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REVIEW

Cellular and molecular mechanisms of bacterial adhesion to respiratory mucosa

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Cellular and molecular mechanisms of bacterial adhesion to respiratory mucosa. M.C. Plotkowski, O. Bajolet-Laudinat, E. Puchelle. ©ERS Journals Ltd 1993.

ABSTRACT: Different bacterial species adhere avidly to respiratory mucus. Such adhesion, when followed by ciliary clearance, represents an important stage of the airway defence system. However, in pathological conditions, the mucociliary clearance may be severely reduced, and mucus-associated bacteria may multiply and infect the underlying epithelium. Only a few bacteria have been shown to adhere to ciliary membranes of functionally active ciliated cells. Therefore, the first way in which most of the respiratory pathogens associate with the airway epithelium is likely to be by their adhesion to mucus. Some bacteria also secrete products that may affect ciliary function and/or cause cell death and epithelial disruption. Respiratory pathogens that do not bind to normal ciliated cells may readily adhere to injured epithelial cells, or to the unmasked extracellular matrix. Furthermore, following injury, epithelial respiratory cells in the process of migration, in order to repair the wounds, may present receptors to which bacteria adhere. The adhesion to all of these epithelial receptors may contribute to the chronicity of many bacterial respiratory infections.

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Micro-organisms which come into contact with mammalian tissues need a firm anchorage to mucous membranes before colonizing or infecting the underlying epithelium. Otherwise, they would surely be removed by host defence mechanisms. Bacteria have developed a wide range of adhesion mechanisms: many strains produce lectin-like substances, which may take the form of fimbriae, or may be embedded in the outer membrane of the bacterial cell wall, or even in the exopolysaccharide which often surrounds the bacterial cells [1]. On the other hand, mammalian cells are surrounded by numerous potential receptors for bacterial adhesins: the saccharide residues from cell surface glycoproteins, glycolipids and proteoglycans [2]. Besides the ability to recognize receptors on eukaryotic cells, bacterial adhesins may play a substantial role in determining the outcome of a host parasite interaction. The intimate contact between microbial and epithelial cells allows bacterial toxic products to reach concentrations sufficient to damage the host cells. Moreover, adhesins may even be toxins, and may mediate cellular toxicity by presentation of the enzymatically active toxic subunit directly to the eukaryotic cells [3].

Toxic products secreted by the microorganisms, prior to their adhesion to the mammalian cell receptors, may affect the outcome of the host bacteria interaction and favour bacterial persistence, by disturbing host defence mechanisms, or by unmasking potential receptors for the microbial adhesins.

Bacterial interaction with respiratory tract mucosa

The lower respiratory tract is sterile from the first bronchial division. That this is so, despite the frequent inhalation of pathogens, is due to host defences. Bacterial colonization of the airways usually results from previous damage to host defence mechanisms, rather than bacterial virulence in itself overcoming them.

The surface of human airways is covered by a gel-like mucous blanket, which is propelled by ciliary beat from the small bronchi towards the trachea. This means that bacterial interaction with mucus precedes any binding between microbial adhesins and cell receptors. Therefore, in order to persist successfully as a respiratory pathogen, bacteria have to be well-equipped to rapidly penetrate the mucous layer, to find the host cell surface receptors (possibly by chemotactic attraction by taxins emanating from the mucosa, as has been shown in intestinal mucosa [4]), and to attach to them. Because respiratory infections are rare events in healthy people, we have to assume that only a few bacterial species fulfil these requirements.

Even though the entrapment of inhaled bacteria by the mucous layer, and their elimination by ciliary beating, play a major role in preventing respiratory infections, in pathological conditions the ciliary clearance of mucus may be severely reduced, due to a decrease in the number and activity of ciliated cells, changes in the biochemical and

rheological properties of the mucus, or modifications in the surface properties of the respiratory mucosa [5]. Under these conditions, mucus-associated bacteria may multiply, degrade the mucus and infect the underlying epithelium. Therefore, not only adhesion to epithelial cell surface receptors favours bacterial colonization of respiratory mucosa, but also their adhesion to non-transported mucus (fig. 1).

Bacterial interaction with respiratory mucus

Mucins are the main molecules of respiratory mucus. They form a group of complex glycoproteins comprising almost 80% sugars, and have a molecular weight which may exceed 10^6 Da. Therefore, airway mucus is rich in potential carbohydrate receptors for bacteria [6].

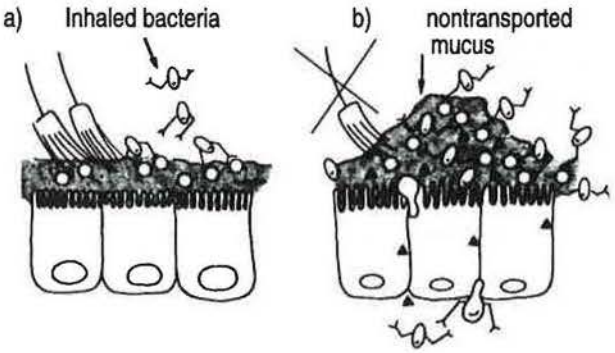

Fig. 1. – a) Bacteria adherent to carbohydrate residues of respiratory mucin are cleared by mucociliary transport. b) In pathological conditions, the ciliary clearance of mucus with attached bacteria may be severely reduced. Adherent bacteria may multiply, penetrate the mucous layer, attach to cell surface receptors and invade the epithelial barrier. ○: carbohydrate residues; ▲: bacterial toxins.

In fact, different micro-organisms demonstrate an affinity for airway mucus: *Streptococcus pneumoniae* [7], *Haemophilus influenzae* [8, 9], and *Staphylococcus aureus* [10] are common respiratory pathogens that do not adhere to normal ciliated epithelium, but bind avidly to respiratory mucus instead. Thus, the first way in which these micro-organisms associate with normal respiratory mucosa is likely to be by their adhesion to mucus. Some of the respiratory pathogens elaborate and release products that stimulate the secretion of more mucin and/or interfere with the mucociliary transport by slowing or disorganizing ciliary beating, or even by damaging the epithelium (table 1). Bacteria may, therefore, contribute to the development of an environment favouring their growth and persistence in the infected airways.

Mechanisms allowing the interaction between respiratory pathogens and mucus have been partially elucidated for *P. aeruginosa*. VISHWANATH and RAMPHAL [21] showed that *P. aeruginosa* adhere significantly more to purified human mucin than do members of the family *Enterobacteriaceae*. Since sialic acid and N-acetylglucosamine (GlcNac) residues inhibit the adhesion of *P. aeruginosa*, and the exposure of mucin to the influenza virus inhibits further binding of bacteria, the authors speculated that sialic acid may be part of the *P. aeruginosa* receptor in mucin.

