a limited accessibility to the target site (112). The gp41 footprints of HK20 and D5 and the global structural principles employed by the two antibodies are similar. Since HK20 targets HR1 instead of MPER or glycans in this region, it has the conceptual advantage over 4E10 and 2F5 of avoiding potential autoreactivity (112). HK20 presents an intermediate breadth of neutralization, preferentially to clades A and C, which did not correspond with the subtype of the infected patient, which was CRF02_AG as described in reference 1. Cross-reactive antibodies to the NHR have also been detected in HIV-1-positive sera, indicating its immunogenic potential (104). Moreover, the NHR trimer-specific D5 NAb was generated from a native single-chain variable fragment library. In the same way as the T-20 peptide, D5 inhibits the assembly of the fusion intermediate *in vitro* (83). D5 was derived from B cells of HIV-naïve subjects and has not been subject to extensive somatic hypermutation, with only seven non-complementarity-determining region amino acid changes from germ line sequences. Unlike b12, 2F5, and 4E10, D5 IgG does not have an atypically long heavy chain CDR3. Unlike 2G12, D5 does not require a "domain-swapped" structure for neutralization activity. D5 retains antiviral activity against primary HIV isolates when converted to an IgG1 format. Finally, and most importantly, D5 was elected by binding to IZN36 and 5H, which are synthetic antigens with well-defined structures mimicking the 6-helix bundle structure. In addition, new NAbs, such as 8K8 and DN9, with limited potency against the NHR trimer of HIV-1, have been isolated by screening of a phage display library (94). Antigen binding and monoclonal Ab competition experiments using 8K8, DN9, and D5 strongly suggest that the epitopes of 8K8 and DN9 are closely related but differ from the D5 epitope. D5 preferentially binds to NHR mimetics in the absence of CHR peptide, thus indicating a significant cross-reactivity of D5 with immobilized 6-helix (77, 83). Similarly, C34 HR2 peptide competes efficiently with 8K8 and DN9 binding to immobilized NHR mimetics (e.g., 5-helix and IZN36), but not with D5. The 3764 Fab has also been shown to bind to the NHR region with the same efficiency for the free mimetics of the NHR coiled-coil-like N35CCG-N13 (94) and for the interaction with CHR peptide in the form of a 6-helix bundle (42, 43).

As described above, gp41 comprises several functional regions which play a major role in fusion of the virus and host cell membranes. These regions are not static during the infection process but retain a degree of flexibility. For instance, HR regions form a six-helix coiled-coil bundle playing an important role in both the early and late stages of the membrane fusion process (16, 58, 74, 76, 90) including the formation of the fusion pore (90, 123, 124). The Fab 3674 is directed against the helix bundle and neutralizes diverse laboratory adapted B strains of HIV-1 and primary isolates of subtypes A, B, and C (43). This structure was described for other NAbs and shows neutralizing activity against primary isolates (142). MPER contains the epitopes of three well-known bNAbs, 2F5, 4E10, and Z13 (12, 161). Recently, MPER-specific antibodies were characterized for an individual chronically infected with subtype C (CAP206). These antibodies present a great breadth of neutralization and were targeted to the distal MPER centered on the D674 asparagine residue (40). Isolation of a novel MPER-specific NAb (CAP206-CH12) from the same individual has also been achieved through the amplification of Ig gene fragments from single memory B cells (87). This MAb overlapped the 4E10 and Z13e1 epitopes and neutralized the same subset of viruses sensitive to the plasma antibodies. CAP206-CH12 used the same VH and VK Ig gene families as the 4E10 NAb, and its CDRH3 sequence showed strong similarities with that of Z13e1 as a result of shared J gene usage. CAP206-CH12 presents a low neutralization activity and limited potency, similar to Z13e1, and reacted with viruses of subtypes C and B. While the CAP206-CH12 MAb was polyreactive, unlike 2F5 and 4E10, it did not bind lipids with high avidity. These data suggest the possibility of convergent evolution among HIV-1 gp41 MPER MAbs. CAP206-CH12 utilized VH1-69 and VK3-20 in a manner similar to that seen with gp41 antibody 4E10. Other gp41 antibodies, such as D5 and HK20 that bind to the stalk of gp41, also utilize VH1-69. VH1-69-derived antibodies are hydrophobic and seem to show a preference for regions of virus envelopes that are in close proximity to viral membranes. CAP206-CH12 shares the same YYYYMD motif in its CDRH3 as Z13e1 and has a shorter CDRH3 that presents some

flexible residues adjacent to the Tyr motif but lacks the hydrophobic residue W or F present in 4E10 and the Z13e1 CDRH3 apex crucial for neutralization (11, 60). MPER-specific Abs present in naturally infected patients are not as broadly neutralizing as 2F5 or 4E10 (8, 15, 89).

Recent studies using chimeric pseudoviruses presenting epitopes in a context more closely related to the MPER structure suggest that MPER-specific NAbs are relatively rare or absent during natural infection. However, the fact that new NAbs map to a different region in MPER than 2F5 and 4E10 provides hope by suggesting that NAbs may be vaccine induced (49, 69). In animal models, 2F5 has been shown to interrupt HIV-1 mucosal transmission and confers protection against SHIV infection (79). The 2F5 antibody was isolated from human immortalized B cells and recognizes an epitope lying between glutamic acid 662 and alanine 667 (ELDKWA). Studies have shown, with an expression library and protection against protease experiments, that this epitope is in fact wider (EQELLELDKWASLWN). By addition of a leucine at each side (LELDKWASL), the affinity of 2F5 for its epitope is increased more than 2,000 times (82, 95, 100, 134). In models studying the involvement of the viral membrane using POPC (1-oleyl-2-palmolityl-*sn*-glycero-3-phosphocholine) and POPC/cholesterol in the recognition and binding to its epitope, it has been shown that 2F5 does not interact with the membrane before fixation (139). In the absence of a hydrophobic environment, 2F5 and 4E10 are unable to bind to the MPER region (95). Recently, it was shown that the binding of 2F5 causes peptide docking on the membrane and increases the ability of the MAb to be intrusive (33). 2F5 induces a confined local disorder in the membrane which can promote exposure and interaction with gp41. The 2F5 epitope contains a β-turn conformation and immunogen which when presented in a membrane context may better mimic the epitope and consequently improve the binding of this Ab (134). Moreover, the polyspecificity of 2F5 and 4E10 and autoreactivity by binding to cardiolipin could explain the low activity of these antibodies *in vivo* since the immune system depletes all B cells which produce autoreactive Abs (47) or induce tolerance mechanisms (140), even if occasionally, some autoreactivity can be related to the development of HIV bNAbs at the cellular level and at the level of serum antibodies, as is the case for 9G4 bNAbs that present self-reactivity to systemic lupus erythematosus (SLE) and neutralizing properties (63).

