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The Pseudomonas Autoinducer N-(3-Oxododecanoyl) Homoserine Lactone Induces Cyclooxygenase-2 and Prostaglandin E₂ Production in Human Lung Fibroblasts: Implications for Inflammation

Roger S. Smith,* Rodney Kelly,§ Barbara H. Iglewski,* and Richard P. Phipps²*

Pseudomonas aeruginosa causes lethal lung infections in immunocompromised individuals such as those with cystic fibrosis. The lethality of these infections is directly associated with inflammation and lung tissue destruction. P. aeruginosa produces several acylated homoserine lactones (AHL) that are important in the regulation of bacterial virulence factors. Little is known about the effects of AHLs on human cells. In this work we report that the AHL N-(3-oxododecanoyl) homoserine lactone (3O-C₁₂-HSL) from P. aeruginosa induces cyclooxygenase (Cox)-2, a seminal proinflammatory enzyme. When primary normal human lung fibroblasts were exposed to 3O-C₁₂-HSL, an 8-fold induction in mRNA and a 35-fold increase in protein for Cox-2 were observed. In contrast, there was no substantial change in the expression of Cox-1. We also demonstrated that the induction of Cox-2 was regulated by 3O-C₁₂-HSL activation of the transcription factor NF-κB. 3O-C₁₂-HSL also stimulated an increase in the newly discovered inducible membrane-associated PGE synthase but had no effect on the expression of the cytosolic PGE synthase. We also demonstrated that 3O-C₁₂-HSL stimulated the production of PGE₂. PGE₂ is known to induce mucus secretion, vasodilation, and edema, and acts as an immunomodulatory lipid mediator. We propose that 3O-C₁₂-HSL induction of Cox-2, membrane-associated PGE synthase, and PGE₂ likely contributes to the inflammation and lung pathology induced by P. aeruginosa infections in the lung. These studies further reinforce the concept that bacterial AHLs not only regulate bacterial virulence but also stimulate the activities of eukaryotic cells important for inflammation and immune defenses. The Journal of Immunology, 2002, 169: 2636–2642.

The bacterium Pseudomonas aeruginosa causes many infections in humans with altered host defenses. The most prominent and deadly are the pulmonary infections. Patients with cystic fibrosis (CF) or HIV or those on mechanical ventilators have increased susceptibility to Pseudomonas lung infections (1). Due to its ubiquitous nature, exposure to P. aeruginosa in the hospital setting is also high, making it one of the most common nosocomial infections (2). Using a mechanism of gene regulation called quorum sensing, P. aeruginosa coordinates turns on and off genes that promote its ability to colonize the host and subvert immune surveillance. Quorum sensing uses the production of acylated homoserine lactones (AHL) to detect bacterial densities. P. aeruginosa predominately produces two AHLs, N-(3-oxododecanoyl) homoserine lactone (3O-C₁₂-HSL; also referred to as PAI-1 or OdDHL) and butyryl homoserine lactone (C₄-HSL), also referred to as PAI-2 or BHL) (3, 4). Although both of these AHLs are diffusible in and out of the bacteria, 3O-C₁₂-HSL is also actively pumped out by the MexAB OprM efflux system (5). As the concentration of P. aeruginosa increases, the amount of AHLs in the environment will also increase. When a threshold concentration is reached, the AHLs bind to and activate transcriptional regulators that subsequently turn on and off genes important for virulence and also lead to the increased production of additional AHLs (6, 7). For a complete review of Pseudomonas quorum sensing and virulence see Ref. 8.

