

# Host Defense Functions of Pulmonary Surfactant

Jo Rae Wright

Department of Cell Biology, Duke University Medical Center, Durham, N.C., USA

## Key Words

Pulmonary surfactants · Collectins · Immunity · Surfactant protein A · Surfactant protein D

## Abstract

Surfactant is a complex of lipids and proteins that reduces surface tension at the air/liquid interface of the lung and regulates immune cell function. Surfactant immune function is primarily attributed to two proteins: SP-A and SP-D. SP-A and SP-D are members of a protein family known as 'collectins', which are distinguished by their N-terminal collagen-like region and their C-terminal lectin domain. The lectin domain binds preferentially to sugars on the surface of pathogens and thereby opsonizes them for uptake by phagocytes. The collectins also modulate the functions of cells of the adaptive immune network including dendritic cells and T lymphocytes. In addition, recent studies show that bacterial products degrade surfactant. In summary, surfactant plays an important role in lung host defense. Surfactant degradation or inactivation may contribute to enhanced susceptibility to lung inflammation and infection.

Copyright © 2004 S. Karger AG, Basel

Supported by NIH, HL 51134, ML 61285 and HL 30923.

## KARGER

Fax +41 61 306 12 34  
E-Mail [karger@karger.ch](mailto:karger@karger.ch)  
[www.karger.com](http://www.karger.com)

© 2004 S. Karger AG, Basel

Accessible online at:  
[www.karger.com/bon](http://www.karger.com/bon)

## Surfactant Is a Complex of Lipids and Proteins That Reduces Surface Tension and Participates in Host Defense against Infection and Inflammation

Surfactant components are synthesized primarily by two types of airway cells: the alveolar type II cell, which produces surfactant lipids and all four surfactant proteins, SP-A, SP-B, SP-C, and SP-D [1, 2], and the airway Clara cell which also synthesizes surfactant proteins, SP-A, SP-B and SP-D, but does not produce surfactant lipids [3–5]. It is not known if the secretory products of both cells carry out similar functions, although it seems reasonable to speculate that the surfactant produced by alveolar type II cells is primarily responsible for reducing surface tension since the Clara cell does not appear to synthesize surfactant lipids. In addition, message has been detected in a variety of non-pulmonary tissues including trachea, brain, testis, salivary gland, heart, prostate gland, kidney, and pancreas [6–8], although it is not yet clear if all of these organs synthesize significant amounts of protein.

Surfactant has two distinct functions. First, it reduces surface tension at the air-liquid interface of the lung. This function requires an appropriate mix of surfactant lipids and the hydrophobic proteins, SP-B and SP-C [9]; SP-B deficiencies have been associated with infant mortality due to respiratory distress syndrome [10, 11]. Second, surfactant also plays a role in host defense against infection

© Free Author Copy - for personal use only

ANY DISTRIBUTION OF THIS ARTICLE WITHOUT WRITTEN CONSENT FROM S. KARGER AG, BASEL IS A VIOLATION OF THE COPYRIGHT.

Written permission to distribute the PDF will be granted against payment of a permission fee, which is based on the number of accesses required. Please contact [permission@karger.ch](mailto:permission@karger.ch)

Jo Rae Wright  
Department of Cell Biology  
Box 3809, Duke University Medical Center  
Durham, NC 27710 (USA)  
Tel. +1 919 684 8040, Fax +1 919 684 8106, E-Mail [j.wright@cellbio.duke.edu](mailto:j.wright@cellbio.duke.edu)

and inflammation. Two of the surfactant proteins, SP-A and SP-D, are members of a family of innate immune proteins, known as collectins [12, 13]. Collectins, which include the liver-derived serum-mannose-binding lectin (MBL), all have an N-terminal collagen-like region and a C-terminal lectin domain that binds carbohydrates in a calcium-dependent manner. The preferential binding sites for the lectin domains are non-host oligosaccharides, such as those found on bacterial and viral surfaces [14]. The collectins play a key role in lung host defense by regulating cells of both the innate and adaptive immune systems.

### **Innate and Acquired Immunity Are Important in Pulmonary Host Defense**

The enormous surface area of the lung epithelium is constantly exposed to inhaled pathogens, particulates, allergens, and oxidant gases. Because it is optimally designed for gas exchange, this portal of entry to the entire body is a thin, delicate barrier that is susceptible to injury if an immune challenge is not contained and a proinflammatory state ensues. Thus, the presence of a pulmonary immune system is critical to lung host defense and maintenance of normal lung function.

*Innate Immunity.* The first line of host defense is provided by innate immunity, a phylogenetically ancient system utilizing germ-line-encoded proteins such as lysozyme, complement, and the collectin family of proteins which includes surfactant proteins SP-A and SP-D. Collectins interact with pathogens and immune cells to facilitate pathogen clearance by enhancing phagocytosis and by regulating production of cell-derived mediators (reviewed in [13, 15–17]).

