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Diverse *Pseudomonas aeruginosa* Gene Products Stimulate Respiratory Epithelial Cells to Produce Interleukin-8

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Abstract

Respiratory epithelial cells play a crucial role in the inflammatory response during *Pseudomonas aeruginosa* infection in the lungs of patients with cystic fibrosis. In this study, we determined whether the binding of specific *Pseudomonas* gene products (pilin, flagellin) to their receptors on respiratory epithelial cells would result in production of the neutrophil chemotaxant IL-8. Piliated wild-type organisms, purified pilin, or antibody to the pilin receptor (asialoGM1) evoked significant production of IL-8 by immortalized airway epithelial cells, whereas nonpiliated organisms were less able to bind to respiratory epithelial cells and stimulated much less IL-8 secretion (P < 0.01). A piliated, nonflagellated strain was also associated with decreased binding and a diminished level of IL-8 production when compared to wild-type organisms. Isogenic, nonadherent *rpoN* mutants, lacking pilin and flagellin, did not bind or elicit an IL-8 response. In addition, the IL-8 response was four-fold higher in a cystic fibrosis cell line compared with its corrected cell line. The *Pseudomonas* autoinducer, an exoprotein secreted during chronic infection, was found to stimulate IL-8 in a dose-dependent manner. *P. aeruginosa* adhesins, which are necessary for initial infection, directly stimulate IL-8 production by respiratory epithelial cells and therefore play a major role in the pathogenesis of *Pseudomonas* infection in patients with cystic fibrosis. The inflammatory response is subsequently perpetuated by *Pseudomonas* autoinducer which is secreted during chronic infection. (J. Clin. Invest. 1995. 96:2204–2210). Key words: *Pseudomonas aeruginosa* • adherence • interleukin-8 • pilin • autoinducer

Introduction

Infection with *Pseudomonas aeruginosa* is closely associated with the development and progression of pulmonary disease in cystic fibrosis (CF). The features of this infection are unique to CF patients: environmental strains of *Pseudomonas* infect the CF respiratory tract and induce a florid host response characterized by the influx of neutrophils into the airway (1). Pilin-mediated adherence to the airway epithelium is important in the initial stages of infection (2); however, in the chronic phases, the organisms no longer adhere directly to the epithelial cells, but remain superficial within the secretions of the airway lumen (3). The toxic effects of neutrophil components, elastase, oxygen radicals (4, 5), and the release of DNA and actin (6) all contribute to the formation of inspissated secretions and symptomatic pulmonary disease in CF. Chronic infection develops despite what appears to be a normal immune system.

The mechanisms by which the genetic defect in CF and the resultant abnormal cystic fibrosis transmembrane regulator (CFTR) (7) lead to chronic infection are not as yet fully delineated. Recent studies demonstrate that CF airway epithelial cell surfaces have a higher than normal concentration of asialylated glycolipids, to which *P. aeruginosa* pilin binds (8). This finding is consistent with data demonstrating that CFTR dysfunction, by limiting acidification within the trans Golgi, can result in diminished sialylation of cell glycoconjugates (9). *Pseudomonas* pilin binds to the increased asialoGM1 which is present on the CF epithelial cells, but not the sialylated form of this glycolipid that is found on normal cells (8).

We postulated that specific *Pseudomonas* gene products might directly elicit an inflammatory response by binding to asialylated glycolipid receptors and result in production of pro-inflammatory cytokines by the respiratory epithelium. To test this hypothesis, *P. aeruginosa* mutants defective in the expression of adhesins and/or specific exoproteins were tested for their ability to bind to epithelial cells and stimulate IL-8 production, the major chemokine associated with neutrophil migration across the airway epithelium (10). This cytokine is found in increased amounts in the lungs of CF patients (11). Pilin, a major adhesin, as well as flagellin, each stimulated substantial IL-8 production. Moreover, a novel *P. aeruginosa* gene product, the *Pseudomonas* autoinducer (PAI), which is expressed in late stationary phase growth typical of chronic infection, also evoked an IL-8 response by epithelial cells.

