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Review

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Surfactant proteins SP-A and SP-D in human health and disease

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Summary

Surfactant proteins A (SP-A) and D (SP-D) are lung surfactant-associated hydrophilic proteins that have been implicated in surfactant homeostasis and pulmonary innate immunity. They are collagen-containing C-type (calcium-dependent) lectins, called collectins, and are structurally similar to mannose-binding protein of the lectin pathway of the complement system. Being carbohydrate pattern-recognition molecules, they recognize a broad spectrum of pathogens and allergens via the lectin domain, with subsequent activation of immune cells via the collagen region, thus offering protection against infection and allergenic challenge. SP-A and SP-D have been shown to be involved in viral neutralization, clearance of bacteria, fungi, and apoptotic and necrotic cells, down-regulation of allergic reaction, and resolution of inflammation. Studies on single-nucleotide polymorphism, protein levels in broncho-alveolar lavage, and gene knock-out mice have clearly indicated an association between SP-A and SP-D and a range of pulmonary diseases. In addition, recent studies using murine models of allergy and infection have raised the possibility that the recombinant forms of SP-A and SP-D may have therapeutic potential in controlling pulmonary infection, inflammation, and allergies in humans.

Key words: **lung • surfactant • innate immunity • pathogen • allergy • disease • pregnancy**

Abbreviations: **SP** – surfactant protein, **rhSP-D** – a recombinant fragment of human SP-D containing homotrimeric neck and C-type lectin domains, **ABPA** – allergic bronchopulmonary aspergillosis, **IPA** – invasive pulmonary aspergillosis, **BALF** – bronchoalveolar lavage fluid, **IAV** – influenza A virus, **HSV** – herpes simplex virus, **RSV** – respiratory syncytial virus, **COPD** – chronic obstructive pulmonary disease, **PAP** – pulmonary alveolar proteinosis, **IPF** – interstitial pulmonary fibrosis, **IPCD** – interstitial pneumonia with collagen vascular disease, ***Afu*** *–Aspergillus fumigatus*, **RDS** – respiratory distress syndrome, **ARDS** – adult RDS, **CF** – cystic fibrosis, **CRD** – carbohydrate recognition domain, **DPPC** – dipalmitoylphosphatidylcholine, **LPS** – lipopolysaccharide, **PBMC** – peripheral blood mononuclear cell, **PC** – phosphatidylcholine, **PI** – phosphatidylinositol, **GERD** – gastroesophageal reflux disease, **TB** – tuberculosis.

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INTRODUCTION

The alveoli are lined with a thin layer of a multifunctional aqueous film of which the key component is pulmonary surfactant, which is secreted by cells lining the distal pulmonary epithelia. The surfactant is a mixture of varied lipids, phospholipids, and proteins that forms a phospholipid-enriched membrane at the air-liquid interface[103]. The thin film readily spreads and is resistant to pressure-induced compression during breathing, which results in respiratory expansion and alveoli contraction. Thus the ability of surfactant to form the membrane results in a low alveoli surface tension, making breathing easier and mechanically sustainable[111].

Four surfactant proteins (SPs), SP-A, SP-B, SP-C, and SP-D, are found to be associated with surfactant lipids and have a distribution of 5.3, 0.7, 0.4, and 0.6%, respectively[59]. SP-B (14 kDa) and SP-C (6 kDa) are small hydrophobic proteins that have important roles in phospholipid packaging, overall organization and adsorption to the air-liquid interface, and in lowering the surface tension at the air-liquid interface in the peripheral air space following expiration. SP-A and SP-D are large hydrophilic proteins and, like SP-B and SP-C, they have important functions in surfactant homeostasis[59] (Table 1). SP-D levels in surfactant lining of the alveolar epithelia are significantly less (~10-fold) than that of SP-A. Approximately 75% of SP-D is generally in the aqueous bronchoalveolar lavage fluid (BALF). Their primary structure includes an N-terminal triple-helical collagen region and a homotrimeric ligand-recognition domain, called "C-type lectin" or the "carbohydrate recognition domain" (CRD). SP-A specifically and avidly binds to the major surfactant phospholipid, dipalmitoylphosphatidylcholine (DPPC) and is considered to have a major role in surfactant turnover and homeostasis[49, 59]. SP-D preferentially binds to phosphatidylinositol (PI) and also to glucosylceramide, both of which contain sugar moieties and are minor components of surfactant. Binding of SP-D to glucosylceramide involves interactions between the CRD region and the glucosyl moiety. SP-D interacts with the inositol moiety of PI via the CRD and neck regions[60, 99]. In addition to their role in surfactant homeostasis, SP-A and SP-D have recently been shown to be important host defense components against respiratory pathogens and allergens[49, 58, 59] (Table 2).

Table 1. Surfactant-related functions of SP-A

Surfactant homeostasis
Receptor-mediated inhibition of surfactant secretion from alveolar type II cells
Receptor-mediated enhanced uptake of surfactant phospholipids by alveolar type II cells
Surfactant biophysical activity
Enhanced phospholipid adsorption to the monolayer
Prevention of protein inhibition by proteinaceous pulmonary edema
Maintenance of tubular myelin
Phospholipase A_2 inhibition
Myosin clearance

Table 2. Immune functions of SP-A and SP-D

Endotoxin clearance and regulation of LPS-induced inflammation
Recognition, agglutination and phagocytosis of viral, bacterial and fungal pathogens
Macrophage and neutrophil activation and chemotaxis
Microbial growth inhibition
Non-specific defense molecules in tears, saliva and body secretions against pathogens
Protection against intrauterine infection and inflammatory reactions
Control of inflammation
Recognition and clearance of apoptotic and necrotic cells
Anti-proliferative effects on B and T lymphocytes
Pattern recognition of glycoprotein allergens
Inhibition of IgE-allergen cross-linking
Suppression of histamine release from basophils and mast cells
Modulation of Th cytokine profile
Modulation of maturation and antigen presentation by dendritic cells

The primary site of SP-A and SP-D production is the lung. Within the lung, SP-A and SP-D are synthesized by alveolar type II cells[20, 104, 129] and non-ciliated bronchial epithelial cells (Clara cells) of the terminal bronchioles and conducting airways[21, 129]. The proteins are packaged into intracellular organelles called lamellar bodies and tubular myelin and are secreted into the alveolar lining layer. There is also evidence to suggest extra-pulmonary existence of SP-A and SP-D. They have also been detected in the serous glands of proximal human trachea, in the endocytic compartment of macrophages, rat intestinal epithelia, human and rat mesentery, and human inner ear. Low levels of material antigenically similar to SP-D are found in normal human serum, and animal studies indicate the presence of SP-D, or SP-D-like proteins, in gastric mucosa, tracheobronchial, lacrymal, and salivary glands. Their presence in tear and saliva and epithelial lining appears to suggest that SP-A and SP-D are general scavenging defense molecules in body secretions with a plausible role in modulation of mucosal immunity.

STRUCTURAL ORGANIZATION OF SP-A AND SP-D

SP-A and SP-D belong to a family of mammalian C-type lectins, called collectins, containing collagen regions[49]. Their primary structure is organized into 4 regions: 1) a cysteine-containing N-terminus

(required for disulfide-dependant oligomerization) that is linked to 2) a triple-helical collagen region composed of repeating Gly-X-Y triplets (associated with maintaining the molecules shape, dimension, stability, and oligomerization), followed by 3) an α-helical coiled coil neck region (whose main function is protein trimerization), and 4) a globular structure at the C-terminal comprising a C-type lectin or CRD (that mediates calcium-dependent ligand-binding to entities such as pathogens, carbohydrates, phospholipids, etc.; Fig. 1). The C-type CRDs are spaced in a trimeric orientation at the end of triple--helical collagen region[59]. The CRD region engages pathogens due to its carbohydrate pattern-recognition properties, whereas the collagen region interacts with putative receptors present on type II or immune cells in order to bring about effector functions.

SP-A and SP-D are large oligomeric structures, each assembled from multiple copies of a single polypeptide chain (with the exception of human SP-A, which has two, closely related, α_2 and α_3 chains; Fig. 1). SP-A has a hexameric structure in which 6 structural subunits, of 105 kDa each, associate to yield a molecule of 630 kDa. Each structural subunit is composed of three 35-kDa polypeptide chains that are held together by disulfide bonds located in the N-terminal halves of the chains. The overall shape of SP-A is very similar to that of the serum complement protein C1q, both molecules appearing in the electron microscope as a bouquet-like structure with 6 globular heads linked by collagen-like strands to a fibril-like central core (Fig. 1). The mature forms of SP-A are composed of 248 amino acids (aa), which include: an N-terminal segment (7 aa), a collagen-like region (73 aa), the neck region (34 aa), and a CRD domain (123 aa).

SP-D is composed of oligomers of a 130-kDa subunit comprising three identical polypeptide chains of 43 kDa, each containing an N-linked oligosaccharide structure at Asn[69]. Human SP-D is assembled into a 520-kDa tetrameric structure with 4 of the 130-kDa homotrimeric subunits linked via their N-terminal regions, but trimers, dimers, and monomers of the 130-kDa subunit are also present in SP-D preparations. Up to 8 of the 520-kDa tetrameric structures can undergo further oligomerization to give SP-D multimers having a large array, of up to 96 (8×12), CRDs. The degree of subunit oligomerization probably affects the recognition of and binding strength to the carbohydrate ligands on the surfaces of pathogens[49]. For instance, the binding constant of a trimeric CRD unit to a monosaccharide ligand (10^{-3} M) is significantly less than binding of collectin trimers (10^{-8} M) and oligomers (10^{-11} M) to polyvalent ligands[60].

INTERACTION OF SP-A AND SP-D WITH LUNG PATHOGENS

Pathogen recognition by SP-A and SP-D results from binding of terminal monosaccharide residues present on many pulmonary pathogens. The broad selectivity of the monosaccharide binding site and the geometrical arrangement of the multiple CRDs allow SP-A and SP-D to bind tightly to arrays of carbohydrate structures normally found on the surfaces of the microorganisms[49, 59]. These collectins bind mannose and glucose residues, which are part of most microbial ligands, more avidly than galactose, fructose, and sialic acid, which are common components of glycoproteins of higher eukaryotes. SP-A and SP-D, as innate immune molecules, can perform a wide range of immune functions (Table 2) aimed at offering resistance to pathogen and allergen exposure[49, 58, 59]. They can readily agglutinate a broad spectrum of lung pathogens, inhibit microbial growth, and recruit phagocytes to the site of infection. These proteins also interact with phagocytic cells such as macrophages and neutrophils and enhance their chemotactic, phagocytic, and oxidative properties[49, 59].

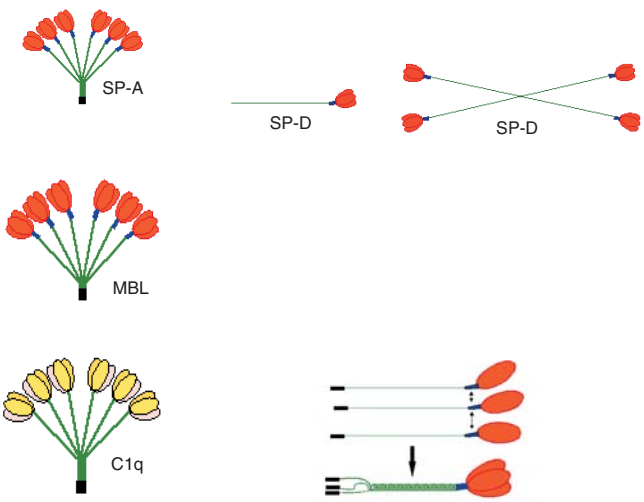

Figure 1. Domain organization and assembly of the collectin molecules. The primary structure of SP-A and SP-D is organized into 4 regions: an N-terminal, non collagen region involved in the formation of inter-chain disulfide bonds, a collagen region composed of Gly-X-Y repeats (where X and Y can be any amino acids), a neck peptide, and a C-terminal C-type CRD domain. SP-A and SP-D are large oligomeric structures, each assembled from multiple copies of a single polypeptide chain (SP-A has two types of chains). The C-type CRDs are spaced, in a trimeric orientation, at the end of triple helical collagen stalks. SP-D has a cruciform-like appearance in the electron microscopy, with 4 arms of equal length ending in globular head. SP-A resembles serum complement proteins C1q and mannose-binding lectin (MBL) in their overall organization.

Aggregation and neutralization of viruses by SP-A and SP-D

Both SP-A and SP-D bind influenza A (IAV) and herpes simplex viruses (HSV) *in vitro*[38, 39, 127]. SP-A is known to act as an opsonin for HSV type I via alveolar macrophages; however, this interaction involves the attachment of virus to complex oligosaccharides on SP-A[128]. SP-A can bind IAV via its sialic acid residues and neutralize the virus[10]. Preincubation of IAV with SP-A enhances the ability of the virus to stimulate respiratory burst of neutrophils[39]. SP-D, a more potent inhibitor of IAV infectivity than SP-A, induces massive aggregation of IAV particles. SP-D CRD regions bind to high-mannose oligosaccharides associated with the globular hemagglutinin (HA) domain, very close to the sialic acid binding site on HA, resulting in inhibition of HA activity[40]. SP-D also binds to the neuraminidase of IAV and inhibits its enzymatic activity. Furthermore, the susceptibility of various IAV strains to neutralization by SP-D correlates with the number of glycoconjugates expressed on the HA molecule[110].