*Adaptive Immunity.* The adaptive or acquired (e.g. antibody-mediated) immune system is a second line of host defense initiated when antigen is presented to lymphocytes either via the major histocompatibility complex class I (MHC I), which is present on all cells of the body, or class II (MHC II) which is present only on antigen presenting cells. Antigen-presenting cells internalize exogenous antigens, process and present peptide via MHC class II to T lymphocytes which respond by proliferating and producing effector cytokines that stimulate B cell division and differentiation into antibody-secreting plasma cells. In contrast, endogenous peptides derived from the cytoplasmic content of cells (e.g. viral peptides) are loaded on MHC class I, transported to the cell surface and presented to cytotoxic T killer cells which results in host cell death.

The adaptive immune response is much slower to mature than the innate response but once it is mature, it can result in rapid recruitment of inflammatory cells. Also, the adaptive response, unlike the innate response, is improved by repeat exposure which results in enhanced antibody levels.

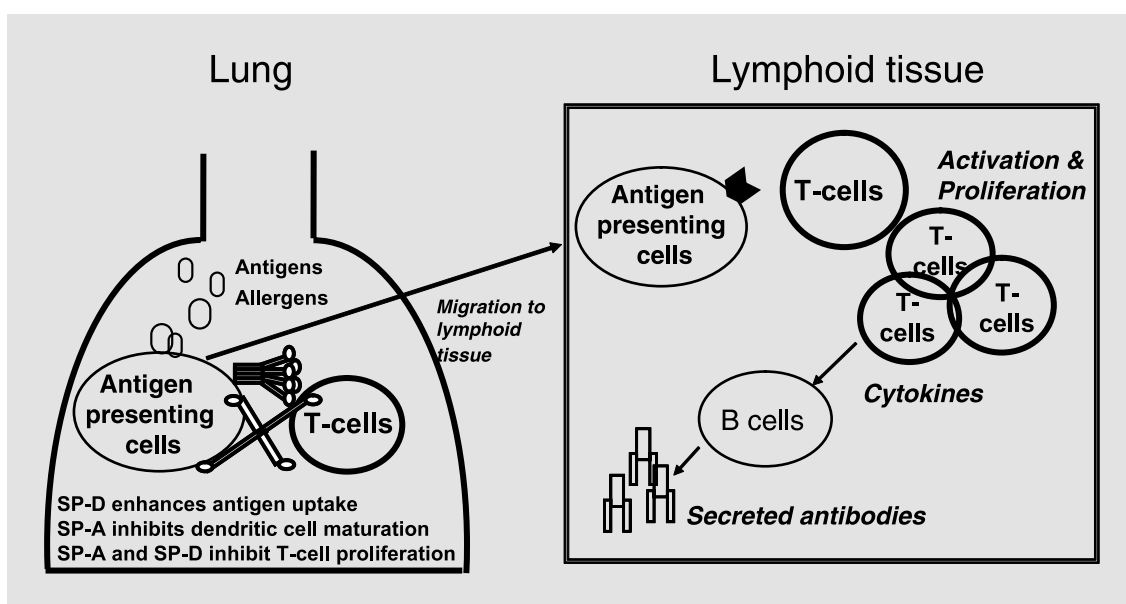
*Cross-Talk between Innate and Acquired Immunity.* Although innate and adaptive immunity have long been regarded as two separate and distinct host defense systems, several lines of evidence show that there is cross-talk between the two systems [18]. This cross-talk is mediated by the cells and regulatory molecules of the two immune systems including the collectins.

### **A Variety of Cells Participate in Innate and Adaptive Immunity in the Lung**

*Macrophages.* Macrophages are the primary immune cells in the alveolar space of the normal lung comprising approximately 95% of lavage cells. Alveolar macrophages are derived primarily from circulating monocytes through poorly understood mechanisms. Macrophages are highly phagocytic and clear a variety of inhaled pathogens including bacteria, viruses, particulates, allergens, as well as internalizing and degrading surfactant (reviewed in [15]). Although some tissue macrophages are effective at antigen presentation, alveolar macrophages are relatively poor antigen-presenting cells [19].

*Neutrophils, Eosinophils, Mast Cells.* During infection or inflammation the alveolar cell population increases by as much as 15-fold; in many inflammatory diseases the primary infiltrating cell is the neutrophil [20]. Neutrophils, also known as polymorphonuclear cells (PMNs) because of their irregularly shaped nuclei, are recruited by chemotactic factors such as complement and cytokines. They are highly phagocytic and contribute significantly to pathogen eradication by both oxidant dependent and independent pathways. Numbers of eosinophils and mast cells also increase during an immune challenge and these cells play an important role in allergic diseases such as asthma.