A recent study from RAMPHAL and co-workers [22] showed that both nonmucoid and mucoid *P. aeruginosa* strains bind to type 1 (galactose β1–3 N-acetylglucosamine) and type 2 (galactose β1–4 N-acetylglucosamine) disaccharide units, commonly found in mucin as part of its oligosaccharide backbone, and in peripheral locations. Even though they found that sialylation of neoglycolipids containing these two saccharide receptor units did not interfere with bacterial binding, alpha 2–6 linked sialic acid blocked *P. aeruginosa* adhesion. It is therefore suggested [22] that the role of sialic acid in *P. aeruginosa* receptors may be to maintain the conformation of the

Table 1. – Respiratory pathogens release factors which stimulate mucus secretion and/or adversely affect ciliated epithelium

Bacteria	Product	Effect	[Ref.]
H. influenzae	Supernatant fluids	Ciliostasis, loss of cilia, cell sloughing	[11]
H. influenzae	Lipo-oligosaccharide	Loss of ciliary activity	[12]
H. influenzae *S. aureus*	Bacterial filtrates	Mucin hypersecretion	[13]
H. influenzae, *S. pneumoniae*	Bacterial filtrates	Mucin hypersecretion	[14]
S. pneumoniae	Pneumolysin	Ciliary slowing, epithelial cell damage	[15]
P. aeruginosa	Bacterial filtrates	Mucin hypersecretion	[16]
P. aeruginosa	Elastase and alkaline protease	Cilia disruption	[17]
P. aeruginosa	Pyocyanin and 1-hydroxyphenazine	Ciliary slowing, ciliostasis	[18]
P. aeruginosa	Rhamnolipid	Mucus hypersecretion, ciliary slowing	[19, 20]

oligosaccharide chains, or to increase the affinity of the adhesion, and not to react directly with bacterial adhesins.

P. aeruginosa persistently colonizes the airways in cystic fibrosis (CF) patients. To explain the specificity of *P. aeruginosa* as a respiratory pathogen in CF on the basis of its affinity for airway mucus, the presence of some component of respiratory mucins unique to this genetic disease may be postulated. However, studies carried out by RAMPHAL and co-workers [23] showed that most mucin-like glycopeptides from CF sputa allowed less adhesion of *P. aeruginosa* than did glycopeptides from the respiratory secretions of bronchitic patients. In addition, SAJJAN *et al.* [24] recently found that *P. aeruginosa* did not bind to CF mucin any more avidly than to non-CF mucin, to bovine serum albumin, or to many structurally unrelated glycoproteins. Since, in their studies, the bacterial adhesion to CF and non-CF mucins could be inhibited by an agent disrupting hydrophobic interactions, they concluded that nonspecific hydrophobic interactions might mediate the *P. aeruginosa* binding to mucin [24].

The presence of chemotactic factors for *P. aeruginosa* in mucins from CF patients has been reported by NELSON *et al.* [25]. However, the observation that both nonmotile and nonchemotactic mutants of *P. aeruginosa* also adhered to mucin, suggests that adhesion is not dependent on motility or chemotaxis [25]. Chemotaxis of other bacterial species towards mucin has previously been shown to be an important step in the association of pathogens with host mucosa [4, 26]. In CF, both hypersecretion of mucus and severe reduction of the mucociliary transport have been described [27]. Thus, we may speculate that initial colonization of the respiratory tract in CF patients by *P. aeruginosa* could be assisted by chemotaxis towards, and adhesion to, respiratory mucin (fig. 2).

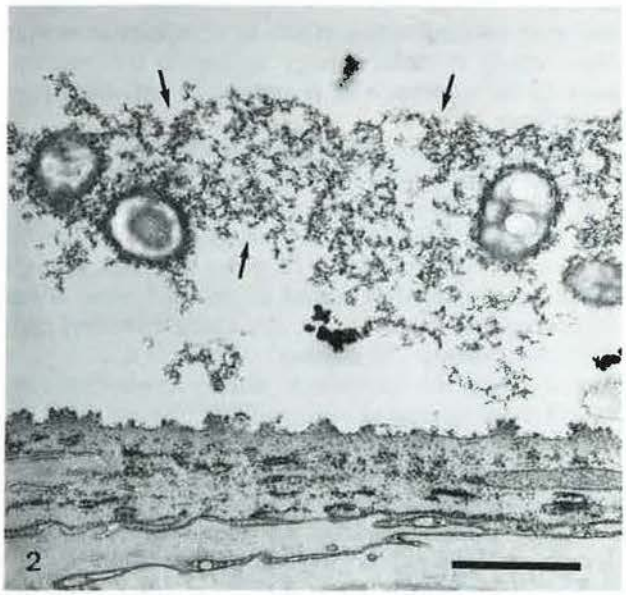

Fig. 2. – Nonmucoid *Pseudomonas aeruginosa* associated with respiratory cells in primary culture from a CF patient are surrounded by a matrix-like material (arrows), which is much more abundant than the matrix found in cell cultures from non-CF patients. CF: cystic fibrosis. (Bar = 1 μm).

Secretory respiratory cells have been reported to package mucins in secretory granules, and to discharge them at the cell surface by exocytosis [28, 29]. However, part of the secreted mucin remains tightly associated with the plasma membrane [28, 30]. By using a monoclonal antibody against respiratory mucin, we detected the presence of secreted mucin at the tips of microvilli, and along cilia, as well as in a matrix-like material which surrounded aggregated *P. aeruginosa* associated with human epithelial respiratory cells in primary culture [31]. Nevertheless, we were unable to detect *P. aeruginosa* adhering to mucin-coated cilia. The recent finding of SAJJAN *et al.* [24] that *P. aeruginosa* react more easily with mucin when it is in solution than when it is immobilized in microtitre wells suggests that structural conformation may affect the *P. aeruginosa*/mucin interaction. We may, therefore, speculate that *P. aeruginosa* adhesion to mucin depends on conformationally specific determinants, not exposed in mucins, which cover the luminal border of the respiratory epithelium.

Adhesins by which *P. aeruginosa* adhere to different substrata include fimbriae [32–36], the mucoid exopolysaccharide [37–39], haemagglutinins [40], internal lectins [41, 42] and exoenzyme S [43–45]. The adhesion of mucoid *P. aeruginosa* to mucin has been reported to be mediated by the mucoid exopolysaccharide, whilst fimbriae mediate the adhesion of nonmucoid strains [46]. However, isogenic nonfimbriated *P. aeruginosa* strains were recently shown to retain their adhesiveness to type 1 and 2 disaccharide units, the putative receptors in mucin for *P. aeruginosa* adhesion [22]. Therefore, it is conceivable that another of the many ligands that *P. aeruginosa* produce can participate in the bacterial adhesion to respiratory mucus, such as the 16 kDa nonpilus protein component of *P. aeruginosa* cells recently described by REDDY [47].