SP-A is known to bind the fusion glycoprotein of respiratory syncytial virus (RSV) in a Ca^{2+}-dependent manner and cause reduced viral infectivity[31]. RSV is responsible for a significant proportion of viral lung infections in neonates and infants. SP-A acts as an opsonin for RSV and offers a portal of entry for the virus so as to infect the target cells[45]. Furthermore, the attachment of SP-A to RSV and subsequent phagocytosis appears important for the infectivity. SP-A-mediated uptake of RSV significantly enhances tumor necrosis factor α (TNF-α) production by peripheral blood mononuclear cells (PBMCs) and also reverses the RSV-induced suppression of TNF-α by U937 macrophages[8]. Native SP-D and a recombinant fragment of human SP-D containing homotrimeric neck and CRD regions (rhSP-D) can bind G protein of RSV and inhibit viral infectivity in a cell culture system[43]. Intranasal administration of rhSP-D to RSV-infected mice has been shown to inhibit viral replication in the lungs and reduce viral load[44].

Clearance of bacteria by SP-A and SP-D

Both SP-A and SP-D bind to a broad spectrum of Gram-positive and Gram-negative bacteria[127]. SP-A can enhance attachment of *Staphylococcus aureus* to macrophages without causing phagocytosis. SP-A enhances the phagocytosis of *Escherichia coli* J 5 (containing O-antigen-deficient rough lipopolysaccharide – LPS), but not *E. coli* O 111 (with O-antigen-containing smooth LPS), by macrophages, suggesting that its binding to Gram-negative bacteria is dependent on LPS structure[128]. SP-A has been reported to bind *Streptococcus pneumoniae* and other Streptococci (group A and group B *Streptococcus*). SP-A binds, aggregates, and promotes phagocytosis by macrophages, of *Hemophilus influenzae* via outer membrane protein[90]. Low binding to *H. influenzae* type b without aggregation and phagocytosis has also been reported. SP-A enhances agglutination, phagocytosis, and killing of *Klebsiella pneumoniae* K21a strain (capsule containing Man α_1 Man sequences), either via opsonization or through activation of macrophages via the mannose receptor[55]. *Mycoplasma pulmonis,* considered to be involved in pneumonia and exacerbation of asthma and chronic obstructive pulmonary disease (COPD), is bound by SP-A, leading to phagocytosis and killing by interferon (IFN)-γ-activated murine alveolar macrophages via generation of peroxynitrite[46].

Binding of SP-A or SP-D to Gram-negative bacteria, which involves rough LPS and the lectin domain, induces aggregate formation[128]. Binding of SP-A to the lipid A moiety of LPS is calcium- dependent and is not inhibited by mannan or by removal of the N-linked carbohydrate. The recombinant form of truncated SP-D, rhSP-D, has been shown to bind to LPS derived from *K. pneumoniae*, *Pseudomonas aeruginosa*, and *E. coli*[58]. Human SP-A has also been shown to enhance phagocytosis of *E. coli*, *P. aeruginosa*, and *Staph. aureus* by alveolar macrophages. SP-D selectively binds to smooth forms of LPS expressed by O-serotypes of *K. pneumoniae* with mannose-rich repeating units in their O-polysaccharides. SP-D is more potent in agglutinating unencapsulated phase variants of O-serotypes, which leads to inhibition of pathogen adhesion to lung epithelial cells[116]. SP-D, but not SP-A, has been shown to bind, via the CRD region, lipoteichoic acid (of *Bacillus subtilis*) and peptidoglycan (of *Staph. aureus*), both being cell wall components of the Gram positive bacteria[125].

SP-A binds BCG, via its CRDs, and brings about phagocytosis by phagocytic cells[135]. Both SP-A and SP-D bind to and modulate phagocytosis of *Mycobacterium tuberculosis* by macrophages. SP-A binds to and enhances attachment of *M. tuberculosis* to alveolar macrophages[30, 102]. Such interactions may favor the pathogen by enhancing the potential of *M. tuberculosis* to gain access to its intracellular niche[30]. Attachment of *M. tuberculosis* to alveolar macrophages is an important step in the pathogenesis of infection, which may be enhanced by SP-A. Interestingly, SP-A levels are increased in the lungs of HIV-infected individuals, and attachment of *M.*

tuberculosis to alveolar macrophages inversely correlates with peripheral blood $CD4^+$ lymphocyte counts. Thus in AIDS patients the elevated concentrations of SP-A may predispose this patient group to tuberculosis (TB)[22]. SP-D has also been shown to bind to the lipoarabinnomannan moiety on the surface of *M. tuberculosis* bacilli through CRD, and this interaction results in reduced uptake of bacteria by alveolar macrophages[25, 26]. However, the inhibition of *M. tuberculosis* phagocytosis by SP-D does not depend on the formation of bacterial aggregates[26].

Anti-fungal properties of SP-A and SP-D

SP-A and SP-D can also recognize higher order pathogens. For example, they contribute to the clustering of *Pneumocystis carinii in vivo* by interacting with glycoprotein 120 (gpA), a mannose- and glucose-rich glycoprotein expressed on cysts and trophozoites[101, 139]. In addition, β-glucans have also been implicated in the interactions of SP-D with *Pneumocystis*. It is likely that the characteristic cyst aggregates observed in lung biopsies of patients with pneumocystis infection are contributed by these collectins.

SP-A and SP-D bind to and induce immune responses against clinically relevant fungal pathogens, such as *Cryptococcus neoformans*, *Candida albicans*, and *Aspergillus fumigatus* (*Afu*). Collectively, these three fungi are by far the most prominent and lethal fungal pathogens worldwide. *C. neoformans* is an opportunistic pathogen that is able to cause serious infections in immunosuppressed or immunocompromised host. The inhalation of acapsular or sparsely encapsulated *C. neoformans* cells into the lungs can cause local infection followed by dissemination, leading to meningitis. Both SP-A and SP-D agglutinate acapsular *C. neoformans*, but only SP-D-agglutinated *C. neoformans* are phagocytozed[120]. Unlike SP-D, phagocytosis of the aggregates of *C. neoformans* is not enhanced by SP-A[130], implying that SP-D has a more important role in resisting *C. neoformans* infection.

Interaction of SP-D with *C. albicans* profoundly inhibits fungal growth and hyphal production. The growth inhibition and restricted pseudohyphal/ /hyphal growth is accompanied by inhibition of phagocytosis of *C. albicans* by alveolar macrophages[112]. However, SP-A does not seem to serve as an opsonin for the phagocytosis of *C. albicans* by alveolar macrophage[113]. Instead, it prevents the excessive release of pro-inflammatory cytokines by alveolar macrophages and monocytes challenged with *C. albicans*[113].

Aspergillus species are the most prominent cause of fungal respiratory infections worldwide. Both human SP-A and SP-D bind and agglutinate *Afu* conidia, and this interaction enhances phagocytosis and killing of germinating conidia by human neutrophils and alveolar macrophages[77]. Using an immunosuppressed murine model of invasive pulmonary aspergillosis (IPA) challenged intranasally with *Afu* spores, the protective effects of intranasal administration of an empirical anti-fungal drug, Amphotericin B (AmB), SP-A, SP-D, and rhSP-D have been examined[80]. Mice treated with AmB, SP-D, and rhSP-D showed a survival rate of 80, 60, and 80%, respectively, compared with 100% mortality in the untreated group. However, SP-A treatment did not have a significant effect on mortality, highlighting a more important anti-fungal role for SP-D. In a recent study, treatment of IPA mice with various doses of SP-D or rhSP-D was found to lower CFU counts in the lung tissues and reduce dramatically fungal hyphae, consistent with raised levels of TNF-α and IFN-γ in the BALF of treated mice (Kishore, unpublished).

ROLE OF SP-A AND SP-D IN PROTECTION AGAINST ALLERGENS AND PULMONARY HYPERSENSITIVITY

SP-A and SP-D can bind via their CRD region to allergenic extracts derived from pollen grains[83], house dust mite[132], and *Afu*[78], inhibit specific IgE binding to allergens, and block allergen-induced histamine release from sensitized basophils[78, 132]. SP-A and SP-D can reduce the proliferation of PBMC isolated from mite-sensitive asthmatic children[133]. SP-D has a suppressive effect on the secretions of interleukin 2 (IL-2) by PBMCs[12], whereas SP-A can inhibit IL-8 production by eosinophils[16]. Recently, *in vivo* therapeutic trials of SP-A, SP-D, and rhSP-D in a murine model of *Afu*-induced pulmonary hypersensitivity yielded interesting results[79]. *Afu* is an opportunistic fungal pathogen that is most commonly implicated in causing both IgE-mediated and non-IgE-mediated hypersensitivity in immunocompetent human subjects, leading to development of allergic bronchopulmonary aspergillosis (ABPA). ABPA is clinically characterized by episodic bronchial obstruction, positive immediate skin reactivity, elevated *Afu*-specific IgG and *Afu*-specific IgE antibodies in serum, peripheral and pulmonary eosinophilia, central bronchiectasis, and expectoration of brown plugs or flecks[60, 79]. Other important features of ABPA are activated Th2 cells and asthma, and patients may develop localized pulmonary fibrosis at later stages of the disease. The murine model resembles the human disease immunologically, exhibiting high levels of specific IgG and IgE, peripheral blood and pulmonary eosinophilia, and a Th2 cytokine response.

Intranasal administration of SP-A, SP-D, or rhSP-D using 3 doses on consecutive days significantly lowered eosinophilia and specific IgG and IgE antibody levels in the mice. This therapeutic effect persisted up to 4 days in the SP-A-treated ABPA mice and up to 16 days in the SP-D- or rhSP-D-treated ABPA mice. Lung sections of the ABPA mice showed extensive infiltration of lymphocytes and eosinophils, which were considerably reduced following treatment with SP-D or rhSP-D. The levels of IL-2, IL-4, and IL-5 were decreased, while IFN-γ levels increased in supernatants of the cultured spleen cells, suggesting a shift in the cytokine profile from pathogenic Th2 to protective Th1 response[79]. The protective effects of SP-D have also been observed in murine models of lung allergy/inflammation induced with house dust mite allergens[121] and ovalbumin[123]. Thus, SP-A and SP-D appear to suppress the Th2 responses, probably via their ability to modulate functions of antigen-presenting cells, such as macrophages[123] and dendritic cells[14, 15, 58] (Kishore, unpublished) which may eventually lead to an induction of IL-12-dependent Th1 responses. Since IgE cross-linking, histamine release, lymphocyte proliferation, and antigen presentation are central steps in the development of allergic asthma, the possibility of using exogenous SP-A and SP-D (or their recombinant fragments) as therapy for allergic disorders merits further investigation[60, 79].

BIOLOGICAL FUNCTIONS OF SP-A AND SP-D IN HUMAN PREGNANCY AND REPRODUCTIVE TISSUES

Surfactant lipids and proteins are important in human reproductive biology, not only because of the essential role of surfactant in the respiratory function of the newborn during the abrupt transition of the fetal lung from an aqueous intrauterine environment to extrauterine air, but because of the possible involvement of surfactant components in the control of parturition and innate defense mechanisms. Pulmonary surfactant is secreted from the fetal lung into the amniotic fluid where it accumulates during the third trimester of pregnancy. Surfactant in amniotic fluid is a complex mixture containing approximately 85% phospholipids, 5% non-polar lipids, and 10% apoproteins by weight[9]. Phosphatidylcholine (PC; also known as lecithin) constitutes about 80% of surfactant phospholipids and palmitate about 70% of surfactant phospholipid fatty acid. Surfactant is nearly unique amongst mammalian tissue extracts or secretions because most of the PC is a single molecular species, DPPC. Other phospholipids include phosphatidylethanolamine, phosphatidylglycerol, phosphatidylserine, and PI. DPPC is required for the surface tension-lowering properties of surfactant, and the measurement of this lipid in amniotic fluid (lecithin/sphingomyelin ratio and other chromatographic assays) has been the basis for the clinical assessment of fetal lung maturation for decades.