Methods

**Bacterial strains and culture conditions.** The *P. aeruginosa* strains, listed in Table 1 (12–21) were grown with aeration in M9 media supplemented with glutamine as necessary. PAO/MP was grown in M9 + 0.5% methionine assay medium (Difco Laboratories, Inc. Detroit, M1). PAO-R1 (lasR) contains an inserional mutation in lasR, affecting the expression of PAI, elastase, alkaline protease, and neumaminidase (20, 22, 23). PAO/MP was constructed by allelic exchange in which the wild-type gene *pilA* was replaced by a homologous piece of DNA containing a tetracycline resistance (Tc+) cartridge within the coding sequence. The construction was confirmed by Southern hybridization and the phenotype documented by resistance to the pilus-specific phase F116 (24). Synthetic PAI and analogues of the autoinducer were generously provided by B. Iglewski, University of Rochester (Rochester, NY).

**Bacterial cell fractionation and cell components.** *P. aeruginosa*
components were isolated to determine which gene products are associated with IL-8 stimulation. Exoproducts were obtained by harvesting supernatants from organisms grown for 18 h in M9 media, concentrated 100-fold with polyethylene glycol, and dialyzed exhaustively versus phosphate buffer pH 7.4. Outer membrane proteins and periplasmic contents were isolated using standard methods (25, 26). Lipopolysaccharide from Escherichia coli and P. aeruginosa, neuraminidase from Vibrio cholera, and phospholipase C from Clostridium perfringens were commercially obtained (Sigma Chemical Co., St. Louis, MO). P. aeruginosa elastase was obtained from Nagase Chemical Co., (Tokyo, Japan).

Pili were isolated from the hyperpiliated PAO1 mutant DB2 using the method of Frost and Paranchych (21). In brief, DB2 was grown overnight on large M9 agar plates. The cells were scrapped off, resuspended in SSC: 15% sucrose solution and filtered through a fine sieve to remove agar. The solution was stirred overnight at 4°C then pulsed at the lowest speed in a blender (Waring Commercial, New Hartford, CT) to shear off the pili. The cells were removed by centrifugation (SS34 8000 rpm for 20 min., Sorvall Instruments Division, Newton, CT) and the supernatant was dialyzed against sterile water at 4°C for 16 h. The pilin protein was precipitated with 50% ammonium sulfate at 4°C overnight and the resulting pellet dissolved in buffer. This was precipitated with 20% ammonium sulfate, resuspended in SSC-15% sucrose and loaded on a discontinuous sucrose gradient, then centrifuged for 20 h at 4°C (SW27 rotor 20,000 rpm, L7 ultracentrifuge; Beckman Instruments, Inc., Fullerton, CA). The protein band was dialyzed and repurified on a CaCl2 gradient. The remaining protein was electrophoresed on an SDS-polyacrylamide gel and visualized with Coomassie blue as a single 15-kD band. Antibody to pilin was obtained by rabbit inoculation (Poocono Rabbit Farm and Laboratory, Inc., Canadensis, PA) and negatively purified by repeated incubation with suspensions of PAO/DP at 37°C.

Flagellin isolation. Flagella from PAO/DP were isolated using the method of Montie et al. (27), and the band corresponding to 53 kD was eluted into Tris 0.5 M buffer. Purity was checked by SDS-polyacrylamide gel electrophoresis demonstrating a single 53-kD band.

IL-8 assays. IL-8- cells, SV40 immortalized human airway epithelial cells, whose epithelial properties have been well characterized (28), were assayed for the production of IL-8 in response to incubation with bacteria or bacterial components. Confluent monolayers of 1HAEo- cells were grown in 96-well plates in DMEM-F12 containing fetal calf serum, and then serum starved for 18 h preceding stimulation. IB3 cells, an adeno-associated virus transformed human bronchial epithelial cell line derived from a patient with CF (ΔF508/W1282X), and the rescued line (C-38 cells) which expresses a plasmid encoded copy of a normal CFTR (29) were similarly grown to confluence in 96-well plates in LHC-8 media (Biofluids, Inc., Rockville, MD).

