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N-Acylhomoserine Lactone-Mediated Quorum Sensing: A Twist in the Tail and a Blow for Host Immunity

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Communication through quorum sensing (QS) enables bacterial populations to coordinate their behavior. Recent work on *N*-acylhomoserine lactone-mediated QS has revealed that some soil bacteria exploit host-derived substrates to generate an alternative *N*-substituted homoserine lactone. New light has also been shed on the mechanism by which *N*-(3-oxo-dodecanoyl)-L-homoserine lactone modulates host inflammatory signaling pathways to promote bacterial survival.

Introduction

Bacteria display complex social behaviors and form communities of cells coordinating their activities through chemical communication. Because such cooperative behavior is often dependent on cell population density, it is usually referred to as "quorum sensing" (QS). Mechanistically, QS involves the activation of a receptor by a diffusible signal molecule. Consequently, the concentration of QS signal reflects the number of bacterial cells and the sensing of a threshold level of signal indicates that the population is "quorate"; that is, ready to make a collective decision (Fuqua and Greenberg, 2002; Lazdunski et al., 2004; Williams, 2007). Although QS was originally used to describe *N*-acylhomoserine lactone (AHL)-dependent cell-to-cell communication in Gram-negative bacteria, QS signal molecules exhibit significant chemical diversity (Figure 1). Where such molecules are responsible for inducing their own biosynthesis, they are referred to as "autoinducers." Although no "universal" bacterial QS language has yet been discovered, the autoinducer-2(AI-2)/LuxS QS system is shared by both Gram-negative and Gram-positive bacteria. In addition, many bacteria employ more than one QS signal molecule from the same or a different chemical class, the activities of which may be coordinated via interacting QS systems, each of which incorporates a signal molecule synthase and a sensor/receptor (Fuqua and Greenberg, 2002; Lazdunski et al., 2004; Williams et al., 2007).

QS controls secondary metabolism, bioluminescence, protein secretion, motility, virulence factor production, plasmid transfer, and biofilm maturation in diverse bacteria (Fuqua and Greenberg, 2002; Williams, 2007). As a result, QS has attracted considerable industrial interest, particularly as a target for novel antibacterial agents that attenuate bacterial virulence rather than growth (Bjarnsholt and Givskov, 2007). Because bacteria cohabit ecosystems with many other organisms, it is perhaps not surprising to discover that QS signals can modulate the behavior of both bacteria other than the QS signal producer itself and of higher organisms, in ways advantageous for bacterial survival (Williams, 2007). Conversely, higher organisms manipulate QS by producing signal mimics, by modulating QS pathways through the action of cytokines, by blocking QS through the deployment of inhibitors, or via the enzymatic inactivation of QS signals.

Two recent publications in this rapidly expanding field provide exciting new insights into the chemical diversity, evolution, and adaptability of AHL-dependent QS signal transduction pathways (Schaefer et al., 2008) and their effects on the host immune response (Kravchenko et al., 2008).

N-Alkanoyl- and *N*-aroyl-Homoserine Lactone-Dependent QS

AHL-mediated QS appears to be employed exclusively by Gram-negative bacteria, including beneficial and pathogenic species, because no AHL-producing Gram-positive bacteria have yet been identified (Williams, 2007; Williams et al., 2007). Most AHL producers synthesize multiple AHLs that are characterized by a homoserine lactone ring, unsubstituted in the β and γ positions, which is *N*-acylated with an acyl chain incorporating variable saturation levels and oxidation states. AHLs are exemplified by compounds with an acyl chain of 4–18 carbons that belong to either the *N*-acyl, *N*-(3-oxoacyl), or *N*-(3-hydroxyacyl) classes (Figure 1; Chhabra et al., 2005). AHLs with one or two double bonds in an acyl chain have also been described. The stereochemistry at the α-center of the homoserine lactone (HSL) ring is L, and the corresponding D-isomers are, in common with ring-open compounds, inactive (Chhabra et al., 2005). Consequently, it is the acyl chain moiety that confers QS signal specificity.

