

**SERIES 'INTERACTION OF BACTERIA AND AIRWAY EPITHELIAL CELLS'**  
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***Pseudomonas aeruginosa* adherence to remodelling  
respiratory epithelium**

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*Pseudomonas aeruginosa* adherence to remodelling respiratory epithelium. S. de Bentzmann, P. Roger, E. Puchelle. ©ERS Journals Ltd 1996.

**ABSTRACT:** *Pseudomonas aeruginosa* is an opportunistic organism, which frequently colonizes the respiratory tract of patients with impaired host defence. In cystic fibrosis (CF) patients, this pathogen causes a progressive destructive bronchitis and bronchiolitis and is responsible for high mortality.

Normal respiratory epithelium is protected against bacteria via mucus and mucociliary clearance. Alteration of mucociliary clearance and of glycosylation of mucins in CF facilitates the access of bacteria to the underlying airway epithelial cells. Intact respiratory epithelium does not bind *P. aeruginosa*, whereas injured respiratory epithelium is highly susceptible to *P. aeruginosa* adherence. We found that the high affinity of respiratory epithelium, from CF and non-CF sources, for *P. aeruginosa*, during the wound repair process is related to the apical expression of asialo ganglioside M1 (aG<sub>M1</sub>). The affinity of repairing respiratory epithelium for *P. aeruginosa* is time-dependent, and is related to transient apical expression of aG<sub>M1</sub> at the surface of repairing respiratory epithelial cells. CF respiratory epithelial cells apically express more aG<sub>M1</sub> residues with relation to an increased affinity for *P. aeruginosa* than non-CF cells.

High epithelial damage followed by repair represents a major cause of *P. aeruginosa* adherence to airway epithelium in cystic fibrosis. However, *P. aeruginosa* adherence and colonization are not restricted to cystic fibrosis disease and *P. aeruginosa* pneumonia may also occur in severely immunocompromised patients, suggesting that epithelial injury and decreased host-response favour the colonization of the airways by *P. aeruginosa*.

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Among various bacteria able to colonize airways, *Pseudomonas aeruginosa* is an opportunistic microorganism often recovered in the airways of patients with an impairment in their host defence. Among the populations having a high risk to develop *P. aeruginosa* pneumonia, patients admitted to intensive care units with respiratory assistance represent the most exposed population. Another exposed population includes patients undergoing chemotherapy following cancer. The cystic fibrosis (CF) population represents a group for which *P. aeruginosa* infection is particularly important. Although *P. aeruginosa* has been associated with CF disease for a long time, it is of interest that recent reports from the National Nosocomial Infections Surveillance System in the United States demonstrated that *P. aeruginosa* is the most frequent pathogen causing nosocomial pneumonia [1]. This clearly demonstrates that cystic fibrosis transmembrane conductance regulator (CFTR) mutation leading to CF disease does not represent the only factor which allows *P. aeruginosa* to colonize airways.

In CF, *P. aeruginosa* infection of patients generally appears at the age of 10–14 yrs; other pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus*, appear earlier. After this age, nearly 98% of the CF population is colonized by *P. aeruginosa*. The eradication of *P. aeruginosa* by

antimicrobial therapy is rarely possible, leading to a progressive destructive bronchitis, and bronchiolitis with ultimate respiratory failure and severe deterioration of the patient's clinical status. Due to the impairment of mucociliary transport and to an increased production of *P. aeruginosa* and neutrophil elastases combined with other bacterial exoproducts, the mucous barrier and the respiratory epithelium may be severely damaged and remodelled. These particular environmental conditions in CF may expose neoreceptors for *P. aeruginosa* adhesins. The initial step of bacterial infection, preceding chronic colonization is the adherence of the bacteria to the epithelial cells. In normal conditions, the epithelial cells are protected by the airway mucus. In contrast, during impairment of host defence, an easier access of bacteria to epithelial cells could be initiated, particularly during the process of wound repair. Therefore, the limitation or the prevention of bacterial adherence are probably the most important means to prevent colonization by *P. aeruginosa* in exposed populations.

