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Asialo GM1 Is a Receptor for *Pseudomonas aeruginosa* Adherence to Regenerating Respiratory Epithelial Cells

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We investigated the implication of asialo GM1 as an epithelial receptor in the increased *Pseudomonas aeruginosa* affinity for regenerating respiratory epithelial cells from cystic fibrosis (CF) and non-CF patients. Human respiratory epithelial cells were obtained from nasal polyps of non-CF subjects and of CF patients homozygous for the ΔF 508 transmembrane conductance regulator protein (CFTR) mutation and cultured according to the explant-outgrowth model. At the periphery of the outgrowth, regenerating respiratory epithelial cells spreading over the collagen I matrix with lamellipodia were observed, characteristic of respiratory epithelial wound repair after injury. *P. aeruginosa* adherence to regenerating respiratory epithelial cells was found to be significantly greater in the ΔF 508 homozygous CF group than in the non-CF group ($P < 0.001$). In vitro competitive binding inhibition assays performed with rabbit polyclonal antibody against asialo GM1 demonstrated that blocking asialo GM1 reduces *P. aeruginosa* adherence to regenerating respiratory epithelial cells in ΔF 508 homozygous CF cultures ($P < 0.001$) as well as in non-CF cultures ($P < 0.001$). Blocking of asialo GM1 was significantly more efficient in CF patients than in non-CF subjects ($P < 0.05$). Distribution of asialo GM1 as determined by preembedding labelling and immunoelectron microscopy clearly demonstrated the specific apical membrane expression of asialo GM1 by regenerating respiratory epithelial cells, whereas other cell phenotypes did not apically express asialo GM1. These results demonstrate that (i) asialo GM1 is an apical membrane receptor for *P. aeruginosa* expressed at the surface of CF and non-CF regenerating respiratory epithelial cells and (ii) asialo GM1 is specifically recovered in regenerating respiratory epithelium. These results suggest that in CF, epithelial repair represents the major event which exposes asialo GM1 for *P. aeruginosa* adherence.

Pseudomonas aeruginosa is closely associated with a dramatic clinical evolution in cystic fibrosis (CF) patients. This opportunistic pathogen accounts for recurrent or chronic pulmonary infections of CF patients resulting in respiratory insufficiency. Although the irreversible transformation of nonmucoid colonizing strains to the mucoid phenotype undoubtedly contributes to the bacterial persistence, the exact mechanisms involved in the chronic colonization of CF airways by *P. aeruginosa* are not yet clear. *P. aeruginosa* has been shown to bind specifically to disaccharide subunits containing the Galβ1-3GlcNAc and Galβ1-4GlcNAc sequences (19) frequently identified in tracheobronchial mucins (13). In CF, mucus dehydration is associated with cystic fibrosis transmembrane conductance regulator protein (CFTR) mutations. Hypersecretion of respiratory mucus, tracheobronchial clearance deficiency, and mucus dehydration all present optimal conditions for bacterial proliferation and penetration through the mucus barrier, favoring close contact between bacteria and the underlying respiratory epithelium. Several in vitro and ex vivo studies have found poor adherence of *P. aeruginosa* to functional and intact respiratory epithelia (15, 18). In contrast, this bacterium has been shown to avidly bind to injured epithelia (24, 26, 29). The CF tracheobronchial mucosa is continuously exposed to multilesional events, leading to respiratory epithelial shedding. Epithelial regeneration, a basic response to injury and essential for the restoration of the epithelial barrier integrity, begins with the spreading and migration of the viable respiratory epithelial cells edging the wound (27). We have previously demonstrated the unique affinity of *P. aeruginosa* for regenerating respiratory epithelial cells with a spreading shape similar to that observed for cells edging a wound (8, 17).

Glycolipids and, in particular, gangliotetraosylceramide containing the GalNAcβ1-4Gal sequence (asialo GM1) have been described as receptors for *P. aeruginosa* (12). The bacterial pilus and, more precisely, the C-terminal region of the pilin protein are involved in the attachment to the glycolipid (14, 23). It has been recently reported by Saiman and Prince (22) that asialo GM1 was more often identified in CF respiratory epithelial cells than in non-CF respiratory epithelial cells, and Imundo et al. (11) have shown the implication of asialo GM1 as a receptor for *P. aeruginosa* adherence to CF respiratory epithelial cell lines. Moreover, *P. aeruginosa* exoproducts are able to increase the number of asialo GM1 receptors available on CF respiratory epithelial cells but not on non-CF cells (22). The purpose of this study was to determine whether the extreme affinity of a piliated *P. aeruginosa* strain for CF or non-CF regenerating respiratory epithelial cells in primary culture could be related to the presence of asialo GM1 at the apical surface of these regenerating cells.

