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V_H1-69 antiviral broadly neutralizing antibodies: genetics, structures, and relevance to rational vaccine design

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Broadly neutralizing antibodies (bnAbs) are potential therapeutic molecules and valuable tools for studying conserved viral targets for vaccine and drug design. Interestingly, antibody responses to conserved epitopes can be highly convergent at the molecular level. Human antibodies targeting a number of viral antigens have often been found to utilize a restricted set of immunoglobulin germline genes in different individuals. Here we review recent knowledge on *V_H1-69*-encoded antibodies in antiviral responses to influenza virus, HCV, and HIV-1. These antibodies share common genetic and structural features, and often develop neutralizing activity against a broad spectrum of viral strains. Understanding the genetic and structural characteristics of such antibodies and the target epitopes should help advance novel strategies to elicit bnAbs through vaccination.

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Introduction

Most vaccines to viral pathogens rely on the induction of neutralizing antibody (nAb) responses for host protection. For highly variable viruses such as influenza virus, HCV, and HIV-1, a broadly effective or universal vaccine that can cross-protect against the diverse spectrum of known viral strains and subtypes remains elusive. This failure so far may be exacerbated by various mechanisms that these viruses use for immune evasion such as high genetic diversity, fast mutation rates facilitated by the error-prone RNA polymerase, glycan shielding of the viral envelope glycoproteins, conformational flexibility of the glycoproteins, and limited accessibility of conserved epitopes (reviewed in Refs. [1–4]). However, broadly nAbs (bnAbs) to such hypervariable RNA viruses have in fact been isolated from infected or vaccinated individuals, demonstrating the ability of the human immune system to overcome some of the viral escape mechanisms and recognize conserved epitopes on the viruses. Thus, in principle, it is possible for a broad vaccine to be designed against these viruses.

In addition to viral escape, host genetic factors such as interleukin genotypes and human leukocyte antigen (HLA) alleles are known to modulate antiviral immune responses, resulting in individual differences in surviving infection [5–7]. Single-nucleotide polymorphisms (SNPs) in HLA, cytokine and cytokine receptor genes have been reported to influence antibody responses during infection and vaccination [8,9]. The recent interest in understanding antiviral bnAbs and the conditions to elicit them in vaccination have led to the study of antibody responses at the genetic level. It is found that some antibodies against a particular epitope share a restricted set of immunoglobulin heavy chain variable region (V_H) genes and are often quite similar in overall structure [10]. For example, influenza antibodies against the hemagglutinin (HA) stem region have so far been found to predominantly utilize *V_H1-69*, *V_H1-18*, and *V_H6-1* [11–14]; HCV antibodies targeting the E2 antigenic region 3 (AR3) mainly derive from *V_H1-69* [15–17]; HIV-1 antibodies against the gp120 CD4 binding site (CD4bs) tend to be encoded by *V_H1-2* and *V_H1-46* [18,19], and by *V_H1-69* when against the coreceptor-binding site on gp120 [also known as CD4-induced (CD4i) binding site] or the conserved heptad repeat 1 (HR1) region and membrane proximal external region (MPER) of gp41 [20,21]; rotavirus antibodies against virus protein 6 (VP6) preferentially use *V_H1-46* [22–24]. Such antibodies can be produced by multiple individuals and often exhibit broadly neutralizing activity, and thus, represent immunological solutions to induce reproducible bnAbs in the general human population by vaccination. Here, we focus on the current knowledge on *V_H1-69*-encoded bnAbs against influenza, HCV and HIV-1, and discuss how their genetic and structural information can inform rational vaccine design.

Conserved epitopes targeted by V_H1-69 bnAbs

The *V_H1-69* gene encodes two hydrophobic residues at the tip of the heavy-chain complementarity-determining region 2 (CDRH2) loop that provide a structural basis for epitope recognition. It is, therefore, preferentially used by antibodies that target conserved hydrophobic regions of viral envelope glycoproteins.

HA is a homotrimeric glycoprotein (trimer of HA1-HA2 dimers) on the envelope of influenza A and B viruses that is responsible for viral receptor attachment and membrane fusion activities. It can be functionally and structurally separated into two domains: a hypervariable membrane-distal head and a conserved membrane-proximal stem [2] (Figure 1a). The head domain is composed only from HA1 and contains the binding site for its sialic acid receptor, while the stem consists of both HA1 and HA2 and houses the fusion machinery. Current seasonal influenza viruses and annual vaccines normally generate strain-specific antibody responses to the HA head. In the last decade, however, the discovery and isolation of human bnAbs to influenza virus have identified an increasing number of antibodies that are able to neutralize a broad spectrum of heterosubtypic influenza viruses (reviewed in Ref. [25•]). Surprisingly, these antibodies (e.g. CR6261, A6, F10, 27F3 and CR9114) exhibit restricted usage of *V_H1-69* and mainly target epitopes that correspond to hydrophobic pockets in the HA stem [11,26–28,29••] (Table 1 and Figure 1a). A small proportion of antibodies (e.g. 2G1, 8M2, and F045-092) that also originate from *V_H1-69* are directed at or near the receptor-binding site (RBS) on the HA head (Table 1 and Figure 1b) [30–32].

