

Surfactant Protein A (SP-A)–Mediated Bacterial Clearance

SP-A and Cystic Fibrosis

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Pulmonary Innate Defenses

The constant challenge to the lung of inhaled microbes and noxious substances is largely kept in check by the innate and adaptive immune systems. Whereas the adaptive immune system is acquired and variable depending on previous challenges, the endogenous innate immune system is constitutively present and rapidly responds to inhaled particles. The principal function of innate immunity is to rapidly clear inhaled substances to prevent the establishment of an inflammatory process. Substances escaping the innate nasopharyngeal and tracheobronchial clearance mechanisms encounter a complex array of phagocytic and immunomodulatory cells and defensive molecules in the more distal lung. Defensins, lactoferrin, lysozyme, secretory leukoprotease inhibitor (SLPI), secretory phospholipase A2, and cathelicidin LL-37 are recognized bacteriocidal molecules (see Refs. 1–4 for review). Surfactant proteins A and D (SP-A and SP-D) are components of the innate defenses of the lung that act in concert with phagocytes to enhance clearance of invading microbes and other substances. SP-A and SP-D, produced and secreted by lung epithelial and tracheal gland cells, belong to the collectin family. Collectins share the same general protein structure of an amino terminal collagenous domain, a neck domain, and a carbohydrate recognition domain. Collectin genes have similar exon/intron junctions with the collagen domain encoded by several small exons and the carbohydrate recognition domain encoded by a single exon. Several collectin genes, including SP-A and SP-D, and at least one of the mannose binding lectin genes, are tightly linked on loci syntenic between the mouse and humans (5, 6). A considerable body of *in vitro* and *in vivo* studies support a role for SP-A and SP-D in pulmonary defense. Excellent recent reviews describe, in detail, functional and structural characteristics of the lung collectins (see Refs. 7–16 for review). This review is limited mainly to studies demonstrating SP-A–mediated mechanisms of bacterial clearance with some comparisons to SP-D–mediated processes.

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Abbreviations: airway surface fluid, ASF; bronchoalveolar lavage, BAL; Bacillus Calmette-Guerin, BCG; cystic fibrosis, CF; lipopolysaccharide, LPS; secretory leukoprotease inhibitor, SLPI; surfactant protein, SP.

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SP-A–Mediated Bacterial Clearance

Evidence generally supports at least four nonexclusive SP-A–mediated mechanisms. (1) SP-A binds and enhances uptake of bacteria by macrophages and neutrophils; (2) SP-A acts as an activation ligand, directly acting on macrophages or neutrophils to enhance phagocytosis; (3) SP-A enhances production of microbicidal free radicals by cells; and (4) SP-A is a chemoattractant for phagocytes. Mechanisms and bacteria studied are summarized in Table 1.

Binding and Enhancement of Bacterial Uptake

SP-A binds and enhances uptake of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, Group B streptococci, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, and *M. pulmonis* by phagocytes (Table 1). Bacterial binding frequently, but not always, correlates with enhanced uptake. Binding to bacteria may vary with species and types of microorganisms. Consistent with studies on types of LPS, SP-A binds and enhances phagocytosis of *E. coli* J5 (rough LPS) but not *E. coli* 0111 (smooth LPS) (23, 24). Type a but not type b *H. influenzae* (28), and live but not heat killed *P. aeruginosa* (26) bind SP-A. *K. pneumoniae* K21a, with a mannose-containing capsule, but not *K. pneumoniae* K2, with a capsule that does not contain mannose, binds SP-A (36). SP-A enhanced uptake of log phase but not stationary phase *E. coli*, while uptake of *P. aeruginosa* was not altered by growth phase (19). Both gram-negative and gram-positive organisms interact with SP-A, but the capacity to bind and enhance phagocytosis varies with the strain, LPS type, the growth phase, or heat treatment of the bacteria. *In vivo*, mice with a null mutation of the SP-A locus, generated by targeted gene inactivation, had decreased clearance and macrophage phagocytosis of intratracheally injected Group B streptococci, *P. aeruginosa*, and *H. influenzae* (27, 29, 30).

