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Antibody responses to viral infections: a structural perspective across three different enveloped viruses

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Abstract

Antibodies serve as critical barriers to viral infection. Humoral immunity to a virus is achieved through the dual role of antibodies in communicating the presence of invading pathogens in infected cells to effector cells and interfering with processes essential to the viral lifecycle, chiefly entry into the host cell. For individuals that successfully control infection, virus-elicited antibodies can provide lifelong surveillance and protection from future insults. One approach to understand the nature of a successful immune response has been to utilize structural biology to uncover the molecular details of the antibodies derived from vaccines or natural infection and how they interact with their cognate microbial antigens. The ability to isolate antigen specific B-cells and rapidly solve structures of functional, monoclonal antibodies in complex with viral glycoprotein surface antigens has greatly expanded our knowledge of the sites of vulnerability on viruses. In this review, we compare the adaptive humoral immune responses to HIV, influenza, and filoviruses, with a particular focus on neutralizing antibodies. The pathogenesis of each of these viruses is quite different, providing an opportunity for comparison of immune responses: HIV causes a persistent, chronic infection; influenza an acute infection with multiple exposures during a lifetime and annual vaccination; and filoviruses, a virulent, acute infection. Neutralizing antibodies that develop under these different constraints are therefore sentinels that can provide insight into the underlying humoral immune responses and important lessons to guide future development of vaccines and immunotherapeutics.

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Introduction

Enveloped viruses are found across diverse viral families and cause some of the deadliest diseases known to man. Despite a spectrum of differences in their biology and pathogenesis, all enveloped viruses share two commonalities: a lipid bilayer envelope co-opted from host cells upon viral egress and the presence of surface-exposed viral glycoproteins for host cell recognition and entry. These viral glycoproteins or 'spike' proteins are exposed to the adaptive immune response and are the main targets of host antibodies, often being the only exposed antigen. Naturally, viruses have developed mechanisms to avoid such responses through rapid evolution of antibody-targeting epitopes, steric shielding of epitopes by glycan post-translational modifications, immune decoys such as soluble antigens that share viral spike epitopes, and immunosuppression to evade host recognition upon cellular entry. In response to the viral arms race, antibodies have in turn developed many creative solutions to overcome viral evasion, including unique structural adaptations that allow antibodies to more readily penetrate the viral armor and exploit sites of vulnerability.

The nature of the enveloped viral surface has major consequences for viral entry and, consequently, how the adaptive immune system responds. For enveloped viruses, entry hinges on the mechanics of the viral glycoprotein. Vital to the function of glycoproteins is their ability to fuse viral and host membranes together. Generally, this is achieved through a complex mechanism, where initial binding to a specific cellular host receptor usually results in drastic changes in the structure of the viral spike that enable transition to the post-fusion conformation. Successful triggering of post-fusion glycoprotein forms provides the energy necessary to fuse the viral and cellular membranes, thereby forming pores that allows the viral genomes to gain entry into the cytoplasm[1,2]. Once entry is achieved, viral replication takes place using a variety of strategies, both in the cytoplasm and/or nucleus, but concludes in assembly and budding of new viral progeny at the host-cell surface. For enveloped viruses, a neutralizing antibody (nAb) disrupting the viral lifecycle almost always involves binding to the viral glycoprotein, which can then disrupt entry by blocking one or more of the processes described above. Additionally, antibodies can also block viral egress by binding to glycoproteins on the infected cell surface, thus preventing viruses from budding. Thus, neutralization is achieved by preventing progeny from infecting a new cell and is a distinct mechanism from neutralization achieved from blocking entry (Figure 1). The pathogenesis of the three viruses that we describe here represent gradations of antigenic exposure to the adaptive immune system and also greatly influences the molecular nature of the antibodies formed in response to infection, which we will discuss at length later. Specific details regarding the lifecycle and pathogenesis of these viruses are summarized in Table 1. While nAbs represent the overwhelming majority of our understanding of the antibody-based immune response to enveloped viruses, especially with respect to their structure, some non-neutralizing antibodies have also been shown to provide protection *in vivo*; however, this review will majorly focus on nAbs.

Due to the potency, specificity, and tolerability of antibodies, they have become popular as therapeutics for viral disease and are also the intended outcome of most vaccines. It is therefore vital to understand not only where antibodies bind to viruses, but the molecular nature of the interaction. Structural biology has led the way in describing how antibodies

interact with viral glycoproteins. While x-ray crystallography has long been the primary technique for solving high resolution structures, single particle electron microscopy has recently also become a powerful and increasingly used method for determining structures of viral antigen-antibody complexes, now reaching resolutions comparable to x-ray crystallography. These data are critical to structure-based design of the next generation of immunogens to elicit neutralizing and protective antibodies and in the selection and design of improved antibody therapeutics.

In this Review, we summarize recent advances in our understanding of neutralizing antibody responses to enveloped viruses, particularly from a structural perspective, with a focus on HIV, influenza, and filoviruses, for which there is a wealth of structural and immunological data. We first describe the overlapping and unique characteristics of each viral lifecycle, glycoprotein structure and pathogenesis. We highlight how structural biology has delineated the immunogenic landscape of these viruses and how antibodies have adapted to target these sites of vulnerability. Next, we discuss common and divergent themes among immune responses to these viruses and, importantly, how this information is being utilized to inform design of vaccines, therapeutics and diagnostics. Finally, we briefly comment on the role of antibodies in the recruitment of the mediators of cellular immunity, the emerging role of Fc-variants, and how lessons learned translate to other viruses and can address future outbreaks.

Neutralizing antibody and glycoprotein structures

Immunoglobulins (Ig) are produced in a wide variety in humans, each with different roles in the immune response. The Ig gamma (G) form is by far the most extensively studied class of Ig, due to its extremely important role in immunotherapeutics as well as in viral immunity. Although there are very few structures of intact IgG[3–6], hundreds of structures of antibody variable domains have been described in the literature at length, usually as fragment antigen binding (Fab) alone and in complex with cognate antigen[7,8]. IgGs can be segregated into two identical Fab domains, which are responsible for binding and recognizing specific antigens, and a single fragment crystallizable (Fc) domain, which is responsible for communicating the presence of pathogens through binding to a limited set of cellular receptors known as Fc gamma receptors (FcγRs) (Figure 2).

The overwhelming majority of antibodies that provide sterilizing protection from infection target the viral spike. Therefore, the structure of the glycoprotein greatly influences how antibodies are selected, matured and specifically interfere with the mechanics of viral entry. Many enveloped glycoprotein structures have been solved, including those of the viruses discussed here, and have provided extensive insight into their mechanism of entry, as discussed below. Such details have led to enveloped virus membrane fusion proteins being classified into three groups based on their structure and mechanism of entry[2]. For the purposes of this review, we will focus on the type-I fusion glycoproteins, which compose the glycoproteins of HIV, influenza and filoviruses. However, while these three viruses are well-characterized examples, they do not represent the complete list of viruses that have contributed to our understanding of the antibody-based immune response to viruses containing type-I glycoproteins.

Type-I glycoproteins are expressed as single polypeptides that, after proteolytic cleavage, give rise to the pre-fusion, metastable viral spikes that are present on the surface of viruses. Type-I glycoproteins are composed of heterodimers arranged in a homotrimeric arrangement. Each protomer is identical and includes an ectodomain consisting of an N-terminal portion, the receptor binding domain (RBD) that houses the receptor binding site (RBS), and a largely C-terminal portion containing the fusion machinery, a transmembrane anchor (TM), and often some type of internal C-terminal tail. Within the fusion machinery, a hydrophobic linear sequence of amino acids, known as the 'fusion peptide' or 'internal fusion loop', is sequestered or partially buried, waiting to be released upon receptor engagement. In each of the viruses discussed here, binding to specific host-cell receptors is not sufficient to activate viral fusion. Rather, there are multiple steps required before glycoproteins become fusion competent. Once the fusion peptide is released, it is thought that it inserts itself into the host membrane and anchors the viral and host membranes together[2]. Helices within the fusion domain below combine to form an elongated helix and thereby 'zip' into their post-fusion form, creating an extended coiled-coil intermediate. The extended intermediate eventually collapses into a 6–helical bundle, bringing the inserted N-terminal fusion-peptides and C-terminal TM regions together to create the post-fusion form of the glycoprotein. This structural rearrangement provides the necessary energy to bring the viral and host membranes close together, forming a pore sufficient for the genetic material to enter into the cytoplasm. Pre-and post-fusion structures representing influenza virus[9,10] HIV[11–14] and Ebola virus[15,16] have all been described and were instrumental in detailing the processes described above.