The 4E10 NAb is also a promising MPER-specific Ab that has been isolated from B cells of HIV patients. It binds a deeper epitope than the 2F5 epitope in the MPER and presents neutralizing activities against primary isolates and laboratory strains of HIV-1. 4E10 binds to a highly conserved linear epitope on MPER ^{671}NWF(D/N)IT^{676} (161). Crystallographic structures of the epitope have been characterized and form a large helical shape with the important residues on the same side of the helix (44). The antigen reacts only with residues of the base and center of the loop of CDRH3. However, much of this loop is not involved in binding to the antigen. The top of the loop of CDRH3 of 4E10 forms a flat hydrophobic surface which suggests a possible interaction with the membrane of the virus. Structural models predict formation of the epitope of 2F5 and 4E10 to be highly dependent on the presence of membrane, since affinity of 4E10 increases in the presence of lipids (75). The W^{672}, F^{673}, and T^{676} residues seem to be crucial for 4E10 binding since their replacement with alanine residues drastically reduces affinity (10). The ^{671}NWFDITNWLWYIK^{683} sequence is optimal for 4E10 recognition. In resistant strains, the pattern NWF(N/D)IT indicates that the 4E10 epitope is complex and would even be discontinuous (44). Mass spectrometry studies reveal that 4E10 binds to the N terminus of gp120 and gp41 on the native conformation (130). It has been suggested that the pattern NWF(N/D)IT forms a cryptic epitope accessible only during the intermediate stages of the merger/fusion. It seems that 4E10 has a higher affinity for the membrane than 2F5, thus conferring the capacity to promote peptide extraction from the bilayer lipid membrane (33). The 4E10 Ab also has a polyspecificity for autoantigens, such as histone or double-stranded DNA (dsDNA). The Z13 Fab is also a derived MPER-specific Ab. This Ab, derived from an expression library, recognizes an epitope located on the C terminus of the 2F5 epitope, which overlaps the 4E10 epitope. The epitope of Z13 is centered on the sequence ^{671}NWFDIT^{676}, but this sequence is dependent on the conformation of the motif and changes such as N glycosylation of asparagine 674 (D^{674}) or exposure to a native protein. This Ab is able to weakly neutralize several clades, such as B, C, and D (161).

In several *in vivo* pharmacokinetic studies and phase I/II clinical trials, intravenous high doses of the three neutralizing antibodies, 4E10, 2F5, and 2G12, were given in combination to 14 HIV-1-infected individuals at weekly intervals over 3 months. The results showed that these three Abs are able to maintain an undetectable viral load in patients in whom infection had been suppressed by antiviral therapy (59). These studies also showed that 4E10 administration is not highly immunogenic, with rare IgM raised against 4E10 but no IgG. Clearance and half-life of 4E10 are identical to those of other classical therapeutic Abs.

CONCLUSIONS

Passive administration of rare human Env-specific monoclonal bNAbs to rhesus macaques can protect them against SHIV challenge (52–54, 78, 80, 84). Detection of high gp120-specific NAb titers was correlated to protection in 31% of vaccinated humans in the recent RV144 Thai trial (48, 109). Furthermore, clues gleaned from the breakthroughs from the protocol G and the very recent Center for HIV/AIDS Vaccine Immunology (CHAVI) studies have confirmed that a major goal of HIV-1 vaccine development is to design highly immunogenic Env antigens capable of inducing NAbs (24, 80, 127). Data from human clinical trials demonstrate that the first generation of soluble protein and vectored Env immunogens are safe and immunogenic. However, the Abs generated are only effective on highly sensitive strains. From the recent literature on HAADs and/or PG NAbs, and their cross-reactivity among circulating viruses, gp120 seems to be an important target immunogen even if the coverage of neutralization is not complete and despite the fact that the glycan shield could evolve and mask potential epitopes. Using gp41 as a target for a prophylactic vaccine can be another important alternative. However, targeted gp41 epitopes are accessible during a short time window occurring while the conformational changes triggered by the fusion process are ongoing. The recently described HK20 epitope may be considered a serious candidate for an anti-HIV vaccine approach. Indeed, conserved Env epitopes targeted by NAbs are poorly immunogenic because they either are masked by the glycan shield (3, 149), appear transiently (34), are sterically hindered (67, 119), or must overcome entropy for Ab binding. Another important point is that conserved epitopes (particularly in gp41) present homologies with self-proteins that could trigger tolerance mechanisms

(47, 50, 141). Finally, even when Abs can neutralize infecting strains, their effect is transient due to escape mutations (107, 149). To date, immunization of nonhuman primates and humans with HIV-1 Env monomers or trimers has failed to induce bNAbs. Induced Abs are mainly effective on easily neutralized tier-1 strains but have weak neutralization abilities for tier-2 and tier-3 strains (35, 80). Poor cross-reactivity of vaccine-elicited NAbs appears to be related to the restricted repertoire of induced Abs and to the complexity of the native viral spike structure. Strikingly, most of the NAbs described in the literature are IgG. The study of neutralizing responses in the mucosal compartment is crucial for the challenge to design a new vaccine. Several studies have described the potential role of mucosal secretory IgA to locally block infection. The involvement of anti-Env secretory IgA that blocks both transcytosis and infection has been well described (5, 25, 138). Secretory IgA from parotid saliva or milk that was also described to recognize different epitopes on gp160, such as V1V2 and/or gp41, could also have neutralization cross-reactive properties (39, 143). However, the role of IgA remains controversial, and seric IgA seems to be also associated with a loss of response in the Thai trial.

