

Review

Linking surfactant protein SP-D and IL-13: Implications in asthma and allergy

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ABSTRACT

Surfactant protein D (SP-D) is an innate immune molecule that plays a protective role against lung infection, allergy, asthma and inflammation. In vivo experiments with murine models have shown that SP-D can protect against allergic challenge via a range of mechanisms including inhibition of allergen-IgE interaction, histamine release by sensitised mast cells, downregulation of specific IgE production, suppression of pulmonary and peripheral eosinophilia, inhibition of mechanisms that cause airway remodelling, and induction of apoptosis in sensitised eosinophils. SP-D can also shift helper T cell polarisation following in vivo allergenic challenge, from pathogenic Th2 to a protective Th1 cytokine response. Interestingly, SP-D gene deficient (–/–) mice show an IL-13 over-expressing phenotype. IL-13 has been shown to be involved in the development of asthma. Transgenic mice over-expressing IL-13 in the lung develop several characteristics of asthma such as pulmonary eosinophilia, airway epithelial hyperplasia, mucus cell metaplasia, sub-epithelial fibrosis, charcot-Leyden-Like crystals, airways obstruction, and non-specific airways hyper-responsiveness to cholinergic stimulation. Although both IL-4 and IL-13 are capable of inducing asthma like phenotype, the effector activity of IL-13 appears to be greater than that of IL-4. SP-D –/– mice seem to express considerably higher levels of IL-13, which is consistent with increased sensitivity and exaggerated immune response of the mice to allergenic challenge. Allergenic exposure also induces elevation in SP-D protein levels in an IL-4/IL-13-dependent manner, which prevents further activation of sensitised T cells. This negative feedback loop seems essential in protecting the airways from inflammatory damage after allergen inhalation. Here, we examine this link between IL-13 and SP-D, and its implications in the progression/regulation of asthma and allergy.

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1. Introduction

Surfactant protein SP-A and SP-D both belong to the collectin (collagen containing lectin) subgroup of the C-type lectin super family and are carbohydrate pattern recognition molecules. They interact with non-self glycoconjugates and lipids present on the surface of microorganisms mainly via homotrimeric carbohydrate

recognition domains (CRDs) or C-type lectin domains (Kishore et al., 2006). SP-A and SP-D are large oligomeric structures, each assembled from multiple copies of single polypeptide chains (except the human SP-A) (Lu et al., 1993) (Fig. 1) SP-A and SP-D are considered as the first line immune defense in the lungs. SP-A gene-deficient (–/–) mice have normal lung histology, but lack of SP-A can lead to increased susceptibility to pulmonary infection due to bacteria and viruses (Korfhagen et al., 1998a). In contrast, SP-D –/– mice develop a spontaneous emphysematous change and pulmonary fibrosis, which reveals the important role played by SP-D in controlling lung inflammation (Botas et al., 1998). The immunomodulatory properties also make SP-D an interesting molecule of innate and adaptive immunity in the lungs (Nayak et al., 2012). SP-D has been shown to modulate allergic response and probably resist repeated allergen provocation (Forbes and Haczku, 2010; Kishor et al., 2002; Madan et al., 2001a; Malherbe et al., 2005) SP-D enhances antigen presentation to dendritic cells (Brinker et al., 2003), inhibits

Abbreviations: AHR, airway hyper-responsiveness; Afu, *Aspergillus fumigatus*; IL, interleukin; SP, surfactant protein; Th, T helper; MCh, methacholine; IgE, immunoglobulin E; LTA, lymphotoxin A; IL-4R, interleukin 4 receptor; KO, knock-out; NFAT, MMP, matrix metalloproteinase; rhSP-D, recombinant human surfactant protein D; IFN- γ , interferon gamma; CTSB, cathepsin B; CTSK, cathepsin K; CTSL, cathepsin L; CTSS, cathepsin S; CTSZ, cathepsin Z.