Covering the surface of all glycoproteins are a variety of glycans, which are typically found to be more concentrated in the N-terminal RBD domains. The extent of coverage, type of glycan and attachment, and role in the viral lifecycle varies between viruses and is not completely understood; however, one of the most commonly attributed purposes of glycans is for immune shielding[17–20], as well as prevention of proteolysis[10]. For determination of some glycoprotein structures, glycans are trimmed back by glycosidases or occasionally deleted to facilitate crystallization and therefore are often not structurally characterized or incompletely characterized. However, in other structures, these inherently flexible glycans can be explicitly resolved in both x-ray and cryo-electron microscopy (EM) structures, particularly when bound to antibodies[20]. Recent efforts to model glycans *in silico* and by mass spectrometry[21], as well as cryo-EM studies[22], have also given a much greater understanding of the extent of glycosylation. Comparing structures of the type-I glycoproteins discussed here emphasizes the common structural themes among them (Figure 3). These structural motifs greatly influence the immune response and are reflected in the antibodies that are elicited, resulting in common themes among nAbs, which will be discussed in greater detail below. It should be noted that while HIV and filoviruses only have one glycoprotein on their surface, influzenza viruses have two, including hemagglutinin (HA) and neuraminidase (NA), which aids in viral egress. While neutralizaing antibodies against NA are significant and can provide protection, we will not discuss them in this review.

Common and divergent themes of antibody binding to enveloped glycoproteins

Antibodies can provide sterilizing protection against viral pathogens and finding antibodies that are potent against diverse strains of related viruses is highly desirable. Once such antibodies are identified and isolated, structural elucidation of their epitopes can reveal regions on the viral surface that can be targeted by vaccines and immunotherapeutics. One driver of antibody epitope mapping has been the utilization of electron microscopy to rapidly survey large number of antibodies through docking crystal structures into lower resolution EM maps[23–27], and now to map polyclonal antibody responses in sera[28,29]. Structural studies have collectively revealed that antibodies utilize a plethora of strategies to recognize the wide range of surfaces and epitope locations on viral antigens, highlighting weak spots on the viral armor. Below we outline some of these sites of vulnerability and point out common and divergent themes.

Receptor binding site and structural mimicry—All viruses utilize a host receptor in some capacity to gain entry into cells. Therefore, it is not surprising that the receptor binding site (RBS) is a major site of vulnerability for many viruses, since this region is an obligate component of the viral lifecycle and, therefore, relatively conserved even under immune pressure (Figure 4, Row 1). Viruses have evolved unique ways to escape this pressure. For example, HIV requires both a primary receptor (CD4) that is sterically difficult for antibodies to access[11,12], as well as a co-receptor (CCR5/CXCR4), whose binding site is sequestered and only formed and accessible after CD4 binds[30]. Nevertheless, many bnAbs have now been identified that target the CD4-binding site[31,32]. Influenza virus' sialic acid binding site is easily accessed on the HA head[33], and is relatively well conserved, although some mutational variability can be tolerated within this region[34,35], and RBS-adjacent regions can actually vary substantially[36,37]. Efforts to engineer broader specificity into RBS-targeting antibodies has been met with difficulty since RBDs occupy relatively small patches on the viral glycoprotein surface compared to the size of an antibody footprint[38] and, therefore, there are many places where the virus can escape neutralization by changing sequence in regions surrounding the RBS. Thus, strain specificity is more typical[39], and truly broadly neutralizing antibodies (bnAbs) to the RBS are rare [40–42]. The ebolaviruses on the other hand require massive proteolytic remodeling for their RBS to become exposed within the late-endosomal compartment and, therefore, the RBS is hidden from the immune system on free virions[43–45]. However, conservation of the RBS on all filoviruses, and the differential exposure of the RBS on Marburg virus (MARV)[46,47], has shown that pan-filoviral antibodies are possible[24,48] .

Just as viruses have evolved complementary grooves on their protein surfaces for receptor binding, antibodies have also evolved into molecular mimics that can to some extent imitate these receptors. In such cases, binding can be quaternary in nature, involving loops that contain specific sequences or motifs similar to the actual receptor[30,33,38,40,49–54]. This mimicry may involve one or more complementarity determining region (CDR) loops. The structure of an antibody is thus well-suited for performing receptor mimicry, and there are examples for each of the viruses discussed here (Figure 4A–C).

HIV relies upon interaction with its receptor CD4 in a conserved binding pocket near the base of gp120 to achieve structural changes required for interaction with secondary receptors[30,55]. Similar to IgG, CD4 is also composed of the Ig-fold; thus, antibodies are predisposed for recognition of the CD4 epitope[56], although they consist of twin Ig domains rather than one for CD4. The VRC01-class of antibodies target the CD4 binding site (Figure 4A), and are typically very broadly reactive and potent[57,58]. Although these antibodies mimic some aspects of CD4 binding to receptor[55], they do not induce the large structural changes associated with receptor binding. The mAb b12 from a long-term survivor, although less potent and broad, more closely emulates the CD4 receptor in that it does induce some structural changes but not to the same extent as CD4 binding[30,59].

Each Influenza HA monomer has a binding pocket that contacts sialic acid moieties on the cell surface as its receptor prior to entry and relies on this interaction for endocytosis. While most of the known bnAbs to influenza virus target the more conserved HA stalk, some antibodies that contact the HA head also have some breadth in their binding and neutralizing capacity[33,38,40,51–53]. For example, the crystal structure of F045–092 (Figure 4B), as well as other mAbs, reveals that such antibodies likely block entry by mimicking the way sialic acid binds by inserting a CDRH3 loop into the RBS on the HA head[38]. On this and other antibodies, a dipeptide on CDRH3 contains an Asp residue that directly mimics the carboxyl on sialic acid[38,53], and a hydrophobic residue that binds in the same hydrophobic pocket as the acetamido moiety of sialic acid[33,38,42,54]. Occasionally CDR2 inserts into the binding site instead of CDRH3[52]. While the strength of the monovalent interaction is often low, the added avidity of an IgG that can span HAs on the surface can subtantially boost affinity and activity[52]. Similarly[51], many other antibodies also contact the HA RBS, but have larger footprints[38] that extend into less conserved regions adjacent to the RBS and are therefore more strain-specific and less broadly neutralizing[33,51,53].

A remarkable example of structural mimicry was recently demonstrated for filoviruses. Antibody MR78 (Figure 4C), which was isolated from a panel of MARV human survivor mAbs with potent neutralizing activity and targets the RBS[24], was shown to bind directly to the Niemann Pick C1 (NPC1) binding site, blocking receptor binding[48]. The motif displayed by MR78 includes hydrophobic residues that closely mimic the loops inserted by NPC1[49]. Ebolaviruses have evolved a more structured glycan cap[15,60–62], in contrast to the one found on marburgviruses[47], that occupies the RBS on the viral surface prior to cleavage by host proteases. The ebolavirus glycan cap, NPC1, and the MR78 CDR loops, all bind to GP in a similar fashion. This three-way type of mimicry demonstrates a unique way that the immune system has been able to take advantage of vulnerable sites on GP and mimic structures that ebolaviruses have evolved to avoid antibody recognition[63].