Role of fetal surfactant lipids

Respiratory failure is a major cause of neonatal death and handicap in preterm babies born with immature lungs, especially those delivered before 32 weeks of gestation. The survival of the newborn baby is closely associated with the development of surfactant, and this has led to the hypothesis[75] that the spontaneous onset of labor at term may be triggered by signals from the fetal lung, thus linking the process of birth with the process of fetal lung maturation. Surfactant is likely to be the carrier of these signals. Parturition is associated with increased intrauterine prostaglandin release[57], and studies on the human amnion obtained during labor have shown that non-esterified arachidonate, which is the obligatory precursor for prostaglandin synthesis, is released selectively from amniocyte diacyl phosphatidylethanolamine by phospholipase A_2 and from PI by the sequential actions of phospholipase C and diacylglycerol lipase[100]. Although quantitatively the most important lipid in fetal pulmonary surfactant is PC and the predominant fatty acid is palmitate, fetal surfactant also contains 4% of arachidonate, which can be rapidly transferred from surfactant arachidonylphosphatidylcholine to amniocyte phosphatidylethanolamine and PI. Thus, surfactant provides the amniotic epithelium with a source of arachidonate that can be used for prostaglandin synthesis during labor. Moreover, surfactant is degraded directly by enzymes (phospholipase C and diacyl- and monoacyl-glycerol lipases) released from the fetal lung and other tissues into the amniotic cavity, and contributes to the pool of free arachidonate in amniotic fluid[76].

Protection by SP-A and SP-D against intrauterine infection

SP-A and SP-D can be detected in human amniotic fluid as early as 26 weeks gestation, and there is a clear rise in SP-A levels towards term, reaching 2–8 μg/ml by 40 weeks gestation[92]. The rise in SP-D levels with advancing gestation is less pronounced, so that the ratio SP-A/SP-D rises from 1:1 at 26–34 weeks to 6:1 at 38–40 weeks. SP-A and SP-D have been localized by immunohistochemistry in the fetal membranes (amniotic epithelium and chorionic membrane) and the choriodecidual layer of the late pregnant uterus, but there was no staining in placental tissue[92]. It is likely that SP-A and SP-D are involved in the recognition and clearance of pathogens from the fetal membranes and amniotic fluid using their cognate

receptors on amnion cells or decidual macrophages[84]. Moreover, the direct bacteriostatic and fungistatic effects of SP-A and SP-D[89, 105, 138] would help prevent intrauterine infections, a particularly threatening complication that can lead to very early preterm birth. Infection-associated preterm birth may result from inflammatory responses in the decidua-fetal membranes area triggered by bacterial products, with increased release of prostaglandins, cytokines, and other inflammatory mediators. This clinical situation is termed chorioamnionitis, and is much more common at 26–32 weeks of gestation (when SP-A and SP-D levels in amniotic fluid are low) than after 37 weeks of gestation[65]. Thus, the low levels of SP-A and SP-D in the amniotic cavity before 32 weeks of gestation may predispose to infection and preterm birth. Conversely, the sharp increase in SP-A towards the end of gestation may have a protective effect. Given the wide spectrum of pathogens involved in chorioamnionitis, SP-A and SP-D are particularly suited as a non-specific, antibody-independent microbe removal system. Moreover, deficiencies in SP-A and SP-D are associated with lung infections in very premature neonates[5]. The protective role of SP-A and SP-D against intrauterine infection in late pregnancy may be complemented by the lubricating effect of the surface-active phospholipid components of amniotic fluid surfactant, which help maintain the mechanical integrity of the fetal membranes[47] and prevent their premature rupture, which would otherwise expose the amniotic cavity to vaginal microbes.

Protection against intrauterine inflammatory reactions

Human labor is associated with increased release of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by intrauterine tissues, notably the decidua[95]. Tissue macrophages are responsible for most of the decidual $PGF_{2\alpha}$ production in labor, and there is a strong autocrine up-regulation by other macrophage products, especially TNF-α[96]. SP-A is likely to have a further protective effect against the premature activation of the pro-labor prostaglandin cascade by inhibiting TNF-α activity in decidual macrophages, as demonstrated in lung macrophages[1]. SP-A and SP-D (as well as mannose-binding lectin, which is also abundant in human amniotic fluid)[84] participate in the clearance of apoptotic and necrotic cells[126]. By facilitating the removal of dead cells and debris in tissues near uterine smooth muscle, SP-A and SP-D are likely to reduce the risk of contraction-provoking inflammatory reactions.

A labor-initiating role for SP-A?

In mice, the gestational increase in SP-A in amniotic fluid is accompanied by increased expression of IL-1β and nuclear factor-κB (NF-κB) in intrauterine macrophages. Moreover, SP-A has a direct stimulatory effect on IL-1β and NF-κB expression by macrophages isolated from amniotic fluid near the end of gestation. Since both IL-1β and NF-κB stimulate prostaglandin synthesis pathways, they are considered pro-labor agents, and in this species, SP-A may have a role in the initiation of parturition[19]. Injection of SP-A into the amniotic sac of pregnant mice causes premature labor, whereas injection of an antibody against SP-A delays labor[19]. It is possible that SP-A has different roles in human and mouse pregnancy, because the endocrine control of parturition is very different in the two species. In small rodents, pregnancy is dependent for its entire duration on the production of progesterone by the corpus luteum of the ovary, and labor starts when luteolysis is triggered by uterine $PGF_{2\alpha}$ release, resulting in progesterone withdrawal. In women, the placenta takes over progesterone production from the corpus luteum early in gestation, and there is no evidence of progesterone withdrawal at term.

SP-D and tissue remodeling

The biological role of SP-D in human pregnancy is not fully understood, and there is evidence to suggest that it may be involved in the control of tissue remodeling. This is due to the interaction between SP-D and decorin[93]. Decorin belongs to the family of small, leucine-rich proteoglycans that bind collagen and other extracellular matrix components. SP-D and decorin interact in two ways: the CRD region of SP-D binds carbohydrate moieties of decorin, whereas the decorin core protein binds the collagen region of SP-D. Decorin is one of the most abundant proteoglycans in fetal membranes[91] and the uterine cervix[137]. Decorin induces the expression of matrix metalloproteases (MMP) and disrupts collagen fibrils to facilitate cervical ripening and dilatation during labor; it may also be involved in regulating the tensile strength of the fetal membranes at the end of pregnancy. Decorin co-purifies with SP-D in human amniotic fluid and the concentration of the two proteins is inversely related[93]. Thus, the SP-D-decorin interaction may have a role in intrauterine tissue remodeling during parturition.

SP-D in female reproductive tissues

SP-D has been localized in many epithelial and glandular surfaces. In women, SP-D is present in theca and granulosa cells of the ovarian follicles, and in theca-lutein cells and granulosa cells of the corpus luteum[67]. SP-D is also present in the luminal epithelium of the Fallopian tubes, in epithelial cells of

glands in the uterine cervix, and in squamous epithelial cells of the vagina. During the menstrual cycle, SP-D is present in the endometrium of the secretory, but not the proliferative phase[67]. The extensive localization of SP-D in the female reproductive tract suggests an important role for this collectin in local defense mechanisms[98], especially during the secretory phase of the cycle, when it is necessary to protect uterine receptivity for the implantation of the embryo. SP-A and SP-D have been shown to enhance phagocytosis of chlamydial pathogens by a macrophage cell line[97]. SP-D, being resident in the human female reproductive system, has been shown to inhibit *Chlamydia trachomatis* infection of cervical epithelial cells[98]. *C. trachomatis* is one of the most prevalent infectious diseases of the cervix that may lead to infertility. Because of its presence in the Fallopian tubes[67], it is speculated that SP-D may also be involved in facilitating sperm and ovum transport and fertilization.

STUDIES WITH MICE GENETICALLY DEFICIENT IN SP-A OR SP-D GENE AND THEIR RELEVANCE TO HUMAN DISEASE

The experiments carried out using mice genetically deficient in SP-A or SP-D have revealed a key role played by SP-A and SP-D in surfactant homeostasis and pulmonary immune response. Mice lacking SP-A mRNA and protein *in vivo*, generated by gene knock-out technology, survive and breed normally. They have normal levels of SP-B, SP-C, and SP-D, phospholipid composition, secretion and clearance, and incorporation of phospholipid precursors. Although there is a complete absence of tubular myelin in SP-A-deficient (–/–) mice, it does not appear to have a significant physiologic effect[2, 53]. SP-A$^{-/-}$ mice show increased bacterial proliferation and systemic dissemination following intratracheal inoculation with Group B *Streptococci*, and defective clearance of *S. aureus*, *P. aeruginosa* and *K. pneumoniae*[63]. These mice also show increased susceptibility to RSV, *M. pneumoniae*, and *Pneumocystis* than the wild-type mice. Killing of group B *Streptococcus* and *H. influenza* is significantly reduced in SP-A$^{-/-}$ mice, which is accompanied by increased inflammation of the lung, decreased oxidant production, and decreased macrophage phagocytosis[72, 74]. SP-A$^{-/-}$ mice exposed to LPS have elevated levels of TNF-α. Furthermore, the exogenous administration of SP-A leads to normalized levels of TNF-α, confirming an association of SP-A and the control of inflammation. These studies on SP-A$^{-/-}$ mice essentially reaffirm the role of SP-A as an important regulator of pulmonary inflammation[71, 72].

Mice bred after disruption of the SP-D gene (SP-D$^{-/-}$ mice) have shown remarkable abnormalities in surfactant homeostasis and alveolar cell morphology. The SP-D$^{-/-}$ mice exhibit a progressive accumulation of surfactant lipids and apoproteins in the alveolar space, hyperplasia of alveolar type II cells with massive enlargement of intracellular lamellar bodies, and an accumulation of foamy alveolar macrophages[13]. These mice spontaneously develop emphysema and fibrosis of the lungs, which suggests continuous inflammatory reaction associated with abnormal oxidant metabolism and MMP activity[136]. These phenotypes are quite similar to those observed in the mice deficient in granulocyte-macrophage colony stimulating factor[23]. SP-D deficiency results in a low SP-A pool size, rapid conversion from large-aggregate to small-aggregate surfactant, increased uptake into alveolar type II cells and recycling[13, 136]. The lack of SP-D is associated with a significantly more severe lung inflammation than that of SP-A$^{-/-}$ mice. Thus, SP-A and SP-D$^{-/-}$ mice have distinct phenotypes with respect to microbial challenge and the inflammatory response[70, 71]. SP-D$^{-/-}$ mice infected with bacteria or viruses have increased lung inflammation compared with infected wild-type strains, suggesting an anti-inflammatory role for SP-D.

Mice deficient in both SP-A and SP-D genes (double knock-out) show a progressive increase in BAL phospholipid, protein, and macrophage content through 24 weeks of age[41]. The double knock-out phenotype is characterized by the excessive accumulation of surfactant lipid in the alveolar space, increased numbers of foamy alveolar macrophages with up-regulation of MMP-12, and emphysema[41]. The absolute increase in macrophage number and the extent of MMP up-regulation by macrophages was greater in the double knock-out mice compared with the SP-D$^{-/-}$ mice. The double knock-out mice are likely to shed more light on a possible overlap between SP-A and SP-D functions. It is worthwhile to note that so far no human subject has been reported to have a complete deficiency of either SP-A or SP-D.

SP-A AND SP-D AS DIAGNOSTIC MARKERS FOR RESPIRATORY INFECTION AND DISEASE

SP-A and SP-D are constitutively synthesized and secreted into the airspace of the lungs by alveolar type II and non-ciliated bronchiolar epithelial cells[21, 104, 129]. The synthesis and secretion of both collectins increase with acute injury and epithelial activation[106]. Biochemical surfactant abnormalities, including alterations in BALF levels of SP-A and SP-D, have been reported in lung diseases such as asthma, bronchiolitis, COPD, lung transplantation, adult respiratory distress syndrome (ARDS), pulmonary edema, chronic lung disease of prematurity, and SP-B defi-

ciency, sarcoidosis, idiopathic pulmonary fibrosis (IPF), hypersensitivity pneumonitis, pulmonary alveolar proteinosis (PAP), cystic fibrosis (CF), pneumonia, and pulmonary infections caused by IAV, Mycoplasma, and *P. carinii* during AIDS[64]. For instance, *P. carinii* pneumonia is associated with raised levels of alveolar SP-A and SP-D, probably as a result of increased expression and accumulation of these collectins[4]. In all these pathological conditions, pneumonia and septic complications are a major cause of death. Interestingly, cigarette smoking, which is associated with an increased risk of COPD, pneumonia, and other lung infections, appears to reduce SP-A and SP-D BALF levels in healthy smokers[11, 51]. However, it is presently unclear if these deficiencies are a cause or consequence of the disease.