Recently, it has become apparent that 3O-C₁₂-HSL is not only important in the regulation of bacterial virulence genes but can also interact with eukaryotic cells and stimulate an immune response. 3O-C₁₂-HSL stimulation of human lung structural cells, such as fibroblasts and bronchial epithelial cells, induces production of the chemokine IL-8 (9, 10). This induction of IL-8 is regulated by the activation of a mitogen-activated protein kinase pathway that subsequently leads to the induction of the transcription factor NF-κB (10). When human lung cells were stimulated with other AHLs there was no induction in IL-8, indicating that this induction was structurally specific (9, 10). 3O-C₁₂-HSL also activates classic immune cells. For example, when LPS-activated mouse peritoneal exudate cells were cultured with 3O-C₁₂-HSL, there was a significant inhibition in the production of the cytokine IL-12 (11). 3O-C₁₂-HSL was also recently shown to directly stimulate T cells to produce the cytokine IFN-γ (12). These data demonstrate that 3O-C₁₂-HSL is a potent activator of multiple different eukaryotic cells and that the production of 3O-C₁₂-HSL by P. aeruginosa may greatly affect its ability to cause disease.
The initial steps in PG production involve the release of arachidonic acid from the plasma membrane of cells by phospholipases. Arachidonic acid is then converted to PGH₂ by the enzyme cyclooxygenase (Cox), also called PGH synthase. There are two known isoforms of Cox, Cox-1 and Cox-2 (13). Cox-1 is constitutively expressed in most tissues and is thought to be responsible for physiological functions such as platelet aggregation and renal function (14, 15). Cox-2 is usually only expressed when induced by bacterial products or cytokines. The induction of Cox-2 production is an early hallmark of inflammation. The overexpression of Cox-2 leads to the enhanced production of PGs. Inhibition of Cox-2 with selective inhibitors reduces inflammation, fever, pain, and possibly certain cancers (16). PGH₂ produced by Cox is further converted to one of many prostanoids by specific synthases. PGE synthase (PGES) is the terminal step in the conversion of PGH₂ to PGE₂. There are two known isoforms of PGES, a constitutive cytosolic PGES (cPGES) and an inducible membrane-associated PGES (mPGES) (17). Recent evidence indicates that the mPGES is linked to the Cox-2 enzyme and that the cPGES is linked to the Cox-1 enzyme (18, 19). These data support the concept that induction of Cox-2 leads to increased production of PGE₂. PGE₂ has been shown to cause fever and permeability of vascular endothelium, enhance mucus secretion, and induce pain (20). PGE₂ also synergizes with IL-8 to enhance neutrophil migration (21). It also acts as an immunoregulator by inhibiting IL-12 production, a cytokine that stimulates T cells to produce IFN-γ. Therefore, the production of PGE₂ skews the immune response by promoting type-2 responses (e.g., IL-4 production) at the expense of type-1 responses (e.g., IFN-γ production) (22). PGE₂ also has anti-inflammatory effects in the lung. In asthmatic patients PGE₂ inhibits the release of mediators from mast cells and migration of eosinophils and induces bronchodilation (23). These data indicate that PGE₂ is a potent molecule that affects multiple functions in the body and thus its production may have substantial effects on the pathogenesis of P. aeruginosa.

The ability of 3O-C₁₂-HSL to regulate Cox-2 and PGs in human cells is the focus of this study. In mouse pneumonia and thermal injury models, it has been demonstrated that P. aeruginosa are potent inducers of PGE₂ (24, 25). In this manuscript we demonstrate that the P. aeruginosa quorum sensing molecule 3O-C₁₂-HSL is a potent inducer of Cox-2 and PGE₂ production in human lung fibroblasts.

Materials and Methods

Reagents

3O-C₁₂-HSL and C₁₀-HSL are organically synthesized in our laboratory as previously described (3, 4). These molecules are structurally and functionally identical to the natural molecule produced by P. aeruginosa. Each lot of 3O-C₁₂-HSL and C₁₀-HSL is shown to be pure by HPLC. No detectable levels of endotoxin were found in preparations of AHLs using a Limulus amebocyte lysate assay (Cape Cod Associates, Falmouth, MA). SC58125, a Cox-2 selective inhibitor (26), was a gift from Dr. P. Isakson (Searle, Skokie, IL). Indomethacin, a general Cox inhibitor, was obtained from Sigma-Aldrich, St. Louis, MO. SN50, a NF-κB inhibitor (27) or SN50 M (mutant inhibitor) for 1 h before stimulation with 100 μM SC58125. SC58125.