Although not as prevalent as *P. aeruginosa*, *Psedomonas cepacia* is another opportunistic respiratory pathogen in some CF patients. Recently, SAJJAN and co-workers [48] found that *P. cepacia* binds to respiratory mucin from CF patients, as well as to normal human intestinal mucins. Mucin receptors for these bacteria seem to include N-acetylglucosamine and N-acetylgalacto-samine residues, probably linked together as part of the core oligosaccharide structures. *P. cepacia* adhesin for mucin was reported to be a protein, located on fimbriae, present over the entire surface of the bacterial cell [49].

Bacterial adhesion to normal respiratory epithelium

If only a few pathogens have developed the ability to infect the respiratory mucosa, the bacterial species that adhere directly to normal ciliated cells are still more limited. In fact, only *Mycoplasma pneumoniae* and *Bordetella pertussis*, two aetiological agents of community-acquired infections of the airways mucosa, have been observed to adhere along ciliary membranes of functionally active ciliated cells [50–54].

Besides respiratory ciliated cells, *M. pneumoniae* adhere to human erythrocytes and to a variety of other cell types,

usually *via* a polar tip structure [55]. Erythrocyte receptors for *M. pneumoniae* are long chain sialo-oligosaccharides of I and i antigen type (*i.e.* oligosaccharides of poly N-acetyllactosamine series, presenting sialic acid joined by alpha 2–3 linkage to their penultimate galactose residues) that occur on glycoproteins and on different glycolipids [56, 57]. Recently, LOVELESS and FEIZI [58] showed these sialo-oligosaccharide receptors to be highly concentrated on human cilia, and at the apical microvillar domain of bronchial ciliated cells. The reported selective attachment of *M. pneumoniae* to ciliated cells [53, 55] is explained by the lack of this sialo receptor in the membranes of other mature epithelial cells from the respiratory mucosa, such as the secretory cells, or in the mucus they produce [58]. The lack of the sialo long-chain receptor structure in the secreted mucus seems to be an important factor favouring bacterial adhesion to ciliated cells, rather than adhesion to mucus and clearance by the mucociliary transport [59].

In contrast to *M. pneumoniae*, which adheres to a number of cell types, *B. pertussis* infects only human ciliated cells. By transmission electron microscopic observation of segments of human bronchial mucosa incubated with *B. pertussis* suspensions, bacteria were seen to adhere to cilia both by direct apposition, and by filaments coursing between cilia and the micro-organisms [52]. *B. pertussis* did not adhere to the body of ciliated cells or to nonciliated cells, but were seen characteristically localized in the proximal portion of the ciliary tuft, adjacent to both cilia and microvilli. By working on mutant *B. pertussis* strains, each deficient in one of several bacterial virulence factors, TUOMANEN and WEISS [60] observed loss of adhesion to ciliated cells, which was associated with a lack of secretion of two surface antigens: the filamentous haemagglutinin and pertussis toxin. These deficient strains regained adhesion if the missing proteins were supplied exogenously. Therefore, the authors hypothesized that filamentous haemagglutinin and pertussis toxin act together as adhesins, by establishing a bridge between bacteria and one or more carbohydrate-containing receptors on cilia. TUOMANEN [61] also evaluated the effect of the presence of these two adhesins, bound to the surface of cilia, in the adhesion of other respiratory pathogens. When either ciliated cells or *S. pneumoniae*, *S. aureus* or *H. influenzae* were pretreated with filamentous haemagglutinin and pertussis toxin, bacteria acquired the ability to adhere to normal cilia, *in vitro* and *in vivo*. This "piracy" of adhesins may facilitate the bacterial respiratory superinfections, which are common in complicated cases of whooping cough.

Fimbriae, the classic structures related to the adhesion of pathogenic bacteria, have been identified on strains of *B. pertussis* that possess agglutinogen 2 (types 1,2,3 and 1,2) [62], but they do not appear to mediate the attachment of *B. pertussis* to mammalian cells [60, 63].

Although the definitive structure of receptors for *B. pertussis* on human cilia still requires confirmation, some of their biochemical details are beginning to emerge. Galactose, N-acetylglucosamine, lactose and different complex carbohydrates containing lactosamine residues can inhibit the adherence of *B. pertussis* to human cilia *in vitro* [64]. Masking of the receptor on cilia can be achieved by preincubation of ciliated cells with lectins or anti-carbohydrate monoclonal antibodies recognizing galactose-glucose structure. Reagents directed against β1-4 configuration are effective inhibitors. This suggests that the core structure of many blood group antigens, such as A, B, H and Lewis could serve as receptors for *B. pertussis*, a fact that would explain why virtually all people are susceptible to whooping cough. The binding of *B. pertussis* to purified lactosylceramide in thin layer chromatography binding studies suggests that receptors for *B. pertussis* on cilia are galactose-glucose-containing glycolipids [60].

In marked contrast to *M. pneumoniae* and *B. pertussis*, which have been found enmeshed in respiratory cilia from infected hosts, other airway pathogens, such as *H. influenzae*, *S. pneumoniae* and *S. aureus*, as well as nonmucoid *P. aeruginosa*, do not bind to normal ciliated cells. Instead, bacterial adhesion is dependent on a prior injury of the respiratory mucosa.

Bacterial adhesion to injured respiratory epithelium

In human nasopharyngeal tissue incubated with both encapsulated and nonencapsulated strains of *H. influenzae*, FARLEY *et al.* [8] showed that damage to ciliary activity by bacterial products occurs 6 h after infection, being associated with the sloughing of ciliated cells, to which *H. influenzae* were not attached. *H. influenzae* were attached to nonciliated cells of damaged mucosa or to surface mucus. Similarly, READ *et al.* [9] could not detect adherent *H. influenzae* on normal areas of human nasopharyngeal epithelium, but found bacteria adhering to structurally damaged ciliated and nonciliated cells, 24 h after infection. Studies carried out by others [65–68] have also detected marked damage to respiratory mucosa following infection with a variety of *H. influenzae* strains. Thus, airway epithelial damage appears to be a requirement for the association of *H. influenzae* with respiratory epithelium.

