Eosinophil-Associated Lung Diseases
A Cry for Surfactant Proteins A and D Help?

Julie G. Ledford[1,2], Kenneth J. Addison[1], Matthew W. Foster[1], and Loretta G. Que[1]

[1]Department of Medicine, Division of Pulmonary, Allergy and Critical Care, and [2]Department of Cell Biology, Duke University Medical Center, Durham, North Carolina

Abstract

Surfactant proteins (SP)-A and SP-D (SP-A/-D) play important roles in numerous eosinophil-dominated diseases, including asthma, allergic bronchopulmonary aspergillosis, and allergic rhinitis. In these settings, SP-A/-D have been shown to modulate eosinophil chemotaxis, inhibit eosinophil mediator release, and mediate macrophage clearance of apoptotic eosinophils. Dysregulation of SP-A/-D function in eosinophil-dominated diseases is also not uncommon. Alterations in serum SP-A/-D levels are associated with disease severity in allergic rhinitis and chronic obstructive pulmonary disease. Furthermore, oligimerization of SP-A/-D, necessary for their proper function, can be perturbed by reactive nitrogen species, which are increased in eosinophilic disease. In this review, we highlight the associations of eosinophilic lung diseases with SP-A and SP-D levels and functions.

Keywords: eosinophil; surfactant; collectin; surfactant protein-A; surfactant protein-D

Clinical Relevance

This review summarizes the functions of surfactant proteins A and D (SP-A/-D) in the lung and proposes several mechanisms by which SP-A/-D may regulate eosinophil activity and function in inflammatory airway diseases. Understanding how SP-A/-D regulate airway inflammation will help us to develop novel strategies to treat lung diseases in which eosinophils play an important role in disease pathogenesis.

Surfactant proteins (SP-A, SP-B, SP-C, and SP-D) make up 5 to 10% of all pulmonary surfactant. They differ from one another in their synthesis, oligomerization, and function. Whereas SP-B and SP-C are hydrophobic and function to prevent alveolar collapse by reducing surface tension in the distal lung, SP-A and SP-D are hydrophilic and play unique and important roles in lung host defense. In this review we focus on SP-A and SP-D. SP-A and SP-D belong to the collectin (collagen-like lectins) family of proteins. Collectins have four unique regions: an N-terminal segment with one to three cysteine residues, a collagen-like region, an α-coiled neck region, and a C-terminal carbohydrate recognition domain (CRD). SP-A and SP-D monomers can undergo trimerization by disulfide crosslinking of their N-terminal domains and additional noncovalent hydrogen bonding. In addition, the neck region of SP-D, which is an α-helical coiled-coil with a centrally place tyrosine ring, can mediate oligomerization of its three CRDs into a trimeric assembly (1). Upon complete oligomerization, SP-D forms a cruciform-like structure composed of four-trimeric subunits (dodecamer), which can participate in higher orders of multimerization to form astral bodies (2), and SP-A assembles into a bouquet-like structure of six-trimeric subunits (octadecamer). Unlike SP-A, the collagen-like tails of SP-D are buried under normal conditions.

The CRDs of SP-A and SP-D mediate binding to a variety of ligands that exhibit glycosylation motifs, including pathogens, lipids, cells, and receptors. Despite the similarities and some shared functions of SP-A and SP-D,

(Received in original form March 10, 2014; accepted in final form June 17, 2014)

This work was supported by National Institutes of Health grants HL111151, AI81672, and HL107590.

Author Contributions: Conception and design: J.G.L., L.G.Q. Drafting and interpretation: J.G.L., K.J.A., M.W.F., L.G.Q. Final approval of edited version: J.G.L., K.J.A., M.W.F., L.G.Q.

Correspondence and requests for reprints should be addressed to Loretta G. Que, M.D., Department of Internal Medicine, Division of Pulmonary, Allergy and Critical Care, Duke University medical Center, 203 Research Drive, CB #2641, Durham, NC. E-mail: Loretta.Que@dm.duke.edu

Am J Respir Cell Mol Biol Vol 51, Iss 5, pp 604–614, Nov 2014

Originally Published in Press as DOI: 10.1165/rcmb.2014-0095TR on June 24, 2014
Internet address: www.atsjournals.org

they have distinct characteristics and roles as well (3). For decades, the most notable immune function of SP-A and SP-D was attributed to their opsonic activity to bind and aggregate pathogens via their CRDs and to aid in pathogen uptake by immune cells, such as macrophages and neutrophils. More recently, SP-A/-D have been shown to act as direct mediators of cellular signal transduction. Altered SP-A/-D levels are commonly observed as markers of airway and lung diseases. However, the clinical associations with disease are ambiguous as to whether SP-A/-D dysregulation/dysfunction is important for disease pathology or rather a byproduct of the diseased lung environment (4). In this review we highlight the association of altered surfactant protein levels in eosinophil-dominated lung diseases and speculate on possible mechanisms by which SP-A/-D may regulate or be regulated by eosinophil functions, thus highlighting a lesser-appreciated area of SP-A/-D immunobiology.

Roles of SP-A/-D in Cell-Mediated Immunity

Numerous studies have examined the roles of SP-A and SP-D as opsonins and their participation in pathogen phagocytosis (5–15). Most often, increased phagocytosis of opsonized pathogens is beneficial to the host as a mode of clearance. However, in some instances, pathogens are able to subvert the normal phagocyte clearance machinery and utilize the protective environment of the phagocyte. In these rare cases, enhanced SP-A/-D binding may be a risk factor contributing to worsening of disease and increased pathogen burden (16–19). As an example, SP-D–deficient mice infected with the fungus *Cryptococcus neoformans* (CN) have decreased pulmonary eosinophils and lower IL-5 levels in lung lavage fluid compared with control wild-type (WT) mice. Moreover, CN-infected SP-D mice have decreased fungal burden and improved survival compared with the WT controls (20). These findings support the concept that SP-D is a virulence factor for CN and facilitates its infection in mice. This is of clinical interest in the HIV-seropositive population, who are disproportionately infected by this fungus. Studies examining SP-D levels in lung fluid have found significantly higher levels of SP-D in HIV-seropositive/AIDs subjects with low CD4 T cell counts ($<$ 200 cells/μl) as compared with those with high CD4 T cell counts ($>$ 200 cells/μl) (21). Additionally, IL-5 is often reported to be elevated in HIV-seropositive individuals (22), further suggesting a link between SP-D regulation of IL-5 and risk for CN infection in HIV-seropositive individuals (22).

In addition to aiding in clearance of bacteria and viruses via opsonization, surfactant proteins bind to other biological/abiotic particles and to various cell populations and participate in their clearance from the pulmonary environment. SP-A has been shown to enhance phagocytosis of IgG-opsonized particles (23) and complement-coated particles (24) and to preferentially bind apoptotic neutrophils, which aides in their removal from the inflamed lung (25, 26). SP-D is known to aggregate and aid in the removal of pollen starch granules (27, 28), to bind and enhance clearance of genomic DNA and apoptotic cells (29), and to aggregate and remove nanoparticles (30).

Apart from the more traditional role of phagocytosis, neutrophils can extrude neutrophil extracellular traps (NETs) as a last mode of defense to directly combat microbes (31). NETs are composed of decondensed chromatin fibers coated with antimicrobial histones and granular proteins and can be released upon stimulation by a variety of agents (e.g., protozoa, fungi, viruses, bacteria, and endotoxin) (reviewed in Reference 32). Recent studies have demonstrated that SP-D can bind to NET-DNA and to bacteria simultaneously, thereby promoting bacterial trapping by the NETs (33). SP-D–NET binding is also thought to promote NET clearance by macrophages. Extracellular DNA traps can also be generated from eosinophils (eosinophil extracellular traps [EETs]) (34) and have been found in several eosinophil-associated diseases, including bronchial asthma, contact dermatitis, spirochetosis, and scabies (reviewed in Reference 35). Thus far, the function of SP-A/-D in mediating EETs has not been defined. However, SP-A/-D bind eosinophils, and, during allergic inflammation when functional SP-A/-D levels are decreased, the association with and regulation of eosinophils may be diminished. In the absence of functional SP-A/-D, we speculate that EETs will then be stimulated more readily by factors commonly elevated in the allergic lung environment, such as IL-5, thymic stromal lymphopoietin, eotaxin, and C5a (reviewed in Reference 35). Additionally, if either SP-A or SP-D aides in EET-DNA removal by macrophages, lack of functional SP-A/-D may lead to prolonged presence of EETs, which could contribute to worsened symptoms due to extracellular localization of cytotoxic granular agents.

SP-A/-D Role in Signaling

Although the globular head domain of SP-A/-D binds to pathogens and pathogen-associated molecular patterns, their collagen-like tails are left exposed to initiate phagocytosis by interactions with phagocytes. However, the interaction of the SP-A/-D tail region is also capable of initiating cellular signaling cascades by specific receptor interactions. Gardai and colleagues (36) showed that SP-A and SP-D bind SIRP-α through the globular head region to initiate a signaling pathway that attenuates proinflammatory cytokine production. In contrast, the collagenous tail, by interacting with CD91/calreticulin, stimulates proinflammatory mediator production.