Cell culture

L828 cells are a normal, nontransformed human lung fibroblast strain previously isolated in our laboratory (27). These cells were maintained in MEM (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS (HyClone Laboratories, Logan, UT) and 50 μM gentamicin (Life Technologies). Cells were passaged every 4–5 days using 0.05% trypsin with 0.1% EDTA to dissociate adherent cells. Fibroblasts were used between passages 5 and 15.

RNA isolation and quantification

L828 cells were grown in six-well plates until confluent (~5 × 10⁶ cells/well). Cells were serum starved for 18 h before stimulation with a titration of 3O-C₁₂-HSL. Cells were stimulated for various times and RNA was extracted with TriReagent (Molecular Research Center, Cincinnati, OH) as per manufacturer’s procedures. cDNA was prepared from RNA using reverse transcriptase and random hexamers (PE Applied Biosystems, Foster City, CA). Primers (forward, GTTTGTTGACTACCATCACTAAT; reverse, ACCTTGAAGGATCCAGCATTAG) and probe CGCAACCGTATTGCCAGGAG and the Cox-2 primers (forward, GTTGTGTAATCCAGATACATTGA; reverse, GAGAGGCCTCCAGCGCTT GTA) and probe TGACAGTCXACCACACTTCAAGCTGACTAG were used to amplify DNA templates in a TaqMan 7700 (PE Applied Biosystems) using FAM/TAQMAN dyes for the probes. The probe for cova spans the junction between exons 3 and 4 of the cova-2 gene (i.e., it straddles the third intron); therefore, there is no chance that there was any interference from genomic DNA. Ribosomal (18S) RNA was amplified and used as a control. After 40 cycles the Ct (related to the cycle number at which signal appears) for the cova RNA (FAM signal) and the 18S were recorded. The absolute relative quantification was achieved using the formula 2-DDCt, which relates the amount of Cox cDNA to the 18S internal control and a standard Cox reference CDNA derived from U937 monocytes.

Protein isolation and Western blot analysis

Cells were cultured as described above and then extracted with cold lysis buffer (150 mM NaCl, 50 mM Tris (pH 8), 1 mM EDTA, 1% IGEPAL (Sigma-Aldrich), and 10% protease inhibitor mixture (Sigma-Aldrich)). Protein extracts were quantified using the bicinechonic acid assay (Pierce, Rockford, IL). Total protein was resolved on 10% SDS-PAGE gels and transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Non-specific binding was blocked by incubating the blots with 10% skim milk in PBS with 0.1% Tween 20 for 2 h at room temperature. Immunoreactive proteins were detected by incubating the blots with mouse anti-human Cox-1 or Cox-2 Abs or with rabbit anti-human PGES Abs (Cayman Chemical, Ann Arbor, MI) overnight at 4°C. Between each step the nitrocellulose was washed three times for 5 min with PBS/0.1% Tween 20. Bound Abs were detected with an anti-mouse IgG or anti-rabbit IgG conjugated to HRP (Santa Cruz Biotechnology, Santa Cruz, CA). Specific bands were visualized when nitrocellulose blots were incubated with ECL reagents (Amersham Pharmacia Biotech) and exposed to Kodak X-Omat film (Kodak, Rochester, NY).

