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Intoxication vs. infection

A decade of studying *Burkholderia pseudomallei* virulence in a simple infection model

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"He was indeed a deplorable spectacle. In the dim light of a foggy November day the sick room was a gloomy spot, but it was that gaunt, wasted face staring at me from the bed which sent a chill to my heart. His eyes had the brightness of fever, there was a hectic flush upon either cheek, and dark crusts clung to his lips; the thin hands upon the coverlet twitched incessantly, his voice was croaking and spasmodic."

From The Adventure of the Dying Detective by Sir Arthur Conan Doyle, 1913

In *The Adventure of the Dying Detective*, Dr Watson provides this detailed description of a seemingly gravely ill Sherlock Holmes. However, it is later revealed that the great detective was actually feigning an exotic, life-threatening illness called "Tapanuli fever" in order to trap a would-be assassin from Sumatra. The inspiration for Doyle's contrived Asian illness has been postulated to be meliodosis,[1] which had been reported by the British pathologist Captain Alfred Whitmore and his assistant C.S. Krishnaswami one year before the publication of *The Adventure of the Dying Detective*.[2] While meliodosis is now understood to have considerable clinical diversity,[3] Doyle may have drawn inspiration from Whitmore and Krishnaswami's initial description of the life-threatening illness, characterized by chronic pneumonia, deep organ abscesses and sepsis in the emaciated opium addicts of Rangoon, Burma.[2] With a mortality rate approaching 40%, limited treatment options due to both inherent and acquired antibiotic resistance and an expanding geographic distribution, meliodosis remains a serious concern today.[3]

The causative agent of meliodosis is *Burkholderia pseudomallei*, a Gram-negative soil saphrophyte endemic to Southeast Asia and northern Australia.[3] Several attributes of *B. pseudomallei*, including its low infectious dose, ease of laboratory culture and ability to cause disease through multiple routes including inhalational exposure, has led to its classified as a Category B select agent by the Centers for Disease Control and Prevention. Nevertheless, the biology of *B. pseudomallei* in the natural environment and the virulence mechanisms important for human infection by *B. pseudomallei* remain only partially understood a century after its original discovery.[4] In recent years, diverse laboratory models of *B. pseudomallei* infection, including small animals (mice and hamsters), invertebrates (wax moths), free-living protozoa (Acanthamoeba) and plants (tomatoes), have been used to explore *B. pseudomallei* biology and virulence mechanisms.[4,5] Included among these systems is a *Caenorhabditis elegans*-based infection model, which has been used both to replicate a natural host/pathogen (predator/prey) relationship and also as a surrogate model for human infection.[6-8]

At least five different mechanisms of *C. elegans* killing by microbial pathogens have been described: toxin-mediated killing, transient or persistent intestinal infection, direct invasion and biofilm formation.[9] In initial studies reported by O'Quinn et al. a decade ago, nematodes fed *B. pseudomallei* were observed to develop impaired locomotion, reduced feeding behavior and egg-laying abnormalities before succumbing to infection, leading to the hypothesis that nematode death was due to a neurotoxin or paralytic agent. However, no toxin was identified.[6] Additional work by Gan et al. demonstrated that a relatively short (12 h) period of exposure to *B. pseudomallei* was sufficient to kill a substantial portion of the nematode population but that live bacteria were required for maximal killing.[7] Furthermore, Gan et al. also identified two novel virulence genes in *B. pseudomallei* (encoding a putative amino acid transporter and a hypothetical protein), which led to attenuated nematode killing in deletion mutants, and showed that environmental factors, such nutrient composition, had an effect on virulence, a finding that was recently confirmed by Lee et al.[7,8] Finally, like O'Quinn et al., Gan et al. also suggested that nematode killing may be due in part to a soluble toxin.[7] By contrast, recent work by Lee et al. shows evidence to the contrary—that nematode killing by *B. pseudomallei* is not due to a diffusible or heat killed toxin but requires direct and prolonged contact with the organism.[8] Taken together, these studies established the *C. elegans-B. pseudomallei* infection model as a promising tool to explore *B. pseudomallei* virulence mechanisms and biology, but many questions remain regarding *B. pseudomallei* infection in nematodes, the nature of the toxin (if present) and its ultimately its relevance to human infection and/or *B. pseudomallei* fitness in the environment.