MATERIALS AND METHODS

Patient population. Twenty-one subjects were recruited for this study. Eleven were non-CF subjects, and 10 were CF patients homozygous for the ΔF 508 CFTR mutation. Respiratory tissue was obtained from nasal polyps after polypectomy due to nasal obstruction.

For the CF population, FEV_1/FVC ratios (ratios of the forced expiratory

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volume in 1 s to the forced vital capacity; determined within 3 months), Shwachman scores, *P. aeruginosa* colonization of the airways, and antibiotic therapy were specified for each patient. All these data are presented below (see Table 1). For the non-CF population, the allergic status was noted. No respiratory function analysis had been performed, since these subjects had no symptom of airway disease. Allergic status was determined according to criteria including signs of blood eosinophilia, abnormal range of serum immunoglobulin E (IgE), positive radioallergosorbent tests, and positive skin-prick tests.

Bacteria. A nonmucoid piliated strain of *P. aeruginosa*, PO 10, isolated from the respiratory tract of a non-CF patient, was grown in Trypticase soy broth medium for 18 h at 28°C in order to favor fimbria production (6). For each experiment, a bacterial culture was obtained from one isolated colony. Just before the experiment, the bacterial suspension was centrifuged at 3,000 rpm for 15 min, and the pellet was resuspended in RPMI 1640 medium (Seromed Polylabo, Strasbourg, France) containing 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid). The bacterial suspension was adjusted to a final concentration of 5×10^8 CFU/ml. Bacterial self-aggregation was limited by extruding the ex tempo bacterial suspension through a narrow needle just before contact with the cultured respiratory epithelial cells.

Respiratory epithelial cell culture. Respiratory epithelial cells were cultured according to a technique developed by Chevillard et al. (2). The nasal polyps were cut into small explants (2 mm^2) and seeded onto a collagen I matrix in a defined RPMI 1640 medium supplemented with hormones and growth factors. RPMI 1640 medium, which is a serum-free medium (Gibco, Grand Island, N.Y.), is supplemented with insulin (1 μg/ml) (Sigma Chemical Co., St. Louis, Mo.), transferrin (1 μg/ml) (Serva, Heidelberg, Germany), epidermal growth factor (10 ng/ml) (Serva), hydrocortisone (0.5 μg/ml) (Sigma), and retinoic acid (10 ng/ml) (Sigma). At day 3 of culture, the outgrowth surface was sufficient for statistical quantitation. Moreover, after this period of culture, well-differentiated as well as nondifferentiated cells (in the center and at the periphery of the outgrowth, respectively) were identified.

Bacterial adherence. *P. aeruginosa* adherence to regenerating respiratory epithelial cells was analyzed with primary cultures for 10 ΔF 508 homozygous CF patients (patients 1 to 10) and 11 non-CF subjects (subjects 11 to 21). On day 3 of the culture, *P. aeruginosa* was incubated with the respiratory epithelial cells for 1 h at 37°C.

Preparation for scanning electron microscopy (SEM) observation. After the nonadherent bacteria were removed by repetitive rinsing with 0.1 M phosphate buffer, the cultures were fixed in 0.1 M phosphate buffer (pH 7.2) containing 2.5% glutaraldehyde for 1 h at 4°C. The cultures were then dehydrated through graded concentrations of ethanol, critical-point dried with CO_2, stuck on stubs, and coated with 15-nm-diameter gold-palladium beads.

Quantitation of bacterial adherence. Bacterial adherence to regenerating respiratory cells with a spreading shape was quantified by computer-assisted SEM observation. We used a Philips XL 30 SEM with a Biocom 500 image analyzer. At the extreme periphery of the outgrowth, 35 nonconsecutive fields of 1,530 μm^2 each at a constant magnification of ×2,700 were analyzed for each cell culture. The aggregates of bacteria were counted with the help of software that we developed (9). It was based on the results of a two-dimensional stereological study performed with more than 30 different transmission electron microscopy sections of *P. aeruginosa* aggregates, which allowed us to determine the filling rate of these aggregates (9). This filling rate of 50% means that 50% of the total volume of the bacterial aggregate is effectively occupied by bacteria. Isolated bacteria and aggregates were numbered and reported as the total number of adherent bacteria per square micrometer of regenerating respiratory epithelial cell surface (3).

Competitive binding inhibition assay. The implication of the asialo GM1 receptors in *P. aeruginosa* adherence to regenerating respiratory epithelial cells was studied by preincubation of cell cultures for four ΔF 508 homozygous CF patients (patients 7 to 10) and five non-CF subjects (subjects 17 to 21) with a rabbit polyclonal antibody against asialo GM1 (Wako Chemicals, Neuss, Germany) at a concentration of specific IgG of 10 μg/ml in RPMI 1640 medium before addition of bacteria. Incubation with the anti-asialo GM1 antibody was performed for 1 h at 37°C. Then the culture medium was replaced by the *P. aeruginosa* suspension at 5×10^8 CFU/ml for 1 h at 37°C. The nonadherent bacteria were removed by repetitive rinsing with 0.1 M phosphate-buffered saline (pH 7.2) and prepared for SEM observation. Controls were performed by preincubating cell cultures in RPMI 1640 medium with nonimmune rabbit IgG at 10, 50, and 100 μg/ml (Sigma) before addition of the bacteria.