Immunocytochemistry

L828 fibroblasts were grown in eight-well Permanox chamber slides (Nalge Nunc, Naperville, IL) at a density of 2 × 10⁶ cells per well. Cells were serum starved for 24 h before incubation with medium only or with a titration of 3O-C₁₂-HSL for 24 h. Cells were washed twice with PBS and incubated with 6 μM Hoechst 33258 (Biological Industries, Israel) for 30 min on ice and washed twice with PBS and fixed with 4% formaldehyde for 15 min. Fixed cells were incubated with 0.1% Triton X-100 for 30 min, blocked with 10% horse serum in PBS, at room temperature for 1 h. Cells were dried using the bicinchoninic acid assay (Pierce, Rockford, IL). Total protein was resolved on 10% SDS-PAGE gels and transferred to nitrocellulose membranes (Amersham Pharmacia Biotech, Piscataway, NJ). Nonspecific binding was blocked by incubating the blots with 10% skim milk in PBS with 0.1% Tween 20 for 2 h at room temperature before overnight incubation at 4°C with 10 μg/ml mouse anti-human Cox-1 or Cox-2 Abs or an isotype control mouse IgG2b or IgG1 Ab (Caltag Laboratories, Burlingame, CA). Between each step cells were washed three times with PBS containing 0.05% Tween 20. Cells were incubated at room temperature for 1 h with a 1/20 dilution of a biotin-labeled anti-mouse IgG secondary Ab (Vector Laboratories, Burlingame, CA). Streptavidin HRP (Jackson ImmunoResearch Laboratories, West Grove, PA), at a 1/1000 dilution in PBS, was incubated with cells for 1 h at room temperature. Aminoethyl carbazole (Zymed Laboratories, Burlingame, CA) was then added for 30 min at room temperature. Cells were counterstained with hematoxylin and mounted with Immobion (Sigma-Aldrich). In experiments examining the role of NF-κB, cells were incubated with 50 μM SN50 (NF-κB inhibitor) or SN50 M (mutant inhibitor) for 1 h before stimulation with 100 μM 3O-C₁₂-HSL. For NF-κB mobilization studies cells were stimulated for 24 h with 100 μM 3O-C₁₂-HSL and then stained with 2 μg/ml of an anti-NF-κB p65 Ab (Santa Cruz Biotechnology) as previously described (10).
AUTOINDUCER 3O-C12-HSL INDUCES Cox-2, PGES, AND PGE2

Statistics
A Wilcoxon signed rank test was used to determine statistical significance. Error bars indicate the SD of the mean.

Results
Stimulation of human lung fibroblasts with 3O-C12-HSL induces Cox-2 mRNA

L828 fibroblasts were stimulated with 1–100 μM 3O-C12-HSL and RNA extracts were examined using real-time RT-PCR. Increases in steady-state Cox-2 mRNA levels occurred as early as 4 h after stimulation with a maximal induction at 8 h. This maximal induction was 8-fold over that found in nonstimulated fibroblasts (Fig. 1). After 8 h of stimulation, Cox-2 mRNA levels decreased and returned to that of nonstimulated cells. The optimal concentration of 3O-C12-HSL that consistently induced significant Cox-2 mRNA expression was 100 μM. 3O-C12-HSL stimulation had no significant effect on the level of Cox-1 mRNA, which was constitutively expressed at very low levels in these cells (Fig. 1).

3O-C12-HSL stimulation of human lung fibroblasts increases the expression of Cox-2 protein but not Cox-1 protein