The altered expressions of SP-A and SP-D during health and disease can be useful specific markers for lung diseases[64]. Tests to measure the levels of these SPs are considered diagnostic for a number of respiratory infections and disorders. SP-A and SP-D production and lung maturity in neonates and infants with RDS are measured using amniotic fluids and tracheal aspirates, respectively[18, 108]. The SP-A and/or SP-D concentrations in BALF are significantly decreased in patients with ARDS, in patients at risk of developing ARDS, in neonates with bronchopulmonary dysplasia (BPD), and in children with CF. Conversely, significant increases in these proteins in BAL and sputum is diagnostic for PAP[64, 82]. ARDS is caused by surfactant deficiency at birth due to immaturity and a lack of differentiation of the alveolar epithelial cells involved in surfactant synthesis and secretion. Abnormal levels of SP-A and SP-D in the BALF have been reported in infant and ARDS patients[7]. However, a progressive increase in SP-A levels has been observed in patients with ARDS on mechanical ventilation[6]. Serum SP-D has been identified as a valuable biomarker for ARDS patients since SP-A and SP-D levels increase in the serum of ARDS patients[32]. SP-A has also been found useful as a marker for respiratory distress and alveolar injury[24, 82]. For example, a decrease in SP-A levels in BALF and lung biopsies has been associated with chronic lung injury in a familial form of interstitial lung disease[3]. The concentrations of SP-A and SP-D in BALF from patients with IPF and interstitial pneumonia with collagen vascular diseases (IPCD) are markedly lower than those in healthy controls, and the SP-A/phospholipid ratio may be a useful marker of survival prediction[52, 122].

In CF, which is associated with chronic lung inflammation and a high rate of superimposed *P. aeruginosa* lung infections, SP-A and SP-D BALF levels are lowered when combined with infection, which is inversely related to pulmonary inflammation[52, 94, 107]. Thus, in patients with CF, both inflammation (neutrophil-derived proteases) and infection (*Pseudomonas*-secreted proteases virulence factors) can potentially degrade and functionally alter SP-A and SP-D, contributing to the increased risk of infection. *P. aeruginosa* secretes a number of proteases that contribute to its virulence, and protease IV has been shown to degrade SP-A, SP-D, and SP-B, thereby limiting their biophysical and immunological properties[85]. Similarly, SP-D is a target of *P. aeruginosa* elastase that cleaves within the C-type lectin domain, rendering the molecule incapable of binding and aggregating lung pathogens[2]. The neutrophil serine proteases cathepsin G, elastase, and proteinase-3 have also been shown to cause degradation of SP-A[115]. Neutrophil serine proteinases have been shown to cleave SP-D within its CRD region and thus inactivate it functionally[48].

As described earlier, SP-A and SP-D have been shown to be involved in the modulation of pulmonary inflammatory responses and resistance to allergen-induced airway hypersensitivity[60, 79, 121, 123]. Abnormal levels of SP-A and SP-D in the BALF have been reported in hypersensitivity lung diseases[107]. Asthmatics show increased amounts of SP-A and SP-D in BALF compared with those in controls, and serum SP-D levels for two allergic patients have been shown to decrease following corticosteroid therapy[17].

The extent of oligomerization of SP-A and SP-D may be a contributory factor to lung disease. This is evident in the case of gastroesophageal reflux disease (GERD), which is often associated with chronic, severe lung damage coupled with episodes of recurrent infections in children. Comparison of the BALF from GERD patients and a control group who were not suffering from any respiratory disease showed differences in the macromolecular organization of SP-A and SP-D. The more active high-molecular-weight oligomers were massively diminished, particularly SP-D, and a marked increase in the smaller-sized forms was noted in children with GERD[33]. It is possible that functional impairment of SP-A and SP-D contributes to the GERD pathogenesis, which involves reflux-induced lung injury. Patients with birch pollen allergy and PAP show a shift towards lower oligomeric forms of SP-A with probable alteration of biological function in comparison with healthy volunteers[43]. Therefore, extrinsic factors that contribute to the maintenance or destabilization of multimeric structures of SP-A and SP-D need to be identified.

Table 3. Serum concentrations of SP-A in patients with various lung diseases*

Patients	SP-A in ng/ml (mean ±SD)
Healthy volunteers	24.9±9.60
Idiopathic pulmonary fibrosis (IPF)	67.9±42.5
Interstitial pneumonia with collagen vascular disease (IPCD)	55.3±7.90
Pulmonary alveolar proteinosis (PAP)	74.0±45.7
Sarcoidosis	23.7±13.6
Tuberculosis	37.5±19.3
Bronchial asthma	24.1±9.00
Chronic pulmonary emphysema	28.3±14.2
Diffuse panbronchiolitis	35.9±16.0
Bacterial pneumonia	32.5±21.7

* SP-D also appears in serum of patients with interstitial lung diseases. The mean SP-D level in serum from healthy volunteers is 48.7 ng/ml. Although the amount of SP-D (0.88±0.13 μg/ml) recovered in BAL fluids is less than that of SP-A (3.5±1.1 μg/ml) in the alveolar space, the SP-D concentration in serum is clearly higher than the serum SP-A concentrations. The absolute values of serum SP-D in patients with PAP, IPF and IPCD have been found to be 461, 339 and 208 ng/ml, respectively. There are no significant increases of serum SP-D levels in patients with bronchial asthma, bacterial pneumonia, diffuse panbronchiolitis and chronic pulmonary emphysema.

SP-A and SP-D also appear in the circulation in specific lung diseases (Table 3). SP-A and SP-D serum concentrations are significantly increased in patients with PAP, IPF, IPCD, and ARDS[88, 122]. High serum concentrations of SP-A in usual interstitial pneumonia compared with non-specific interstitial pneumonia is a good diagnostic marker[54]. SP-A is also a marker for lung adenocarcinomas and can be used to differentiate lung adenocarcinomas from other forms of metastatic cancers that have spread to the lungs[124]. The protein is also used to detect metastasis of lung adenocarcinomas. Recently, serum SP-D level has been reported to be significantly higher in allergic patients than in controls [mean serum SP-D concentration: 62.7 (55.5, 70.0) in allergic patients vs. 49.5 (36.7, 62.3) ng/ml in non-allergic controls]. In addition, baseline serum SP-D appeared to be an independent predictor for the magnitude of the late asthmatic response after allergen challenge[61]. Thus, the detection of SP-A and SP-D in sera may be a useful and non-invasive new diagnostic tool for a range of lung diseases[50]. The successive monitoring of serum levels of SP-A and/or SP-D may predict disease activity, although they do not always correlate with lung function tests. However, the mechanisms by which SP-A and SP-D increase in patients' sera are not clear. It could be attributed to type II cell hyperplasia and/or greater synthesis of these collectins, combined with a breakdown of epithelial barrier due to lung injury, fibrosis, and exaggerated inflammation.

ROLE OF GENETIC VARIANTS OF SP-A AND SP-D IN VARIOUS LUNG ASSOCIATED DISEASES

Respiratory diseases (such as allergy, asthma, and infectious diseases) are multi-factorial and caused by multiple genes' variants interacting with each other and in combination with environmental factors. Identifying and characterizing the genes involved in these disorders is a difficult task because of the reliance on substantial resources such as large collections of family data, highly informative genetic markers that span the genome, statistical approaches specifically developed to deal with multi-factorial disease, and the means to create physical maps. The use of candidate genes has increased the ability to identify genetic factors involved in diseases with complex etiology.

There has been interest in whether genetic variations in SP-A or SP-D correlate with an increased susceptibility to respiratory diseases. It is possible that the pathogenesis of respiratory diseases, where the function of SP-A and SP-D is altered due to environmental factors, impact more severely on genetically predisposed individuals. The SP-A and SP-D genes are located on chromosome 10q22–q23. SP-A product is encoded by two highly homologous genes that are referred to as SP-A1 and SP-A2, whereas the SP-D protein is expressed by a single gene[49]. The SP-A alleles may have potential for qualitative differences in SP-A structure and function[117, 131]. A recombinant human SP-A, consisting of a single gene product, is less efficient in stimulating TNF-α production by alveolar macrophages. SP-A consisting of single gene products do not form high-molecular-weight multimers compared with those composed of SP-A1 and SP-A2 alleles. In addition, ozone-induced oxidation differs significantly between SP-A1 and SP-A2 alleles. It is likely that certain polymorphisms in SP-A and SP-D genes may result in the production of proteins with impaired function(s), which in turn increases the likelihood for infection by respiratory pathogens. Several single-nucleotide polymorphisms (SNPs) are present in the SP-A1, SP-A2, and SP-D genes. Interestingly, the SP-A gene locus has been shown to be sufficiently polymorphic among various populations[109] (Saxena, unpublished).

Association with ARDS

The association between the SP-A allelic genotypes and the risk of RDS appears to be dependent on the SP-B genotype and is significantly influenced by the degree of prematurity, antenatal glucocorticoid therapy, multiple birth, and birth order[37]. An association of SP-A alleles with RDS has been reported using

control (n=83) and RDS (n=82) patients in a >8-week white population[56]. Differences between the two groups were observed for the 1A0 allele and 1A0 genotypes. Moreover, a significant synergistic positive association was observed between 1A0 allele + SP-B polymorphic variant and RDS. In an allele association study of 19 polymorphisms in SP-A1, SP--A2, SP-B, and SP-D genes in ARDS in a Caucasian population, multivariate analysis revealed significant differences only for the C/T (1580) polymorphism of SP-B, suggesting that SP-B or a linked gene contributes to susceptibility to ARDS[73]. When genotype analyses on 684 prematurely born neonates, of whom 184 developed RDS, were performed, neither of the two SP-B polymorphisms (Ile^{131}Thr and length variation of intron 4) associated directly with RDS or with prematurity[35]. Instead, it was observed that the previously identified association between SP-A alleles [susceptibility to RDS (6A(2), 1A(0)], or protection against it [6A(3), 1A(2)] and RDS, was dependent on the SP-B Thr/Thr genotype. Floros et al.[27] have genotyped black and white subjects with and without RDS for the SP-B intron 4 size variants (invariant, deletion, insertion) and for four [–18 (A/C), 1013 (A/C), 1580 (C/T), 9306 (A/G)] SP-B SNPs. It was also determined whether specific SP-B variants interacted with RDS susceptibility, or protective SP-A variants so as to enhance or reduce the risk for RDS. The SP-B intron 4 deletion variant appears a greater RDS risk factor for white male subjects, the SP-B intron 4 insertion variant for black female subjects. In white subjects, SP-A1 [6A(2)/6A(2)] or SP-A2 (1A(0)/1A(0) or 1A(0)/*) genotypes in subjects of certain GA and with a specific SP-B genotype (9306 (A/G) or del/*) are associated with an enhanced risk for RDS. However, in black subjects, SP-A1 (6A3/6A(3) or 6A(3)/*) genotypes in subjects of 31 weeks <or =GA< or =35 weeks and with the SP-B [1580 (T/T)] genotype are associated with a reduced risk for RDS.

A set of 76 father-mother-offspring trios for transmission disequilibrium from parents to affected offspring and a set of 31 trios have been studied for allele transmission from parents to hypernormal offspring born very prematurely (<32 weeks)[36]. SP-A1--A2 haplotype 6A(2)-1A(0) showed significant excess transmission to affected infants and SP-A1 allele 6A(2) decreased transmission to the hypernormals. This study substantiated a role of the SP-A alleles as genetic predisposers to RDS in premature infants. Marttila et al.[86] genotyped 441 premature singleton infants and 480 twin or multiple infants for SP-A1, SP-A2, and SP-B exon 4 polymorphisms and intron 4 size variants in a homogeneous white population. SP-A1 allele 6A2 (p=0.009) and the homozygous genotype 6A2/6A2 (p=0.003) were over-represented in RDS of singletons when the SP-B exon 4 genotype was Thr/Thr, and under-represented in RDS of multiples when the SP-B genotype was Ile/Thr (p=0.012 for 6A2 and p=0.03 for 6A2/6A2) or Thr/Thr (p=0.12 for 6A2 and p=0.018 for 6A2/6A2). In another study, the associations between SP-A allelic variants and RDS in a population consisting of 198 premature twin pairs were also evaluated[87]. The main SP-A1 allele 6A2 (p=0.030), genotype 6A2/6A2 (p=0.0042), and haplotype 6A2-1A(0) (p=0.016) were over-represented in healthy premature twin infants compared with RDS twins. The homozygous genotype 6A2/6A2 was over-represented in twin pairs of whom both were healthy compared with twins concordant for RDS (odds ratio (OR)=0.18, confidence intervals (CI)=0.06, 0.6, p=0.0016) and born between 32 and 36 weeks with birth weight sum higher than the median (OR=0.15, CI=0.04, 0.6, p=0.0025). Thus, 1) the association between SP-A polymorphism and RDS is different in twins from that seen in premature singleton infants and 2) SP-A genotype-specific susceptibility to RDS is related to the size of the uterus and the length of gestation at birth.

Association with BPD, COPD, and IPF

Bronchopulmonary dysplasia (BPD), the most common chronic lung disease in infants, is influenced by a number of antenatal and postnatal risk factors and is mostly preceded by RDS in the newborn. An association between SP-A1 and SP-A2 alleles and the risk of BPD in Caucasian preterm infants has been examined[134]. A significantly increased frequency of the SP-A1 polymorphism 6A6 was observed in infants with BPD compared with controls. An association study between SP-A, SP-B, SP-C, and SP-D gene variations and BPD revealed that the frequency of only the SP-B intron 4 deletion variant allele was increased in BPD versus controls (p=0.008, OR=2.0), and this variation did not associate with RDS[114]. Genotype analysis of SP-A, SP-B, SP-C--linked microsatellite, and SP-D marker alleles revealed several COPD susceptibility alleles: AA62_A (SP-A), B1580_C (SP-B), D2S388_5 (SP-D), based on an odds ratio (OR>2.5). The predictive ability of this model for developing COPD was reported to be good (c=0.926)[34].

