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).

https://doi.org/10.1016/j.tim.2019.05.011

References

1. Zanotto, P.M.A. and Leite, L.C.C. (2018) The challenges imposed by dengue, Zika, and chikungunya to Brazil. *Front. Immunol.* 9, 1964
2. Carleton, M. *et al.* (1997) Role of glycoprotein PE2 in formation and maturation of the Sindbis virus spike. *J. Virol.* 71, 1558–1566
3. Mulvey, M. and Brown, D.T. (1995) Involvement of the molecular chaperone BiP in maturation of Sindbis virus envelope glycoproteins. *J. Virol.* 69, 1621–1627
4. Helenius, A. (1995) Alphavirus and flavivirus glycoproteins: structures and functions. *Cell* 81, 651–653
5. Li, L. *et al.* (2010) Structural changes of envelope proteins during alphavirus fusion. *Nature* 468, 705–708
6. Voss, J.E. *et al.* (2010) Glycoprotein organization of Chikungunya virus particles revealed by X-ray crystallography. *Nature* 468, 709–712
7. Zhang, R. *et al.* (2018) Mxra8 is a receptor for multiple arthritogenic alphaviruses. *Nature* 557, 570–574
8. Song, H. *et al.* (2019) Molecular basis of arthritogenic alphavirus receptor MXRA8 binding to chikungunya virus envelope protein. *Cell* Published online May 9, 2019. https://doi.org/10.1016/j.cell.2019.04.008
9. Basore, K. *et al.* (2019) Cryo-EM structure of chikungunya virus in complex with the Mxra8 receptor. *Cell* Published online May 9, 2019. https://doi.org/10.1016/j.cell.2019.04.006

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

independently a week apart showing that bacterial type IV pili are a promising therapeutic target.

Antivirulence as a Solution to Antibiotic Resistance?

Antibiotic resistance has been recognized as a major problem for many years and is now frequently encountered in clinical settings. Available efficient and broad-spectrum antibiotics are increasingly limited in number. Industrial attempts to develop new molecules are thwarted by the time and effort needed, and also precisely because of their limited use due to the rapid emergence of resistance. Endeavors coming from academic laboratories to fully describe molecular mechanisms of infection provide valuable leads towards the development of innovative therapies. In addition, therapies designed to block virulence rather than killing bacteria may slow down the emergence of resistance [1]. Virulence-related strategies likely impose less selective pressure on bacterial communities by affecting only a limited number of species and by restricting survival of bacteria only in specific environments. In addition, given the increasing realization of the importance of commensal bacteria, treatment that preserves these bacterial communities should be favored.

Bacterial Nanomachines as a Target for Innovative Antivirulence Therapeutics

Disease-causing bacteria have a number of properties that allow them to colonize specific niches in the host, cross barriers in tissues, and avoid the immune system. Such properties are made possible by the expression of a diverse set of virulence factors. Of particular interest from the therapeutic point of view, bacterial pathogens have evolved a number of surface-localized complex nanomachines to interact with their host, which broadly fall into two classes: secretion and piliation systems [2]. Release of proteins, as well as other types of molecules, through secretion systems into the extracellular milieu and/or directly into host cells allows pathogens to modulate their environment to their benefit. Several inhibitors of type III and type IV secretion systems have been identified [2]. Piliation systems that allow bacteria to interact with each other, to adhere to and move on cellular surfaces, have also been targeted. Pilicides are a typical example of such inhibitors that affect the biogenesis of the chaperone-usher class of pili by blocking the function of chaperones necessary for the insertion of subunit components into the pilus fiber [3].

Type IV Pili Are Essential Virulence Factors in Numerous Human Pathogens

Despite their broad distribution, inhibitors of type IV pili had not been identified until two recent studies [4,5]. These adhesive filamentous structures (Figure 1) have the unique property of being highly dynamic by undergoing repeated and rapid cycles of extension and retraction [6]. Type IV pili a priori constitute a target of choice as they are necessary for the infectious process of a long list of human pathogens. For instance, the key role of type IV pili is well documented in *Vibrio cholerae* during gut colonization in an infant mice model as well as in humans [7,8]. In a subcutaneous route of infection with *Francisella tularensis*, the agent of tularemia, infection doses required to cause a lethal infection with nonpiliated mutants is 10^5-fold greater than the wild-type strain [9]. In a xenograft-based animal model of infection with *Neisseria meningitis,* type IV pili are essential for vascular colonization and disease progression [10]. In the closely related bacterium *Neisseria gonorrhoeae*, piliation is required for infection of human male volunteers [11]. Other human pathogens, including enterotoxigenic *E. coli*, enteropathogenic *E. coli*, and *Pseudomonas aeruginosa*, also express type IV pili. Potential applications of type IV pili inhibitors are thus very broad.