***P. aeruginosa* binding to respiratory mucins**

In normal airways, the surface epithelium is covered by a thin mucous layer, which functions as a filtration

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barrier in trapping exogenous particles (including bacteria), hydration and clearance. Mucins, which represent the major glycoprotein component of mucus, have been described as representing a source of various carbohydrate chains able to specifically bind to bacteria. Nonmucoid as well as mucoid strains of *P. aeruginosa* have been reported to bind to specific disaccharide sequences, such as Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc and Gal $\beta$ 1-4GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc [2]. The observation that nonpiliated *P. aeruginosa* mutants bind to mucins suggests that the adherence of *P. aeruginosa* to mucins may be mediated by nonpilus adhesins.

Several reports have suggested that the mucus glycoproteins secreted by respiratory epithelial cells from CF patients are altered [3–5], and that, at least for salivary mucins, the carbohydrate composition and therefore the *P. aeruginosa* binding are altered in CF [6, 7]. Recent results from CARNOY *et al.* [8] clearly indicate that several proteins of the outer membrane from *P. aeruginosa* have a common affinity both for mucins and lactoferrin, via a common carbohydrate sequence Gal $\beta$ 1-4GlcNAc. These findings suggest that the carbohydrate diversity of mucins in association with other antibacterial proteins, such as transferrin, may in normal conditions protect the airway cells from being colonized. The bacteria entrapped in the gel phase of mucus are then rapidly cleared out from the airways by the ciliary activity. In pathological conditions, such as in CF, the impairment of mucociliary clearance, and possible alteration of the glycosylation profile of the mucins associated with stasis of mucus represent conditions providing easy access to the underlying airway epithelial cells.

#### *How does P. aeruginosa persist within the respiratory tract?*

In normal airways, active mucociliary clearance and antibacterial secretory proteins present in respiratory mucus, maintain the sterility of the airways. In pathological conditions, as is the case in cystic fibrosis, many events occur which may favour colonization by *P. aeruginosa*. Mucus hypersecretion, decrease of mucociliary clearance and inflammation represent optimal conditions for *P. aeruginosa* colonization. Until recently, little was known about the chronology of infection with *P. aeruginosa* and the specific implication of cystic fibrosis transmembrane conductance regulator (CFTR) protein mutations in *P. aeruginosa* infection and colonization. Interestingly, recent reports support the idea that neutrophil-dominated inflammation precedes infection, thus preparing the respiratory tract for chronic bacterial infection by the release of cytokines [9]. Several factors predispose to *P. aeruginosa* replication in CF. In addition to the impairment of mucociliary clearance, mainly ascribed to dehydration and hyperviscosity of respiratory mucus, it has also been demonstrated that the local ionic concentrations, and sodium concentration, in particular could favour this bacterial replication because of the decrease of bactericidal activity of macrophages in depleted extracellular concentrations of sodium [10]. Thus, *P. aeruginosa* is able to be in contact with the respiratory epithelial cell surface and infection and colonization are, therefore, initiated. Firstly, *P. aeruginosa* by way of its adhesins is

able to recognize and adhere to the corresponding epithelial receptors. *P. aeruginosa* adhesins include fimbriae [11–15], the mucoid exopolysaccharide [16–18], haemagglutinins [19], internal lectins [20], the exoenzyme S [21, 22], and nonpilus adhesins [23]. Pilus adhesins are reported to be responsible for *P. aeruginosa* adherence to epithelial cell surface, whereas nonpilus adhesins are more implicated in adhesion to mucins or in interaction with phagocytic cells.

