Perspective

Distinct Roles for Lung Collectins in Pulmonary Host Defense

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It has long been recognized that the lung contains resident components of the innate immune system that provide a first-line defense against infectious challenge. Recent studies have provided considerable evidence that two surfactant associated proteins, surfactant protein (SP)-A and SP-D, play important roles in pulmonary host defense (1). SP-A and SP-D are members of a larger family of proteins known as collectins (collagenous C-type lectins), which also includes the serum collectins mannose-binding lectin (MBL) and conglutinin (2). Members of this family are characterized by common N-terminal collagen-like domains and C-terminal carbohydrate recognition domains (CRD). They have the capacity to bind a variety of macromolecules, including carbohydrates, phospholipids, and proteins.

Numerous *in vitro* and *in vivo* studies during the past decade have supported the hypothesis that SP-A and SP-D contribute to the containment of infections caused by a wide variety of respiratory pathogens, including bacteria, viruses, and fungi, through modulation of pathogen clearance, immune cell function, and lung inflammation (reviewed in Refs. 1–5). Similar effects have been noted for SP-A and SP-D in the innate immune response. However, specific differences in function have suggested that each collectin may play a very specific role in defense against different microbial pathogens. Although both SP-A and SP-D can bind to, aggregate, and/or promote uptake of certain pathogens by immune cells in the airway (summarized in Table 1), each collectin behaves in a very specific manner, which may define the outcome of infection. For example, SP-A has been shown to function as an efficient opsonin for a variety of bacteria such as *Pseudomonas aeruginosa* and mycobacteria (Table 1; reviewed in Refs. 3–7), and promote uptake of the complexes by alveolar macrophages. In contrast, SP-D is not a very effective opsonin, and only moderately enhances the phagocytosis of *P. aeurginosa* by alveolar macrophages (8), and inhibits phagocytosis of *Mycobacterium tuberculosis* (9). SP-A also enhances the phagocytic capacity of macrophages (10), and increases ingestion of nonopsonized bacteria such as *M. tuberculosis* (11) and *Klebsiella* (12). Although SP-A has been reported to promote aggregation of certain viruses and bacteria, SP-D forms large aggregates compared with the microscopic aggregates in the presence of SP-A. These large SP-D-associated aggregates may promote airway mucociliary clearance as well as internalization by pulmonary phagocytic cells (3).

The differences in the oligomeric structures of these collectins may contribute to their interaction with various microbial pathogens. SP-A and SP-D are both assembled as oligomers of trimeric subunits (1, 3). Each subunit contains a short amino-terminal disulfide crosslinking domain, a triple helical collagen domain, a short trimeric coiled-coil linking domain, and a C-terminal CRD. SP-A exists predominantly as octadecamers in "bouquet-like" oligomers, with closely spaced CRDs. SP-D is assembled as dodecamers with long crosslinking domains, resulting in more widely spaced CRDs. Because the separation of CRDs in SP-D is fivefold greater than for SP-A (100 nm versus 20 nm), SP-D has a greater capacity to link interactions between binding sites on different particulate ligands, potentially contributing to the higher levels of aggregation of microbial ligands seen with SP-D.

Key recent studies using mice deficient in SP-A or SP-D support a role for these collectins in pulmonary host defense (summarized in Table 2). Work from several groups using SP-A$^{-/-}$ mice has shown increased bacterial load following infection with group B streptococcus (13), and defective clearance of *Hemophilus influenzae* (14), *P. aeruginosa* (15), and *Mycoplasma pulmonis* (16). SP-A$^{-/-}$ mice also show increased susceptibility to challenge with respiratory syncyticial virus (RSV) (17). In most instances, the decreased microbial clearance can be reversed by addition of exogenous SP-A. Because these mice show normal respiratory function and surfactant lipid metabolism (20), the defects in microbial defense appear to be primarily attributable to SP-A. Mice lacking SP-A also show variable increases in proinflammatory mediators and decreases in anti-inflammatory cytokines, with an overall net proinflammatory environment. In contrast, evaluation of the role of SP-D using SP-D–deficient mice has been complicated by abnormal surfactant homeostasis and altered alveolar macrophage function (21). Nevertheless, in the absence of SP-D, several studies have examined infection of SP-D$^{-/-}$ mice with bacterial and viral pathogens, including group B streptococcus (14), *H. influenzae* (14), and influenza A virus (IAV) (19). Several differences in response to *in vivo* challenges with these microbes by SP-A$^{-/-}$ and SP-D$^{-/-}$ mice have been reported. First, SP-D$^{-/-}$ mice show no change in bacterial load following group B streptococcus and *H. influenzae* challenge compared with reduced clearance in SP-A–deficient mice (14). Second, whereas production of reactive oxygen species (ROS) by alveolar macrophages

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Abbreviations: carbohydrate recognition domains, CRD; hemagglutinin; HA; influenza A virus, IAV; mannose-binding lectin, MBL; reactive oxygen species, ROS; respiratory syncytial virus, RSV; surfactant protein, SP.

