Surfactant Protein A Blocks Recognition of *Pseudomonas aeruginosa* by CKAP4/P63 on Airway Epithelial Cells

Mariette Barbier, Inmaculada Martínez-Ramos, Paul Townsend, and Sebastián Alberti

We used isogenic mutant strains that were deficient or over-expressed capsule to study the function of the alginate exopolysaccharide in the interaction of *Pseudomonas aeruginosa* with the human airway epithelial cells (AEC) in the presence or absence of surfactant protein A (SP-A). SP-A prevented the invasion of AEC by alginate-producing *P. aeruginosa* strains because of a direct effect on the AEC. Monoclonal antibodies to CKAP4/P63, the principal SP-A-binding receptor on AEC, or inhibition of its expression using specific siRNA reduced the invasion of both highly encapsulated and poorly encapsulated strains, but not the invasion of the acapsular mutant. Treatment of AEC with SP-A, monoclonal antibodies to CKAP4/P63, or CKAP4/P63-specific siRNA decreased the binding of purified alginate exopolysaccharide to AEC. Alginate binding to AEC reduced SP-A release by these cells. Because the alginate exopolysaccharide is surface-exposed, levels of SP-A may be crucial to modulate the interaction of *P. aeruginosa* with AEC.

*Pseudomonas aeruginosa* (PA) is a major opportunistic pathogen responsible for a wide range of nosocomial and community-acquired infections. This bacterium frequently causes acute respiratory infection in patients receiving mechanical ventilation [1] and establishes severe chronic respiratory tract infection in immunocompromised hosts, such as patients with cystic fibrosis (CF) [2]. PA colonizes the lung of patients with bronchiectasis or chronic obstructive pulmonary disease (COPD) and is responsible for a worsening of symptoms and treatment failure [3, 4]. After a period of intermittent colonization, the microorganism becomes permanently established and is rarely eliminated, despite an exuberant host inflammatory response [2, 5]. The persistence of PA in the lung of chronically infected patients with CF and COPD correlates with the apparition of colony variants that can be dwarf, nonmobile, Lipopolysaccharide (LPS) deficient, and highly resistant to commonly used antibiotics [5, 6]. A decisive hallmark in the transition to chronic colonization is the expression of high amount of alginate exopolysaccharide [7], which correlates with a worsening of the symptoms and a poorer prognosis of chronically colonized patients. Alginate exopolysaccharide is a negatively charged copolymer of partially O-acetylated β-1,4-linked D-mannuronic acid and α-L-glucuronic acid, which provides the bacterium with a protection against opsonization, phagocytosis, and reactive oxygen species [8].

Airway epithelial cells (AEC) play an important role during the establishment of PA respiratory infection. In vitro and in vivo experiments have shown that PA attaches to and enters AEC, triggering the release of IL-1, IL-6, IL-8, IL-16, TNF-α, and INF-γ, which orchestrates the innate immune response in the lung by activating and recruiting lymphocytes on the site of infection [9, 10, 11]. This early recognition of the pathogen by the AEC is crucial to initiate an effective innate immune response that allows the clearance of the microorganism from the lung.
The dynamics of PA interactions with AEC are most probably modulated by the presence of pulmonary surfactant, in which surfactant protein A (SP-A) is the most abundant protein. SP-A is a main player of the innate immunity in the lung and is involved in PA opsonization and direct killing through bacterial membrane permeabilization [12]. It has been shown that, in the lung of wild-type mice, PA is cleared more readily than in the lung of SP-A−/− mice, mainly because of an enhanced macrophage phagocytosis in wild-type mice [13], indicating that SP-A is crucial for the control of PA infection in the lung. Other than these observations, there is no evidence of the possible involvement of SP-A in modulation of PA interaction with AEC. In this study, we used isogenic mutants that over-expressed or were deficient in alginate polysaccharide to investigate the function of SP-A in the interplay of mucoid and nonmucoid strains of AEC. As a result of this investigation, we found that PA alginate exopolysaccharide and SP-A share a common receptor on the AEC.

MATERIALS AND METHODS

Bacterial Strains and Cell Lines

Bacterial strains used in this study and their relevant features are listed in Table 1. Bacterial cells were grown in Luria Bertani (Scharlau) broth at 37°C with shaking or were solidified with 1.5% agar.

