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A complex relationship: the interaction among symbiotic microbes, invading pathogens, and their mammalian host

MM Curtis[1] and V Sperandio[1]

Symbiosis between microbes and their mammalian host is vital to maintaining homeostasis. Symbiotic microbes within the gastrointestinal tract provide an array of benefits to the host, including promotion of host immunity. A coordinated effort of the host and symbiotic microbes deters the colonization and survival of many invading pathogens. However, pathogens have devised strategies to overcome these mechanisms. Furthermore, some pathogens can hijack host hormones and bacterial autoinducers to induce virulence traits. Intra- and inter-species (bacteria/bacteria) and interkingdom (bacteria/host) communication orchestrates the complex relationship among symbiotic microbes, invading pathogens, and their mammalian host. Insight into this communication will provide a foundation for the development of targeted antimicrobial therapies.

INTRODUCTION

Infectious diseases wreak havoc on mankind. The mammalian immune system has in place a line of defense specialized in recognizing and eradicating invading pathogens; however, sometimes the pathogen evades these mechanisms and establishes disease in its host. Therapies like antibiotics and vaccination abet the immune system in its fight against pathogenic microbes. Over time, resistance to antibiotics has developed because of the intense selective pressure the antibiotics place on bacteria. Furthermore, although a number of vaccines have been successful, far too many infectious diseases still do not have efficacious vaccines. A desperate call for new therapeutics exists.

Understanding the complex relationship among the host, symbiotic microbes, and invading pathogens will provide important insight for the rational design of therapeutics. Bacteria can communicate with one another through hormone-like signals to modulate their gene expression[1] in a process termed quorum sensing (QS).[2] Additionally, these bacterial signals can modify mammalian cell-signal transduction,[3] and likewise host hormones can cross signal to regulate bacterial gene expression[4] in a process termed interkingdom signaling. Interference with the cell-to-cell signaling pathways that control bacterial virulence offers a promising new strategy in the treatment of bacterial infections. This review will discuss both the mechanisms employed by the host and symbiotic bacteria to impair pathogen virulence, as well as the conserved cell-to-cell signaling pathways implemented by pathogens that allow for exploitation of their host environment.

ANTIMICROBIAL STRATEGIES ENLISTED BY THE HOST AND SYMBIONTS

The human gut hosts an estimated 500–1,000 species of bacteria.[5,6] A mutually beneficial relationship exists between the human intestine and many of its symbionts: the human intestine provides nutrients to the resident bacteria, whereas bacteria aid in the digestion of food and absorption of nutrients, produce vitamins such as biotin and vitamin K, regulate immune system function, and hinder the colonization of pathogenic microorganisms.[7] Two major bacterial phyla, *Firmicutes* and *Bacteroidetes*, and five minor bacterial phyla, *Proteobacteria*, *Actinobacteria*, *Fusobacteria*, *Cyanobacteria*, and *Verrucomicrobia*, compose the intestinal gut flora in adult humans.[8] Variations in gut microbiota, however, can result from genetic and environmental factors such as diet, living conditions, and birthplace.[6]

In a concerted effort, the host and symbiotic bacteria employ the use of physical, chemical, and cell-mediated antimicrobial strategies to prevent or impair pathogen survival and virulence. Physical barriers provide the first line of resistance against pathogens. The intestinal mucosal barrier, composed of a thick mucus layer, a layer of epithelial cells, and an underlying layer of cells composed predominantly by immune cells, provides both a physical and chemical barrier through the

[1]Department of Microbiology, University of Texas Southwestern Medical Center, Dallas, Texas, USA. Correspondence: V Sperandio (Vanessa.Sperandio@utsouthwestern.edu)

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secretion of mucins, immunoglobulins, antimicrobial peptides, and lectins.[9] Especially important in barrier function, immunoglobulins secreted by B-lymphocytes aid in the opsonization of microbes and prevent microbial penetration of the mucosal layer.[9] Resident bacteria provide another crucial line of defense against the colonization of pathogens by competing for nutrition and attachment sites to the colonic epithelium, a mechanism known as the "barrier effect."[10]