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TABLE 1
*Comparison of the interaction of SP-A and SP-D with microbial pathogens**

Microbe	Binding	Aggregation	Collectin-enhanced uptake
K. pneumoniae			
SP-A	Yes	Yes	Yes[†]
SP-D	Yes	Yes	
P. aeruginosa			
SP-A	Yes		Yes
SP-D	Yes	No	Yes
H. influenza			
SP-A	Yes	Yes	Yes
SP-D	Yes	Yes	
E. coli			
SP-A	Yes	Yes	Yes
SP-D	Yes	Yes	
Group A strep			
SP-A	Yes	Yes	Yes
Group B strep			
SP-A	Yes	Minimal	Yes
SP-D	Yes	Yes	No
S. aureus			
SP-A	Yes	Yes	Yes
SP-D	Yes	Yes	
S. pneumoniae			
SP-A	Yes		Yes
P. carinii			
SP-A	Yes		
SP-D	Yes	Yes	
A. fumigatus			
SP-A	Yes		Yes
SP-D	Yes		Yes
C. neoformans			
SP-A	Yes		
SP-D	Yes		
BCG			
SP-A	Yes		Yes
M. tuberculosis			
SP-A	Yes		Yes[†]
SP-D	Yes		No[‡]
Influenza A virus			
SP-A	Yes	Minimal	
SP-D	Yes	Yes	No (macrophages) Yes (neutrophils)
RSV			
SP-A	Yes		Yes
SP-D	Yes		
CMV			
SP-A	Yes		Yes[†]

* Summarized from References 1–5.
[†] Nonopsonic uptake.
[‡] Inhibition of uptake.

from SP-A$^{-/-}$ mice following infection with group B streptococcus and *H. influenzae* was decreased, increased ROS production was found in SP-D$^{-/-}$ mice (14). Taken together, these *in vitro* and *in vivo* studies support an important role for both collectins in pulmonary host defense against a variety of bacterial, viral, and fungal pathogens, and suggest that SP-A and SP-D may play specific roles that are dependent on the nature of the pathogen.

Recent studies examining the differences in SP-A and SP-D in viral host defense have provided additional clues as to how these collectins might act independently and specifically in pulmonary host defense. In 1994, Malhotra and coworkers demonstrated that SP-A could bind to IAV (22), and Hartshorn and colleagues reported that SP-A neutralizes certain strains of IAV through direct attachment (23). In addition, SP-A can act as an opsonin and enhance IAV and herpes simplex virus uptake by alveolar macrophages (24, 25), as well as inhibit IAV and RSV infectivity and IAV HA activity (26). In *in vivo* studies, LeVine and colleagues reported that SP-A–deficient mice showed decreased clearance of RSV (17).

In 1997, Hartshorn and colleagues demonstrated that both SP-A and SP-D neutralize IAV infectivity through inhibition of the hemagglutinin (HA) activity (27). However, SP-A was significantly less potent as an inhibitor, and the inhibition was not blocked by mannan, whereas SP-D reduced HA activity at much lower concentrations through a CRD-dependent mechanism. The authors concluded from their studies that IAV strains with less extensive high mannose carbohydrate modifications were more sensitive to SP-A, and that the IAV HA bound to the accessible sialic acid–containing carbohydrate side chains on the SP-A molecule. In contrast, IAV strains with high level of exposed mannose groups interacted with SP-D through a classical CRD-mannose binding interaction, leading to formation of large aggregates. Furthermore, SP-D promoted internalization of IAV by neutrophils. LeVine and colleagues recently extended the study of IAV clearance to an *in vivo* model using SP-D–deficient mice (19). Viral clearance was reduced in SP-D$^{-/-}$ mice, and addition of exogenous recombinant SP-D enhanced clearance. Results from these *in vivo* and *in vitro* studies suggest that SP-A and SP-D may play very different roles in IAV host defense, and that SP-D may be the collectin primarily involved in enhancement of clearance of the virus by pulmonary phagocytic cells.