Immunoelectron microscopic detection of asialo GM1 receptors. Immunoelectron microscopic detection of asialo GM1 receptors was performed with non-CF respiratory epithelial cells in culture from subjects 18 to 21. The cell cultures were incubated with freshly prepared fixative containing 0.01 M metaperiodate sodium, 0.075 M L-lysine, and 2% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 1 h at room temperature. The cell cultures were then rinsed in 0.1 M phosphate buffer (pH 7.2) for 10 min and in 0.1 M phosphate buffer (pH 7.2) containing 1% bovine serum albumin (BSA) (Sigma). The cell cultures were incubated for 1 h with rabbit anti-asialo GM1 antibody at a concentration of 1 μg of specific IgG per ml in 0.1 M phosphate buffer (pH 7.2) containing 1% BSA. Controls were performed by either incubating cell cultures with nonimmune rabbit IgG at 10 μg/ml or omitting the incubation with the primary antibody. The cell cultures were then rinsed twice with 0.1 M phosphate buffer and with 0.1 M phosphate buffer containing 1% BSA for 10 min and thereafter with biotinylated anti-rabbit secondary antibody (Amersham, Les Ulis, France) (1:50) at room temperature for 1 h. After three washes, the cell cultures were exposed to streptavidin–10-nm-diameter gold particle complex (Tebu, Le Perray en Yvelines, France) at a 1:10 dilution for 1 h at room temperature and rewashed in phosphate buffer and distilled water. The cell cultures were then dehydrated through graded concentrations of ethanol and embedded in agar resin 100 (Agar Scientific, Orsay, France). Ultrathin sections were stained with uranyl acetate and lead citrate and then observed in a Hitachi 300 transmission electron microscope operating at 75 kV.

Statistical analysis. Data were expressed as median values and ranges.

A nonparametric Mann-Whitney test was used to compare *P. aeruginosa* adherence to regenerating respiratory epithelial cells from ΔF 508 homozygous CF patients and non-CF subjects.

Each treatment of epithelial cells (controls and incubation with anti-asialo GM1 antibody or with nonimmune rabbit IgG at 10, 50, and 100 μg/ml) was performed on six different explants for each patient. The percent reduction in *P. aeruginosa* adherence in the presence of anti-asialo GM1 antibody was calculated by subtracting the adherence of bacteria in the control cultures and in the cultures treated with the anti-asialo GM1 antibody and dividing by the adherence of bacteria in the control cultures for each patient. We compared the effects of asialo GM1 treatment on non-CF and CF cultures with a nonparametric Wilcoxon test. A nonparametric Mann-Whitney test was used to compare the percent reduction in *P. aeruginosa* adherence to regenerating respiratory epithelial cells from ΔF 508 homozygous CF patients and non-CF subjects.

TABLE 1. Clinical description of the ΔF 508 homozygous CF population

Patient[a]	Age (yr)	Sex[b]	FEV_1/FVC (%)	Shwachman score[c]	*P. aeruginosa* colonization	Antibiotic therapy
1	12	M	81	85	+	−
2	11	M	65	72	+	+
3	7	M	63	?	+ (transplanted)	+
4	9	M	76	95	−	−
5	20	F	75	90	+ (transplanted)	−
6	19	M	71	80	+	+
7	12	F	81.4	88	−	−
8	11	F	75	90	+ (transplanted)	−
9	22	F	40	75	+	+
10	30	M	43	50	+	+

[a] Patients 1 to 10 were studied for bacterial adherence. Patients 7 to 10 were further studied for adherence after asialo GM1 was blocked.
[b] M, male; F, female.
[c] Maximal score, 100.

RESULTS

Patients. All the data concerning the ΔF 508 homozygous CF recruited population are presented in Table 1.

The ΔF 508 homozygous CF population of 10 patients consisted of six males and four females from 7 to 30 years old. Their pulmonary function, expressed as the ratio of FEV_1/FVC in terms of a percentage of the predicted value, ranged from 40 to 81.3%. Their Shwachman scores ranged from 50 to 95 (of 100). Of the 10 patients, 8 had already been colonized by *P. aeruginosa*. Two had never been colonized by *P. aeruginosa*. Three had received lung transplants at the moment of the polypectomy. They were colonized by *P. aeruginosa* before the transplants. After the transplants, *P. aeruginosa* was still recovered from their airways. Five of the 10 patients were in an infectious exacerbation period at the moment of the polypectomy and received antibiotic therapy.

