Chapter 3
HIV Broadly Neutralizing Antibodies: VRC01 and Beyond

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Abstract Developing an effective prophylaxis HIV-1 vaccine is likely to require the elicitation of broadly neutralizing antibodies (bnAbs). As the HIV-1 envelope (Env) glycoprotein – the sole target of bnAbs – has evolved multiple mechanisms to evade antibody neutralization, the processes for bnAb generation are highly selective and time-consuming. Benefiting from antibody isolation technologies of single B cell culturing and direct single B cell sorting and cloning, a new generation of monoclonal bnAbs has been isolated since 2009, exhibiting remarkable breadths and potencies, thus breaking through a nearly 20-year-long limit of four monoclonal bnAbs with moderate breadth and potency. The discovery of a long list of monoclonal bnAbs has provided in-depth understanding of the sites of vulnerability on the HIV-1 Env and the complexity of human B cell immunology to generate such responses, thus presenting both guidance and challenges to move the Env immunogen design effort forward.

Keywords HIV-1 · VRC01 · Monoclonal antibody · Neutralization

3.1 Introduction

The development of HIV-1 broadly neutralizing antibodies (bnAbs) in a subset of infected individuals has demonstrated the ability of the human immune system to mount effective antibody responses against the virus (Simek et al. 2009; Doria-Rose et al. 2010). As the HIV-1 envelope (Env) glycoprotein is the only viral protein expressed on the surface of the virion, bnAbs target the HIV-1 Env to block viral infection. Because HIV-1 Env has evolved a number of mechanisms to evade antibody neutralization (Wei et al. 2003; Moore et al. 2009), the processes of bnAb generation are proven highly selective and time-consuming. Nonetheless, HIV-1 bnAbs with 50% breadth are developed in half of HIV-1 chronically infected individuals (Hraber et al. 2014), supporting the feasibility of inducing a similar

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spectrum of antibody responses with optimal Env immunogens. To inform Env immunogen design and improve our understanding of human B cell immunology to mount an effective antibody response, the isolation of monoclonal bnAbs has become critical.

Though high in demand, there were only four monoclonal HIV-1 bnAbs isolated before 2009, largely due to limitations in methods for monoclonal antibody (mAb) isolation and lack of knowledge for HIV-1 bnAbs at that time. Since 2009, improved methods of single B cell culturing and direct single B cell cloning were introduced and applied to HIV-1-infected samples, leading to a major breakthrough in identifying a new generation of bnAbs with remarkable breadths and potencies. The new era of HIV-1 monoclonal bnAb discovery and understanding of these antibodies opened up new avenues for basic research in immunology, structural biology, and vaccinology, as well as in translational and clinical research for HIV-1 infection. As we contributed to the isolation of the first HIV-1 monoclonal bnAb VRC01 by singe B cell sorting and cloning, I review the critical path and scientific thoughts that drove the progress of the project and eventually led to the discovery of this exciting antibody.

3.2 HIV-1 Neutralization by Polyclonal Sera

Sera or plasma profiling, as a straightforward analysis, usually takes place first by directly sampling the polyclonal antibodies in donor sera or plasma for binding specificities to antigens and for antibody functions against pathogens. Since the identification of HIV as the cause of AIDS in the early 1980s (Barre-Sinoussi et al. 1983; Gallo et al. 1983, 1984), polyclonal sera and plasma samples from HIV-infected individuals have been rigorously tested for binding specificities to various viral antigens and for activity to neutralize the virus. Initial neutralization studies used lab-adapted strains such as IIIB, MN, HXB2, and NL4-3 and found that sera and plasmas from vaccinated or infected donors potently neutralized lab-adapted strains (Mascola et al. 1994; Kovacs et al. 1993; Schwartz et al. 1993). However, subsequent studies using HIV-1 primary isolates showed high levels of resistance to sera and plasma neutralization from vaccinated and infected donors (Moore et al. 1995; Parren et al. 1998). This difference in neutralization sensitivity indicated that the Envs presented on the surface of lab-adapted strains were different from those presented on primary isolates. To categorize the neutralization sensitivity among lab-adapted strains and primary isolates, a “tiered” system (Seaman et al. 2010) was introduced with tier 1A representing the easy-to-neutralize lab-adapted strains, followed by tier 1B, then tier 2 representing the typical primary isolates, and finally tier 3 representing the most difficult-to-neutralize strains.