As shown by Nguyen and colleagues (37), SP-A and surfactant lipids up-regulate IRAK-M, a negative regulator of Toll-like receptor (TLR)-mediated inflammation, and inhibit LPS-induced cytokine production in human macrophages. More recently, SP-A has been shown to mediate LPS–TLR-4 signaling by interactions with β-arrestin 2 (38). By enhancing the colocalization of TLR-4 with the post-Golgi compartment in alveolar macrophages, SP-A significantly reduces the LPS-induced colocalization of TLR-4 with the early endosome antigen, leading to attenuation of the proinflammatory response. SP-A is unable to exert this affect in β-arrestin $2^{-/-}$ mice, which demonstrates that SP-A modulates LPS-induced TLR-4 trafficking by interacting with β-arrestin 2.

Increases in exhaled nitric oxide (NO) are associated with eosinophilic inflammation and correlate with other indices of inflammation in asthma. Under these conditions, posttranslational modifications of SP-D appear to modulate the inflammatory properties of SP-D. For example, NO can modify key cysteine residues in the tail domain of SP-D, which leads to a dissociation of SP-D multimers into trimers, thus exposing the S-nitrosylated N-termini (*see* discussion below). This exposed S-nitrosylated tail domain can then bind to the calreticulin/CD91 receptor complex and potentiate this proinflammatory pathway, which occurs via phosphorylation of p38 and NF-κB activation (39).

Metabolism of SP-A/-D in the Normal Lung

The majority of SP-A is synthesized by alveolar type (AT)II cells in the distal airways. Newly synthesized SP-A is packaged into lamellar bodies by the ATII cells for storage and ensuing secretion via regulated exocytosis (40). Upon release of SP-A into the extracellular milieu, it begins to form tubular myelin (lipid transport system unique to the lungs) in conjunction with lipids (41–43). SP-A is not only synthesized and secreted from the ATII cells; it is also predominantly recycled by them (44, 45). Although SP-D is synthesized and secreted constitutively by ATII cells, it is also produced by nonciliated Clara cells in the upper airways (46). Similar to SP-A, SP-D release is regulated by granule exocytosis (47, 48). Although the majority of surfactant phospholipid and protein are removed from the alveolus by ATII cell uptake (49–51), evidence suggests that alveolar macrophages are also important participants in the uptake and degradation of exhausted surfactant protein (52–54). Uptake by macrophages and ATII cells is mediated predominantly by endocytosis via clathin-coated pits (55, 56).

Metabolism of SP-A/-D May Be Altered in Eosinophilic Diseases

Accumulating evidence suggests that dysregulated SP-A/-D metabolism and signaling play key roles in the pathobiology of lung diseases. Indeed, changes in SP-A/-D levels in serum (57) and bronchoalveolar lavage (BAL) are seen in a variety of lung diseases (Table 1). Below, we focus on eosinophilic diseases in which aberrant SP-A/-D levels or function have recently been implicated.

Asthma

Asthma is a common chronic disease of the airways characterized by inflammation and reversible airflow obstruction caused by a combination of genetic and environmental factors. Based on studies in murine models of allergic inflammation and in studies of patients with asthma, it has become increasingly evident that SP-A and SP-D play important regulatory roles in allergic airways diseases. Mice challenged with OVA, house dust mite (HDM), or fungi have alterations in SP-A/-D levels at the height of eosinophilia (58–60). An increase in SP-A/-D during eosinophilic inflammation is likely a key defense mechanism for eosinophil regulation: SP-D inhibits eosinophil chemotaxis, SP-A and SP-D bind eosinophils and attenuate degranulation, and SP-A suppresses IL-8 production from eosinophils (61–63). In support of these findings, when mice lacking SP-A or SP-D are challenged with allergen, they develop severely enhanced eosinophilia compared with WT control mice and display worsened symptoms of allergic airways inflammation tied to Th2-dominant disease (64, 65).

In studies of segmental antigen challenge in atopic and individuals with asthma, the ability of total surfactant (lipoproteins and proteins) extracted from the antigen-challenged lobe was found to be dysfunctional in its ability to maintain airway patency as compared with surfactant from a control (saline challenged) lobe (66, 67). Normal activity could be achieved by removing water-soluble inhibitors from the extracted surfactant, which was attributed to leakage of plasma proteins into the lumen during inflammation. Studies have demonstrated that plasma proteins, albumin, and fibrinogen impair surfactant function at physiological concentrations (68). Additionally, a product released from activated eosinophils, eosinophil cationic protein, had a profound effect on the arrangement of phospholipids within the surfactant biofilm due to what appeared to be unwinding of the lamellar bodies (68). Normally, SP-A will partially protect from this surfactant-inhibiting effect. However, in chronic inflammatory lung conditions such as asthma, functional SP-A levels may be decreased and unable to adequately regulate this interaction. Indeed, SP-A isolated from subjects with asthma has been shown to be less effective at inhibiting Muc5AC and IL-8 production by stimulated epithelial cells versus SP-A isolated from normal healthy control subjects (69). Although the mechanisms for asthma-derived SP-A dysfunction are unclear, the increased eosinophilia and eosinophil-derived factors (eosinophil peroxidase, eosinophil cationic protein, eosinophil associated RNase, and major basic protein) associated with Th2-predominant asthma may alter SP-A oligomerization and render SP-A incapable of carrying out normal host protective functions.

Acute Eosinophilic Pneumonia

Acute eosinophilic pneumonia (AEP) is a rare disease of unknown etiology characterized by acute respiratory failure, bilateral infiltrates, hypoxemia ($Pa_{O_2} < 60$ mm Hg), and eosinophilic infiltration of the lung (70). Unfortunately, no animal models for AEP exist. Although the pathophysiology of AEP is unknown, eosinophils are believed to play a role because they comprise greater than 25% of BAL cells and because IL-5 and IL-1rα are detected at increased levels in the BAL of affected patients (71). Levels of SP-A and SP-D in BAL and serum are reported to be significantly elevated in patients with AEP compared with healthy control subjects (72). It is not clear what the implications of these observations are for the pathobiology of AEP. One may speculate that, during AEP, the increase in eosinophilia would lead to SP-A/-D breakdown and dysfunction, and therefore, as a compensatory mechanism, more SP-A/-D would be produced and secreted in an attempt to regulate eosinophil activities.