HCV envelope glycoproteins E1 and E2 form heterodimers on the viral surface and play essential roles in viral entry, assembly, fusion and budding [33,34]. E2 is the receptor binding protein that directly interacts with the host receptors tetraspanin CD81 and the scavenger receptor class B member 1 (SR-B1) [35,36•], while E1 is suggested to help modulate the E2-receptor interactions and carry out fusion with the host cell membrane [37,38]. HCV bnAbs have been mapped onto the E2 neutralizing face, a predominantly hydrophobic surface formed by the front layer and the tip of the CD81 binding loop [36•] (Figure 1c), as well as the E1E2 heterodimer (reviewed in Ref. [39•]). Biased use of *V_H1-69* has been reported in anti-E2 antibody responses in multiple studies [15–17,40–43]. Most of these antibodies (e.g. AR3-class antibodies, HC84-class antibodies, HEPC3/74-class, HC-1-class, and AT12-009) recognize the overlapping neutralizing sites on the E2 neutralizing face (Table 1, Figure 1c and d), and neutralize diverse HCV genotypes by blocking E1E2 binding to CD81 (Table 1) [44–46,47••].

HIV-1 envelope glycoprotein (Env) consists of two non-covalently associated subunits that form trimers of the receptor binding glycoprotein gp120 and the membrane-anchored fusion protein gp41. The fusion and entry of HIV-1 to host cells are initiated by binding of gp120 to the CD4 receptor and then to CCR5 or CXCR4 co-receptor, which promotes conformational changes and leads to exposure of the gp41 hydrophobic N-terminal fusion peptide [2,48]. bnAbs against HIV-1 recognize all major exposed surfaces of the prefusion-closed Env trimer (reviewed in Refs. [49,50••]) (Figure 1e). *V_H1-69* has been found to be over-represented in HIV-1 CD4i antibodies [20,21]. These antibodies, exemplified by 17b and 412d, however, have no or limited neutralization by themselves (Table 1). Some *V_H1-69*-encoded nAbs have been mapped to gp41: 4E10 and CAP206-CH12 to linear epitopes in the MPER [51–53], while HK20 and D5 to the highly conserved HR1 region [54–56] (Table 1 and Figure 1e). Recently, a CD4bs-directed antibody VRC13 originating from *V_H1-69* was isolated and demonstrated potent neutralizing activity [19] (Table 1 and Figure 1f).

