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Disarming the Invader

Type III secretion systems are used by many gram-negative bacterial pathogens of animals and plants to deliver essential virulence factors into targeted host cells. The identification of chemical compounds that block the function of these systems is the first step toward developing chemical attenuation as an effective method for the treatment of infectious disease.

Over the past decade it has become abundantly clear that despite vastly different disease outcomes caused by pathogenic bacteria, common mechanisms exist for targeting specific virulence factors to host sites. Type III secretion (TTS) systems are essential for virulence of many gram-negative pathogens of animals including species of *Bordetella, Chlamydia, Pseudomonas, Salmonella, Shigella*, and *Yersinia* [1]. In humans, these bacteria cause a variety of diseases such as whooping cough, plague, and several forms of gastroenteritis. Moreover, several plant diseases, which have had great economic impact, are caused by bacteria that utilize TTS systems such as *Erwinia* spp., *Pseudomonas syringae*, *Ralstonia solanacearum*, and *Xanthomonas campestris* [1]. TTS systems function in many cases only when the pathogen is intimately associated with a host cell. In this context, the physical interaction between the bacterium and the host cell induces the TTS system to deliver virulence proteins in a single step from the bacterial cytosol into the cytosol of the cell. This is a remarkable task when one considers that the delivery of proteins by gram-negative bacteria into a eukaryotic cell demands transport across three biological membranes. The particular set of proteins delivered by different pathogens is highly divergent, but the bacterial machinery composing the TTS systems is quite conserved. Thus, many gram-negative bacteria that cause disease have in common TTS systems, which can be targeted for the development of chemical compounds to block an essential virulence activity and effectively disarm this group of bacterial invaders.

While TTS systems are required for survival of bacteria during infection, they are dispensable for bacteria that have a free-living stage in their life cycle. Thus, a compound that blocks TTS will not necessarily inhibit bacterial growth. Traditionally, antibiotics are developed to interfere with an activity, such as synthesis of DNA, RNA, peptidoglycans, or proteins, which is essential for bacterial growth or survival [2]. This approach has been very productive and has changed the fate of humanity

with penicillin, tetracycline, and numerous other compounds used in medicine today. However, the emergence of multiple antibiotic-resistant organisms has demanded that scientists focus on alternative approaches. In this issue, a group of researchers led by Mikael Elofsson at Umeå University in Sweden present the result of their approach to identify compounds that specifically inhibit the function of a TTS system. This provides scientists with the platform to test the revolutionary idea that these compounds may effectively attenuate virulence during an infection. Chemical attenuation is a departure from the traditional approach to the development of antibiotics because the target is not necessarily required for bacterial viability. Instead, targeting TTS is likely to limit the bacterium's ability to subvert the host immune system, thereby effectively removing its armament. This would allow the host to efficiently clear the infection.

To identify compounds that inhibit TTS, Elofsson and colleagues designed a high throughput screen to examine more than 9000 unique compounds. The screen was based on the ability to rapidly and efficiently monitor the expression of the gene *yopE* in the bacterium *Yersinia pseudotuberculosis*, which is highly expressed only when the Ysc TTS system is functional. The choice to use *Y. pseudotuberculosis* as a model organism was well founded because the Ysc TTS system has been extensively studied and can be induced to secrete proteins in the absence of host cell contact by culturing the bacteria in growth medium depleted of calcium [3]. Expression of *yopE* was monitored by fusing a copy of the *yopE* promoter to *luxAB*, genes originating from *Vibrio harveyi* that encode luciferase. This provided the opportunity to grow *Y. pseudotuberculosis* under conditions where the Ysc TTS system was induced and to monitor the effect of test compounds on *yopE* expression. Using an arrayed format, a library of chemical compounds was screened, with 12 candidate TTS inhibitors being identified.

To follow up on these initial lead compounds, several secondary screening protocols were used to evaluate possible targets. These screens included evaluating the effect of candidate compounds for effects on bacterial growth, expression of other genes that are involved in Ysc TTS functions, and evaluating whether candidate compounds affected flagellar-dependent motility. Evaluating motility was a particularly clever screen since biogenesis of bacterial flagella involves an independent TTS system that is homologous to "contact-dependent" TTS systems [4]. These secondary screens reduced the group of lead compounds to a few strong candidates. Nine of the compounds affected bacterial growth and, therefore, probably affected Ysc TTS indirectly. These compounds may be valuable new candidates for the traditional development of an antibiotic but do not fit the criteria for a compound that could be used for chemical attenuation.

The other three compounds did not significantly affect bacterial growth and appear to be bona fide inhibitors of the Ysc TTS system. Interestingly, each of these compounds appears to have a different target. One compound is clioxanide, an *O*-acetyl salicylanilide that appears to block the Ysc TTS system by targeting an as yet unknown bacterial component required for the expression of the Ysc TTS master regulatory gene *lcrF*. These results indicate clioxanide may be a candidate compound for evaluating whether chemical attenuation can be used to treat infections caused by pathogenic *Yersinia* and other pathogens that control TTS in a similar manner. The other two compounds appear to target protein export functions of the Ysc TTS system. One of these two compounds belongs to a family of haloid-containing sulfonamidobenzamides. This compound did not appear to affect expression of *lcrF* but interfered with protein export by the Ysc TTS. These are properties that may indicate this family of compounds will provide several prospects for future development. The other compound was an acylated salicylaldehyde hydrazone, which interfered with Ysc TTS and also affected flagellar-mediated motility. This result is very exciting because it suggests this compound inhibits the activity of closely related proteins required for the function of distantly related TTS systems. Acylated salicylaldehyde hydrazones and related compounds are therefore excellent candidates to test for inhibitory effects on TTS systems of other pathogens.

In the broader perspective, Elofsson and colleagues have provided proof of concept that identifying chemicals that inhibit TTS systems of bacterial pathogens is viable. Chemical attenuation could also be used to target other virulence properties of pathogenic bacteria. The use of chemical attenuation is a new concept that opens the door to a new horizon on the battle against infectious diseases. Much work still needs to be completed to evaluate whether this approach will be generally applicable to treatment of infections caused by bacteria that utilize TTS systems. Scientists still need to test whether chemical attenuation will be effective once a disease develops. However, it should not be overlooked that this is the first step in the attempt to disarm the invader.

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