Derangement in pulmonary surfactant or its components and alveolar collapse are common observations in IPF. The associations between IPF and genetic polymorphic variants of SP-A1, SP-A2, SP-B, SP-C, and SP-D have been examined in a Mexican population[119]. One SP-A1 (6A(4)) allele and SNPs that

characterizes the 6A(4) allele, and one SP-B (B1580_C) were found with higher frequency ($p \leq 0.01$) in nonsmoker and smoker IPF (n=84) subgroups, respectively, compared with healthy controls (n=194). However, the SP-C and SP-D SNPs and SP--B-linked microsatellite markers did not associate with IPF.

Association with pulmonary TB

A case-control association study by Floros et al.[27] in TB patients of Mexican origin indicated involvement of SP alleles in TB pathogenesis. Regression analyses of the TB and the tuberculin skin test-positive groups showed that DA11_C (SP-D) and GATA_3 (SP-B) are TB susceptibility alleles and (AAGG_2; SP-B) is a protective marker allele. Similarly, between TB patients and general population control subjects, susceptibility 1A(3) (SP-A2), 6A(4) (SP-A1), and B1013_A (SP-B) and protective AAGG_1 (SP-B), and AAGG_7 (SP-B) marker alleles were observed. Moreover, interactions were seen between alleles 6A(2) (SP-A1) and 1A(3) (SP-A2; p=0.0064) and between 1A(3) (SP-A2) and B1013_A (SP-B; p=0.036).

The relationship between polymorphisms in the collagen regions of SP-A1 and SP-A2 genes and pulmonary TB has been investigated in an Indian population[81]. Two intronic polymorphisms, SP-A1C1416T (p=0.0000, OR=20.767) and SP-A2C1382G (p=0.0054, OR=3.675), showed significant association with pulmonary TB. A redundant SNPA1660G of SP-A2 gene showed significant association with pulmonary TB. This polymorphism, when existing along with a non-redundant polymorphism, SP--A2G1649C ($Ala^{91}Pro$), resulted in a stronger association with pulmonary TB (p=0.000, OR=16.3).

Various regions of the SP-D gene were also screened in TB patients and controls of Indian origin (Vaid, unpublished). The SP-D gene from Indian subjects significantly differed from Caucasian subjects at 15 nucleotide positions. Comparative analysis of the allele frequencies of TB patients and healthy control subjects showed that SNP G459A (p=0.01, OR=3.04; redundant polymorphism in exon 7 encoding CRD) and T3130G (p=0.00, OR=13.75; intron 6) were significantly associated with TB in the Indian population.

Association with RSV infection

The frequency of the allele of SP-D coding for Met^{11} (p=0.033) has been shown to be increased in a severe RSV group of 84 Finnish infants hospitalized for the treatment of RSV bronchiolitis in comparison with 93 healthy controls[66]. Conditional logistic regression analysis further confirmed this association (p=0.028).

Association with allergy and asthma

A possible association of polymorphisms in the collagen region of SP-A2 with ABPA patients and its clinical markers has been examined[118]. A significantly higher frequency of the AGA allele (A1660G) of SP--A2 was observed in patients with ABPA in comparison with control subjects (p=0.0156, OR=4.78, 95% CI=1.23<OR<18.52). This polymorphism, when existing long with a nonredundant polymorphism, SP-A2 G1649C ($Ala^{91}Pro$), resulted in a stronger association with ABPA (A1660G and G1649C: p=0.0079, OR=10.4, 95% CI=1.62< OR<66.90). The ABPA patients containing GCT and AGG alleles showed significantly high levels of total IgE and percentage eosinophilia versus patients with ABPA with CCT and AGA alleles. Thus, SP-A2 G1649C and SP-A2 A1660G polymorphisms in the collagen region of SP-A2 could be one of the contributing factors to genetic predisposition and severity of clinical features of ABPA. In a recently concluded study (Saxena, unpublished), SNPs involving SP-A1 (C1101T, T3192C, and T3234C) and SP-A2 (A3265C) have been found to be associated with constitutional susceptibility to high-altitude pulmonary edema, a disease characterized by increased capillary permeability due to exaggerated inflammation and free radical-mediated lung injury. This is consistent with a protective role assigned to SP-A in controlling inflammation and oxidative damage.

Table 4 summarizes the observed alleles of SP-A and SP-D in association with various diseases. Different alleles of these genes seem to predispose the individuals to various diseases. A logical explanation seems to be that different SNPs lead to different alterations in function or expression. However, common SNPs predispose Caucasians to RDS and Mexicans to TB. Similarly, common SNPs predispose the Indian population to ABPA and TB. Furthermore, Met^{11} SP-D allele is predisposing Mexicans to TB and Finns to RSV infection. It is also interesting to note that some of the alleles of SP-A interact with other alleles of SP-A and SP-B and thus increase the susceptibility of subjects to a disease. These variants probably would serve as markers to identify subgroups of patients at risk, facilitating diagnosis and specific therapies. Studies examining the impact of gene polymorphisms in SP-A and/or SP-D genes in disease with concomitant measurement of a proximal phenotype are beginning to be reported. Leth-Larsen et al.[68] have

Table 4. SP-A and SP-D alleles associated with various diseases

Polymorphism		Disease association, population, type of study	Reference
gene	allele		
SP-A2	1A0	susceptibility, RDS, Caucasian	27, 36, 56
SP-A1	6A2	susceptibility, RDS, Caucasian	36, 56
SP-A1	6A3	protection, RDS, Caucasian	56
SP-A2	1A2	protection, RDS, Caucasian	56
SP-A1	6A3	protection, RDS, Negroids	27
SP-A1, SP-A2	6A2-1A0	susceptibility, RDS, Caucasian, family	36
SP-A1	6A2	protection, RDS, Caucasian, twins	86
SP-A1, SP-A2	6A2-1A0	protection, RDS, Caucasian, twins	87
SP-A1	6A6	susceptibility, BPD, Caucasian	134
SP-A	AA62_A	susceptibility, COPD, Mexican	34
SP-D	D2S388_5	susceptibility, COPD, Mexican	34
SP-A1	6A1	susceptibility, IPF, Mexican	119
SP-D	DA11_C	susceptibility, TB, Mexican	28
SP-A2	1A3	susceptibility, TB, Mexican	28
SP-A1	6A4	susceptibility, TB, Mexican	28
SP-A1, SP-A2	6A2-1A0	susceptibility, TB, Mexican	28
SP-A1	C1416T	susceptibility, TB, Indian	81
SP-A2	C1382G	susceptibility, TB, Indian	81
SP-A2	A1660G-G1649C	susceptibility, TB, Indian	81
SP-D	G459A	susceptibility, TB, Indian	Vaid, unpublished
SP-D	T3130G	susceptibility, TB, Indian	Vaid, unpublished
SP-D	Met 11	susceptibility, RSV, Finnish	66
SP-A2	A1660G-G1649C	susceptibility, ABPA, Indian	118

genotyped three SNPs altering amino acids in the mature protein in codon 11 ($Met^{11}Thr$), 160 ($Ala^{160}Thr$), and 270 ($Ser^{270}Thr$) of the SP-D gene and related to the SP-D levels in serum, Thr/Thr^{11} having lower SP-D serum levels than Met/Met^{11} genotypes. Interestingly, the recombinant and serum Met/Met^{11} variant of SP-D contains both multimeric as well as single-subunit proteins, compared with Thr/Thr^{11} protein species that primarily comprise low-molecular-weight monomers. The high- and low--molecular-weight species also show differences in their ligand binding properties[68]. The SNPs involving $Ala^{160}Thr$ and $Ser^{270}Thr$ do not seem to affect the oligomeric state of serum or recombinant SP-D. Recently, Heidinger et al. reported a frequently occurring specific haplotype (SFTPD*03 allele) in Caucasians that is associated with low serum SP-D levels[42]. The identification of a fragment-negative variant of the SFTPD gene provides another basis for the analysis of the functions of SP-D in pulmonary diseases. Thus it is obvious that critical studies that combine quantitative measurements of SP-A and SP-D and the functional consequences of various SNPs are urgently required.

PERSPECTIVES AND CONCLUSIONS

It is now clearly established that SP-A and SP-D play important roles in restricting pulmonary infection, lung allergy, and inflammation. The anti-microbial properties of SP-A and SP-D revolve around their ability to agglutinate a variety of pathogens, recruit and activate neutrophils and macrophages, and enhance killing via phagocytosis and/or production of superoxide radicals. These collectins also have direct inhibitory effect on microbial growth. SP-A and SP-D appear to offer resistance to allergen challenge by interfering with allergen-IgE interaction, mast cell/basophil degranulation, cellular infiltration, and helper T cell polarization. They are also involved in manipulating cytokine and chemokine profiles during inflammation due to infection, allergen challenge, or apoptotic and necrotic cells. The range of functions performed by these multifaceted and versatile proteins is probably achieved by their ability to engage a number of cell types. Thus, a number of putative candidate receptor molecules have been identified[49, 59].

The control of inflammation by SP-A and SP-D appears to reflect on the dichotomy in the mechanisms and regulatory pathways involved. Gardai et al. demonstrated that SP-A and SP-D can act as either pro-inflammatory or anti-inflammatory agents[29], depending on the binding orientation of the protein and availability of the CRDs to interact with macrophage Signal Regulating Protein α (SIRPα), a transmembrane protein associated with signal transduction. SP-A or SP-D CRD regions are able to bind to SIRPα, and the net effect is the inhibition of pro-inflammatory mediator production.

However, an interaction between the collagen region and calreticulin/CD91 has the opposite effect, in which pro-inflammatory mediators are produced. Thus, inflammation in the healthy lung is restricted by the physical association of the CRD of SP-A and SP-D with SIRPα exposed on the surface of resident cells. However, in the presence of pathogens or apoptotic/necrotic cells, the CRD region instead binds to molecular patterns displayed on the surface of microorganisms or damaged cells, leading to the formation of aggregates. This may present the collagen regions to calreticulin/CD91, thereby stimulating phagocytosis and pro-inflammatory responses.

Inflammation in the lung may be considered as a double-edged sword. The process may be critical in preventing serious infections. However, chronic inflammation may ultimately damage the lung. Therefore, this important host response to allergens and pathogens would appear to require a stringent control. Invariably, the average human lungs inhale a plethora of potential pathogens and allergens. However, the naïve healthy lung is not inflamed, which is probably achieved by anti-inflammatory properties of SP-A and SP-D that would thus clear pathogens/allergens/apoptotic cells during the initial innate immune response. In some individuals, the allergen/pathogen is able to overcome this initial host defense, after which the pathogen starts to proliferate and invade the lung, bringing a pro-inflammatory response into play. Here, SP-A and SP-D adopt a different function, in which they are associated with aiding induction of a pro-inflammatory response.

The therapeutic aspects of SP-A and/or SP-D appear to be an interesting proposition. For example, murine studies involving allergy suggest that administration of these proteins, especially SP-D or their recombinant fragments, can potentially serve as useful therapeutic agents, in conjunction with the established artificial surfactant mixtures already in clinical use. Specific agents currently being developed for the treatment of allergic inflammation include inhibitors of eosinophilic inflammation, drugs that inhibit allergen presentation, and inhibitors of Th2 cells. Thus, SP-A- and SP-D-based therapy are likely to modulate various components of allergic inflammatory response. In addition, because of their anti-inflammatory and anti-oxidant properties, SP-A, SP-D, or their recombinant fragments (containing functional lectin domains) may have potential therapeutic use in conditions such as RDS, BPD, CF, and chronic infections which involve exaggerated inflammation, defective clearance of cell debris, and generation of free radicals. However, most of these diseases are multifactorial and involve a hierarchy of etiology. Therefore, careful considerations should be given to translational research. There are very few murine studies reported so far where the assessment of the prophylactic or therapeutic properties of SP-A and SP-D have been fully described in terms of the protective mechanisms involved.

Murine studies involving *Afu* infection are also informative and of promising clinical relevance. *Aspergillus* species are increasingly being recognized as major fungal pathogens in immunocompromised or neutropenic patients. *Afu* is responsible for nearly 90% of cases of IPA, which is a prominent cause of infectious morbidity and mortality in patients with hematological malignancies and in recipients of stem cell and organ transplants. As incidences of AIDS, aplastic anemia, and organ transplantation are increasing and the use of long courses of corticosteroids as well as aggressive anti-neoplastic chemotherapeutic regimens are becoming frequent, the number of patients susceptible to *Aspergillus* infection is rising. In the immunocompromised or neutropenic patients, IPA, the commonest form of the disease, is characterized by hyphal invasion and destruction of pulmonary tissue. Dissemination of *Aspergillus* infection to other organs occurs in approximately 20% of IPA cases. Despite correct diagnosis and treatment, IPA results in mortality of >80% of the patients (up to 95% mortality rate among patients of bone marrow transplantation). The gold standard anti-fungal therapy using AmB is limited because of toxicities associated with dosage and inadequate effects in severely immunosuppressed hosts. Because IPA is extremely rare in immunocompetent individuals, therapy aimed at strengthening the host's immune response to the organisms offers a promising new approach in the treatment of this disease. The therapeutic use of SP-A and SP-D can be specially advantageous when the infection involves *Afu* strains resistant to AmB and/or itraconazole.