Human bronchoepithelial immortalized cells (16HBE14o−) [17] and human lung carcinoma cells (A549; ATCC CCL185) derived from type II pneumocytes were used in the present study. The cells were propagated in Earl's minimal essential medium plus 1% L-glutamine culture medium or in RPMI 1640 plus 1% hydroxyethyl piperazine ethane sulphonic acid (HEPES; all from Labclinics), respectively, supplemented with 10% fetal calf serum (Labclinics) plus penicillin and streptomycin (Sigma). Cells were incubated at 37°C and 5% CO2 until confluence.

Table 1. Pseudomonas aeruginosa Strains Used in this Study

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant features</th>
<th>Alginete exopolysaccharide productiona</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1</td>
<td>Reference strain</td>
<td>2.1 ± 0.2</td>
<td>Laboratory collection</td>
</tr>
<tr>
<td>PAO1ΔalgD</td>
<td>Alginate negative unencapsulated transposon algD mutant derived from PAO1 (PA3540-C03::ISphoA/hah)</td>
<td>Not detected</td>
<td>[14]</td>
</tr>
<tr>
<td>PAOMA</td>
<td>Highly encapsulated mucA mutant derived from PAO1</td>
<td>18.9 ± 1.2</td>
<td>[15]</td>
</tr>
<tr>
<td>PAA2</td>
<td>Clinical isolate from acute pneumonia</td>
<td>3.4 ± 0.7</td>
<td>[16]</td>
</tr>
<tr>
<td>PAC20</td>
<td>Clinical isolate from chronic respiratory infection</td>
<td>12.9 ± 1.1</td>
<td>[16]</td>
</tr>
</tbody>
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* Determined by the carbazole assay and expressed as µg/10⁹ cfu.

Reagents

Native human surfactant protein A was purified from bronchoalveolar lavage (BAL) specimens from patients with alveolar proteinosis according to the method previously described [18]. The alginate exopolysaccharide was extracted as previously described [19]. Alginate was labeled with fluorescein isothiocyanate (FITC) in 2% glutaraldehyde, 100 mM Na₂CO₃, and 100 mM NaCl (pH, 8.0) buffer for 1 hour at 4°C and was dialyzed against water for 24 hours at 4°C. Alginate concentration was determined using the carbazole assay [20].

Cell Culture Assays

Monolayers of 16HBE14o− and A549 were grown to confluence in 24-well tissue culture plates and used for standard internalization assays. In brief, cells (~5 × 10⁴ cells/well) were washed 3 times with PBS and incubated for 1 hour at 37°C in 5% CO₂ with a suspension of bacterial cells resuspended in cell culture medium at a multiplicity of infection (MOI) of 10:1 or 50:1, corresponding to a dose of 5 × 10⁵ cfu and 2.5 × 10⁶ cfu, respectively, depending on the experiment. After incubation, the monolayers were washed 3 times with PBS to remove unattached bacteria and then incubated for 1 hour with fresh medium containing gentamicin (100 µg/mL) to kill extracellular bacteria. After a washing step, the epithelial cells were lysed by the addition of 0.5% Triton X-100 (Sigma), and intracellular bacteria were quantified by plating appropriate dilutions on Luria Bertani agar plates. Invasion data are expressed as the number of viable cfu/mL of lysis buffer.

To study the effect of SP-A in PA interaction with AEC, we used 3 different protocols. In the first protocol, cell monolayers were infected with bacteria as described above in the presence or absence of SP-A (8 µg/mL). In the second one, before infection, bacteria were pre-incubated in Veronal buffer (Bio-Mérieux), containing 8 µg/mL of SP-A or PBS as control. Bacteria were then washed 3 times with PBS to remove unattached SP-A, resuspended in warm culture medium, and used
effect to infect the cells. Finally, in the third protocol, before the internalization assays, monolayers were pretreated with 8 µg/mL of SP-A or PBS as control—both dissolved in warm cell culture medium—for 1 hour at 37°C with 5% CO₂. Cytotoxicity was estimated by using the Lactodehydrogenase Cytotoxicity Detection kit (Roche) according to the manufacturer’s instructions.

In the blocking experiments, AECs were pretreated with anti-CKAP4/P63 (anti-CLIMP 63, clone G1/296; 10 µg/mL; Alexis Biochemicals) or anti-CD44 (clone SFF-2; 10 µg/mL; Alexis Biochemicals) for 1 hour at 37°C and then coincubated with bacterial cells in the presence of the blocking antibodies. After 1 hour of incubation, intracellular bacteria were quantified as described above.