Allergic Bronchopulmonary Aspergillosis

The ability of SP-A and SP-D to interact with the glycosylated antigens and

Table 1. Bronchoalveolar Lavage Surfactant Proteins in Human Lung Disease

Lung Diseases	Change in SP-A/-D	Number of Subjects	Reference
Lung diseases with increased SP-A/-D	–		
Asthma*	↑↑SP-A asthma (6.1 ± 0.8 μg/ml); versus healthy control subjects (4.4 ± 0.5 μg/ml); and	*n* = 10 patients with asthma; *n* = 11 healthy control subjects	139
	↑↑SP-D asthma (0.62 ± 0.04 μg/ml) versus healthy control subjects (0.42 ± 0.06 μg/ml)	*n* = 10 patients with asthma; *n* = 11 healthy control subjects	139
Asthma (segmental challenge)*	↑↑SP-D	*n* = 23 patients with asthma; *n* = 10 healthy control subjects	140
	‖SP-A		
AEP*	↑↑SP-A AEP (3.461 μg/ml ± 2.449) versus healthy control subjects (1.209 ± 28 μg/ml)	*n* = 5 patients with AEP; *n* = 7 healthy control subjects	72
	↑↑SP-D AEP (1.693 μg/ml ± 2.233) versus healthy control subjects (0.196 ± 0.0024 μg/ml)	*n* = 5 patients with AEP; *n* = 7 healthy control subjects	72
Pulmonary Langerhans cell histiocytosis*	↑↑SP-A versus healthy control subjects or COPD	*n* = 1 each	141
HP*	↑↑SP-A (8 ± 0.7 μg/ml) HP versus healthy control subjects (4 ± 0.3 μg/ml)	*n* = 10 patients with HP; *n* = 21 healthy control subjects	91
PAP	↑↑SP-A PAP (39.3 ± 12.5 μg/l) versus healthy control subjects (3.5 ± 1.1 μg/ml)	*n* = 6 patients with PAP; *n* = 13 healthy control subjects	142
Lung diseases with decreased SP-A/-D			
Asbestos exposure	↑↑SP-D PAP (19.3 ± 9.3 μg/ml) versus healthy control subjects (0.88 ± 0.13 μg/ml)	*n* = 9 patients with PAP; *n* = 28 healthy control subjects	143
ARDS	↓↓SP-A ARDS (29.88 ± 6.18 μg/ml) versus healthy control subjects (123.64 ± 118.04 μg/ml)	*n* = 67 patients with ARDS; *n* = 29 healthy control subjects	144
COPD/smoker	↓↓SP-D never-smoker (0.558 ± 309 μg/ml) versus smoker/COPD (0.235 ± 0.203 μg/ml)	*n* = 65 never-smokers; *n* = 22 COPD/ smokers	99
IPCD	≈SP-D IPCD (0.62 ± 0.16 μg/ml) versus healthy control subjects (0.88 ± 0.13 μg/ml)	*n* = 7 patients with IPCD; *n* = 28 healthy control subjects	143
IPF*	≈SP-D IPF (0.58 ± 0.06 μg/ml) versus healthy control subjects (0.88 ± 0.13 μg/ml)	*n* = 33 patients with IPF; *n* = 28 healthy control subjects	143
	↓↓SP-A IPF (1.13 ± 0.252 μg/ml) versus healthy control subjects (1.53 ± 0.136 μg/ml)	*n* = 34 patients with IPF; *n* = 25 healthy control subjects	93
Sarcoidosis	≈SP-D sarcoidosis (0.97 ± 0.08 μg/ml) versus healthy control subjects (0.88 ± 0.13 μg/ml)	*n* = 60 patients with sarcoidosis; *n* = 28 healthy control subjects	143
	↑↑SP-A sarcoidosis (9.0 ± 1.7 μg/ml) versus healthy control subjects (4.0 ± 0.3 μg/ml)	*n* = 35 patients with sarcoidosis; *n* = 21 healthy control subjects	91
Smokers	↓↓SP-A smoker (1.8 ± 0.4 μg/ml) versus nonsmoker (3.1 ± 0.4 μg/ml)	*n* = 10 nonsmokers; *n* = 8 smokers	100
	↓↓SP-D smoker (0.5 ± 0.1 μg/ml) versus nonsmoker (1.3 ± 0.2 μg/ml)	*n* = 12 nonsmokers; *n* = 7 smokers	100
	↓↓SP-D never-smoker (0.558 ± 309 μg/ml) versus smoker (0.287 ± 0.204 μg/ml)	*n* = 65 never-smokers; *n* = 23 smokers	99

Definition of abbreviations: AEP, acute eosinophilic pneumonia; ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; HP, hypersensitivity pneumonitis; IPCD, interstitial pneumonia with collagen disease; IPF, idiopathic pulmonary fibrosis; PAP, pulmonary alveolar proteinosis; SP-A/-D, surfactant proteins A and D.
*Eosinophilic disease.

allergens of the fungal pathogen *Aspergilous fumigatus* (AFU) to inhibit specific IgE binding to these allergens makes them an attractive therapeutic target for AFU-associated diseases (73–75). Murine models of pulmonary hypersensitivity induced by AFU immunologically resemble the human disease allergic bronchopulmonary

aspergillosis (ABPA) a condition characterized by serum and pulmonary eosinophilia, hypersensitivity to AFU, and increased total IgE. These models have successfully established a protective role for SP-A and SP-D in the treatment of ABPA. Expression of SP-D is increased in BAL in AFU-sensitized mice (76) and in serum of humans with ABPA (77). Ablation of SP-A and SP-D leads to enhanced eosinophilia and increases total IgE in AFU-sensitized mice (65, 78). Additionally, evidence is accumulating that genetic polymorphisms of SP-A1 and SP-A2 are associated with increased susceptibility to ABPA (79, 80). Moreover, treatment of AFU-sensitized WT and SP-A– and SP-D–deficient mice with "rescue" SP-A and SP-D, respectively, has been shown to suppress IgE levels, eosinophilia, cellular inflammation in the lung, and shift the pathogenic TH2 cytokine profile to a protective TH1 profile (75, 81).

Allergic Rhinitis

Allergic rhinitis (AR) is an IgE-mediated chronic inflammatory disease characterized by the recruitment of eosinophils, basophils, and T cells expressing TH2 cytokines to the nasal mucosa (82). In a murine model of AR, treatment with exogenous SP-A decreased eosinophil number in nasal epithelium, corrected the TH1/TH2 imbalance, and blocked ovalbumin (OVA)-specific IgE (83); these findings strongly suggest a protective role for SP-A in AR.

In humans, SP-A, -B, -C, and -D are components of healthy nasal mucosa and have been shown to increase with inflammation, with the exception of SP-C (84). Expression of SP-A is much higher in patients with AR and nasal polyps than in control subjects, and the level of SP-A positively correlates with eosinophil number within the basement membrane of epithelium (85). Because these findings are somewhat in contrast to studies in mice showing that a decrease in eosinophilia results when exogenous SP-A is given, one may speculate that the increased level of SP-A detected in the patients with AR is compensating for defective SP-A, which is unable to adequately regulate eosinophil functions. This potentially defective SP-A may be a direct product of the inflammatory milieu associated with activated eosinophils and their proteolytic mediators that can alter the oligomeric structure of SP-A. A lack of protective SP-A would likely lead to localized enhanced production of SP-A in an attempt to compensate for the decreased immune function.

To date, only SP-A expression in nasal mucosa has been shown to correlate with severity of disease as measured by the Rhinitis Symptom Utility Index in patients with AR (86), suggesting that it plays a key role in the inflammatory process regulating AR and nasal polyp formation in these patients. Indeed, early studies using liposomes, which consist of phospholipids that make up 75% of the nasal surfactant layer, have been found to be comparable to cromoglycolate therapy in the treatment of patients with AR (87). In patients with chronic rhinosinusitis associated with cystic fibrosis, expression of SP-A, SP-B, and SP-D is also increased (88, 89).

Idiopathic Lung Disease

Interstitial lung diseases are a heterogeneous group of lung diseases resulting from damage to lung parenchyma by varying patterns of inflammation and fibrosis. These diseases involve not only the interstitium of the lung but also the vessels and airways and are frequently associated with increased BAL eosinophils and peripheral blood eosinophilia (90). Hypersensitivity pneumonitis (HP), also known as extrinsic allergic alveolitis, is an immune mediated interstitial lung disease induced by inhalation of antigens to which a person is already sensitized. The clinical presentation and radiographic imaging findings of HP overlap broadly with other interstitial lung diseases, making it difficult to diagnosis, especially because identification of the antigen exposure is often challenging. Elevated serum SP-A levels have been reported in patients diagnosed with HP and idiopathic pulmonary fibrosis (IPF), an interstitial lung disease of unknown etiology (91–93); however, SP-A levels are not consistently elevated in the BAL of these patients (93). Findings in IPF show a significant negative correlation between BAL SP-A levels and the presence of BAL eosinophils. Low levels of BAL SP-A in subjects with enhanced eosinophilia associated with IPF suggest that SP-A may be involved in the regulation of eosinophil recruitment, survival, or resolution in the lung in response to environmental stresses (94). An alternative explanation could be that in patients with IPF with associated eosinophilia, degradation of SP-A occurs, lowering the detectable levels of SP-A.

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease (COPD) is generally considered to be a neutrophilic disease. However, there is increasing evidence to suggest that a subgroup of patients with stable COPD exists that have chronic airway eosinophilia and steroid responsive disease (95, 96).

Cigarette smoke is the major risk factor for COPD and is known to adversely affect surfactant (97, 98). Decreased levels of BAL SP-A and SP-D have been detected in healthy smokers compared with nonsmoking control subjects. The decreased concentration of SP-A and SP-D in lung lavage in smokers (99, 100) is speculated to impair the host defense functions of surfactant in the peripheral airways and may contribute to the development of chronic obstructive lung disease.

In patients with COPD, sputum SP-A/-D (101) and serum SP-D levels associate with lung function and with health status (102) and increase significantly during COPD exacerbations (103), suggesting that SP-D may be a biomarker of disease severity for COPD. In a recent candidate gene association study by Kim and colleagues, SP-D was identified as one of two risk loci for COPD from among circulating COPD biomarkers measured in the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) study (104). These findings support the relevance of SP-D in the pathogenesis of COPD. There is no direct evidence linking changes in SP-A/-D expression/function and eosinophil activity in COPD. However, cigarette smoke has been shown to lead to disruption of the quaternary structure

of SP-D (105), which supports the "translocation hypothesis" that dissociation of pulmonary SP-D into smaller subunits can lead to rapid increases in serum SP-D. Because SP-D has been shown to inhibit eosinophil chemotaxis (62), a loss of pulmonary-associated SP-D may facilitate subsequent recruitment of eosinophils into the airspace and induced sputum, as observed in smokers with COPD (106).