Fusion peptide—Recent work has revealed the fusion peptide at the N-terminus of the membrane-proximal envelope glycoprotein as a common site of vulnerability[64–66]. This short, hydrophobic sequence is vital to the fusion process and must be liberated from its buried or partially sequestered location in the stalk or stem of glycoproteins to contact the host cell membrane. This makes it a difficult, but an ideal target for nAbs due to the necessary sequence conservation within a viral family. Structures of antibodies reactive with the fusion peptide have been shown for HIV[66–68] (Figure 4D), influenza[23,64,69,70] (Figure

4E) and ebolaviruses[65,71–73] (Figure 4F). In each case, antibodies that engage the fusion peptide do so either directly or partially[23] and are broadly reactive.

The glycoprotein stalk and membrane-proximal external region (MPER)—The viral stalk or stem emerges from the transmembrane anchor of glycoproteins, and contains the membrane fusion machinery. This domain is composed of the second of the two glycoproteins (HA2 for influenza virus, gp41 for HIV and GP2 for filoviruses) as well as the N and C-terminal regions of the first and membrane-distal glycoprotein (HA1 for influenza virus, gp120 for HIV and GP1 for filoviruses). This central functional role also makes it an important site of vulnerability that is often highly conserved in sequence. With the exception of HIV-1 Env, access to this region was at one time thought to be difficult or impossible, due to proximity to the hydrophobic viral membrane and spike density[74,75]. However, many potently neutralizing antibodies have now been discovered that can access this region for HIV (Figure 4G) and filoviruses (Figure 4I)[73,76–81]. The influenza stem is larger than in HIV and filoviruses and is now appreciated as a more common site of vulnerability than once thought (Figure 4H), prompting the pursuit of potential vaccine candidates that focus on this region[80,82–88].

The MPER connects the heptad repeat region 2 (HR2) to the transmembrane region and is involved in the fusion machinery. Remarkably, HR2/MPER-directed mAbs for filoviruses contact epitopes nearly buried within the viral membrane (Figure 4I) and, in HIV-1, such mAbs have additionally evolved to interact with lipids in the membrane [89,90] or access transiently exposed hydrophobic residues[76] (Figure 4G). There is not a well-defined MPER in influenza HA and consequently no antibodies to this specific region as for the other viruses is discussed here; however, the portion of HA that sits proximal to the membrane is referred to as the stem and does have spatially analogous regions to the MPER [91].

Virus-specific sites of vulnerability—In addition to the inherently common structural features that underlie all type-I viral glycoproteins, there are many unique features that can also serve as hotspots for eliciting potent antibodies. Determination of the structures of antibodies in complex with HIV Env, in particular, has uncovered some of the most unique examples[40,92]. The duration of an HIV infection allows for a long-term arms race to commence among the increasingly diverse viral population and the host immune system[20,93–96]. This is aided, in part, by an extensive array of glycans on the surface Env, which can help shield the underlying protein surface from immune surveillance. Strikingly though, this overabundance has given rise to antibodies that can bind directly and specifically to glycans that are highly conserved due to their high density that protects them from secondary processing [20,25,26,97–100]. For example, antibody 2G12 exclusively recognizes immature high mannose glycans on HIV gp120[27,101,102]. While some glycan components of influenza and filoviral epitopes have been identified, they are not to the extent of HIV-directed mAbs.

HIV Env also houses many important epitopes that are buried within the central core of the viral spike, only exposed transiently through ‘conformational masking[103], or exposed only after CD4 is bound[30]. At the apex of the HIV viral spike are several variable loops, including V1/V2 and V3, that can widely vary in sequence, but that change position after

CD4 binding and participate in co-receptor binding[11,12,30]. These hidden epitopes are still accessible by antibodies, often through non-classical methods of antibody binding. For example, broadly neutralizing antibodies PG9 and PGT145 (Figure 4J) bind to the apex through very long CDR_{H3} loops (>30 residues) that can penetrate deep into recesses in the apex at the trimer axis. These Abs also bind asymmetrically where only a single Ab binds at the apex compared to most other HIV antibodies where three Fabs can be accommodated per trimer[98,104]. Some more recently identified antibodies can however bind to the Env apex with shorter CDRs and with a stoichiometry of up to two Fabs per trimer[105,106]. A common theme among such antibodies is the quaternary nature of their epitopes, requiring an intact trimeric spike and at least two protomers for binding, which is a mode of antibody binding that was not fully appreciated until the first structures of trimeric Env were determined[11,12]. To achieve such complicated acrobatics, these antibodies have often undergone extensive somatic hypermutation (SHM) that involves stabilizing mutations in framework regions, CDR loops that support the structure of the long inserting loops, such as CDR H3, in the paratope, and extended secondary structure within the CDR loops themselves. Conversely, some of the most potently neutralizing antibodies to influenza have far less SHM[107–110] and typically much shorter CDRH3 loops, likely reflecting the much more acute nature of these infections and the possibility of first responder or "SOS-like" antibodies in the human immune repertoire that evolved as a rapid defensive against infection[111,112].

On ebolaviruses, a structure referred to as the glycan cap, which covers the RBS, has been attributed to eliciting several neutralizing antibodies, despite its cleavage and removal upon entry[62]. The glycan cap and portions of the core GP are also shared with a second *GP* product known as sGP, which is secreted abundantly during infection[113,114]. Consequently, antibodies, such as 13C6, that react with the glycan cap are often cross-reactive to sGP[115–117] (Figure 4K). Therefore, the mechanism behind how such antibodies neutralize ebolaviruses is not well understood, and marburgviruses do not produce sGP or have a defined glycan cap, rather leaving their RBS exposed on virions[47].

Antibody allostery—Antibodies have also been shown to provide allosteric influence on glycoproteins, where binding in one location essentially alters a distal site. For example, HIV bnAb PGT151 binding to two sites on the HIV trimer induces asymmetry within the pliable Env structure, such that the third binding site on Env becomes inaccessible[76]. In a different but similar manner, antibodies have also been shown to exhibit cooperative binding, such as the binding of the Ebola virus (EBOV) antibodies FVM09 and m8C4, which do not offer effective neutralization or protection alone, but can potently neutralize EBOV in combination[118]. In this case, it is hypothesized that the binding of one alters the epitope of another, such that the epitope becomes more accessible.

Structures illuminate sites-of-vulnerability—The structures described here indicate that there are essentially no surfaces on viral glycoproteins that cannot be targeted by the adaptive immune response (Figure 5, Table S1). The diversity of antibody responses that occurs during infection demonstrate the extraordinary ability of the adaptive immune system to uniquely overcome viral obstacles. These studies provide valuable information that

inform the development of next-generation therapeutics and vaccines that can mirror these activities.

Implications for vaccine, therapeutic and diagnostic development

Structural data that have been amassed for enveloped viral glycoproteins in the past few decades have informed a more fundamental understanding of the complex viral lifecycle, but have also been used to directly and significantly advance efforts to generate and improve vaccines, therapeutics and diagnostics for these viruses. These efforts have been recently reviewed[119]; therefore, we will briefly highlight the most significant advances in each of these areas.

Efforts to generate an effective HIV-1 vaccine have been slow and challenging. Traditional routes to vaccination do not produce the bnAbs that are necessary to protect against the hugely diverse range of viral strains that are circulating in different parts of the world[120–123]. Failure has largely been fueled by the complex biology of HIV Env, which is highly glycosylated, is metastable, and undergoes larges structural changes during entry at the cell surface, and which can withstand large numbers of amino-acid substitutions within the so-called “hypervariable” regions[124]. To develop bnAbs, the adaptive response must overcome these obstacles through repeated exposure to a more diverse population of viruses, such as in long-term survivors[93,125–127]. Being relatively rare, it took many years to identify and isolate such bnAbs in reasonable numbers, and less than a handful were available in the 1990s[128–131]. Understanding how these mAbs recognize Env was further thwarted for many years by the lack of a stable and soluble form of Env that was suitable for structural studies. However, significant progress in antigen engineering resulted in the SOSIP antigen[132–136] that enabled solving the first antibody-Env structures by X-ray crystallography and cryo-EM[11,12].