**Western Blots**

To study the binding of SP-A to PA, bacterial cells were incubated for 1 hour at 37°C with human SP-A (8 µg/mL). After incubation, cells were washed with PBS, resuspended in Laemmli buffer, boiled for 5 minutes, and resolved by SDS-PAGE. Separated proteins were transferred to INMOBILON-P membranes (Millipore). After transfer, membranes were blocked for 2 hours at room temperature with 5% nonfat dry milk in PBS buffer and incubated overnight at 4°C with a specific polyclonal anti-human SP-A antibody (Chemicon). The membranes were subsequently washed with PBS and incubated for 1 hour at room temperature with an alkaline phosphatase-conjugated goat anti-rabbit IgG secondary antibody.

Finally, membranes were revealed using the 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium kit (Sigma). To evaluate the expression of CKAP4/P63, AEC proteins were extracted with RIPA buffer (Santa Cruz Biotechnology), and protein concentration of solubilized proteins samples was measured using a Coomassie-staining protein assay kit according to the manufacturer’s instructions (Biorad). The solubilized proteins were then boiled for 5 minutes in Laemmli buffer; each lane was loaded with 20 µg of proteins, and proteins were separated, transferred, and immunodetected as described above. CKAP4/P63 and β-actin were detected using a rabbit polyclonal anti-CKAP4/P63 (Sigma) and with mouse monoclonal anti-β-actin (Sigma) antibodies, respectively. Both polyclonal and monoclonal antibodies were detected with alkaline phosphatase-conjugated secondary antibodies. Finally, membranes were developed as described above.

**Immunofluorescence Microscopy**

AECs were plated at 2 × 10⁴ cells/well on 8-well slide chambers and grown to confluence as described above. After 48 hours, the cells were fixed using 3.7% paraformaldehyde for 15 minutes at room temperature, washed with PBS, and incubated with rabbit polyclonal anti-CKAP4/P63 antibodies (Alexis Biochemical) or with isotype-matched antibodies for 1 hour at 37°C. Cells were then washed with PBS and further incubated with a goat anti-rabbit IgG tetramethyl rhodamine iso-thiocyanate (TRITC)–labeled antibody (Sigma) for 1 hour at 37°C. Cytoskeleton was stained green by incubation for 30 minutes at 37°C with Alexa fluor 488 Phalloidin (Invitrogen). Negative controls for the method included cells incubated with the secondary antibody alone.

Samples were examined using a Leika LS230 confocal laser scanning microscope.

**siRNA**

A549 epithelial cells were plated at 2.5 × 10⁵ cells/well in 6-well tissue culture plates. After 24h, cells were transfected with 25 nM of specific pooled CKAP4/P63 siRNAs or scrambled nonspecific siRNAs as control (Dharmacon) diluted in 1 mL of Opti-MEM (Invitrogen) containing 5 µL of Oligofectamine Transfection Reagent (Invitrogen) according to the instructions of the manufacturer. After 72 hours, cells were used in standard internalization assays as described above. No changes in the cell proliferation were observed because of the treatment of the cells with CKAP4/P63 siRNA.

**Cell-Alginate Binding Assays**

Monolayers of A549 were grown to confluence as described above in 96-well tissue culture plates. Cells were washed and incubated in the presence of a solution of purified human SP-A ranging from 0 to 1 µg/well in Veronal Buffer for 1 hour at 37°C. After 3 PBS washes, 5 µg of FITC-labeled alginate in PBS were added to each well and incubated with the cells for 1 hour at 37°C. Monolayers were finally washed 3 times and covered in PBS. Fluorescence was detected at 521 nm after excitation at 495 nm.

**SP-A Secretion**

Monolayers of A549 were grown to confluence in 6-well tissue culture plates (~2.5 × 10⁵ cells/well) as described above. Cells were washed and incubated in the presence of purified alginic exopolysaccharide (1 µg/well) in RPMI plus HEPES for 10 hours at 37°C. Supernatants were collected, filtered to remove cellular debris, and used to determine the levels of SP-A by Western blot analysis, as described above. No changes in cell viability were observed in the cells treated with alginate, compared with the untreated cells.

**RESULTS**

**Effect of Surfactant Protein A on Bacterial Internalization by Airway Epithelial Cells**

The function of SP-A in opsonization and killing of PA have been widely studied [12, 21]. However, little is known about the function of SP-A in the interaction of this pathogen with AEC. To study directly the role of SP-A on the bacterium-epithelial cells interaction, we investigated the ability of the
16HBE14o− and A549 epithelial cells to internalize PAO1, its derived isogenic unencapsulated and highly encapsulated mutants PAO1ΔalgD and PAOMA, and 2 clinical PA isolates, in the presence or absence of a sublethal concentration of SP-A (Figure 1).