Tested against various panels of tier 2 strains, many chronically infected polyclonal sera and plasma samples have been ranked based on their neutralization breadths and potencies. Though most samples lacked activities to neutralize genetically diverse tier 2 strains, small factions (10–20%) were identified with such activities (Simek et al. 2009; Doria-Rose et al. 2010). These donors have

been referred to as "broad neutralizers" and "elite neutralizers" if ranked in the top 1%. The identification of broad and elite neutralizers was exciting because they demonstrated that the human immune system can mount effective antibody responses against the virus, thus providing a scientific basis for Env immunogen design to elicit similar antibody responses to block infection. The first step to implement this strategy was to understand the antibody specificities that mediated the observed neutralization breadths, i.e., where did the antibodies bind on the HIV Env to block viral entry? As the plasma neutralization test itself did not inform antibody specificities or targets, the analyses followed were sera mapping (Walker et al. 2010; Binley et al. 2008; Gray et al. 2009). The caveat was that, because sera and plasmas are mixes of polyclonal antibodies, it has been extremely difficult to dissect complex antibody specificities and precisely map the epitopes and targets mediating neutralization. Before the isolation of monoclonal bnAbs, there had been an extensive debate on whether polyclonal antibodies or mAbs mediated neutralization breadth. To address this question and to precisely map the epitopes targeted by bnAbs, the solution was to isolate monoclonal bnAbs. For this purpose, samples from the identified broad and elite neutralizers received the highest priority and were selected for the isolation of mAbs that could account for donor's serological activities.

3.3 mAb Isolation and the First Generation of Monoclonal bnAbs

Before 2009 only four mAbs, b12, 2G12, 2F5, and 4E10, all isolated in the early 1990s (Table 3.1), demonstrated superior neutralization spectrums over the other mAbs available at that time. For nearly 20 years, these four mAbs had been used for almost all bnAb-related basic, pre-clinical and clinical research, including epitope mapping, Env structural analysis, Env immunogen design, passive immunization in nonhuman primates (Mascola et al. 1999, 2000), and a clinical trial to treat HIV-infected individuals (Trkola et al. 2005). Because of their wide uses for such a prolonged period, the four mAbs became well known for their role in defining HIV-1 monoclonal bnAbs. Given the fact that a mAb only binds to a single epitope on the Env antigen, a single mAb may not be expected to neutralize a broad range of circulating strains. Based on a conservative expectation, the relatively narrow breadths (30–60%) and weak potencies (mean or median IC50s >2 μg/ml) of the four mAbs (Walker et al. 2009) were accepted by the research community as the limit of neutralization for a single mAb. Meanwhile, the breadths and potencies displayed by a number of well characterized broadly neutralizing plasmas from HIV-1 chronically infected donors (Li et al. 2007), including the NIH donor 45 from whom VRC01 was later isolated, greatly exceeded those displayed by the four mAbs. Thus, there was a discrepancy in the observed neutralization breadths between the polyclonal plasmas and the four known monoclonal bnAbs. Because of this discrepancy, HIV neutralization breadth was thought to be mediated by polyclonal antibodies and not