SP-A Is an Activation Ligand

Pretreatment of macrophages was reported to be sufficient for enhancing macrophage uptake of *E. coli* K12, *S. aureus*, and *P. aeruginosa* (19, 21). Although SP-A binds the K21a capsule type of *K. pneumoniae*, direct pretreatment of macrophages with SP-A also produced increased uptake. The direct effect of SP-A was inhibited by mannan, suggesting that SP-A increased activity of the mannose receptor (36). Although SP-A binds *M. tuberculosis*

TABLE 1
SP-A mechanisms of bacterial clearance

Bacteria	SP-A binds	SP-A enhances attachment or uptake	SP-A enhances killing
<i>S. aureus</i>	Yes (17, 18)	Yes (monocytes, macrophages) (17–21)	No (17, 18)
"	No (19, 21)	—	—
"	Yes (22)	Yes (neutrophils) (22)	—
<i>E. coli</i> J5	Yes (23, 24)	Yes (macrophages) (24)	Yes (24)
" 0111	No (23, 24)	—	—
" K12	No (19, 21)	Yes (macrophages) (19, 21)	—
" K12	Yes (22)	Yes (neutrophils) (22)	—
<i>P. aeruginosa</i>	Yes (26)	Yes (macrophages) (19, 21, 26, 27)	Yes (27)
"	No (19, 21, 25, 26)	No* (25)	—
<i>S. pneumoniae</i>			
Type 25	Yes (18)	No (18)	—
Type 4	Yes (22)	Yes (neutrophils) (22)	—
Type 19	—	Yes (neutrophils) (22)	—
Type 23	—	Yes (neutrophils) (22)	—
Not typed	Yes (25)	Yes (macrophages) (25)	—
<i>H. influenzae</i>			
Type a	Yes (28)	Yes (macrophages) (28)	Yes (28)
Type b	Weakly (28)	No (28)	No (28)
Not typed	Yes (25)	Yes (macrophages) (25)	Yes (29)
Group B			
Streptococci	Yes (30)	Yes (macrophages) (30)	Yes (30)
<i>M. tuberculosis</i>	Yes (31)	Yes (macrophages) (31, 32, 33)	—
BCG	Yes (34)	Yes (macrophages) (34)	Yes (35)
<i>K. pneumoniae</i>			
K21a	Yes (36)	Yes (macrophages) (36)	Yes (36)
K2	No (36)	No (36)	—
<i>M. pulmonis</i>	Yes (37)	—	Yes (37, 38)

Phagocytic cells studied and reference numbers are indicated by parentheses; — indicates not reported.
* Heat-killed *P. aeruginosa* were not phagocytosed (25), whereas live bacteria were phagocytosed (19, 21, 26).

and Bacillus Calmette-Guerin (BCG), SP-A was also reported to enhance adherence of *M. tuberculosis* to macrophages by enhancing activity of the mannose receptor (33). Pretreatment of neutrophils was also sufficient to enhance uptake of tested bacteria (22). Evidence is accumulating that SP-A can alter phagocytosis by direct interactions with phagocytes, enhancing phagocytic activity by altering activity of some receptors.