Intestinal epithelial cells produce two major classes of antimicrobial peptides: defensins and cathelicidins.[11,12] Defensins function by embedding into the microbial membrane to form pores that allow for the efflux of essential ions and nutrients.[13] In addition to their antimicrobial activity, defensins and cathelicidins modulate the host immune response,[14] in large part by forming local chemotactic gradients that promote the mobilization of leukocytes.[12] Angiogenins represent another important class of antimicrobial proteins. Whereas mouse Ang1 and human ANG exhibit bactericidal and fungicidal activity against systemic pathogens, Ang4 acts selectively in the gut against enteric pathogens. Secreted by Paneth cells into the gut lumen, Ang4 expression is induced by the intestinal bacteria *Bacteroides thetaiotamicron*.[15] Also influenced by the intestinal flora, the mouse C-type lectin RegIIIγ and its human counterpart HIP/PAP exert their bactericidal activity against Gram-positive bacteria via an interaction with peptidoglycan[16] and are vital to antimicrobial protection in the mammalian gut.[17]

Symbiotic microbes provide another source of antimicrobial molecules within the mucosal barrier. Through the production of butyrate, a short-chain fatty acid that is the only known stimulus for cathelicidin expression,[18] the enteric microflora aids in the stimulation of host innate immunity. The short-chain fatty acid has also been shown to decrease Shiga toxin expression in enterohemorrhagic *Escherichia coli* (EHEC).[19] Additionally, symbiotic microbes can interfere with pathogen survival and growth through the production of potent toxins called bacteriocins. For example, *Lactobacillus salivarius* UCC118 produces the bacteriocin ABP-118 that is active against food-borne pathogens, including *Bacillus*, *Listeria*, *Enterococcus*, and *Staphylococcus* species.[20,21]

Although both the host and symbiotic microbes have evolved mechanisms to prevent pathogen invasion and colonization, similarly pathogens have devised means to subvert and even exploit their environment.

QUORUM SENSING

Bacteria respond to hormone-like molecules called autoinducers to regulate specific target genes, a process known as QS.[1,2] To date, four main categories of cell-to-cell signaling systems have been studied in detail. Gram-negative bacteria communicate in response to autoinducer-1 (AI-1) and autoinducer-3 (AI-3), whereas Gram-positive bacteria use an autoinducing polypeptide system.[22] Autoinducer-2 (AI-2) acts as a "universal" signal for interspecies communication and is found in both Gram-negative and Gram-positive cells.[23] Because of space constraints, only the AI-1 and AI-3 cell signaling systems will be discussed in this review.

The LuxI/LuxR System

The foundation of QS, AI-1 and its cognate signaling system, the LuxI/LuxR system, was initially discovered in the bioluminescent marine bacterium *Vibrio fisheri* and its host, the Hawaiian Bobtail Squid *Euprymna scolopes*.[1,24–26] LuxI synthesizes AI-1, an *N*-acyl homoserine lactone (AHL) that can diffuse freely across the bacterial membrane. The simultaneous production of AI-1 by a growing population of bacteria increases the local concentration of AI-1, and at high population density, AI-1 diffuses back into the cell. Inside the cell, AI-1 binds the transcriptional activator LuxR that, in its bound state to AI-1, can activate the transcription of the *luxCDABEGH* operon.[27]

Homologs to the LuxI/LuxR system have been identified in many Gram-negative bacteria. For example, the opportunistic human pathogen, *Pseudomonas aeruginosa*, has two homologous LuxI/LuxR systems: the LasI/LasR system (**Figure 1**) and the RhlI/RhlR system.[28] *P. aeruginosa* produces two AHLs: 3-oxo-C12 homoserine lactone (HSL) that acts on the *las* system and C4-HSL that acts on the *rhl* system. LasI produces 3-oxo-C12-HSL to activate LasR[29–31] that leads to the production of virulence factors like elastase[30] and pyoverdin.[32] RhlI synthesizes C4-HSL to activate RhlR,[33–35] allowing for the production of rhamnolipid biosurfactants[36] and a number of other virulence factors important in biofilm formation and pathogenesis.[37]

Interestingly, both *E. coli* and Salmonella typhimurium encode for a LuxR homolog named SdiA but do not have a LuxI homolog.[38,39] The absence of a LuxI homolog indicates that neither *E. coli* nor *S.* typhimurium can produce its own AI-1. Instead, presence of SdiA may allow for these bacteria to respond to AI-1 made by other bacteria, including AI-1 produced by the resident flora in the gastrointestinal tract where both of these pathogens colonize.