Fimbriae have been postulated to facilitate *H. influenzae* adhesion to human buccal cells [68–70]. However, the role of these adhesins in the association of *H. influenzae* with ciliated epithelium is controversial. As shown by READ *et al.* [9], fimbriated *H. influenzae* transformant failed to associate with normal or damaged areas of the respiratory epithelium to a greater extent than their nonfimbriated parents. In contrast, WEBER *et al.* [71] have recently found the colonization density of nonfimbriated *H. influenzae* inoculated intranasally in neonatal monkeys to be tenfold less than that of their isogenic fimbriated parent. Similarly, STERK *et al.* [72] found that monoclonal antibodies specific for *H. influenzae* fimbriae inhibited the bacterial binding to human ciliated mucosa. Finally, FARLEY *et al.* [67] observed that fimbriated and nonfimbriated *H. influenzae* strains differed in their pattern of adhesion to damaged respiratory mucosa, and in their ability to invade the damaged mucosa. Mucosal penetration occurred to a much greater extent with the nonfimbriated strain. Therefore, it may be hypothesized that,

as the binding of nonfimbriated *H. influenzae* to respiratory mucosa is of a low affinity, bacteria may easily invade areas of mucosal damage and necrosis. In contrast, fimbriated *H. influenzae* bind tightly to the epithelial surface and are less readily displaced.

Whilst the minimal structure of the receptor for the fimbriae of *H. influenzae* on human oropharyngeal epithelial cells and erythrocytes was determined to be sialyl-lactosylceramide residues [73], nothing is known concerning the nature of the receptors on damaged ciliated mucosa.

S. pneumoniae have been shown to adhere to human pharyngeal epithelial cells *in vitro* [74], and glycoconjugates containing the disaccharide unit N-acetylglucosamine β1-3 galactose were suggested as their pharyngeal cell receptors [75]. Epithelial cells from patients with recurrent respiratory infections by *S. pneumoniae* have been shown to be more susceptible to bacterial adhesion, than cells from patients without underlying pulmonary disease [76]. These findings suggest that the cell content of surface receptors for *S. pneumoniae* adhesins may vary, and may influence the pathogenesis of bronchopulmonary infections.

Very few studies have been carried out on *S. pneumoniae* adhesion to ciliated respiratory epithelium, but the existing data point to the lack of adhesion to normal respiratory cells. LUNDBERG *et al.* [77], looking for the adhesion of *S. pneumoniae* to nasopharyngeal cells from children, found that bacteria adhered more frequently to desquamated cells in mucus than to normal ciliated or nonciliated cells. No bacteria were found attached to ciliated cells. Whilst evaluating whether Influenza A virus infection could enhance *S. pneumoniae* adhesion to respiratory epithelium, and so favour bacterial superinfection, we could never detect *S. pneumoniae* attached to uninfected tracheal epithelium, or to damaged cells in virus infected tissues [78]. In contrast, bacteria were seen adherent to differentiating cells repairing the injured tracheal mucosa. This finding led us to speculate that the presence of receptors for microbial adhesins may vary during epithelial regeneration and cell differentiation after injury. The failure of *S. pneumoniae* to adhere to either normal or damaged respiratory cells was recently reported by FELDMAN *et al.* [79]. Analysing the interaction of *S. pneumoniae* with human respiratory epithelium *in vitro*, the authors observed epithelial damage occurring after 24 h following tissue infection. Bacteria, rather than adhering to epithelial cell surfaces, were seen embedded in a mucinous layer, overlaying damaged epithelium, mainly where ciliary beating was not detected.

P. aeruginosa adhesion to respiratory epithelium

In the literature, contradictory results are reported on the adhesion of nonmucoid *P. aeruginosa* to normal respiratory epithelium. Studies carried out on functionally active animal models have shown that bacteria do not adhere to respiratory epithelium, even after long incubation periods, unless it has first been injured in some way [37, 80–83]. In contrast, *P. aeruginosa* were shown to adhere to cilia of human cells, obtained by tracheal brushing [33, 34, 84]. This adhesion has been shown to be promoted by bacterial fimbriae [34, 35], since it could be inhibited by purified fimbriae, by fragments of the carboxy terminal end of the native molecule, or by their synthetic peptide analogues [35, 85]. The discrepancy between the results obtained with animal and human models may be attributed to differences between them in the distribution of receptors for *P. aeruginosa* adhesins. Alternatively, it may result from the fact that exfoliated cells expose molecules which are not normally accessible. Other evidence seems to corroborate this hypothesis: for example, we recently observed that exfoliated ciliated cells found in primary culture of human epithelial respiratory cells were intensely reactive with the Ricimus communis (RCA) lectin, which contrasted sharply with normal cells, which were nonreactive (unpublished results). Studies from ZOUTMAN *et al.* [83], in which *P. aeruginosa* were observed attached to the cilia and lateral edge of exfoliated tracheal ciliated cells and not to healthy cells, lend further weight to this theory.

To evaluate the susceptibility of functionally active ciliated cells to *P. aeruginosa* adhesion, we have exposed human epithelial respiratory cells in primary culture to nonmucoid *P. aeruginosa* suspensions. With scanning and transmission electron microscopy, bacteria were never seen adherent along ciliary membranes or in the interciliary spaces. In the vast majority of cases, *P. aeruginosa* associated with ciliated cells were present as aggregates of countless bacterial cells. The aggregated adherent micro-organisms were seen to be surrounded by a mucin-containing fibrillar material, which appeared to establish the interaction between bacteria and cilia (fig. 3) [86].

The affinity of *P. aeruginosa* for mucosa of injured airways has been reported by several authors. RAMPHAL and co-workers [87] found bacteria to adhere only to desquamated cells of tracheae from mice infected with the Influenza A virus or from ferrets submitted to endotracheal intubation. RAMPHAL and PYLE [81], working on acid-treated mice tracheae, reported that bacteria adhered to injured tracheal cells, but not to normal control tracheae. PLOTKOWSKI and co-workers [82] found *P. aeruginosa* to adhere to granules of recently secreted mucus, to desquamated cells, and to the exposed extracellular matrix of respiratory epithelium treated by human leucocyte elastase, but not to undamaged cells. Finally, ZOUTMAN *et al.* [83] observed that bacteria adhered to exfoliating cells, and to exposed basement membranes, of SO_2-treated canine tracheae.

The adhesion of mucoid strains to acid-treated mice tracheae was reported to be mediated by the mucoid exopolysaccharide [37], whilst fimbriae seemed to mediate the adhesion of nonmucoid strains [32].

P. aeruginosa fimbriae are composed of pilin protein subunits, encoded by one chromosomal gene. Fimbriae of *P. aeruginosa* strains PAO and PAK have been purified, and the complete amino acid sequence of the PAK pilin subunits determined [88]. Recently, the disulphide loop at the carboxy terminal end of the pilin molecule has been shown to be the specific binding domain of the pilin subunits [35].