The role of NAbs in the containment of viral replication in infected individuals seems to be uncertain, and in most cases, NAbs do not protect against disease progression (1). However, NAbs may neutralize the virus during the first step of infection and may help in the induction of adaptive immunity against HIV to prevent or delay the progression to AIDS. Detection of NAbs depends on the *in vitro* neutralization assays used, and standardization of the assays is essential in order to be able to compare the magnitudes and qualities of NAb responses from sera or other fluids from HIV-infected patients, uninfected HIV-1 exposed persons, and vaccinated humans. Viral mechanisms to prevent neutralization include high variability and extensive glycosylation of the envelope proteins, envelope trimerization and shedding, as well as late exposure of functionally important entry domains by conformational changes induced upon CD4 binding. These are also the difficulties encountered in the design of immunogens able to induce NAbs upon vaccination. Finally, solutions to improve access of neutralizing molecules, such as antibody heavy chain or Fab, to crucial residues or key regions can be found by looking at what occurs in animal models. Recently, camelid Abs, which consist of a unique heavy chain and have some properties superior to those of classical antibodies, have been described. Llama heavy chain antibody fragment (VHH) that neutralizes and recognizes epitopes on CD4bs has been characterized (131). The VHH antibodies are small enough to interfere during formation of the viral synapsis and thus inhibit the crucial early steps of infection. Other animals producing Abs with the same properties, such as sharks and cartilaginous fishes (131, 132), could provide new insights into anti-HIV vaccine development.

The development of an HIV vaccine has proven to be a formidable scientific challenge given the extreme genetic variability of the virus, lack of good animal models, lack of knowledge of all of the mechanisms involved in immunity, and certain limitations in current technology. However, existing and recent data would appear to suggest that the ability of a candidate vaccine to generate a bNAb response against multiple epitopes in the Env glycoprotein will be of high importance. Faced with a very accomplished escape mechanism with the sole aim of thwarting and circumventing the entire spectrum of immune strategies employed by our immune system, we must distinguish between immunogenicity and antigenicity and every aspect of the immune response. Recent advances in the development of new immunogens or new bNAbs in combination with the deepening of our knowledge of the structure of the envelope glycoprotein and its interaction with neutralizing antibodies can show the way for the development and design of a new and effective vaccine able to prevent or contain HIV infection and/or progression to AIDS.

REFERENCES

1. **Balla-Jhagjhoorsingh SS, et al.** 2011. Characterization of neutralizing profiles in HIV-1 infected patients from whom the HJ16, HGN194 and HK20 mAbs were obtained. PLoS One **6**:e25488. doi:10.1371/journal.pone.0025488.
2. **Belair M, et al.** 2009. The polymorphic nature of HIV type 1 env V4 affects the patterns of potential N-glycosylation sites in proviral DNA at the intrahost level. AIDS Res. Hum. Retroviruses **25**:199–206.
3. **Binley JM, et al.** 2010. Role of complex carbohydrates in human immunodeficiency virus type 1 infection and resistance to antibody neutralization. J. Virol. **84**:5637–5655.
4. **Binley JM, et al.** 2004. Comprehensive cross-clade neutralization analysis of a panel of anti-human immunodeficiency virus type 1 monoclonal antibodies. J. Virol. **78**:13232–13252.
5. **Bomsel M, et al.** 2011. Immunization with HIV-1 gp41 subunit virosomes induces mucosal antibodies protecting nonhuman primates against vaginal SHIV challenges. Immunity **34**:269–280.
6. **Bonsignori M, et al.** 2011. Analysis of a clonal lineage of HIV-1 envelope V2/V3 conformational epitope-specific broadly neutralizing antibodies and their inferred unmutated common ancestors. J. Virol. **85**:9998–10009.
7. **Bonsignori M, et al.** 2012. Two distinct broadly neutralizing antibody specificities of different clonal lineages in a single HIV-1-infected donor: implications for vaccine design. J. Virol. **86**:4688–4692.
8. **Braibant M, et al.** 2006. Antibodies to conserved epitopes of the HIV-1 envelope in sera from long-term non-progressors: prevalence and association with neutralizing activity. AIDS **20**:1923–1930.
9. **Briggs JA, et al.** 2009. Structure and assembly of immature HIV. Proc. Natl. Acad. Sci. U. S. A. **106**:11090–11095.
10. **Brunel FM, et al.** 2006. Structure-function analysis of the epitope for 4E10, a broadly neutralizing human immunodeficiency virus type 1 antibody. J. Virol. **80**:1680–1687.
11. **Bryson S, Julien JP, Hynes RC, Pai EF.** 2009. Crystallographic definition of the epitope promiscuity of the broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2F5: vaccine design implications. J. Virol. **83**:11862–11875.
12. **Buchacher A, et al.** 1994. Generation of human monoclonal antibodies against HIV-1 proteins; electrofusion and Epstein-Barr virus transformation for peripheral blood lymphocyte immortalization. AIDS Res. Hum. Retroviruses **10**:359–369.
13. **Burton DR, et al.** 2011. Limited or no protection by weakly or nonneutralizing antibodies against vaginal SHIV challenge of macaques compared with a strongly neutralizing antibody. Proc. Natl. Acad. Sci. U. S. A. **108**:11181–11186.
14. **Calarese DA, et al.** 2003. Antibody domain exchange is an immunological solution to carbohydrate cluster recognition. Science **300**:2065–2071.
15. **Chan DC, Fass D, Berger JM, Kim PS.** 1997. Core structure of gp41 from the HIV envelope glycoprotein. Cell **89**:263–273.
16. **Chan DC, Kim PS.** 1998. HIV entry and its inhibition. Cell **93**:681–684.
17. **Changela A, et al.** 2011. Crystal structure of human antibody 2909 reveals conserved features of quaternary structure-specific antibodies that potently neutralize HIV-1. J. Virol. **85**:2524–2535.
18. **Chen B, et al.** 2005. Structure of an unliganded simian immunodeficiency virus gp120 core. Nature **433**:834–841.
19. **Chen L, et al.** 2009. Structural basis of immune evasion at the site of CD4 attachment on HIV-1 gp120. Science **326**:1123–1127.
20. **Corti D, et al.** 2010. Analysis of memory B cell responses and isolation of novel monoclonal antibodies with neutralizing breadth from HIV-1-infected individuals. PLoS One **5**:e8805. doi:10.1371/journal.pone.0008805.
21. **Curlin ME, et al.** 2010. HIV-1 envelope subregion length variation