Table 3.1 Summary of unique HIV-1 monoclonal bnAbs

#	mAb ID	Donor (viral clade)	Env target, B cell probe	V-genes (hypermutation)	CDR3 length (amino acids)	References
First generation of bnAbs						
1	b12	(B)	CD4bs[a]	VH1-3 (13%), VK3-20 (14%)	H3: 18, L3: 9	Burton et al. (1994)
2	2G12	(B)	gp120 glycan cluster	VH3-21 (21%), VK1-5 (14%)	H3: 14, L3: 9	Trkola et al. (1995)
3	2F5	(B)	MPER[a]	VH2-5 (14%), VK1-13 (12%)	H3: 22, L3: 9	Muster et al. (1993)
4	4E10	(B)	MPER	VH1-69 (14%), VK3-20 (7%)	H3: 18, L3: 9	Buchacher et al. (1994)
New generation of bnAbs isolated by HIV-1 Env probes						
1	VRC01	NIH45 (B)	CD4bs, RSC3	VH1-2 (32%), VK3-20 (18%)	H3: 12, L3: 5	Wu et al. (2010)
2	3BNC117	RU3 (B)	CD4bs, gp120 core	VH1-2 (26%), VK1-33 (16%)	H3: 10, L3: 5	Scheid et al. (2011)
3	12A12	IAVI57	CD4bs, gp120 core	VH1-2 (23%), VK1-33 (19%)	H3: 13, L3: 5	Scheid et al. (2011)
4	1B2530	RU1 (B)	CD4bs, gp120 core	VH1-46 (28%), VL1-47 (18%)	H3: 16, L3: 11	Scheid et al. (2011)
5	8ANC131	RU8 (B)	CD4bs, gp120 core	VH1-46 (26%), VK3-20 (19%)	H3: 16, L3: 9	Scheid et al. (2011)
6	8ANC195	RU8 (B)	gp120-gp41, gp120 core	VH1-3 (28%), VK1-5 (16%)	H3: 20, L3: 9	Scheid et al. (2011)
7	VRC-PG04	IAVI74 (AD)	CD4bs, RSC3	VH1-2 (30%), VK3-20 (19%)	H3: 14, L3: 5	Wu et al. (2011)
8	VRC-CH31	CH0219 (A)	CD4bs, RSC3	VH1-2 (24%), VK1-33 (15%)	H3: 13, L3: 5	Wu et al. (2011)
9	3 BC176	RU3 (B)	trimer, cell BaL gp140	VH1-2 (24%), VL2-23 (15%)	H3: 19, L3: 10	Klein et al. (2012)
10	VRC-PG19	IAVI23	CD4bs, RSC3	VH1-2 (23%), VL2-14 (14%)	H3: 11, L3: 5	Zhou et al. (2013)
11	VRC23	NIH-127/C (B)	CD4bs, RSC3	VH1-2 (22%), VK3-15 (15%)	H3: 12, L3: 5	Georgiev et al. (2013)
12	CH103	CH505 (C)	CD4bs, RSC3	VH4-61 (17%), VL3-1 (11%)	H3: 13, L3: 10	Liao et al. (2013)
13	VRC13	NIH44 (B)	CD4bs, RSC3	VH1-69 (34%), VL2-14 (24%)	H3: 21, L3: 6	Zhou et al. (2015)
14	VRC16	NIH-C38 (B)	CD4bs, RSC3	VH3-23 (18%), VK1-39 (19%)	H3: 20, L3: 9	Zhou et al. (2015)
15	VRC18	NIH-C38 (B)	CD4bs, RSC3	VH1-2 (27%), VK3-20 (18%)	H3: 10, L3: 5	Zhou et al. (2015)
16	VRC27	NIH-Z258 (B)	CD4bs, RSC3	VH1–2 (30%), VK1–33 (27%)	H3: 13, L3: 5	Zhou et al. (2015)
17	179NC75	EB179 (B)	CD4bs, gp120 core	VH3–21 (28%), VL3-1 (22%)	H3: 24, L3: 10	Freund et al. (2015)

(continued)

Table 3.1 (continued)

#	mAb ID	Donor (viral clade)	Env target, B cell probe	V-genes (hypermutation)	CDR3 length (amino acids)	References
18	DRVIA7	DRVI01 (B′)	CD4bs, RSC3	VH1–2 (19%), VK1–5 (17%)	H3: 11, L3: 5	Kong et al. (2016a)
19	N123-VRC34	NIH-N123 (B)	gp120-gp41/FP[a], SOSIP	VH1–2 (15%), VK1–9 (10%)	H3: 13; L3: 9	Kong et al. (2016b)
20	ACS202	D12950 (B)	gp120-gp41/FP, SOSIP	VH3-30 (16%), VK1-33 (16%)	H3: 22; L3: 9	van Gils et al. (2016)
21	PCDN-33A	IAVI-PC76 (C)	N332 supersite, gp120	VH4-34 (12%), VK3-20 (11%)	H3: 20; L3: 8	MacLeod et al. (2016)
22	N90-VRC38.01	NIH-N90 (B)	V1V2 apex, VLP[a]	VH3-13 (18%), VK2-28 (9%)	H3: 16; L3: 10	Cale et al. (2017)
New generation of bnAbs isolated by B cell culture and micro-neutralization						
23	PG9	IAVI24 (A)	V1V2 apex	VH3-33 (13%), VL2-14 (6%)	H3: 28, L3: 11	Walker et al. (2009)
24	CH01	CH0219 (A)	V1V2 apex	VH3-20 (13%), VK3-20 (10%)	H3: 24, L3: 9	Bonsignori et al. (2011)
25	PGT121	IAVI17 (A)	N332 supersite	VH4-59 (17%), VL3-21 (18%)	H3: 24, L3: 12	Walker et al. (2011a)
26	PGT128	IAVI36 (AG)	N332 supersite	VH4-39 (19%), VL2-8 (9%)	H3: 19, L3: 10	Walker et al. (2011a)
27	PGT135	IAVI39 (C)	N332 supersite	VH4-39 (17%), VK3-15 (16%)	H3: 18, L3: 9	Walker et al. (2011a)
28	PGT145	IAVI84 (A or D)	V1V2 apex	VH1-8 (18%), VK2-28 (16%)	H3: 31, L3: 9	Walker et al. (2011a)
29	10E8	NIH-N152 (B)	MPER	VH3-15 (21%), VL3-19 (14%)	H3: 20, L3: 12	Huang et al. (2012)
30	VRC24	NIH-N27 (B)	N332 supersite	VH4-4 (23%), VL1-15 (18%)	H3: 24, L3: 9	Georgiev et al. (2013)
31	CAP256-VRC26	CAP256 (C)	V1V2 apex	VH3-30 (14%), VL1-51 (10%)	H3: 37, L3: 12	Doria-Rose et al. (2014)
32	PGT151	IAVI31 (C)	gp120-gp41/FP	VH3-30 (20%), VK2-29 (12%)	H3: 26, L3: 9	Falkowska et al. (2014)
33	35O22	NIH-N152 (B)	gp120-gp41	VH1-28 (35%), VL2-14 (24%)	H3: 14, L3: 10	Huang et al. (2014)
34	CH235	CH505 (C)	CD4bs	VH1-46 (8%), VK3-15 (5%)	H3: 13, L3: 8	Gao et al. (2014)
35	N6	NIH-Z258 (B)	CD4bs	VH1-2 (31%), VK1–33 (25%)	H3: 13, L3: 5	Huang et al. (2016)
36	CAP248-2B	CAP248 (C)	gp120-gp41/MPER	VH4-31 (22%), VL2-14 (14%)	H3: 15, L3: 19	Wibmer et al. (2017)
New generation of bnAbs isolated by other methods						
37	HJ16	242315 (B)	CD4bs	VH3-30 (29%), VK4-1 (20%)	H3: 19, L3: 8	(Corti et al. (2010)