Polymorphism of *V_H1-69* gene and its effect on bnAb expression

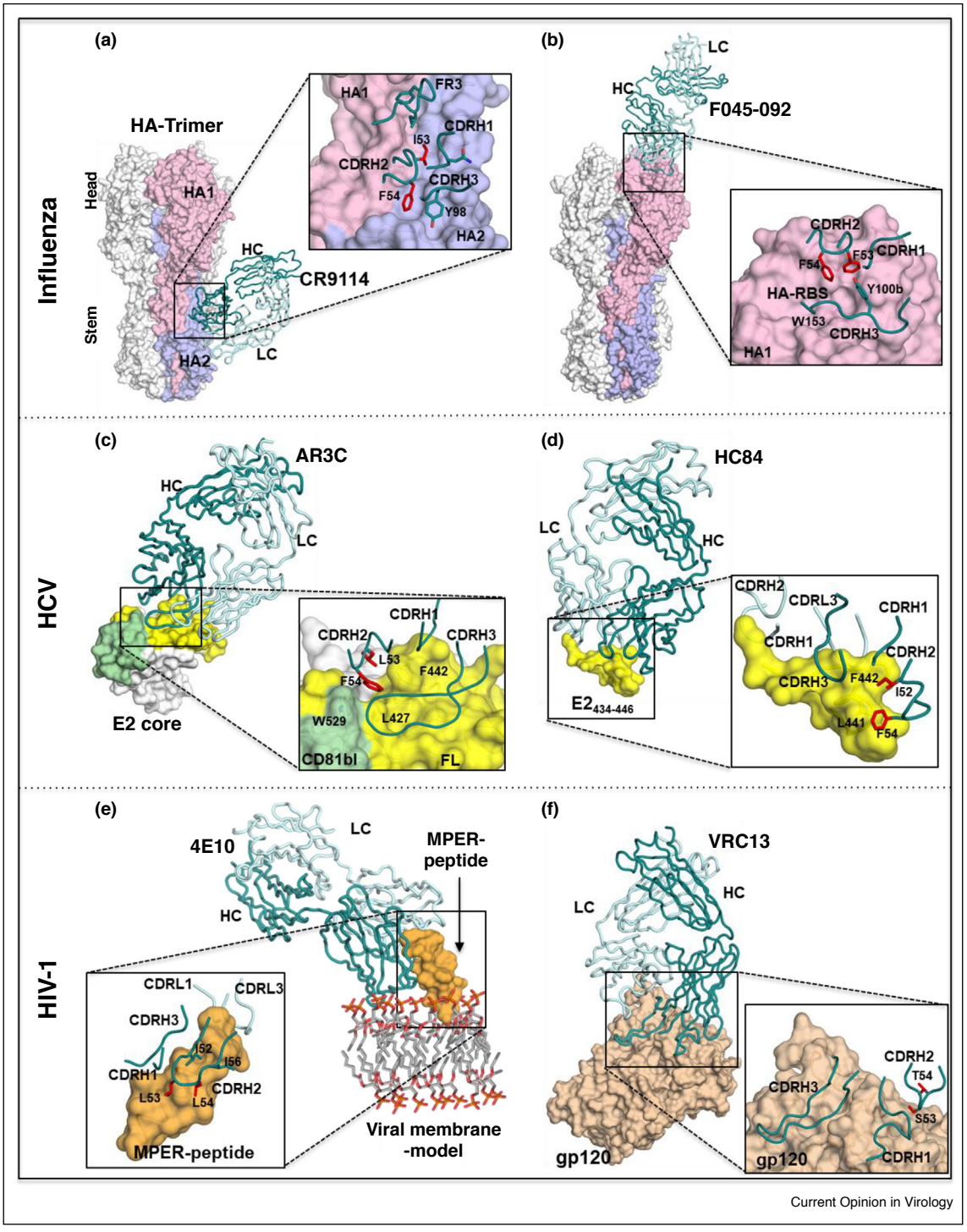
V_H1-69 is one of the most polymorphic loci within the human V_H gene cluster (14q32.33), exhibiting both allelic and copy number variation [57,58]. There are 17 alleles known to be associated with this gene: ten have a phenylalanine at amino acid position 54 (Kabat numbering) in CDRH2 (F alleles) and the remaining seven have a leucine (L alleles) (Figure 2a). The V_H1-69 bnAbs often originate from F alleles, mainly *V_H1-69*01* and *V_H1-69*06* (Table 1 and Figure 2), whereas weak or non-nAbs (e.g. HIV-1 antibodies 17b and CAP206-CH12 and HCV antibody AR1A) tend to be encoded by L alleles [15,20,52]. One exception is 4E10, a bnAb against HIV-1, which is encoded by the L allele *V_H1-69*10* [52]. The *V_H1-69* F/L polymorphism strongly affects serum bnAb titers to the influenza HA stem [14,59•], and the copy number also impacts the IgM V_H1-69 bnAb response and overall bnAb memory B cell pool [59•].

Interestingly, in the general population, around 33% of individuals are homozygous for F alleles (*F*/*F*), 56% are heterozygous (*F*/*L*), and 11% are homozygous for L alleles (*L*/*L*) [12]. Such *V_H1-69* polymorphism appears to play a role in shaping global *V_H* germline gene utilization [59•] and might explain in part the difference in an individual's capacity to mount an bnAb response to viruses during infection and vaccination.

Genetic features of V_H1-69 bnAbs

As noted above, one unique feature of V_H1-69 bnAbs is an unusually hydrophobic CDRH2 loop that serves as a crucial anchor for interaction with conserved hydrophobic epitopes. Typically, CDRH2 contains a canonical hydrophobic residue at position 53 (isoleucine for most influenza antibodies and somatically mutated hydrophobic residues for others) and a critical phenylalanine at