In this issue Li and coworkers extend these studies to specifically examine the contribution of SP-A in host defense against infection with IAV using SP-A–deficient mice challenged with an IAV strain lacking the major SP-D attachment site (18). The authors report that the mice exhibited decreased survival at all viral concentrations tested, and that viral loads at Days 2 and 6 were not significantly different between −/− and +/+ mice (Table 2). These observations suggest that SP-A does not contribute to virus clearance, and support the *in vitro* and *in vivo* studies discussed above suggesting that SP-D may play a more important role in limiting IAV replication. Findings from other studies support this role for SP-D in IAV infection. First, several laboratories have reported that SP-D levels are increased following IAV infection (18, 19). In addition, human lung washings contain sufficient SP-D to inhibit IAV HA activity, and depletion of SP-D from human bronchoalveolar lavage fluid decreases the inhibitory activity (27). The specific structural features of SP-A and SP-D and how they interact with the virus may explain their differing roles in IAV infection (27). IAV binds to the sialic residues on SP-A; due to this rather unique pathogen-SP-A interaction and the structure of the SP-A oligomer, small aggregates are formed that may not be recognized efficiently by pulmonary phagocytic cells. On

TABLE 2
Comparison of microbial clearance in SP-A$^{-/-}$ and SP-D$^{-/-}$ mice

Microbe	Clearance	# PMNs	Proinflammatory cytokines			ROS production	Rescue by A or D	Ref.
			TNF	IL-6	MIP2			
Group B strep								
SP-A	Decreased	No change	Inc	Inc	N.C.	Decreased	Yes	13
SP-D	No change	No change	Inc	Inc	N.C.	Increased	N.D.	14
P. aeruginosa								
SP-A	Decreased							15
H. influenzae								
SP-A	Decreased	Increased	Inc	Inc	Inc	Decreased	N.D.	14
SP-D	No change	No change	Inc	Inc	N.C.	Increased	N.D.	14
M. pulmonis								
SP-A	Decreased							16
RSV								
SP-A	Decreased	Increased	Inc	Inc	Inc		Yes	17
IAV								
SP-A	No change	Increased	N.D.	Dec	Inc		N.D.	18
SP-D	Decreased	Increased	Inc	Inc	Inc	Decreased	Yes	19

the other hand, IAV is bound through the CRD on SP-D. This interaction and the structure of SP-D appear to promote formation of very large aggregates that might then be effectively internalized by host cells.

The study by Li and colleagues also suggests that the primary role for SP-A in IAV infection may be to control the host inflammatory response, thus reducing local tissue damage (18). Similar to other studies of viral clearance in SP-A–deficient mice, Li and colleagues found that the inflammatory cytokine milieu was altered following IAV infection, with higher levels of MIP-2 mRNA and protein, and increased neutrophilic influx (Table 2). In addition, IAV-infected mice lacking SP-A had increased airway epithelial injury and higher alveolar cellular infiltrates compared with infected SP-A$^{+/+}$ mice. An increased inflammatory response has also been reported in SP-A$^{-/-}$ mice following challenge with RSV (17). Although broadly in agreement, the specific nature and magnitude of the responses by SP-A$^{-/-}$ mice to challenge by bacteria and viruses varies considerably. For example, levels of IL-6 were decreased following IAV challenge (18), whereas infection with RSV, group B streptococcus, and *H. influenzae* resulted in enhanced IL-6 production in SP-A$^{-/-}$ mice (12, 14, 19). Li and coworkers found that MIP-2 levels were increased at all time points following IAV challenge (18), whereas LeVine and cowrkers found no differences in this cytokine in RSV challenge (17). Although the reasons for these discrepancies are not entirely clear, differences may be related to levels of microbes used and times of assay. Taken together, studies of both bacterial and viral challenge using SP-A$^{-/-}$ mice show enhanced neutrophilic influx, greater airway tissue damage, and enhanced proinflammatory cytokine production, suggesting that SP-A functions to regulate the inflammatory response and limit local tissue damage. In contrast, at least in the setting of IAV infection, SP-A does not appear to contribute to viral clearance.

In summary, both SP-A and SP-D interact with a variety of microbial pathogens, and alter host defense in settings of infection by these pathogens. However, a number of observations from both *in vitro* and *in vivo* studies support the hypothesis that SP-A and SP-D play very distinct roles in pulmonary host defense, and that these differences are dependent on the nature of the pathogen. For example, SP-D does not alter clearance of GBS or *H. influenzae*, but is involved in promoting both IAV and RSV clearance in mice. SP-A does not appear to contribute to clearance of an SP-D–resistant IAV strain, but does enhance clearance of other viruses such as RSV. Differences in SP-A and SP-D structures may define the specific binding interaction of each collectin with specific pathogens. This interaction, especially in the case of SP-D–microbe interactions, may result in formation of large aggregates leading to more efficient clearance. Finally, although SP-A appears to modulate the proinflammatory environment in both bacterial and viral infections, SP-D also regulates levels of proinflammatory mediators in response to certain microbes, underscoring the overlap in functions of these collectins in the lung. More extensive comparative studies using SP-A– and SP-D–deficient mice should provide additional insights into the relative contribution of these collectins, specifically during viral infection and more generally in pulmonary host defense.

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