In the absence of SP-A, we detected an inverse relationship between the amount of capsule produced by the strain and its capacity to enter both AEC lines. Thus, PAO1ΔalgD entered more efficiently than did PAO1 (P = .0099 in 16HBE14o−; P = .0324 in A549), and PAO1 entered more efficiently than did PAOMA (P = .0327 in 16HBE14o−; P = .0364 in A549). These differences were also observed in the presence of SP-A: PAO1ΔalgD versus PAO1 (P < .0001 in 16HBE14o−; P = .0005 in A549) and PAO1 versus PAOMA (P = .0432 in 16HBE14o−; P = .0386 in A549).

The number of highly encapsulated bacterial cells internalized by both lines of epithelial cells was decreased to one-half log–fold lower when incubated with SP-A than without it. Similar results were obtained with the parental strain PAO1 and with the clinical isolates PAA2 and PAC20. This reduction was not observed with the unencapsulated mutant using a similar MOI used for the other strains (50:1) or using a lower MOI (10:1) to obtain a similar rate of internalization to those observed with the other strains in the absence of SP-A. These results indicate that SP-A reduces the internalization of alginate-producing PA strains by AEC.

We next investigated whether the reduced efficiency observed when alginate-producing bacteria were coincubated with the epithelial cells and SP-A was attributable to the opsonization of the bacteria or to an alteration of the epithelial cell surface molecule expression. For this purpose, bacteria were preopsonized with SP-A and subsequently incubated with the epithelial cells. The conditions used to preincubate the bacteria led to deposition of both dimeric (64-kDa) and monomeric (32-kDa) forms of SP-A on the surface of the bacteria (Figure 2A). However, the unencapsulated mutant and PAO1 bound SP-A more efficiently than did the highly encapsulated mutant. Despite all strains bound to SP-A, we did not observe a reduction of the intracellular bacteria into the 16HBE14o− cells (Figure 2B) or the A549 cells (Figure 2C), indicating an effect of SP-A on the epithelial cells rather than on the bacteria.

Preincubation of 16HBE14o− cells (Figure 2D) or the A549 cells (Figure 2E) with SP-A, followed by the internalization assay, confirmed our hypothesis. The internalization rate of PAO1 and the highly encapsulated mutant decreased after incubation of the cells with SP-A but not the internalization rate of the unencapsulated mutant. These results indicate that the unencapsulated mutant did not exhibit a decreased rate of internalization by the AEC in the presence of SP-A, because although SP-A efficiently opsonized it, the effect of SP-A was on the epithelial cell and specific for the alginate-producing strains.

**CKAP4/P63 Mediates Internalization of Alginate-Producing PA by Airway Epithelial Cells**

Our data suggest that alginate-producing strains interact with an AEC receptor that mediates bacterial uptake into the cells and can be blocked by SP-A. Several specific receptors have been identified for SP-A interaction with AEC [22–24], including CKAP4/P63, recently identified on the surface of human lung epithelial cells.
of type II pneumocytes and responsible for the SP-A-mediated surfactant production inhibition effect in those cells [25–27].

We examined the cell surface expression of CKAP4/P63 on both epithelial cell lines, 16HBE14o− cells, and the A549 cells. Immunofluorescence analysis confirmed the expression of

**Figure 2.** The effect of preincubation with SP-A. (A). Representative Western blot analysis of SP-A monomers (32 kDa) and dimmers (64 kDa) deposited on PA PAO1, its derived highly encapsulated and unencapsulated mutants PAOMA and PAO1ΔalgD after preincubation with purified human SP-A. Purified SP-A, used as control, is in the last lane on the right. Molecular weight markers (in kilodaltons) are indicated on the right side of the panel. (B, C, D, and E). The effect of preincubation of bacterial cells (B and C) or epithelial cells (C and D) with SP-A (white bars) or PBS (black bars) on the internalization of PAO1, the highly encapsulated PAOMA mutant and the isogenic unencapsulated PAO1ΔalgD mutant by human bronchoepithelial cells 16HBE14o− (B and D), or type II pneumocytes A549 (C and E). Multiplicity of infection (MOI) is indicated in brackets. Data are representative of 3 different experiments. Results significantly different from control are denoted with an asterisk (**P < .01; ***P < .001, 2-tailed t test).
CKAP4/P63 on the surface of the bronchoepithelial cells (Figure 3A) and on the pneumocytes (Figure 3B).