Production of Cox-1 protein in L828 lung fibroblasts was found to be constitutive in nonstimulated cells and, when cells were cultured with increasing amounts of 3O-C12-HSL, there was no significant change in the amount of Cox-1 protein expressed (Fig. 2). In contrast, Western blots for Cox-2 protein demonstrated that there was no expression in nonstimulated fibroblasts; however, when they were stimulated with 3O-C12-HSL there was a significant induction in Cox-2 protein. Stimulation with 1–10 μM 3O-C12-HSL induced a 2- to 5-fold increase in Cox-2 protein, whereas stimulation with 100 μM led to a 35-fold induction (Fig. 2). Cells were also stimulated with 3O-C12-HSL and then stained with anti-human Cox Abs to demonstrate Cox-1 and Cox-2 expression in individual fibroblasts. Similar to what was observed in Western blot experiments, nonstimulated L828 fibroblasts constitutively expressed Cox-1 and this expression was unaffected by 3O-C12-HSL (Fig. 3). When fibroblasts were stained with an anti-Cox-2 Ab there was no Cox-2 expression in nonstimulated cells, but with increasing concentrations of 3O-C12-HSL the amount of Cox-2 staining also increased. Faint staining for Cox-2 was observed in cells stimulated with 1 μM 3O-C12-HSL, with maximal staining in cells stimulated with 10 or 100 μM (Fig. 3). A similar induction in Cox-2 protein expression was found with other human lung fibroblast strains and with a human bronchial epithelial cell line (data not shown). To determine whether other AHLs could similarly stimulate an induction of both Cox-2, fibroblasts were cultured with C4-HSL, a second AHL produced by P. aeruginosa. When 100 μM C4-HSL was added to L828 fibroblasts there was only a slight induction in Cox-2 expression, while Cox-1 was constitutively expressed, even in nonstimulated cells (Fig. 3). Other concentrations of C4-HSL were unable to induce any expression of Cox-2 protein (data not shown). These data indicate that 3O-C12-HSL induces Cox-2 expression and that another AHL produced by P. aeruginosa was unable to activate human lung fibroblasts as effectively.
**NF-κB is essential for 3O-C_{12}-HSL induction of Cox-2**

The human *cox-2* gene contains consensus sequences for the binding of the transcription factors NF-κB, NF-IL-6, and cAMP response element (28). In human lung epithelial cells, Cox-2 induction is primarily mediated by the transcription factor NF-κB (29). We previously demonstrated that 3O-C_{12}-HSL stimulation of human bronchial epithelial cells induces NF-κB, which subsequently leads to the production of the chemokine IL-8 (10). To determine whether NF-κB also regulates 3O-C_{12}-HSL induction of Cox-2, specific inhibitors for NF-κB translocation were used. In nonstimulated lung fibroblasts NF-κB is found sequestered in the cytoplasm in an inactive state by the IκB inhibitory proteins. When cells become activated by an inflammatory signal, IκB proteins are phosphorylated and degraded, allowing the translocation of NF-κB to the nucleus and gene transcription. A cell-permeable peptide (SN50) that contains the nuclear translocation sequence for NF-κB was used to inhibit NF-κB activity. This peptide has been shown to specifically inhibit nuclear translocation of NF-κB and thus inhibit its ability to induce the transcription of genes (30, 31). In nonstimulated L828 fibroblasts, NF-κB staining was found only in the cytoplasm, but when the fibroblasts were stimulated with 3O-C_{12}-HSL, NF-κB staining was also found in the nucleus (Fig. 4). The addition of the peptide inhibitor SN50 to cultures blocked 3O-C_{12}-HSL-induced translocation, but when SN50M, a peptide that contains a mutation in the nuclear translocation sequence, was added to 3O-C_{12}-HSL-stimulated cultures there was no effect on NF-κB translocation (Fig. 4). Having shown that 3O-C_{12}-HSL induced the translocation of NF-κB in these cells and that SN50 inhibited this induction, we next wanted to examine what effect this induction had on Cox-2 production. When SN50 was added with 3O-C_{12}-HSL to L828 cultures and the cells were stained with an anti-Cox-2 Ab, it was observed that there was a substantial decrease in expression of Cox-2 protein. The addition of the mutant peptide SN50 M to cultures stimulated with 3O-C_{12}-HSL resulted in no effect on Cox-2 induction (Fig. 4). As a control, fibroblasts were also cultured with SN50 or SN50 M, and in the absence of stimulation there was no effect on NF-κB or Cox-2 expression (data not shown). These data indicate that the induction of NF-κB by 3O-C_{12}-HSL is essential for the production of Cox-2 in these cells.