Alterations of SP-A/-D in Eosinophil-Predominant Diseases

SP-A and SP-D come into contact with a wide range of inhaled allergens. Binding of SP to these foreign allergens leads to a competitive interaction for cell-sequestered IgE, resulting in an attenuation of mast cell degranulation. HDM allergens, a major cause of allergic asthma in developed countries, have cysteine protease activity that leads to degradation of SP-A and SP-D under physiological conditions (107). More generally, SP-A and SP-D can be degraded by a range of host- and pathogen-derived proteases, including *Pseudomonas aeruginosa* and human neutrophil elastases (108, 109), cathepsin-S (110), and matrix metalloproteinase-9 (111), and it is therefore plausible that increased proteolytic activity might serve to inactivate SP-A in eosinophil-dominated diseases.

SP-D and SP-A oligomeric structures can be altered by HDM protease, and SP-D is altered by many of the neutrophil derived proteinases (elastase, proteinase-3, and cathepsin-G) (107, 112). These altered oligomers limit the SP ability to bind carbohydrates and agglutinate bacteria. In addition, reactive oxygen and nitrogen species may also affect posttranslational modifications of SP-D that alter its structure and function at sites of inflammation (39, 113). For example, NO, produced locally by airway epithelial cells and increased in asthma (114), has been shown to S-nitrosylate two free Cys thiols in the N-terminus of dodecameric SP-D (Cys-15 and Cys-20) and to affect dissociation into SP-D trimers (115). Consequences of this structural perturbation include the activation of SP-D proinflammatory chemoattractant function and the loss of control TLR4 blockade (39). Tyrosine nitration of SP-A and SP-D and Tyr-Tyr crosslinking of SP-D have been also found in protein exposed *in vitro* to peroxynitrite (116–119) and in inflammatory lung diseases (120). Nitration of SP-A has been detected at Tyr164, Tyr166, and Tyr220 (116), and these modifications decrease lectin activity (117, 121). Reactive nitrogen species cause nitration of SP-D at Tyr341 and crosslinking of Tyr228 (119). In addition to increases in FeNO, increased tyrosine nitration has been demonstrated in allergic asthma (122), which may be a specific activity of eosinophils and eosinophil peroxidase (123–125). Thus, although the effects associated with posttranslational modification of Tyr have not been link to a particular oligomeric state, these data nonetheless suggest an additional mechanism for modulation of SP-D activity under conditions of eosinophilic inflammation.

Modification of SP-D oligimerization and Tyr crosslinking have been investigated in the context of allergic asthma (126). SP-D in BAL fluid collected from subjects with asthma before and after allergen challenge or the combination of allergen and LPS challenge had increases in both monomeric and in tyrosine-crosslinked SP-D. These effects, which were correlated with high

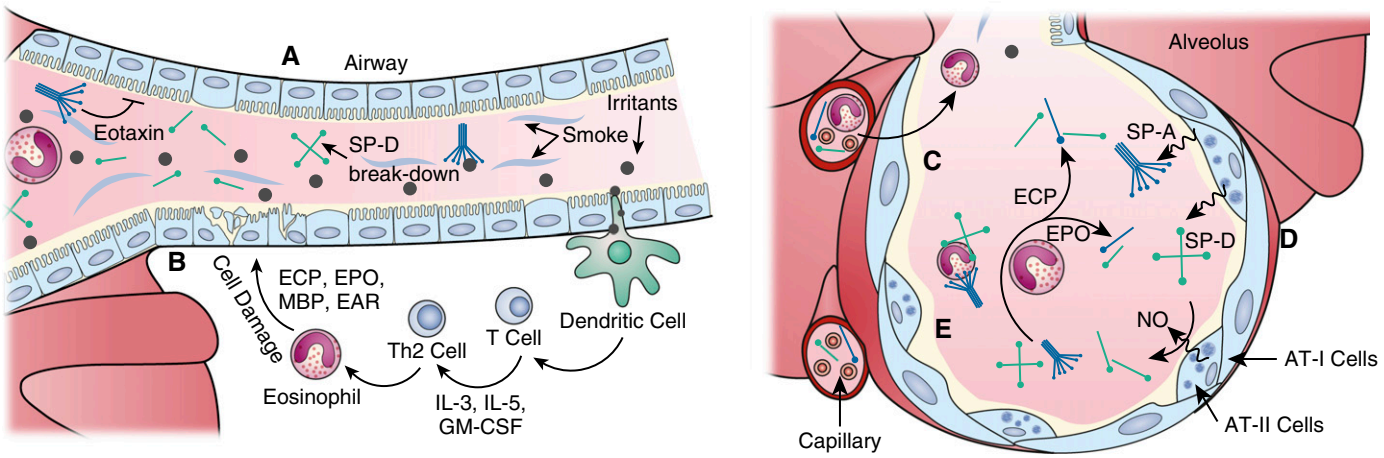

Figure 1. Proposed model of eosinophil associated lung disease and surfactant protein A and D regulation. (*A*) In the airway, irritants are bound and cleared by surfactant protein (SP)-A, and cigarette smoke can alter the multimeric structure of SP-D. (*B*) Cytokines (IL-3, IL-5, and granulocyte macrophage-colony stimulating factor [GM-CSF]) released by T cells recruit eosinophils to the lung. Unbound eosinophils release proteolytic enzymes (eosinophil cationic protein [ECP], eosinophil peroxidase [EPO], major basic protein [MBP], and eosinophil associated RNase [EAR]) that cleave the multimeric structure of SP, resulting in free trimmers, and modify SP-A/-D activity. (*C*) In the distal airway, the inflammatory lung milieu (increased NO production) and the degradative enzymes released from the eosinophils (i.e., ECP, EPO) impair epithelial barrier permeability and SP-A/-D leak from the lumen into the tissue. (*D*) In an attempt to modulate eosinophilia, AT II cells increase synthesis and secretion of SP-A/-D. (*E*) Carbohydrate recognition domains of SP-A/-D multimeric structures (SP-A, octadecamer; SP-D, dodecamer) bind to eosinophils and prevent degranulation and cytokine production.

levels of BAL eosinophils, NO, SNO–SP-D, and Th2-dominant cytokines, were not observed after challenge of nonasthmatics or with LPS alone. These data suggest, for the first time, a correlation between regulatory posttranslational modifications of SP-D and allergic airway inflammation.

In addition to posttranslational modification, surfactant levels are altered after subsegmental allergen challenge in asthma (67). Also, purified eosinophil cationic protein has been shown to alter surfactant structure and function (68, 127), although these *in vitro* studies used Alveofact (Boehringer Ingelheim, Ingelheim, Germany), a natural bovine surfactant devoid of SP-A (128). Leakage of plasma and serum proteins in the airway space, which is more likely during lung inflammation, can accelerate the conversion of functional surfactant large aggregates into poorly functional small aggregates (129). This conversion is typically regulated by SP-A and is consistent with dysfunctional SP-A in asthma.

Possibility of Therapeutic Surfactants in Eosinophilic Diseases

Although surfactant therapy in preterm neonates had dramatically changed their long-term outcome, the current formulation is devoid of SP-A and SP-D. Additionally, preterm neonates often suffer from respiratory distress syndrome and have a higher risk for development of infection and bronchopulmonary dysplasia. Given our current knowledge of the protective roles of SP-A and SP-D in these areas, clinicians using new surfactant therapies may consider the addition of SP in the treatment of these patients.

Much of what we know about the potential use of SP-A/-D therapy has arisen from studies using SP-A– or SP-D–deficient mice that are given exogenous SP as a "rescue" treatment. Although the oligomeric structure of SP-A and SP-D is thought to be essential for some SP function, several research publications have also found specific regions and fragments with activity. An animal model using AFU-allergen in SP-D–deficient mice shows that exogenous SP-D treatment given to the mice can rescue their allergic phenotype (75, 130). Additional studies have determined that SP-D given as a full-sized dodecamer oligomer or as a shorter fragment containing the trimeric subunit and CRD are capable of alleviating allergic inflammation in an OVA mouse model (131). Likewise, SP-D fragments were capable of reducing early airway responses to AFU allergen and led to significantly decreased airway hyperresponsiveness, eosinophilia, and histamine levels as compared with placebo (132). In fact, studies examining the mechanisms behind the difference in allergic airways phenotype between Balb/c and C57Bl/6 mice revealed that SP-D levels were significantly elevated in the C57BL/6 mice as compared with Balb/c mice, which could account for the attenuation of response (133).

Fewer studies have examined the consequence of SP-A rescue therapy in allergic airways. Although SP-D is commonly synthesized recombinantly from cell lines, SP-A is typically extracted from the lavage of patients with alveolar proteinosis. Several studies have given SP-A from APP as a rescue in other murine models, including the bleomycin model of pulmonary fibrosis and in LPS-driven inflammation. In both cases, exogenous given SP-A led to "rescue" of the innate and adaptive immune phenotypes observed in SP-A–deficient mice (134–137). More recently, Awasthi and colleagues has found that a specific fragment of SP-A (20-mer) has the ability to inhibit LPS-induced cytokine production and lung inflammation by interaction with TLR-4 (138). This finding suggests the possibility of other SP-A fragments that may be active against a specific receptor target for which SP-A is known to associate, such as TLR-2, SIRP-α, and CD91. Because SP-A and SP-D are known to bind the FCgRII/III (CD16/32) complex, this receptor should also be examined in SP-A/-D–mediated eosinophil activities, including cytokine regulation, degranulation, apoptosis, and chemotaxis.