*Antigen-Presenting Cells.* The dendritic cell is the most potent antigen-presenting cell especially for initiating an adaptive immune response to naïve T cells. In the normal lung dendritic cells are found in airway epithelium, the lung parenchyma, the visceral pleura and the alveolar air-space [21–24]. There are relatively few dendritic cells in the alveolar space of the normal lung but challenge with ovalbumin antigen or bacillus Calmette-Guérin results in



**Fig. 1.** SP-A and SP-D modulate dendritic cell and T lymphocyte function. We propose a model in which SP-A and SP-D act specifically in the pulmonary compartment to both enhance antigen uptake and inhibit T lymphocyte proliferation. Also SP-A inhibits dendritic cell maturation. Migration and maturation of dendritic cells will result in their movement to the lymphoid tissue compartment where they can activate T cells and contribute to the adaptive immune response.

a 60-fold increase in dendritic cells (e.g. approximately  $2.5 \times 10^5$ ) recovered by lung lavage in rats [21, 25]. Dendritic cell function varies with the state of maturation [26]. Immature dendritic cells, such as those found in lung tissue, are highly phagocytic. When stimulated by cytokines, such as granulocyte-monocyte colony-stimulating factor (GM-CSF) or by bacterial products such as lipopolysaccharide (LPS), dendritic cells mature, become less phagocytic and exhibit increased expression of MHC molecules and a concomitant increase in the ability to present antigen. In vivo and in vitro studies suggest that dendritic cells in the alveolar space and the interstitium internalize inhaled antigen and migrate to lung lymph nodes where they can present antigen to T cells residing there [24, 27, 28]. The role of dendritic cells in allergic inflammation has been demonstrated [29–31] and was recently reviewed [32].

**Lymphocytes.** The total number of T cells in the lung is estimated to be greater than the total number of T cells in peripheral blood [33]. The alveolar pool in humans is estimated to be  $5 \times 10^8$  cells [34] and the number of alveolar lymphocytes increases in response to an immune or inflammatory challenge. It has been documented that lymphocytes can migrate from the alveolar space to the

draining bronchial lymph nodes where they can interact with antigen-presenting cells [35]. Ansfield et al. [36] reported more than two decades ago that lymphocytes in the alveolar compartment are hyporesponsive with respect to their ability to proliferate when stimulated with mitogen compared to lymphocytes from circulating blood. Part of this hyporesponsiveness is due to the fact that many of the lavage lymphocytes are memory T cells [37, 38]. However, part of this hyporesponsiveness is also mediated by surfactant phospholipids [36] and proteins [39–41], secretory products of alveolar macrophages (e.g. prostaglandin  $E_2$ , superoxide and vitamin D metabolites [19, 42]) and unidentified secretory products of type II cells [43]. An important recent study by Seitzman et al. [44] confirmed that lung lymphocytes proliferate minimally in vivo in response to an antigen challenge. Since lymphocyte proliferation is a key factor in propagation and expansion of the adaptive immune response [45], it has been proposed that suppression of lymphocyte proliferation in the alveolar space protects the host from tissue damage and inflammation that would occur if the T cells were constantly activated [46]. In fact, excessive T cell responses have been associated with a variety of lung diseases including asthma and sarcoidosis [47, 48].

**Table 1.** SP-A and SP-D affect the functions of a variety of immune cells

Cell type	Collectin	Function	Reference
Macrophages	SP-A and SP-D	phagocytosis, chemotaxis, cytokine and radical production	reviewed in 14, 15
Neutrophils	SP-A and SP-D	phagocytosis, chemotaxis, cytokine and radical production	reviewed in 14, 15
Eosinophils	SP-A	IL-8 production	64
Lymphocytes	SP-A and SP-D	inhibits proliferation induced by mitogen and allergens	39–41, 63, 87
Dendritic cells	SP-D	enhances antigen uptake and presentation	61
	SP-A	inhibits dendritic cell maturation	62

### Surfactant Proteins Play an Important Role in Pulmonary Host Defense

Studies with SP-A- and SP-D-deficient mice show the lung collectins enhance bacterial and viral clearance and facilitate the resolution of lung injury and inflammation. For example, SP-A deficient mice infected with group B streptococcus [49, 50], *Pseudomonas aeruginosa* [51], or respiratory syncytial virus [52] clear the pathogens more slowly and have higher levels of proinflammatory cytokines in lavage fluid than do wild-type mice. In addition, both SP-A- and SP-D-deficient mice are more susceptible to acute lung injury induced by lipopolysaccharide (LPS) [53, 54].

Although the precise mechanisms by which the collectins mediate these responses are not known, a number of in vitro studies have shown that many of the immune cells in the lung are affected by collectins (table 1). For example, SP-A and SP-D stimulate phagocytosis and chemotaxis and regulate cytokine and free radical production by multiple immune cells. There is, however, some controversy surrounding these studies; it has been reported that SP-A both enhances and inhibits the production of inflammatory mediators such as TNF- $\alpha$  and nitric oxide. Although the sources of these conflicts are not totally clear, the type of cell and its state of activation may contribute to differential responses to SP-A [55]. In addition, the presence and type of pathogen will affect the cell's response to SP-A and possibly to SP-D [56, 57]. A recent important study by Gardai et al. [59] determined that the different effects of the collectins are mediated by different receptors. Both SP-A and SP-D were found to block inflammatory mediator production by binding to

signal regulatory protein  $\alpha$  (SIRP $\alpha$ ) via their lectin domains. In contrast, the collagen-like stalks of SP-A and SP-D stimulate proinflammatory mediator production by binding to calreticulin/CD91. Furthermore, the binding to calreticulin/CD91 was enhanced when the lectin domains were engaged by foreign particles or apoptotic cells. Thus, it appears that depending on the environment and associated pathogens SP-A and SP-D can elicit either pro- or anti-inflammatory effects.