These early results helped to shape next-generation of Env subunit vaccine designs, and have yielded two parallel efforts, exemplified mostly by the HIV and influenza fields. The first approach aims to present stable, idealized bnAb epitopes, wherein sites of vulnerability on the viral glycoprotein are presented to the immune system in an idealized way. This typically is achieved in one of two ways: 1) presentation of multiple epitopes on an intact, trimeric viral spike that is engineered for stability and that limits exposure of known non-neutralizing epitopes[137–141] and 2) presentation of individual epitopes that are grafted onto protein scaffolds in order to immune-focus the antibody response to a particular site of vulnerability. Both of these approaches have advantages and disadvantages[142]. The epitope-focused approach has shown success as a proof-of-concept study with respiratory syncytial virus (RSV)[142] and has since been expanding to show success in eliciting desired antibodies for HIV[143–145], but has yet to show clinical success. However, epitope-focused vaccine designs have thus far not induced bnAbs[146–148]. Activity against the more complex and quaternary epitopes found on the intact, trimeric viral antigens can be lost or produce obstacles for antibodies elicited by monomeric designs. For HIV, more stabilized and engineered versions of a “native-like” trimer indeed induced stronger neutralizing antibodies titers[149–151] but the responses generally lack breadth, only inducing antibodies that target the autologous immunogen. For influenza, the most broadly potent antibodies have been consistently

mapped to the HA stalk. Therefore, an epitope focused approach has been much more successful, where only the HA stalk is presented to the immune system[84,87,88,152–156] or presented in a chimeric form with different HA heads to focus the immune system on the conserved stem region[152,153,156]. In these cases, neutralizing responses can be relatively high and contain a level of breadth. One possible explanation is that the HA stalk has a larger degree of sequence conservation across the viral landscape than HIV Env, and less glycosylation.

The second approach to vaccines is to design immunogens that can stimulate specific precursor B-cells that can evolve into bnAbs. Thus, these immunogens target the non-somatic germline genes that first recombine to form the B-cell receptor (BCR) and aim to emulate the early stages of infection to initiate an immune response that has the potential to mature to breadth and potency over time[143–145,149,157,158]. This approach has arisen from analyzing structures of known bnAbs and engineering immunogens to bind germline versions of these bnAbs. Thus, vaccination occurs in stages, by priming bnAb precursors and then "guiding" these populations through subsequent vaccinations with immunogens that progressively drive somatic hypermutation toward bnAbs. The most advanced demonstration of these efforts has been shown by Schief and colleagues who have used state-of-the-art animal models and analytics, such as next generation sequencing (NGS), to study immune responses in great detail[159,144,160,161]. The germline targeting approach has two major obstacles. First, not all individuals necessarily make a high frequency, or any at all, of the naïve B-cell populations to be targeted[162,163]. Encouragingly though, some germline bnAb precursors have been found in naïve humans at low but sufficient frequency to initiate a response[144,164,165]. Indeed, as more bnAbs are found against particular epitopes, the frequency of the particular germlines in the naïve population can be taken into account in germline-targeting approaches. Additionally, longitudinal studies of the sequences and structures of bnAb evolution can provide clues as to how to better design immunogens that shepherd bnAb responses[166–171]. The second hurdle of this approach is that targeting human germline genes is difficult to test in animal models. Here, humanized and knock-in mice have provided a valuable tool for at least providing proof-of-concept for this approach[172–174].

The B-cell origins of broadly neutralizing mAbs tend to be restricted to particular germline sequences. For example, the CD4 binding site bnAbs of HIV and the stem-directed bnAbs of influenza are often constrained to particular germline genes, namely V_H1–2/V_H1–46[32,167,175] and V_H1–69[108], respectively. Interestingly, broadly potent V_H1–69-derived nAbs are also found against a variety of other viral epitopes, including those found on ebolaviruses[73,79,115,116] and HIV-1[32,167,176,177]. This commonality does not have an obvious underlying reason other than the CDRH2 of V_H1–69 antibodies has a hydrophobic tip that can insert into hydrophobic surfaces in viral antigens, particularly fusion domains[23,81,108,109]. The prevalence of V_H1–69 does suggest a possible conserved immunological approach to targeting enveloped pathogens, which have evolved alongside humans for millennia[111,112]. Conversely, immune responses are often very diverse, particularly within a single patient, as observed in those infected by filoviruses[24,115,116]. In these cases, SHM tends to be low, and the polyclonal response may be more vital to overcoming more acute filoviral or seasonal influenza infections with no or limited antigenic

variability. Conversely, effective responses to the hypervariable HIV reflect the longer exposure to the virus, where SHM is high within one or more predominant clonal lineages of antibodies. Influenza virus infection represents an acute infection but can occur in an individual multiple times over a lifetime through seasonal variants, and antibody responses to seasonal strains are a mixture of recall and *de novo* antibody responses[85]. Stem antibodies have greater promise for neutralization breadth due to conservation in the stem of HA, but are restricted to only a few antibody lineages, although not restricted to VH1–69. These lessons from natural infection provide important clues as to how to approach vaccine and therapeutic design, and can be tuned to the particular virus.

Vaccine and therapeutic development for filoviruses has experienced a surge in research due to a recent and unprecedented outbreak in Western Africa from 2013–2106[178]. Outbreaks of filovirus infection are relatively rare and most often isolated to sub-Sahara Africa; therefore, there was little information on the antibody-based response to exposure. Prior to the 2013 outbreak, there was evidence to suggest that neutralizing immune responses to infection were rare and that immunotherapy may not be possible or that vaccination may be difficult[179]. However, several studies that appeared during the last major outbreak showed that humanized mouse-derived mAbs, when used in combination, could provide protection. Two of these therapies, ZMab[180] and MB-003[181], were recombined into the tri-mAb cocktail ZMapp™,[182], which demonstrated complete protection in non-human primates at late stages of infection[183]. Conducting trials of ZMapp™ were met with some difficulties due to the waning outbreak, and demonstration of protective efficacy in humans could not be fully substantiated[184]. One vaccine trial, however, did show great promise[185] and there is evidence for long-term sustained protection in survivors of natural infection[186]. A new outbreak of EBOV in 2018 in the Democratic Republic of the Congo has seen the use of ZMapp™ as well as another tri-mAb cocktail from Regeneron [187] and as the single antibody mAb114[188] and all three of these treatments are being evaluated in clinical trials in an effort to produce an FDA-approved treatment for EBOV infection.

Many new anti-Ebola Abs have been recently described[24,65,71,79,115,118], including 26 new structures of antibodies/GP complexes that delineated major sites of vulnerability including the IFL, glycan cap, base, head and HR2 region of GP[189]. These studies demonstrate the polyclonal diversity of anti-filovirus antibodies. Additionally, several human-derived antibodies have been isolated that show broad cross-reactivity across ebolaviruses or marburgviruses and are actively being evaluated for use as immunotherapies. As for HIV and influenza, these bnAbs will likely be more restricted to particular germlines or sets of germlines. By understanding the mechanism of broadly neutralizing antibodies, it is hoped that this information will lead to pan-filoviral therapies that could replace the single species-focused ZMapp™, which would not be effective against the many other filoviral species that are pathogenic to humans.

Humanized mice offer an alternative platform to study for antibody reponses than human survivors of pandemic and epidemic viral infection, A recent study in which VelocImmune mice[190] were vaccinated with either DNA-encoded or soluble EBOV GP (Makona variant) demonstrated that fully human IgGs could be produced in mice that bind to common sites of vulnerability on filoviruses that are targeted by the human immune system. Remarkably,

these antibodies provide comparable protection to other therapies, including the ability to engage human Fcγ receptors, providing some level of protection through non-neutralizing means, similar to c13C6 from ZMapp™[183]. This study shows that, in principal, these mice can be vaccinated against any pathogen to produce antibodies that may offer protection in humans, but on a much faster timeline, making them an attractive option to learn how to combat other emerging infectious diseases.

