Usurping bacterial virulence factors as self-delivery vehicles for therapeutic use

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Type III secretion systems (T3SS) as virulence factors were initially discovered in Yersinia and are now widely recognized in many bacterial pathogens that infect animals and plants.1-3 It was observed about 20 y ago that the conserved machinery of T3SS allows the transport of heterologous T3SS proteins from other species.4 With this knowledge, approaches to deliver vaccines and therapeutics using T3SS-based systems have been examined. For this to work, native effector proteins must be deleted to attenuate their virulence functions. Alternatively, anti-infective therapeutics have targeted effector proteins for inhibition. This approach must be tailored for pathogen specificity, as there is little conservation between effectors of different bacteria. However, our expanding knowledge of host responses to the T3SS pore hampers the use of these systems over concern of unwanted stimulation of immune responses.

In a recent article by Grabowski et al. in Virulence, an approach is presented that turns this idea on its head, reversing the thinking that Yersinia outer protein (Yop) effector proteins must always be pathogenic.5 Collectively, the Yops are toxins - even for the deadly plague pathogen Yersinia pestis, deletion of the T3SS results in greater than 8-log loss of virulence. But individual Yops are almost harmless, in many cases, little to no loss of virulence occurs when an individual Yop is deleted. Many of the effectors work together to control inflammation through the concerted acts of individual proteins on host signal transduction. Furthermore, and independently of the T3SS pore for delivery, individual Yops have activities that have therapeutic potential. This concept, ie the use of toxins in human medicine, is not unprecedented, particularly in the area of vaccines where inactive toxins have been tested as antigens or even adjuvants due to the potent ability of the innate immune system to recognize intoxication and stimulate the adaptive immune response.6

The technology is predicated on a molecular property inherent to some of the T3SS effectors: cell-penetrating peptides or epitopes (CPE) encoded within the protein that facilitate autonomous translocation across membranes. Given the ease of constructing recombinant molecules with an insertion of a CPE where necessary, the concept to use secreted virulence factors as self-limiting therapies promises to be adaptable to virtually any disease. While 10–20 species of bacterial pathogens depend on T3SSs for virulence, types IV, VI and VII secretion systems also inject proteins that disrupt cellular functions.7-10 Collectively, these systems encode for the secretion of potentially thousands of effectors, most of which act on host proteins that are important to human diseases. While some of the Yersinia Yops and other T3SS effector proteins have been well-characterized, comparatively less is known about those injected by types IV, VI and VII secretion systems, where computational identification of effectors predominates, and in some cases, with little to no experimental validation.11

In Yersinia, 7 effector proteins are believed injected into host cells, only a subset of these carrying cell-penetrating peptides that allow for delivery in the absence of a translocation pore. Other systems, for example the highly related Pseudomonas, are believed to inject at least 50 proteins into host cells.12 Collectively, secreted effector proteins have known functions that perturb host cell death, actin rearrangement, vesicle trafficking, autophagy and transcriptional regulation of the inflammatory response. Furthermore, continued advances in understanding molecular mechanisms of pathogenesis may yield the identification of additional cell penetrating peptides and intracellular mechanisms that impact delivery efficiency, intracellular trafficking and stability. For
example, *Yersinia* YopM induces cell penetration through endosomal membranes whereas the CPEs of other YopM family members induce translocation across the plasma membrane.

Within the many known effector protein classes are sequence variations believed to determine intracellular target specificity, protein stability or pathogen-specific functions, making an individual member of a protein class potentially a better drug. The well-known YopJ family of T3SS effectors is defined as diverse effector proteins with a cysteine protease catalytic domain that has acetyltransferase activity, and there are more than 20 family members that encode homologous domains in animal and plant pathogens. The recently reported crystal structure for HopZ1a, a YopJ homolog from *Pseudomonas syringae*, revealed that its transferase activity is stimulated by conformational changes caused by the binding of inositol hexakisphosphate (IP6), a signaling molecule associated with the plasma membrane. This suggests biologically relevant enrichment of acetyl transferase activity on membrane localized host proteins which could ultimately improve or finely tune the therapeutic applications of HopZ1.

A detailed molecular understanding of the effector proteins, their interactions with host and bacterial proteins, stability within host cells and contributions to disease will be necessary to understand optimization of delivery and activity as well as the potential risks or unwanted effects. For example, many type III, IV and VII effector proteins are known to act in combination, conferring specificity and/or regulation of their activities. Furthermore, innate and adaptive immune responses to these protein antigens should be characterized to determine the potential toxicity as well as generation of immunity to CPEs or the cargo to which they are attached. Additional challenges lie in the poor pharmacokinetic and pharmacodynamic profiles of protein therapies, but the potential that specific cells could be targeted and specific functions inactivated raises a seemingly limitless set of therapeutic approaches for human and animal medicine.

Combined with advances in targeted delivery of proteins using nanoparticles or viral particles, it may be further possible to limit the activity of CPE-tethered proteins to specific host cells, which will improve their safety. We’ve only breached the surface of the potential therapeutic power of known effectors of bacterial secretion systems, collectively offering an untapped pool of naturally occurring pharmaceuticals. Although much research is still needed before this technology could be used in humans, it is an approach that takes advantage of the exquisite specificity and potency that results from the co-evolution of pathogens with their hosts.

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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