SP-A and SP-D have also recently been shown to have direct anti-microbial activity [59]. Wu and colleagues [59] demonstrated that the lung collectins inhibit the growth of several strains of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes*. At least part of the growth inhibition was a consequence of increased membrane permeability induced by the C-terminal domains. A similar anti-fungal effect was also observed for *Histoplasma capsulatum* [60].

SP-A and SP-D both affect cells of the adaptive immune system including dendritic cells and T cells. Antigen uptake and presentation is enhanced by SP-D [61], whereas SP-A inhibits dendritic cell maturation [62]. Both SP-A and SP-D inhibit proliferation of lymphocytes induced by mitogens and allergens [63]. SP-A also inhibits the release of IL-8 by eosinophils [64]. A possible role for SP-A and SP-D in the pathogenesis of allergic inflammation was suggested by a report showing that SP-A and SP-D inhibit lymphocyte proliferation induced by dust mite allergen [63]. Importantly, this inhibition was observed with lymphocytes from stable asthmatic children and their age-matched controls, but the ability of SP-A and SP-D to inhibit proliferation was decreased when activated lymphocytes from asthmatic children with acute

asthmatic attacks were tested. In addition, SP-A and SP-D bind to water-extractable, allergenic glycoproteins from dust mites and purified allergens from *Aspergillus fumigatus*, both of which are associated with allergic disorders [63]. SP-A and SP-D inhibit binding of allergen-specific IgE to house dust mite extracts and inhibit histamine release from cells obtained from whole blood of asthmatic children [63], leading the authors to speculate that SP-A and SP-D may participate in modulation of allergen sensitization and/or the development of allergic reactions [3].

These studies in aggregate suggest that lung collectins contribute to pulmonary host defense by mediating cell specific responses of both the innate and adaptive immune systems.

### **Surfactant Levels Are Altered in Diseases Including Acute Lung Injury and Infection**

Surfactant levels are known to be altered in patients with a variety of lung diseases (reviewed in [65]). For example, SP-A is decreased in bronchoalveolar lung fluid of patients with acute respiratory distress syndrome (ARDS) [66], lung trauma [67], pneumonia [68] and pulmonary fibrosis [69] but is increased in patients with sarcoidosis [70], asbestosis [71] and hypersensitivity pneumonitis [72]. SP-D levels are decreased in patients with sarcoidosis, lung fibrosis and ARDS [73]. SP-B levels are also decreased in ARDS [66] and pneumonia [74]. Surfactant lipids are variably altered in a variety of lung diseases (reviewed in [65]). In aggregate, these data suggest that changes in surfactant pool sizes are not just a consequence of damage to the alveolar epithelium which would result in diminutions in all surfactant components. Rather, the data suggest that the metabolism of the different surfactant components is differentially affected by the disease process. To date, it is not clear if the changes that occur in disease are a cause or a consequence of the disease or more likely a combination of both.

Surfactant is specifically altered in patients with cystic fibrosis (CF) who are commonly colonized with *P. aeruginosa*, a major cause of morbidity and mortality in patients with cystic fibrosis and bronchiectasis (reviewed in [75]). For example, Postle et al. [76] reported that SP-A levels were decreased by approximately 5-fold in CF patients whereas SP-D levels were decreased by approximately 50-fold. In contrast, phospholipid levels and content were unaffected. Likewise, Griese and co-workers also reported diminished levels of SP-A in lung lavage fluid of CF patients with lung infections [77] and degradation prod-

ucts of SP-A were detected [78]. Meyer et al. [79] reported decreased association of SP-A with the lipid components of surfactant in patients with CF, although total SP-A levels were comparable in CF patients and normal volunteers. In contrast, Hull et al. [80] reported increased SP-A in lavage of CF patients with lung infections. Although the reasons for the differences are not known, the fact that the patients in Hull et al.'s studies were very young with a mean age of 23 months and the fact that the samples were collected by a smaller volume of lung lavage may have affected the outcome. Surfactant levels have also been reported to be altered in a rodent model of *P. aeruginosa* pneumonia [81].

Our recent studies suggest that reductions in surfactant protein levels during infection may be due in part to degradation by bacterial products. We found that both SP-A and SP-D are degraded by *P. aeruginosa* supernatants and that the activity can be attributed to elastase and other enzymes [82]. It has also been shown that human leukocyte elastase degrades SP-D [83]. In normal healthy individuals, inhaled *P. aeruginosa* is cleared by the mucociliary system and by uptake by phagocytes [84]. SP-A enhances the uptake of *P. aeruginosa* by phagocytes in both in vitro studies [85, 86] as well as in vivo studies demonstrating that SP-A-deficient mice are more susceptible to infection and inflammation induced by *P. aeruginosa* than are wild-type, SP-A replete mice [51]. Our findings that *P. aeruginosa* degrades SP-A suggest that the pathogen has evolved a novel virulence mechanism in the pathogenesis of pseudomonas infections.