AHL biosynthesis mostly depends on LuxI family proteins that use *S*-adenosylmethionine (SAM) and an acyl carrier protein (ACP) charged with the appropriate fatty acid as sources of the homoserine lactone (HSL) and acyl chain, respectively (Moré et al., 1996; Fuqua and Greenberg, 2002; Figure 2). Although one or two compounds usually predominate, most LuxI homologs produce a range of AHLs, indicating that they exhibit some flexibility and can accept a number of different acyl-ACP donors (Ortori et al., 2007). In LuxI-driven AHL biosynthesis, two separate chemical events occur. The irst is the transfer of the acyl group from the ACP to the amino group of SAM, followed by lactonization and the release of the AHL and methylthioadenosine (Figure 2A). In this reaction mechanism, SAM acts as a homoserine rather than as a methyl donor (Moré et al., 1996; Fuqua and Greenberg, 2002). Structural studies of EsaI and LasI (which produce predominantly *N*-[3-oxohexanoyl]- L -homoserine lactone and *N*-[3-oxododecanoyl]- L -homoserine

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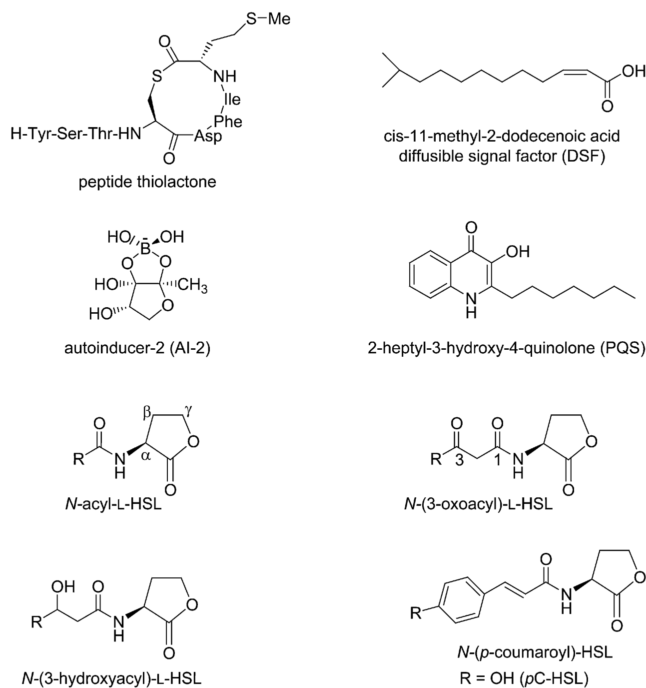

Figure 1. Structures of Some Bacterial QS Signal Molecules

lactone [3-oxo-C12-HSL], respectively) have highlighted a substrate-binding pocket for SAM and a common binding site for the acyl-ACP phosphopantetheine group, which constitutes a tunnel passing through the enzyme in LasI. This is occluded in EsaI, a finding that provides a partial explanation for the acyl chain specificities of the two enzymes (Watson et al., 2002; Gould et al., 2004).

Once produced, AHLs diffuse across the cell envelope and subsequently accumulate within the extracellular environment until a feedback concentration sufficient to activate a target LuxR family protein has been achieved. AHLs bind to and activate LuxR proteins directly such that the LuxR/AHL complex activates or represses one or more target genes that often include the AHL synthase gene, thus establishing a positive autoinduction circuit in which the AHL signal also controls its own biosynthesis (Williams, 2007).