Bacteria were grown to log-phase, washed, and resuspended in PBS pH 7.4. An aliquot of 10^7 CFU/ml of bacteria was incubated for 60 min at 37°C with a confluent monolayer of 1HAEo- cells. The monolayers were washed three times with PBS and reincubated with 200 pg/ml anti-MIF (100 μg/ml of gentamicin) for 14 h. Bacterial supernatants were sterilized by the addition of gentamicin 40 μg/ml and similarly incubated with the epithelial cell monolayer for 1 h. Comparable experiments were done using aliquots of the purified cell components, pili, flagella, and the commercially obtained exoproducts, all at concentrations of 10 μg/ml. Protein concentrations were measured by the method of Bradford (Bio-Rad Laboratories, Richmond, CA). IL-1/20 ng/ml (R&D Systems, Minneapolis, MN) was used as a positive control for stimulation of IL-8 from the epithelial cells. Antibody to asialoGMI was purchased from Wako Pure Chemical Industries, Ltd. (Chuo-Ku, Japan) and was tested on the IB3 and C-38 cell lines. The integrity of the monolayer before IL-8 assay was assessed by visualization, and cell viability after stimulation was confirmed by trypan blue exclusion. The uniformity of the monolayer was determined by quantifying the cell number/well.

IL-8 in the epithelial cell supernatant was assayed 18 h after stimulation by ELISA kit (R&D Systems, Inc.) or by ELISA plates coated with mAb-IL-8 generously provided by Drs. I. Lindley and A. Rot (Sandoz, Vienna, Austria). IL-8 kinetics studies showed that peak IL-8 production from 1HAEo- cells occurs at 8–24 h after stimulation with P. aeruginosa (data not shown). Each data point was performed at least in duplicate, and each experiment was performed at least three times. Statistical analysis was performed using ANOVA analysis (Microsoft Excel 5.0 software; Microsoft Corp., Seattle, WA).

Northern blot analysis. 1HAEo- cells were analyzed for IL-8 gene expression 4 h after incubation with bacteria. Total cellular RNA was extracted by the single-step guanidine thiocyanate-phenol-chloroform extraction method (30) from 25-cm² culture flasks of confluent 1HAEo- cells exposed either to control media or P. aeruginosa. The RNA pellet was precipitated with isopropanol, washed with 70% ethanol, vacuum dried, and redissolved in DEPC-treated water. Aliquots of 10 μg total RNA (determined by spectrophotometry, 260-nm wavelength) were run on a denaturing formaldehyde 1% agarose gel. The RNA was transferred to a nylon membrane and UV crosslinked to the membrane. Filters were hybridized at 57°C with a 32P end-labeled oligonucleotide probe with the sequence 5' GTT-GGC-GCA-GTG-TGG-TCC-CTC-AAT-CAC-3' (31). Blots were washed twice for 60 min at 57°C in 2 × SSC, then 0.5 × SSC/1% SDS. To assess the relative amounts of RNA loaded, blots were rehybridized for glyceraldehyde 3-phosphate dehydrogenase (GAPDH) after stripping of the original probe.

Bacterial adherence assay. The number of P. aeruginosa adhering to the epithelial monolayers was quantified as has been previously described (8). The P. aeruginosa strains were grown to late log phase, metabolically labeled with 35S methionine, washed with 10 mM NaCl and a 0.1-ml inoculum of 5 × 10^7 CFU/ml was added to each well of