Conclusions

Pulmonary surfactant proteins are critical in mediating a variety of immune and physiological responses during health and disease. Many lung diseases associated with eosinophilia also have dysregulated SP-A/-D metabolism, as detected by altered levels in serum, BAL, or both. Although the etiology of altered levels of SP-A/-D is unclear in each of the diseases mentioned, SP-A and SP-D bind eosinophils and regulate their degranulation. Additionally, SP-D inhibits eosinophil chemotaxis, and SP-A binds apoptotic eosinophils and aides in their engulfment by macrophages. A proposed model (Figure 1) suggests that a common underlying mechanism for dealing with enhanced eosinophilia may be to up-regulate SP-A/-D production. Alternatively, eosinophil-derived proteases, environmental irritants, or NO produced in the inflamed lung milieu may alter the oligomeric structures and render SP-A/-D dysfunctional.

Although rescue treatments that give exogenous full-length or peptides of SP-A or SP-D have shown promising results in allergic animal models, to the best of our knowledge, no studies have examined the therapeutic potential of purified SP-A/-D or targeted SP-A/-D peptides in human lung diseases in which eosinophils are thought to play an important role in pathogenesis. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

References

1. Hakansson K, Lim NK, Hoppe HJ, Reid KB. Crystal structure of the trimeric alpha-helical coiled-coil and the three lectin domains of human lung surfactant protein D. *Structure* 1999;7:255–264.
2. Crouch E, Persson A, Chang D, Heuser J. Molecular structure of pulmonary surfactant protein D (SP-D). *J Biol Chem* 1994;269: 17311–17319.
3. Wright JR. Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 2005;5:58–68.
4. Winkler C, Hohlfeld JM. Surfactant and allergic airway inflammation. *Swiss Med Wkly* 2013;143:w13818.
5. LeVine AM, Elliott J, Whitsett JA, Srikiatkhachorn A, Crouch E, DeSilva N, Korfhagen T. Surfactant protein-d enhances phagocytosis and pulmonary clearance of respiratory syncytial virus. *Am J Respir Cell Mol Biol* 2004;31:193–199.

6. Ofek I, Mesika A, Kalina M, Keisari Y, Podschun R, Sahly H, Chang D, McGregor D, Crouch E. Surfactant protein D enhances phagocytosis and killing of unencapsulated phase variants of Klebsiella pneumoniae. *Infect Immun* 2001;69:24–33.
7. Restrepo CI, Dong Q, Savov J, Mariencheck WI, Wright JR. Surfactant protein D stimulates phagocytosis of Pseudomonas aeruginosa by alveolar macrophages. *Am J Respir Cell Mol Biol* 1999;21:576–585.
8. Schagat TL, Tino MJ, Wright JR. Regulation of protein phosphorylation and pathogen phagocytosis by surfactant protein A. *Infect Immun* 1999;67:4693–4699.
9. LeVine AM, Kurak KE, Wright JR, Watford WT, Bruno MD, Ross GF, Whitsett JA, Korfhagen TR. Surfactant protein-A binds group B streptococcus enhancing phagocytosis and clearance from lungs of surfactant protein-A-deficient mice. *Am J Respir Cell Mol Biol* 1999;20:279–286.
10. Tino MJ, Wright JR. Surfactant protein A stimulates phagocytosis of specific pulmonary pathogens by alveolar macrophages. *Am J Physiol* 1996;270:L677–L688.
11. Geertsma MF, Nibbering PH, Haagsman HP, Daha MR, van Furth R. Binding of surfactant protein A to C1q receptors mediates phagocytosis of Staphylococcus aureus by monocytes. *Am J Physiol* 1994;267:L578–L584.
12. Sakai K, Kweon MN, Kohri T, Kishino Y. Effects of pulmonary surfactant and surfactant protein A on phagocytosis of fractionated alveolar macrophages: relationship to starvation. *Cell Mol Biol* 1992;38:123–130.
13. Manz-Keinke H, Plattner H, Schlepper-Schafer J. Lung surfactant protein A (SP-A) enhances serum-independent phagocytosis of bacteria by alveolar macrophages. *Eur J Cell Biol* 1992;57: 95–100.
14. van Iwaarden JF, van Strijp JA, Ebskamp MJ, Welmers AC, Verhoef J, van Golde LM. Surfactant protein A is opsonin in phagocytosis of herpes simplex virus type 1 by rat alveolar macrophages. *Am J Physiol* 1991;261:L204–L209.
15. Tenner AJ, Robinson SL, Borchelt J, Wright JR. Human pulmonary surfactant protein (SP-A), a protein structurally homologous to C1q, can enhance FcR- and CR1-mediated phagocytosis. *J Biol Chem* 1989;264:13923–13928.
16. Geunes-Boyer S, Oliver TN, Janbon G, Lodge JK, Heitman J, Perfect JR, Wright JR. Surfactant protein D increases phagocytosis of hypocapsular Cryptococcus neoformans by murine macrophages and enhances fungal survival. *Infect Immun* 2009;77:2783–2794.
17. Gaynor CD, McCormack FX, Voelker DR, McGowan SE, Schlesinger LS. Pulmonary surfactant protein A mediates enhanced phagocytosis of Mycobacterium tuberculosis by a direct interaction with human macrophages. *J Immunol* 1995; 155:5343–5351.
18. Koziel H, Phelps DS, Fishman JA, Armstrong MY, Richards FF, Rose RM. Surfactant protein-A reduces binding and phagocytosis of pneumocystis carinii by human alveolar macrophages in vitro. *Am J Respir Cell Mol Biol* 1998;18:834–843.
19. Yong SJ, Vuk-Pavlovic Z, Standing JE, Crouch EC, Limper AH. Surfactant protein D-mediated aggregation of Pneumocystis carinii impairs phagocytosis by alveolar macrophages. *Infect Immun* 2003;71:1662–1671.
20. Holmer SM, Evans KS, Asfaw YG, Saini D, Schell WA, Ledford JG, Frothingham R, Wright JR, Sempowski GD, Perfect JR. Impact of surfactant protein D, interleukin-5, and eosinophilia on Cryptococcosis. *Infect Immun* 2014;82:683–693.
21. Jambo KC, French N, Zijlstra E, Gordon SB. AIDS patients have increased surfactant protein D but normal mannose binding lectin levels in lung fluid. *Respir Res* 2007;8:42.
22. Paganelli R, Scala E, Mazzone AM, Rosso R, Mattiacci G, Dell'Anna L, Mezzaroma I, Aiuti F. Th2-type cytokines, hypereosinophilia, and interleukin-5 in HIV disease. *Allergy* 1997;52:110–111.
23. Wofford JA, Wright JR. Surfactant protein A regulates IgG-mediated phagocytosis in inflammatory neutrophils. *Am J Physiol Lung Cell Mol Physiol* 2007;293:L1437–L1443.
24. Watford WT, Smithers MB, Frank MM, Wright JR. Surfactant protein A enhances the phagocytosis of C1q-coated particles by alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2002;283:L1011–L1022.
25. Schagat TL, Wofford JA, Wright JR. Surfactant protein A enhances alveolar macrophage phagocytosis of apoptotic neutrophils. *J Immunol* 2001;166:2727–2733.
26. Reidy MF, Wright JR. Surfactant protein A enhances apoptotic cell uptake and TGF-beta1 release by inflammatory alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2003;285:L854–L861.
27. Erpenbeck VJ, Malherbe DC, Sommer S, Schmiedl A, Steinhilber W, Ghio AJ, Krug N, Wright JR, Hohlfeld JM. Surfactant protein D increases phagocytosis and aggregation of pollen-allergen starch granules. *Am J Physiol Lung Cell Mol Physiol* 2005;288: L692–L698.
28. Winkler C, Huper K, Wedekind AC, Rochlitzer S, Hartwig C, Muller M, Braun A, Krug N, Hohlfeld JM, Erpenbeck VJ. Surfactant protein D modulates pulmonary clearance of pollen starch granules. *Exp Lung Res* 2010;36:522–530.
29. Palaniyar N, Clark H, Nadesalingam J, Hawgood S, Reid KB. Surfactant protein D binds genomic DNA and apoptotic cells, and enhances their clearance, in vivo. *Ann N Y Acad Sci* 2003; 1010:471–475.
30. Kendall M, Ding P, Mackay RM, Deb R, McKenzie Z, Kendall K, Madsen J, Clark H. Surfactant protein D (SP-D) alters cellular uptake of particles and nanoparticles. *Nanotoxicology* 2013;7: 963–973.
31. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. *Science* 2004;303:1532–1535.
32. Cheng OZ, Palaniyar N. NET balancing: a problem in inflammatory lung diseases. *Front Immunol* 2013;4:1.
33. Douda DN, Jackson R, Grasemann H, Palaniyar N. Innate immune collectin surfactant protein D simultaneously binds both neutrophil extracellular traps and carbohydrate ligands and promotes bacterial trapping. *J Immunol* 2011;187:1856–1865.
34. Yousefi S, Gold JA, Andina N, Lee JJ, Kelly AM, Kozlowski E, Schmid I, Straumann A, Reichenbach J, Gleich GJ, *et al*. Catapult-like release of mitochondrial DNA by eosinophils contributes to antibacterial defense. *Nat Med* 2008;14:949–953.
35. Yousefi S, Simon D, Simon HU. Eosinophil extracellular DNA traps: molecular mechanisms and potential roles in disease. *Curr Opin Immunol* 2012;24:736–739.
36. Gardai SJ, Xiao YQ, Dickinson M, Nick JA, Voelker DR, Greene KE, Henson PM. By binding SIRPalpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 2003;115:13–23.
37. Nguyen HA, Rajaram MV, Meyer DA, Schlesinger LS. Pulmonary surfactant protein A and surfactant lipids upregulate IRAK-M, a negative regulator of TLR-mediated inflammation in human macrophages. *Am J Physiol Lung Cell Mol Physiol* 2012;303: L608–L616.
38. Sender V, Lang L, Stamme C. Surfactant protein-A modulates LPS-induced TLR4 localization and signaling via beta-arrestin 2. *PLoS ONE* 2013;8:e59896.
39. Atochina-Vasserman EN. S-nitrosylation of surfactant protein D as a modulator of pulmonary inflammation. *Biochim Biophys Acta* 2012;1820:763–769.
40. Fisher AB, Dodia C, Ruckert P, Tao JQ, Bates SR. Pathway to lamellar bodies for surfactant protein A. *Am J Physiol Lung Cell Mol Physiol* 2010;299:L51–L58.
41. Williams MC, Hawgood S, Hamilton RL. Changes in lipid structure produced by surfactant proteins SP-A, SP-B, and SP-C. *Am J Respir Cell Mol Biol* 1991;5:41–50.
42. Poulain FR, Allen L, Williams MC, Hamilton RL, Hawgood S. Effects of surfactant apolipoproteins on liposome structure: implications for tubular myelin formation. *Am J Physiol* 1992;262:L730–L739.
43. Froh D, Ballard PL, Williams MC, Gonzales J, Goerke J, Odom MW, Gonzales LW. Lamellar bodies of cultured human fetal lung: content of surfactant protein A (SP-A), surface film formation and structural transformation in vitro. *Biochim Biophys Acta* 1990;1052:78–89.