In the pregenomics age, screening for AHLs was often undertaken using biosensors that typically contained a *luxR* homolog plus a target QS-dependent promoter fused to a reporter gene such as *lacZ* or *lux* and maintained in *Escherichia coli* (a non-AHL producer) (Swift et al., 1993). By introducing a chromosomal gene bank into such biosensors and examining clones for DNA fragments that activated the reporter, novel *luxI* homologs could be identified (Swift et al., 1993). New *luxI* and *luxR* homologs are now more likely to be identified via genome-sequencing projects. Indeed Schaefer et al. (2008) noted the presence of a chromosomal *luxIR* pair (*rpaIR*) in the genome of the photosynthetic bacterium *Rhodopseudomonas palustris*. This soil bacterium degrades a variety of aromatic compounds including *p*-coumarate (i.e., *p*-hydroxycinnamate), a major constituent of the lignin polymers abundant in plant cell walls. Growth of *R. palustris* on *p*-coumarate as a sole carbon source resulted in the upregulation of *rpaI*, implying a link between QS and *p*-coumarate degradation. However, no activity was observed in AHL-biosensor assays, implying the presence of a novel class of compounds. Because *rpaI* expression was enhanced by growth on *p*-coumarate, the authors fused the *rpaI* promoter to *lacZ* and screened culture supernatants for activating compounds. Only extracts prepared from *R. palustris* grown in the presence of *p*-coumarate efficiently activated *rpaI::lacZ*, whereas bacteria cultured with other related aromatic compounds such as *m*-coumarate or cinnamate exhibited weak activity and AHLs were inactive. Spectroscopic analysis of the active compound purified from culture supernatants was consistent with *N*-(*p*-coumaroyl)-L-HSL (*p*C-HSL) (Figure 1). The function of RpaI as an aroyl-HSL (the correct nomenclature for this acyl moiety is "aroyl" rather than "aryl") synthase was initially established by showing that deletion of *rpaI* rendered *R. palustris* unable to synthesize *p*C-HSL whereas the expression of *rpaI* in *E. coli* or *Pseudomonas aeruginosa* resulted in *p*C-HSL production, but only when exogenous *p*-coumarate was supplied. This is particularly interesting given that it implies a potential role for the host in facilitating QS signal synthesis and consequently might impact pathogenic or beneficial host-bacterial interactions if such systems prove to be much more widespread than currently appreciated. The purified RpaI protein was also demonstrated to catalyze *p*C-HSL biosynthesis from SAM and *p*-coumaroyl-CoA (Schaefer et al., 2008). A charged ACP is the preferred substrate for most LuxI-type proteins, but CoA-derivatives can be used less efficiently. Whereas *p*-coumaroyl-CoA is formed in crude extracts of *R. palustris*, the existence of a *p*–coumaroyl-ACP has yet to be confirmed. Because RpaI is a LuxI homolog, it would be interesting to determine the substrate specificity of this synthase and whether it can also catalyze in vitro the formation of AHLs from SAM and the appropriately fatty acid charged ACPs or CoAs (Figure 2B).

Given that RpaR is a repressor of *rpaI* expression and is derepressed on exposure to *p*C-HSL, it is clear that the RpaIR system constitutes a novel QS circuit that depends on an exogenous substrate produced by a different organism. The contribution of *p*C-HSL-dependent QS to the lifestyle of *R. palustris* has not been fully elucidated, although a microarray analysis following growth on *p*-coumarate identified 17 significantly upregulated genes including *rpaI* itself and several putative chemotaxis genes (Schaefer et al., 2008). This could imply a role for RpaIR QS in directing this soil bacterium toward plant roots. Although the RpaIR regulon appears to be much more limited than the QS regulon of bacteria such as *P. aeruginosa,* this may be a consequence of the growth conditions employed. Furthermore, *p*C-HSL-dependent QS may not be unique to *R. palustris* because the compound was detected in spent supernatants from two other environmental bacterial species (*Bradyrhizobium* and *Silicibacter*) whose genomes contain *luxI* homologs. Whether these bacteria only make small amounts or whether *p*-coumarate is only related to the natural substrate remains to be determined.

Despite the large number of LuxI protein sequences available, these often exhibit as little as 30% homology. Phylogenetic analysis shows that the majority of these proteins cluster within the same family rather than by the AHL produced. Sequence comparisons coupled with site-specific mutagenesis revealed the presence of 10 conserved amino acid residues within the