Figure 1



Recognition of influenza, HCV and HIV-1 envelope glycoproteins by representative antiviral V_H1-69 bnAbs.
(a)+**(b)** Binding of the influenza HA stem-specific CR9114 ((a), PDB 4FQI) and RBS-specific F045-092 ((b), PDB 4O58) bnAbs to the influenza HA-trimer. One HA protomer is colored in light pink and light purple for the HA1 and HA2 subunits, respectively. For clarity only one mAb is shown in each illustration. **(c)**+**(d)** Binding of AR3C to HCV E2 antigen region 3 ((c), PDB 4MWF) and HC84-1 to $E2_{434-446}$ peptide ((d), PDB 4JZN) on the neutralizing face. The E2 front layer (FL) is colored in yellow and CD81 binding loop (CD81bl) in green. **(e)**+**(f)** Binding of 4E10 to HIV-1 membrane-proximal-external region (MPER) on gp41 ((e), PDB 4XCF), and VRC13 to CD4 binding site (CD4bs) on HIV-1 gp120 that does not use a hydrophobic motif at the tip of CDRH2. ((f), PDB 4YDJ). In each complex, the side chains of the hydrophobic residues at the tip of CDRH2 are shown in sticks and colored in red. The heavy chain (HC) and light chain (LC) CDRs that contribute to the binding are shown in a narrow tube representation.