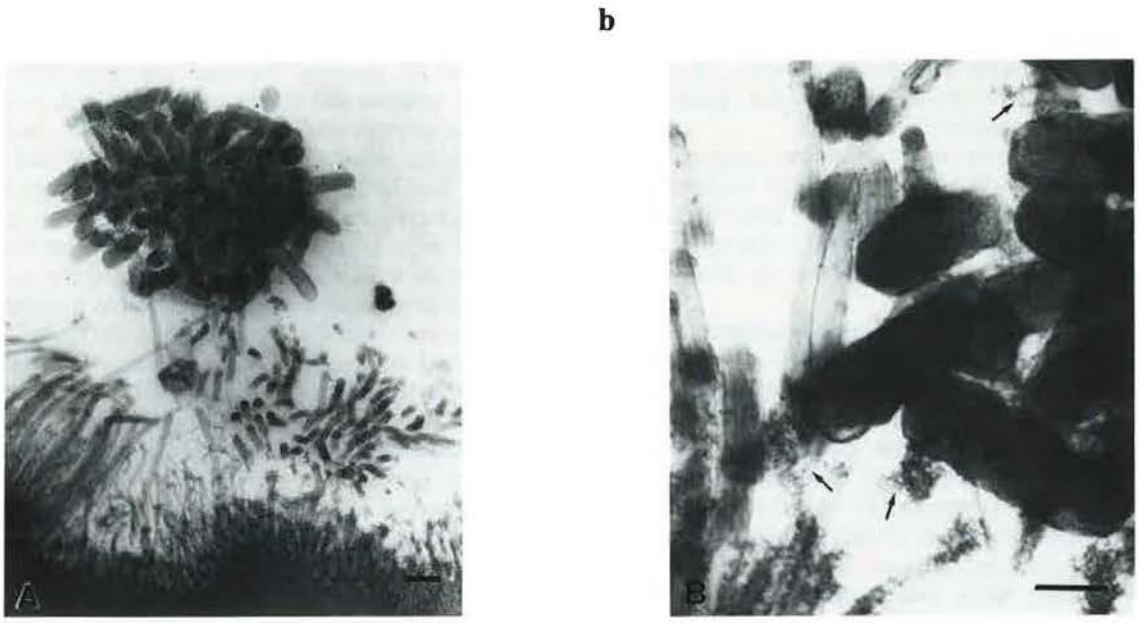

Fig. 3. – a) Aggregates *Pseudomonas aeruginosa* associated with human epithelial respiratory cells in primary culture. b) A higher magnification showing the presence of a matrix-like material surrounding aggregated bacteria (arrows), which appears to establish the interaction between aggregated bacteria and cilia (Bars = 1 and 0.5 μm in a and b, respectively).

ZOUTMAN *et al.* [83], working on *P. aeruginosa* strains engineered to express either the pilin gene of *P. aeruginosa* PAO or PAK, observed that PAO-bearing strains adhered in greater numbers to injured tracheal cells than did PAK-bearing strains, suggesting that the adhesiveness of bacteria may depend on the primary structure of the subunits of their fimbriae.

In contrast with what is known about *P. aeruginosa* adhesins, the receptors on human injured tissue for *P. aeruginosa* adhesion are still unknown. Since *P. aeruginosa* treatment with crude ganglioside preparations significantly decreased their adhesion to acid-treated mice tracheae, RAMPHAL and PYLE [89] supposed the receptor for *P. aeruginosa* adhesion to be a glycolipid.

Glycosphingolipids have been reported to be membrane receptors for different pathogenic microorganisms [90]. The binding of *P. aeruginosa* to gangliotetraosylceramide (asialo GM1) and to other glycolipids containing terminal or internal GalNAc β1–4Gal sequence has also been reported [91, 92]. Recently, LINGWOOD *et al.* [44] described the specific binding of exoenzyme S, one of the many adhesins produced by *P. aeruginosa*, both to asialo GM1 and GM2. Interestingly, asialo GM1 occurs in substantial amounts in human lung tissues [93].

P. aeruginosa has been shown to bind avidly to injured corneal tissues [94, 95]. Scarified mouse corneas, treated with lipase solution, significantly reduced *P. aeruginosa* adhesion [96], as did bacterial treatment with asialo GM1 solution. Although all these data suggest that membrane glycolipids may be the attachment site for *P. aeruginosa* adhesion in injured tissues, it is still unknown whether damaged respiratory cells differ in their surface glycolipids from normal cells.

P. aeruginosa adhesion to respiratory cells from cystic fibrosis patients

Cystic fibrosis (CF) patients are most susceptible to *P. aeruginosa* respiratory infection. Epithelial cells from CF patients differ from those of non-CF patients in permeability to chloride ions and in releasing high molecular weight glycoconjugates, which exhibit greater sulphation than those from the cells of non-CF patients [97]. This high sulphur content of the cell surface glycoconjugates could play a role in the interaction of the bacteria with the airway surface in CF patients [97, 98]. Alternatively, it is known that CF results in defective glycosylation of the cystic fibrosis transmembrane conductance regulator protein (CFTR) [99]. Defective glycosylation of cell surface glycoconjugates could also occur and impart specific adhesive properties for *P. aeruginosa* to epithelial respiratory cells in CF patients. To test this hypothesis, we compared respiratory cells from five CF patients with different genotypes and four non-CF patients in their susceptibility to *P. aeruginosa* adhesion [31]. Quantitation of bacterial adhesion by scanning electron microscopy showed no difference between the cells from CF and non-CF patients. In both cases, bacteria did not adhere to the membrane of ciliary shafts but were detected trapped at the tips of cilia, mainly as large bacterial aggregates, surrounded by a matrix-like material reactive with a monoclonal antibody raised against respiratory mucin [31]. Such surrounding mucin-containing material was much more abundant in cultures from CF patients. This finding may explain the discrepancy between our results and recent data from SAIMAN *et al.* [100], who observed more radiolabelled *P. aeruginosa* bound to respiratory cells from CF patients than to control cells. Interestingly, in

our study, the percentage of ciliated cells from the only patient homozygous for ΔF508 presenting associated bacteria was significantly higher than that of control cells (unpublished observation). However, even here, associated bacteria were always found trapped at the extremities of cilia, surrounded by the matrix-like material, and never observed to interact directly with the epithelial cell surface. Therefore, the chronic colonization of the airways of CF patients cannot be explained by an increased affinity between *P. aeruginosa* and the respiratory cell surface receptors. Data published by BALTIMORE *et al.* [101], on the immunohistopathological localization of *P. aeruginosa* in airways from patients with CF, further support the concept that these micro-organisms do not adhere to intact respiratory mucosa. In their study, neither *P. aeruginosa* interaction with intact epithelium nor bacteria entangled within cilia were ever noted. *P. aeruginosa* were always seen to remain intraluminally, sequestered within a surrounding exudate. In contrast, wherever there was erosion of the epithelium, bacteria were seen attached to the denuded membranes.

