Small Things Considered

No Bacterium Is An Island
http://schaechter.asmblog.org/schaechter/2013/05/no-bacterium-is-an-island.html by S. Marvin Friedman

To paraphrase an old adage, no bacterium is an island. Indeed, bacteria in nature exist as polymicrobial communities where interactions between individuals influence activities of the entire population. This is especially true of pathogenic bacteria. Nevertheless, we frequently prescribe antibiotic therapy based upon isolation of a single species from an infection site—a practice that yields an incomplete picture of the infection dynamics. A recent study of coinfection systems revealed interesting details of how synergy with neighboring organisms can contribute to a pathogen’s virulence.

*Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen that often synergistically colonizes chronic wounds along with gram-positive bacteria. Previous work had found that production of the virulence factor pyocyanin (the pigment that gives the colonies of this organism their characteristic green color) is enhanced by coinfection with gram-positive bacteria. The N-acetylglucosamine (NAG) moiety in peptidoglycan fragments shed from the gram-positive cell wall was shown to be necessary and sufficient for enhanced pyocyanin formation. Next the researchers sought to isolate a mutant that produces normal levels of pyocyanin in the absence of NAG but not more pyocyanin in its presence. Using transposon inactivation, they found such a mutant with an insertion at gene PA0601. This gene encodes a two-component response regulator with sequence homology to the Lux family of quorum sensing regulators. Once again, the themes of virulence and quorum sensing converge.

Several other virulence factors of *P. aeruginosa* are coregulated with pyocyanin production, e.g., the extracellular protease elastase. The autoinducer for this kind of quorum sensing is the *Pseudomonas* Quinolone Signal (PQS or 2-heptyl-3-hydroxy-4(1H)-quinolone). PQS is an antistaphylococcal agent, as are pyocyanin and elastase. The researchers hypothesized, and then demonstrated, that the increase in the levels of pyocyanin and elastase when stimulated by NAG is due to enhanced production of PQS. It all fits: quorum sensing becomes a surveillance mechanism for *Pseudomonas* to detect its bacterial neighbors and respond by producing antimicrobial factors.

The researchers then turned to an in vivo model of infection. In *Drosophila* flies, ingestion of *P. aeruginosa* resulted in colonization of the flies’ crop and subsequent death. With wild type (WT) bacteria, 50% of the flies died by 2 days. Flies fed the PA0601 mutant lived significantly longer although the mutant was able to colonize and persist in the fly’s crop. The authors hypothesized that this difference was due to peptidoglycan shed by the large number of gram-positive bacteria present in the crops. To test this, flies were fed a mixture of antibiotics that selectively and effectively killed the gram-positive biota. The treated flies survived longer following infection by either the WT or the PA0601 mutant. Moreover, peptidoglycan sensing by WT *P. aeruginosa* resulted in about a 1,000-fold reduction in colonization of the flies’ crop and subsequent death. With wild type (WT) bacteria, 50% of the flies died by 2 days. Flies fed the PA0601 mutant lived significantly longer although the mutant was able to colonize and persist in the fly’s crop. The authors hypothesized that this difference was due to peptidoglycan shed by the large number of gram-positive bacteria present in the crops. To test this, flies were fed a mixture of antibiotics that selectively and effectively killed the gram-positive biota. The treated flies survived longer following infection by either the WT or the PA0601 mutant. Moreover, peptidoglycan sensing by WT *P. aeruginosa* resulted in about a 1,000-fold reduction in colonization of the flies’ crop, while the PA0601 mutant had a much smaller effect.

Was the delayed killing of antibiotic-treated flies due to a lack of peptidoglycan in the fly crop? Sure enough, feeding peptidoglycan abolished this effect in the WT, but not in the PA0601 mutant. So, does the enhancement of PQS and thus PQS-controlled virulence factors by peptidoglycan play a role in vivo? To test this, the researchers extracted RNA from crops colonized by WT *P. aeruginosa* and, using reverse transcriptase PCR, measured the expression of *psqA*, the first gene in the PQS regulon. *psqA* levels were reduced about threefold in antibiotic-treated flies compared to controls. Feeding peptidoglycan to antibiotic-treated flies restored transcription levels of *psqA* transcripts to those in the controls. Furthermore, these effects required transport of NAG, suggesting that NAG is the peptidoglycan component that is sensed.

The results presented in this paper clearly show that in a polymicrobial environment, commensal gram-positive bacteria can potentiate the virulence of a gram-negative opportunistic pathogen. The mechanism? Cell wall fragments shed by the gram-positives enhance virulence factor production via a QS system. As an aside, it’s curious that both some bacteria and the host detect invading bacteria through their shedding of cell wall fragments. But then, peptidoglycan (or its fragments) is a good choice for a calling card, being that this compound is quite unique in biochemistry and is restricted to bacteria.

These findings suggest that therapeutic strategies targeting gram-positive bacteria may be helpful for treating *P. aeruginosa*-dominated polymicrobial infections, including those of chronic wounds. Or, to say the least, it emphasizes that the polymicrobial nature of bacterial infections must always be carefully considered.