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Potent Antibody Protection against an Emerging Alphavirus Threat

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Chikungunya virus recently caused large outbreaks world-wide. In this issue of *Cell*, Fox et al. describe several potently neutralizing antibodies against multiple alphaviruses. The structure of the virus in complex with one of the antibodies reveals the antibody-induced rearrangement and crosslinking of the viral surface proteins that result in neutralization.

The alphavirus Chikungunya virus (CHIKV) was originally isolated in Africa. Although it caused sporadic large outbreaks in Africa, it largely flew under the scientific radar. In 2004, CHIKV emerged as a global pathogen when it generated a much larger pandemic of millions of cases and a number of deaths in countries around the Indian Ocean (Schwartz and Albert, 2010). CHIKV was first reported in the Americas in 2013 and since then has spread rapidly in the New World, with more than a million cases in at least 43 countries, including the United States (Johansson, 2015). CHIKV is transmitted by mosquito vectors, and the emergence of this pathogen has been fueled by its adaptation to new mosquito species and by the spread of these vectors into new areas. CHIKV causes fever and arthritis, with resultant joint problems that can linger for years. There are currently no licensed vaccines or antiviral therapies for CHIKV or for other alphavirus pathogens, including Eastern and Western equine encephalitis viruses. Those two viruses are endemic in the United States, where they cause low numbers of cases but have high fatality rates. A better understanding of the immune response against the array of alphavirus pathogens would promote the development of necessary vaccines and immunotherapeutics. Given the high mutation rate of RNA viruses, broadly reactive antibody strategies may be of particular importance.

In this issue of *Cell*, a multidisciplinary team of investigators led by Michael Diamond screened a panel of mouse and human monoclonal antibodies raised against CHIKV and found that, of the 60 that neutralized CHIKV, 19 also bound to other alphaviruses such as the African O'nyong'nyong virus (86% identical to CHIKV) and South American Mayaro virus (60% identical to CHIKV), with differing abilities to neutralize these disparate viruses (Fox et al., 2015). The most potent of these broadly reactive mAbs, termed CHK-265, also protected mice from CHIKV, O'nyong'nyong, and Mayaro virus challenge.

Alphaviruses are small enveloped viruses with highly organized structures and infect host cells by receptor-mediated endocytosis and low-pH-triggered membrane fusion (Kuhn, 2013). On the surface are two transmembrane glycoproteins, the class II fusion protein E1 and the receptor-binding protein E2. These proteins are arrayed symmetrically on the viral surface to form 80 spikes, each a trimer of E2-E1 heterodimers. E2 has three domains with immunoglobulin-like folds: a central domain A, a distal domain B, and a membrane-proximal domain C. E2 covers much of the E1 protein on the viral surface and clamps the fusion loop at the membrane-distal tip of E1 between its domains A and B. E2 regulates E1's fusion activity, and a key step in fusion is the low-pH-triggered dissociation/rearrangement of the E2-E1 dimer, thus allowing E1 to insert into the endosome membrane and refold to the hairpin conformation that drives fusion (Gibbons et al., 2004). A first step in this process is the "uncapping" of the E1 fusion loop by the movement of the E2 B domain (Li et al., 2010; Voss et al., 2010). E2 is the principal target of neutralizing antibodies, which have been mapped to locations across its outer surface, while the E2 A and B domains are implicated in receptor interaction (reviewed in Voss et al., 2010).

Here, Fox et al., perform cryoelectron microscopy on CHK-265 Fabs in complex with virus particles. The resulting structures illustrate that binding of this potent neutralizing antibody occurs with a concomitant structural rearrangement of the envelope proteins.