during disease progression. PLoS Pathog. **6**:e1001228. doi:10.1371/journal.ppat.1001228.
22. **Davenport TM, et al.** 2011. Binding interactions between soluble HIV envelope glycoproteins and quaternary-structure-specific monoclonal antibodies PG9 and PG16. J. Virol. **85**:7095–7107.
23. **Davis KL, et al.** 2009. High titer HIV-1 V3-specific antibodies with broad reactivity but low neutralizing potency in acute infection and following vaccination. Virology **387**:414–426.
24. **de Souza MS, et al.** 2012. The Thai phase III trial (RV144) vaccine regimen induces T cell responses that preferentially target epitopes within the V2 region of HIV-1 envelope. J. Immunol. **188**:5166–5176.
25. **Devito C, et al.** 2002. Cross-clade HIV-1-specific neutralizing IgA in mucosal and systemic compartments of HIV-1-exposed, persistently seronegative subjects. J. Acquir. Immune Defic. Syndr. **30**:413–420.
26. **Dimitrov AS, et al.** 2007. Exposure of the membrane-proximal external region of HIV-1 gp41 in the course of HIV-1 envelope glycoprotein-mediated fusion. Biochemistry **46**:1398–1401.
27. **Diskin R, et al.** 2011. Increasing the potency and breadth of an HIV antibody by using structure-based rational design. Science **334**:1289–1293.
28. **Doria-Rose NA, et al.** 2010. Breadth of human immunodeficiency virus-specific neutralizing activity in sera: clustering analysis and association with clinical variables. J. Virol. **84**:1631–1636.
29. **Dwyer JJ, et al.** 2003. The hydrophobic pocket contributes to the structural stability of the N-terminal coiled coil of HIV gp41 but is not required for six-helix bundle formation. Biochemistry **42**:4945–4953.
30. **Eckert DM, Kim PS.** 2001. Mechanisms of viral membrane fusion and its inhibition. Annu. Rev. Biochem. **70**:777–810.
31. **Falkowska E, et al.** 2012. PG V04, an HIV-1 gp120 CD4 binding site antibody, is broad and potent in neutralization but does not induce conformational changes characteristic of CD4. J. Virol. **86**:4394–4403.
32. **Forthal DN, Moog C.** 2009. Fc receptor-mediated antiviral antibodies. Curr. Opin. HIV AIDS **4**:388–393.
33. **Franquelim HG, et al.** 2011. Anti-HIV-1 antibodies 2F5 and 4E10 interact differently with lipids to bind their epitopes. AIDS **25**:419–428.
34. **Frey G, et al.** 2008. A fusion-intermediate state of HIV-1 gp41 targeted by broadly neutralizing antibodies. Proc. Natl. Acad. Sci. U. S. A. **105**:3739–3744.
35. **Gilbert P, et al.** 2010. Magnitude and breadth of a nonprotective neutralizing antibody response in an efficacy trial of a candidate HIV-1 gp120 vaccine. J. Infect. Dis. **202**:595–605.
36. **Gnann JW, Jr, Nelson JA, Oldstone MB.** 1987. Fine mapping of an immunodominant domain in the transmembrane glycoprotein of human immunodeficiency virus. J. Virol. **61**:2639–2641.
37. **Gorny MK, et al.** 2005. Identification of a new quaternary neutralizing epitope on human immunodeficiency virus type 1 virus particles. J. Virol. **79**:5232–5237.
38. **Gorny MK, et al.** 1991. Production of site-selected neutralizing human monoclonal antibodies against the third variable domain of the human immunodeficiency virus type 1 envelope glycoprotein. Proc. Natl. Acad. Sci. U. S. A. **88**:3238–3242.
39. **Granados-González V, et al.** 2009. Role of the HIV-1 gp120 V1/V2 domains in the induction of neutralizing antibodies. Enferm. Infecc. Microbiol. Clin. **27**:523–530. (In Spanish.)
40. **Gray ES, et al.** 2009. Broad neutralization of human immunodeficiency virus type 1 mediated by plasma antibodies against the gp41 membrane proximal external region. J. Virol. **83**:11265–11274.
41. **Guglietta S, Pantaleo G, Graziosi C.** 2010. Long sequence duplications, repeats, and palindromes in HIV-1 gp120: length variation in V4 as the product of misalignment mechanism. Virology **399**:167–175.
42. **Gustchina E, et al.** 2010. Structural basis of HIV-1 neutralization by affinity matured Fabs directed against the internal trimeric coiled-coil of gp41. PLoS Pathog. **6**:e1001182. doi:10.1371/journal.ppat.1001182.
43. **Gustchina E, Louis JM, Lam SN, Bewley CA, Clore GM.** 2007. A monoclonal Fab derived from a human nonimmune phage library reveals a new epitope on gp41 and neutralizes diverse human immunodeficiency virus type 1 strains. J. Virol. **81**:12946–12953.
44. **Hager-Braun C, Tomer KB.** 2005. Determination of protein-derived epitopes by mass spectrometry. Expert Rev. Proteomics **2**:745–756.
45. **Hallenberger S, et al.** 1992. Inhibition of furin-mediated cleavage activation of HIV-1 glycoprotein gp160. Nature **360**:358–361.