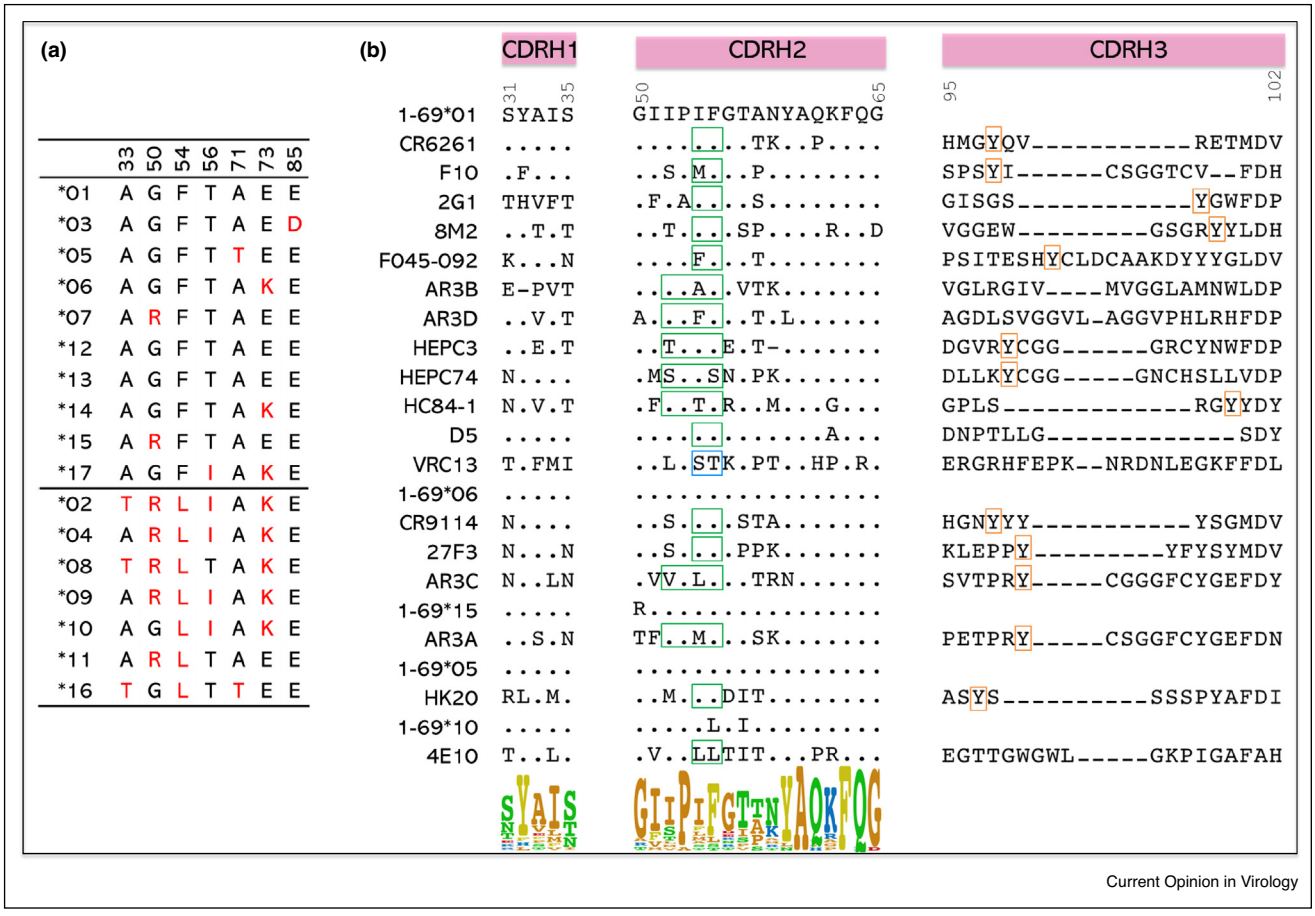
Table 1

Properties of representative V_H1-69 antiviral antibodies

Virus	Antibody	V_H1-69 allele	Genetic features	CDRH3 length[a]	Epitope	Recognition properties	Neutralization breadth	PDB	Reference
Influenza	CR6261	F	Hydrophobic Ile-Phe (IF) sequence at the tip of CDRH2 [Met-Phe (MF) for F10] with a conserved Tyr (Y) in CDRH3 to give an IFY motif	12	HA stem	Heavy chain only binding; CDRH2 dominant	Influenza A group 1	3GBM	[27]
	F10	F		15				3FKU	[11]
	CR9114	F		12				4FQI	[28]
	27F3	F		14		Heavy chain dominant; CDRH2 dominant	Influenza A group 1 and 2 viruses	5WKO	[29••]
	2G1	F	Hydrophobic IF (FF for F045-092) at the tip of CDRH2	11	HA head RBS	Heavy chain dominant; CDRH2 and H3 mediated	Influenza A group 1	4HG4	[32]
	8M2	F		14				4HFU	[32]
	F045-092	F		23		Heavy chain only binding; CDRH3 dominant	Influenza A group 1 and 2	4O58; 4O5I	[30,70]
HCV	AR3A	F	Hydrophobic I/V-P-X-F motif at the tip of CDRH2 for AR3-class and HC84-class antibodies; T/S-P-I-F/S motif for HEPC3/74-class antibodies	18	E2 AR3	Heavy chain only binding; CDRH2 and CDRH3 mediated	Broadly neutralizing	6BKB	[15,60••]
	AR3B	F		19				6BKC	[15,60••]
	AR3C	F		18				4MWF	[15,44,60••]
	AR3D	F		22				6BKD	[15,60••]
	HEPC3	F		17				6MEI; 6MEJ; 6MEK	[47••]
	HEPC74	F		18				6MEH	[47••]
	HC84-1	F		10	E2 domain D	Heavy chain dominant; CDRH2 dominant	Broadly neutralizing	4JZN	[45]
	HC84-27	F		10				4JZO	[45]
	HC84-26	F		10				5ERW	[46]
HIV-1	4E10	L	Hydrophobic LL at the tip of CDRH2	18	gp41 MPER and membrane lipid	Heavy chain dominant; CDRH2 dominant	Broadly neutralizing	2FX7, 4XCF	[51,53,75,75]
	HK20	F	Hydrophobic IF at the tip of CDRH2	13	gp41 HR1	Heavy chain dominant; CDRH2 dominant	Less breadth and potency	2XRA	[56]
	D5	F		10				2CMR	[55]
	17b	L	Hydrophobic IL at the tip of CDRH2	19	gp120 coreceptor-binding site	Heavy chain dominant; CDRH2 and H3 mediated	Limited neutralizing	1RZ8 1GC1, 1G9N, 1G9M	[20,77,78]
	VRC13	F	Polar ST at the tip of CDRH2	21	gp120 CD4bs	CDRH3 dominant	Broadly neutralizing	4YDJ	[19]

[a] On the basis of Kabat numbering, in which the CDRH3 length is 2 amino acids shorter than in the IMGT definition.

Figure 2



V_H1-69 allelic polymorphism and heavy-chain CDR sequences of representative V_H1-69 antibodies.
(a) Polymorphic amino-acid residues encoded by *V_H1-69* alleles. **(b)** Alignment of amino-acid sequences of the heavy-chain CDRs of representative V_H1-69 antibodies to viral antigens and their germline gene alleles. The hydrophobic motif in CDRH2 tip for each antibody is boxed in green (except for VRC13, which is polar and boxed in blue), and the conserved tyrosine in CDRH3 is boxed in orange. Kabat numbering is indicated. The sequence logo shows the amino-acid composition at each position in CDR H1 and H2.

position 54 (Figure 2b). Some influenza V_H1-69 antibodies also have a conserved tyrosine in CDRH3, which, together with the IF residues in CDRH2, represents the IFY motif that is important for binding to the HA stem (Figure 2b).

A hallmark of HIV-1 bnAbs is their extraordinary levels of somatic hypermutation (SHM) (13–32% nucleotide mutations for V_H) [49]. Strikingly, most V_H1-69 bnAbs (except VRC13) carry low to moderate SHM (5–16% nucleotide mutations) [12,16,17,45,46,52,60••], although not all of the mutations are required for antibody function [12,61]. This implies functionally mature V_H1-69 antibodies can be generated in fewer cell replication cycles compared to the highly mutated anti-HIV antibodies. As SHM levels over 20% (nucleotide) may be difficult to achieve by vaccination [62–66], this feature of low genetic barrier in bnAb formation indicates *V_H1-69* as a promising gene to target for rational vaccine design.