New Additions to the Antivirulence Therapeutic Toolbox

Two studies seized this opportunity by providing several inhibitors of type IV pili [4,5]. In one case the authors fortuitously observed that certain members of the phenothiazine family of molecules, including trifluoroperazine and thioridazine, induce the disassembly of type IV pili (Figure 1). In the second study, by Aubey *et al.* [4], compounds such as P4MP4 (1-[(piperidin-4-yl)methyl]piperidin-4-ol, called compound B in the publication) were identified by a phenotypic screen on the ability of *N. meningitidis* to adhere to endothelial cells. In both cases treatment of bacteria with the drugs lead to a decrease in the number of pili at the bacterial surface. The effect of both compounds is strikingly rapid, taking place within a few minutes, and it reflects the unusually rapid dynamics of type IV pili, which undergo cycles of extension and retraction within minutes. Accordingly, a *pilT* mutant that fails to retract its pili is resistant to all of these new compounds. The exquisite dynamics of type IV pili that lead to bacterial motility on surfaces, liquid-like aggregate formation, and competence, turns out to also be its Achilles' heel [6].

Mechanism of Action of the Type IV Pili-disrupting Drugs

P4MP4 targets the PilF ATPase. Pilin subunits of type IV pili are stored in the inner membrane and can then be captured by the assembly complex composed of about 15 proteins, leading to its incorporation into the helical pilus fiber. The PilF ATPase provides the energy required for the assembly of pili. P4MP4 does not, however, inhibit the PilT retraction ATPase, thus locking the system in a retraction-only mode and eventually leading to a bacterium without pili. The drug thus affects the delicate balance between extension and retraction of the pili without affecting bacterial viability. Future structural studies will reveal where the

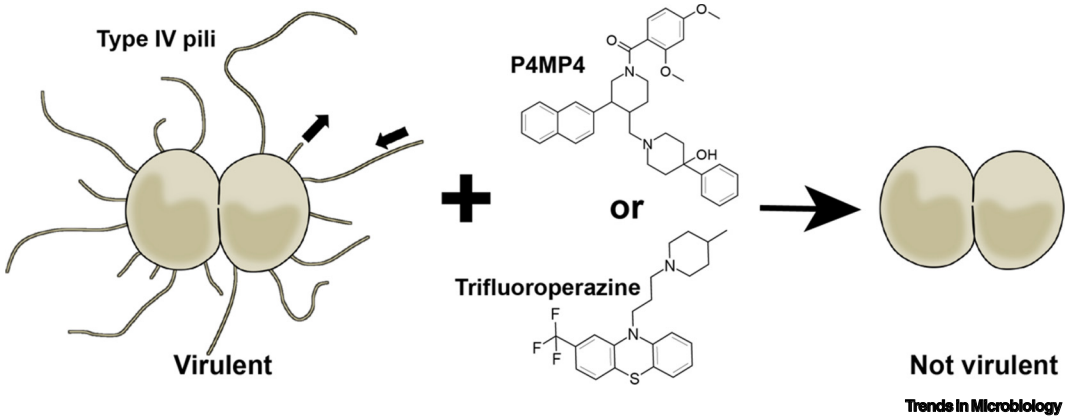

Figure 1. New Drugs Lead to Type IV Pili Disassembly in Several Pathogenic Bacteria.

compound is binding on PilF and how this prevents the function of this complex hexameric enzyme.

The phenothiazine derivatives function in a different way, at least initially. They affect the function of the Na^+-pumping NADH: quinone oxidoreductase (Na^+-NQR) [12]. Because trifluoroperazine is partially toxic to bacteria at concentrations triggering type IV pili disassembly, Denis *et al.* [5] elegantly identified this transporter by selecting mutants that survive this treatment. Bacterial toxicity and pilus disassembly are thus tied together in this case, although the authors provide ample evidence that antibiotic-mediated bacterial killing is not sufficient to trigger pilus retraction and block pilus-mediated phenotypes. The reaction catalyzed by Na^+-NQR is similar to the one carried out by the H^+-pumping NADH:quinone oxidoreductase from the respiration complex I found in mitochondria for instance. In both cases, these enzymes participate in the generation of the electrochemical gradient that is essential to subsequently drive ATP synthesis. These two respiratory enzymes are not homologous, however, and likely have a different mechanism of action. Importantly, the Na^+-NQR enzyme can be found in the genome of numerous pathogenic bacteria but has no eukaryotic homolog, underlining its potential as a therapeutic target. Trifluoroperazine would thus first induce pilus retraction, perhaps by lowering intracellular stores of ATP, eventually altering the bacterium's viability. Exactly how the Na^+-NQR transporter modulates type IV pilus dynamics, and whether the balance between PilF and PilT activities is affected, remains to be determined.