46. **Hartley O, Klasse PJ, Sattentau QJ, Moore JP.** 2005. V3: HIV's switch-hitter. AIDS Res. Hum. Retroviruses **21**:171–189.
47. **Haynes BF, et al.** 2005. Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. Science **308**:1906–1908.
48. **Haynes BF, et al.** 2012. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N. Engl. J. Med. **366**:1275–1286.
49. **Haynes BF, Montefiori DC.** 2006. Aiming to induce broadly reactive neutralizing antibody responses with HIV-1 vaccine candidates. Expert Rev. Vaccines **5**:579–595.
50. **Haynes BF, Moody MA, Verkoczy L, Kelsoe G, Alam SM.** 2005. Antibody polyspecificity and neutralization of HIV-1: a hypothesis. Hum. Antibodies **14**:59–67.
51. **Hemming A, et al.** 1994. Identification of three N-linked glycans in the V4-V5 region of HIV-1 gp 120, dispensable for CD4-binding and fusion activity of gp 120. Arch. Virol. **134**:335–344.
52. **Hessell AJ, et al.** 2007. Fc receptor but not complement binding is important in antibody protection against HIV. Nature **449**:101–104.
53. **Hessell AJ, et al.** 2009. Effective, low-titer antibody protection against low-dose repeated mucosal SHIV challenge in macaques. Nat. Med. **15**:951–954.
54. **Hessell AJ, et al.** 2009. Broadly neutralizing human anti-HIV antibody 2G12 is effective in protection against mucosal SHIV challenge even at low serum neutralizing titers. PLoS Pathog. **5**:e1000433. doi:10.1371/journal.ppat.1000433.
55. **Hu G, Liu J, Taylor KA, Roux KH.** 2011. Structural comparison of HIV-1 envelope spikes with and without the V1/V2 loop. J. Virol. **85**:2741–2750.
56. **Huber M, Trkola A.** 2007. Humoral immunity to HIV-1: neutralization and beyond. J. Intern. Med. **262**:5–25.
57. **Huskens D, Van Laethem K, Vermeire K, Balzarini J, Schols D.** 2007. Resistance of HIV-1 to the broadly HIV-1-neutralizing, anti-carbohydrate antibody 2G12. Virology **360**:294–304.
58. **Johnson WE, Sauvron JM, Desrosiers RC.** 2001. Conserved, N-linked carbohydrates of human immunodeficiency virus type 1 gp41 are largely dispensable for viral replication. J. Virol. **75**:11426–11436.
59. **Joos B, et al.** 2006. Long-term multiple-dose pharmacokinetics of human monoclonal antibodies (MAbs) against human immunodeficiency virus type 1 envelope gp120 (MAb 2G12) and gp41 (MAbs 4E10 and 2F5). Antimicrob. Agents Chemother. **50**:1773–1779.
60. **Julien JP, Bryson S, Nieva JL, Pai EF.** 2008. Structural details of HIV-1 recognition by the broadly neutralizing monoclonal antibody 2F5: epitope conformation, antigen-recognition loop mobility, and anion-binding site. J. Mol. Biol. **384**:377–392.
61. **Kim M, Qiao Z, Yu J, Montefiori D, Reinherz EL.** 2007. Immunogenicity of recombinant human immunodeficiency virus type 1-like particles expressing gp41 derivatives in a pre-fusion state. Vaccine **25**:5102–5114.
62. **Klein JS, Webster A, Gnanapragasam PN, Galimidi RP, Bjorkman PJ.** 2010. A dimeric form of the HIV-1 antibody 2G12 elicits potent antibody-dependent cellular cytotoxicity. AIDS **24**:1633–1640.
63. **Kobie JJ, et al.** 2012. 9G4 autoreactivity is increased in HIV-infected patients and correlates with HIV broadly neutralizing serum activity. PLoS One **7**:e35356. doi:10.1371/journal.pone.0035356.
64. **Koch M, et al.** 2003. Structure-based, targeted deglycosylation of HIV-1 gp120 and effects on neutralization sensitivity and antibody recognition. Virology **313**:387–400.
65. **Krachmarov C, et al.** 2011. Characterization of structural features and diversity of variable-region determinants of related quaternary epitopes recognized by human and rhesus macaque monoclonal antibodies possessing unusually potent neutralizing activities. J. Virol. **85**:10730–10740.
66. **Kwong PD, Mascola JR, Nabel GJ.** 2011. Rational design of vaccines to elicit broadly neutralizing antibodies to HIV-1. Cold Spring Harbor Perspect. Med. **1**:a007278. doi:10.1101/cshperspect.a007278.
67. **Labrijn AF, et al.** 2003. Access of antibody molecules to the conserved coreceptor binding site on glycoprotein gp120 is sterically restricted on primary human immunodeficiency virus type 1. J. Virol. **77**:10557–10565.
68. **Law M, Cardoso RM, Wilson IA, Burton DR.** 2007. Antigenic and immunogenic study of membrane-proximal external region-grafted gp120 antigens by a DNA prime-protein boost immunization strategy. J. Virol. **81**:4272–4285.
69. **Li B, et al.** 2006. Evidence for potent autologous neutralizing antibody titers and compact envelopes in early infection with subtype C human immunodeficiency virus type 1. J. Virol. **80**:5211–5218.