Another feature of many human bnAbs is a long CDRH3 loop, which helps gain access to epitopes that are partially obscured, especially by surrounding glycans, or which require an extensive interacting surface with generally flat (or undulating) epitopes, even if exposed [44,60••,67,68]. Long CDRH3s are found in V_H1-69 HCV AR3-class and HEPC3/74-class antibodies, influenza RBS-specific antibody F045-092, and HIV-1 antibodies 4E10 and VRC13 (17-23 amino acids, as defined by Kabat numbering) (Table 1 and Figure 2b), compared to the normal range of 9–15 residue CDRH3s in healthy blood donors [19,60••,69]. However, this long CDRH3 feature is not observed for most influenza stem-specific and many RBS-specific V_H1-69 bnAbs, which are

characterized by shorter CDRH3s of 11–14 residues [12,32], and for HCV HC84-class antibodies, which have even shorter CDRH3s of only 10 residues [45] (Table 1 and Figure 2b).

Structural basis for V_H1-69 bnAb recognition

The binding modes for influenza stem-specific V_H1-69 antibodies, such as F10, CR6261, CR9114, and 27F3, have been structurally well defined [11,27,28,29••], and revealed common binding features for this group of antibodies: (1) the majority of the buried surface and the interactions with the stem groove are mediated by the heavy chain through CDRs H1, H2, framework 3 (FR3) and H3 (for antibodies 23F3, CR6261, and CR9114, very few to no light chain interactions were observed); and (2) a conserved IFY motif (or similar MFY motif for antibody F10) arising from CDRs H2 (IF) and H3 (Y) anchors the antibody to the hydrophobic groove in the stem and provides the basis for HA stem-specific V_H1-69 antibody recognition [12] (Figures 1a and 2 b). Despite the conservation of heavy-chain contacts, there are significant differences in the rotation of the antibody V_H domains relative to the HA stem, which may influence the potency and breadth of these antibodies [29••]. Beside the stem-specific antibodies, three RBS-specific V_H1-69 antibodies, 2G1, 8M2, and F045-092, have also been structurally characterized [32,70]. These antibodies (as for antibodies encoded by other V_H genes [71••]) primarily use their heavy chain CDRs (CDRH2 or CDRH3) to insert into the RBS, mimic some of the features of the sialic acid receptor, and hence block virus entry [70,72]. Like the stem-specific antibodies, they have identical IF hydrophobic residues (FF for antibody F045-092) at the tip of CDRH2 that interact with a conserved hydrophobic pocket at the base of the RBS (Figure 1b). For 8M2 and 2G1, although the heavy-chain and light-chain CDRs interact with the HA RBS, the recognition of the RBS is still dominated by the heavy chain, where CDRs H2 and H3 make extensive contacts with the receptor binding pocket [32]. Unlike the stem-specific antibodies, a tyrosine residue in CDRH3, Y100 for 2G1 and Y100d for 8M2, makes no interaction with the RBS. In contrast, F045-092 binds HA RBS only through the heavy-chain CDRs and the principal contacts are via CDRH3 (the hydrophobic CDRH2 makes fewer contacts with the HA); in this case, Y100b in CDRH3 makes a hydrogen bond with the RBS (Figures 1b and 2 b). The long CDRH3 loop (23 amino acids) of F045-092 with an internal disulfide reaches deeper and more extensively into the RBS and leads to enhanced receptor mimicry [70] (Figure 1b). This mode of receptor mimicry is presumably the basis for the neutralization breadth of the F045-092 and other RBS-specific bnAbs [71••].