Future perspectives

The plethora of structural information that has been generated in the past decade has opened many doors for understanding how the adaptive immune system recognizes and neutralizes enveloped and other viruses, resulting in exciting new vaccine and antibody therapeutic development opportunities. One trait that is shared amongst nearly all neutralizing antibodies examined thus far and reviewed here is the restricted range of angles (90°) of approach to viral spikes (with respect to the viral membrane). The majority of neutralizing antibodies approach at a steeper angle nearly perpendicular to the membrane (PG9, PGT145, CAP256, CH01–4, VRC38, C05, 13C6), while others approach at a nearly parallel angle to the membrane (VRC01, FI6, CR8781, 10E8, KZ52, 4G7, ADI-16061). These allowable angles then likely reflect the window in which naïve B-cells receptors can successfully engage the viral membrane-bound antigen for the prolonged periods required for activation, with the viral membrane a providing some constraint and limiting angles beyond 90°. Upward angles are unfavorable for soluble IgGs as the membrane provides a steric constraint for approaching glycoproteins for binding[141], although strain-specific neutralizing antibodies that approach GP at such an angle are not unheard of for filoviruses[61,182,189]. Additionally, the density of viral spikes on the surface of a virus can facilitate bivalent binding, which is possible for GP[191] and HA[38,51,52,192], but perhaps less so for HIV, where the spike density can be quite low[75,193–195]. In this case, engineering bivalent binding within a single antigen may be an effective approach to overcoming lower monovalent binding affinity to broadly reactive epitopes[195,196].

The examples above demonstrate the near infinite capacity of the adaptive immune system to evolve in response to diverse antigenic insults that humans and animals encounter. The diverse epitopes targeted by acute ebolavirus infection[115] demonstrate how a polyclonal antibody response with low SHM can be very effective, while the incredible breadth and potency of monoclonal bnAbs isolated from chronically infected HIV patients reveal the extremes of SHM that antibodies can accommodate to overcome huge antigenic diversity[197–200]. Despite this adaptive potential, the immune system still has a difficult time to keep pace with antigenically variable viruses like influenza and HIV that have high mutations rates[94]. Superficially, one would expect influenza to be an easier target for antibodies, particularly with yearly boosts via seasonal infection or vaccination. Yet, bnAbs are rare and typically do not persist and means that the world-wide human population is under constant threat of a new influenza pandemic. On the other hand, ebolaviruses may well be a relatively easy target for the adaptive immune system, but its spectacularly rapid pathogenesis normally results in mortality before effective antibodies can made. We sometimes take for granted the wonderful arsenal of vaccines that have already been developed, largely by empirical methods, and which result in lasting immunity with

impressive potency. Of course, most of the viruses for which these vaccines are aimed at have little variability. Ironically, we have a relatively limited understanding of the sequence and molecular of antibody responses to historical vaccines and a much greater understanding of the antibody responses to pathogens that continue to outpace current vaccines. Even then, the monoclonal antibodies that have been successfully isolated and structurally characterized are almost certainly underrepresent the true diversity of immune responses. Thus, the pursuit of new antibodies, and therefore new pathways to bnAbs, remains highly valuable. Understanding polyclonal responses to large antigenic surfaces during infection/vaccination [29] also holds future promise for improving vaccines that increase on-and decrease off-target responses.

As noted above, structure-based design for HIV has already generated some encouraging results in animal models that demonstrate it is possible to drive the path of antibody evolution towards a neutralizing but not a broadly neutralizing response at present by vaccination with candidate immunogens[144,145,159,164,165,173]. Here, vaccine design increasingly benefits from a deeper understanding of the basic biological processes that happen in B-cell germinal centers[165,201,202], as well as antigen display and uptake.

There is also renewed interest in the role of the innate immune pathways in antibody-based protection, as well as the role of non-neutralizing antibodies[118,203–206]. Fc-mediated protection has been shown to play at least some part in providing protection from all viruses discussed here. Even neutralizing antibodies have often been found to rely on some level of Fc-mediated function to realize their full potency[207,208]. While there is a basic understanding of the antibody-based innate immune response[209–212], there is still much to be learned about the subtleties of the molecular nature of effector cell activation. Future studies to address the role of Fc-mediated protection in individuals that effectively control HIV replication, as well as in those that survive filovirus infection, will enhance vaccine and therapeutic research. A more detailed molecular understanding of the immune activation complex, and what type of antibody-antigen interaction results in a potent innate immune response, could help to improve antibody therapeutic selection and engineering. Further, these types of studies may provide information that will guide antibody designs that can specifically recruit effector cell subsets and immune responses, such as NK cells and ADCC, which have shown great promise in augmenting antibody neutralization[213].

Based on the incredible advances described above, it is conceivable that, in the not so distant future, we will be able to rationally design vaccines that elicit antibodies with epitope specificity and broad antigen reactivity. An exciting new challenge will be the design of vaccines that also elicit antibodies that can also potently and specifically recruit desired effector functions. Integrating lessons from different viruses, including those described here and others, will continue to provide insights to arm researchers in their quest to vanquish the most formidable of pathogens.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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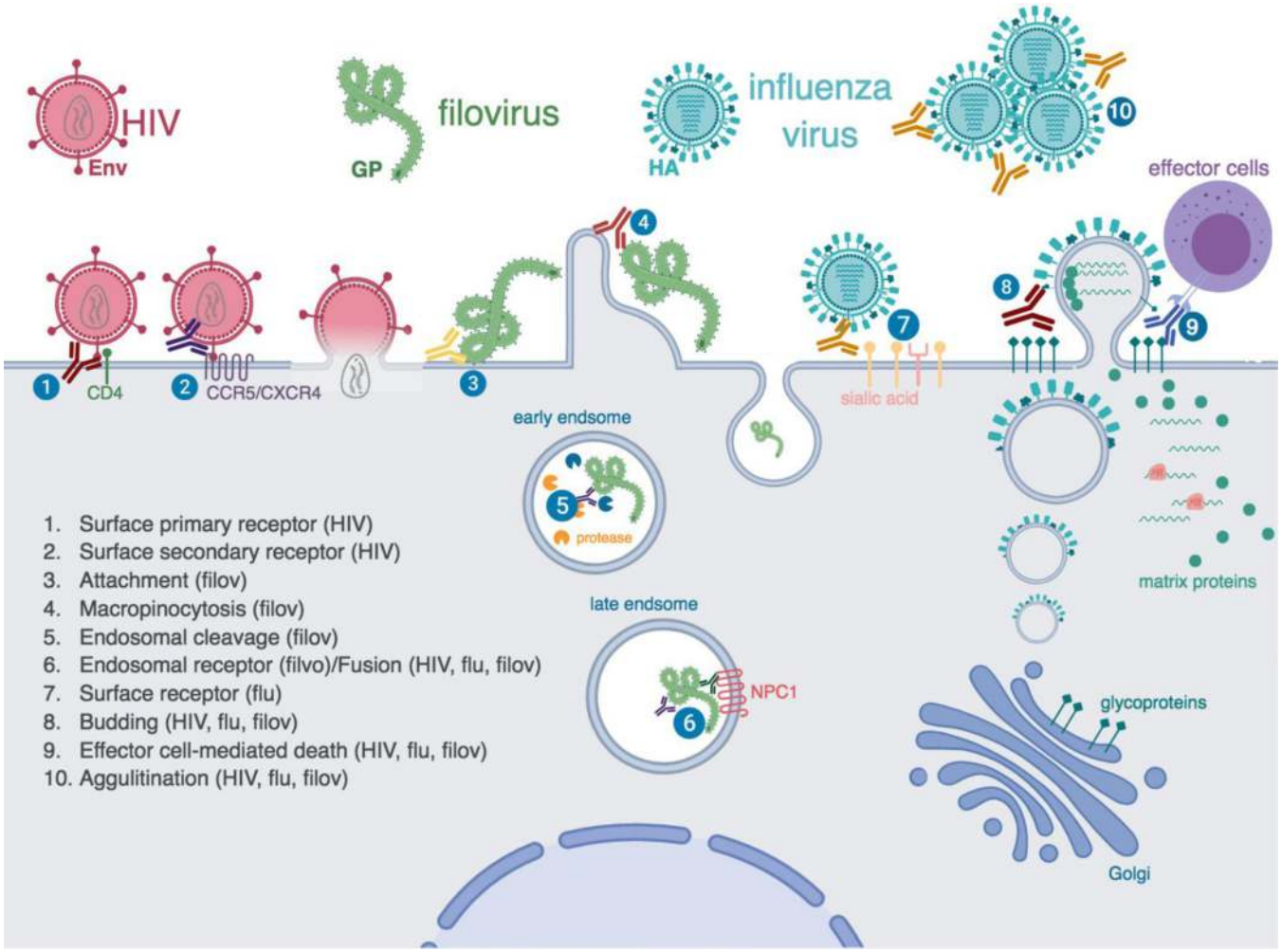

Figure 1. Points of antibody blockade to enveloped virus entry and egress.