Bacterial invasion of epithelial cells

A few recent studies have shown that respiratory pathogens which are not generally considered intracellular parasites, such as *B. pertussis* [102], *H. influenzae* [66, 103, 104] and *P. aeruginosa* [83, 105, Plotkowski MC, unpublished observation], can invade epithelial cells and reside intracellularly (fig. 4). Even though the *in vitro* entry of bacteria into epithelial cells may suggest an *in vivo* mechanism for the evasion of host defences, allowing bacterial persistence in the respiratory tract, whether these findings bear any relationship to pathogenesis remains to be established.

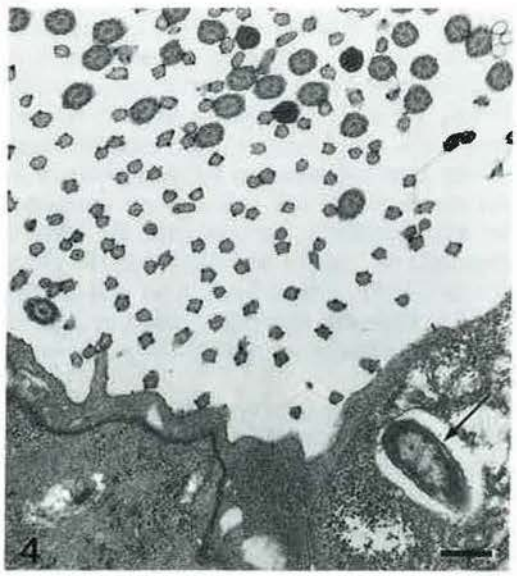

Fig. 4. – Transmission electron micrograph of human epithelial respiratory cells in primary cultures exposed to a nonmucoid *Pseudomonas aeruginosa* strain. Note the presence of intracellular bacteria (arrow). Bar = 0.5 μm).

Bacterial interaction with extracellular matrix

Following epithelial injury and cell desquamation induced by infectious, mechanical or inflammatory factors, the extracellular matrix becomes unmasked. Different respiratory pathogens have been reported to adhere to exposed basement membranes: during our study of the effect of Influenza A virus infection on *S. pneumoniae* adhesion to mouse tracheae, we observed bacteria adhering in high numbers to denuded basement membranes, following virus-induced epithelial cell sloughing [78]. *H. influenzae* have also been shown to adhere to, and even invade, epithelial basement membranes [9, 67]. During our evaluation of the effect of human leucocyte elastase on *P. aeruginosa* adhesion to respiratory mucosa, we also observed bacteria binding avidly to extracellular matrix exposed following enzymatically-induced epithelial cell exfoliation [82]. Several authors have also reported the affinity of *P. aeruginosa* for exposed basement membranes [83, 95]. In order to characterize *P. aeruginosa* affinity for extracellular matrix components further, we evaluated their adhesion to a matrix extracted from the tumour of Engelbreth-Holm-Swarm (EHS), known to contain the major components of basement membranes, *i.e.* laminin, type IV collagen and proteoglycans [106]. All *P. aeruginosa* strains studied, both mucoid and nonmucoid, adhered significantly to EHS-coated glass coverslips (table 2), [107] and the majority of the strains adhered at least as well as a *Staphylococcus aureus* Cowan I strain, a bacterium known to show a high affinity for laminin and fibronectin. *P. aeruginosa* also adheres significantly to a matrix rich in type I collagen [86], a major component of submucosal underlying connective tissues.

Specific receptors for extracellular matrix components have been found on cells that invade internal tracts of the body, such as tumour cells, granulocytes, lymphocytes, as well as on some pathogenic micro-organisms.

Table 2. – Adhesion of *Pseudomonas aeruginosa* to glass coverslips coated with a reconstituted basement membrane matrix extracted from the tumour of Engelbreth-Holm-Swarm (EHS) or to control uncoated coverslips

P. aeruginosa strains	EHS adhesion %	Control adhesion %
M1	16±3	8±1
M3	45±4	17±2
M5	24±2	8±1
M24	42±3	23±2
NM2	49±4	35±1
NM4	17±2	4±1
NM13	35±4	15±1
NM27	34±1	24±1
NM167	24±2	11±1

The adhesion of [35S]-methionine labelled bacteria was expressed as the percentage of radioactivity remaining on the coverslips. Values are mean±SD; $p<0.001$ compared to control. M: mucoid strains; NM: nonmucoid strains.

It is conceivable, therefore, that in chronic respiratory diseases, *P. aeruginosa* may bind to specific receptors of the extracellular matrix which has been exposed following tissue injury. Such affinity may account for the tendency of these opportunistic bacteria to infect injured tissues, as well as for their propensity to disseminate from the primary site of infection. However, injured respiratory epithelium may exhibit specific receptors for *P. aeruginosa* adhesins that are different from the extracellular matrix proteins.

P. aeruginosa adhesion to repairing respiratory epithelium

Epithelial regeneration is a basic response to injury, fundamental for the maintenance of epithelial barrier function. Regeneration of hamster tracheal epithelium *in vivo* [108], as well as human respiratory epithelium *in vitro* [109], has been shown to begin by the spreading and migration of viable cells neighbouring the wound margins, to cover the denuded lesions. Glycoconjugates from cellular plasma membranes are known to be altered on migrating cells [110, 111]. Accordingly, we wondered whether epithelial cells participating in the regeneration of injured respiratory epithelium would possess membrane receptors for *P. aeruginosa*. To test this hypothesis, we used the *in vitro* model of respiratory epithelium repair developed by ZAHM *et al.* [109]. *P. aeruginosa* adhesion to cells migrating to repair the epithelial wounds was significantly higher than the adhesion to nonmigrating ciliated or nonciliated cells [112] (fig. 5).