SP-A Stimulates Free Radical Production

Reactive oxygen and nitrogen species are known to effect intracellular and extracellular bacterial killing (39–41). SP-A-coated zymosan, but not free SP-A, induced an enhanced production of oxygen free radicals (42). In contrast, in a separate report, free SP-A was found to induce oxygen radical production by rat alveolar macrophages and *S. aureus*, opsonized with SP-A, generated an additive response to that produced by either single component. Alveolar macrophages only, and not peritoneal macrophages, neutrophils, or monocytes were induced by SP-A (20). SP-A was also found to increase macrophage nitric oxide (43) and increase BCG-induced nitric oxide and tumor necrosis factor- α production by rat bone marrow macrophages, leading to enhanced BCG killing. Interestingly, an antibody to SPR210, a putative SP-A receptor, blocked the SP-A enhancement of nitric oxide production, suggesting that these effects are mediated through a single SP-A receptor (35). SP-A also induced nitric oxide production by interferon- γ -activated macrophages, leading to killing of *M. pulmonis* (37). Although

specific effects on bacteria were not reported, SP-A and SP-D enhanced nitric oxide production by neutrophils stimulated by *Aspergillus* conidia (44).

In vivo, alveolar macrophages of mice lacking SP-A did not produce increased oxygen free radicals following group B streptococcal infection (30). The level of nitric oxide produced, following intratracheal injection of LPS, was decreased in the SP-A null mice (45). SP-A null mice were also deficient in clearance of *M. pulmonis*, a process requiring peroxynitrite generation (38). Evidence is accumulating that SP-A enhances free radical production *in vitro* and *in vivo* leading to enhanced killing of certain bacterial species.

SP-A Induces Chemotaxis

Testing in microchemotaxis chambers revealed that both SP-A and SP-D are chemoattractants for human neutrophils and rat alveolar macrophages (44, 46). Increased F-actin polymerization and actin-filled filopodia, indicating directional actin polymerization, were detected in SP-A- and SP-D-treated macrophages (46). The chemoattractant properties of lung collectins support a model where increased accumulation of collectins in areas of infection, either by binding to microbes or increased localized production, serve as signals to attract phagocytes.

In this issue, Khubchandani and colleagues revisit and further characterize processes by which SP-A enhances macrophage uptake of *P. aeruginosa* (47). Using the THP-1 monocyte/macrophage cell line, the authors demonstrated dose and temperature dependency of SP-A-mediated up-

take of *P. aeruginosa*, PAO1, a nonmucoid strain. Although most of the studies were performed with PAO1, uptake of a mucoid clinical isolate by the THP-1 cells was also enhanced by SP-A, supporting the previous study of Mariencheck and colleagues that SP-A enhanced uptake of a live mucoid *P. aeruginosa* by rat alveolar macrophages (26). Low levels of contaminating LPS and LPS added to SP-A preparations did not effect the SP-A-mediated uptake in the THP-1 cells. Mannose, when added during the assay, inhibited SP-A-mediated uptake, supporting previous studies that mannose blocked SP-A binding to a mucoid *P. aeruginosa* (26). The presence of SP-A was required during the entire assay.

Perhaps the most interesting new finding in the Khubchandani study is the narrow range of sodium chloride concentrations over which SP-A enhanced *P. aeruginosa* uptake by the THP-1 cells. Concentrations of 100 or 150 mM (but not 10, 50, or 200 mM) NaCl permitted SP-A-mediated uptake. These data suggest that if NaCl concentrations are altered in infected lungs, the role of SP-A in enhancing bacterial clearance could be compromised.

SP-A Levels in Pulmonary Inflammation

Decreased levels of SP-A are associated with various causes of pulmonary inflammation. Lavage fluid of patients with bacterial pneumonia was reported to contain about 3–9 fold less SP-A than lavage fluid of patients with idiopathic pulmonary fibrosis or healthy patients (48). Tracheal aspirates from children with pneumonia had significantly decreased SP-A/protein concentrations compared with aspirates from patients with adult respiratory distress syndrome or recovering from cardiopulmonary bypass surgery (49). Pathogens identified in these studies (48, 49) included *H. influenzae*, *P. aeruginosa*, *S. aureus*, and *S. pneumoniae*. Adult patients with respiratory failure and pneumonia were also reported to have reduced bronchoalveolar lavage (BAL) SP-A, but not SP-B, concentrations (50). In a ventilated neonatal premature baboon model, SP-A levels in lavage were significantly less in animals with naturally acquired infection compared with animals without infection. Some of the pathogens isolated from the infected lungs are known to interact with SP-A (Table 1), including *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* (51).