[a]CD4bs, CD4-binding site; MPER, membrane proximal external region; FP, fusion peptide; VLP, virus-like particle

by mAbs. This view would disfavor vaccine development strategies to target a conserved site or site of vulnerability on the HIV Env. To address the discrepancy between broadly neutralizing plasmas and few known monoclonal bnAbs, it was necessary to identify additional monoclonal bnAbs, should they exist.

Historically mAbs were isolated by phage display, Epstein-Barr virus (EBV) transformation, electrofusion, and hybridomas. Despite limitations and weaknesses, these technologies dominated the field since their discoveries in the 1970s to 1990s. Before 2009, HIV-1-specific mAbs had been isolated by a few labs that specialize in mAb isolation using these technologies. Disappointingly these technologies suffered from low efficiency when applied to HIV-1-infected samples. Another limiting factor was the mAb screening process that was not based on virus neutralization but instead was based on binding affinities to gp120 monomers, gp120 peptides, or gp41 peptides, thus further reducing the number of bnAbs yielded from these efforts. Despite numbering only four, the discoveries of monoclonal bnAbs still provided tremendous knowledge about HIV-1 neutralization. For example, epitope mapping indicated that b12 targets the CD4-binding site (CD4bs) on gp120, that 2G12 targets a cluster of glycans on gp120, and that 2F5 and 4E10 each binds to a peptide in the membrane-proximal external region (MPER) of gp41. Therefore, we learned that the CD4bs, a cluster of gp120 glycans, and MPER are sites of vulnerability on the HIV-1 Env. Additionally, passive immunizations using a single or combinations among the four mAbs demonstrated protection in nonhuman primate models (Mascola et al. 1999, 2000), thus providing a basis for developing Env immunogens aiming to elicit similar bnAbs. The four monoclonal bnAbs also provided antibody sequences that hinted at common genetic features of HIV-1 bnAbs such as high levels of somatic hypermutation and relatively long heavy chain CDR3 lengths. Nonetheless, after intensive research on the four mAbs for such a long period of time, there was an increasing urgency to identify additional monoclonal bnAbs to verify and address critical questions such as: (1) Were the four monoclonal bnAbs generalizable to other infected donors? If so, could similar mAbs be identified from those donors? (2) Were there mAbs that account for neutralization breadths and potencies observed in donor plasmas? (3) Was HIV-1 neutralization breadth mediated by polyclonal antibodies or mAbs?