Like a wrench thrown into the gears of a machine, antibodies are able to neutralize or protect against viruses by blocking one or more biological processes during viral entry or exit. The mechanism of neutralization depends largely on which epitope the antibody targets, and this subsequently determines which process is slowed or inhibited completely. For HIV, antibodies may block primary CD4 receptor (1) or secondary (2) CCR5/CXCR4 co-receptor binding. Filoviruses and influenza do not enter cells at the surface, but may be blocked by preventing attachment (3) or pinocytosis (4). For filoviruses, after endocytosis, antibodies must stay attached and survive acidification and subsequently prevent endosomal cleavage (5) or block endosomal receptor binding (6). For influenza, receptor binding can be blocked at the cell surface (7). In all cases, if receptor binding occurs, antibodies may directly block fusion through binding to fusion machinery. If viruses enter the cell, antibodies can potentially block egress of progeny virus (8). Non-neutralizing pathways to antibody-based protection include tagging cells for destruction by effector cells before viruses have a chance to exit (9), for example by antibody-dependent cellular cytotoxicity (ADCC) or by complement, or through agglutination of virions which can then be destroyed by effector

cells (10). Additional abbreviations for human immunodeficiency virus 1, influenza virus and filovirus are HIV, flu and filov, respectively. This image was made using BioRender.

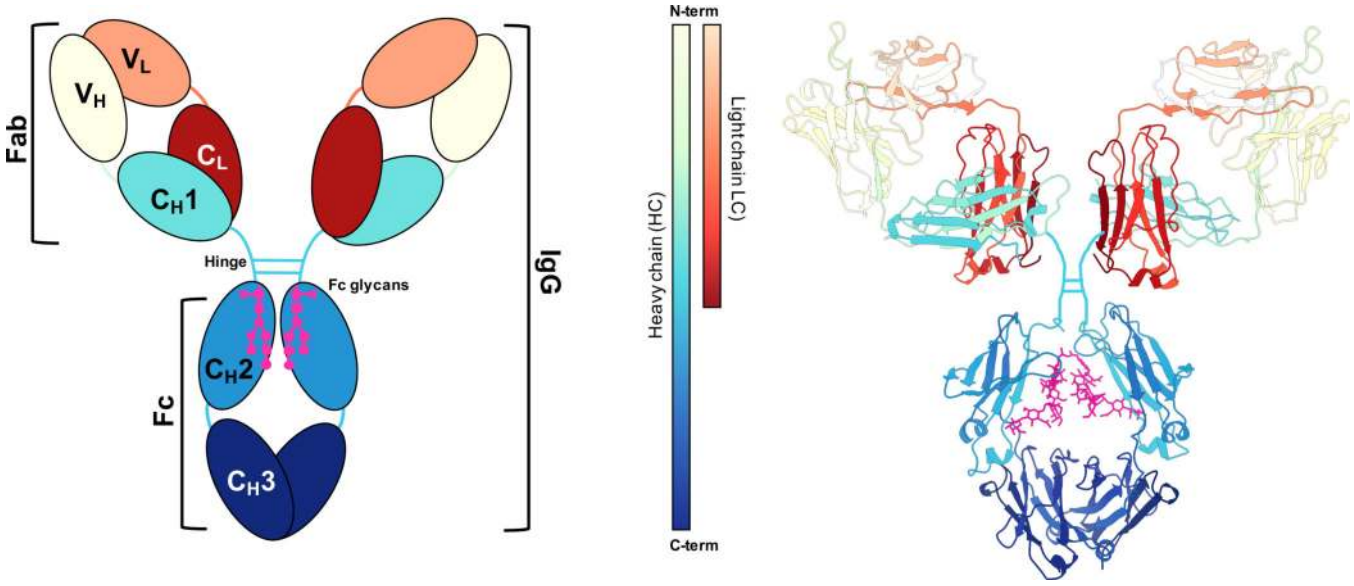

Figure 2. Antibody structure and domain topology.

When discussing antibodies in the context of viral immunity, we are generally referring to the IgG isotype, and particularly IgG1, which makes up the majority of serum antibodies and is primarily responsible for protection against infection. IgGs are heterodimers with two identical heavy chains (HC) and two identical light chains (LC) linked by disulfide bonds at a flexible hinge region that separates the Fab domains and Fc domain. The HC, named for its larger size, is a single polypeptide that contains four Ig domains, including the two Fab HC domains (V_H and C_H1), the hinge-region, and the Fc domain (C_H2 and C_H3), while the LC is composed of two LC Ig domains (V_L and C_L). The Fc region is responsible for linking antigens to effector cells and communicating their presence to the host through binding to FcγRs on effector cells, which exist in a variety of isotypes and are expressed at varying levels and compositions depending on the particular effector cell and activation state.