70. **Li Y, et al.** 2007. Broad HIV-1 neutralization mediated by CD4-binding site antibodies. Nat. Med. **13**:1032–1034.
71. **Li Y, et al.** 2011. Mechanism of neutralization by the broadly neutralizing HIV-1 monoclonal antibody VRC01. J. Virol. **85**:8954–8967.
72. Reference deleted.
73. **Li Y, et al.** 2009. Analysis of neutralization specificities in polyclonal sera derived from human immunodeficiency virus type 1-infected individuals. J. Virol. **83**:1045–1059.
74. **Liu S, et al.** 2005. Different from the HIV fusion inhibitor C34, the anti-HIV drug Fuzeon (T-20) inhibits HIV-1 entry by targeting multiple sites in gp41 and gp120. J. Biol. Chem. **280**:11259–11273.
75. **Lorizate M, et al.** 2006. Recognition and blocking of HIV-1 gp41 pre-transmembrane sequence by monoclonal 4E10 antibody in a Raft-like membrane environment. J. Biol. Chem. **281**:39598–39606.
76. **Lu M, Blacklow SC, Kim PS.** 1995. A trimeric structural domain of the HIV-1 transmembrane glycoprotein. Nat. Struct. Biol. **2**:1075–1082.
77. **Luftig MA, et al.** 2006. Structural basis for HIV-1 neutralization by a gp41 fusion intermediate-directed antibody. Nat. Struct. Mol. Biol. **13**: 740–747.
78. **Mascola JR.** 2002. Passive transfer studies to elucidate the role of antibody-mediated protection against HIV-1. Vaccine **20**:1922–1925.
79. **Mascola JR, Frankel SS, Broliden K.** 2000. HIV-1 entry at the mucosal surface: role of antibodies in protection. AIDS **14**(Suppl. 3):S167–S174.
80. **Mascola JR, Montefiori DC.** 2010. The role of antibodies in HIV vaccines. Annu. Rev. Immunol. **28**:413–444.
81. **McLellan JS, et al.** 2011. Structure of HIV-1 gp120 V1/V2 domain with broadly neutralizing antibody PG9. Nature **480**:336–343.
82. **Menendez A, Chow KC, Pan OC, Scott JK.** 2004. Human immunodeficiency virus type 1-neutralizing monoclonal antibody 2F5 is multispecific for sequences flanking the DKW core epitope. J. Mol. Biol. **338**:311–327.
83. **Miller MD, et al.** 2005. A human monoclonal antibody neutralizes diverse HIV-1 isolates by binding a critical gp41 epitope. Proc. Natl. Acad. Sci. U. S. A. **102**:14759–14764.
84. **Montefiori DC, Mascola JR.** 2009. Neutralizing antibodies against HIV-1: can we elicit them with vaccines and how much do we need? Curr. Opin. HIV AIDS **4**:347–351.
85. **Moore PL, et al.** 2011. Potent and broad neutralization of HIV-1 subtype C by plasma antibodies targeting a quaternary epitope including residues in the V2 loop. J. Virol. **85**:3128–3141.
86. **Moore PL, et al.** 2009. Limited neutralizing antibody specificities drive neutralization escape in early HIV-1 subtype C infection. PLoS Pathog. **5**:e1000598. doi:10.1371/journal.ppat.1000598.
87. **Morris L, et al.** 2011. Isolation of a human anti-HIV gp41 membrane proximal region neutralizing antibody by antigen-specific single B cell sorting. PLoS One **6**:e23532. doi:10.1371/journal.pone.0023532.
88. **Moulard M, et al.** 2002. Broadly cross-reactive HIV-1-neutralizing human monoclonal Fab selected for binding to gp120-CD4-CCR5 complexes. Proc. Natl. Acad. Sci. U. S. A. **99**:6913–6918.
89. **Mühlbacher M, Spruth M, Siegel F, Zangerle R, Dierich MP.** 1999. Longitudinal study of antibody reactivity against HIV-1 envelope and a peptide representing a conserved site on Gp41 in HIV-1-infected patients. Immunobiology **200**:295–305.
90. **Muñoz-Barroso I, Durell S, Sakaguchi K, Appella E, Blumenthal R.** 1998. Dilation of the human immunodeficiency virus-1 envelope glycoprotein fusion pore revealed by the inhibitory action of a synthetic peptide from gp41. J. Cell Biol. **140**:315–323.
91. **Muñoz-Barroso I, Salzwedel K, Hunter E, Blumenthal R.** 1999. Role of the membrane-proximal domain in the initial stages of human immunodeficiency virus type 1 envelope glycoprotein-mediated membrane fusion. J. Virol. **73**:6089–6092.
92. **Muster T, et al.** 1993. A conserved neutralizing epitope on gp41 of human immunodeficiency virus type 1. J. Virol. **67**:6642–6647.
93. **Nelson JD, et al.** 2007. An affinity-enhanced neutralizing antibody against the membrane-proximal external region of human immunodeficiency virus type 1 gp41 recognizes an epitope between those of 2F5 and 4E10. J. Virol. **81**:4033–4043.
94. **Nelson JD, et al.** 2008. Antibody elicited against the gp41 N-heptad repeat (NHR) coiled-coil can neutralize HIV-1 with modest potency but non-neutralizing antibodies also bind to NHR mimetics. Virology **377**: 170–183.
95. **Ofek G, et al.** 2004. Structure and mechanistic analysis of the anti-human immunodeficiency virus type 1 antibody 2F5 in complex with its gp41 epitope. J. Virol. **78**:10724–10737.