Pathogenesis in the Gut

The gastrointestinal tract is a diverse and dynamic environment, home to large communities of bacterial flora, and constantly threatened by opportunistic and pathogenic microbes. Within the gastrointestinal tract, bacteria communicate with one another and with their host to coordinate expression of key genes. Hormonal communication between the host and microorganisms is termed inter-kingdom signaling.[40] One example of an inter-kingdom signaling system is the AI-3/epinephrine/norepinephrine signaling system.[4]

Commensal *E. coli*, as well as the intestinal bacterial species EHEC, enteropathogenic *E. coli*, *Klebsiella pneumoniae*, *Shigella* sp., *Salmonella* sp., and *Enterobacter cloacae,* produce AI-3.[41] The mammalian hormones epinephrine and norepinephrine are also found in the intestine.[42] Although the primary roles of epinephrine and norepinephrine are to modulate smooth muscle contraction, submucosal blood flow, and chloride and potassium secretion,[43] enteric pathogens like EHEC O157:H7 can hijack these host hormones to induce virulence genes and promote colonization in the intestine.[4]

The QseBC (quorum sensing *E. coli* regulators B and C) two-component QS system can detect AI-3, epinephrine, and norepinephrine.[44] Upon sensing its signal, the histidine sensor

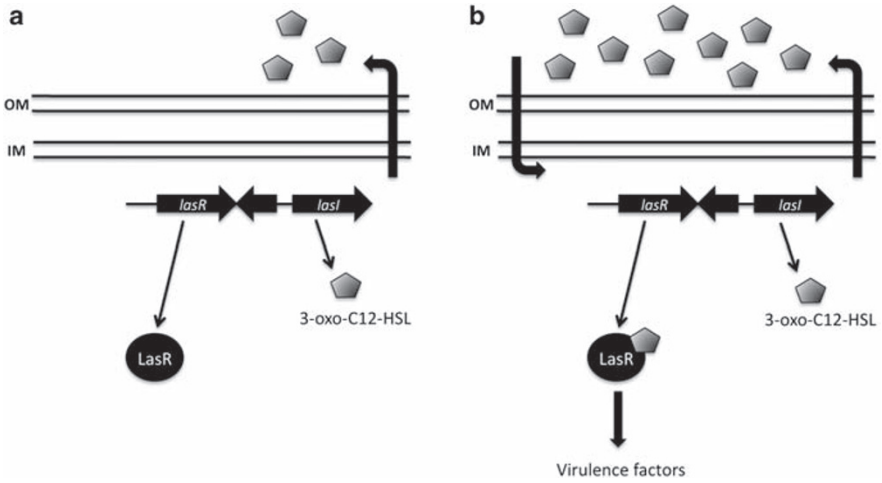

Figure 1 The LasI/LasR quorum-sensing system in *Pseudomonas aeruginosa*. (**a**) LasI synthesizes 3-oxo-C12 homoserine lactone (HSL), an *N*-acyl homoserine lactone (AHL) that freely diffuses across the bacterial membrane at low cell density. (**b**) At high cell density, 3-oxo-C12 HSL diffuses back into the cell, binds to the transcriptional activator LasR, and induces the expression of virulence genes.

