Δ*cydA M. tuberculosis* strain [3]. These results suggest that C10 does not target cytochrome bd. As inactivation of cytochrome bd increases susceptibility to cytochrome bc1 inhibitors (e.g., Q203), C10 does not target cytochrome bc1 either, as inhibitors of bc1 show increased activity in *cydAB* mutants due to no compensatory pathway availability.

Although mutations in various DNA sequences have been found in INH-resistant clinical isolates of *M. tuberculosis*, only mutations in *katG* and *inhA* have been correlated with INH resistance [7]. The proportion of mutations in *katG* and *inhA* varies geographically, but it is estimated that at least 80% of all INH-resistant clinical isolates have either the mutated Ser315 codon of *katG* or the C-15 T nucleotide substitution in the *inhA* promoter [7]. Accordingly, the C10 would be effective only to treat patients infected with *M. tuberculosis* INH-resistant strains harboring *katG* mutations. Notwithstanding, the *in vivo* mechanism of INH resistance appears not to be reflected by *in vitro* experiments [8]. Neither of the two most common *in vivo* mutations were found in seven *in vitro*-selected INH-resistant strains as insertions and deletions (frameshifts) as well as missense mutations (W328L, A172T, and A144E) in the *katG* gene were identified [3]. To reach a specific drug target in *M. tuberculosis*, the chemical agent must be transported from the blood compartment to a nonvascularized pulmonary lesion, diffuse into necrotic foci and the caseum, permeate the lipid-rich cell envelope of bacilli, bind to its intended target at adequate concentrations and act upon it for a required time frame [9]. Several physiological barriers need thus to be overcome when drugs are orally administered, including first-pass metabolism, adequate permeability in the lungs, and uptake into *M. tuberculosis* to reach the intracellular target(s) [9]. Furthermore, chemical stability under different physiological conditions of the multicellular structures that are characteristic of TB pathology, such as necrotizing or caseum granulomas, must be considered [10]. Accordingly, further efforts should be pursued to translate the C10 compound into a chemotherapeutic agent to treat TB infection in human hosts infected with INH-resistant strains of *M. tuberculosis* harboring *katG* mutations. Elucidation of the mode of action of C10 may unveil novel targets that can be valuable for the development of new chemotherapeutic agents to treat TB.

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[1]Instituto Nacional de Ciência e Tecnologia em Tuberculose (INCT-TB), Centro de Pesquisas em Biologia (CPBMF), Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), Porto Alegre, Rio Grande do Sul, Brazil
[2]Escola de Ciências da Saúde, PUCRS, Porto Alegre, Rio Grande do Sul, Brazil
[3]Escola de Ciências, PUCRS, Rio Grande do Sul, Brazil

*Correspondence:
pablo.machado@pucrs.br (P. Machado) and
luiz.basso@pucrs.br (L.A. Basso).

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Spotlight

Structures Unveil the Invasion Mechanism of Chikungunya Virus

Qiu Sun,[1] Xiao Du,[2,3,*] and Wei Cheng[1,*]

Structures of the multiple arthritogenic alphavirus receptor MXRA8 as well as MXRA8 in complex with chikungunya virus (Song *et al.*, *Cell*, 2019; Basore *et al.*, *Cell*, 2019) have revealed the mechanism underlying viral invasion and could facilitate the development of novel vaccines and entry inhibitors.

Alphaviruses are enveloped, single-stranded RNA viruses transmitted primarily by mosquitoes; they include Sindbis virus (SINV), Semliki Forest virus (SFV), Ross River virus (RRV), chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV), Barmah Forest virus (BFV), o'nyong-nyong virus (ONNV), and Mayaro virus (MAYV). These viruses cause endemic diseases and widespread epidemics. However, no specific therapeutic approaches have been developed to treat infections caused by alphaviruses [1]. The arthritogenic alphavirus CHIKV is the causal agent of an emerging widespread outbreak of a debilitating human disease with symptoms varying from fever or rash to severe arthritis. Similar to other alphaviruses, the membrane fusion-related envelope glycoproteins of CHIKV,