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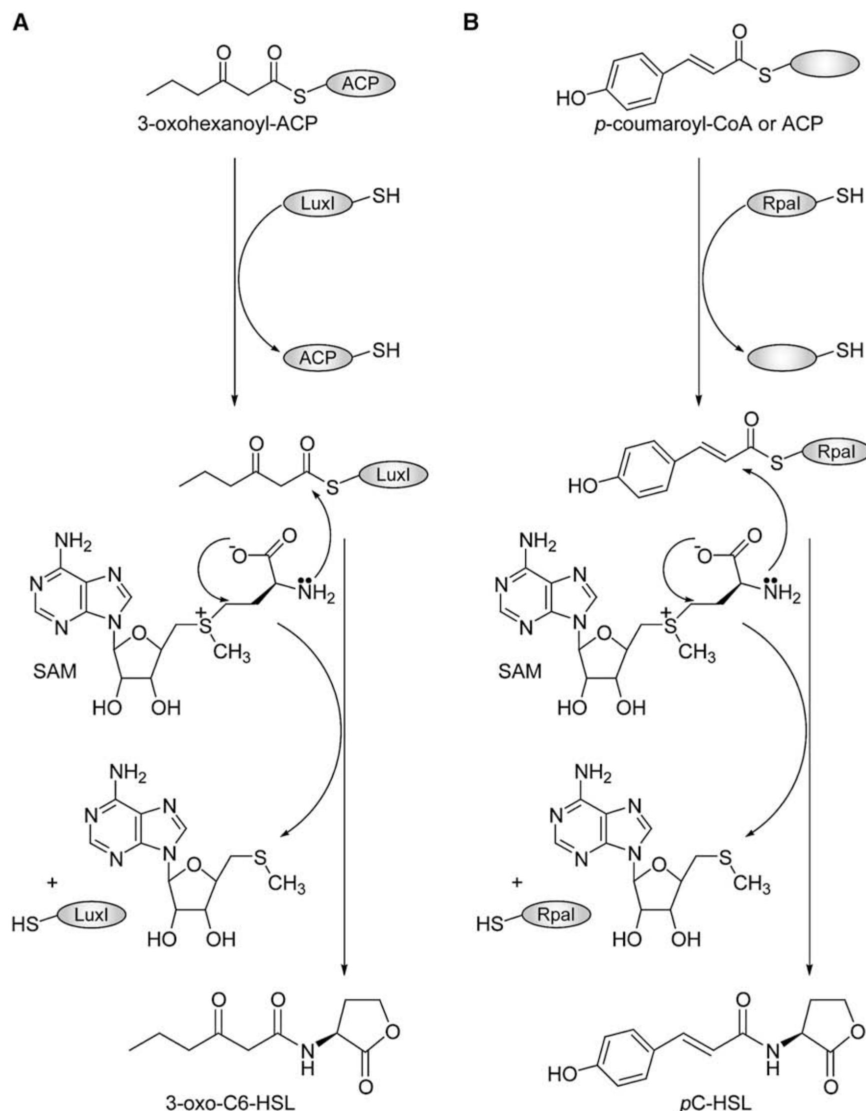

Figure 2. Biosynthesis of 3-oxo-C6-HSL and pC-HSL

(A) Biosynthesis of *N*-(3-oxohexanoyl)homoserine lactone (3-oxo-C6-HSL) via the charged acyl carrier protein and either LuxI or RpaI.

(B) Biosynthesis of pC-HSL via the charged acyl carrier protein and either LuxI or RpaI.

N-terminal half of the LuxI protein family (Fuqua and Greenberg, 2002). RpaI possesses nine of these and would have been expected to synthesize an AHL. Given that RpaI instead synthesizes an aroyl-HSL, these data raise some interesting questions about the evolution of both RpaI and RpaR given that the latter, as the cognate receptor, had to coevolve to respond to *p*C-HSL. Interestingly, the specificity of LuxR proteins can be modified by directed or forced evolution to generate proteins with either relaxed or altered AHL recognition (Collins et al., 2006). Similar approaches have been taken with LuxI proteins (Brader et al., 2005), indicating that both LuxR and LuxI homologs are evolutionarily pliable and suggesting that many more *N*-acyl and *N*-aroyl-HSL variants await discovery.

Interestingly, *N*-(cinnamoyl)- L-HSL, a des-hydroxy analog of *p*C-HSL, has previously been synthesized by Geske et al. (2008), as have other *N*-acyl, *N*-sulfonyl, *N*-aroyl, and *N*-alkanoyl-HSLs. Structure-activity relationship (SAR) studies with these analogs were determined by screening their activity as agonists or antagonists against three LuxR proteins (TraR, LasR and LuxR). AHLs with acyl groups containing aromatic functionality with electron withdrawing groups such as *N*-(*p*-cinnamoyl)- L-HSL generally acted as antagonists. Conversely, derivatives with electron-donating substituents such as hydroxyl on the aromatic ring were agonists, as was the case with RpaR and *p*C-HSL. Clearly there is a fine balance in RpaR substrate specificity, particularly in terms of electronic and steric requirements.