3.4 Sera Mapping and the CD4-Binding Site (CD4bs)

If HIV-1 neutralization breadth were mediated by polyclonal antibodies and not by mAbs, searches for monoclonal bnAbs would likely fail. Though the chance of success was small, there had been sera mapping data strongly supporting the presence of monoclonal bnAbs in some chronically infected donors. Using antibody adsorption and elution from gp120 proteins with and without a point mutation D368R that knocks out CD4 binding, Li et al. mapped the neutralizing specificities of two broadly reactive sera, the NIH donors 1 and 45, to the CD4bs (Li et al. 2007). The fractioned plasmas were tested for neutralization against four HIV-1 strains, with

similar results obtained. Therefore, antibodies bound to the D368 site neutralized at least four genetically distant strains, supporting an antibody specificity that targeted the conserved D368 residue and mediated neutralization against different strains.

The rationale for sera mapping studies focusing on the CD4bs was based on the fact that HIV depends on CD4 to initiate viral entry and infection; thus the CD4bs must be functionally conserved. Structurally the CD4bs has been defined at the atomic level in a liganded complex of a gp120 core bound with a 2-domain soluble CD4, along with a mAb 17b antigen-binding fragment (Fab) directed at the co-receptor binding site (Kwong et al. 1998). The crystal structure revealed three domains of gp120 core, an inner domain, an outer domain, and a bridging sheet that connects the two. The gp120 residues in contact with CD4 were discontinuous and spread out in all three domains but were more concentrated in the outer domain. Notably the spans of 365–371, later termed the CD4-binding loop, and 425–430 at β20–21, as part of the bridging sheet, contributed 57% of the total CD4 contact area on gp120 (Kwong et al. 1998). The residue D368 in the middle of the CD4-binding loop was identified as one of the key contact residues for CD4 interaction, and the D368R mutation specifically knocked out CD4 binding. Sera mapping using gp120 proteins with and without the D368R point mutation indicated that novel antibodies to this site were elicited in some HIV-1-infected individuals, and exposure of this conserved site to memory B cells in these individuals might probe the B cells expressing such antibodies. The CD4bs-targeting monoclonal bnAb b12 (Burton et al. 1994) also supported the CD4bs as a site of vulnerability.

3.5 b12

The mAb b12 was isolated in the year 1994 by phage display using PBMCs from an HIV-1 clade B-infected individual (Burton et al. 1994). The phage display library lost antibody heavy and light chain pairing information and produced Fabs with randomly paired heavy and light chains, thus capturing antibodies not "naturally" produced in donor plasmas. This had raised questions and criticism in the field. Moreover, panning of phage display libraries relied on monomeric gp120 binding. Hence, the phage display effort largely yielded mAbs that were capable of binding to monomeric gp120 but not necessarily neutralizing the virus. Nonetheless, mAb b12 exhibited an overall 35% neutralization breadth against cross-clade HIV strains and a preferential 58% breadth against clade B strains (Walker et al. 2009). Competition ELISA with CD4-Ig indicated that b12 efficiently competed with CD4 to bind to gp120, suggesting that the b12 epitope overlaps with the CD4bs (Moore and Sodroski 1996).

The b12 Fab atomic level crystal structure in complex with HXB2 core was solved in 2007, revealing that only the b12 heavy chain interacted with gp120 and that the vast majority of b12 epitope was on the outer domain (Zhou et al. 2007). The b12 heavy chain CDR1, CDR2, and CDR3 "grasped" all around the CD4-binding loop, making direct contact with each of the 10 consecutive residues from 364 to 373. As

expected, the D368R mutation in the CD4-binding loop knocked out b12 binding. Though both primarily targeted the CD4-binding loop, CD4 only formed contacts with one side (six residues), yet b12 grasped on both sides (all ten residues) of the CD4-binding loop (Zhou et al. 2007). Therefore, b12-escape mutations commonly arose on the four residues dispensable for CD4 interaction in the CD4-binding loop (Wu et al. 2009). When the footprints of b12 and CD4 were superimposed on gp120 core, they overlapped primarily on the outer domain, defining an appealing target that was functionally conserved, structurally stable, and antibody accessible. Therefore, new designs of gp120 core proteins to specifically present this region of gp120 to memory B cells in chronically infected donors might probe the B cells expressing antibodies targeting this region. With a powerful single B cell sorting and cloning platform introduced to the field (Tiller et al. 2008; Scheid et al. 2009), new opportunities for functional mAb discovery appeared on the horizon particularly suited to the quest of identifying novel monoclonal bnAbs against HIV.