Despite rapid growth in the field in last decade, there are significant gaps in our understanding of many aspects of research involving pulmonary collectins. For example, the regulatory physiological and molecular mechanisms that control SP-A and/or SP-D expression during health and disease are not well defined. It is also not clear what hierarchies of immune mechanisms are triggered by SP-A and SP-D during persistent infection, allergy and inflammation. With the advent of genomics and proteomics it is envisaged that the next few years will provide a great deal of information towards

understanding relationships between SP-A and SP-D and other cytokine/chemokine pathways which together are likely to orchestrate pulmonary homeostasis of surfactant and immunity. Moreover, the roles of SP-A and SP-D in reproductive tissues need further investigation. It will also be of considerable interest to know how well the results from murine experiments (using wild-type and gene knock-out mice) involving allergy, infection, inflammation, and emphysema correlate in the context of human disease before a possible clinical trial can be undertaken.

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REFERENCES

1. Alcorn J. F. and Wright J. R. (2004): Surfactant protein A inhibits alveolar macrophage cytokine production by CD14-independent pathway. Am. J. Physiol. Lung Cell Mol. Physiol., 286, L129–L136.

2. Alcorn J.F. and Wright J.R. (2004): Degradation of pulmonary surfactant protein D by *Pseudomonas aeruginosa* elastase abrogates innate immune function. J. Biol. Chem., 279, 30871–30879.

3. Amin R. S., Wert S. E., Baughman R. P., Tomashefski J. F. Jr., Nogee L. M., Brody A. S., Hull W. M. and Whitsett J. A. (2001): Surfactant protein deficiency in familial interstitial lung disease. J. Pediatr., 139, 85–92.

4. Atochina E. N., Beck J. M., Scanlon S. T., Preston A. M. and Beers M. F. (2001): *Pneumocystis carinii* pneumonia alters expression and distribution of lung collectins SP-A and SP-D. J. Lab. Clin. Med., 137, 429–439.

5. Awasthi S., Coalson J. J., Yoder B. A., Crouch E. and King R. J. (2001): Deficiencies in lung surfactant proteins A and D are associated with lung infection in very premature neonatal baboons. Am. J. Respir. Crit. Care Med., 163, 389–397.

6. Balamugesh T., Kaur S., Majumdar S. and Behera D. (2003): Surfactant protein-A levels in patients with acute respiratory distress syndrome. Indian J. Med. Res., 117, 129–133.

7. Ballard P. L., Merrill J. D., Godinez R. I., Godinez M. H., Truog W. E. and Ballard R. A. (2003): Surfactant protein profile of pulmonary surfactant in premature infants. Am. J. Respir. Crit. Care Med., 168, 1123–1128.

8. Barr F. E., Pedigo H., Johnson T. R. and Shepherd V. L. (2000): Surfactant protein A enhances uptake of respiratory syncytial virus by monocytes and U937 macrophages. Am J Respir Cell Mol Biol., 23, 586–592.

9. Bayer H., Bonnar J., Phizackerley P. J., Moore R. A. and Wylie F. (1973): Amniotic fluid phospholipids in normal and abnormal pregnancy. J. Obstet. Gynaecol. Br. Commonw., 80, 333–337.

10. Benne C. A., Kraaijeveld C. A., van Strijp J. A., Brouwer E., Harmsen M., Verhoef J., van Golde L. M. and van Iwaarden J. F. (1995): Interactions of surfactant protein A with influenza A viruses: binding and neutralization. J. Infect. Dis., 171, 335–341.

11. Betsuyaku T., Kuroki Y., Nagai K., Nasuhara Y. and Nishimura M. (2004): Effects of ageing and smoking on SP-A and SP-D levels in bronchoalveolar lavage fluid. Eur. Respir. J., 24, 964–970.

12. Borron P. J., Crouch E. C., Lewis J. F., Wright J. R., Possmayer F. and Fraher L. J. (1998): Recombinant rat surfactant-associated protein D inhibits human T lymphocyte proliferation and IL-2 production. J. Immunol., 161, 4599–4603.

13. Botas C., Poulain F., Akiyama J., Brown C., Allen L., Goerke J., Clements J., Carlson E., Gillespie A. M., Epstein C. and Hawgood S. (1998): Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. Proc. Natl. Acad. Sci. USA, 95, 11869–11874.

14. Brinker K. G., Garner H. and Wright J. R. (2003): Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells. Am. J. Physiol. Lung Cell Mol. Physiol., 284, L232–L241.

15. Brinker K. G., Martin E., Borron P., Mostaghel E., Doyle C., Harding C. V. and Wright J. R. (2001): Surfactant protein D enhances bacterial antigen presentation by bone marrow-derived dendritic cells. Am. J. Physiol. Lung Cell Mol. Physiol., 281, L1453–L1463.

16. Cheng G., Ueda T., Nakajima H., Nakajima A., Kinjyo S., Motojima S. and Fukuda T. (1998): Suppressive effects of SP-A on ionomycin-induced IL-8 production and release by eosinophils. Int. Arch. Allergy Immunol., 117 (suppl. 1), 59–62.

17. Cheng G., Ueda T., Numao T., Kuroki Y., Nakajima H., Fukushima Y., Motojima S. and Fukuda T. (2000): Increased levels of surfactant protein A and D in bronchoalveolar lavage fluids in patients with bronchial asthma. Eur. Respir. J., 16, 831–835.

18. Chida S., Fujiwara T., Konishi M., Shimada S. and Takahashi A. (1997): Surfactant proteins and stable microbubbles in tracheal aspirates of infants with respiratory distress syndrome: relation to the degree of respiratory failure and response to exogenous surfactant. Eur. J. Pediatr., 156, 131–138.

19. Condon J. C., Jeyasuria P., Faust J. M. and Mendelson C. R. (2004): Surfactant protein secreted by the maturing mouse fetal lung acts as a hormone that signals the initiation of parturition. Proc. Natl. Acad. Sci. USA, 101, 4978–4983.

20. Crouch E., Parghi D., Kuan S. F. and Persson A. (1992): Surfactant protein D: subcellular localization in nonciliated bronchiolar epithelial cells. Am J Physiol., 263, L60–L66.

21. Crouch E., Rust K., Marienchek W., Parghi D., Chang D. and Persson A. (1991): Developmental expression of pulmonary surfactant protein D (SP-D). Am. J. Respir. Cell Mol. Biol., 5, 13–18.

22. Downing J. F., Pasula R., Wright J. R., Twigg H. L. 3rd and Martin W. J. 2nd. (1995): Surfactant protein A promotes attachment of *Mycobacterium tuberculosis* to alveolar macrophages during infection with human immunodeficiency virus. Proc. Natl. Acad. Sci. USA, 92, 4848–4852.

23. Dranoff G., Crawford A. D., Sadelain M., Ream B., Rashid A., Bronson R. T., Dickersin G. R., Bachurski C. J., Mark E. L. and Whitsett J. A. (1994): Involvement of granulocyte-macrophage colony-stimulating factor in pulmonary homeostasis. Science, 264, 713–716.

24. Eisner M. D., Parsons P., Matthay M. A., Ware L. and Greene K. (2003): Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. Thorax, 58, 983–988.

25. Ferguson J. S., Voelker D. R., McCormack F. X. and Schlesinger L. S. (1999): Surfactant protein D binds to *Mycobacterium tuberculosis* bacilli and lipoarabinomannan via carbohydrate-lectin interactions resulting in reduced phagocytosis of the bacteria by macrophages. J. Immunol., 163, 312–321.

26. Ferguson J. S., Voelker D. R., Ufnar J. A., Dawson A. J. and Schlesinger L. S. (2002): Surfactant protein D inhibition of human macrophage uptake of *Mycobacterium tuberculosis* is independent of bacterial agglutination. J. Immunol., 168, 1309–1314.

27. Floros J., Fan R., Diangelo S., Guo X., Wert J. and Luo J. (2001): Surfactant protein (SP) B associations and interactions with SP-A in white and black subjects with respiratory distress syndrome. Pediatr. Int., 43, 567–576.

28. Floros J., Lin H. M., Garcia A., Salazar M. A., Guo X., DiAngelo S., Montano M., Luo J., Pardo A. and Selman M. (2000): Surfactant protein genetic marker alleles identify a subgroup of tuberculosis in a Mexican population. J. Infect. Dis., 182, 1473–1478.

29. Gardai S. J., Xiao Y. Q., Dickinson M., Nick J. A., Voelker D. R., Greene K. E. and Henson P. M. (2003): By binding SIRPα or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. Cell, 115, 13–23.

30. Gaynor C. D., McCormack F. X., Voelker D. R., McGowan S. E. and Schlesinger L. S. (1995): Pulmonary surfactant protein A mediates enhanced phagocytosis of *Mycobacterium tuberculosis* by a direct interaction with human macrophages. J. Immunol., 155, 5343–5351.

31. Ghildyal R., Hartley C., Varrasso A., Meanger J., Voelker D. R., Anders E. M. and Mills J. (1999): Surfactant protein A binds to the fusion glycoprotein of respiratory syncytial virus and neutralizes virion infectivity. J. Infect. Dis., 180, 2009–2013.

32. Greene K. E., Wright J. R., Steinberg K. P., Ruzinski J. T., Caldwell E., Wong W. B., Hull W., Whitsett J. A., Akino T., Kuroki Y., Nagae H., Hudson L. D. and Martin T. R. (1999): Serial changes in surfactant-associated proteins in lung and serum before and after onset of ARDS. Am. J. Respir. Crit. Care Med., 160, 1843–1850.

33. Griese M., Maderlechner N., Ahrens P. and Kitz R. (2002): Surfactant proteins A and D in children with pulmonary disease due to gastroesophageal reflux. Am. J. Respir. Crit. Care Med. 165, 1546–1550.

34. Guo X., Lin H. M., Lin Z., Montano M., Sansores R., Wang G., DiAngelo S., Pardo A., Selman M. and Floros J. (2001): Surfactant protein gene A, B, and D marker alleles in chronic obstructive pulmonary disease of a Mexican population. Eur. Respir. J., 18, 482–490.

35. Haataja R., Marttila R., Uimari P., Lofgren J., Ramet M. and Hallman M. (2001): Respiratory distress syndrome: evaluation of genetic susceptibility and protection by transmission disequilibrium test. Hum. Genet., 109, 351–355.

36. Haataja R., Ramet M., Marttila R. and Hallman M. (2000): Surfactant proteins A and B as interactive genetic determinants of neonatal respiratory distress syndrome. Hum. Mol. Genet., 9, 2751–2760.

37. Hallman M., Haataja R. and Marttila R. (2002): Surfactant proteins and genetic predisposition to respiratory distress syndrome. Semin. Perinatol., 26, 450–460.

38. Hartshorn K. L., Crouch E. C., White M. R., Eggleton P., Tauber A. I., Chang D. and Sastry K. (1994): Evidence for a protective role of pulmonary surfactant protein D (SP-D) against influenza A viruses. J. Clin. Invest., 94, 311–319.

39. Hartshorn K. L., Reid K. B. M, White M. R., Jensenius J. C., Morris S. M., Tauber A. I. and Crouch E. C. (1996): Neutrophil deactivation by influenza A viruses: mechanisms of protection after viral opsonization with collectins and hemagglutination-inhibiting antibodies. Blood, 87, 3450–3461.

40. Hartshorn K. L., White M. R., Voelker D. R., Coburn J., Zaner K. and Crouch E. C. (2000): Mechanism of binding of surfactant protein D to influenza A viruses: importance of binding to haemagglutinin to antiviral activity. Biochem J., 351, 449–458.

41. Hawgood S., Ochs M., Jung A., Akiyama J., Allen L., Brown C., Edmondson J., Levitt S., Carlson E., Gillespie A. M., Villar A., Epstein C. J. and Poulain F. R. (2002): Sequential targeted deficiency of SP-A and -D leads to progressive alveolar lipoproteinosis and emphysema. Am. J. Physiol. Lung Cell Mol. Physiol., 283, L1002–1010.

42. Heidinger K., Konig I.R., Bohnert A., Kleinsteiber A., Hilgendorff A., Gortner L., Ziegler A., Chakraborty T. and Bein G. (2005): Polymorphisms in the human surfactant protein D (SFTPD) gene: strong evidence that serum levels of surfactant protein D (SP-D) are genetically influenced. Immunogenetics, 57, 1–7.