44. Madsen J, Tornoe I, Nielsen O, Koch C, Steinhilber W, Holmskov U. Expression and localization of lung surfactant protein A in human tissues. *Am J Respir Cell Mol Biol* 2003;29:591–597.
45. Wong CJ, Akiyama J, Allen L, Hawgood S. Localization and developmental expression of surfactant proteins D and A in the respiratory tract of the mouse. *Pediatr Res* 1996;39:930–937.
46. Crouch EC. Collectins and pulmonary host defense. *Am J Respir Cell Mol Biol* 1998;19:177–201.
47. Crouch E, Parghi D, Kuan SF, Persson A. Surfactant protein D: subcellular localization in nonciliated bronchiolar epithelial cells. *Am J Physiol* 1992;263:L60–L66.
48. Voorhout WF, Veenendaal T, Kuroki Y, Ogasawara Y, van Golde LM, Geuze HJ. Immunocytochemical localization of surfactant protein D (SP-D) in type II cells, Clara cells, and alveolar macrophages of rat lung. *J Histochem Cytochem* 1992;40: 1589–1597.
49. Chander A, Reicherter J, Fisher AB. Degradation of dipalmitoyl phosphatidylcholine by isolated rat granular pneumocytes and reutilization for surfactant synthesis. *J Clin Invest* 1987;79: 1133–1138.
50. Fisher AB, Chander A, Reicherter J. Uptake and degradation of natural surfactant by isolated rat granular pneumocytes. *Am J Physiol* 1987;253:C792–C796.
51. Fisher AB, Dodia C, Chander A. Alveolar uptake of lipid and protein components of surfactant. *Am J Physiol* 1991;261: L334–L340.
52. Bates SR, Fisher AB. Surfactant protein A is degraded by alveolar macrophages. *Am J Physiol* 1996;271:L258–L266.
53. Gurel O, Ikegami M, Chroneos ZC, Jobe AH. Macrophage and type II cell catabolism of SP-A and saturated phosphatidylcholine in mouse lungs. *Am J Physiol Lung Cell Mol Physiol* 2001; 280:L1266–L1272.
54. Wright JR. Clearance and recycling of pulmonary surfactant. *Am J Physiol* 1990;259:L1–L12.
55. Baritussio A, Alberti A, Armanini D, Meloni F, Bruttomesso D. Different pathways of degradation of SP-A and saturated phosphatidylcholine by alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol* 2000;279:L91–L99.
56. Jain D, Dodia C, Fisher AB, Bates SR. Pathways for clearance of surfactant protein A from the lung. *Am J Physiol Lung Cell Mol Physiol* 2005;289:L1011–L1018.
57. Griese M. Pulmonary surfactant in health and human lung diseases: state of the art. *Eur Respir J* 1999;13:1455–1476.
58. Wang JY, Shieh CC, Yu CK, Lei HY. Allergen-induced bronchial inflammation is associated with decreased levels of surfactant proteins A and D in a murine model of asthma. *Clin Exp Allergy* 2001;31:652–662.
59. Haley KJ, Ciota A, Contreras JP, Boothby MR, Perkins DL, Finn PW. Alterations in lung collectins in an adaptive allergic immune response. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L573–L584.
60. Kasper M, Sims G, Koslowski R, Kuss H, Thuemmler M, Fehrenbach H, Auten RL. Increased surfactant protein D in rat airway goblet and Clara cells during ovalbumin-induced allergic airway inflammation. *Clin Exp Allergy* 2002;32: 1251–1258.
61. Ledford JG, Mukherjee S, Kislan MM, Nugent JL, Hollingsworth JW, Wright JR. Surfactant protein-A suppresses eosinophil-mediated killing of Mycoplasma pneumoniae in allergic lungs. *PLoS ONE* 2012;7:e32436.
62. von Bredow C, Hartl D, Schmid K, Schabaz F, Brack E, Reinhardt D, Griese M. Surfactant protein D regulates chemotaxis and degranulation of human eosinophils. *Clin Exp Allergy* 2006;36: 1566–1574.
63. Cheng G, Ueda T, Nakajima H, Nakajima A, Arima M, Kinjyo S, Fukuda T. Surfactant protein A exhibits inhibitory effect on eosinophils IL-8 production. *Biochem Biophys Res Commun* 2000;270:831–835.
64. Pastva AM, Mukherjee S, Giamberardino C, Hsia B, Lo B, Sempowski GD, Wright JR. Lung effector memory and activated CD4+ T cells display enhanced proliferation in surfactant protein A-deficient mice during allergen-mediated inflammation. *J Immunol* 2011;186:2842–2849.
65. Madan T, Reid KB, Singh M, Sarma PU, Kishore U. Susceptibility of mice genetically deficient in the surfactant protein (SP)-A or SP-D gene to pulmonary hypersensitivity induced by antigens and allergens of Aspergillus fumigatus. *J Immunol* 2005;174: 6943–6954.
66. Jarjour NN, Enhorning G. Antigen-induced airway inflammation in atopic subjects generates dysfunction of pulmonary surfactant. *Am J Respir Crit Care Med* 1999;160:336–341.
67. Hohlfeld JM, Ahlf K, Enhorning G, Balke K, Erpenbeck VJ, Petschallies J, Hoymann HG, Fabel H, Krug N. Dysfunction of pulmonary surfactant in asthmatics after segmental allergen challenge. *Am J Respir Crit Care Med* 1999;159:1803–1809.
68. Schmiedl A, Krug N, Hohlfeld JM. Influence of plasma and inflammatory proteins on the ultrastructure of exogenous surfactant. *J Electron Microsc (Tokyo)* 2004;53:407–416.
69. Wang Y, Voelker DR, Lugogo NL, Wang G, Floros J, Ingram JL, Chu HW, Church TD, Kandasamy P, Fertel D, *et al*. Surfactant protein A is defective in abrogating inflammation in asthma. *Am J Physiol Lung Cell Mol Physiol* 2011;301:L598–L606.
70. Shorr AF, Scoville SL, Cersovsky SB, Shanks GD, Ockenhouse CF, Smoak BL, Carr WW, Petruccelli BP. Acute eosinophilic pneumonia among US military personnel deployed in or near Iraq. *JAMA* 2004;292:2997–3005.
71. Allen JN, Liao Z, Wewers MD, Altenberger EA, Moore SA, Allen ED. Detection of IL-5 and IL-1 receptor antagonist in bronchoalveolar lavage fluid in acute eosinophilic pneumonia. *J Allergy Clin Immunol* 1996;97:1366–1374.
72. Daimon T, Tajima S, Oshikawa K, Bando M, Ohno S, Sugiyama Y. KL-6 and surfactant proteins A and D in serum and bronchoalveolar lavage fluid in patients with acute eosinophilic pneumonia. *Intern Med* 2005;44:811–817.
73. Wright JR. Immunomodulatory functions of surfactant. *Physiol Rev* 1997;77:931–962.
74. Reid KB. Interactions of surfactant protein D with pathogens, allergens and phagocytes. *Biochim Biophys Acta* 1998;1408: 290–295.
75. Madan T, Kishore U, Singh M, Strong P, Clark H, Hussain EM, Reid KB, Sarma PU. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by Aspergillus fumigatus antigens and allergens. *J Clin Invest* 2001;107: 467–475.
76. Haczku A, Atochina EN, Tomer Y, Chen H, Scanlon ST, Russo S, Xu J, Panettieri RA Jr, Beers MF. Aspergillus fumigatus-induced allergic airway inflammation alters surfactant homeostasis and lung function in BALB/c mice. *Am J Respir Cell Mol Biol* 2001;25:45–50.
77. Krane M, Griese M. Surfactant protein D in serum from patients with allergic bronchopulmonary aspergillosis. *Eur Respir J* 2003;22:592–595.
78. Kishore U, Kojouharova MS, Reid KB. Recent progress in the understanding of the structure-function relationships of the globular head regions of C1q. *Immunobiology* 2002;205: 355–364.
79. Saxena S, Madan T, Shah A, Muralidhar K, Sarma PU. Association of polymorphisms in the collagen region of SP-A2 with increased levels of total IgE antibodies and eosinophilia in patients with allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 2003;111:1001–1007.
80. Madan T, Kaur S, Saxena S, Singh M, Kishore U, Thiel S, Reid KB, Sarma PU. Role of collectins in innate immunity against aspergillosis. *Med Mycol* 2005;43:S155–S163.
81. Strong P, Reid KB, Clark H. Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of Aspergillus fumigatus. *Clin Exp Immunol* 2002;130:19–24.
82. Broide DH. Allergic rhinitis: pathophysiology. *Allergy Asthma Proc* 2010;31:370–374.
83. Ren J, Deng Y, Xiao B, Wang G, Tao Z. Protective effects of exogenous surfactant protein A in allergic rhinitis: a mouse model. *Ann Otol Rhinol Laryngol* 2013;122:240–246.