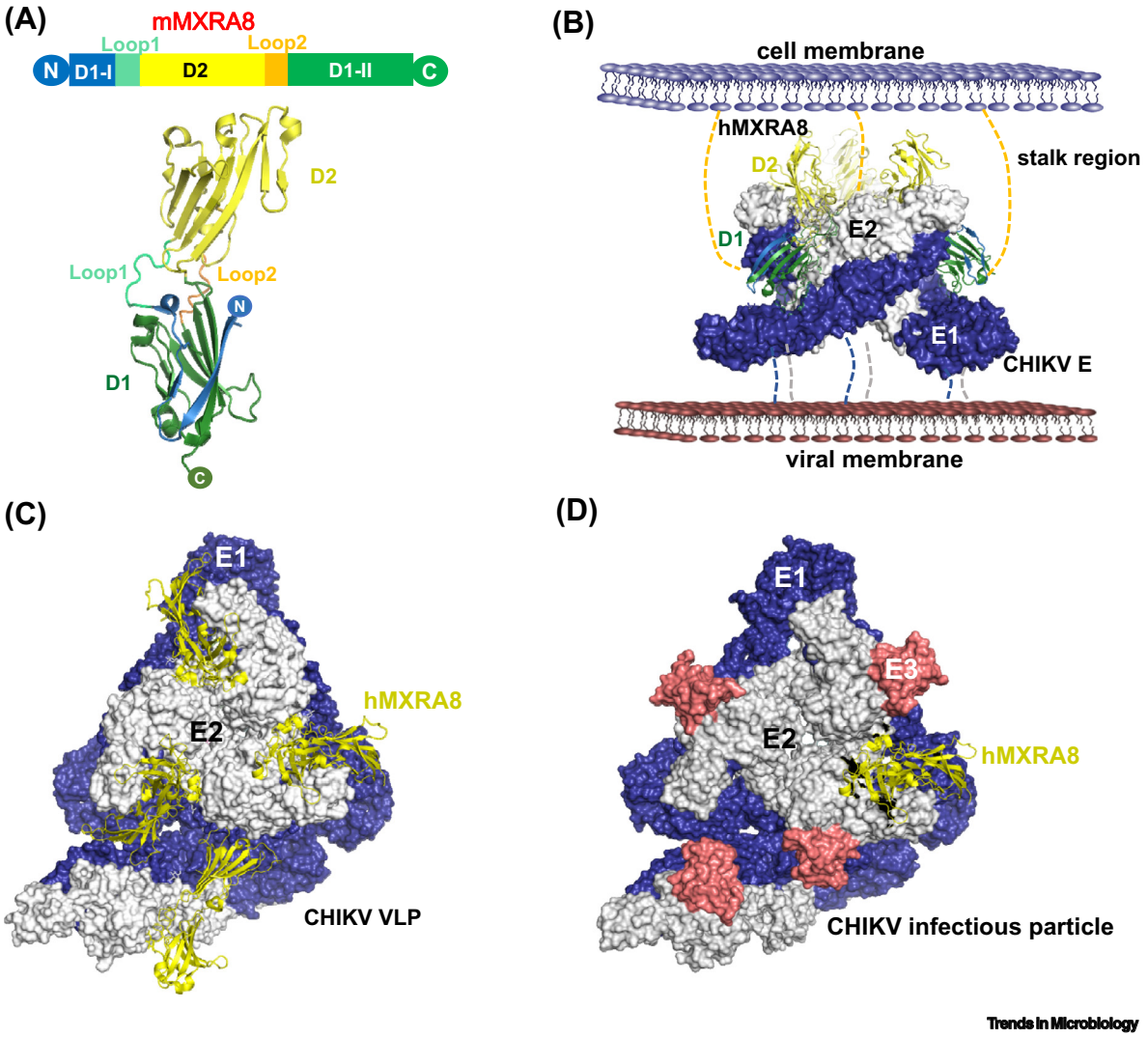

Figure 1. Structures of the MXRA8 Receptor Alone and in Complex with Chikungunya Virus (CHIKV). (A) Architecture of the ectodomain of mouse MXRA8 (mMXRA8, PDB: 6NK3). Ribbon model of the two Ig-like domains. The discrete D1-I and D1-II regions are highlighted in blue and green, respectively. D2 domain is colored in yellow. (B) The model of human MXRA8 (hMXRA8) in complex with CHIKV E protein (PDB: 6JO8) on the cell surface. The length of each membrane-proximal stalk of CHIKV E1, E2, or MXRA8 is represented by broken lines. The CHIKV E1 and E2 proteins are colored in deep blue and gray, respectively. The three hMXRA8 molecules are shown as cartoons. (C) An overall cryo-EM structure of the hMXRA8–CHIKV virus-like particle (VLP) complex (EM Data Bank: EMD-9857). The CHIKV E1 and E2 proteins are colored in blue and gray, respectively. The three hMXRA8 molecules are shown as cartoons and are colored in yellow. (D) An overall cryo-EM structure of chikungunya virus in complex with the mMXRA8 receptor (EM Data Bank: EMD-9395). The CHIKV E1, E2, and E3 proteins are colored in deep blue, gray, and pink, respectively. The mMXRA8 molecule is presented as a cartoon and is colored in yellow.

especially E1 and E2, expressed on the surface of the virion, mediate the processes of entry and viral cell–cell spread. The E3 glycoprotein facilitates the proper folding of p62 (precursor of E2) and the formation of the p62–E1 heterodimer but dissociates during maturation and does not exist in the mature spike [2–4]. Crystallographic studies of alphavirus surface glycoprotein complexes [5,6] have provided substantial insights into the organization of the E protein, the acid-induced conformational change of the virus particle, and the built-in mechanism of inhibition in the immature viral complex. To further determine the host factors required for alphavirus entry, a genome-wide CRISPR-Cas9-based screen was performed. A cell-adhesion molecule, Mxra8, was identified as an entry receptor for multiple emerging arthritogenic alphaviruses [7]. Mxra8 is believed to bind to the surface-exposed region of E2 and facilitate virus attachment and internalization

in cells. However, the detailed mechanism by which Mxra8 engages the alphavirus spike protein is not clear owing to the lack of structural information for Mxra8 and the Mxra8–E protein complex.