43. Hickling T. P., Bright H., Wing K., Gower D., Martin S. L., Sim R. B. and Malhotra R. (1999): A recombinant trimeric surfactant protein D carbohydrate recognition domain inhibits respiratory syncytial virus infection *in vitro* and *in vivo*. Eur J Immunol., 29, 3478–3484.

44. Hickling T. P., Malhotra R., Bright H., McDowell W., Blair E. D. and Sim R. B. (2000): Lung surfactant protein A provides a route of entry for respiratory syncytial virus into host cells. Viral Immunol., 13, 125–135.

45. Hickling T. P., Malhotra R. and Sim R. B. (1998): Human lung surfactant protein A exists in several different oligomeric states: oligomer size distribution varies between patient groups. Mol. Med., 4, 266–275.

46. Hickman-Davis J., Gibbs-Erwin J., Lindsey J. R. and Matalon S. S. (1999): Surfactant protein A mediates mycoplasmacidal activity of alveolar macrophages by production of peroxynitrite. Proc. Natl. Acad. Sci. USA, 96, 4953–4958.

47. Hills B. A. (1994): Further studies of the role of surfactant in premature rupture of the membranes. Am. J. Obstet. Gynecol., 170, 195–201.

48. Hirche T. O., Crouch E. C., Espinola M., Brokelman T. J., Mecham R. P., DeSilva N., Cooley J., Remold-O'Donnell E. and Belaaouaj A. (2004): Neutrophil serine proteinases inactivate surfactant protein D by cleaving within a conserved subregion of the carbohydrate recognition domain. J. Biol. Chem., 279, 27688–27698.

49. Holmskov U., Thiel S. and Jensenius J. C. (2003): Collectins and ficolins: humoral lectins of the innate immune defense. Annu. Rev. Immunol., 21, 547–578.

50. Honda Y., Kuroki Y., Matsuura E., Nagae H., Takahashi H., Akino T. and Abe S. (1995): Pulmonary surfactant protein D in sera and bronchoalveolar lavage fluids. Am. J. Respir. Crit. Care Med., 152, 1860–1866.

51. Honda Y., Takahashi H., Kuroki Y., Akino T. and Abe S. (1996): Decreased contents of surfactant proteins A and D in BAL fluids of healthy smokers. Chest, 109, 1006–1009.

52. Hull J., South M., Phelan P. and Grimwood K. (1997): Surfactant composition in infants and young children with cystic fibrosis. Am. J. Respir. Crit. Care Med., 156, 161–165.

53. Ikegami M., Korfhagen T. R., Bruno M. D., Whitsett J. A. and Jobe A. H. (1997): Surfactant metabolism in surfactant protein A-deficient mice. Am. J. Physiol., 272, L479–L485.

54. Ishii H., Mukae H., Kadota J., Kaida H., Nagata T., Abe K., Matsukura S. and Kohno S. (2003): High serum concentrations of surfactant protein A in usual interstitial pneumonia compared with non-specific interstitial pneumonia. Thorax, 58, 52–57.

55. Kabha K., Schmegner J., Keisari Y., Parolis H., Schlepper-Schaeffer J. and Ofek I. (1997): SP-A enhances phagocytosis of *Klebsiell*a by interaction with capsular polysaccharides and alveolar macrophages. Am. J. Physiol., 272, L344–352.

56. Kala P., Ten Have T., Nielsen H., Dunn M. and Floros J. (1998): Association of pulmonary surfactant protein A (SP-A) gene and respiratory distress syndrome: interaction with SP-B. Pediatr. Res., 43, 169–177.

57. Keirse M. J. (1978): Biosynthesis and metabolism of prostaglandins in the pregnant human uterus. Adv. Prostaglandin Thromboxane Res., 4, 87–102.

58. Kishore U., Madan T., Sarma P. U., Singh M., Urban B. C. and Reid K. B. M. (2002): Protective roles of pulmonary surfactant proteins, SP-A and SP-D, against lung allergy and infection caused by *Aspergillus fumigatus*. Immunobiology, 205, 610–618.

59. Kishore U. and Reid K. B. M. (2001): Structures and functions of mammalian collectins. Mammalian carbohydrate recognition proteins. Results Probl. Cell Differ., 33, 225–248.

60. Kishore U., Wang J. Y., Hoppe H. J. and Reid K. B. M. (1996): The α-helical neck region of human lung surfactant protein D is essential for the binding of the carbohydrate recognition domains to lipopolysaccharides and phospholipids. Biochem J., 318, 505–511.

61. Koopmans J. G., van der Zee J. S., Krop E. J., Lopuhaa C. E., Jansen H. M. and Batenburg J. J. (2004): Serum surfactant protein D is elevated in allergic patients. Clin. Exp. Allergy, 34, 1827–1833.

62. Korfhagen T. R., Bruno M. D., Ross G. F., Huelsman K. M., Ikegami M., Jobe A. H., Wert S. E., Stripp B. R., Morris R. E., Glasser S. W., Bachurski C. J., Iwamoto H. S. and Whitsett J. A. (1996): Altered surfactant function and structure in SP-A gene targeted mice. Proc. Natl. Acad. Sci. USA, 93, 9594–9599.

63. Korfhagen T. R., LeVine A. M. and Whitsett J. A. (1998): Surfactant protein A (SP-A) gene targeted mice. Biochim. Biophys. Acta, 1408, 296–302.

64. Kuroki Y., Takahashi H., Chiba H. and Akino T. (1998): Surfactant proteins A and D: disease markers. Biochim. Biophys. Acta, 1408, 334–345.

65. Lahra M. M. and Jeffery H. E. (2004): A fetal response to chorioamnionitis is associated with early survival after preterm birth. Am. J. Obstet. Gynecol., 190, 147–151.

66. Lahti M., Lofgren J., Marttila R., Renko M., Klaavuniemi T., Haataja R., Ramet M. and Hallman M. (2002): Surfactant protein D gene polymorphism associated with severe respiratory syncytial virus infection. Pediatr. Res., 51, 696–699.

67. Leth-Larsen R., Floridon C., Nielsen O. and Holmskov U. (2004): Surfactant protein D in the female genital tract. Mol. Hum. Reprod., 10, 149–154.

68. Leth-Larsen R., Garred P., Jensenius H., Meschi J., Hartshorn K., Madsen J., Tornoe I., Madsen H.O., Sorensen G., Crouch E. and Holmskov U. (2005): A common polymorphism in the SFTPD gene influences assembly, function, and concentration of surfactant protein D. J. Immunol., 174, 1532–1538.

69. LeVine A. M., Gwozdz J., Stark J., Bruno M., Whitsett J. and Korfhagen T. (1999): Surfactant protein-A enhances respiratory syncytial virus clearance *in vivo*. J. Clin. Invest., 103, 1015–1021.

70. LeVine A. M., Hartshorn K., Elliott J., Whitsett J. and Korfhagen T. (2002): Absence of SP-A modulates innate and adaptive defence responses to pulmonary influenza infection. Am. J. Physiol. Lung Cell Mol. Physiol., 282, L563–L572.

71. LeVine A. M., Whitsett J. A., Gwozdz J. A., Richardson T. R., Fisher J. H., Burhans M. S. and Korfhagen T. R. (2000): Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. J. Immunol., 165, 3934–3940.

72. LeVine A. M., Whitsett J. A., Hartshorn K. L., Crouch E. C. and Korfhagen T. R. (2001): Surfactant protein D enhances clearance of influenza A virus from the lung *in vivo*. J. Immunol., 167, 5868–5873.

73. Lin Z., Pearson C., Chinchilli V., Pietschmann S. M., Luo J., Pison U. and Floros J. (2000): Polymorphisms of human SP-A, SP-B, and SP-D genes: association of SP-B $Thr^{131}Ile$ with ARDS. Clin. Genet., 58, 181–191.

74. Linke M. J., Harris C. E., Korfhagen T. R., McCormack F. X., Ashbaugh A. D., Steele P., Whitsett J. A. and Walzer P. D. (2001): Immunosuppressed surfactant protein A-deficient mice have increased susceptibility to *Pneumocystis carinii* infection. J. Infect. Dis., 183, 943–952.

75. López-Bernal A., Newman G. E., Phizackerley P. J. and Turnbull A. C. (1988): Surfactant stimulates prostaglandin E production in human amnion. Br. J. Obstet. Gynaecol., 95, 1013–1017.

76. López-Bernal A. and Phizackerley P. J. (2000): Fetal surfactant as a source of arachidonate in human amniotic fluid. Prostaglandins Other Lipid Mediat., 60, 59–70.

77. Madan T., Eggleton P., Kishore U., Strong P., Aggrawal S. S., Sarma P. U. and Reid K. B. M. (1997): Binding of pulmonary surfactant proteins A and D to *Aspergillus fumigatus* conidia enhances phagocytosis and killing by human neutrophils and alveolar macrophages. Infect. Immun., 65, 3171–3179.

78. Madan T., Kishore U., Shah A., Eggleton P., Strong P., Wang J. Y., Aggrawal S. S., Sarma P. U. and Reid K. B. M. (1997): 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., 110, 241–249.

79. Madan T., Kishore U., Singh M., Strong P., Clark H., Hussain E. M., Reid K. B. M. and Sarma P. U. (2001): Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. J. Clin. Invest., 107, 467–475.

80. Madan T., Kishore U., Singh M., Strong P., Hussain E. M., Reid K. B. and Sarma P. U. (2001): Protective role of lung surfactant protein D in a murine model of invasive pulmonary aspergillosis. Infect. Immun., 69, 2728–2731.

81. Madan T., Saxena S., Murthy K. J., Muralidhar K. and Sarma P. U. (2002): Association of polymorphisms in the collagen region of human SP-A1 and SP-A2 genes with pulmonary tuberculosis in Indian population. Clin. Chem. Lab. Med., 40, 1002–1008.

82. Maeda H., Fujita M. Q., Zhu B. L., Ishida K., Quan L., Oritani S. and Taniguchi M. (2003): Pulmonary surfactant-associated protein A as a marker of respiratory distress in forensic pathology: assessment of the immunohistochemical and biochemical findings. Leg. Med. (Tokyo)., 5 (suppl. 1), S318–S321.

83. Malhotra R., Haurum J., Thiel S., Jensenius J. C. and Sim R. B. (1993): Pollen grains bind to lung alveolar type II cells (A549) via lung surfactant protein A (SP-A). Biosci. Rep. 13, 79–90.

84. Malhotra R., Willis A. C., Lopez Bernal A., Thiel S. and Sim R. B. (1994): Mannan-binding protein levels in human amniotic fluid during gestation and its interaction with collectin receptor from amnion cells. Immunology, 82, 439–444.

85. Malloy J. L., Veldhuizen R. A., Thibodeaux B. A., O'Callaghan R. J. and Wright J. R. (2005): *Pseudomonas aeruginosa* protease IV degrades surfactant proteins and inhibits surfactant host defense and biophysical functions. Am. J. Physiol. Lung Cell Mol. Physiol., 288, L409–418.

86. Marttila R., Haataja R., Guttentag S. and Hallman M. (2003): Surfactant protein A and B genetic variants in respiratory distress syndrome in singletons and twins. Am. J. Respir. Crit. Care Med., 168, 1216–1222.

87. Marttila R., Haataja R., Ramet M., Pokela M. L., Tammela O. and Hallman M. (2003): Surfactant protein A gene locus and respiratory distress syndrome in Finnish premature twin pairs. Ann. Med., 35, 344–352.

88. McCormack F. X., Gibbons R., Ward S. R., Kuzmenko A., Wu H. and Deepe G. S. Jr. (2003): Macrophage-independent fungicidal action of the pulmonary collectins. J. Biol. Chem., 278, 36250–36256.

89. McCormack F. X., King T. E. Jr., Voelker D. R., Robinson P. C. and Mason R. J. (1991): Idiopathic pulmonary fibrosis. Abnormalities in the bronchoalveolar lavage content of surfactant protein A. Am. Rev. Respir. Dis., 144, 160–166.

90. McNeely T. B. and Coonrod J. D. (1994): Aggregation and opsonization of type A but not type B *Hemophilus influenzae* by surfactant protein A. Am. J. Respir. Cell Mol. Biol., 11, 114–122.

91. Meinert M., Eriksen G. V., Petersen A. C., Helmig R. B., Laurent C., Uldbjerg N. and Malmstrom A. (2001): Proteoglycans and hyaluronan in human fetal membranes. Am. J. Obstet. Gynecol., 184, 679–685.

92. Miyamura K., Malhotra R., Hoppe H. J., Reid K. B. M., Phizackerley P. J., Macpherson P. and Lopez Bernal A. (1994): Surfactant proteins A (SP-A) and D (SP-D): levels in human amniotic fluid and localization in the fetal membranes. Biochim. Biophys. Acta, 1210, 303–307.