QS and the Immune Response

Like *R. palustris*, *P. aeruginosa* is an environmental Gram-negative bacterium found in soil; however, is also an opportunistic human pathogen. *P. aeruginosa* employs both AHLs (primarily 3-oxo-C12-HSL and *N*-butanoylhomoserine lactone [C4-HSL] synthesized via the LuxI homologs LasI and RhlI, respectively) and 2-alkyl-4-quinolones in a sophisticated QS network that controls secondary metabolite production, biofilm maturation, and virulence (Williams et al., 2007). With respect to the latter, the contribution of QS to the pathogenesis of *P. aeruginosa* infections has been extensively demonstrated in experimental animal infection models (Rumbaugh et al., 2000). Apart from their role as QS signal molecules, 3-oxo-C12-HSL and 2-heptyl-3-hydroxy-4-quinolone (PQS) also modulate inflammation and immune responses in mammals (Williams, 2007). Interestingly, the converse also appears to be true: *P. aeruginosa* responds to host immune molecules, including cytokines, by upregulating QS (Wu et al., 2005). Although *P. aeruginosa* is regarded as an opportunistic pathogen in humans that is responsible for acute infections, it can, once established in susceptible individuals such as those with cystic fibrosis, become chronic and difficult to eradicate with conventional antibiotic chemotherapy. It has been postulated that QS signal molecules facilitate chronic *P. aeruginosa* infections both by regulating virulence factor expression and by modifying host inflammatory and immune responses (Rumbaugh et al., 2000). It is the mechanism of this secondary activity that has recently been clarified for 3-oxo-C12-HSL. Although PQS modulates inflammation and immune responses (Hooi et al., 2004), its mechanism of action remains unclear except that it differs from that of 3-oxo-C12-HSL. By contrast, there are numerous reports of the effects

of 3-oxo-C12-HSL on mammalian cells, and recent advances in defining the mammalian intracellular targets of 3-oxo-C12-HSL have shed significant light on the mechanisms involved.

Many of the effects of 3-oxo-C12-HSL on mammalian cells occur at concentrations (10–50 μM) that are higher than those that have been demonstrated in human infections. In planktonic *P. aeruginosa* culture supernatants, 3-oxo-C12-HSL is present at submicromolar levels, although concentrations of up to 600 μM 3-oxo-C12-HSL have been reported in biofilms (Charlton et al., 2000). In sputa and lung tissues from *P. aeruginosa*-infected cystic fibrosis patients, 3-oxo-C12-HSL concentrations are at nanomolar concentrations (Erickson et al., 2002). However, the presence of lactonases in lung tissue (Ozer et al., 2005) and the lability of 3-oxo-C12-HSL and other AHLs under physiological pH and temperature conditions (Yates et al., 2002) can reduce the detectable levels of AHLs, such that the actual local concentrations in the tissues might be much higher. Whether 3-oxo-C12-HSL can diffuse out of the lung and influence systemic immune responses has yet to be established, although the lipophilic nature of the molecule suggests that it could possibly disseminate through the lymphatic system if not via the bloodstream.