CHK-265 is primarily directed toward domain B of the E2 protein, and binding of the antibody induces a slight rotation of domain B from its unbound position. Unexpectedly, binding of CHK-265 also causes a concomitant large repositioning of domain A up and out of each envelope trimer, involving a $\sim$20 Å translation and 70° rotation about E1 (Figure 1). Ultimately, each copy of CHK-265 bridges domain B of one spike to domain A of a neighboring trimer on the viral surface, with each Fab binding 19 residues of domain B and 4 residues of the neighboring trimer's domain A. The effect of CHK-265 binding is a cross-linking network across the virus surface. Fab fragments of CHK-265 were less potent than IgG, suggesting that the IgG could induce additional cross-linking and perhaps a steric blockage of viral entry as well.

Although the orientation of domain A is changed radically, E2's receptor-binding activity is unchanged. Notably, CHIKV still

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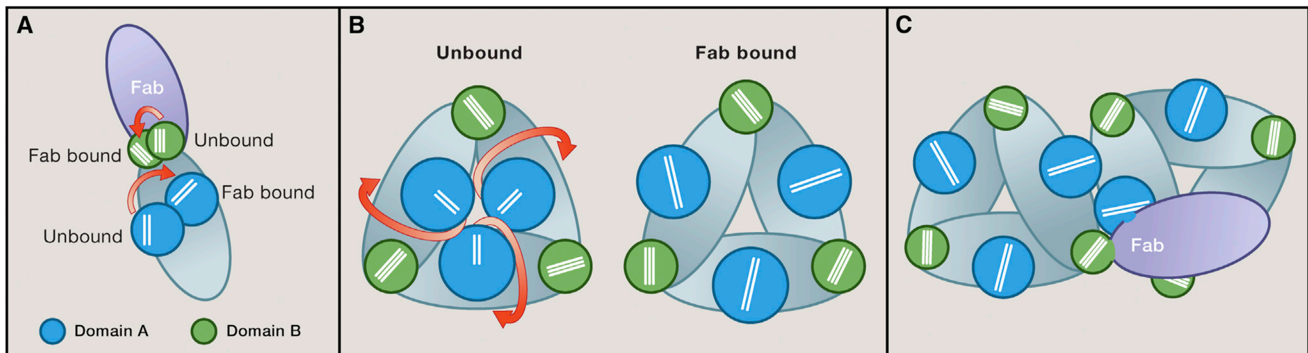

Figure 1. Antibody-Induced Rearrangements of Chikungunya Virus Envelope Proteins
(A) Binding of Fab CHK-265 induces a rearrangement of domains A and B of envelope protein E2. The rest of E2 and the E1 protein are both colored as a single gray oval for simplicity. Illustrated is one monomer. White lines represent the axes of major β strands in the domain structure.
(B) Domain rotation illustrated for an envelope trimer, before and after binding of Fab CHK-265.
(C) Fab CHK-265 bridges domain A of one copy of E2 to domain B of a different copy of E2 belonging to a neighboring trimer. Figure: Christina Corbaci, TSRI.

attaches to cells in the presence of CHK-265, so the block achieved by this antibody is not at receptor engagement but, rather, at events downstream. CHK-265 partially inhibits virus fusion and partially inhibits egress: its total dampening of infectivity may result from a sum of separate functions at separate steps. Based on the structure, CHK-265 could function by inhibiting the uncapping step by domain B or by "clamping" the E2-E1 dimer to impede its dissociation. Alternatively, antibody-mediated crosslinking of adjacent spikes could inhibit more global rearrangements of the virus particle surface that occur during fusion, as has been previously observed for West Nile virus (Kaufmann et al., 2010).

Unlike viruses with structurally related fusion proteins, such as Dengue virus, there is to date no compelling evidence that antibodies to the alphavirus envelope proteins cause antibody-dependent enhancement of infection. Vaccine candidates, including those based on virus-like particles, measles virus chimeras, or attenuated CHIKV, are under development (reviewed in Cassone, 2015; Weaver et al., 2012). The correlations shown in this paper between broadly neutralizing CHIKV antibodies and the structure of the epitopes on the viral particle may prove important to developing and evaluating these vaccines. While the CHIKV antibodies discussed here did not cross-neutralize the single encephalitic alphavirus tested, development of a potent neutralizing antibody could be an important strategy against these viruses as well.

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