3.6 VRC01 and Its Class

The platform of single B cell sorting and cloning was first used to recover influenza-specific mAbs from the plasmablasts of vaccinated individuals (Wrammert et al. 2008) and had not been applied to HIV-infected samples before 2009. With PBMCs from broad neutralizers and a Yu2 gp140 foldon trimer protein readily available, the single B cell sorting platform was first applied and attempted to isolate novel monoclonal bnAbs in 2009. Using the Yu2 gp140 as bait, Scheid et al. processed PBMC samples from four broad neutralizers, including the NIH donor 45, and recovered more than 500 gp140-binding mAbs (Scheid et al. 2009). However, none of the isolated mAbs was broadly neutralizing against HIV-1. It was unclear at that time why the recovered gp140-binding mAbs were not broadly neutralizing, and this result would have supported the view that polyclonal antibodies but not mAbs mediate HIV-1 neutralization breadth. Though inexperienced, our group decided to test the single B cell sorting and cloning platform in our hands using a modified gp120 core as bait.

As mentioned above, the overlapping footprint of b12 and CD4 on gp120 core revealed the portion of CD4bs on the outer domain to be functionally conserved, structurally stable, and antibody accessible; thus new designs of gp120 core proteins to specifically present this region might specifically probe the B cells expressing antibodies targeting this region. With designs from William Schief at the Scripps Institute, Yang and colleagues at the Vaccine Research Center expressed and tested a series of proteins that preserved the part of CD4bs on the outer domain but altered the rest of the protein surface to non-HIV-1 (Wu et al. 2010). These modified gp120 core proteins were termed resurfaced stabilized core (RSC) proteins. Among the expressed RSC proteins, RSC3 performed the best in retaining b12 binding but reducing binding to other non-neutralizing mAbs. Because the inner domain and bridging sheet were altered, RSC3 lost stable binding to CD4, thus abrogating CD4

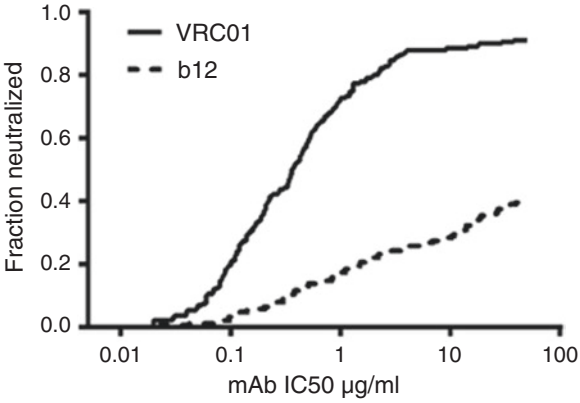

Fig. 3.1 Neutralization breadth (y-axis) is plotted at the corresponding potency (x-axis) for VRC01 and b12 using the IC50 data from Wu et al. 2010 against a total of 190 HIV-1 Env-pseudotyped viruses representing strains circulating globally

interference. Using RSC3 along with a negative control ΔRSC3, which deleted a single residue I371 in the CD4-binding loop, we stained a PBMC sample from the NIH donor 45 and sorted individual IgG-expressing B cells that stained RSC3 + ΔRSC3-. From the sorted individual B cells, we recovered three monoclonal bnAbs, VRC01, VRC02, and VRC03, which belonged to the same B cell lineage and same class of bnAbs – the VRC01-class – and recapitulated the RSC3 + ΔRSC3-binding profile (Wu et al. 2010).

Because the prototype mAb for RSC3 design was b12, we compared the gp120 binding and viral neutralization profiles of VRC01 to b12 (Wu et al. 2010). VRC01 clearly targeted the CD4bs, displaying all known binding characteristics of mAbs directed to the CD4bs, including efficient competition with CD4-Ig and b12 and reduced binding with the D368R mutation. Importantly, VRC01 exhibited superior neutralization breadth and potency than b12 (Fig. 3.1), reaching an overall breadth of 90% with a geometric mean IC50 of 0.33 µg/ml against global circulating strains. Furthermore, VRC01 accounted for a major fraction of the neutralization activities measured in the donor plasma (Wu et al. 2010). Therefore the isolation of VRC01 supported the presence of highly conserved sites of vulnerability on the HIV-1 Env and that neutralization breadths observed in the donor plasma were in a large part mediated by mAbs. Crystal structure analysis of the VRC01 Fab in complex with gp120 core revealed that the VRC01 epitope overlapped with CD4 on the outer domain (Zhou et al. 2010), precisely targeting the site that RSC3 was designed to expose to B cells. Therefore, the RSC3 bait and VRC01 isolation demonstrated an example of successful protein design and engineering. The atomic structure also indicated that the heavy chain CDR2 of VRC01 partially mimicked the CD4 interaction with gp120, forming contacts with only one side of the CD4-binding loop, thus avoiding escape mutations arising from the other side of the CD4-binding loop, a strategy exploited by the virus to escape b12. This mimicry of CD4 by VRC01 partially explained its broader neutralization spectrum than that of b12. Later studies determined that the virus mainly mutates gp120 loop D and V5 to escape VRC01 neutralization (Li et al. 2011).