For the non-CF population, 2 of 11 subjects were allergic.

Regenerating respiratory epithelial cells. As shown in Fig. 1, cells present at the periphery of the outgrowth exhibit a particular phenotype. These cells are flattened and do not exhibit microvilli on their apical surface but have thin cytoplasmic expansions running over the collagen matrix, such as lamelli-

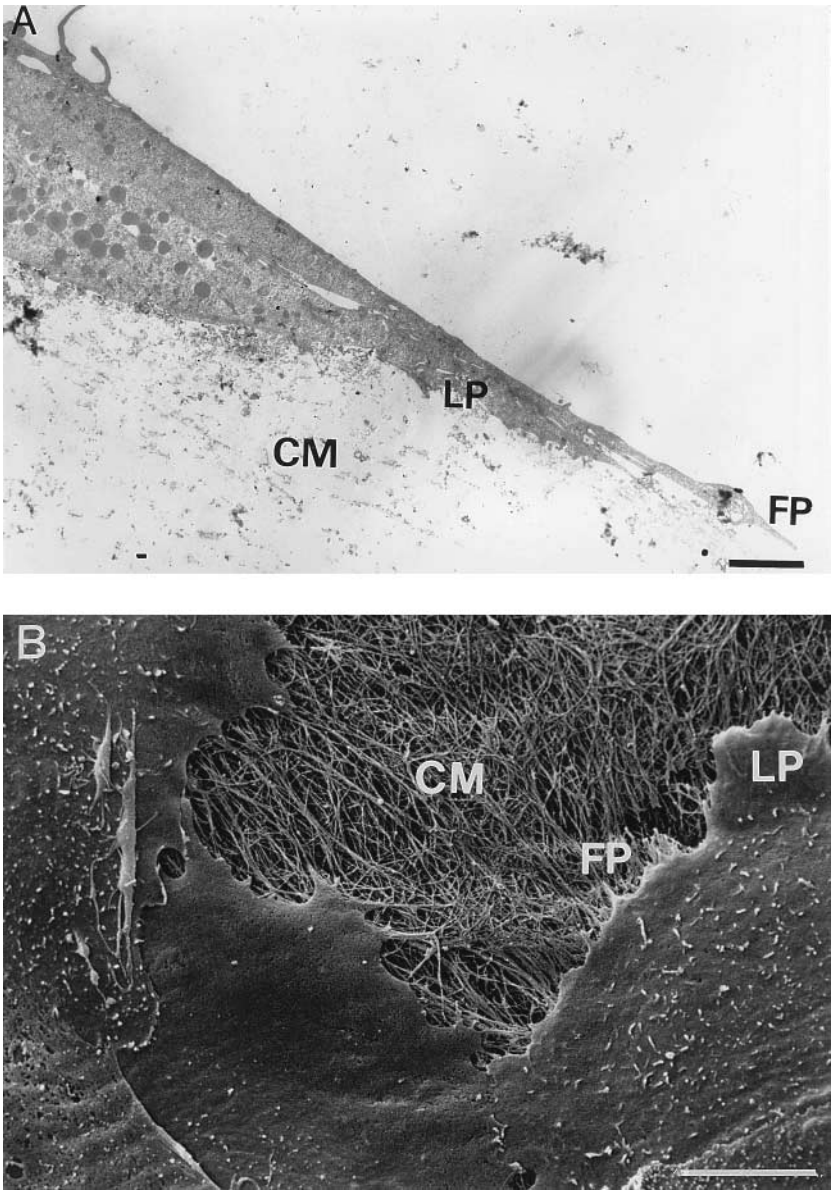

FIG. 1. Regenerating respiratory epithelial cells. (A) Transmission electron microscopic observation of regenerating respiratory epithelial cells showed the flattened shape of these cells spreading over the collagen matrix (CM). Cytoplasmic expansions on the free edge of the cell are lamellipodia (LP) and filopodia (FP). Bar = 5 μm. (B) SEM observation demonstrated the flattened aspect of these cells without any or with very small microvilli, with lamellipodia and filopodia, associated with the collagen matrix fibrils. Bar = 5 μm.

podia and filopodia. Figure 1A represents the cytoplasmic expansion of the regenerating respiratory epithelial cells edging the collagen matrix observed by transmission electron microscopy. By SEM observation (Fig. 1B), the surface of the regenerating respiratory epithelial cells is flat and lamellipodia and filopodia are closely linked to the collagen matrix fibrils. The total exposed surface of the regenerating respiratory epithelial cells is greater than the surface of the other respiratory epithelial cells present in the outgrowth. These regenerating respiratory epithelial cells are able to coexpress various differentiation markers of epithelial cells, such as cytokeratin 14 (a marker of basal cells), cytokeratin 18 (a marker of ciliated and secretory cells) (4), and vimentin (a marker of mesenchymal cells) (data not shown), whereas the other respiratory epithelial cells in the outgrowth express either cytokeratin 14 or cytokeratin 18 and rarely vimentin.