To understand the interaction between MXRA8 and CHIKV envelope glycoproteins, Song *et al.* have recently reported the crystal structures of mouse MXRA8 (mMXRA8) and the human MXRA8 (hMXRA8)–CHIKV E protein complex as well as the cryo-electron microscopy structures of hMXRA8 and CHIKV virus-like particles [8]. Interestingly, MXRA8 consists of two Ig-like domains with a unique topological structure (Figure 1A), unlike typical two-domain Ig-like molecules. Domain 1 (D1) consists of two discontinuous fragments, and domain 2 (D2) is inserted between the two fragments of D1, resulting in two hinge loop connections as well as an interdomain disulfide bond connection between D1 and D2. The crystal structure of hMXRA8 in complex with the CHIKV E protein further elucidated the mechanism by which MXRA8 binds to CHIKV E (Figure 1B). MXRA8 and E3–E2–E1 proteins adopt a unique 3:3 binding mode. Three MXRA8 proteins form a very tight bond with the trimeric spike protein, and each MXRA8 wedges into a cleft between one CHIKV E1–E2 heterodimer and extends to connect to an adjacent heterodimer. Apart from E3, both E1 and E2 are involved in hMXRA8 binding.

The complex structures of hMXRA8 and CHIKV virus-like particles (VLPs) were subsequently resolved by cryo-electron microscopy (Figure 1C). These results were consistent with the complex crystal structure of the hMXRA8–CHIKV E protein complex, while in the cryo-EM density map, the E3 protein was absent in the hMXRA8–VLP complex. Song *et al.* [8] performed site-directed mutagenesis and subsequent surface plasmon resonance experiments to verify the key interaction residues. Notably, R69A and R98A, as well as the disruption of the interdomain disulfide bond, completely destroyed virion binding. Additionally, the biological function of the MXRA8 stalk region was elucidated (Figure 1B). MXRA8 is different from other type I transmembrane proteins owing to its unique topological arrangement. The N terminal D1 domain of MXRA8 is the most membrane-proximal domain; other type I transmembrane proteins show the opposite pattern. In particular, the D1 domain of MXRA8 is deeply embedded in a cleft of the CHIKV spike, requiring sufficient length and flexibility of the stalk region near the membrane. Thus, the hMXRA8 stalk region is likely necessary for CHIKV virus binding and entry. Furthermore, a series of biological assays, including truncations and mutations of the hMXRA8 stalk region, have demonstrated the critical role of the stalk region in the process of virus invasion.

Basore *et al.* have published the X-ray crystal structure of MXRA8 and the cryo-EM structures of MXRA8 with CHIKV VLPs and infectious viruses [9]. Combined with mutational and epitope analysis, they obtained similar conclusions to those of Song *et al.* concerning the structural features of MXRA8 and the mechanism underlying the interaction between MXRA8 and CHIKV E. Moreover, two classes of binding sites were defined on the basis of their binding affinity. Of note, the E3 protein, to a great extent, affects the binding mode of MXRA8. The retention of the E3 protein in infectious CHIKV decreases the occupancy of MXRA8 (Figure 1D), which leads to binding only at high-affinity binding sites, whereas MXRA8 occupies both high-affinity and low-affinity binding sites on VPLs lacking E3 (Figure 1C).

In summary, MXRA8 is a multiple arthritogenic alphavirus receptor, and the results of Song *et al.* and Basore *et al.* provide an important basis for the development of new vaccines and broad-spectrum neutralizing antibodies targeting multiple arthritogenic alphaviruses. Their findings confirm that MXRA8 is a novel Ig-like receptor with a unique topological structure and interdomain assembly. Furthermore, they expand our understanding of the detailed mechanism underlying the interaction between MXRA8 and CHIKV.

[1]Division of Respiratory and Critical Care Medicine, West China Hospital, Sichuan University, No. 37, Guo Xue Xiang, Chengdu 610041, Sichuan, China
[2]Department of Gastrointestinal Surgery, West China Hospital, Sichuan University, No. 37, Guo Xue Xiang, Chengdu 610041, Sichuan, China
[3]Department of General Surgery, Yaan People's Hospital, Yaan 625000, Sichuan, China

*Correspondence:
duxiao_home@163.com (X. Du) and
chengwei669@scu.edu.cn (W. Cheng).

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Forum

Type IV Pili as a Therapeutic Target

Guillaume Duménil [1,*]

In the age of antibiotic resistance, strategies targeting virulence traits of bacteria are the focus of intense study. Two such studies came out