*P. aeruginosa* is able to synthesize and secrete a large variety of virulence factors, including elastase alkaline protease, phospholipase C and neuraminidase but also lipopolysaccharide (LPS), alginate, exoenzyme S, exotoxin A and pyocyanin, creating optimal local conditions in which to persist and replicate [24]. The release of virulence factors contributes to the pathogenesis of the bacteria, facilitating the disruption of the epithelial integrity.

Recently, PRINCE *et al.* [25] demonstrated that *P. aeruginosa* adherence via pili and flagella leads to the production of interleukin (IL)-8 by CF respiratory epithelial cells, which contributes to creating optimal conditions for bacterial colonization. According to the local environment, including local concentrations of iron, phosphate and phosphatidylcholine, *P. aeruginosa* is then able to regulate the production of its virulence factors. Understanding the molecular and the cellular events that allow the adherence of *P. aeruginosa* to respiratory epithelial cells remains fundamental to eradication of this opportunistic pathogen, due to the fact that these bacterial species can become resistant to antibiotics.

#### *Pseudomonas aeruginosa adherence to respiratory epithelium*

An extensive literature has been devoted to the adherence of *P. aeruginosa* to the respiratory epithelium of various species. Interestingly, intact respiratory epithelium has never been shown to bind *P. aeruginosa* [26–28]. In contrast, it has been clearly shown that injured respiratory epithelium is highly susceptible to *P. aeruginosa* adherence [26, 27]. Desquamated cells [29], as well as the denuded extracellular matrix (ECM) [26, 30], and in particular components of the ECM, including fibronectin [31] and laminin [32], either belonging to the basement membrane or secreted [30] in the extracellular milieu, represent preferential targets for *P. aeruginosa* attachment.

Five years ago, our attention was drawn to respiratory epithelial cells undergoing the process of repair and the specific targets they represent for *P. aeruginosa* adherence [30, 33]. The respiratory epithelial cells near the edge of the wound modify their phenotype. After injury, they dedifferentiate, spread, migrate and proliferate in order to close the wound. They have been identified and characterized by ZAHM and co-workers [34, 35] in an *in vitro* respiratory epithelial wound repair model. Multi-lesional events, such as caused by viruses, bacteria, chemicals or mechanical forces, can, *in vivo*, create damage. In CF, the emergence of *P. aeruginosa*, following infections by other microorganisms, suggests that partially exposed epithelial CF receptors may always be present due to constant remodelling and injury of the airways. Such a scenario could explain the recurrent *P. aeruginosa* infection in CF (fig. 1).