The in vitro effects of 3-oxo-C12-HSL generally fall into two categories: immunosuppressive or antiinflammatory effects at concentrations below 10 μM, and proinflammatory or proapoptotic effects at concentrations of 20 μM and above. The earliest report of immune modulation by 3-oxo-C12-HSL (Telford et al., 1998) revealed that concentrations of 3-oxo-C12-HSL below 10 μM could reduce the LPS-induced production of the proinflammatory cytokine IL-12 by monocytes in vitro. Such inhibition of IL-12 production would exert a dampening effect on innate immunity, and also, because monocytes can present antigen to T cells, could modulate the outcomes of the adaptive immune response. Telford et al. (1998) also showed that 3-oxo-C12-HSL inhibited mitogen-induced proliferation of lymphocytes, suggesting that it directly inhibited lymphocyte immune responses. This was confirmed by later reports showing that 3-oxo-C12-HSL could inhibit the proliferation and function (cytokine production) of both mitogen-stimulated (e.g., Chhabra et al., 2003; Ritchie et al., 2003) and antigen-stimulated (Ritchie et al., 2005) T lymphocytes, and modulate antibody production by B lymphocytes (Telford et al., 1998; Ritchie et al., 2003). Overall, the outcome of this modulation during *P. aeruginosa* infection would tend to generate a less effective antibody-mediated, rather than a more effective cell-mediated, adaptive immune response to the bacteria, and could thus facilitate persistence. A SAR study of 3-oxo-C12-HSL indicated that, like QS activity, immune modulatory activity requires an intact HSL ring, L-configuration at the chiral center, and an acyl chain of 11–13 carbons (Chhabra et al., 2003).

Other studies have investigated the effects of 3-oxo-C12-HSL on respiratory epithelial cells and fibroblasts, cell types likely to be exposed to *P. aeruginosa* during lung infections, and also likely to be exposed to the highest in vivo concentrations of 3-oxo-C12-HSL. At concentrations above 20 μM, 3-oxo-C12-HSL induced production of the neutrophil-attracting chemokine, CXCL8 (interleukin-8, IL-8), by a range of cell types, including respiratory fibroblast and epithelial cell lines (Smith et al., 2001). However, Kravchenko et al. (2006) reported that primary respiratory epithelial cells showed no such induction. 3-oxo-C12-HSL is also chemotactic for neutrophils (Zimmermann et al., 2006). In contrast to IL-8 production and neutrophil attraction, both of which would tend to promote inflammation, 3-oxo-C12-HSL induces apoptosis in both neutrophils and monocytes (Tateda et al., 2003), an effect confirmed in fibroblasts by Shiner et al. (2006). This would tend to reduce inflammation by reducing the number of effective phagocytes and the mediators they produce. However, it has also been argued that together with an increase in inflammatory mediators leading to host cell damage, the induction of apoptosis in inflammatory cells would favor spread of invading bacteria and promote persistence of infection (Rumbaugh et al., 2000).

Although descriptive studies of the varied effects of 3-oxo-C12-HSL on mammalian cells in vitro have been numerous, it is only in the last 5 or 6 years that significant advances have been made in elucidating the mechanisms involved. 3-oxo-C12-HSL freely enters mammalian cells and retains intracellular activity (Ritchie et al., 2007; Shiner et al., 2004), but in vivo it is likely that physiological pH (Yates et al., 2002) and mammalian enzymes (Ozer et al., 2005) will substantially reduce extracellular levels. These findings suggest that the most likely target for 3-oxo-C12-HSL would be an intracellular rather than a cell surface receptor. A number of studies have provided evidence to support this hypothesis, although only recently has a direct target for 3-oxo-C12-HSL been identified.

Smith et al. (2002) found that 3-oxo-C12-HSL induced activation of the proinflammatory signaling components Cox-2 and NF-κB in transformed cell lines, although Kravchenko et al. (2006) suggested that this does not occur in primary cells. 3-oxo-C12-HSL also inhibited STAT3 activity in a breast cancer cell line (Li et al., 2004), an interesting finding given that STAT3 is an important component of the signal transduction pathway leading to IL-8 synthesis, and is present in many cell types including immune cells. 3-oxo-C12-HSL also induces phosphorylation of MAPK p38 and eIFα, both promoters of inflammatory signaling, but this does not occur through canonical pathogen pattern recognition receptors such as toll-like receptor 4 (TLR4), the receptor for LPS, or its downstream components (Kravchenko et al., 2006). These findings suggested the possibility of a novel pathogen pattern recognition pathway. However, an interaction between TLRs and the activity of 3-oxo-C12-HSL was first suggested by a comparison of the results of Tateda et al. (2003), who showed that 3-oxo-C12-HSL induced inflammatory cytokine production by non-LPS stimulated macrophages, and Telford et al. (1998), who showed that LPS-stimulated monocyte IL-12 production was inhibited. These data indicated that the effects of 3-oxo-C12-HSL on mammalian cells might be modulated by the presence of other bacterial products, such as LPS, that signal through TLRs. Another indication of the complexity of the effects of 3-oxo-C12-HSL on mammalian cells was suggested by Shiner et al. (2006), who showed that whereas induction of apoptosis in fibroblasts was dependent on calcium flux into cells, induction of cytokine production in these cells was not, implying that at least two different intracellular pathways were affected.