Bacterial adherence. The study of bacterial adherence focused on regenerating respiratory epithelial cells localized at the periphery of the outgrowth culture and characterized by a spreading shape. Very few bacteria adhered to the other respiratory epithelial cells of the outgrowth and of the explant. The adherence of *P. aeruginosa* was significantly greater in the ΔF 508 homozygous CF group (median, 4.4×10^{-2} bacteria per μm^2; range, 2.9×10^{-2} to 7.9×10^{-2} bacteria per μm^2) than in the non-CF group (median, 3.0×10^{-2} bacteria per μm^2; range, 0.9×10^{-2} to 4.9×10^{-2} bacteria per μm^2) ($P < 0.001$) (Fig. 2A). The coefficients of variation were 33.2 and 34.5% for the CF and non-CF groups, respectively. Cell cultures for two subjects exhibiting opposite results for in vitro *P. aeruginosa* adherence on regenerating respiratory epithelial cells (Fig. 2A) are shown as examples in Fig. 2B and C. In the cultures for CF patient 1 (Fig. 2B), a great number of bacteria

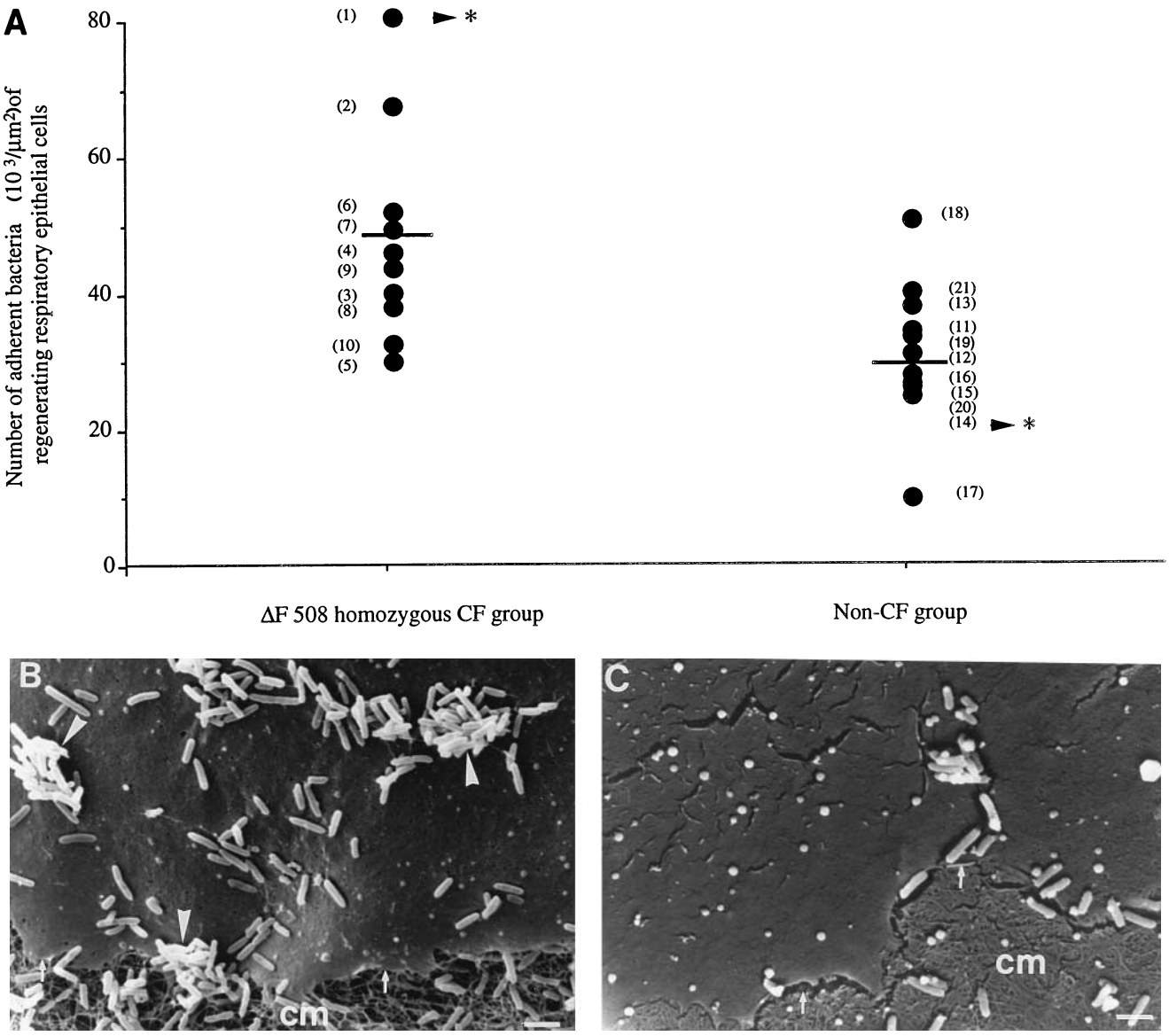

FIG. 2. *P. aeruginosa* adherence to CF and non-CF regenerating respiratory epithelial cells in 10 ΔF 508 homozygous CF patients and in 11 non-CF subjects (numbers in parentheses) was studied. Adherence was found to be significantly ($P < 0.001$) higher in the ΔF 508 homozygous CF group than in the non-CF group (A). The SEM views of *P. aeruginosa* adherence (B and C) refer to data reported in panel A (asterisks). In panel B, note the numerous aggregated bacteria (arrowheads) or isolated bacteria adhered to the regenerating respiratory epithelial cells from the selected ΔF 508 homozygous CF patient. At the free edge of the cells spreading over the collagen matrix (cm) (arrow), the bacteria were also found attached to the exposed collagen matrix fibrils. In panel C, bacteria, either isolated or aggregated, adhering to the regenerating respiratory epithelial cells from the selected non-CF patient were present in lesser quantities. Bars = 2.5 μm.

were adhered to regenerating respiratory epithelial cells as isolated or aggregated bacteria. In the non-CF selected cultures for patient 14 (Fig. 2C), bacteria were also adhered to regenerating respiratory epithelial cells but in lesser quantities. The exposed collagen matrix also trapped *P. aeruginosa* along its fibrils.