96. **Palker TJ, et al.** 1987. A conserved region at the COOH terminus of human immunodeficiency virus gp120 envelope protein contains an immunodominant epitope. Proc. Natl. Acad. Sci. U. S. A. **84**:2479–2483.
97. **Pancera M, et al.** 2010. Structure of HIV-1 gp120 with gp41-interactive region reveals layered envelope architecture and basis of conformational mobility. Proc. Natl. Acad. Sci. U. S. A. **107**:1166–1171.
98. **Pantophlet R, Burton DR.** 2006. GP120: target for neutralizing HIV-1 antibodies. Annu. Rev. Immunol. **24**:739–769.
99. **Parker CE, et al.** 2001. Fine definition of the epitope on the gp41 glycoprotein of human immunodeficiency virus type 1 for the neutralizing monoclonal antibody 2F5. J. Virol. **75**:10906–10911.
100. **Pejchal R, et al.** 2011. A potent and broad neutralizing antibody recognizes and penetrates the HIV glycan shield. Science **334**:1097–1103.
101. **Phogat S, Wyatt R.** 2007. Rational modifications of HIV-1 envelope glycoproteins for immunogen design. Curr. Pharm. Des. **13**:213–227.
102. **Platt EJ, Gomes MM, Kabat D.** 2012. Kinetic mechanism for HIV-1 neutralization by antibody 2G12 entails reversible glycan binding that slows cell entry. Proc. Natl. Acad. Sci. U. S. A. **109**:7829–7834.
103. **Pollakis G, et al.** 2001. N-linked glycosylation of the HIV type-1 gp120 envelope glycoprotein as a major determinant of CCR5 and CXCR4 coreceptor utilization. J. Biol. Chem. **276**:13433–13441.
104. **Poumbourios P, McPhee DA, Kemp BE.** 1992. Antibody epitopes sensitive to the state of human immunodeficiency virus type 1 gp41 oligomerization map to a putative alpha-helical region. AIDS Res. Hum. Retroviruses **8**:2055–2062.
105. **Prabakaran P, et al.** 2006. Structural mimicry of CD4 by a cross-reactive HIV-1 neutralizing antibody with CDR-H2 and H3 containing unique motifs. J. Mol. Biol. **357**:82–99.
106. **Reeves JD, et al.** 2005. Enfuvirtide resistance mutations: impact on human immunodeficiency virus envelope function, entry inhibitor sensitivity, and virus neutralization. J. Virol. **79**:4991–4999.
107. **Richman DD, Wrin T, Little SJ, Petropoulos CJ.** 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. Proc. Natl. Acad. Sci. U. S. A. **100**:4144–4149.
108. **Ringe R, Phogat S, Bhattacharya J.** 2012. Subtle alteration of residues including N-linked glycans in V2 loop modulate HIV-1 neutralization by PG9 and PG16 monoclonal antibodies. Virology **426**:34–41.
109. **Rolland M, Gilbert P.** 2012. Evaluating immune correlates in HIV type 1 vaccine efficacy trials: what RV144 may provide. AIDS Res. Hum. Retroviruses **28**:400–404.
110. **Roux KH, Taylor KA.** 2007. AIDS virus envelope spike structure. Curr. Opin. Struct. Biol. **17**:244–252.
111. **Rusert P, et al.** 2011. Interaction of the gp120 V1V2 loop with a neighboring gp120 unit shields the HIV envelope trimer against cross-neutralizing antibodies. J. Exp. Med. **208**:1419–1433.
112. **Sabin C, et al.** 2010. Crystal structure and size-dependent neutralization properties of HK20, a human monoclonal antibody binding to the highly conserved heptad repeat 1 of gp41. PLoS Pathog. **6**:e1001195. doi: 10.1371/journal.ppat.1001195.
113. **Salzwedel K, West JT, Hunter E.** 1999. A conserved tryptophan-rich motif in the membrane-proximal region of the human immunodeficiency virus type 1 gp41 ectodomain is important for Env-mediated fusion and virus infectivity. J. Virol. **73**:2469–2480.
114. **Saphire EO, et al.** 2007. Structure of a high-affinity "mimotope" peptide bound to HIV-1-neutralizing antibody b12 explains its inability to elicit gp120 cross-reactive antibodies. J. Mol. Biol. **369**:696–709.
115. **Sattentau QJ.** 2011. Vaccinology: a sweet cleft in HIV's armour. Nature **480**:324–325.
116. **Scanlan CN, et al.** 2002. The broadly neutralizing anti-human immunodeficiency virus type 1 antibody 2G12 recognizes a cluster of alpha1→2 mannose residues on the outer face of gp120. J. Virol. **76**:7306–7321.
117. **Scheid JF, et al.** 2009. Broad diversity of neutralizing antibodies isolated from memory B cells in HIV-infected individuals. Nature **458**:636–640.
118. **Scheid JF, et al.** 2011. Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding. Science **333**:1633–1637.
119. **Schief WR, Ban YE, Stamatatos L.** 2009. Challenges for structure-based HIV vaccine design. Curr. Opin. HIV AIDS **4**:431–440.
120. **Seaman MS, et al.** 2010. Tiered categorization of a diverse panel of HIV-1 Env pseudoviruses for assessment of neutralizing antibodies. J. Virol. **84**:1439–1452.