Recently, two seminal papers have shed light on the mechanism of 3-oxo-C12-HSL action in mammalian cells. Kravchenko et al. (2008) demonstrated that in the absence of LPS, 3-oxo-C12-HSL alone did not affect NF-κB-mediated signaling, but

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did induce phosphorylation of mitogen-activated protein kinase (MAPK) p38, which could result in cytokine production. They also showed that, through its effects on MAPKs, 3-oxo-C12-HSL also potentiates TNFα-induced poly(adenosine 5′-diphosphate-ribose) (PARP) cleavage, which is a biochemical marker of apoptosis. However, in LPS-stimulated cells, 3-oxo-C12-HSL inhibited the LPS-mediated induction of phosphorylation of the NF-κB associated protein IκB. Because nonphosphorylated IκB retains NF-κB in the cytoplasm, preventing its translocation to the nucleus to initiate gene transcription, the effect of 3-oxo-C12-HSL would be to reduce the intensity of NF-κB signaling and consequent production of inflammatory mediators, although some cytokine production could still be induced through 3-oxo-C12-HSL activation of MAPKs (Kravchenko et al., 2008). The kinetics of these effects are complex, but the overall effect of 3-oxo-C12-HSL is to attenuate LPS-induced inflammation, which is likely to contribute to the establishment of persistent infection by *P. aeruginosa*. Thus, it is possible that whereas 3-oxo-C12-HSL induces some cytokine production and/or apoptosis in the absence of other bacterial products, this might be cell-type specific, but in the presence of other bacterial products that signal via TLRs and NF-κB, 3-oxo-C12-HSL exerts net antiinflammatory effects.

In their comprehensive study, Kravchenko et al. (2008) did not identify a direct binding target of 3-oxo-C12-HSL in mammalian cells. However such a target, the peroxisome proliferator activated receptors (PPARs) that belong to the nuclear hormone receptor family has recently been identified (Jahoor et al., 2008). The PPAR subfamily has broad roles in inflammation and lipid metabolism and binds a range of endogenous and exogenous lipids. There are three PPAR isoforms, each of which has different tissue distributions and ligand specificities, but all modulate inflammation (Ricote and Glass, 2007). This occurs through the direct regulation of transcription by the formation of a ligand-dependent heterodimer with the retinoid X receptor, which binds to PPAR response elements in a number of genes. Direct interactions with transcription factors including NFκB and AP-1 (MAPK pathway) have also been reported, as has *trans*-repression involving a range of corepressors and coactivators (Ricote and Glass, 2007).

Jahoor et al. (2008) used a number of in vitro techniques to demonstrate that 3-oxo-C12-HSL binds to at least two isoforms of PPAR (PPARγ and PPARβ/δ) and could modulate the formation of the PPARγ signaling complex with the retinoid X receptor and its binding to DNA to initiate transcription. Significantly, a principal mechanism by which PPARs regulate inflammation is by modulating TLR and NF-κB signaling (De Bosscher et al., 2006) and inducing phosphorylation of p38 MAPK (Gardner et al., 2005). Thus, it seems likely that 3-oxo-C12-HSL modulates NF-κB signaling through a direct interaction with PPARs, and that the effects of 3-oxo-C12-HSL on PPARs and hence on NF-κB signaling could in turn be modulated by concomitant signaling through other pathogen recognition receptors, including TLRs (Figure 3). Modulation of a subset of TLR pathways by PPARs has been demonstrated (Ogawa et al., 2005), and an example of such selective modulation was shown by Gurley et al. (2008) who reported that in brain macrophages PPARγ agonists inhibited LPS-induced TNFα production, but augmented LPS-induced production of macrophage inflammatory protein 2a, a homolog of IL-8. To add to the possibilities for flexibility, 3-oxo-C12-HSL also differentially modulated signaling though different PPAR isoforms: it appeared to act as an antagonist for PPARγ and an agonist for PPARβ/δ (Jahoor et al., 2008). Because PPAR isoform expression is cell-type specific, the outcome of 3-oxo-C12-HSL interference with mammalian cell signal transduction pathways might also be to some extent cell-type specific, and its effects on cellular function are likely to depend on the relative expression of different PPAR isoforms in different cell types.