In this issue of *Virulence*, Ooi et al. build upon earlier work, further exploring how *B. pseudomallei* kills *C. elegans*.[10] The study focuses on the intestinal luminal

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colonization of *B. pseudomallei* and is among the most thorough investigations of nematode intestinal lumen colonization not aided by electron microscopy reported to date. Using a combination of fluorescence microscopy and quantification of intestinal tract colony forming units (CFU), the authors demonstrate that nematodes do not become fully colonized even 28 h follow exposure to *B. pseudomallei* and that the number of live organisms contained within the intestinal tract of infected worms are considerably lower than observed with other pathogens. In addition, they examined colonization using a mutant strain of *C. elegans* [*tnt-3 (aj3)*] that is defective in the ability to physically disrupt ingested microbes. While many more *tnt-3(aj3)* animals were fully colonized along the length of their intestinal tract with *B. pseudomallei* compared with wild-type nematodes, the number of viable *B. pseudomallei* CFU in the lumen was 2–3 log less than that recovered from nematodes exposed to *P. aeruginosa* (as an example of a pathogen that kills nematodes through a mechanism involving intestinal lumen colonization). Furthermore, intraluminal CFU counts of *B. pseudomallei* did not appreciably increase in mutant animals following extended incubation.

Several potential reasons for the lack of intestinal colonization by *B. pseudomallei* were investigated. First, the authors observed that *C. elegans* pharyngeal pumping rates were reduced when nematodes were exposed to *B. pseudomallei*. To determine if this was the source of poor intestinal colonization, the media was supplemented with serotonin, which increased the pharyngeal pumping rates. Despite this, no increase in intraluminal CFU was observed in serotonin-exposed nematodes. Next, defecation rates were measured in *B. pseudomallei*-exposed animals and were found to be consistently lower than to those exposed to a non-pathogenic food source. Thus, they conclude that the minimal nematode intestinal tract colonization by *B. pseudomallei* was not the result of altered pharyngeal pumping or defecation rates. This comprehensive evaluation suggests that there may be virulence mechanisms involved in *B. pseudomallei* killing of *C. elegans* distinct from intraluminal colonization but that direct bacterial contact may be required in order to achieve a maximal effect.

Previous work has suggested that nematode killing by *B. pseudomallei* may be due in part to a diffusible toxin that can pass through a 0.22 μm nitrocellulose filter.[7] Nematodes exposed to the conditioned media following removal of the filter were killed, albeit at a reduced rate compared with when live bacteria was present.[7] However, when this same procedure was repeated by other investigators, killing via a diffusible or heat stable toxin could not be demonstrated.[6,8] Ooi et al. further explore the possibility that a toxin is involved in nematode killing by looking at the induction of *C. elegans pgp-5* as a marker of toxin production by *B. pseudomallei*. *pgp-5* encodes an ATP-binding cassette (ABC) P-glycoprotein transporter that provides protection by actively exporting toxins that diffuse into the cell. Previous work has shown that *pgp-5* is induced to varying degrees during both bacterial infections and exposure to heavy metals such as cadmium, in a TIR-1/p38 MAP kinase-dependent manner.[11] In addition, *pgp-5* mutant *C. elegans* have decreased survival when exposed to *P. aeruginosa* or *Salmonella* Typhimurium, suggesting PGP-5 plays an important role in the worms defense against bacterial infection.[11] Ooi et al. found *pgp-5* transcription was highly and, among the P-glycoprotein gene superfamily, specifically induced following exposure to *B. pseudomallei*, as evaluated both by RT-PCR and florescence microscopy using *pgp-5::gfp* transgenic worms. Furthermore, RNAi *pgp-5* knock-down worms were found to be more susceptible to infection by *B. pseudomallei*, further suggesting PGP-5 is important for *C. elegans* host defense against *B. pseudomallei*.

The authors conclude that their findings suggest that *B. pseudomallei* secretes a toxin (or toxins) into cells that mediate nematode killing. A secreted toxin is one possible explanation for these findings but the results are by no means conclusive. Limited intestinal lumen colonization by *B. pseudomallei* compared with other intestinal infection-associated pathogens certainly suggests an alternative or more complex mechanism of virulence and cellular detoxification by PGP-5 appears to play a contributory role in nematode defenses against *B. pseudomallei*. However, it should be noted that PGP-5 influences nematode survival during intestinal colonization-associated infections with *P. aeruginosa* and *S.* Typhimurium, which suggests that the cellular detoxification processes mediated by PGP-5 enhance nematode fitness during infections by pathogens that have more complex, multifactorial and redundant virulence mechanisms than toxin production alone. Furthermore, maximal killing of *C. elegans* by *B. pseudomallei* requires continued exposure to the bacteria, suggesting either a continuously produced toxin or some other direct effect of the bacteria on the nematode.

