

# Inhibitory Antibodies Targeting Emerging Viruses: Advancements and Mechanisms

Jing Jin, Graham Simmons

Blood Systems Research Institute, San Francisco, California, USA, and University of California, San Francisco, San Francisco, California, USA

From Ebola virus outbreaks in Western Africa to the introduction of chikungunya and Zika viruses in the Americas, new and neglected viruses continue to emerge and spread around the world. Due to a lack of existing vaccines or specific therapeutics, little other than supportive care and attempts to interrupt transmission can be provided during initial outbreaks. This has prompted a shift in vaccine design and development to identify novel epitopes and mechanisms of protection that may offer a broader range of protection against groups or whole families of viruses. Receptor-binding domains and other motifs within viral envelope proteins represent one excellent opportunity to target communal epitopes shared by related viruses. Similarly, for viruses where envelope participates in driving viral egress from infected cells, shared epitopes need to be identified to guide the development of broadly protective antibodies and vaccines. Here, we discuss recent advances in our understanding of broadly protective humoral responses for emerging viruses.

## RECENT EMERGING VIRUS THREATS DEMONSTRATE A NEED FOR NOVEL RESPONSES

Since 2012, there have been at least four major viral epidemics of infections by novel or reemerging pathogens, covering four different viral families. A novel pathogenic coronavirus emerged in 2012 in the Middle East, while the devastating outbreak of Ebola virus (EBOV) infections in three West African countries in 2013 signaled a new stage in the emergence of this highly pathogenic virus and demonstrated that, in an age of international travel and increasing urbanization, infectious agents can rapidly spread and establish epidemics in new regions. Similarly, first chikungunya virus (CHIKV) rapidly spread throughout much of the Americas in 2013 to 2014 and then Zika virus (ZIKV) did so in 2015 to 2016. All four epidemics were foreshadowed by previous outbreaks of related viruses, demonstrating that, while it is impossible to accurately predict the nature of new epidemics, preparations can be made. The apparent success of antibody (Ab) cocktails, such as that of Z-Mapp during the 2013 EBOV outbreak, renewed interest in antibody-based therapeutics as antivirals and highlighted in particular the idea that development of broadly protective therapeutics would be beneficial. For example, stockpiling of immunotherapeutics able to treat EBOV would be more practical and attractive if the antibodies were able to offer protection against all filoviruses—particularly as the particular species may not be immediately identified. Likewise, it is unlikely that a CHIKV vaccine would be deployed *en masse*; however, employment of a pan-alphavirus vaccine able to stimulate protective or even partially protective responses to multiple pathogenic alphaviruses is a much more appealing prospect. ZIKV was a relatively neglected mosquito-borne flavivirus prior to establishing a foothold in Brazil in 2015, and the only likely circumstance under which specific treatments for controlling such an unstudied virus would have been in place would be one in which broadly acting therapeutics were available, including pan-flavivirus protective antibodies, pan-flavivirus inhibitors, or non-pathogen-specific immune therapies. In order to achieve broadly protective immunotherapeutics and vaccine immunogens, novel approaches are required to aid identification of important epitopes. Here, we focus on a discussion of

recent advances in the identification of broadly protective antibodies and epitopes.

## EFFECTIVE CROSS-REACTIVE RESPONSES TO FLAVIVIRUSES

A traditional approach to combating viral diversity between related viruses is exemplified by a new tetravalent vaccine for treatment of dengue virus (DENV) infections. This vaccine provides a degree of protective immunity against infections by DENV type 1 (DENV-1) through DENV-4 by inclusion of all four serotypes on chimeric attenuated yellow fever virus backbones. However, protection is not uniform across all the serotypes and requires three vaccinations across 12 months. The limitations seen with the tetravalent vaccine suggest that complementary approaches may be appropriate, and indeed, recent publications have highlighted the possibility of pan-serotype neutralization (1, 2). These findings suggest that both the engineering of single universal immunogens able to stimulate specific, cross-reactive responses and the effective use of passive immunotherapy with potent broadly neutralizing monoclonal antibodies (MAbs) may be possible. Thus, DENV can be held to be a model for development and optimization of novel therapeutics and vaccines for new and emerging viruses.

DENV belongs to the *Flavivirus* genus, which includes West Nile virus (WNV), yellow fever virus, ZIKV, and several other viruses that may cause encephalitis. Flaviviruses have a complex quaternary structure that can adopt different morphologies with a temperature shift. Furthermore, the viruses undergo a series of complex rearrangements in their surface glycoproteins (GPs)—both during virus maturation after virus assembly/budding in the endoplasmic reticulum and after viral entry in acidified endosomes. Flaviviruses consist of 180 premembrane (prM) and 180

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Address correspondence to Graham Simmons, gsimmons@bloodsystems.org.