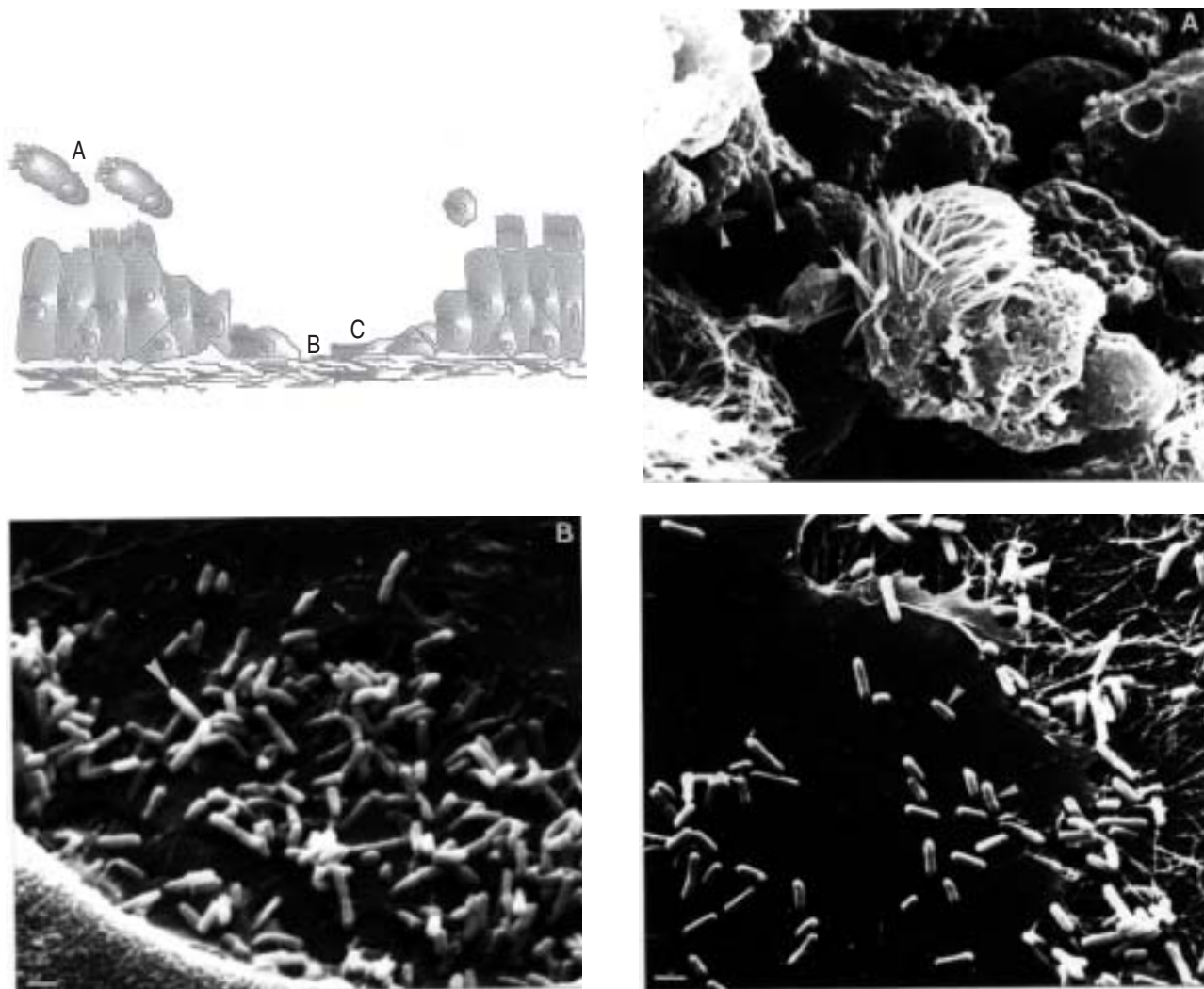


Fig. 1. — Injury of the respiratory epithelium represents propitious conditions for adherence of *Pseudomonas aeruginosa*. Schematic representation and electron micrographs showing A) Desquamated cells; B) denuded extracellular matrix; and C) respiratory epithelial cells engaged in the process of repair represent the main targets for adherence of *P.aeruginosa* (arrowheads). (Internal scale bar=1.5  $\mu$ m).

#### Epithelial receptors involved in *P. aeruginosa* adherence to respiratory epithelium

Epithelial receptors involved in the adherence of *P. aeruginosa* remain, up to now, poorly defined, even though research in this field has progressed significantly during the last 5 yrs. In CF in particular, the understanding of the epithelial receptors involved in *P. aeruginosa* adherence *in vivo* remains a challenge. One crucial and unanswered question is whether a specific receptor at the surface of cystic fibrosis respiratory epithelial cells is capable of recognizing *P. aeruginosa*. The adherence of *P. aeruginosa* to epithelial cells from the respiratory tract [36, 37], as well from other organs such as cornea [38], has been attributed to the glycosphingolipids and, in particular, to those which contain a specific disaccharide sequence of GalNAc $\beta$ 1-4Gal occurring in asialo ganglioside M1 (aG<sub>M1</sub>) and in asialo ganglioside M2 (aG<sub>M2</sub>). The corresponding bacterial adhesin reported to bind to this carbohydrate moiety is the bacterial pilin, which is the major component of the pilus adhesin, and, in particular,

the C-terminal part of the pilin protein [39]. Recent studies have demonstrated that aG<sub>M1</sub> occurs in greater amounts at the surface of CF respiratory epithelial cells, compared to non-CF respiratory epithelial cells. Furthermore *P. aeruginosa*, by its neuraminidase activity, is able to increase the amount of available aG<sub>M1</sub> on the surface of CF respiratory epithelial cells, in contrast to non-CF cells [36].