Following the successful isolation of VRC01 by single B cell sorting and cloning with a specific Env bait, more HIV-1 monoclonal bnAbs have been isolated using similar methods. From the B cells sorted with Yu2 gp140, re-PCRs with improved primers yielded VRC01-class of monoclonal bnAbs (Scheid et al. 2011), indicating that the PCR primers used in the first study missed these highly mutated bnAbs because the original primers did not account for possible mutations at the start of the mAb-coding region. To date the VRC01-class of bnAbs has been isolated from more than 10 donors (Table 3.1), rendering it a category of antibody response generalizable across individuals.

In addition to the VRC01-class, six other classes of bnAbs have been identified that target the CD4bs as exemplified by mAbs HJ16, 8ANC131, CH103, VRC13, VRC16, and 179NC75 (Table 3.1). A collection of crystal structures of the CD4bs-targeting bnAbs in complex with gp120 cores revealed substantially overlapping epitopes and different modes of gp120 recognition (Zhou et al. 2013; Zhou et al. 2015), with the VRC01-class and 8ANC131-class partially mimicking the CD4 interaction with gp120. Though the CD4bs itself is not glycosylated, it is surrounded by glycans, and the bnAbs targeting this site all avoided or accommodated glycans to reach their epitopes. Also, because the CD4bs of gp120 is always readily available to interact with CD4, the Env trimer packaging and conformational changes have minimal impact on bnAbs that target this site. However, this is not the case for non-neutralizing mAbs. Since current vaccines induced non-neutralizing mAbs to the CD4bs, it will be important to consider and adress how to modify and adjust the mode or angle of antibody binding to gp120 in Env immunogen design.

3.7 Antibody Genetic Composition and Next-Generation Sequencing (NGS)

Because each unique B cell receptor has its own VDJ recombined composition, bnAb nucleotide sequences are essential to establish the corresponding B cell lineages. For mAbs identified by single B cell cloning, their variable region nucleotide sequences are obtained for paired heavy and light chains. As illustrated in Fig. 3.2 using heavy chain as an example, mAbs are usually characterized by their V-gene usage, somatic hypermutation, and CDR3 length. While the antibody's primary antigen interacting regions CDR1 and CDR2 are coded within the V-gene, its CDR3 is composed of VD junction, D gene, DJ junction, and part of the J gene. Thus, CDR3 is the most diverse region of the antibody. The VRC01-class of bnAbs from multiple donors shared a sequence signature of IGHV1-2 gene usage, high levels of somatic hypermutation, and a short light chain CDR3 length of five amino acids (Zhou et al. 2013; West et al. 2012). This distinct sequence signature has been used to select naïve B cells with the potential to evolve and mature to become the VRC01-class of bnAbs (Jardine et al. 2016).

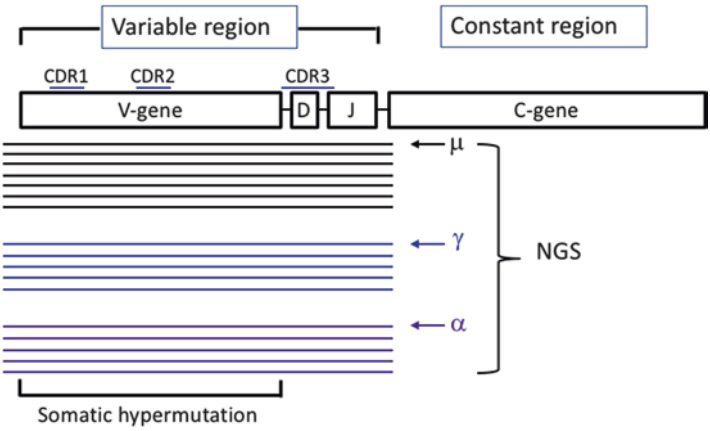

Fig. 3.2 A schematic of the gene structure for antibody heavy chain showing characteristic elements of V-gene usage, somatic hypermutation, and CDR3. For repertoire studies using next-generation sequencing (NGS), reverse primers annealing to the start of the constant region distinguish between μ chain for IgM, γ chain for IgG, and α chain for IgA