**Table 1.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Description</th>
<th>Source (Reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAK/NP</td>
<td>Prototype, non-mucoid, laboratory strain</td>
<td>S. Lory (12, 13)</td>
</tr>
<tr>
<td>PAK/N1</td>
<td>Pil* constructed by gene replacement with pilA interrupted by a Te³ cassette</td>
<td>S. Lory (19)</td>
</tr>
<tr>
<td>PAK/fla</td>
<td>rpoN constructed by gene replacement</td>
<td>S. Lory (14)</td>
</tr>
<tr>
<td>PAK/fla</td>
<td>fla*, constructed by gene replacement</td>
<td>G. Jacoby</td>
</tr>
<tr>
<td>PAK152</td>
<td>Flr*, Pil* mutant isolated as nonmotile after chemical mutagenesis</td>
<td>T. Montie (16)</td>
</tr>
<tr>
<td>PAK/MP</td>
<td>Pilin adhesin domain interrupted by the insertion of a CAT cassette</td>
<td>W. Paranchych (15)</td>
</tr>
<tr>
<td>PAK/DB2</td>
<td>Hyperpiliated mutant</td>
<td>W. Paranchych (21)</td>
</tr>
<tr>
<td>PAK/MP</td>
<td>Pil*, constructed by gene replacement as for PAK</td>
<td>This study</td>
</tr>
<tr>
<td>PAO1</td>
<td>PAK/MP mutant isolated as nonmotile after chemical mutagenesis</td>
<td>M. Vasil (18)</td>
</tr>
<tr>
<td>PAO/DB2</td>
<td>Hyperpiliated mutant</td>
<td>D. Frank (17)</td>
</tr>
<tr>
<td>PAO/DB2</td>
<td>Paranchych (21)</td>
<td></td>
</tr>
</tbody>
</table>

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a confluent monolayer of the IHAEO- cell line for 60 min at 37°C. Nonadherent organisms were removed with three PBS rinses. The monolayers were solubilized in 200 μl of 2% SDS and scintillations counted. Each point was performed in triplicate and a mean and standard deviation were determined.

Results

P. aeruginosa induces IL-8 gene expression. Northern blot analysis demonstrated IL-8 mRNA from epithelial cells exposed to P. aeruginosa PAO (Fig. 1). There was no evidence of endogenous IL-8 mRNA from control epithelial cells under the conditions used, in contrast to abundant amounts of mRNA specific for the constitutively expressed gene GAPDH.

Adherent P. aeruginosa elicit IL-8 production. The adherence of two different wild-type piliated strains of P. aeruginosa (PAK, PAO1) were compared (Fig. 2 A). The more adherent strain, PAO1, evoked greater IL-8 expression by the epithelial monolayers (Fig. 2 B) and relative adherence was found to correlate roughly with the relative amounts of IL-8 produced by epithelial cells incubated with the organisms. The nonadherent rpoN mutant which expresses neither pil nor flagella and may be missing putative "nonpilus adhesins" (19, 32) did not elicit a detectable IL-8 response (Fig. 2, A and B), suggesting that bacterial contact was important in triggering the IL-8 response. To determine if pilin itself was necessary for IL-8 stimulation, or if mere juxtaposition of the organisms was sufficient to allow a different gene product to come in contact with the epithelial cell, IL-8 assays were performed with several pil mutants as well as purified pilin.

Pilin evokes an IL-8 response. Pil- mutants of PAO1 and PAK were significantly less adherent and less efficient in triggering IL-8 expression (P < 0.01) than the parental strains (Fig. 2, A and B). PAO/MP, a PAO1 mutant with a CmR cassette interrupting the carboxy terminus of the adhesin domain of the pilin, but otherwise normal pilin structure (15), was less adherent, correspondingly less effective in eliciting an IL-8 response (P < 0.01), and was associated with IL-8 release virtually identical to that elicited by the Pil- mutant. The TcR marker used to construct the Pil- strains PAO/NP and PAK/NP had no effect on the IL-8 response, as a control strain, PAO/SRN with the identical TcR marker in an irrelevant gene (phospholipase C) had attachment and IL-8 stimulatory properties identical to that of the parental PAO1 (data shown below).