In summary, the lung collectins have been shown both in vitro and in vivo to enhance pathogen clearance and to modulate the production of inflammatory mediators. These effects are likely to be a consequence of cell-specific regulation that is due at least in part to interaction of the collectins with specific receptors. Levels of surfactant proteins and lipids are altered in a variety of disease states and surfactant proteins are degraded by both bacterial and immune cell proteases. A decrease in the pool of functional surfactant will be likely to lead to altered lung homeostasis including decreased lung compliance and increased susceptibility to infection and inflammation. Inhibition of these processes and/or restoration of a functional pool of surfactant during acute lung injury or bacterial infections are possible mechanisms by which lung function may be restored.

## References

- 1 Wright JR, Clements JA: Metabolism and turnover of lung surfactant. *Am Rev Respir Dis* 1987;135:426-444.
- 2 Weaver TE: Pulmonary surfactant-associated proteins. *Gen Pharmacol* 1988;19:361-368.
- 3 Voorhout WF, Veenendaal T, Kuroki Y, Ogasawara Y, van Golde LMG, 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.
- 4 Kalina M, Mason RJ, Shannon JM: Surfactant protein C is expressed in alveolar type II cells but not in Clara cells of rat lung. *Am J Respir Cell Mol Biol* 1992;6:594-600.
- 5 Wang JX, Souza P, Kuliszewski M, Tanswell AK, Post M: Expression of surfactant proteins in embryonic rat lung. *Am J Respir Cell Mol Biol* 1994;10:222-229.
- 6 Rubio S, Lacaze-Masmonteil T, Chailley-Heu B, Kahn A, Bourbon JR, Ducroc R: Pulmonary surfactant protein A (SP-A) is expressed by epithelial cells of small and large intestine. *J Biol Chem* 1995;270:12162-12169.
- 7 Lin Z, Phelps D, deMello D, Page M, Coltun W, Floros J: Both human SP-A1 and SP-A2 genes are expressed in small and large intestine. *Am J Respir Crit Care Med* 2000;161:A43.
- 8 Madsen J, Kliem A, Tornoe I, Skjodt K, Koch C, Holmskov U: Localization of lung surfactant protein D on mucosal surfaces in human tissue. *J Immunol* 2000;164:5866-5870.
- 9 Whitsett JA, Nogee LM, Weaver TE, Horowitz AD: Human surfactant protein B: Structure, function, regulation, and genetic disease. *Physiol Rev* 1995;75:749-757.
- 10 Hamvas A, Nogee LM, deMello DE, Cole FS: Pathophysiology and treatment of surfactant protein-B deficiency. *Biol Neonate* 1995;67:18-31.
- 11 Williams GD, Christodoulou J, Stack J, Symons P, Wert SE, Murrell MJ, Nogee LM: Surfactant protein B deficiency: Clinical, histological and molecular evaluation. *J Paediatr Child Health* 1999;35:214-220.
- 12 Crouch E, Wright JR: Surfactant proteins A and D and pulmonary host defense. *Annu Rev Physiol* 2001;63:521-554.
- 13 Crouch E, Hartshorn K, Ofek I: Collectins and pulmonary innate immunity. *Immunol Rev* 2000;173:52-65.
- 14 Crouch EC: Collectins and pulmonary host defense. *Am J Respir Cell Mol Biol* 1998;19:177-201.
- 15 Wright JR: Immunomodulatory functions of surfactant. *Physiol Rev* 1997;77:931-962.
- 16 Crouch EC: Modulation of host-bacterial interactions by collectins. *Am J Respir Cell Mol Biol* 1999;21:558-561.
- 17 Lawson PR, Reid KB: The roles of surfactant proteins A and D in innate immunity. *Immunol Rev* 2000;173:66-78.
- 18 Janeway CA Jr: Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 1989;54:1-13.
- 19 Lyons CR, Ball EJ, Towes GB, Weissler GC, Stastyn P, Lipscomb BF: Inability of human alveolar macrophages to stimulate resting T cells correlates with decreased antigen-specific T cell-macrophage binding. *J Immunol* 1986;137:1173-1180.
- 20 Xing Z, Kirpalani H, Torry D, Jordana M, Gaudie J: Polymorphonuclear leukocytes as a significant source of tumor necrosis factor-alpha in endotoxin-challenged lung tissue. *Am J Pathol* 1993;143:1009-1015.
- 21 Havenith CE, Breedijk AJ, Hoefsmit EC: Effect of Bacillus Calmette-Guerin inoculation on numbers of dendritic cells in bronchoalveolar lavages of rats. *Immunobiology* 1992;184:336-347.
- 22 Gong JL: Intraepithelial airway dendritic cells: A distinct subset of pulmonary dendritic cells obtained by microdissection. *J Exp Med* 1992;175:797-807.
- 23 Sertl K, Takemura T, Tschachler E, Ferrans V, Kaliner M, Shevach E: Dendritic cells with antigen-presenting capability reside in airway epithelium, lung, parenchyma, and visceral pleura. *J Exp Med* 1986;163:436-451.