Binding inhibition assays. Pretreatment of the regenerating respiratory epithelial cells with rabbit anti-asialo GM1 antibody significantly reduced *P. aeruginosa* adherence (Fig. 3) in the ΔF 508 homozygous CF group (median, 68%; range, 65 to 83%; $P < 0.001$) (patients 7 to 10) as well as in the non-CF group (median, 62% reduction in *P. aeruginosa* adherence; range, 54 to 67%; $P < 0.001$) (patients 17 to 21). The decrease in *P. aeruginosa* adherence following treatment with antibody against asialo GM1, expressed as the percent reduction in *P. aeruginosa* adherence, was significantly greater in the ΔF 508 homozygous CF group than in the non-CF group ($P < 0.05$).

Pretreatment of the regenerating respiratory epithelial cells with nonimmune rabbit IgG at different concentrations ranging from 10 to 100 μg/ml did not modify *P. aeruginosa* adherence (data not shown).

Immunoelectron microscopy. A total of 10 to 15 ultrathin sections derived from four non-CF subjects (subjects 18 to 21) were observed. Preembedding immunolocalization of asialo GM1 revealed that the cell surface of regenerating respiratory epithelial cells at the periphery of the outgrowth was labelled (Fig. 4A). The asialo GM1 labelling on the surface of the regenerating cells was heterogeneous: the lamellipodium re-

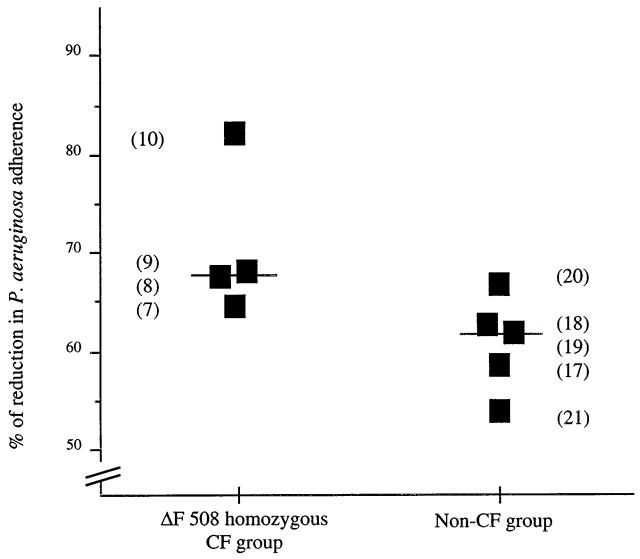

FIG. 3. Effect of blocking asialo GM1 receptors on *P. aeruginosa* adherence. The percent reduction in *P. aeruginosa* adherence to regenerating respiratory epithelial cells from ΔF 508 homozygous CF patients ($n = 4$; patients 7 to 10) and from non-CF patients ($n = 5$; patients 17 to 21) after treatment with rabbit polyclonal anti-asialo GM1 antibody was significantly higher ($P < 0.05$) for the ΔF 508 homozygous CF group than the non-CF group.

gion was negative for asialo GM1, whereas the posterior region was positive. Poor immunoreactivity was observed for cells localized near the explant, which could be identified as ciliated or nonciliated cells (Fig. 4B). Apical expression of asialo GM1 in regenerating respiratory epithelial cells and, in contrast, no expression of asialo GM1 at the apical surface of the other cells, ciliated or nonciliated, were representative of all the observed cultures. Controls with the omission of the primary antibody or preincubation with nonimmune IgG did not show any immunogold reactivity (data not shown).