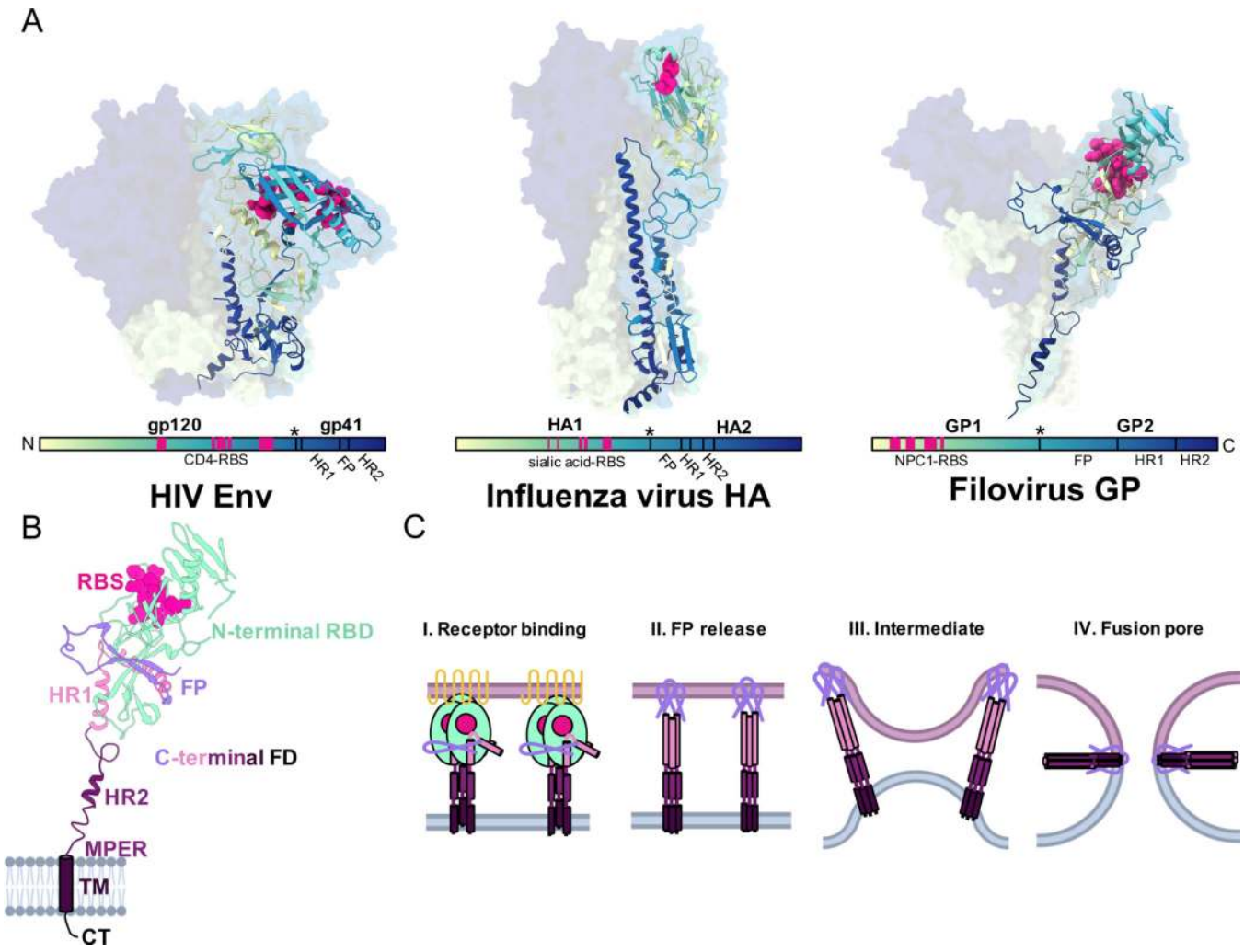

Figure 3. Shared structural features of type I glycoproteins.

A) The glycoproteins of HIV (PDB 5V7J), influenza (PDB 3KU3) and filoviruses (PDB 5JQ3) are widely divergent in sequence, size and shape but do share common structural features that are conserved in type I fusion mechanisms of enveloped virus entry. Here we show representative structures of glycoproteins from each of these viruses that are gradient colored from the N-terminus (light yellow) to the C-terminus (dark blue). This allows a comparison of where potential common sites of vulnerability exist on each of these structures in relation to divergent viruses, including the RBS, fusion loop, and stem. B) The sub-domain architecture of a type-I viral fusion protein is shown with this example of a $GP_{1,2}$ subunit of Ebola virus GP (PDB 5JQ3). The N-terminal receptor binding domain (RBD, light green) is positioned above the C-terminal fusion domain (FD) and houses the receptor binding site (RBS, magenta). The FD contains a fusion peptide (FP, lavender), two heptad repeats (HR1 in pink and HR2 in purple), a hydrophobic membrane proximal external region (MPER, dark purple), and a transmembrane (TM, dark purple) anchor and C-terminal tail (CT, black). C) Type-I membrane fusion occurs in distinct stages. I) N-terminal RBDs bind to their cognate receptors, beginning the process of fusion. II) Receptor binding releases the FP, which pierces the host membrane and causes HR1 and HR2 to form an

extended 3-helix bundle. III) During intermediate stages, it is thought that groups of viral spikes cluster to induce membrane buckling, allowing viral and host membranes to come close to each other. IV) Finally, HR2 collapses upon HR1, forming a 6-helix bundles that draws the TM domains together with the FPs, causing mixing of host and viral membranes and the formation of the fusion pore, which then permits the viral genome to exit into the host cytoplasm.

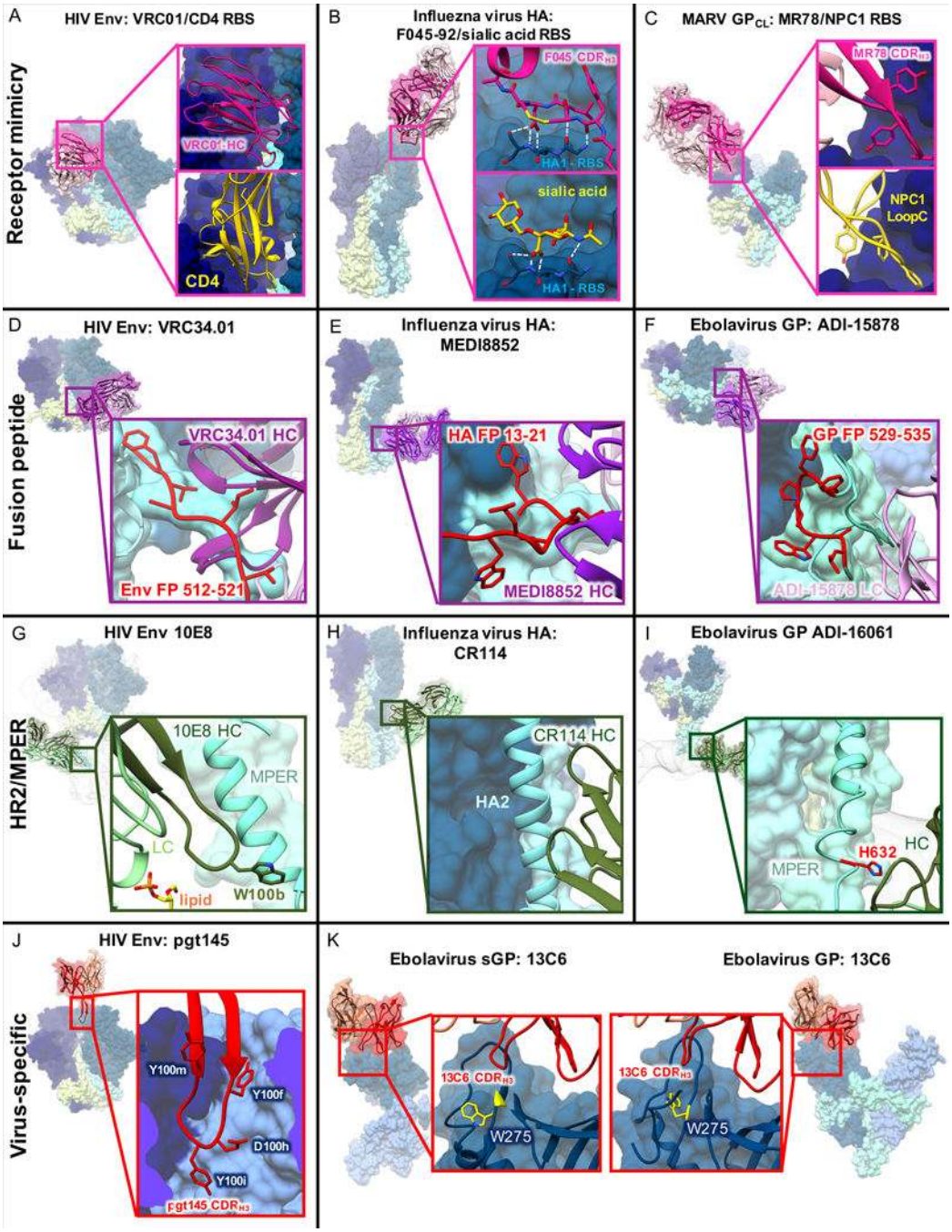

Figure 4. Examples of enveloped virus common and divergent sites of vulnerability targeted by neutralizing antibodies.

A) CD4 binding site (CD4bs) antibodies, such as VRC01 (PDB 5FYJ), bind in between HIV Env protomers using their HCs (top) and mimic the immunoglobulin fold (Ig) of the actual receptor (bottom). B) CDRH3 of F045–92 (PDB 4O58), and others like it, reach into the sialic acid binding pocket (top) and closely mimic the natural ligand (bottom). C) The antibody MR78 (PDB 5UQY) uses an extended hydrophobic CDR_{H3} (top) to bind to the NPC1 receptor binding site (RBS). D) The fusion peptide (FP) of Env sits near the base and is contacted largely by the HC of VRC34.01 (PDB 5I8H). E) The HA FP also sits near the

base on the HA stalk and similarly is contacted largely by the HC of MEDI8852 (PDB 5JW3). F) For ADI-15878 (PDB 6DZL), contacts are made across HR1 with the HC and the FP is mostly contact by the LC. G) The HIV antibody 10E8 (Env PDB 5V7J and 10E8 PDB 5T85 fit into EMDB 3312) has evolved to partially contact portions of the viral lipid membrane. H) HA stalk antibodies typically have the broadest neutralizing paratopes and CR9114 (PDB 4FQI) contacts large portions of the extended HA2 alpha-helix with its HC. I) bNab ADI-16061 (GP PDB 5JQ3 and example Fab PDB 5HJ3 fit into EMD 8698) binds far below the base of GP, contacting conserved hydropbobic residues within HR2 and the MPER. J) The long-term exposure of the immune system to HIV allows for extensive SHM and antibody evolution, producing antibodies like PGT145 (PDB 5V8L), with an extended CDRH3 that is rigidified by a beta-hairpin structure with hydrophobic residues and sulfated tyrosines. This allows the antibody to reach deep into the apex pocket of Env. K) Ebolaviruses have two glycoproteins, the viral GP trimer and the soluble GP (sGP) dimer, which is expressed in large abundance during infection and is thought to be a type of immune decoy. The first 296 amino acids of GP and sGP are shared and the protective antibody 13C6 binds to both GP (left, PDB 5KEL) and sGP (right, PDB 5KEN) near a highly conserved residue (W275).