Cystic Fibrosis and SP-A

Cystic Fibrosis (CF) is associated with chronic lung inflammation and a high rate of superimposed *P. aeruginosa* lung infections. Infants with CF (mean age 22.7 mo), and evidence of lung inflammation, were reported to have about a 2-fold greater SP-A concentration in BAL than a control group or patients with CF but without inflammation (52).

An older group of children (mean age 90 mo) were reported to have about a 5-fold decrease in SP-A concentration in BAL compared with an unaffected control group. In this same study, SP-D was also significantly decreased and SP-D was undetectable in many CF patients (53). In another report, young adults (mean age 23 yr) were found to have significantly decreased surfactant-associated SP-A without decreases in total BAL. This data suggest that the normal SP-A function of surfactant lipid association may be dramati-

cally altered in the CF lung (54). BAL from CF patients (ages 5.9–20 yr) with increased neutrophils had decreased concentrations of SP-A without changes in SP-B (55). Degradation of SP-A was detected in 15 of 17 BAL samples of CF patients, but not in eight samples from healthy children (56). In a recently reported pilot study, the extent of SP-A degradation was partially reduced by inhalation of α_1 -protease inhibitor (57). Considered together, the clinical studies suggest that SP-A levels are decreased in older patients with CF, particularly in the presence of inflammation. In patients with CF, residual SP-A may be degraded and functionally altered. The alterations in SP-A may contribute to the increased risk of *P. aeruginosa* in the CF population.

Deficient Bacterial Clearance in CF

Identification of defective innate immunity in CF has been the subject of a number of investigations in recent years. As chloride concentrations were increased, bacteriocidal activity against *P. aeruginosa* and other pathogens was noted to be inhibited in airway surface fluid (ASF) collected from cultured airway epithelia (58). Subsequently, defensins, lysozyme, lactoferrin, and SLPI were found to have decreased activity in higher salt concentrations, greater than 75 mM for human B defensin 1 (59), and an ionic strength greater than 65 mM for other peptides (3). Synergistic antibacterial activity against *E. coli* of combinations of lysozyme-lactoferrin, lysozyme-SLPI and lactoferrin-SLPI were also reduced at ionic strengths greater than 65 mM. The activity of SP-A, to enhance phagocytosis of *P. aeruginosa*, as reported in this issue, suggests that SP-A may be active where activity of the antimicrobial peptide activity is impaired (47).

At present, it is difficult to correlate the effects of NaCl concentration on SP-A function because the concentrations of NaCl in ASF are controversial. The NaCl concentration in ASF from patient or mouse samples or epithelial culture models has been reported as hypotonic to isotonic. Moreover, there is no general agreement as to whether salt concentrations in CF airways are different from those in non-CF airways (58, 60–66).

SP-A, *P. aeruginosa*, and CF

As documented in this Perspective, there is considerable *in vitro* and *in vivo* evidence that SP-A enhances clearance of certain types of bacteria as part of the innate defenses of the lung (Table 1). The role of SP-A in enhancing macrophage phagocytosis of *P. aeruginosa* is well-supported (19, 21, 26, 27, 47), and recently SP-D was also reported to enhance *P. aeruginosa* phagocytosis (67). The clinical data demonstrating decreased SP-A or SP-D levels in clinical CF samples (53–55), and suggesting that SP-A is degraded, possibly by proteolysis (56, 57), suggests that the levels of SP-A and SP-D in the CF lung should be considered in correcting innate immune deficiencies caused by CF. Future studies to assess mechanisms of collectin degradation in CF, possible synergistic clearance activities of collectins and microbial peptides, and possible CF-related alterations of collectin-mediated chemotactic functions or ligand activation functions may ultimately benefit patients with CF.

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