93. Nadesalingam J., Bernal A. L., Dodds A. W., Willis A. C., Mahoney D. J., Day A. J., Reid K. B. M. and Palaniyar N. (2003): Identification and characterization of a novel interaction between pulmonary surfactant protein D and decorin. J. Biol. Chem., 278, 25678–25687.

94. Noah T. L., Murphy P. C., Alink J. J., Leigh M. W., Hull W. M., Stahlman M. T. and Whitsett J. A. (2003): Bronchoalveolar lavage fluid surfactant protein A and surfactant protein D are inversely related to inflammation in early cystic fibrosis. Am. J. Respir. Crit. Care Med., 168, 685–691.

95. Norwitz E. R., Lopez Bernal A. and Starkey P. M. (1992): Tumor necrosis factor-α selectively stimulates prostaglandin $F_{2\alpha}$ production by macrophages in human term decidua. Am. J. Obstet. Gynecol., 167, 815–820.

96. Norwitz E. R., Starkey P. M. and Lopez Bernal A. (1992): Prostaglandin D2 production by term human decidua: cellular origins defined using flow cytometry. Obstet. Gynecol., 80, 440–445.

97. Oberley R. E., Ault K. A., Neff T. L., Khubchandani K. R., Crouch E. C. and Snyder J. M.(2004): Surfactant proteins A and D enhance the phagocytosis of *Chlamydia* into THP-1 cells. Am. J. Physiol. Lung Cell Mol. Physiol., 287, L296–L306.

98. Oberley R. E., Goss K. L., Ault K. A., Crouch E. C. and Snyder J. M. (2004): Surfactant protein D is present in the human female reproductive tract and inhibits *Chlamydia trachomatis* infection. Mol. Hum. Reprod., 10, 861–870.

99. Ogasawara Y., McCormack F. X., Mason R. J. and Voelker D. R. (1994): Chimeras of surfactant proteins A and D identify the carbohydrate recognition domains as essential for phospholipid interaction. J. Biol. Chem., 269, 29785–29792.

100. Okita J. R., MacDonald P. C. and Johnston J. M. (1982): Mobilization of arachidonic acid from specific glycerophospholipids of human fetal membranes during early labor. J. Biol. Chem., 257, 14029–14034.

101. O'Riordan D. M., Standing J. E., Kwon K. Y., Chang D., Crouch E. C. and Limper A. H. (1995): Surfactant protein D interacts with *Pneumocystis carinii* and mediates organism adherence to alveolar macrophages. J. Clin. Invest., 95, 2699–2710.

102. Pasula R., Downing J. F., Wright J. R., Kachel D. L., Davis T. E. Jr. and Martin W. J. 2nd. (1997): Surfactant protein A (SP-A) mediates attachment of *Mycobacterium tuberculosis* to murine alveolar macrophages. Am. J. Respir. Cell Mol. Biol., 17, 209–217.

103. Perez-Gil J. (2002): Molecular interactions in pulmonary surfactant films. Biol. Neonate, 81 (suppl. 1), 6–15.

104. Persson A., Rust K., Chang D., Moxley M., Longmore W. and Crouch E. (1988): CP4: a pneumocyte-derived collagenous surfactant-associated protein. Evidence for heterogeneity of collagenous surfactant proteins. Biochemistry, 27, 8576–8584.

105. Piboonpocanun S., Chiba H., Mitsuzawa H., Martin W., Murphy R. C., Harbeck R. J. and Voelker D. R. (2005): Surfactant protein A binds *Mycoplasma pneumoniae* with high affinity and attenuates its growth by recognition of disaturated-phosphatidylglycerols. J. Biol. Chem., 280, 9–17.

106. Pison U., Obertacke U., Seeger W. and Hawgood S. (1992): Surfactant protein A (SP-A) is decreased in acute parenchymal lung injury associated with polytrauma. Eur. J. Clin. Invest., 22, 712–718.

107. Postle, A. D., Mander A., Reid K. B. M., Wang J. Y., Wright S. M., Moustaki M. and Warner J. O. (1999): Deficient hydrophilic lung surfactant proteins A and D with normal surfactant phospholipid molecular species in cystic fibrosis. Am. J. Respir. Cell Mol. Biol., 20, 90–98.

108. Pryhuber G. S., Hull W. M., Fink I., McMahan M. J. and Whitsett J. A. (1991): Ontogeny of surfactant proteins A and B in human amniotic fluid as indices of fetal lung maturity. Pediatr. Res., 30, 597–605.

109. Ramet M., Haataja R., Marttila R., Hamalainen A. M., Knip M. and Hallman M. (2000): Human surfactant protein A gene locus for genetic studies in the Finnish population. Dis. Markers, 16, 119–124.

110. Reading P. C., Morey L. S., Crouch E. C. and Anders E. M. (1997): Collectin-mediated antiviral host defense of the lung: evidence from influenza virus infection of mice. J Virol., 71, 8204–8212.

111. Rooney S. A., Young S. L. and Mendelson C. R. (1994): Molecular and cellular processing of lung surfactant. FASEB J., 8, 957–967.

112. Rosseau S., Guenther A., Seeger W. and Lohmeyer J. (1997): Phagocytosis of viable *Candida albicans* by alveolar macrophages: lack of opsonin function of surfactant protein A. J. Infect. Dis., 175, 421–428.

113. Rosseau S., Hammerl P., Maus U., Gunther A., Seeger W., Grimminger F. and Lohmeyer J. (1999): Surfactant protein A down-regulates proinflammatory cytokine production evoked by *Candida albicans* in human alveolar macrophages and monocytes. J. Immunol., 163, 4495–4502.

114. Rova M., Haataja R., Marttila R., Ollikainen V., Tammela O. and Hallman M. (2004): Data mining and multiparameter analysis of lung surfactant protein genes in bronchopulmonary dysplasia. Hum. Mol. Genet., 13, 1095–1104.

115. Rubio. F, Cooley J., Accurso F. J. and Remold-O'Donnell E. (2004): Linkage of neutrophil serine proteases and decreased surfactant protein-A (SP-A) levels in inflammatory lung disease. Thorax, 59, 318–323.

116. Sahly H., Ofek I., Podschun R., Brade H., He Y., Ullmann U. and Crouch E. (2002): Surfactant protein D binds selectively to *Klebsiella pneumoniae* lipopolysaccharides containing mannose-rich O-antigens. J Immunol., 169, 3267–3274.

117. Sanchez-Barbero F., Strassner J., Garcia-Canero R., Steinhilber W. and Casals C. (2005): Role of the degree of oligomerization in the structure and function of human surfactant protein A. J. Biol. Chem., 280, 7659–7670.

118. Saxena S., Madan T., Shah A., Muralidhar K. and Sarma P.U. (2003): 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., 111, 1001–1007.

119. Selman M., Lin H.M., Montano M., Jenkins A. L., Estrada A., Lin Z., Wang G., DiAngelo S. L., Guo X., Umstead T. M., Lang C. M., Pardo A., Phelps D. S. and Floros J. (2003): Surfactant protein A and B genetic variants predispose to idiopathic pulmonary fibrosis. Hum. Genet., 113, 542–550.

120. Schelenz S., Malhotra R., Sim R. B., Holmskov U. and Bancroft G. J. (1995): Binding of host collectins to the pathogenic yeast *Cryptococcus neoformans*: human surfactant protein D acts as an agglutinin for acapsular yeast cells. Infect. Immun., 63, 3360–3366.

121. Singh M., Madan T., Waters P., Parida S. K., Sarma P. U. and Kishore U. (2003): Protective effects of a recombinant fragment of human surfactant protein D in a murine model of pulmonary hypersensitivity induced by dust mite allergens. Immunol. Lett., 86, 299–307.

122. Takahashi H., Fujishima T., Koba H., Murakami S., Kurokawa K., Shibuya Y., Shiratori M., Kuroki Y. and Abe S. (2000): Serum surfactant proteins A and D as prognostic factors in idiopathic pulmonary fibrosis and their relationship to disease extent. Am. J. Respir. Crit. Care Med., 162, 1109–1114.

123. Takeda K., Miyahara N., Rha Y. H., Taube C., Yang E. S., Joetham A., Kodama T., Balhorn A. M., Dakhama A., Duez C., Evans A. J., Voelker D. R. and Gelfand E. W. (2003): Surfactant protein D regulates airway function and allergic inflammation through modulation of macrophage function. Am. J. Respir. Crit. Care Med., 168, 783–789.

124. Tsutsumida H., Goto M., Kitajima S., Kubota I., Hirotsu Y. and Yonezawa S. (2004): Combined status of MUC1 mucin and surfactant apoprotein A expression can predict the outcome of patients with small-size lung adenocarcinoma. Histopathology, 44, 147–155.

125. van de Wetering J. K., van Eijk M., van Golde L. M., Hartung T., van Strijp J. A. and Batenburg J. J. (2001): Characteristics of surfactant protein A and D binding to lipoteichoic acid and peptidoglycan, two major cell wall components of Gram-positive bacteria. J. Infect. Dis., 184, 1143–1151.

126. Vandivier R. W., Ogden C. A., Fadok V. A., Hoffmann P. R., Brown K. K., Botto M., Walport M. J., Fisher J. H., Henson P. M. and Greene K. E. (2002): Role of surfactant proteins A, D, and C1q in the clearance of apoptotic cells *in vivo* and *in vitro*: calreticulin and CD91 as a common collectin receptor complex. J. Immunol., 169, 3978–3986.

127. van Iwaarden J. F., Pikaar J. C., Storm J., Brouwer E., Verhoef J., Oosting R. S., van Golde L. M. and van Strijp J. A. (1994): Binding of surfactant protein A to the lipid A moiety of bacterial lipopolysaccharides. Biochem. J., 303, 407–411.

128. van Iwaarden J. F., van Strijp J. A., Visser H., Haagsman H. P., Verhoef J. and van Golde L. M. (1992): Binding of surfactant protein A (SP-A) to herpes simplex virus type 1-infected cells is mediated by the carbohydrate moiety of SP-A. J Biol Chem., 267, 25039–25043.

129. Voorhout W. F., Veenendaal T., Kuroki Y., Ogasawara Y., van Golde L. M. and Geuze H. J. (1992): Immunocytochemical localization of surfactant protein D (SP-D) in type II cells, Clara cells, and alveolar macrophages of rat lung. J. Histochem. Cytochem., 40, 1589–1597.

130. Walenkamp A. M., Verheul A. F., Scharringa J. and Hoepelman I. M. (1999): Pulmonary surfactant protein A binds to *Cryptococcus neoformans* without promoting phagocytosis. Eur. J. Clin. Invest., 29, 83–92.

131. Wang G., Bates-Kenney S. R., Tao J. Q., Phelps D. S. and Floros J. (2004): Differences in biochemical properties and in biological function between human SP-A1 and SP-A2 variants, and the impact of ozone-induced oxidation. Biochemistry, 43, 4227–4239.

132. Wang J. Y., Kishore U., Lim B. L., Strong P. and Reid K. B. M. (1996): Interaction of human lung surfactant proteins A and D with mite (*Dermatophagoides pteronyssinus*) allergens. Clin. Exp. Immunol., 106, 367–373.

133. Wang, J. Y., Shieh C. C., You P. F., Lei H. Y. and Reid K. B. M. (1998): 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., 158, 510–518.

134. Weber B., Borkhardt A., Stoll-Becker S., Reiss I. and Gortner L. (2000): Polymorphisms of surfactant protein A genes and the risk of bronchopulmonary dysplasia in preterm infants. Turk. J. Pediatr., 42, 181–185.

135. Weikert L. F., Edwards K., Chroneos Z. C., Hager C., Hoffman L. and Shepherd V. L. (1997): SP-A enhances uptake of *bacillus Calmette-Guerin* by macrophages through a specific SP-A receptor. Am. J. Physiol., 272, L989–L995.

136. Wert S. E., Yoshida M., LeVine A. M., Ikegami M., Jones T., Ross G. F., Fisher J. H., Korfhagen T. R. and Whitsett J. A. (2000): Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. Proc. Natl. Acad. Sci. USA, 97, 5972–5977.

137. Westergren-Thorsson G., Norman M., Bjornsson S., Endresen U., Stjernholm Y., Ekman G. and Malmstrom A. (1998): Differential expressions of mRNA for proteoglycans, collagens and transforming growth factor-β in the human cervix during pregnancy and involution. Biochim. Biophys. Acta, 1406, 203–213.

138. Wu H., Kuzmenko A., Wan S., Schaffer L., Weiss A., Fisher J. H., Kim K. S. and McCormack F. X. (2003): Surfactant proteins A and D inhibit the growth of Gram-negative bacteria by increasing membrane permeability. J. Clin. Invest., 111, 1589–1602.

139. Zimmerman P. E., Voelker D. R., McCormack F. X., Paulsrud J. R. and Martin W. J. 2nd. (1992): 120-kD surface glycoprotein of *Pneumocystis carinii* is a ligand for surfactant protein A. J. Clin. Invest., 89, 143–149.