From this latter study, it becomes evident that the CF respiratory epithelial cells have a significantly increased number of epithelial receptors for *P. aeruginosa*, and that they are more susceptible to *P. aeruginosa* neuraminidase activity [40, 41]. This CF receptor does not explain why *P. aeruginosa* is able to colonize the airways of patients without any CFTR mutation and defect. Moreover, the close affinity of *P. aeruginosa* to respiratory epithelial cells from non-CF patients, particularly to cells under the process of repair, is puzzling. Consequently, the question to address is whether epithelial remodelling, particularly during the wound repair process, could increase the expression of aG<sub>M1</sub> on respiratory epithelial cells engaged in the process of repair.



### *P. aeruginosa* adherence to respiratory epithelium undergoing repair

We also analysed the kinetics of the interaction of *P. aeruginosa* with respiratory epithelial cells under the process of repair. We tried to determine whether aG<sub>M1</sub>, classically described as a *P. aeruginosa* receptor specific to CF cells, could be involved not only in *P. aeruginosa* affinity for respiratory epithelial cells under the process of repair in CF patients but also in non-CF patients. Using the *in vitro* model of wound repair of respiratory epithelium developed in our laboratory by ZAHM and co-workers [34], we studied the adherence of a piliated strain of *P. aeruginosa* during wound closure [42]. In this model, on confluent dissociated respiratory epithelial cells obtained from nasal polyps in culture, chemical wounds were performed; and, for an initial size of 30 mm<sup>2</sup>, the wound closure occurs in 72 h. Wound repair includes spreading, migration and proliferation of respiratory epithelial cells edging the wound. Studies of *P. aeruginosa* adherence were executed by adding a constant inoculum of *P. aeruginosa* of 10<sup>8</sup> colony-forming units (CFU)·mL<sup>-1</sup> after making the wound, at 2, 24 and 48 h, 72 h (wound closure), and 84 h (12 h after the wound closure had occurred). After incubation for 1 h to allow adherence, the different wound tissues were prepared for quantification of adherence by computer-assisted scanning electron microscopy [43, 44].

It appeared that adherence of *P. aeruginosa* to respiratory epithelial cells under the process of repair, was dependent on the time of repair as well as on the distance from the front edge of the wound. The closer to the front edge of the wound, the higher the *P. aeruginosa* adherence. *P. aeruginosa* adherence was strictly limited to the respiratory epithelial cells edging the wound in the process of spreading and migration over the collagen matrix, whereas the other cells belonging to the area undergoing repair or belonging to the confluent layer outside the wound area did not bind *P. aeruginosa*. These respiratory epithelial cells undergoing repair exhibited specific phenotypic characteristics: they were poorly differentiated, nonpolarized; they spread over the collagen matrix with cytoplasmic expansions described as lamellipodia and thinner filipodia; and they had a smooth surface without microvilli. We also observed that adherence of *P. aeruginosa* was maximal at 2 and 24 h of repair decreased significantly by 48 h of repair, and was close to zero when the wound was closed (72 and 84 h of repair). These results strongly suggest that *P. aeruginosa* adherence is closely associated to the spreading phenotype of respiratory epithelial cells undergoing repair, and that, during the repair process, the spreading and repairing respiratory epithelial cells exhibit a different *P. aeruginosa* adherence behaviour.