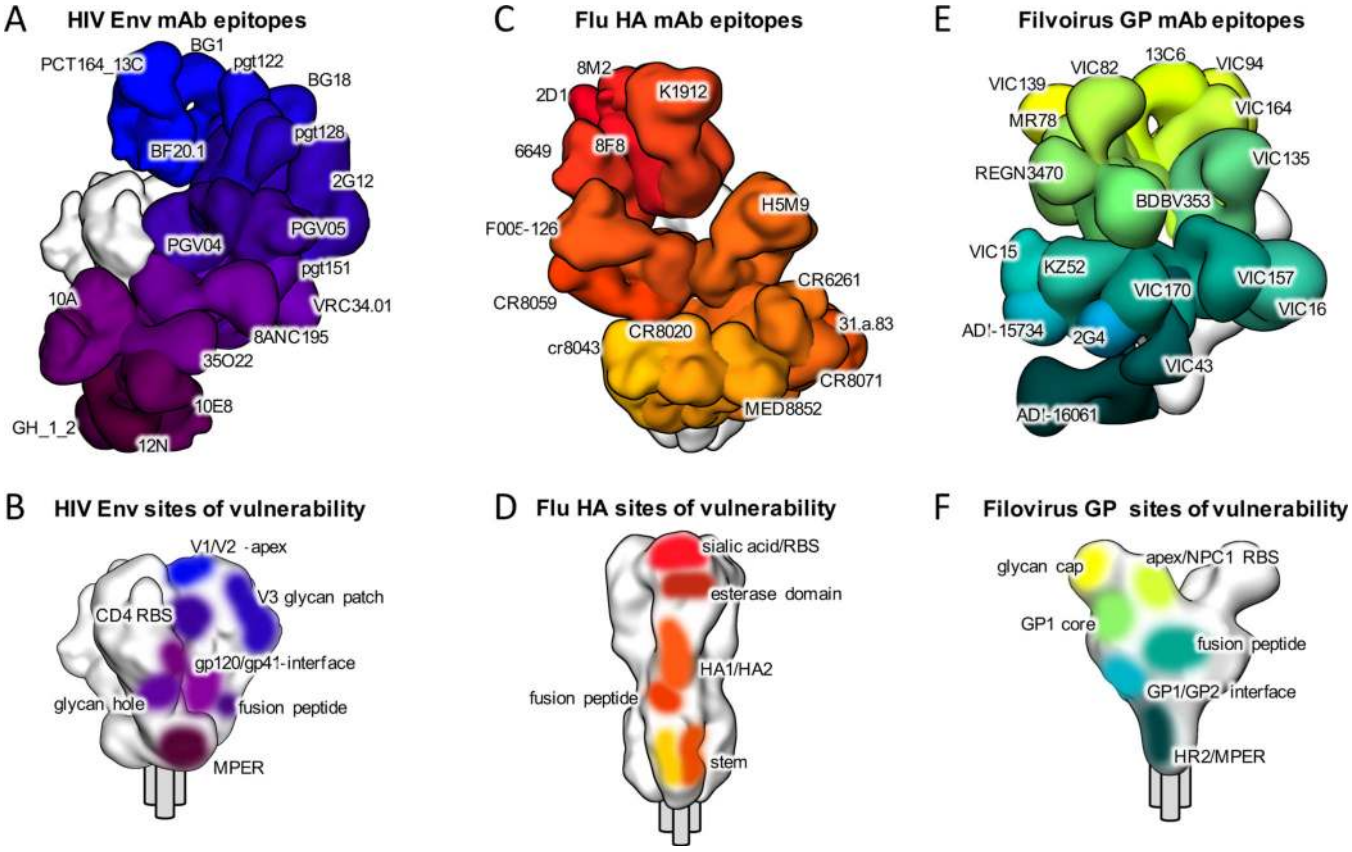

Figure 5. The immunogenic landscape of enveloped viruses illuminated by structural biology.

Overlaying low-pass filtered structures of selected nAbs bound to trimeric glycoproteins from A, B) HIV (blues and purples), C, D) influenza (reds and oranges) and E, F) filoviruses (yellows and greens) reveals the immunogenic landscape of these viral glycoproteins and the 90 degrees of approach angles that antibodies can utilize to probe the glycoprotein surface. Abs bound to a single protomer are shown for clarity. Stripping back these antibodies clarifies how these epitopes are focused into distinct regions of vulnerability, although nearly the entire surface is susceptible to nAb binding. Several of these sites of vulnerability are equivalent across these examples, including the apex, RBS, interface of RBS and fusion domains, FP and viral stem, which includes HR1, HR2 and the MPER domains.

Table 1.

Comparison of HIV, influenza and filoviral biology, taxonomy and pathogenesis.

Glycoprotein	**HIV**	**Influenza**	**Ebolaviruses**
Viral Spike Name	Envelope (Env)	Hemagglutinin (HA)	Glycoprotein (GP)
Receptor Binding Domain	gp120	HA1	GP1
Fusion domain	gp41	HA2	GP2
Predidcted Glycosylation sites			25
Protein size	1,195 amino acids	522 amino acids	667 amino acids
Known Receptor(s)	CD4/CCR5/CXCR5	sialic acid	NPC1
Entry strategy	cell surface receptor	endosomal acidification	endosomal
Classification			
Group	Group VI (+ssRNA-RT)	Group V (-ssRNA)	Group V (-ssRNA)
Order	*Unassigned*	*Unassigned*	*Mononegavirales*
Family	*Retroviridae*	*Orthomyxoviridae*	*Filoviridae*
Subfamily	*Orthoetrovirinae*	n/a	n/a
Genera	*Lentivirus*	*Alphainfluenzavirus* Betainfluenzavirus *Deltainfluenzavirus* *Gammainfluenzavirus*	*Ebolavirus* *Marburgvirus* *Cuevavirus*
Species	*HIV1* *HIV2*	*Influenza A* *Influenza B* *Influenza C* *Influenza D*	*Zaire ebolavirus* *Bundibugyo ebolavirus* *Reston ebolavirus* *Sudan ebolavirus* *Tai Forest ebolavirus* *Marburg marburgvirus* *Lloviu cuevavirus*
Pathogenesis			
Infection route	bodily fluids/blood	respitory	bodily fluids/blood
Latency	weeks to months	days	days
Duration	chronic	acute	acute
Yearly cases	1–2 million	9–50 million since 2010	varied (1–29,000)
Tropism	CD4+ T-cells	epithelial cells	universal

Glycoprotein	HIV	Influenza	Ebolaviruses
FDA approved treatments	40	3	none

[1]The complex biology of HIV, influenza and filoviruses is difficult to categorize and each respective field has developed its own jargon to describe each virus. Here, we summarize the basic aspects of each viruses' biology, taxonomy and pathogenesis to provide a perspective on their commonalties and divergence in a variety of categories relevant to the antibody-based immune responses. This table is not meant to convey all aspects of the viruses but rather to provide broad generalizations that allow some level of comparison.