The research reported by Ooi et al. advances our understanding of *B. pseudomallei* infection in the *C. elegans* model. One limitation to the study is only one strain of *B. pseudomallei* was tested, as other investigators have noted significant strain-to-strain variability in nematocidal activity.[6,8] Future investigation is also warranted to delineate the specific role PGP-5 plays in nematode defense against *B. pseudomallei*. Most importantly, we continue to await the identification of an elusive, perhaps cell-associated *B. pseudomallei* toxin that contributes to nematode killing—a toxin that may need to be continuously produced by live *B. pseudomallei* in the intestinal tract order to have its full pathogenic effect. This conclusion is essentially unchanged since O'Quinn et al. published the first report of the *C. elegans/B. pseudomallei* model system over 10 years ago, when it was noted that "nematode pathogenesis by…*B. pseudomallei* involves an intoxication mechanism plus additional factors that depend upon living bacteria for delivery." Identification of a toxin could lead to important insights into the biology of *B. pseudomallei* in the natural environment and perhaps into the pathogenesis of meliodosis in humans.

References

1. Sodeman WA, Jr.. Sherlock Holmes and tropical medicine: a centennial appraisal. Am J Trop Med Hyg 1994; 50:99-101; PMID:8304578
2. Whitmore A, Whitmore CS. An account of the discovery of a hitherto undescribed infectious disease occurring among the population of Rangoon. Ind Med Gaz 1912; 46:262-7.
3. Wiersinga WJ, Currie BJ, Peacock SJ. Melioidosis. N Engl J Med 2012; 367:1035-44; PMID:22970946; http://dx.doi.org/10.1056/NEJMra1204699
4. Galyov EE, Brett PJ, DeShazer D. Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. Annu Rev Microbiol 2010; 64:495-517; PMID:20528691; http://dx.doi.org/10.1146/annurev.micro.112408.134030
5. Lee YH, Chen Y, Ouyang X, Gan YH. Identification of tomato plant as a novel host model for *Burkholderia pseudomallei*. BMC Microbiol 2010; 10:28; PMID: 20109238; http://dx.doi.org/10.1186/1471-2180-10-28
6. O'Quinn AL, Wiegand EM, Jeddeloh JA. *Burkholderia pseudomallei* kills the nematode *Caenorhabditis elegans* using an endotoxin-mediated paralysis. Cell Microbiol 2001; 3:381-93; PMID:11422081; http://dx.doi.org/10.1046/j.1462-5822.2001.00118.x
7. Gan YH, Chua KL, Chua HH, Liu B, Hii CS, Chong HL, et al. Characterization of *Burkholderia pseudomallei* infection and identification of novel virulence factors using a *Caenorhabditis elegans* host system. Mol Microbiol 2002; 44:1185-97; PMID:12068805; http://dx.doi.org/10.1046/j.1365-2958.2002.02957.x
8. Lee SH, Ooi SK, Mahadi NM, Tan MW, Nathan S. Complete killing of *Caenorhabditis elegans* by *Burkholderia pseudomallei* is dependent on prolonged direct association with the viable pathogen. PLoS One 2011; 6:e16707; PMID:21408228; http://dx.doi.org/10.1371/journal.pone.0016707
9. Sifri CD, Begun J, Ausubel FM. The worm has turned–microbial virulence modeled in *Caenorhabditis elegans*. Trends Microbiol 2005; 13:119-27; PMID: 15737730; http://dx.doi.org/10.1016/j.tim.2005.01.003
10. Ooi SK, Lim TY, Lee SH, Natha S. *Burkholderia pseudomallei* kills *Caenoarhabditis elegans* through virulence mechanisms distinct from intestinal lumen colonization. Virulence 2012; 3:485-96; PMID: 23076282; http://dx.doi.org/10.4161/viru.21808
11. Kurz CL, Shapira M, Chen K, Baillie DL, Tan MW. *Caenorhabditis elegans pgp-5* is involved in resistance to bacterial infection and heavy metal and its regulation requires TIR-1 and a p38 map kinase cascade. Biochem Biophys Res Commun 2007; 363:438-43; PMID: 17888400; http://dx.doi.org/10.1016/j.bbrc.2007.08.190