**3O-C_{12}-HSL induces the expression of PGES**

PGH₂ produced by Cox is converted to various PGs by specific PG synthases. There are two forms of PGES, a cytosolic (cPGES) and a microsomal (mPGES) synthase. Recent data have demonstrated that, in human lung epithelial cells stimulated with IL-1, Cox-2 and mPGES were coordinately regulated (32). To determine whether 3O-C_{12}-HSL up-regulates the expression of PGES, L828 fibroblasts were stimulated with 100 μM 3O-C_{12}-HSL for varying times, and Western blots for Cox-2, cPGES, or mPGES were done. These experiments demonstrated that mPGES was not expressed in nonstimulated cells but was induced as early as 8 h after 3O-C_{12}-HSL stimulation. Expression of mPGES continued to increase even after 96 h of stimulation. When these samples were examined for Cox-2 expression, it was observed that Cox-2 was induced earlier than mPGES. As previously shown, Cox-2 protein was not expressed in nonstimulated cells but induction occurred after 4 h of stimulation with 3O-C_{12}-HSL and maximal expression occurred after 8 h. By 48 h Cox-2 expression had returned to background levels (Fig. 5). Although mPGES was induced with 3O-C_{12}-HSL stimulation, cPGES was constitutively expressed in L828 fibroblasts and was not significantly affected by stimulation with 3O-C_{12}-HSL (Fig. 5).

**FIGURE 5.** 3O-C_{12}-HSL stimulation induces the expression of mPGES. L828 fibroblasts were stimulated for various times with 100 μM 3O-C_{12}-HSL. Protein extracts (*top three panels*) were analyzed for expression of Cox-2, mPGES, or cPGES by Western blot. Densitometry is graphed as the relative sum intensity of each band (bar graph). These data are representative of four separate experiments.
3O-C₁₂-HSL stimulates the production of PGE₂ in human lung fibroblasts

Having demonstrated that 3O-C₁₂-HSL increased the expression of both Cox-2 and mPGES, we next wanted to determine whether PGE₂ levels were also increased. When L828 lung fibroblasts were cultured with a titration of 3O-C₁₂-HSL, a significant amount of PGE₂ could be measured in culture supernatants. Although 50 μM 3O-C₁₂-HSL stimulated a statistically significant induction in PGE₂, the 100 μM concentration induced an 8-fold increase over that of untreated cells (Fig. 6A). The addition of exogenous arachidonic acid to the culture supernatants did not enhance the production of PGE₂ with 3O-C₁₂-HSL stimulation (data not shown). These data indicate that the availability of arachidonic acid in L828 fibroblasts is not a limiting step in the production of PGE₂ with 3O-C₁₂-HSL stimulation. When cells were cocultured with 20 μM indomethacin, an inhibitor of both Cox-1 and 2, or 5 μM SC58125, a Cox-2 selective inhibitor, the amount of PGE₂ produced was reduced to background levels (Fig. 6B). Because the inhibition found with the Cox-2 selective inhibitor was similar to that found with the general Cox inhibitor, indomethacin, these data indicate that the production of PGE₂ with 3O-C₁₂-HSL stimulation occurs through the induction of Cox-2.

Discussion

During the initial colonization of the lung by Pseudomonas, it is proposed that bacteria bind to the ciliated bronchial epithelial surface or the overlying mucus layer. In an immunocompetent individual these bacteria would be cleared from the lung by ciliary movement, mucus flow out of the lung, phagocytosis, and epithelial internalization and desquamation (33). However, in patients that have defects in these innate functions, such as CF patients and patients on mechanical ventilators, these mechanisms are unable to clear the bacteria from the lung. In CF patients, mutations in the chloride channel known as the CF transmembrane conductance regulator result in high salt and thick mucus in the lungs. This environment is conducive to P. aeruginosa colonization and produces a niche in which the bacteria can grow. In the CF lung, P. aeruginosa has been found to grow to very high densities (1–10⁷ CFU/ml sputum) as biofilms, an organized structure consisting of bacteria and polysaccharide (34). A high concentration of bacteria in the lungs of CF patients has been correlated to increased inflammation and tissue destruction.