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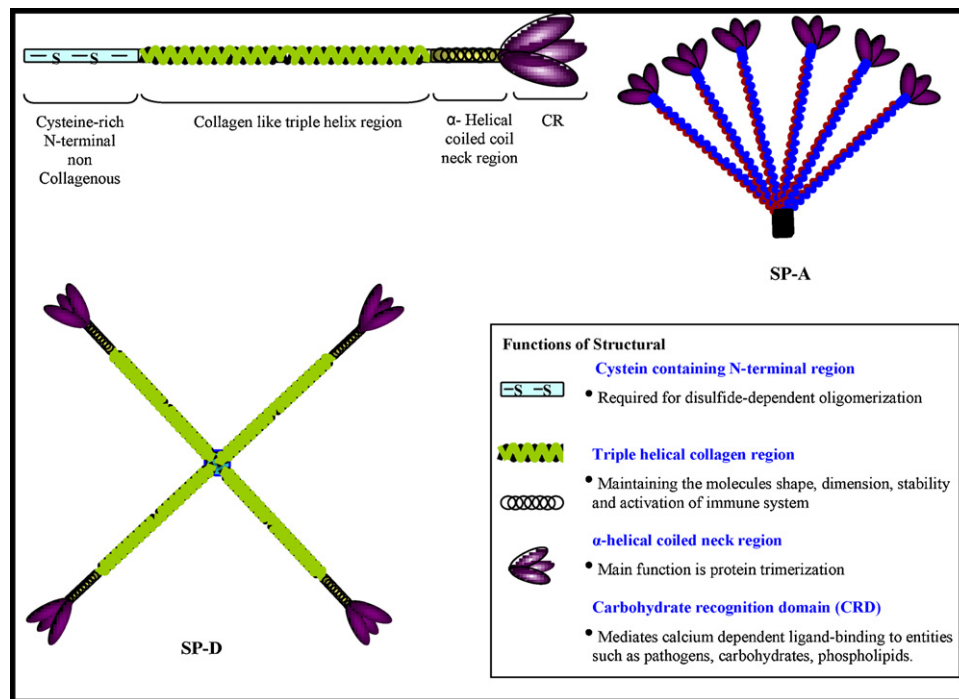


Fig. 1. SP-A and SP-D are large hydrophilic proteins and belong to the collectin (collagen containing lectin) subgroup of the C-type lectin super family. SP-A and SP-D are large oligomeric structures, each assembled from multiple copies of single polypeptide chains (except the human SP-A). SP-A has a hexameric structure with 6 subunits (each composed of three 35 kDa polypeptide chains) of 105 kDa each, which assemble to form a 630 kDa molecule. The shape of SP-A resembles that of the serum complement protein C1q, appearing like a flower bouquet under the electron microscope with 6 globular heads linked by collagen-like strands to a fibril-like central core. SP-D is a 43 kDa glycoprotein, composed of 130-kDa oligomers (each made up of three identical polypeptide chains of 43 kDa). SP-D forms a cruciform-like structure composed of 12 subunits arranged in four trimeric subunits. The primary structures of SP-A and SP-D are organized into 4 regions, a non collagen region involved in the formation of inter-chain disulfide bonds (—SH—SH—), a collagen region composed of Gly-X-Y repeats where X and Y can be any amino acids (generally proline or hydroxyproline), an α -helical coiled-coil neck peptide, and C-type (calcium dependent) carbohydrate recognition domain (CRDs).

IL-2 dependent T lymphocyte proliferation, allergen induced lymphocyte proliferation (Borron et al., 2002), and histamine release from the sensitised basophils of asthmatic children (Madan et al., 1997; Wang et al., 1998). The therapeutic administration of SP-D in a murine model of pulmonary allergy (Madan et al., 2001b) lowers specific IgE levels, with reduction in the splenic levels of pathogenic Th2 cytokines (IL-4 and IL-5) (and an increase in the Th1 cytokines (IFN- γ and IL-12)), pulmonary and blood eosinophilia, and pulmonary infiltration (Table 1). SP-D also results in rapid clearance of apoptotic cells, which is central to the resolution of inflammation and maintenance of immune homeostasis (Clark et al., 2002; Vandivier et al., 2002).

2. SP-D and IL-13

The characteristics of asthma, comprising airway hyper-responsiveness (AHR), airway inflammation, mucus responses and tissue remodelling, are co-ordinated by an immunological network involving both cellular and molecular events (Cohn et al., 2004; Hohlfeld et al., 2002; Homer et al., 2002). IL-13 is produced in large quantities primarily by Th2 CD4+ cells (Akbari et al., 2003; Schmid-Grendelmeier et al., 2002). Human IL-13 gene encoding a 17 kDa glycoprotein is located on chromosome 5q31 along with genes coding for IL-4, IL-3, IL-5, IL-9 and granulocyte-macrophage colony-stimulating factor (GM-CSF) (Doucet et al., 1998; Renauld, 2001; Zhu et al., 1999). IL-13 mediates its effects by interacting with a receptor system comprising the IL-4RA and two IL-13 binding proteins, IL-13RA1 and IL-13RA2. The IL-4RA associates with the IL-13 RA1 to exclusively signal for IL-4 and IL-13, therefore contributing to many of their overlapping functions. The IL-4RA2 is speculated to function as a decoy receptor. Its expression has been shown to be inhibited by IL-13 over expression and vice versa (Daines

and Hershey, 2002; Kawakami et al., 2001). Fig. 2 illustrates the signalling cascade of IL-13. Studies using transgenic and gene deficient mice have validated the relevance of IL-13 in mediating the key events in asthma (Homer et al., 2002; Zhu et al., 1999; Haczku et al., 2006; Fortin et al., 2010; Elias and Lee, 2011; Elias et al., 2003).