Purified pilin was demonstrated as a single band by Coomasie blue stain of SDS-PAGE, and confirmed by Western hybridization with antipilin antibody (Fig. 3). Pilin, when incubated with the IHAEO- cells demonstrated a dose-response effect in stimulating IL-8 production (Fig. 4). Cytochalasin D in various concentrations (0.2–0.25 μg/ml) had no effect on the amount of IL-8 produced under these conditions (data not shown),

Figure 1. Northern blot analysis for IL-8 gene expression. 1. Control IHAEo- cells in serum free media; 2. IHAEo- cells 4 hours after incubation with PAO1.
thus it is unlikely that epithelial uptake of pilin mediated by cytoskeleton components is involved in the IL-8 response.

Cell fractionation studies demonstrated that the outer membrane protein (OMP) fraction had the highest activity, but other cell compartments also triggered IL-8 production although to a lesser extent (Fig. 5). LPS did not elicit IL-8 from the epithelial cell.

**CF cells produce more IL-8 in response to PAO1.** We postulated that pilin act superficially on the epithelial surface by binding to their receptor, a GalNACβ1-4Gal moiety found in asialylated lipids such as asialoGM1 (8). As CF cells have significantly more asialoGM1 available on their cell surfaces, a CF cell line (IB3) and the corrected isogenic line (C-38), were compared for their ability to produce IL-8 in response to incubation with PAO1 (Fig. 6). PAO1 triggered four-fold greater IL-8 expression from the CF cells as compared with the corrected cells \( (P < 0.05) \). Antibody (1:32 dilution) directed against asialoGM1, the pilin receptor, was as effective as PAO1 in eliciting IL-8 expression from the CF cells. The corrected cell line was refractory to stimulation by the identical amount of antibody (Fig. 6), suggesting that the appropriate receptors were unavailable on the corrected cells. This is consistent with the reported 86% increased binding of PAO1 to these CF cells as compared with the corrected cells \( (33) \).

**Flagellin and IL-8 production.** Nonpiliated mutants of *P. aeruginosa* were capable of triggering an IL-8 response, albeit reduced, suggesting that other gene products are also capable of IL-8 stimulation. Recent reports suggest that flagellin can function as an adhesin and recognizes the same GalNACβ1-4Gal receptor as pilin \( (34) \). To determine if flagellin can elicit an IL-8 response, the Fla" mutants AK1152 (PAO) and PAK/fliA were studied (Fig. 2). AK1152 was poorly adherent and unable to stimulate detectable levels of IL-8. Although this strain does produce pilin as determined by Western hybridization (data not shown), it was resistant to the pilus-dependent phage F116 and does not produce functional, retractile pili. The PAK/fliA strain had intermediate levels of attachment and corresponding efficiency in evoking IL-8 expression (Fig. 2). Purified flagellin, demonstrated as a single band on Coomassie stain of SDS-PAGE (Fig. 7), was applied to the epithelial cells and stimulated a dose-dependent IL-8 response which was actually greater on a molar basis, than the pilin response (Fig. 8). These experiments suggest that flagellin contributes to the IL-8 response.
8 response not only by providing motility for the organism, but also by serving as an adhesin for epithelial attachment. Thus, two bacterial components which recognize superficial receptors on epithelial cells trigger IL-8 expression.

*P. aeruginosa exoprotein PAI elicits an IL-8 response.* *P. aeruginosa* express a number of enzymes that modify eukaryotic targets. Human proteases such as neutrophil elastase stimulate IL-8 production (4), suggesting that *P. aeruginosa* proteases might have analogous effects. Mutants with defective expression of specific exoproteins were compared for their ability to stimulate IL-8. Neither the phospholipases nor exoenzyme S appear to contribute to IL-8 production as the PAO/SRN (PLC−) and PAO/ExoS were even more efficient than the parental strain in evoking an IL-8 response (Fig. 9). PAO-R1, however, was significantly impaired in its stimulatory activity (*P* < 0.01). This strain has a mutation in lasR affecting the production of PAI, a homoserine lactone derivative which is a diffusible molecule acting as a cofactor for the transcriptional activator LasR. This molecule is involved in the expression of several genes such as elastase, alkaline protease, and neuraminidase. Purified elastase and neuraminidase had no stimulatory effect on the monolayers (data not shown). However, synthetic autoinducer had a dose-response effect in evoking IL-8 production by the 1HAEo− cells (Fig. 10). Control compounds, homoserine lactone derivatives with very minor changes in the molecular structure, were ineffective in up-regulating IL-8 expression (Fig. 10, insert).