84. Schicht M, Knipping S, Hirt R, Beileke S, Sel S, Paulsen F, Brauer L. Detection of surfactant proteins A, B, C, and D in human nasal mucosa and their regulation in chronic rhinosinusitis with polyps. *Am J Rhinol Allergy* 2013;27:24–29.
85. Deng Y, Zuo J, Tao Z, Kong Y, Xiao B. Expression and significance of SP-A in nasal mucosa of allergic rhinitis and nasal polyp (In Chinese]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2009;23:642–645.
86. Wootten CT, Labadie RF, Chen A, Lane KF. Differential expression of surfactant protein A in the nasal mucosa of patients with allergy symptoms. *Arch Otolaryngol Head Neck Surg* 2006;132:1001–1007.
87. Bohm M, Avgitidou G, El Hassan E, Mosges R. Liposomes: a new non-pharmacological therapy concept for seasonal-allergic-rhinoconjunctivitis. *Eur Arch Otorhinolaryngol* 2012;269: 495–502.
88. Woodworth BA, Wood R, Baatz JE, Schlosser RJ. Sinonasal surfactant protein A1, A2, and D gene expression in cystic fibrosis: a preliminary report. *Head Neck Surg* 2007;137:34–38.
89. Woodworth BA, Wood R, Bhargave G, Cohen NA, Baatz JE, Schlosser RJ. Surfactant protein B detection and gene expression in chronic rhinosinusitis. *Laryngoscope* 2007;117:1296–1301.
90. Allen JN, Davis WB, Pacht ER. Diagnostic significance of increased bronchoalveolar lavage fluid eosinophils. *Am Rev Respir Dis* 1990;142:642–647.
91. Hamm H, Luhrs J, Guzman y Rotaeche J, Costabel U, Fabel H, Bartsch W. Elevated surfactant protein A in bronchoalveolar lavage fluids from sarcoidosis and hypersensitivity pneumonitis patients. *Chest* 1994;106:1766–1770.
92. Sterclova M, Vasakova M, Paluch P, Paulik M. Surfactant protein A in chronic extrinsic allergic alveolitis. *Eur Respir J* 2012;39: 1543–1544.
93. Gunther A, Schmidt R, Nix F, Yabut-Perez M, Guth C, Rosseau S, Siebert C, Grimminger F, Morr H, Velcovsky HG, *et al*. Surfactant abnormalities in idiopathic pulmonary fibrosis, hypersensitivity pneumonitis and sarcoidosis. *Eur Respir J* 1999;14:565–573.
94. Okamoto T, Miyazaki Y, Shirahama R, Tamaoka M, Inase N. Proteome analysis of bronchoalveolar lavage fluid in chronic hypersensitivity pneumonitis. *Allergol Int* 2012;61:83–92.
95. Scott KA, Wardlaw AJ. Eosinophilic airway disorders. *Semin Respir Crit Care Med* 2006;27:128–133.
96. Soter S, Barta I, Antus B. Predicting sputum eosinophilia in exacerbations of COPD using exhaled nitric oxide. *Inflammation* 2013;36:1178–1185.
97. Lusuardi M, Capelli A, Carli S, Tacconi MT, Salmona M, Donner CF. Role of surfactant in chronic obstructive pulmonary disease: therapeutic implications. *Respiration* 1992;59:28–32.
98. Scott JE. The pulmonary surfactant: impact of tobacco smoke and related compounds on surfactant and lung development. *Tob Induc Dis* 2004;2:3–25.
99. Heinrich SM, Griese M. Assessment of surfactant protein A (SP-A) dependent agglutination. *BMC Pulm Med* 2010;10:59.
100. Honda Y, Takahashi H, Kuroki Y, Akino T, Abe S. Decreased contents of surfactant proteins A and D in BAL fluids of healthy smokers. *Chest* 1996;109:1006–1009.
101. Ishikawa N, Hattori N, Tanaka S, Horimasu Y, Haruta Y, Yokoyama A, Kohno N, Kinnula VL. Levels of surfactant proteins A and D and KL-6 are elevated in the induced sputum of chronic obstructive pulmonary disease patients: a sequential sputum analysis. *Respiration* 2011;82:10–18.
102. Ju CR, Liu W, Chen RC. Serum surfactant protein D: biomarker of chronic obstructive pulmonary disease. *Dis Markers* 2012; 32:281–287.
103. Shakoori TA, Sin DD, Ghafoor F, Bashir S, Bokhari SN. Serum surfactant protein D during acute exacerbations of chronic obstructive pulmonary disease. *Dis Markers* 2009;27:287–294.
104. Kim DK, Cho MH, Hersh CP, Lomas DA, Miller BE, Kong X, Bakke P, Gulsvik A, Agusti A, Wouters E. Genome-wide association analysis of blood biomarkers in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012;186:1238–1247.
105. Winkler C, Atochina-Vasserman EN, Holz O, Beers MF, Erpenbeck VJ, Krug N, Roepcke S, Lauer G, Elmlinger M, Hohlfeld JM. Comprehensive characterisation of pulmonary and serum surfactant protein D in COPD. *Respir Res* 2011;12:29.
106. Domagala-Kulawik J, Maskey-Warzechowska M, Kraszewska I, Chazan R. The cellular composition and macrophage phenotype in induced sputum in smokers and ex-smokers with COPD. *Chest* 2003;123:1054–1059.
107. Deb R, Shakib F, Reid K, Clark H. Major house dust mite allergens Dermatophagoides pteronyssinus 1 and Dermatophagoides farinae 1 degrade and inactivate lung surfactant proteins A and D. *J Biol Chem* 2007;282:36808–36819.
108. Mariencheck WI, Alcorn JF, Palmer SM, Wright JR. Pseudomonas aeruginosa elastase degrades surfactant proteins A and D. *Am J Respir Cell Mol Biol* 2003;28:528–537.
109. Liau DF, Yin NX, Huang J, Ryan SF. Effects of human polymorphonuclear leukocyte elastase upon surfactant proteins in vitro. *Biochim Biophys Acta* 1996;1302:117–128.
110. Lecaille F, Naudin C, Sage J, Joulin-Giet A, Courty A, Andrault PM, Veldhuizen RA, Possmayer F, Lalmanach G. Specific cleavage of the lung surfactant protein A by human cathepsin S may impair its antibacterial properties. *Int J Biochem Cell Biol* 2013;45:1701–1709.
111. Bratcher PE, Weathington NM, Nick HJ, Jackson PL, Snelgrove RJ, Gaggar A. MMP-9 cleaves SP-D and abrogates its innate immune functions in vitro. *PLoS ONE* 2012;7:e41881.
112. Hirche TO, Crouch EC, Espinola M, Brokelman TJ, Mecham RP, DeSilva N, Cooley J, Remold-O'Donnell E, Belaaouaj A. Neutrophil serine proteinases inactivate surfactant protein D by cleaving within a conserved subregion of the carbohydrate recognition domain. *J Biol Chem* 2004;279:27688–27698.
113. Atochina-Vasserman EN, Beers MF, Gow AJ. Review: chemical and structural modifications of pulmonary collectins and their functional consequences. *Innate Immun.* 2010;16:175–182.
114. Yamamoto M, Tochino Y, Chibana K, Trudeau JB, Holguin F, Wenzel SE. Nitric oxide and related enzymes in asthma: relation to severity, enzyme function and inflammation. *Clin Exp Allergy* 2012;42:760–768.
115. Guo CJ, Atochina-Vasserman EN, Abramova E, Foley JP, Zaman A, Crouch E, Beers MF, Savani RC, Gow AJ. S-nitrosylation of surfactant protein-D controls inflammatory function. *PLoS Biol* 2008;6:e266.
116. Greis KD, Zhu S, Matalon S. Identification of nitration sites on surfactant protein A by tandem electrospray mass spectrometry. *Arch Biochem Biophys* 1996;335:396–402.
117. Zhu S, Haddad IY, Matalon S. Nitration of surfactant protein A (SP-A) tyrosine residues results in decreased mannose binding ability. *Arch Biochem Biophys* 1996;333:282–290.
118. Haddad IY, Zhu S, Ischiropoulos H, Matalon S. Nitration of surfactant protein A results in decreased ability to aggregate lipids. *Am J Physiol* 1996;270:L281–L288.
119. Matalon S, Shrestha K, Kirk M, Waldheuser S, McDonald B, Smith K, Gao Z, Belaaouaj A, Crouch EC. Modification of surfactant protein D by reactive oxygen-nitrogen intermediates is accompanied by loss of aggregating activity, in vitro and in vivo. *FASEB J* 2009;23:1415–1430.
120. Zhu S, Ware LB, Geiser T, Matthay MA, Matalon S. Increased levels of nitrate and surfactant protein a nitration in the pulmonary edema fluid of patients with acute lung injury. *Am J Respir Crit Care Med* 2001;163:166–172.
121. Zhu S, Kachel DL, Martin WJ II, Matalon S. Nitrated SP-A does not enhance adherence of Pneumocystis carinii to alveolar macrophages. *Am J Physiol* 1998;275:L1031–L1039.
122. Ghosh S, Erzurum SC. Nitric oxide metabolism in asthma pathophysiology. *Biochim Biophys Acta* 2011;1810:1008–1016.
123. MacPherson JC, Comhair SA, Erzurum SC, Klein DF, Lipscomb MF, Kavuru MS, Samoszuk MK, Hazen SL. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. *J Immunol* 2001;166:5763–5772.
124. Iijima H, Duguet A, Eum SY, Hamid Q, Eidelman DH. Nitric oxide and protein nitration are eosinophil dependent in allergen-challenged mice. *Am J Respir Crit Care Med* 2001;163:1233–1240.