- 24 Holt PG, Schon-Hegrad MA, Oliver J: MHC class II antigen-bearing dendritic cells in pulmonary tissues of the rat. *J Exp Med* 1988;167:262-274.
- 25 Lambrecht BN, Carro-Muino I, Vermaelen K, Pauwels RA: Allergen-induced changes in bone-marrow progenitor and airway dendritic cells in sensitized rats. *Am J Respir Cell Mol Biol* 1999;20:1165-1174.
- 26 Mellman I, Turley SJ, Steinman RM: Antigen processing for amateurs and professionals. *Trends Cell Biol* 1998;8:231-237.
- 27 Havenith CE, van Miert PP, Breedijk AJ, Beelen RH, Hoefsmit EC: Migration of dendritic cells into the draining lymph nodes of the lung after intratracheal instillation. *Am J Respir Cell Mol Biol* 1993;9:484-488.
- 28 Havenith CE, Breedijk AJ, van Miert PP, Blijleven N, Calame W, Beelen RH, Hoefsmit EC: Separation of alveolar macrophages and dendritic cells via autofluorescence: Phenotypic and functional characterization. *J Leukoc Biol* 1993;53:504-510.
- 29 van den Heuvel MM, Vanhee DD, Postmus PE, Hoefsmit EC, Beelen RH: Functional and phenotypic differences of monocyte-derived dendritic cells from allergic and nonallergic patients. *J Allergy Clin Immunol* 1998;101:90-95.
- 30 Bellini A, Vittori E, Marini M, Ackerman V, Mattoli S: Intraepithelial dendritic cells and selective activation of Th2-like lymphocytes in patients with atopic asthma. *Chest* 1993;103:997-1005.
- 31 Gelfand EW: Essential role of T lymphocytes in the development of allergen-driven airway hyperresponsiveness. *Allergy Asthma Proc* 1998;19:365-369.
- 32 Masten BJ, Lipscomb MF: Dendritic cells: Pulmonary immune regulation and asthma. *Monaldi Arch Chest Dis* 2000;55:225-230.
- 33 Pabst R, Tschernig T: Lymphocytes in the lung: An often neglected cell. Numbers, characterization and compartmentalization. *Anat Embryol (Berl)* 1995;192:293-299.
- 34 Holt PG, Robinson BW, Reid M, Kees UR, Warton A, Dawson VH, Rese A, Sekon-Hegrad M, Papdimitriou JM: Extraction of immune and inflammatory cells from human lung parenchyma: Evaluation of an enzymatic digestion procedure. *Clin Exp Immunol* 1986;66:188-200.
- 35 Pabst R, Binns R: Lymphocytes migrate from the bronchoalveolar space to regional bronchial lymph nodes. *Am J Respir Crit Care Med* 1995;151:495-499.
- 36 Ansfield MJ, Kaltreider HB, Caldwell JL, Herskowitz FN: Hyporesponsiveness of canine bronchoalveolar lymphocytes to mitogens: Inhibition of lymphocyte proliferation by alveolar macrophages. *J Immunol* 1979;122:542-548.
- 37 Becker S, Harris DT, Koren HS: Characterization of normal human lung lymphocytes and interleukin-2-induced lung T cell lines. *Am J Respir Cell Mol Biol* 1990;3:441-448.
- 38 Saltini C, Kirby M, Trapnell BC, Tamura N, Crystal RG: Biased accumulation of T lymphocytes with 'memory'-type CD45 leukocyte common antigen gene expression on the epithelial surface of the human lung. *J Exp Med* 1990;171:1123-1140.
- 39 Borron P, Veldhuizen RA, Lewis JF, Possmayer F, Caveney A, Inchley K, Mc Fadden RG, Fraher LJ: Surfactant associated protein-A inhibits human lymphocyte proliferation and IL-2 production. *Am J Respir Cell Mol Biol* 1996;15:115-121.
- 40 Borron P, McCormack FX, Elhalwagi BM, Chronoes ZC, Lewis JF, Zhu S, Wright JR, Shepherd VL, Possmayer F, Inchley K, Fraher LJ: Surfactant protein A inhibits T cell proliferation via its collagen-like tail and a 210-kDa receptor. *Am J Physiol* 1998;19:L679-L686.
- 41 Borron PJ, Crouch EC, Lewis JF, Wright JR, Possmayer F, Fraher LJ: Recombinant rat surfactant-associated protein D inhibits human T lymphocyte proliferation and IL-2 production. *J Immunol* 1998;161:4599-4603.
- 42 Holt PG: Alveolar macrophages. II. Inhibition of lymphocyte proliferation by purified macrophages from rat lung. *Immunology* 1979;37:429-436.
- 43 Paine R 3rd, Chavis A, Gaposchkin D, Christensen P, Mody CH, Turka LA, Torlos GB: A factor secreted by a human pulmonary alveolar epithelial-like cell line blocks T-cell proliferation between G<sub>1</sub> and S phase. *Am J Respir Cell Mol Biol* 1992;6:658-666.
- 44 Seitzman GD, Sonstein J, Kim S, Choy W, Curtis JL: Lung lymphocytes proliferate minimally in the murine pulmonary immune response to intratracheal sheep erythrocytes. *Am J Respir Cell Mol Biol* 1998;18:800-812.
- 45 Saltini C, Richeldi L, Holroyd KJ, du Bois RM, Crystal RG: Lymphocytes; in Crystal RG, West JB, Barnes PJ, Chernick NS, Weibel ER (eds): *The Lung: Scientific Foundations*. New York, Raven Press, 1991, pp 459-482.