To investigate whether CKAP4/P63 mediates uptake of PA into the epithelial cells, we used a specific anti-CKAP4/P63 blocking antibody to block the interaction between alginate-producing PA and the epithelial cell surface receptor for SP-A, CKAP4/P63. Internalization of PAO1 and the highly encapsulated mutant PAOMA by both AEC lines 16HBE14o− (A) and A549 (B), respectively. Panels on the left were stained with an anti-CKAP4/P63 mAb antibody, and panels on the right were stained with an isotype-matched antibody as control. Both antibodies were stained red with a TRITC-labeled secondary antibody, and the cytoskeleton was stained green with Alexa fluor 488 Phalloidin. Effect of the treatment of human bronchoepithelial cells 16HBE14o− (C) or type II pneumocytes A549 (D) with blocking anti-CKAP4/P63 antibodies (10 µg/ml) on the internalization of PAO1, the highly encapsulated PAOMA mutant, and the isogenic unencapsulated PAO1ΔalgD mutant. The internalization of P. aeruginosa by cells treated with anti-CKAP4/P63 antibodies is plotted as a percentage of that obtained for cells treated with anti-CD44 antibodies (10 µg/ml) as control. Data are representative of 3 different experiments. Results significantly different from control are denoted with an asterisk (**P<.01, 1-tail t test).

Figure 3. Expression of CKAP4/P63 on airway epithelial cells and blockade of P. aeruginosa internalization by epithelial cells with specific anti-CKAP4/P63 antibodies. Immunofluorescence analysis of CKAP4/P63 expression on the surface of the human bronchoepithelial cells 16HBE14o− (A) and on the pneumocytes type II A549 (B), respectively. Panels on the left were stained with an anti-CKAP4/P63 mAb antibody, and panels on the right were stained with an isotype-matched antibody as control. Both antibodies were stained red with a TRITC-labeled secondary antibody, and the cytoskeleton was stained green with Alexa fluor 488 Phalloidin. Effect of the treatment of human bronchoepithelial cells 16HBE14o− (C) or type II pneumocytes A549 (D) with blocking anti-CKAP4/P63 antibodies (10 µg/ml) on the internalization of PAO1, the highly encapsulated PAOMA mutant, and the isogenic unencapsulated PAO1ΔalgD mutant. The internalization of P. aeruginosa by cells treated with anti-CKAP4/P63 antibodies is plotted as a percentage of that obtained for cells treated with anti-CD44 antibodies (10 µg/ml) as control. Data are representative of 3 different experiments. Results significantly different from control are denoted with an asterisk (**P<.01, 1-tail t test).
Alginate Exopolysaccharide Interacts with CKAPA/P63 on the Airway Epithelial Cells

The results described above show that the expression of the alginate exopolysaccharide is crucial for the interaction of PA with the cell surface receptor CKAP4/P63. To further investigate whether alginate exopolysaccharide is the bacterial ligand involved in the interaction of PA with this receptor, A549 cells were grown in 96-well plates and treated as described above using anti-CKAP4/P63 blocking antibody or anti-CD44 antibodies or with scrambled or specific silencing siRNA. After treatment, cells were washed and incubated serially with SP-A at concentrations ranging from 0 to 1 µg/mL for 1 hour and FITC-labeled alginate. Fluorescence registered in each well indicated that alginate bound to lung epithelial cells treated with anti-CD44 antibodies or with scrambled siRNA and that this binding was reduced in the presence of SP-A in a dose-dependent manner (Figure 5A and 5B, respectively). However, in cells preincubated with CKAP4/P63-blocking antibodies or with specific CKAP4/P63-silencing siRNA, alginate did not bind to the cells (Figure 5A and 5B, respectively).

These results indicate that alginate exopolysaccharide specifically binds to AEC through the cell surface receptor CKAP4/P63.

Alginate Exopolysaccharide Reduces Secretion of SP-A by the Airway Epithelial Cells

To investigate whether alginate exopolysaccharide affects the secretion of SP-A by AEC, A549 cells were grown in 6-well plates and treated with purified alginate (1 µg) or left untreated for 10 hours. After treatment, the amount of SP-A present in the supernatant was quantified by Western blot analysis. Results of these analysis demonstrated that cells treated with alginate secreted less SP-A than did the untreated cells (Figure 6). Densitometric analysis of 4 independent experiments indicated that the secretion of SP-A by the A549 cells decreased up to 28% ± 2% (P = .023; 2-tailed t test) in the cells treated with alginate, compared with the untreated cells.