In association with *P. aeruginosa* adherence, we studied the apical distribution of aG<sub>M1</sub> receptors during the repair process [42]. Interestingly, we observed that aG<sub>M1</sub> was specifically expressed at the surface of spreading and repairing respiratory epithelial cells, whereas no aG<sub>M1</sub> was identified on the surface of nonspreading respiratory epithelial cells belonging to the repairing area, as well as to those belonging to the nonrepairing area. This apical distribution of aG<sub>M1</sub> at the surface of repairing respiratory epithelial cells was dependent on the time of

repair: apical aG<sub>M1</sub> expression onto these cells was maximal after 2 h of repair and decreased to zero after 24, 48, 72 and 84 h of repair.

The importance of aG<sub>M1</sub> receptors in adherence of *P. aeruginosa* to spreading repairing respiratory epithelial cells was examined by blocking these receptors with an antibody against aG<sub>M1</sub> [45]. It was observed that blocking aG<sub>M1</sub>, significantly reduced *P. aeruginosa* adherence to spreading and repairing respiratory epithelial cells from CF and non-CF patients. However, the percentage of reduction in *P. aeruginosa* adherence in the presence of antibody against aG<sub>M1</sub> varied from one patient to another (from 53 to 82%), and was significantly more important in the CF group compared to the non-CF group. In the CF group, the lowest value of reduction in *P. aeruginosa* adherence in the presence of anti-aG<sub>M1</sub> antibody was found for tissue from a ΔF508 homozygous CF patient, who had never been colonized by *P. aeruginosa*, whereas the highest values were found for CF patients who had already been colonized by *P. aeruginosa*.

These results suggest that exoproducts from *P. aeruginosa*, such as neuraminidase, are able to expose new available aG<sub>M1</sub> receptors, thus increasing *P. aeruginosa* adherence as has been demonstrated previously [36, 40, 41].

In conclusion, these results suggest that:

1. Asialo Ganglioside M1 is an epithelial receptor for *P. aeruginosa* present at the surface of respiratory epithelial cells, and is expressed during repair.
2. The apical expression of aG<sub>M1</sub> is closely associated with the spreading phenotype of respiratory epithelial cells appearing during the initial step of wound repair.
3. This apical expression of aG<sub>M1</sub> is transient, and is maximal immediately after the onset of the wound repair, possibly associated with dramatic changes in the cytoskeleton organization and/or in the sorting of molecules towards the apical membrane.
4. The partial inhibition of *P. aeruginosa* adherence to respiratory epithelial cells undergoing repair following blocking of aG<sub>M1</sub> suggests that in addition to aG<sub>M1</sub>, other unidentified receptors are probably involved.
5. High damage followed by repair is certainly a major cause of *P. aeruginosa* adherence to respiratory epithelial cells in CF. The expression of aG<sub>M1</sub> on the surface of CF cells could be due either to undersialylation of glycolipids [36], caused by CFTR mutations leading to defective intracellular acidification [46], or to the high and constant degree of injury and remodelling of the airway epithelium.

The CF airways environment, resulting from the CFTR defect, may exacerbate the adherence of *P. aeruginosa*: the changes in local ionic concentrations (extracellular chloride and sodium) [10]; the increased production of IL-8 by respiratory epithelial cells that have bound *P. aeruginosa* via aG<sub>M1</sub> [25]; the ability of *P. aeruginosa* exoproducts to expose neo-aG<sub>M1</sub> receptors on CF cells [36, 41]; and the defective sialylation of gangliosides, due to decreased activity of α2,6 sialyltransferase [47], may dramatically increase the susceptibility of the CF airway to *P. aeruginosa*. However, *P. aeruginosa* adherence and colonization are not restricted to cystic fibrosis disease and *P. aeruginosa* pneumonia develops in other populations at risk. These populations are generally dramatically immunocompromised or immunosuppressed, which suggests, that injury of their respiratory epithelium associated with decreased host response could

greatly favour the colonization of their airways by *P. aeruginosa*.

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