PAI effect on the 1HAEO− cells was very similar to that described by other investigators who found that a small (< 1 kD) molecular mass compound isolated from late stationary phase culture supernatants of a nonadherent strain of *P. aeruginosa*, PA103, was capable of IL-8 stimulation (35). This reported compound is water soluble and heat stable, characteristics consistent with PAI. The amount of IL-8 expressed by the 1HAEo− cells (6.6 × 10^4±3.2 × 10^5 cells/well) after incubation with PAI, when expressed as a function of the DNA content, was equivalent to 736 pg/μg of DNA. This is approximately one order of magnitude higher than the reported IL-8 concentration detected by incubation of a different cell line (16HAEo− cells) with the small molecular weight compound isolated by Maisson et al. (35).

**Discussion**

As with many infectious diseases, it is the type and severity of the host inflammatory response which is mainly responsible for the clinical outcome. Abundance of inflammatory mediators and products of polymorphonuclear leukocytes are found within the
bronchial tree of CF patients (11). Even young children (< 1 yr old) with CF, without clinically apparent lung disease, have *P. aeruginosa* isolated from pulmonary secretions (Ramsey, B., and A. Smith CFF-Research Development Program, unpublished data), indicating that CF patients have inherent elevated affinity for *P. aeruginosa* infection. It has been unclear why these organisms, and rarely other opportunistic pathogens, are so specifically linked to the pulmonary pathology in CF. Availability of asialylated glycolipid receptors for *P. aeruginosa* adhesins on the CF epithelium may serve not only to provide a niche for attachment but also to initiate a self-perpetuating inflammatory response which ultimately results in the destruction of the CF lung.

During the earliest stages of infection of the CF lung, when *P. aeruginosa* is transiently inspired but not cleared by the usual muco–ciliary mechanisms, they may attach via pili to the GalNAcβ1-4Gal receptors found in significantly increased numbers in CF patients, especially those homozygous for the Δ508 mutation in CFTR (36). The experiments reported here demonstrate that this *P. aeruginosa* binding, as mediated by pili and/or flagella, triggers IL-8 production by respiratory epithelial cells. In fact, IL-8 production elicited by various mutants parallels the adherence pattern of the mutants. IL-8 expression is followed by the infiltration of PMN’s whose elaboration of superoxide and elastase serve to further stimulate the epithelium to produce IL-8 (4). This cycle of inflammation persists during later stages of infection when organisms may no longer express pili or flagella and may not even be directly apposed to the epithelium, but are enmeshed in relatively inert microcolonies surrounded by glycoconjugates of both bacterial and human origin. Under these conditions exoproducts such as the PAI would be secreted by *P. aeruginosa* and continue to evoke IL-8 expression.

Pilin-mediated adherence is an important stimulus for IL-8 production by the epithelium. Nonpiliated mutants were significantly impaired in their ability to trigger IL-8, as were mutants with genetically modified, dysfunctional, pilin adhesion domains. Moreover, antibody to the pilin receptor was effective in eliciting an IL-8 response specifically from CF cells which have increased numbers of available receptor sites. These findings suggest that the nonspecific binding of *P. aeruginosa*, as mediated by nonpilus (and nonflagellar) adhesins may not be associated with as significant pathology. Recent studies using a neonatal mouse model of acute pneumonia have demonstrated that purified pilin specifically induces polymorphonuclear leukocyte–dominated airway inflammation, consistent with an IL-8-mediated response (2).