The Added Value of Drugs Targeting Type IV Pili over Antibiotics

Perhaps the most important finding of the study by Denis *et al.* [5] is the demonstration that, in a humanized animal model, treatment with the phenothiazine family, in addition to antibiotic therapy, performs better than antibiotics alone. During *N. meningitidis* infection of this animal model, antibiotics alone kill the majority of the bacteria but a significant amount of inflammation and coagulation remains. The phenothiazines favor bacterial clearance from the blood and increase mouse survival following infection. Both treatments combined not only lead to complete bacterial clearing but also to reduced coagulation and inflammation. This exciting finding opens the possibility of a synergistic double treatment. Since the phenothiazine family of drugs has been used for years as an antipsychotic drug typically used to treat schizophrenia, a potentially rapid translation towards the clinic can be envisioned.

Concluding Remarks

Two independent studies together make a strong argument that type IV pili are a druggable target and describe several of such inhibitors. Although initially identified for *Neisseria meningitidis*, both studies show that these compounds are efficient on other type IV pili-expressing bacterial pathogens (*Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*), thus offering a broad application for these drugs. Much work is needed to optimize these drugs and understand their precise modes of action but the possibility of making a difference in the treatment of patients is now within reach.

[1]Pathogenesis of Vascular Infections Unit, INSERM, Institut Pasteur, 75015 Paris, France

*Correspondence:
guillaume.dumenil@pasteur.fr (G. Duménil).

https://doi.org/10.1016/j.tim.2019.05.011

References

1. Totsika, M. (2017) Disarming pathogens: benefits and challenges of antimicrobials that target bacterial virulence instead of growth and viability. *Future Med. Chem.* 9, 267–269
2. Steadman, D. *et al.* (2014) Bacterial surface appendages as targets for novel antibacterial therapeutics. *Future Microbiol.* 9, 887–900
3. Svensson, A. *et al.* (2001) Design and evaluation of pilicides: potential novel antibacterial agents directed against uropathogenic *Escherichia coli*. *Chembiochem* 2, 915–918
4. Aubey, F. *et al.* (2019) Inhibitors of the *Neisseria meningitidis* PilF ATPase provoke type IV pilus disassembly. *Proc. Natl. Acad. Sci. U. S. A.* 116, 8481–8486
5. Denis, K. *et al.* (2019) Targeting type IV pili as an antivirulence strategy against invasive meningococcal disease. *Nat. Microbiol.* 4, 972–984
6. Craig, L. *et al.* (2019) Type IV pili: dynamics, biophysics and functional consequences. *Nat. Rev. Microbiol.* Published online April 15, 2019. https://doi.org/10.1038/s41579-019-0195-4
7. Herrington, D.A. *et al.* (1988) Toxin, toxin-coregulated pili, and the *toxR* regulon are essential for *Vibrio cholerae* pathogenesis in humans. *J. Exp. Med.* 168, 1487–1492
8. Taylor, R.K. *et al.* (1987) Use of *phoA* gene fusions to identify a pilus colonization factor coordinately regulated with cholera toxin. *Proc. Natl. Acad. Sci. U. S. A.* 84, 2833–2837
9. Forslund, A.L. *et al.* (2006) Direct repeat-mediated deletion of a type IV pilin gene results in major virulence attenuation of *Francisella tularensis*. *Mol. Microbiol.* 59, 1818–1830
10. Melican, K. *et al.* (2013) Adhesion of *Neisseria meningitidis* to dermal vessels leads to local vascular damage and purpura in a humanized mouse model. *PLoS Pathog.* 9, e1003139
11. Kellogg Jr., D.S. *et al.* (1968) *Neisseria gonorrhoeae*. II. Colonial variation and pathogenicity during 35 months *in vitro*. *J. Bacteriol.* 96, 596–605
12. Juarez, O. and Barquera, B. (2012) Insights into the mechanism of electron transfer and sodium translocation of the Na(+)-pumping NADH:quinone oxidoreductase. *Biochim. Biophys. Acta* 1817, 1823–1832