Structural studies of HCV E2 have been very challenging [36•], nevertheless, the recognition of E2 by V_H1-69 antibodies have been characterized from the structures of E2 core-AR3A-D complexes, truncated E2 ectodomain-HEPC3/74 complexes, and complexes of HC84-1, HC84-27, HC84-26, and HC84-26AM (the affinity-matured HC84-26 antibody) with a linear peptide component ($E2_{434-446}$) of the corresponding epitopes [44–46,47••,60••]. Similar to influenza V_H1-69 antibodies, the hydrophobic CDRH2 tip of HCV AR3-class and HC84-class antibodies contains an I/V-P-X-F motif (Figure 2b) and interacts with a highly conserved hydrophobic pocket on E2. The HC84-class antibodies interact with the E2 front layer L441 and F442, whereas the CDRH2 of the AR3-class antibodies interacts also with front layer L427 and with W529 of the CD81 binding loop (Figure 1c and d). In the CDRH2 motif, residue 53 is hydrophobic for AR3-class antibodies, but can be a polar amino acid for HC84-class antibodies. The CDRH2 tip of HEPC3/74-class antibodies is less hydrophobic and contains the T/S-P-I-F/S motif (for HEPC74, the highly conserved F54 is mutated to serine) (Figure 2b) [47••]. In addition to the hydrophobic interactions (P53 and I54 with Y443 for HEPC3 and I53 with F442 for HEPC74), their CDRH2s form a hydrogen bond with the front layer (P53 with K446 for HEPC3 and N55 with Y443 for HEPC74). Compared to HC84-class antibodies, for which both heavy and light chains interact with the linear component of the epitopes in the E2 front layer [45,46], AR3-class and HEPC3/74 class antibodies bind to a discontinuous epitope where the interactions are mediated mainly by the heavy chain CDRs [44,47••,60••], and the long CDRH3 forms multiple main-chain to main-chain hydrogen bonds with the front layer and the CD81 binding loop. This difference in the binding mode may be a consequence of differences in the CDRH3 length (17–22 residues for AR3-class and HEPC3/74-class antibodies versus 10 residues for HC84-class antibodies).

The MPER-directed HIV-1 bnAb 4E10 also has a hydrophobic CDRH2 loop that extensively interacts with a linear epitope in the highly conserved MPER of gp41 [73] (Figure 1e). Interestingly, 4E10 exhibits some polyreactivity with cardiolipin phospholipids [74], and indeed recognizes an extended epitope that includes the MPER peptide and specific lipids in the virus membrane: it contacts the lipid head groups through CDRH1, and the hydrophobic lipid tails through its CDRH3 [75]. Such lipid binding properties have been proposed to increase the local concentration of these MPER-specific antibodies in the vicinity of the epitopes for virus neutralization [76]. V_H1-69 CD4i antibodies (e.g. 17b and 412d) bind to a hydrophobic surface on gp120 surrounded by basic residues that overlap with the chemokine-receptor-binding site [20,77,78]. The binding is dominated by CDRH2, which interacts with the hydrophobic center of the co-receptor binding site, and a long acidic CDRH3 (19–25 amino acids) that complements the gp120 basic residues. Indeed, CDRH3 might contain a sulfated tyrosine that mimics the co-receptor acidic N-terminus that includes

tyrosine sulfation [79]. V_H1-69 HR1-specific antibodies (e.g. HK20 and D5) bind a conserved hydrophobic pocket in the gp41 fusion intermediate or post-fusion conformation [54–56]. The binding is dominated by CDRH2 and a short CDRH3 (10/13 amino acids, Table 1) with minor contribution of CDRL3. Unlike all the other V_H1-69 bnAbs described in this review, the CDRH2 tip of HIV-1 bnAb VRC13 is not hydrophobic but polar (S53 and T54, Figure 2b). This antibody targets the CD4bs on gp120, with the antibody interface dominated by CDRH3 [19] (Figure 1f).

Developmental pathways for V_H1-69 bnAbs

Recent longitudinal analysis by next-generation sequencing (NGS) has greatly expanded our understanding of B-cell lineage development, that is, how bnAb lineages initially arise and then evolve via SHM to acquire-specific biological functions (i.e. cross-neutralization in this case). The pathways for B cells to develop breadth and potency differ depending on the epitope targeted, but involve initial V(D)J recombination and selection of B cells with a particular V_H gene and/or often a long CDRH3 to generate the 'precursor' or 'ancestral' B cells, followed by subsequent SHM during an active immune response to antigen stimulation [50••,80–82]. To date, only a few studies have defined the developmental pathways of V_H1-69 bnAb lineages targeting influenza HA stem or HCV AR3. Remarkably, these antibodies achieved breadth through a rapid lineage development, with only few mutations [12,17,60••,61]. Indeed, Lingwood *et al.* revealed that affinity maturation of CR6261 requires only seven (7.4%) amino acids in CDRH1 and FR3 of the germline precursor to acquire full activity [61]. Pappas *et al.* reported that in Y98-bearing influenza V_H1-69 antibodies, a single proline to alanine mutation at position 52a in CDRH2 is sufficient to confer high affinity binding of the antibodies to the selecting H1 HA antigen [12]. More recently, we determined that some germline-encoded precursors of HCV AR3-class bnAbs bind E2 with high affinity and show strain-specific neutralization capability, although more mutations are required for cross-reactivity [60••]. These observations strengthen the hypothesis that such V_H1-69 bnAbs could be easier to induce by vaccination than highly mutated bnAbs to viral antigens, and would provide broad protection against genetically variable viruses.