121. **Selvarajah S, et al.** 2005. Comparing antigenicity and immunogenicity of engineered gp120. J. Virol. **79**:12148–12163.
122. **Selvarajah S, et al.** 2008. Focused dampening of antibody response to the immunodominant variable loops by engineered soluble gp140. AIDS Res. Hum. Retroviruses **24**:301–314.
123. **Shnaper S, Sackett K, Gallo SA, Blumenthal R, Shai Y.** 2004. The C- and the N-terminal regions of glycoprotein 41 ectodomain fuse membranes enriched and not enriched with cholesterol, respectively. J. Biol. Chem. **279**:18526–18534.
124. **Shu W, et al.** 2000. Helical interactions in the HIV-1 gp41 core reveal structural basis for the inhibitory activity of gp41 peptides. Biochemistry **39**:1634–1642.
125. **Simek MD, et al.** 2009. Human immunodeficiency virus type 1 elite neutralizers: individuals with broad and potent neutralizing activity identified by using a high-throughput neutralization assay together with an analytical selection algorithm. J. Virol. **83**:7337–7348.
126. **Sougrat R, et al.** 2007. Electron tomography of the contact between T cells and SIV/HIV-1: implications for viral entry. PLoS Pathog. **3**:e63. doi:10.1371/journal.ppat.0030063.
127. **Stamatatos L, Morris L, Burton DR, Mascola JR.** 2009. Neutralizing antibodies generated during natural HIV-1 infection: good news for an HIV-1 vaccine? Nat. Med. **15**:866–870.
128. **Stanfield RL, Dooley H, Flajnik MF, Wilson IA.** 2004. Crystal structure of a shark single-domain antibody V region in complex with lysozyme. Science **305**:1770–1773.
129. **Stanfield RL, Dooley H, Verdino P, Flajnik MF, Wilson IA.** 2007. Maturation of shark single-domain (IgNAR) antibodies: evidence for induced-fit binding. J. Mol. Biol. **367**:358–372.
130. **Stiegler G, et al.** 2001. A potent cross-clade neutralizing human monoclonal antibody against a novel epitope on gp41 of human immunodeficiency virus type 1. AIDS Res. Hum. Retroviruses **17**:1757–1765.
131. **Strokappe N, et al.** 2012. Llama antibody fragments recognizing various epitopes of the CD4bs neutralize a broad range of HIV-1 subtypes A, B and C. PLoS One **7**:e33298. doi:10.1371/journal.pone.0033298.
132. **Teixeira C, Gomes JR, Gomes P, Maurel F.** 2011. Viral surface glycoproteins, gp120 and gp41, as potential drug targets against HIV-1: brief overview one quarter of a century past the approval of zidovudine, the first anti-retroviral drug. Eur. J. Med. Chem. **46**:979–992.
133. **Thenin S, et al.** 2012. Naturally occurring substitutions of conserved residues in human immunodeficiency virus type 1 variants of different clades are involved in PG9 and PG16 resistance to neutralization. J. Gen. Virol. **93**(Pt 7):1495–1505.
134. **Tian Y, et al.** 2002. Structure-affinity relationships in the gp41 ELDKWA epitope for the HIV-1 neutralizing monoclonal antibody 2F5: effects of side-chain and backbone modifications and conformational constraints. J. Pept. Res. **59**:264–276.
135. **Tran EE, et al.** 2012. Structural mechanism of trimeric HIV-1 envelope glycoprotein activation. PLoS Pathog. **8**:e1002797. doi:10.1371/journal.ppat.1002797.
136. **Trkola A, et al.** 1995. Cross-clade neutralization of primary isolates of human immunodeficiency virus type 1 by human monoclonal antibodies and tetrameric CD4-IgG. J. Virol. **69**:6609–6617.
137. **Trkola A, et al.** 1996. Human monoclonal antibody 2G12 defines a distinctive neutralization epitope on the gp120 glycoprotein of human immunodeficiency virus type 1. J. Virol. **70**:1100–1108.
138. **Tudor D, et al.** 2009. HIV-1 gp41-specific monoclonal mucosal IgAs derived from highly exposed but IgG-seronegative individuals block HIV-1 epithelial transcytosis and neutralize CD4(+) cell infection: an IgA gene and functional analysis. Mucosal Immunol. **2**:412–426.
139. **Veiga AS, Castanho MA.** 2006. The membranes' role in the HIV-1 neutralizing monoclonal antibody 2F5 mode of action needs reevaluation. Antiviral Res. **71**:69–72.
140. **Verkoczy L, et al.** 2011. Rescue of HIV-1 broad neutralizing antibody-expressing B cells in 2F5 VH x VL knockin mice reveals multiple tolerance controls. J. Immunol. **187**:3785–3797.
141. **Verkoczy L, et al.** 2010. Autoreactivity in an HIV-1 broadly reactive neutralizing antibody variable region heavy chain induces immunologic tolerance. Proc. Natl. Acad. Sci. U. S. A. **107**:181–186.
142. **Vincent N, et al.** 2008. Antibodies purified from sera of HIV-1-infected patients by affinity on the heptad repeat region 1/heptad repeat region 2 complex of gp41 neutralize HIV-1 primary isolates. AIDS **22**:2075–2085.
143. **Vincent N, Malvoisin E, Pozzetto B, Lucht F, Genin C.** 2004. Detection of IgA inhibiting the interaction between gp120 and soluble CD4 receptor in serum and saliva of HIV-1-infected patients. AIDS **18**:37–43.
144. **Volpe JM, Cowell LG, Kepler TB.** 2006. SoDA: implementation of a 3D alignment algorithm for inference of antigen receptor recombinations. Bioinformatics **22**:438–444.
145. **Walker LM, et al.** 2011. Broad neutralization coverage of HIV by multiple highly potent antibodies. Nature **477**:466–470.
146. **Walker LM, et al.** 2009. Broad and potent neutralizing antibodies from an African donor reveal a new HIV-1 vaccine target. Science **326**:285–289.
147. **Walker LM, et al.** 2010. A limited number of antibody specificities mediate broad and potent serum neutralization in selected HIV-1 infected individuals. PLoS Pathog. **6**:e1001028.
148. **Watkins JD, et al.** 2011. An anti-HIV-1 V3 loop antibody fully protects cross-clade and elicits T-cell immunity in macaques mucosally challenged with an R5 clade C SHIV. PLoS One **6**:e18207.
149. **Wei X, et al.** 2003. Antibody neutralization and escape by HIV-1. Nature **422**:307–312.
150. **Wen M, et al.** 2010. GPI-anchored single chain Fv—an effective way to capture transiently-exposed neutralization epitopes on HIV-1 envelope spike. Retrovirology **7**:79.
151. **Wu X, et al.** 2010. Rational design of envelope identifies broadly neutralizing human monoclonal antibodies to HIV-1. Science **329**:856–861.
152. **Wu X, et al.** 2011. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. Science **333**:1593–1602.
153. **Wyatt R, Sodroski J.** 1998. The HIV-1 envelope glycoproteins: fusogens, antigens, and immunogens. Science **280**:1884–1888.
154. **Yang P, et al.** 2010. The cytoplasmic domain of human immunodeficiency virus type 1 transmembrane protein gp41 harbors lipid raft association determinants. J. Virol. **84**:59–75.
155. **Zanetti G, Briggs JA, Grunewald K, Sattentau QJ, Fuller SD.** 2006. Cryo-electron tomographic structure of an immunodeficiency virus envelope complex in situ. PLoS Pathog. **2**:e83. doi:10.1371/journal.ppat.0020083.
156. **Zhou T, et al.** 2010. Structural basis for broad and potent neutralization of HIV-1 by antibody VRC01. Science **329**:811–817.
157. **Zhou T, et al.** 2007. Structural definition of a conserved neutralization epitope on HIV-1 gp120. Nature **445**:732–737.
158. **Zhu P, et al.** 2006. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature **441**:847–852.
159. **Zolla-Pazner S, Cardozo T.** 2010. Structure-function relationships of HIV-1 envelope sequence-variable regions refocus vaccine design. Nat. Rev. Immunol. **10**:527–535.
160. **Zwick MB, Burton DR.** 2007. HIV-1 neutralization: mechanisms and relevance to vaccine design. Curr. HIV Res. **5**:608–624.
161. **Zwick MB, et al.** 2001. Broadly neutralizing antibodies targeted to the membrane-proximal external region of human immunodeficiency virus type 1 glycoprotein gp41. J. Virol. **75**:10892–10905.
162. **Zwick MB, et al.** 2003. Molecular features of the broadly neutralizing immunoglobulin G1 b12 required for recognition of human immunodeficiency virus type 1 gp120. J. Virol. **77**:5863–5876.