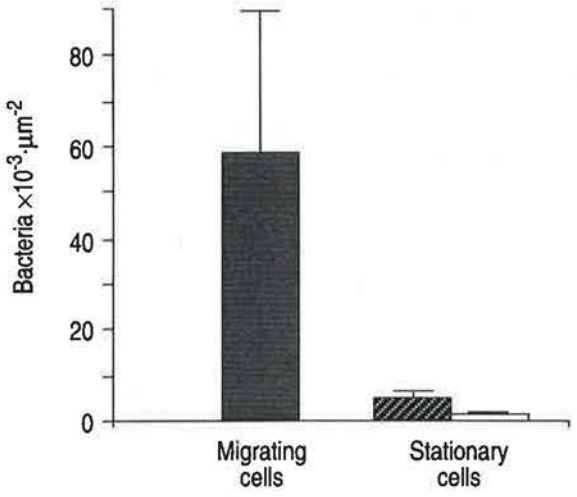

Fig. 5. – Adhesion of *Pseudomonas aeruginosa* to respiratory cells migrating to repair epithelial wounds and to stationary ciliated (CC) and nonciliated (NCC) respiratory cells in primary culture. Data are mean±SD of adherent bacteria observed in eight different cultures. ▩: ciliated cells; □: nonciliated cells.

Molecular basis of *P. aeruginosa* adhesion to migrating epithelial respiratory cells

Cell migration is a complex process requiring cell adhesion to the extracellular matrix and controlled detachment as the cells move. Fibronectin (FN) is a multifunctional glycoprotein present in an insoluble form at the cell surface and on the extracellular matrix, and in a soluble form in plasma and other body fluids [113]. Because of its adhesive properties, FN plays a major role in securing epithelial cells to their underlying substrate [114]. Both the number and the distribution of cell receptors for FN are modulated as cells move [114, 115]. Moreover, migrating cells from the edges of epithelial wounds rapidly synthesize FN and deposit it at the cell basement membrane interface, providing a matrix over which cells can easily displace [116–118].

Fibronectin has been found, in soluble form, in cultures of human epithelial respiratory cells [119], and SUTER *et al.* [120] have shown human bronchial epithelium to be intensely labelled by an anti-FN antibody, revealing the presence of polymerized insoluble FN at the apical surface of bronchial cells. The presence of insoluble polymerized fibronectin at the apical surface of human buccal cells has been shown to correlate inversely with *P. aeruginosa* adhesion [121, 122].

As the expression of FN on epithelial cells is known to be modulated during cell migration, it was hypothesized that the preferential adhesion of *P. aeruginosa* to migrating cells could result from a decrease in the concentration of insoluble fibronectin at the apical surfaces of migrating respiratory cells. However, by immunofluorescent studies, no difference in the content of insoluble fibronectin at the apical surface of migrating and stationary respiratory cells could be found [86]. Therefore, *P. aeruginosa* adhesion to migrating cells could not be correlated with a low content of insoluble fibronectin at the surface of migrating epithelial respiratory cells. In contrast with the poor labelling of epithelial cells with the anti-FN antibody, a FN-containing fibrillar material was seen unevenly distributed over the cell culture. This FN-containing material systematically surrounded aggregated bacteria associated with respiratory cells (fig. 6). This finding led us to hypothesize that FN released by respiratory cells could interact with bacterial receptors, and mediate bacterial adhesion by establishing a bridge between the bacteria and cell surface receptors. To evaluate this hypothesis, we compared the adhesion to migrating cells of control untreated and FN-treated *P. aeruginosa* [123]. The adhesion of FN-treated *P. aeruginosa* to migrating respiratory cells was significantly higher than the adhesion of control microorganisms (fig. 7).

Several different micro-oganisms present specific lig-ands for FN, which facilitate their attachment to both mammalian cells and to FN-coated biopolymers [124–128]. In contrast, polymerized FN was reported to serve as a barrier that blocks the adhesion of *P. aeruginosa*. To support our *P. aeruginosa* soluble FN reactivity hypothesis, two different explanations may be proposed. The first depends on studies from FFRENCH-CONSTANT *et al.* [129], in which the FN secreted by cells at the base of epithelial wounds were shown to present an embryonic pattern.

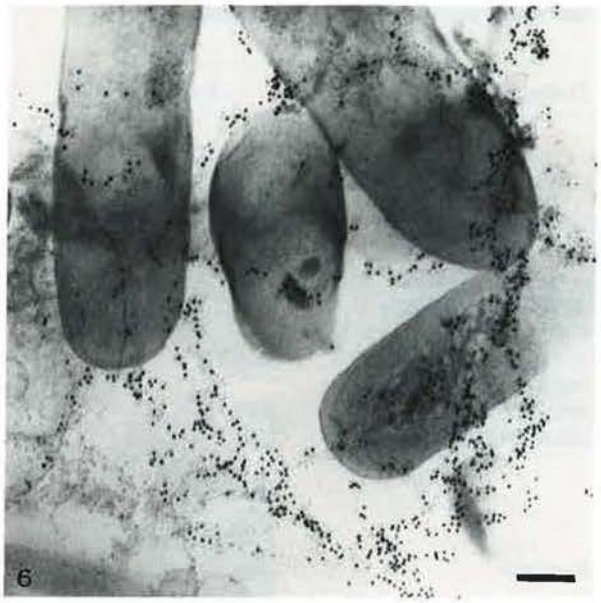

Fig. 6. – Transmission electron micrograph of *Pseudomonas aeruginosa* associated with human respiratory cells in primary culture. Note the presence of a fibrillar material intensely labelled by gold particles, revealing the presence of fibronectin secreted by the respiratory cells in culture. (Bar = 0.25 μm).

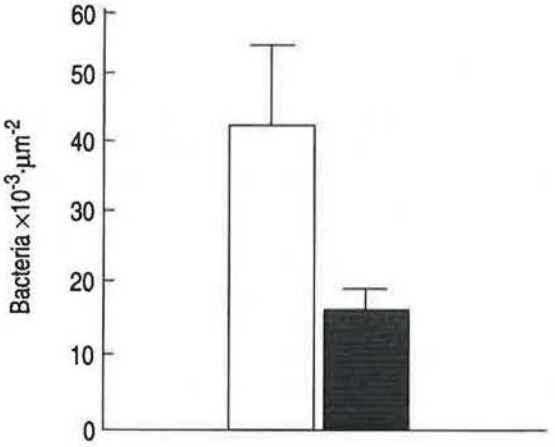

Fig. 7. – Adhesion of fibronectin (FN)-treated *Pseudomonas aeruginosa* to migrating human respiratory cells in primary culture was signficantly higher ($p<0.05$) than the adhesion of control untreated bacteria. Data are mean±SD of adherent bacteria observed in at least 40 scanning electron microscopic fields, in two different assays. □: FN-treated bacteria; ■: control untreated bacteria.