DISCUSSION

Our results comparing the invasion of isogenic highly encapsulated and unencapsulated mutant strains confirmed evidence from earlier studies showing that alginate exopolysaccharide decreases the overall invasiveness of PA to AEC [28] and highlights the role of SP-A as a host innate blocking component for the interaction of mucoid PA with AEC. Both observations are consistent with the clinical and experimental evidences showing that mucoid PA do not interact with AEC but stuck in the airway lumen trapped in the mucus in the lungs of patients and mice with CF [11].

Whereas previous studies identified SP-A as an opsonin increasing PA uptake by alveolar macrophages [21], several works have demonstrated that other surfactant proteins, such as SP-D, are able to reduce PA invasion in corneal epithelial cells [29] and Klebsiella pneumoniae interaction with AEC [30]. Unexpectedly, this study demonstrates that opsonization of PA with SP-A does not alter the invasiveness of the bacterium. By contrast, SP-A pretreatment of the cells is able to block specifically the invasion of those cells by alginate-producing strains. Remarkably, invasion of poorly encapsulated isolates was also significantly reduced by SP-A, indicating that...
this SP-A blockage occurs not only in highly encapsulated strains, such as PAOMA, but also in strains that express nominal amount of capsule, such as PAO1.

Many PA isolates grow as nonmucoid or glossy colonies on solid media; those strains are generally considered as non–alginate-exopolysaccharide producers. In this study, however, SP-A blocked invasion of PAO1, a strain considered traditionally as noncapsulated. These results, together with previous studies that demonstrated that alginate exopolysaccharide production can be higher in vivo [31, 32] suggest that many PA strains considered to be acapsular on the basis of colony morphology and carbazole quantification in vitro may produce amounts of alginate exopolysaccharide sufficient to mediate attachment of PA and, consequently, to be susceptible to blocking by SP-A.

Uptake of PA by AEC may represent a host defense mechanism that contributes to bacterial clearance. Entry of the bacteria into AEC triggers an intense inflammatory response that allows the clearance of the bacteria by the recruited phagocytes [9, 10, 11]. Accordingly, the blockage of PA internalization by SP-A may represent a strategy to modulate the inflammatory response induced by PA, and the low levels or lack of SP-A may result in increased inflammatory response as observed by LeVine et al in mice deficient in SP-A [13].

Alternatively, invasion of AEC by PA may permit the bacteria to penetrate the epithelial cell layer, reach the bloodstream, and disseminate to distant organs. Thus, SP-A blockage may act as a crucial host strategy to avoid systemic infections.

Our experiments using blocking antibodies or specific siRNA clearly show that the cellular receptor for alginate exopolysaccharide is CKAP4/P63. This non glycosylated, type II membrane protein is found on the surface of many cell types, including type II epithelial cells and bronchoepithelial cells. CKAP4/P63 is an endogenous receptor for SP-A, and the interaction of this protein with the receptor is responsible for SP-A–triggered surfactant secretion inhibition and phospholipid uptake by AEC [25]. It is likely that the interaction of PA alginate with CKAP4/P63 could play a role in surfactant homeostasis in the lung. This hypothesis is in agreement with
the study by Wu et al that revealed that mucoid PA is able to reduce both in vivo and in vitro the production of dipalmitoylexophatidylcholine by AEC [33]. In the present study, we revealed that alginate also reduce the secretion of SP-A by AEC. According to these observations, the interaction of alginate exopolysaccharide with CKAP4/P63 could provide the bacterium with an advantage for colonization, reducing the binding of and susceptibility to SP-A bactericidal effects and having some secondary effects on lung homeostasis. Thus, the alginate/CKAP4/P63 interaction represents a novel mechanism together with other strategies used by the bacterium that participate in surfactant degradation, such as the expression of elastase and proteinase IV, which could be responsible for the altered surfactant levels observed in patients chronically infected with this bacterium, such as those with CF [34].

In light of these results, the restoration of constitutive surfactant levels, often altered in patients chronically infected with PA, such as those with CF, could be a great help to control the intense inflammatory response caused by this microorganism and to avoid the impairment of the lung homeostasis that could favor PA persistence.

Notes

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