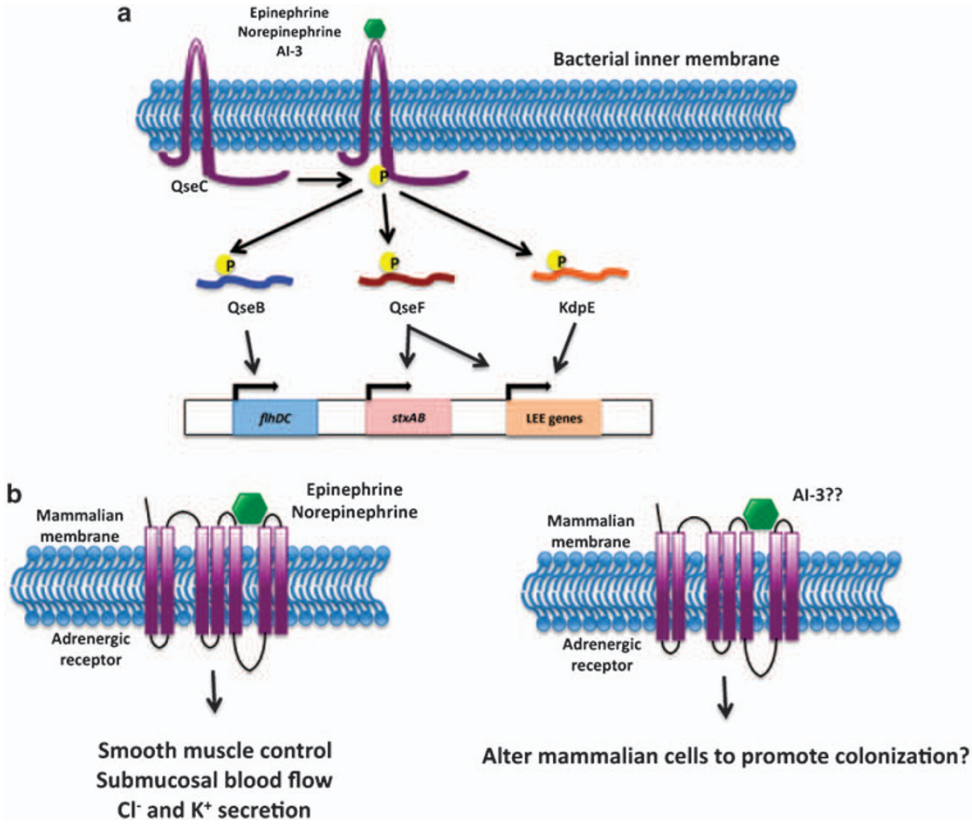

Figure 2 The epinephrine/norepinephrine/autoinducer-3 (AI-3) interkingdom signaling system. (**a**) The histidine sensor kinase QseC senses the mammalian hormones epinephrine and norepinephrine and the bacterial autoinducer AI-3. Activation of QseC stimulates an intrinsic autophosphorylation activity, allowing QseC to then transfer its phosphate group to one of three response regulators: QseB, QseF, and KdpE. The response regulators differentially regulate gene expression. (**b**) Epinephrine and norepinephrine bind to mammalian adrenergic receptors and have a role in smooth muscle control, submucosal blood flow, and chloride (Cl) and potassium (K) secretion. It remains unknown if AI-3 can bind to mammalian adrenergic receptors and alter mammalian function.

kinase QseC stimulates an intrinsic autophosphorylation activity that results in phosphorylation of a conserved histidine residue present in the cytoplasm (**Figure 2a**). The phosphate group is then transferred to a conserved aspartate residue on three response regulators: QseB, KdpE, and QseF.[45] Once phosphorylated, the response regulators differentially regulate gene expression. QseB activates the master flagellar regulator genes *flhDC* to regulate flagella biosynthesis and motility.[46] KdpE

controls potassium uptake, osmolarity, and the formation of AE (attaching and effacing) lesions.[45] QseF regulates the bacterial SOS stress response, as well as the formation of AE lesions.[45] The genes responsible for AE lesion formation are contained within the LEE (locus of enterocyte effacement), a chromosomal pathogenicity island responsible for much of EHEC's virulence; in addition, the LEE encodes for a type III secretion system and secreted effectors.[47–49]

QseC is a functional analog of an adrenergic receptor, and its activity can be blocked by the α-adrenergic antagonist phentolamine.[44] Interestingly, the α_2-adrenergic receptor is expressed at high levels in the proximal and transverse colon of the human intestine, the site at which EHEC and commensal flora colonize.[50] It remains unknown if AI-3 can bind to mammalian adrenergic receptors (**Figure 2b**). If so, this could be another mechanism by which EHEC exploits the intestinal environment to promote its colonization.

The importance of QseC is highlighted by its conservation in a number of bacterial species, including *Salmonella* sp., *Shigella flexneri*, *Vibrio parahaemolyticus*, *Yersinia pestis*, and *Francisella tularensis*.[51] In a mutant strain of *S.* typhimurium deficient in QseC, *S.* typhimurium has impaired flagellar motility and reduced invasion and survival in macrophages.[52] Furthermore, mice deficient in dopamine β-hydroxylase that are unable to produce epinephrine or norepinephrine have different susceptibility to *Salmonella* infection, indicating a role for AI-3/epinephrine/norepinephrine in inter-kingdom signaling.[52]