125. Duguet A, Iijima H, Eum SY, Hamid Q, Eidelman DH. Eosinophil peroxidase mediates protein nitration in allergic airway inflammation in mice. *Am J Respir Crit Care Med* 2001;164:1119–1126.
126. Atochina-Vasserman EN, Winkler C, Abramova H, Schaumann F, Krug N, Gow AJ, Beers MF, Hohlfeld JM. Segmental allergen challenge alters multimeric structure and function of surfactant protein D in humans. *Am J Respir Crit Care Med* 2011;183:856–864.
127. Hohlfeld JM, Schmiedl A, Erpenbeck VJ, Venge P, Krug N. Eosinophil cationic protein alters pulmonary surfactant structure and function in asthma. *J Allergy Clin Immunol* 2004; 113:496–502.
128. Bernhard W, Mottaghian J, Gebert A, Rau GA, von Der HH, Poets CF. Commercial versus native surfactants: surface activity, molecular components, and the effect of calcium. *Am J Respir Crit Care Med* 2000;162:1524–1533.
129. Ueda T, Ikegami M, Jobe A. Surfactant subtypes: in vitro conversion, in vivo function, and effects of serum proteins. *Am J Respir Crit Care Med* 1994;149:1254–1259.
130. Madan T, Kishore U, Singh M, Strong P, Hussain EM, Reid KB, Sarma PU. Protective role of lung surfactant protein D in a murine model of invasive pulmonary aspergillosis. *Infect Immun* 2001;69:2728–2731.
131. Lin KW, Jen KY, Suarez CJ, Crouch EC, Perkins DL, Finn PW. Surfactant protein D-mediated decrease of allergen-induced inflammation is dependent upon CTLA4. *J Immunol* 2010;184: 6343–6349.
132. Erpenbeck VJ, Ziegert M, Cavalet-Blanco D, Martin C, Baelder R, Glaab T, Braun A, Steinhilber W, Luettig B, Uhlig S, *et al*. Surfactant protein D inhibits early airway response in Aspergillus fumigatus-sensitized mice. *Clin Exp Allergy* 2006; 36:930–940.
133. Atochina EN, Beers MF, Tomer Y, Scanlon ST, Russo SJ, Panettieri RA Jr, Haczku A. Attenuated allergic airway hyperresponsiveness in C57BL/6 mice is associated with enhanced surfactant protein (SP)-D production following allergic sensitization. *Respir Res* 2003;4:15.
134. Goto H, Ledford JG, Mukherjee S, Noble PW, Williams KL, Wright JR. The role of surfactant protein A in bleomycin-induced acute lung injury. *Am J Respir Crit Care Med* 2010; 181:1336–1344.
135. Mukherjee S, Giamberardino C, Thomas JM, Gowdy K, Pastva AM, Wright JR. Surfactant protein A modulates induction of regulatory T cells via TGF-beta. *J Immunol* 2012;188: 4376–4384.
136. Mukherjee S, Giamberardino C, Thomas J, Evans K, Goto H, Ledford JG, Hsia B, Pastva AM, Wright JR. Surfactant protein A integrates activation signal strength to differentially modulate T cell proliferation. *J Immunol* 2012;188:957–967.
137. Borron P, McIntosh JC, Korfhagen TR, Whitsett JA, Taylor J, Wright JR. Surfactant-associated protein A inhibits LPS-induced cytokine and nitric oxide production in vivo. *Am J Physiol Lung Cell Mol Physiol* 2000;278:L840–L847.
138. Ramani V, Madhusoodhanan R, Kosanke S, Awasthi S. A TLR4-interacting SPA4 peptide inhibits LPS-induced lung inflammation. *Innate Immun* 2013;19:596–610.
139. Cheng G, Ueda T, Numao T, Kuroki Y, Nakajima H, Fukushima Y, Motojima S, Fukuda T. Increased levels of surfactant protein A and D in bronchoalveolar lavage fluids in patients with bronchial asthma. *Eur Respir J* 2000;16:831–835.
140. Erpenbeck VJ, Fischer I, Wiese K, Schaumann F, Schmiedl A, Nassenstein C, Krug N, Hohlfeld JM. Therapeutic surfactants modulate the viability of eosinophils and induce inflammatory mediator release. *Int Arch Allergy Immunol* 2009;149: 333–342.
141. Ghafouri B, Persson HL, Tagesson C. Intriguing bronchoalveolar lavage proteome in a case of pulmonary langerhans cell histiocytosis. *Am J Case Rep* 2013;14:129–133.
142. Honda Y, Takahashi H, Shijubo N, Kuroki Y, Akino T. Surfactant protein-A concentration in bronchoalveolar lavage fluids of patients with pulmonary alveolar proteinosis. *Chest* 1993;103: 496–499.
143. Honda Y, Kuroki Y, Matsuura E, Nagae H, Takahashi H, Akino T, Abe S. Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. *Am J Respir Crit Care Med* 1995;152:1860–1866.
144. Gregory TJ, Longmore WJ, Moxley MA, Whitsett JA, Reed CR, Fowler AA III, Hudson LD, Maunder RJ, Crim C, Hyers TM. Surfactant chemical composition and biophysical activity in acute respiratory distress syndrome. *J Clin Invest* 1991;88: 1976–1981.