- 46 Strickland D, Kees UR, Holt PG: Regulation of T-cell activation in the lung: Alveolar macrophages induce reversible T-cell anergy in vitro associated with inhibition of interleukin-2 receptor signal transduction. *Immunology* 1996; 87:250–258.
- 47 Poulter LW, Janossy G, Power C, Sreenan S, Burke C: Immunological/physiological relationships in asthma: Potential regulation by lung macrophages. *Immunol Today* 1994;15: 258–261.
- 48 Pinkston P, Bitterman PB, Crystal RG: Spontaneous release of interleukin-2 by lung T lymphocytes in active pulmonary sarcoidosis. *N Engl J Med* 1983;308:793–800.
- 49 LeVine AM, Bruno MD, Huelsman KM, Ross GF, Whitsett JA, Korfthagen TR: Surfactant protein A-deficient mice are susceptible to group B streptococcal infection. *J Immunol* 1997;158:4336–4340.
- 50 LeVine AM, Kurak KE, Wright JR, Watford WT, Bruno MD, Ross GF, Whitsett JA: Surfactant protein-A binds group B streptococcus enhancing phagocytosis and clearance from lungs of surfactant protein-A-deficient mice. *Am J Resp Cell Mol Biol* 1999;20:279–286.
- 51 LeVine AM, Kurak KE, Bruno MD, Stark JM, Whitsett JA, Korfthagen TR: Surfactant protein-A deficient mice are susceptible to *Pseudomonas aeruginosa* infection. *Am J Respir Cell Mol Biol* 1998;19:700–708.
- 52 LeVine AM, Gwozdz J, Stark J, Bruno M, Whitsett J, Korfthagen T: Surfactant protein-A enhances respiratory syncytial virus clearance in vivo. *J Clin Invest* 1999;103:1015–1021.
- 53 Borron P, McIntosh JC, Korfthagen TR, Whitsett JA, Taylor J, Wright JR: Surfactant-associated protein A inhibits LPS-induced cytokine and nitric oxide production in vivo. *Am J Physiol* 2000;278:L840–L847.
- 54 Greene K, Whitsett JA, Korfthagen TR, Fisher JH: SP-D expression regulates endotoxin mediated lung inflammation in vivo. *Am J Resp Crit Care Med* 2000;161:A515.
- 55 Stamme C, Wright JR: Surfactant protein A enhances interferon- $\gamma$  induced nitric oxide but inhibits LPS-induced nitric oxide alveolar macrophages. *Am J Respir Crit Care Med* 2000;161:A515.
- 56 Hickman-Davis J, Gibbs-Erwin J, Lindsey JR, Matalon S: Surfactant protein A mediates mycoplasma activity of alveolar macrophages by production of peroxynitrite. *Proc Natl Acad Sci USA* 1999;96:4953–4958.
- 57 Pasula R, Wright JR, Kachel DL, Martin WJ 2nd: Surfactant protein A suppresses reactive nitrogen intermediates by alveolar macrophages in response to *Mycobacterium tuberculosis*. *J Clin Invest* 1999;103:483–490.
- 58 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.
- 59 Wu H, Kuzmenko A, Wan S, Schaffer L, Weiss A, Fisher JH, Kim KS, McCormack FX: Surfactant proteins A and D inhibit the growth of gram-negative bacteria by increasing membrane permeability. *J Clin Invest* 2003;111: 1589–1602.
- 60 McCormack FX, Gibbons R, Ward SR, Kuzmenko A, Wu H, Deepe GS Jr: Macrophage-independent fungicidal action of the pulmonary collectins. *J Biol Chem* 2003;278:36250–36256.
- 61 Brinker KG, Martin E, Borron P, Mostaghel E, Doyle C, Harding CV, Wright JR: Surfactant protein D enhances bacterial antigen presentation by bone marrow-derived dendritic cells. *Am J Physiol* 2001;281:L1453–L1463.
- 62 Brinker KG, Garner H, Wright JR: Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells. *Am J Physiol* 2003;284:L232–L241.
- 63 Wang JY, Shieh CC, You PF, Lei HY, Reid KB: Inhibitory effect of pulmonary surfactant proteins A and D on allergen-induced lymphocyte proliferation and histamine release in children with asthma. *Am J Respir Crit Care Med* 1998;158:510–518.
- 64 Cheng G, Ueda T, Nakajima H, Nakajima A, Kinjo S, Motojima S, Fukuda T: Suppressing effects of SP-A on ionomycin-induced IL-8 production and release by eosinophils. *Int Arch Allergy Immunol* 1998;117:59–62.
- 65 Hermans C, Bernard A: Lung epithelium-specific proteins. *Am J Respir Crit Care Med* 1999;159:646–678.