DISCUSSION

In this study, we have confirmed the preferential affinity of *P. aeruginosa* for CF and non-CF regenerating respiratory epithelial cells, particularly during the spreading process. We have also demonstrated the implication of asialo GM1 as a receptor for *P. aeruginosa* adherence to regenerating respiratory epithelial cells and the greater implication of asialo GM1 as a receptor for *P. aeruginosa* in CF regenerating respiratory epithelial cells than in non-CF cells. We have also shown the specific apical membrane distribution of asialo GM1 in the regenerating respiratory epithelial cells. The specific tropism of *P. aeruginosa* for the CF respiratory tract remains a key question in the clinical course of CF. *P. aeruginosa* exhibits poor affinity for intact respiratory epithelium, whereas marked adherence to injured respiratory epithelium and to the basement membrane has often been reported (16–18, 20, 24, 26). The major bacterial adhesins implicated in epithelial adherence are pili (23). We and others have reported elsewhere (8, 17, 18) the unique tropism of *P. aeruginosa* for regenerating respiratory epithelial cells characterized by a spreading shape, which was previously identified during respiratory epithelial wound repair after injury (27). Following denudation of the extracellular matrix, the respiratory epithelium is rapidly able to regenerate. The repair process represents a crucial moment at which the regenerating cells and extracellular matrix (17) are susceptible to *P. aeruginosa* adherence. During spreading, which is the first event of epithelial regeneration, CF and non-CF respiratory epithelial cells bind a great number of *P. aeruginosa* organisms, with significantly greater bacterial adherence in CF cells than in non-CF cells. These results are in agreement with our previous data (8) and with the results of Zar et al. (28), who suggested that the CFTR gene mutation leading to CFTR

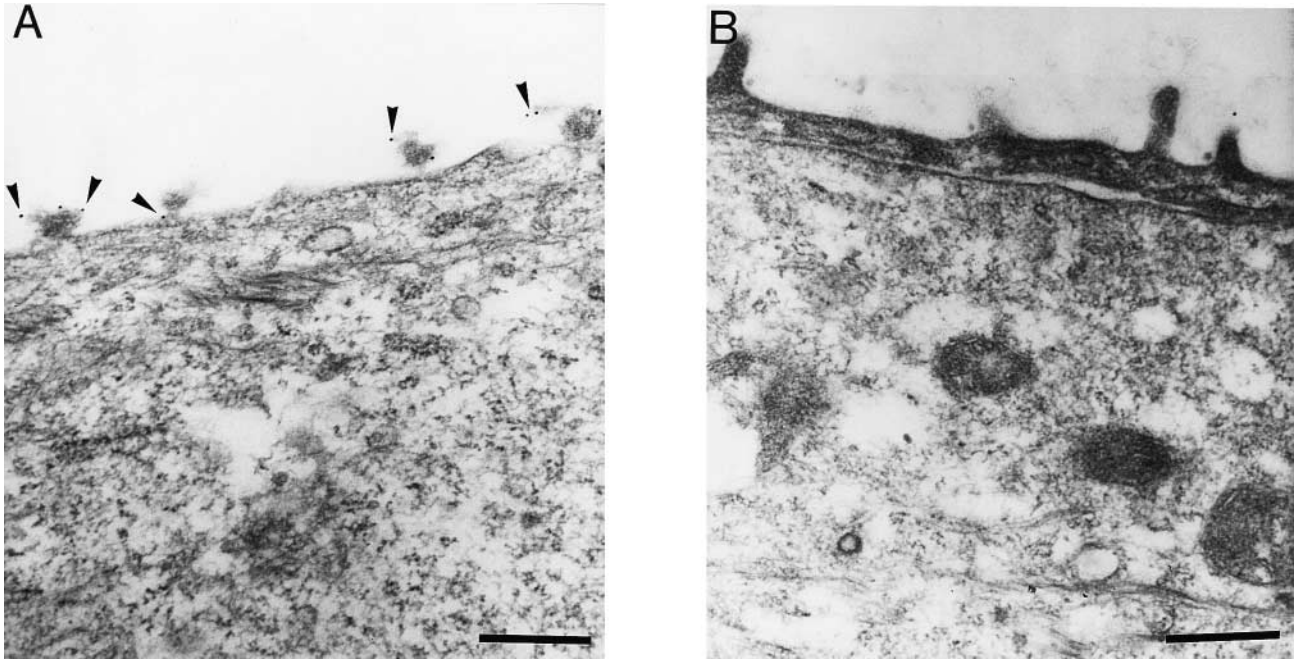

FIG. 4. Immunoelectron microscopic localization of asialo GM1 on respiratory epithelial cells in primary culture. Regenerating respiratory epithelial cells (A) exhibit an anti-asialo GM1 reactivity (arrowheads) over their surface. Well-differentiated respiratory epithelial cells with well-developed microvilli (B) or with cilia (data not shown) were not labelled for asialo GM1. Bars = 0.5 μm.