The precise role of flagella in the pathogenesis of respiratory tract infection is less clear. Flagella confer motility to the organism, therefore, nonmotile strains may have less ability to find an appropriate binding site. Fla" mutants including the strain used here, AK1152, have been demonstrated in other models of infection to be less virulent (16). In addition, it appears that flagellin can act as an adhesin, as inferred from the studies presented here as well as the data of Gehring and Baker (34). Flagellin also contributes to the ability of *P. aeruginosa* to elicit an inflammatory response. This was best illustrated by the results obtained with AK1152, the Pil" - Fla" mutant which was unable to bind and unable to stimulate IL-8, suggesting that flagellin may account for the pilin-independent attachment and IL-8 stimulation associated with PAO/NP (Pil", Fla"), PAK-N1 the rpoN mutant which is defective in the expression of several genes requiring the alternative g34 RNA polymerase, is nonadherent and unable to stimulate an IL-8 response. Other rpoN-dependent genes have been postulated to act as “nonpilus adhesins” that recognize receptors in mucins or other glycoproteins (19). Such adhesins do not appear to be involved in these interactions with epithelial cells. The data presented here suggest that pili and flagella are the major *P. aeruginosa* gene products which interact with the available receptors on the epithelial cell surface to confer attachment and elicit IL-8 release.

The binding process may also serve to facilitate the juxtaposition of additional bacterial component(s) which elicit IL-8 expression. Our data has identified only PAI as a major secreted effector in stimulating an IL-8 response. The earlier report by Masson et al. is also consistent with a single active compound that is not a protein and is present in the cell supernatant. PAI concentration is dependent on bacterial concentration, and would be high in a chronically infected lung with dense bacterial growth. As a small, hydrophobic, highly permeant molecule, PAI is likely to act on the epithelium in a manner distinct from that of pili and flagella and may recognize an intracellular target in eukaryotes.

It is likely that at least two independent mechanisms are operative in the epithelium to activate IL-8 expression; one which involves a superficial event as initiated by pilin (or flagellin) binding to its asialylated receptor and another mechanism which is stimulated by PAI. Unlike the macrophage the respiratory epithelial cell does not express IL-8 in response to LPS, a generic signal of gram-negative organisms. Pilin stimulation of IL-8 production by these respiratory cells may be similar to IL-8 production by other mucosal cells that can be superficially infected by adherent, noninvasive organisms; for example, uropathelial cells express IL-8 after piliated *E. coli* adhere, but not after random interactions with nonpiliated organisms (37). This process is somewhat different from the cytochalasin-inhibitable IL-8 expression elicited from TS4 cells after organisms such as Salmonellae actually invade the cells (38) because it is not inhibited by cytochalasin and it doesn’t require invasion. *P. aeruginosa* behave more like the uropathogenic *E. coli* in that superficial attachment to the epithelium is sufficient to trigger IL-8 synthesis and, as in the uropathelium, a novel pathway of signal transduction, perhaps via ceramide or ganglioside–mediated binding, may be involved (39).

The observed epithelial secretion of IL-8 in response to contact with *P. aeruginosa* and specific exoproducts provides an important link to explain the pathogenesis of *Pseudomonas* infection in CF. Increased *Pseudomonas* attachment is a consequence of abnormal CFTR function; diminished acidification in the trans-Golgi and consequently diminished sialyltransferase activity results in more asialylated receptors for *P. aeruginosa* pil (8). The biological consequence of increased GalNacβ1-4Gal receptors is excessive stimulation of IL-8 secretion as of infection with *P. aeruginosa*. Asialo receptors are triggered by *Pseudomonas* binding, an event which is unlikely in the normal, fully sialylated epithelium but would have a much greater probability of occurring in the CF host. The degree of persistent infection and inflammation would then fluctuate depending upon continued synthesis of bacterial factors such as PAI, viral superinfections, and the immunologic response to these exogenous as well as endogenous mediators. Strategies to block the GalNacβ1-4Gal *P. aeruginosa* pilin receptor exposed on the CF epithelium may be useful to prevent the initial inflammatory response in the CF lung and the ensuing activation of neutrophils.
Acknowledgments

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