Viral escape from V_H1-69 bnAbs

Enveloped RNA viruses such as influenza, HCV and HIV-1 exhibit a remarkable ability to adapt and evade the host immune response. Despite the impressive breadth and potency of bnAbs *in vitro*, these highly mutable viruses may still escape from them in cell culture and in animal models through mutations at critical sites on the target surface glycoproteins. The escape mutations can occur directly in the neutralization epitope leading to reduction in antibody binding, or indirectly outside the targeted epitope altering epitope presentation and accessibility of bnAbs for neutralization [83–88].

For influenza V_H1-69 bnAbs, antibody selection of escape virus resulted in a single indirect escape mutation for CR6261 (H111L) [89] and CR9114 (V466I or R526G) [90], and four single indirect escape mutations for F10 (three in HA and one in neuraminidase) [91]. For HCV V_H1-69 bnAbs, several mutations in E2 (e.g. D431E, F442L and L438F) that conferred resistence to AR3-class antibodies [88,92] or to HC11 [93] were identified in viruses with compromised viral fitness. However, escape virus was not observed for HC84-class antibodies [45,46,93] and HC-1 [93] *in vitro*. For HIV-1 V_H1-69 bnAbs, Env mutants carrying W672G and F673L demonstrated resistant to 4E10 *in vitro*, although the resistance was difficult to achieve because of impaired viral infectivity [94,95].

Although escape viruses have been identified for most bnAbs, given the high conservation of the targeted neutralizing epitopes and the fitness cost of acquiring and maintaining escape mutations in the virus, a vaccine designed to target broadly neutralizing epitopes of genetically diverse viruses will likely be superior to traditional vaccine strategies.

Relevance of V_H1-69 bnAbs in rational vaccine design

The discovery of the frequent occurrence of V_H1-69 bnAbs against viral antigens has led to the identification of several conserved epitopes suitable for rational vaccine designs. Structural information on antibody-epitope complexes and knowledge on the development of bnAbs at the genetic level can serve to guide vaccine design approaches based on epitope or B cell ontogeny.

Epitope-based design involves identification of bnAbs, determination of structures of the epitopes, and design and engineering structural mimics of the target epitopes for immunization [50••,96]. In the case of influenza, the HA stem presents a highly conserved region for bnAbs raising optimism of developing a universal vaccine [27,71••,97,98,99•,100–102]. Several approaches have been employed to elicit bnAb responses against this domain, including design of head-stem chimeric HA, headless HA or mini-HA, and hyperglycosylated HA [71••,103–107]. For HCV, the structural and genetic information on V_H1-69 bnAb-epitope complexes is now enabling immunogen design of vaccine candidates targeting the highly conserved epitopes in the neutralizing face. For HIV-1, identification of the lipid binding sites on 4E10 may aid in a MPER-targeting immunogen design that include a lipid component for eliciting bnAbs. Notably, the V_H1-69 CD4i antibodies, such as E51, 412d and 17b, can enhance the association of Env with CD4bs bnAbs and, hence, improve neutralization potency of the

antibody mixtures [108]. Similar synergistic neutralizing activity has also been reported in HCV bnAb mixtures [109•]. These data support the development of vaccines designed to elicit multiple bnAbs of complementary specificities.

B cell ontogeny-based vaccine design is based on structural information of antibody maturation to guide design of germline-targeting immunogeons to activate a specific antibody lineage [71••,110]. The V_H1-69 bnAbs have been found in different individuals and share common features in gene usage, mode of recognition and developmental pathways, suggesting that appropriately designed immunogens may elicit these antibodies reproducibly in vaccinated populations. Compared to bnAbs that require high levels of SHM for maturation, the low-to-medium SHM rate for most V_H1-69 bnAbs should be more readily achievable through vaccination.

Conclusion

Outstanding progress has been made over the past decade in isolation of human bnAbs against influenza, HCV and HIV-1. Among the numerous bnAbs, the V_H1-69 bnAbs are of great interest. They are dominantly found in protective antibody responses to viral pathogens, and are found repeatedly at the population level. Genetic and structural characterization of these antibodies have revealed several classes of antibodies that recognize the same region in particular antigens, employ the same mode of recognition, and develop through rapid maturation pathways with low-to-medium SHM. These features make the V_H1-69 bnAbs appealing candidates for rational vaccine design. Further studies aimed at designing immunogens to engage a specific B cell lineage to a highly conserved epitope such as the HA stem or HCV neutralizing face, based on the genetic and structural information of V_H1-69 bnAbs summarized here, may provide a means to achieve cross-protection for broadly effective or universal vaccines for these viruses.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

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