Sputum from P. aeruginosa-colonized patients contains transcripts for quorum sensing genes as well as quorum sensing regulated genes (35, 36). More recently it was shown that 3O-C₁₂-HSL and C₄-HSL could be detected in the sputum of CF patients colonized with P. aeruginosa (36). These data indicate that P. aeruginosa produces 3O-C₁₂-HSL in vivo, which actively regulates many bacterial virulence genes. Because 3O-C₁₂-HSL is both diffused and pumped out of P. aeruginosa, it directly interacts with surrounding eukaryotic cells (5). In studies examining the colonization of squid light organs with the bacteria Vibrio fischeri, which produces an AHL similar to 3O-C₁₂-HSL, it was observed that the AHL not only was located around the bacteria but had penetrated into the epithelial layer of the light organ (37). We propose that 3O-C₁₂-HSL produced by P. aeruginosa may have a similar interaction with the epithelium of the lung. Also during chronic P. aeruginosa infections there is destruction of the lung epithelium that exposes the underlying fibroblasts. These data indicate that 3O-C₁₂-HSL would be in direct contact with fibroblasts during P. aeruginosa infections. It has been shown that biofilms of P. aeruginosa grown in vitro can produce ~600 μM 3O-C₁₂-HSL, a concentration that is significantly higher than what has previously been measured in planktonic cultures (1–5 μM) (38). This level of 3O-C₁₂-HSL production is well above the concentrations needed to induce Cox-2 and PGE₂ expression in vitro (10–100 μM).

One of the hallmarks of an aggressive infection in a CF patient is the progressive inflammation that occurs. It is the overzealous induction of this inflammatory response that leads to extensive tissue destruction and ultimately pulmonary failure. Inflammation and production of inflammatory mediators, such as IL-8, are directly correlated to the presence of P. aeruginosa in the lungs of CF patients (39). Sputum samples from P. aeruginosa-colonized CF patients contain increased amounts of PGs (40, 41). In this manuscript, we demonstrated that 3O-C₁₂-HSL produced by P. aeruginosa induced the expression of Cox-2 and mPGES and the production of PGE₂. PGE₂ stimulates mucus secretion and edema, characteristic commonly found in the CF lung (20). It also synergizes with the chemokine IL-8 to enhance migration of neutrophils (21). High numbers of neutrophils in the lung of a CF patient are associated with excessive inflammation and indicative of a poor prognosis. PGE₂ is a potent inhibitor of IL-12 and IFN-γ production and thus stimulates a type-2 phenotype in T cells (22). In studies with LPS-activated mouse peritoneal exudate cells, 3O-C₁₂-HSL inhibited the production of IL-12 (11). Although these investigators did not measure PGE₂ production in their studies, we speculate that the inhibition of IL-12 in these cells was due to 3O-C₁₂-HSL induction of PGE₂.

In burn wounds, colonization with P. aeruginosa results in a significant increase in both Cox-2 and PGE₂ production (25). Inhibition of PGE₂ production with Cox-2 selective drugs prevented P. aeruginosa dissemination from the wound site and resulted in decreased sepsis and mortality (42). Additional studies using the

![FIGURE 6](http://www.jimmunol.org/)