The authors suggested that, during wound-healing, an alternative splicing of FN messenger ribonucleic acid (mRNA) may occur, as a mechanism to generate forms of FN that may be functionally more appropriate for cell migration and proliferation associated with tissue repair. As embryonic FN presents domains that are not present in the normal adult tissue and plasma FN [130], it is conceivable that these two FN forms may react differently with microbial ligands.

The second explanation to support our hypothesis is based on the dramatic physical conformation change that soluble FN undergoes as it polymerizes, and on the fact that these changes may uncover and/or mask potential receptor sites for pathogens [113]. Our results showing the increase of the adhesion to migrating cells following *P. aeruginosa* treatment by soluble FN suggest that bacteria FN interaction occurred to some extent. Very recently we observed the labelling of *P. aeruginosa* cells with a FN-colloidal gold complex [131], confirming the bacterial reactivity with the soluble form of fibronectin. However, the meaning of these findings in the pathogenesis of *P. aeruginosa* infection of injured tissue remains to be established.

Significance of hydrophobicity in the adhesiveness of respiratory pathogens

The belief that the adhesion to mucosal surfaces depends on a specific interaction between microbial adhesins and host cell receptors has to some degree obscured the recognition and the study of other mechanisms promoting bacterial adhesion, such as the electrostatic and hydrophobic interactions. Hydrophobic interaction is believed to favour bacterial adhesion, by overcoming the repulsive forces between the microbial and the host cells [132, 133].

Lipids of different origins represent a high percentage (1–2%) of macromolecules present in the respiratory mucus, the concentrations of which vary during the course of pathological processes [134, 135]. In addition, GIROD *et al.* [136] have recently identified phospholipids in the serous and mucous secretory granules of the respiratory submucosal glands, as well as attached to the glycocalyx of the surface epithelial cells (Girod *et al.* personal communication). These data suggest that, together with the mucins of the mucus, lipids are capable of forming a hydrophobic layer on the surface of the respiratory cells, thereby giving specific surface properties to the tracheobronchial mucosa. Similarly, the presence of osmophilic lamellar structures observed at the interface between the gel and sol mucous layers [5] suggests that the respiratory mucosa may, like the gastric mucosa, be coated by a layer of phospholipids, which simultaneously isolate it from aggressive agents and renders it hydrophobic [137]. In spite of these data, very few studies have been carried out on the role of hydrophobic interaction in bacterial adhesion to the airways mucosa. ROBINSON *et al.* [138] reported high cell surface hydrophobicity among the virulent-phase cells of *B. pertussis* and a marked reduction when cultures were chemically induced to revert to the avirulent phase. FISH *et al.* [139] suggested that the filamentous haemagglutinin, one of the bacterial adhesins to human ciliated cells, is probably the component responsible for the surface hydrophobicity of this pathogen.

Bacterial fimbriae are hydrophobic structures [140]. Fimbriae of *P. aeruginosa* have been reported to mediate bacterial adhesion to mucin [46], and to human [34, 35] and canine [83] ciliated respiratory cells. However, different studies have indicated that hydrophobic interaction does not seem to play a major role in the adhesion of *P. aeruginosa* to epithelial respiratory cells [141–143].

Table 3. – Main sites of adhesion of respiratory pathogens in normal and injured respiratory mucosa

Site of adhesion	Bacteria	Potential receptors	[Ref.]
Mucus	*S. pneumoniae*	?	[7]
	H. influenzae	?	[8, 9]
	S. aureus	?	[10]
	P. aeruginosa	Type I and type II disaccharide units	[22]
		Hydrophobic bounds	[24]
	P. cepacia	Gal NAc and Glc NAc residues	[48]
Normal ciliated respiratory cells	*M. pneumoniae*	Sialo oligosaccharides of I and i antigen type	[56, 57]
	B. pertussis	Lactosylceramide	[60]
		Lactosamine residues	[64]
Desquamated or injured ciliated cells	*H. influenzae*	?	[9, 67]
	S. pneumoniae	?	[77]
	P. aeruginosa	?	[33–35, 81–83]
		Glycolipid	[89]
Extra cellular matrix	*S. pneumoniae*	?	[78]
	H. influenzae	?	[9, 67]
	P. aeruginosa	?	[83, 95, 107]
Repairing respiratory epithelium	*P. aeruginosa*	?	[109, 112]
	S. pneumoniae	?	[78]

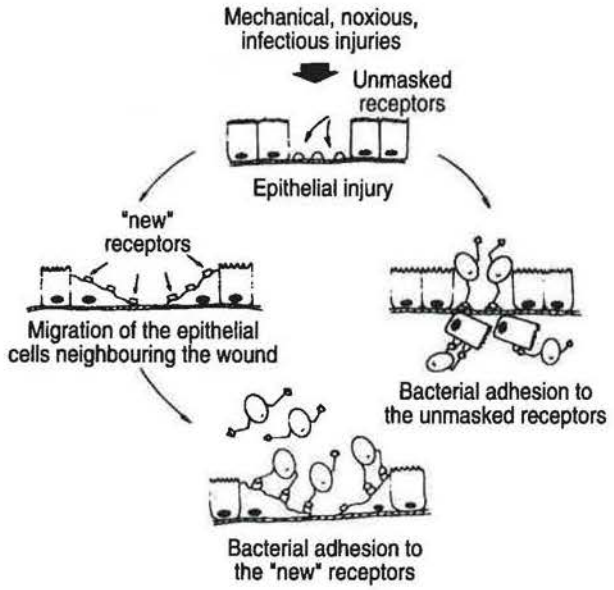

Fig. 8. – Schematic representation of the main receptor sites for the adhesion of micro-organisms in injured respiratory mucosa. Following epithelial injury, receptors for microbial adhesins from desquamated cells and from the underlying extracellular matrix are unmasked. In addition, epithelial respiratory cells migrating to repair the wounds may also expose "new" receptors to which bacteria avidly attach. Such attachment may explain the chronic bacterial colonization of the respiratory tract observed in some patients.

To conclude, we would like to summarize the main receptor sites for the adhesion of micro-organisms in the respiratory tract (table 3). Bacterial adhesion to mucins usually serves a defence function, since attached micro-organisms are removed by ciliary transport. However, if mucociliary clearance is defective, mucin-bound bacteria would persist in stagnant mucus and produce chronic colonization. Membrane glycoconjugates from normal or injured epithelial respiratory cells often play the role of receptors for microbial adhesins. Following epithelial injury and cell desquamation, extracellular matrix receptors are unmasked, allowing bacterial persistence and/or invasion (fig. 8). Finally, during epithelial repair, migrating cells also expose new receptors for the bacterial adhesins, which are not exposed in normal epithelium. The affinity for all these receptors may contribute to the chronicity of many bacterial respiratory infections.

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