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envelope (E) proteins that associate to give a total of 60 trimeric spikes on an immature virus. prM acts as a chaperone, sitting over the fusion loop of E and preventing premature triggering. As the virion exits the cell, prM is proteolytically processed, allowing the E protein to rearrange into 90 head-to-tail homodimers. Conversely, during viral entry, following absorption into cells mediated by domain III of the E protein, flaviviruses are endocytosed and traffic to acidified endosomes. E homodimers are triggered by low pH to rearrange into homotrimers, exposing the hydrophobic fusion loop in domain II of the E protein and driving membrane fusion and viral entry. The primary humoral immune response to DENV is dominated by antibodies targeting prM and domain I and II of E. Many of these antibodies are cross-reactive in that they can bind multiple DENV serotypes. However, they do not represent broadly protective responses because they lack neutralization potency, while possessing the ability to promote antibody-dependent enhancement (ADE) (3, 4). In contrast, domain III of E (EDIII)-specific antibodies constitutes a minor component of the overall human humoral response but may be potentially neutralizing. Recently, Robinson et al. used structure-guided design to optimize an existing EDIII antibody (1). Starting with a potent MAb that targeted a conserved epitope within DENV EDIII, a network map of individual interactions between the paratope and epitope was built. Through experimental mutation of the paratope, in particular, within the complementarity-determining region (CDR)-H1, followed by further structural analysis, the antibody was optimized to maximize affinity. This gave rise to Ab513, a potent pan-serotype MAb that neutralizes all four serotypes with reduced virus-enhancing activity compared to the parental antibody. Importantly, the use of Ab513 is therapeutically effective in animal models of DENV pathogenesis. Such studies have offered an exciting roadmap for careful crafting of paratope/epitope interactions using structure-guided design not only to maximize affinity but also to broaden specificity. For such studies, it is critical to have high-resolution structures of viral particles and/or envelope proteins in native conformations. It is also helpful to have extensive mapping of the antibody-accessible envelope surfaces with large panels of MAbs.

Complex quaternary epitopes are targeted by neutralizing antibodies (NAbs) to prevent flavivirus entry. For example, a recent report of a study of a potent DENV-3-specific MAb described an epitope spanning three separate E protein monomers on the virion (5). Not only do MAbs to these epitopes lock multiple important domains of E protein in place and thus prevent conformational rearrangements, they also require only 60 Fab fragments to fully coat the virus surface due to their unique footprint. Due to the complex quaternary structures targeted by these MAbs, the majority are serotype specific. However, a recent report from Dejnirattisai et al. described the isolation of broadly neutralizing antibodies to DENV (2) based on the complex and highly organized structure of the E protein. These MAbs were shown to target an epitope spanning both E proteins of an E protein dimer on partially mature virus—an epitope that resembles the binding site of prM (6). Antibodies to these E dimer epitopes (EDE) were broadly neutralizing across dengue serotypes and suggest that this epitope may make an ideal immunogen for stimulating a broad, protective response.

The fusion loops of flaviviruses are relatively conserved, and antibodies targeting the fusion loop epitope (FLE) are generally cross-reactive and are able to neutralize different flaviviruses. The

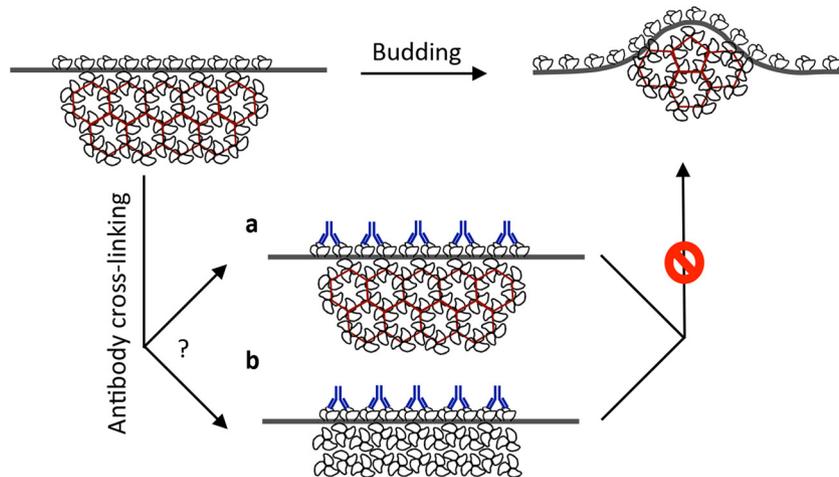
FLE-specific antibodies usually have a lower neutralizing capacity and demonstrate higher ADE than the EDE-specific antibodies (7). A FLE-specific cross-neutralizing antibody, 2A10G6, can neutralize ZIKV and provide *in vivo* protection against ZIKV (8). The structure of ZIKV E protein in complex with 2A10G6 indicates that the antibody binds to immature or partially immature virus particles but not to fully mature virus particles in which the fusion loop is buried in a compact E-dimer arrangement. Interestingly, analysis of cryoelectron microscopy (cryoEM) structures of ZIKV has suggested that ZIKV particles are thermally stable and largely present in the mature form (9). It will be interesting to solve the structure of ZIKV virus particles in complex with 2A10G6 in order to reveal how this FLE-specific antibody neutralizes ZIKV.