- 66 Gregory TJ, Longmore WJ, Moxley MA, Whitsett JA, Reed CR, Fowler AA 3rd, 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.
- 67 Pison U, Obertacke U, Seeger W, Hawgood S: Surfactant protein A (SP-A) is decreased in acute parenchymal lung injury associated with polytrauma. *Eur J Clin Invest* 1992;22:712–718.
- 68 Baumhan RP, Sternberg RI, Hull W, Buchsbaum JA, Whitsett J: Decreased surfactant protein A in patients with bacterial pneumonia. *Am Rev Respir Dis* 1993;147:653–657.
- 69 McCormack FX, King TE, Voelker DR, Robinson PC, Mason RJ: Idiopathic pulmonary fibrosis: Abnormalities in the bronchoalveolar lavage content of surfactant protein A. *Am Rev Respir Dis* 1991;144:160–166.
- 70 Hamm H, Luhrs J, Guzman y Rotaache 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.
- 71 Lesur O, Bernard AM, Begin RO: Clara cell protein (CC-16) and surfactant-associated protein A (SP-A) in asbestos-exposed workers. *Chest* 1996;109:467–474.
- 72 Cormier Y, Israel-Assayag E, Desmeules M, Lesur O: Effect of contact avoidance or treatment with oral prednisolone on bronchoalveolar lavage surfactant protein A levels in subjects with farmer's lung. *Thorax* 1996;51:1210–1215.
- 73 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.
- 74 Kramer HJ, Schmidt R, Gunther A, Becker G, Suzuki Y, Seeger W: ELISA technique for quantification of surfactant protein B (SP-B) in bronchoalveolar lavage fluid. *Am J Respir Crit Care Med* 1995;152:1540–1544.
- 75 Govan JR, Deretic V: Microbial pathogenesis in cystic fibrosis: Mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 1996;60:539–574.
- 76 Postle AD, Mander A, Reid KB, Wang JY, Wright SM, Moustaki M, Warner JO: Deficient hydrophilic lung surfactant proteins A and D with normal surfactant phospholipid molecular species in cystic fibrosis. *Am J Respir Cell Mol Biol* 1999;20:90–98.
- 77 Griese M, Birrer P, Demirsoy A: Pulmonary surfactant in cystic fibrosis. *Eur Respir J* 1997; 10:1983–1988.
- 78 Griese M, von Bredow C, Birrer P: Reduced proteolysis of surfactant protein A and changes of the bronchoalveolar lavage fluid proteome by inhaled alpha-1-protease inhibitor in cystic fibrosis. *Electrophoresis* 2001;22:165–171.
- 79 Meyer KC, Sharma A, Brown R, Weatherly M, Moya FR, Lewandoski J, Zimmerman JJ: Function and composition of pulmonary surfactant and surfactant-derived fatty acid profiles are altered in young adults with cystic fibrosis. *Chest* 2000;118:164–174.
- 80 Hull J, South M, Phelan P, Grimwood K: Surfactant composition in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med* 1997;156:161–165.
- 81 Vanderzwan J, McCaig L, Mehta S, Joseph M, Whitsett J, McCormack DG, Lewis JF: Characterizing alterations in the pulmonary surfactant system in a rat model of *Pseudomonas aeruginosa* pneumonia. *Eur Respir J* 1998;12: 1388–1396.
- 82 Mariencheck WM, Wright JR: A metalloproteinase secreted by *Pseudomonas aeruginosa* degrades pulmonary surfactant protein A (SP-A). *Am J Resp Crit Care Med* 1999;159:A506.
- 83 Griese M, von Bredow C, Birrer P, Schams A: Inhalation of alpha(1)-protease inhibitor in cystic fibrosis does not affect surfactant convertase and surface activity. *Pulm Pharmacol Ther* 2001;14:461–467.
- 84 Prince A: Adhesins and receptors of *Pseudomonas aeruginosa* associated with infection of the respiratory tract. *Microb Pathog* 1992;13: 251–260.
- 85 Khubchandani KR, Oberley RE, Snyder JM: Effects of surfactant protein A and NaCl concentration on the uptake of *Pseudomonas aeruginosa* by THP-1 cells. *Am J Respir Cell Mol Biol* 2001;25:699–706.
- 86 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.
- 87 Madan T, Kishore U, Shah A, Eggleton P, Strong P, Wang JY, Aggrawal SS, Sama PU, Reid KB: Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. *Clin Exp Immunol* 1997;110:241–249.