PATHOGENESIS IN THE LUNGS

P. aeruginosa is an opportunistic human pathogen that colonizes the lungs of cystic fibrosis patients. *P. aeruginosa* produces two AHLs as QS signaling molecules, 3-oxo-C12-HSL and C4-HSL, as discussed previously, that act on the *las* and *rhl* systems, respectively.[29–31,33–35] In addition to the AHL-based QS systems, *P. aeruginosa* uses an autoinducer regulatory system based on 2-alkyl-4(1*H*)-quinolones (AQs). The AQ biosynthetic enzymes of *P. aeruginosa* enable the generation of a diverse range of related AQ molecules: HQNO (2-heptyl-4-hydroxyquinoline N-oxide), HHQ (4-hydroxy-2-heptylquinoline), and PQS (*Pseudomonas* quinolone signal (3,4-dihydroxy-2-heptylquinoline)).[53,54] PQS induces the expression of *pqsABCDE*[55] and is required for the expression of *phzA1-G1*, the gene responsible for pyocyanin production. Release of pyocyanin induces neutrophil apoptosis and epithelial cell damage,[56] allowing *P. aeruginosa* to subvert immune surveillance and gain residency in the lungs. PQS also controls the expression of the *lasB* (elastase) gene,[57] and a synergistic effect is achieved in the presence of both PQS and C4-HSL.[58] Furthermore, PQS increases expression of the *lecA* gene that encodes for PA-I lectin (PA-IL).[59]

In addition to bacterial autoinducers, *P. aeruginosa* activates virulence genes in response to the opioid dynorphin.[60] Endogenous opioids are among the first signals released during times of stress.[61] Similar to the ability of EHEC to respond to the stress hormones epinephrine and norepinephrine, *P. aeruginosa* exploits the host during a weakened state for its own benefit. Dynorphin synergizes with PQS to increase expression of *pqsABCDE* and induce expression of the AQs HQNO and HHQ. Additionally, dynorphin enhances virulence against the probiotic *Lactobacillus* species and the nematode *Caenorhabditis elegans*.[60]

In addition to *P. aeruginosa*, AQs have been identified in a number of species of *Burkholderia,* including *B. ambifaria*, *B. thailandensis*, and *B. pseudomallei*. These organisms produce 3-methyl derivatives of PHQ, HHQ, and NHQ termed 4-hydroxy-3-methyl-2-alkylquinolones.[62] Because AQs also act as antibiotics, as evidenced by the ability of AQs produced by *P. aeruginosa* to inhibit the growth of *Staphylococcus aureus* and *Candida albicans*,[63] it remains to be determined if *Burkholderia* sp. use AQs solely as antibiotics or if they also use AQs as signaling molecules.

CONCLUSIONS

Bacterial communities reside on the skin and on every mucosal surface in the human body. Although many bacteria benefit the host's well-being, opportunistic and pathogenic bacteria await times of stress to exploit their host. Defining pathways unique to opportunistic and pathogenic bacteria will allow for the development of therapies that target specifically the virulence associated with these bacteria.

Since Alexander Fleming's discovery of the antibiotic penicillin in 1928, a number of additional antibiotics have been isolated from living organisms or synthesized. Unfortunately, because of the intense selective pressure placed on bacteria by antibiotics, a number of multidrug-resistant bacteria have emerged. Pathogens utilize an array of virulence strategies to promote colonization and cause disease in their hosts, including expression of adhesins, production of toxins, and secretion of effectors through specialized secretion systems. An alternative strategy to antibiotics is the development of antimicrobial drugs that target microbial virulence instead of growth.[64] Several such drugs are being tested in both laboratory and clinical settings against pathogens like *Bacillus anthracis*,[65,66] *S.* typhimurium, EHEC, *F. tularensis*,[51,64] *S. aureus*,[67] and *P. aeruginosa*.[68,69]

Because many of the QS pathways are conserved across bacterial species, a single therapy could be designed to target multiple pathogens. However, much research remains to be done to evaluate the risk of such therapies on resident flora that may also signal through these pathways. It is crucial that we seek new therapies against microbial pathogens as the incidence of resistance to current antibiotics rapidly rises. As we gain further understanding of the complex relationship among the host, symbiotic microbes, and invading pathogens, we will be better able to rationally design therapies that specifically target virulence traits incurred by pathogens.

DISCLOSURE

The authors declared no conflict of interest.

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