As bnAbs are part of the donor antibody repertoire, the availability of bnAb sequences has inspired and stimulated antibody repertoire analyses by next-generation sequencing (NGS) including IgM, IgG, and IgA (Fig. 3.2) in HIV-1-infected and HIV-1-uninfected donors (Chen et al. 2012; Xiao et al. 2013; Yin et al. 2013; Zhang et al. 2013; He et al. 2014). The NGS data from longitudinal samples of the same donor also allowed for tracking bnAb lineages (Wu et al. 2015; Doria-Rose et al. 2014; Bonsignori et al. 2016; van Gils et al. 2016; MacLeod et al. 2016). However, a caveat to the currently available sequencing platforms is the loss of paired heavy and light chains, and thus antibodies cannot be reconstituted with naturally paired heavy and light chains based on the NGS data alone. Though not yet readily available, new sequencing platforms are being developed to address this issue (DeKosky et al. 2013, 2015). As of today, NGS combining with single B cell sorting and sequencing, which maintains the heavy and light chain pairing information, is still the most practical and comprehensive system to study B cell lineages of interest.

3.8 Other HIV-1 bnAbs

Pioneered by the Burton group using individual B cell cultures with micro-neutralization screening to isolate monoclonal bnAbs PG9 and PG16 (Walker et al. 2009), a similar system has been applied by other laboratories to isolate many more HIV-1 bnAbs targeting Env regions outside of the CD4bs. Along with single B cell sorting using soluble or cellular gp140 trimer and virus-like particle (VLP) baits (Table 3.1), HIV-1 bnAbs targeting Env regions outside of CD4bs have

also been isolated by the single B cell sorting and cloning platform. HIV-1 bnAbs have been extensively reviewed in the literature (Mascola and Haynes 2013; Kwong and Mascola 2012; Kwong et al. 2013; Wu and Kong 2016). Briefly, there are currently seven known categories of bnAbs targeting different regions of the HIV-1 Env. From the Env trimer apex to gp41, known sites of vulnerability include (1) the V1 V2 apex targeted by PG9, CAP256-VRC26, PGT145, N90-VRC38, and others; (2) a gp120 glycan cluster targeted by 2G12; (3) the V3 base glycans targeted by PGT121, PGT128, and others; (4) the CD4bs targeted by VRC01 and others; (5) the gp120 and gp41 interface targeted by 35O22 and others; (6) the fusion peptide targeted by PGT151, VRC34, and ACS202; and (7) the MPER targeted by 10E8, 2F5, and 4E10. These bnAbs play a central role in informing and guiding HIV-1 Env immunogen designs, including defining conserved sites of vulnerability on HIV-1 Env and validating Env immunogens for proper presentation of intact bnAb epitopes. Although still a work in progress, sequential Env variants that evolved during the natural course of infection and stimulated bnAb responses are being identified and cloned (Gao et al. 2014; Bhiman et al. 2015; MacLeod et al. 2016), and the identification of these Envs would rely on the identification of both autologous and heterologous neutralizing antibodies. Additional studies have focused on longitudinal analyses to track bnAb lineages and delineate their maturation pathways (Doria-Rose et al. 2014; Bonsignori et al. 2016; van Gils et al. 2016; MacLeod et al. 2016). As single B cell sorting and cloning has been increasingly included in post-immune analyses (Sundling et al. 2012, 2014; Navis et al. 2014), a relatively complete list of bnAb genetic compositions has been highly informative regarding whether similar bnAb precursors have been engaged by the tested immunizations. The broadest and most potent bnAbs have also been high in demand for research in passive immunization and in HIV-1 treatment and cure.

3.9 Conclusion

With 41 unique bnAbs isolated to date (Table 3.1) that define 7 general categories of effective targets on the HIV-1 Env, current research has moved on to unveil the mechanisms of human B cell responses that produce bnAbs and to investigate the entire bnAb maturation pathway starting from naïve B cell engagement. The pathways for bnAb generation remain mysterious because current knowledge of basic B cell immunology has been gained primarily from mouse studies, yet mouse may not possess the proper B cell repertoire to generate similar highly functional antibodies against HIV (Hu et al. 2015). Since SHIV-infected nonhuman primates have demonstrated the ability to produce HIV-1 bnAbs (Jia et al. 2016; Walker et al. 2011b; Shingai et al. 2012), emphasis on nonhuman primate models may hold promise to elucidate details of HIV-1 bnAb production *in vivo* and fill gaps of knowledge in this regard.

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