dysfunction may be responsible for increased amounts of receptors for *P. aeruginosa* adherence. Saiman and Prince (22) have previously demonstrated increased surface expression of asialo GM1 on ΔF 508 heterozygous CF respiratory epithelial cells compared with that on non-CF cells. Imundo et al. (11) have recently identified asialo GM1 as a receptor for *P. aeruginosa* adherence on CF bronchial cell lines. However, until now, no direct experiment had demonstrated the involvement of asialo GM1 in *P. aeruginosa* adherence to ΔF 508 homozygous CF respiratory cells in primary culture.

Binding competitive inhibition assays clearly demonstrate that asialo GM1 is an epithelial receptor involved in *P. aeruginosa* adherence to regenerating respiratory epithelial cells from ΔF 508 homozygous CF patients as well as from non-CF subjects. Interestingly, asialo GM1 was significantly more implicated in *P. aeruginosa* adherence to ΔF 508 homozygous CF regenerating respiratory epithelial cells than to non-CF cells, suggesting that asialo GM1 receptors could be available at the surface in a greater proportion in CF cells than in non-CF cells, as previously reported by Saiman et al. (21, 22). When asialo GM1 was blocked, the lowest percent reduction in *P. aeruginosa* adherence in the CF group occurred for a patient who had never been infected by *P. aeruginosa*. The increased number of asialo GM1 receptors in CF patients who had already been infected by *P. aeruginosa* might be related to the fact that their cells had been previously exposed in vivo to *P. aeruginosa* exoproducts, particularly neuraminidase (22). After dissociation and in vitro culture, these CF cells may still possess a greater number of available asialo GM1 receptors on their apical surface than the non-CF cells. This suggests that under our culture conditions, *P. aeruginosa* exoproducts do not modify the epithelium, a process which may be more relevant in vivo. To our knowledge, it is the first time that *P. aeruginosa* adherence to respiratory epithelial cells derived from ΔF 508 homozygous CF patients who had never been colonized by *P. aeruginosa* has been studied. Increased quantities of asialylated glycolipids in CF cells compared with those in non-CF cells could be related to abnormal sialyltransferase activity due to abnormal intracellular pH (1), as well as to abnormal α2,6 sialyltransferase distribution (25). It has been reported that CF rescued cells with wild-type CFTR have less available asialo GM1 on their surface and that the CFTR defect is related to undersialylation of glycolipids (11).

The specific apical membrane distribution of asialo GM1 molecules in regenerating respiratory epithelial cells, in contrast to the lack of apical expression of asialo GM1 in other ciliated or nonciliated respiratory epithelial cells, confirmed our previous report describing the specific PNA (lectin from peanut [*Arachis hypogaea*]) reactivity of regenerating respiratory epithelial cells (17). It has been reported that asialo GM1 expression could vary according to the cellular phenotype (5). It is of interest that this apical distribution of asialo GM1 was associated with changes in differentiation markers of respiratory epithelial cells during repair and that much more asialo GM1 was expressed in cells with a double phenotype of mesenchymal and epithelial cells characterized by vimentin and cytokeratin coexpression. Spreading and migrating behavior of regenerating respiratory epithelial cells could explain an increased ability of these cells to synthesize glycolipids (7) or an increased mobilization and recycling of these molecules in membranes (10). A different glycosylation pattern of carbohydrates seems to be strongly correlated to the phenotypical changes of respiratory epithelial cells during repair. The relationship between alterations of the cytoskeleton and glycosylation of glycolipids remains unknown. There is strong evidence that regenerating respiratory epithelial cells exhibit an asialylated profile of glycolipids and that this specific carbohydrate pattern could partly explain the unique affinity of *P. aeruginosa* for regenerating respiratory epithelial cells.

In conclusion, it is a novel finding that asialo GM1 is a receptor for *P. aeruginosa* adherence, which is increased during respiratory epithelial regeneration. The observed increased affinity of *P. aeruginosa* for regenerating respiratory epithelial cells with a spreading phenotype could therefore be related to a specific apical membrane distribution of asialo GM1. Epithelial remodelling and repair are the major events which expose available asialo GM1 for *P. aeruginosa*, in particular in CF cells.

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