### ALPHAVIRUS NEUTRALIZATION

Similarly to flaviviruses, alphavirus envelope glycoproteins (E1 and E2) form icosahedral structures. A total of 240 copies of the E1-E2 heterodimer assemble into 80 spikes on the virion surface. E1 is a class II membrane fusion protein and sits at the base of the trimeric spike, with E2 positioned on top of E1. A fusion loop is located at the distal end of E1 domain II and is protected by E2 domain B at neutral pH. E2 domain A is located in the center of the spike surface and possesses the putative receptor binding site. In acidified endosomes, low pH triggers conformational rearrangements in the envelope glycoproteins. E2 domain B dissociates from the tip of E1 domain II, exposing the fusion loop. E1 then forms a homotrimer, further exposing the fusion loop for virus fusion with the endosomal membrane. In analogy to conformation-dependent anti-flavivirus MAbs, we, and others, have described similar epitopes in alphaviruses that target complex quaternary epitopes and bridge domains to prevent low-pH-induced conformational changes. Thus, for viruses with class II membrane fusion proteins, the development of domain-spanning antibodies may be a highly effective method of protection. Using single-particle cryoEM, we identified epitopes that span domain A and domain B of two separate E2 glycoproteins within one viral spike on the CHIKV particle (10). Similarly, other studies of anti-alphavirus MAbs have shown bridging of domain A and B within the same E2 glycoprotein or cross-linking of E2 proteins from neighboring spikes (11–13). MAbs to these epitopes act to “lock” the conformation of the mature glycoprotein in place by cross-linking domains, thus preventing the low-pH-triggered conformational rearrangements required to expose the fusion loop. Interestingly, a panel of mouse MAbs with broad specificity against multiple alphaviruses were recently identified (14). These cross-reactive MAbs bind to a conserved epitope on E2 domain B and cause conformational rearrangements that reposition E2 domain A, enabling cross-linking of two E2 molecules in neighboring spikes. The epitope targeted by these MAbs is conserved across arthritogenic alphaviruses and led to broad inhibition of multiple alphaviruses by the panel. The results of that study suggest that the conserved B domain antigenic determinants could be targeted against multiple alphaviruses of global concern for vaccine or antibody therapeutic development.

### NEXT-GENERATION FILOVIRUS IMMUNOTHERAPEUTICS

Besides flaviviruses and alphaviruses, another major focus of emerging virus research is humoral responses to EBOV and other filoviruses. Many descriptions of potent neutralizing MAbs and effective immunogens for invoking protective responses have



**FIG 1** Model of virus budding inhibition by antibody cross-linking. Neutralizing antibodies cross-link viral spikes at the cell surface to (a) prevent assembly of pentagons or (b) cause disassembly of spike hexagons.

been published recently. The studies described included several attempts to identify novel MAbs and epitopes able to effectively neutralize the five EBOV-like species and even the more distantly related filoviruses (15–17). The results of analyses of these antibodies emphasize a possible pathway to achieving broadly neutralizing antibodies. In particular, the receptor-binding site (RBS) of most viruses is relatively conserved, due to the requirement for engaging a host cell target. Indeed, all filoviruses, including the diverse Lloviu viruses (LLOV), utilize the same receptor, Niemann-Pick C1 (NPC1), for viral entry. This results in an attractive target for pan-filovirus protective responses and antibody cocktails for use in passive immunotherapies. However, to protect this potential Achilles heel, EBOV and other filoviruses occlude the conserved RBS in several unusual ways (18). First, the EBOV glycoprotein (GP) open reading frame actually encodes a non-structural secreted protein, termed sGP, with a cotranscriptional editing event required to give rise to full-length membrane-bound GP. It is hypothesized that the secretion of large amounts of sGP acts as an immunological decoy, leading to poor neutralizing responses. Second, the membrane distal region of the GP is associated with extensive N- and O-linked glycosylation and thus acts as an immunodominant domain but is not required for functional GP. Finally, following attachment and endocytosis, the virus is trafficked to acidified endosomes. Unlike many other viruses requiring endocytosis, EBOV is not directly pH dependent; rather, GP requires proteolytic activity of pH-dependent host cell proteases in order to remove the glycosylated membrane distal domain and expose the RBS. The RBS then engages NPC1—a cholesterol transporter found only in late endosomal membranes. This unusual mechanism of exposing the RBS within endosomes likely helps protect the virus from neutralizing antibodies targeting this conserved region. Crystal structures of proteolytically processed GP in complex with NPC1 demonstrate a two-step process, with the engagement of a hydrophobic trough in primed GP leading to the conformational rearrangements required to drive membrane fusion (15, 19). The hydrophobic domain in the head of primed GP is highly conserved across the filovirus family, and MAbs directed against this cavity are broadly neutralizing against all filoviruses, although potency varies (15). Thus, the domain is a