Stimulation of L828 fibroblasts with 3O-C₁₂-HSL induces production of PGE₂. A, L828s were stimulated with a titration of 3O-C₁₂-HSL for 24 h and PGE₂ in the culture supernatant was quantified. These data are an average of six separate experiments. B, Fibroblasts were stimulated with 100 μM 3O-C₁₂-HSL along with the addition of indomethacin or SC-58125. These data are an average of four separate experiments. *, p < 0.03.
burn wound model of _P. aeruginosa_ in mice demonstrated that quorum sensing mutants that do not produce 3O-C_{12}-HSL were unable to disseminate and cause mortality (43). These data support the conclusion that production of 3O-C_{12}-HSL by _P. aeruginosa_ and its subsequent induction of Cox-2 and PGE_2 are essential for the bacteria to disseminate and cause mortality. When mice were given lung infections by exposing them to aerosolized _P. aeruginosa_, there was a significant induction of PGE_2 and lung pathology. When these animals were treated with ibuprofen, a general Cox inhibitor, before infection, there was a decrease in both lung inflammation and PGE_2 levels (24). A similar inhibition was observed in ibuprofen-treated rats that had chronic _P. aeruginosa_ infections (44). Collectively these data indicate that infections with _P. aeruginosa_ result in increases in Cox-2 and PGE_2 expression and that when Cox inhibitors are used the pathogenesis of the organism is diminished.

In clinical trials, where CF patients were treated with ibuprofen (a general Cox-1 and Cox-2 inhibitor), the results were mixed. Although these patients showed some improvement in lung function, the ibuprofen treatments had no effect on the bacterial burden found in these patients (45–47). However, the improvements found with ibuprofen treatment warrant addition clinical trials with both general Cox inhibitors as well as new Cox-2 selective drugs (which would have fewer side effects, e.g., gastric irritation) with or without cotreatment with antibiotics.

A novel finding reported herein was that 3O-C_{12}-HSL stimulation increased the expression of mPGES in human lung fibroblasts (Fig. 5). The sequential induction of both Cox-2 and mPGES resulted in a significant increase in the production of PGE_2 found in concert with 3O-C_{12}-HSL stimulation (Fig. 6). The induction of Cox-2 was found to occur more quickly and expression was more ephemeral than that found with mPGES (Fig. 5). This sequential regulation of these enzymes is most likely a method of controlling the levels of PGE_2 production. Stimulation of orbital fibroblasts with IL-1β also induced similar sequential expression of Cox-2 and mPGES (48). We also demonstrated that cPGES and Cox-1 were not induced with 3O-C_{12}-HSL stimulation. Recent data have demonstrated that cPGES is functionally linked to Cox-1 while mPGES is associated with Cox-2 (18, 19). Although we do not discount the role of Cox-1 and cPGES in the production of PGE_2, our data would indicate that 3O-C_{12}-HSL induction of PGE_2 is acting predominately through the induction of Cox-2 and mPGES. Having demonstrated that both Cox-2 and mPGES are induced with 3O-C_{12}-HSL stimulation, inhibition of these enzymes may be important in regulating the inflammation that occurs with _P. aeruginosa_ infections. Drugs that inhibit the various PG synthases are not yet available, but are novel targets for therapeutics treating inflammation.

The use of such drugs in the future may be important in the regulation of _P. aeruginosa_ inflammation. We also demonstrated that 3O-C_{12}-HSL induction of Cox-2 was regulated by the activation of the transcription factor NF-κB (Fig. 4). Previously, we demonstrated that activation of NF-κB was essential for 3O-C_{12}-HSL induction of the chemokine IL-8 (10). Because NF-κB is pivotal in the production of both IL-8 and PGE_2 during _P. aeruginosa_ infections, this transcription factor should be an attractive target for anti-inflammatory therapy. Blocking the activation of the NF-κB transcription factor may prevent the early induction of IL-8 and PGE_2 and thus decrease the inflammation induced by _P. aeruginosa_ infections.

In conclusion, 3O-C_{12}-HSL is not only essential in the regulation of several bacterial virulence factors but also induces key early events in human inflammation, namely the induction of Cox-2, mPGES, and PGE_2. The Cox-2 product PGE_2 likely regulates aspects of innate immunity (e.g., edema and neutrophil migration) and inducible immunity (inhibition of IL-12 and IFN-γ) that influence the immune response to _P. aeruginosa_.

## References