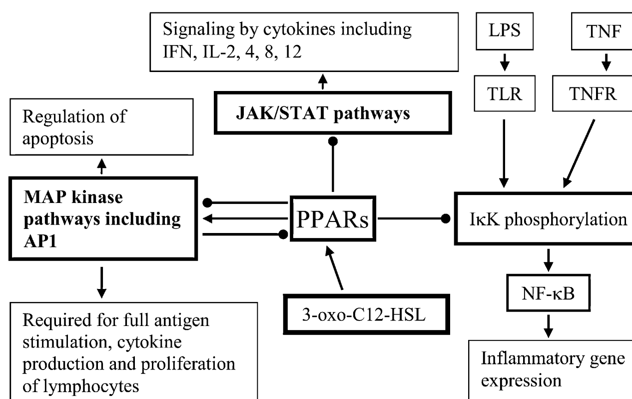

Figure 3. Mechanisms Immune Modulation by 3-oxo-C12-HSL
Proposed mechanisms of immune modulation by 3-oxo-C12-HSL. Arrows indicate positive interactions; lines ending in circles represent negative interactions. Note that reported interactions of PPARs with MAP kinases vary with the PPAR isoform.

The in vitro effects of 3-oxo-C12-HSL on lymphocyte function can also be explained by modulation of PPAR activity. Some PPAR agonists have been reported to inhibit cytokine production and proliferation of lymphocytes (Yang et al., 2000), and if 3-oxo-C12-HSL modulates MAPK or STAT activity in these cells, this would inevitably lead to alterations in lymphocyte activation, proliferation and cytokine production. It is also possible that direct effects of 3-oxo-C12-HSL on dendritic antigen presenting cells in the lung that subsequently migrate to the lymph nodes and spleen could result in indirect modulation of T lymphocyte function, because PPAR agonists have been reported to modulate dendritic cell migration (Angeli et al., 2003), maturation (Gosset et al., 2001) and cytokine production (Faveeuw et al., 2000). Thus, even if significant extracellular 3-oxo-C12-HSL does not disseminate from infected lungs, its effects on antigen presenting cells that migrate out of the lungs after exposure to antigen could lead to modulation of peripheral immune responses.

One of the features of *P. aeruginosa* infection in cystic fibrosis patients is the striking degree of inflammation. Although a number of host and bacterial factors have been suggested to contribute to this, it is also important to note that PPARs are expressed at lower levels in the cells of cystic fibrosis patients than in cells of healthy controls (Reynders et al., 2006; Perez et al., 2008), so any effect of 3-oxo-C12-HSL on PPAR in those cells is likely to be more potent than in normal cells. Interestingly, a recent study that used the PPARγ agonist rosiglitazone as an antiinflammatory treatment in a mouse model of *P. aeruginosa* lung infection showed that the drug was able to attenuate infection-related inflammation in cystic fibrosis (cftr$^{-/-}$) mice but not in normal

mice (Perez et al., 2008). This suggests that modulation of PPAR activity by 3-oxo-C12-HSL produced during *P. aeruginosa* lung infection could contribute to inflammation disproportionately in cystic fibrosis patients compared with other susceptible individuals such as burns patients or the immunocompromised.

In summary, these recent developments in the QS field extend the repertoire of *N*-substituted homoserine lactones now known to be employed as bacterial signal molecules and refine our understanding of the contribution of such molecules to host-pathogen interactions. Paradoxically, 3-oxo-C12-HSL and related molecules have significant therapeutic potential as antiinflammatory or immunosuppressive agents, whereas 3-oxo-C12-HSL-dependent QS remains an attractive target for novel antipseudomonal agents that control infection through the downregulation of bacterial virulence.

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