highly attractive immunogen for potent and pan-filovirus responses.

#### NOVEL TARGETS FOR ANTIBODY INHIBITION

The majority of the MAbs and epitopes described above rely on *in vitro* measures of efficiency. The canonical antiviral mechanism of antibodies is to neutralize virus entry, and analysis of that mechanism traditionally serves as the criterion for antiviral antibody selection. However, for many viruses, *in vitro* potency does not always equate to protection *in vivo*, and functional activity can indeed be demonstrated only *in vivo* for some antibodies. In addition to neutralization, many other factors are involved in antibody control of acute viral infections, including complement and other mechanisms targeting infected cells, as well as antibodies targeting specific pathogenic functions, such as anti-DENV NS1 MAbs. Thanks to advances in various techniques, including single-particle cryoEM, high-throughput antibody repertoire sequencing, and B cell cloning, our understanding of the details of the entry-blocking mechanisms for neutralizing antibodies against almost all virus families, from inhibition of receptor binding to prevention of conformational changes within fusion proteins, has greatly improved. As discussed above, knowledge garnered from these mechanistic studies can successfully guide therapeutic antibody (1) and vaccine design (20).

In our recent studies on the mode of action of a panel of human and mouse MAbs targeting CHIKV E2 glycoprotein, we discovered a novel antiviral mechanism in addition to their classical neutralization of virus entry. Postentry addition of MAbs to CHIKV-infected cells inhibits CHIKV release via bivalent binding, in contrast to the virus entry neutralization by monovalent Fab fragments of these MAbs (10, 14). We demonstrated inhibition of viral release by two human MAbs targeting CHIKV E2 domain A (10) and by a panel of mouse MAbs targeting an epitope within E2 domain B (14). As described above, these domain B MAbs are broadly active against arthritogenic alphaviruses. A nonneutralizing MAb targeting CHIKV E1-domain III was also reported to inhibit CHIKV release (21). Structural studies of human MAb-treated CHIKV-infected cells revealed a block to membrane curvature formation by cross-linking MAbs, leading to the

arrest of assembled nucleocapsid cores beneath the plasma membrane (J. Jin and G. Simmons, unpublished data). This suggests that lateral organization of viral spikes provides the pulling force for CHIKV budding and is disrupted by MAb cross-linking (Fig. 1). This mechanism is different from previously reported mechanisms seen with antibodies able to block viral egress. For example, antibodies can target influenza virus neuraminidase activity required for viral release (22), while nonneutralizing antibodies targeting matrix protein 2 also inhibit viral egress (23), possibly by interfering with virion assembly and budding, and have led to the development of matrix protein 2 as a possible vaccine candidate. Two nonneutralizing Marburg virus glycoprotein-specific MAbs have been reported to prevent virus release by inducing aggregation of progeny virus particles at the cell surface (24). It will be interesting to discover how CHIKV NABs targeting different domains cross-link glycoprotein at the plasma membrane in order to disrupt the critical glycoprotein organization required for membrane bending. For example, the identification of budding specific epitopes would aid the design of therapeutic antibodies and vaccine immunogens for alphaviruses, in a manner similar to that seen with epitopes critical for viral entry. The phenotype of inhibition of viral release by MAbs was also observed for viruses from viral families other than alphaviruses, including bovine leukemia virus, herpesvirus, vaccinia virus, and rubella virus (10), but no mechanism was resolved for these reported phenotypes. It would be interesting to determine whether glycoprotein cross-linking MAbs can block viral budding for those viruses that need glycoproteins to drive membrane curvature formation.

Novel therapeutic and prophylactic answers are required in order to combat a plethora of emerging and reemerging pathogens. Fortunately, the identification of innovative targets and viral pathways reveals new ways to gain humoral protection. Together with novel methods for optimizing and specifically targeting immunotherapeutics and vaccine immunogens, an arsenal of broadly protective measures can be built against emerging viruses.

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