Genetic polymorphisms in the IL-13 gene have been shown to be associated with increased total serum IgE, asthma, bronchial hyper-responsiveness and other asthma related phenotypes (Howard et al., 2001, 2002; Taussig et al., 1989; von Mutius et al., 1998). IL-13 has been shown to be consistently overexpressed in the lungs of human asthmatics (Homer et al., 2002). Although the role of IL-13 in mediating AHR is conclusive, its role in inflammation is controversial. Administration of IL-13 into the airways of mice leads to the infiltration of inflammatory cells such as eosinophils, monocytes/macrophages and lymphocytes. However, blockade of IL-13 does not completely abolish allergen induced inflammation as well as is the inflammatory response compared to that induced by allergen on IL-13 administration (Akbari et al., 2003; Walter et al., 2001). However, the inflammatory cells may play a key role in facilitating and amplifying IL-13 effects due to their ability to express IL-13 receptors. Besides AHR, IL-13 has also been shown to play an equally decisive role in mucus hyper secretion thereby modulating the dynamics of the asthmatic airway (Grunig et al., 1998; Walter et al., 2001; Wills-Karp et al., 1998).

2.1. Airway hyper-responsiveness

AHR is caused by the inflammation of the asthmatic airway resulting in an increased bronchospastic response to as non-specific agonist such as MCh (Pueringer and Hunninghake, 1992; Weinberger, 1993a, 1993b; White, 1993). Although the mechanisms leading to AHR are poorly understood, IL-13 has been shown

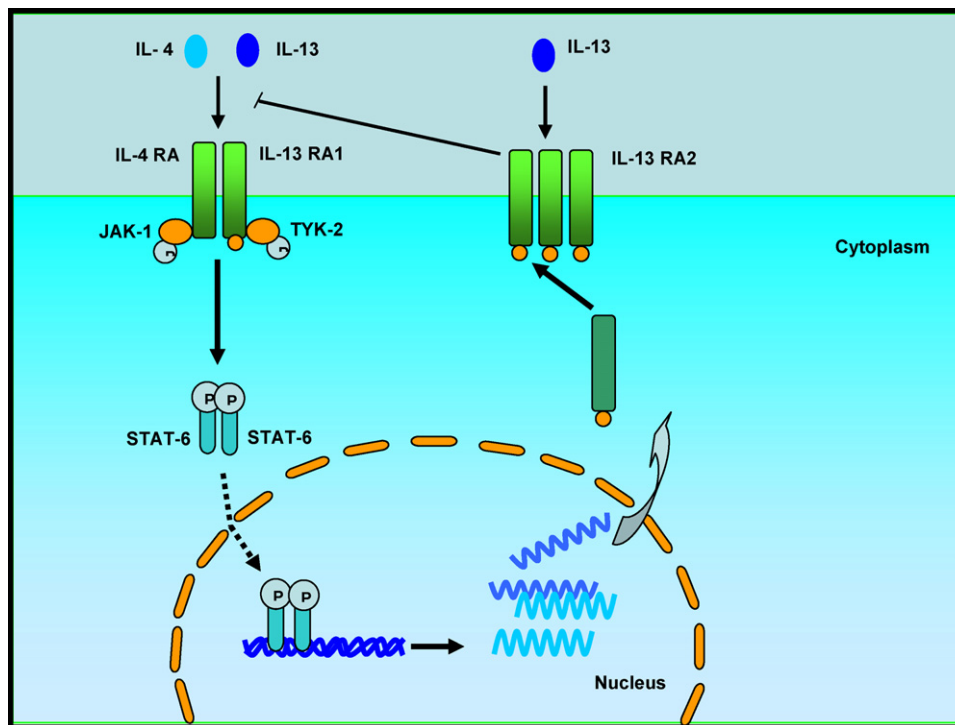


Fig. 2. IL-13 signalling. IL-13 mediates its effects by interacting with a receptor system comprising the IL-4RA and two IL-13 binding proteins, IL-13RA1 and IL-13RA2. IL-4RA is the common receptor for both IL-4 and IL-13. The IL-4RA associates with the IL-4RA1 to exclusively signal for IL-4 and IL-13. In the presence of IL-4 and IL-13, STAT6 activation is maintained indefinitely. Another cognate receptor of IL-13 besides the IL-13RA1 is the IL-4RA2 speculated to function as a decoy receptor. This receptor specifically binds with high affinity to IL-13 but not IL-4. However, binding of IL-13 to this receptor stops further signalling and therefore, reduces the availability of IL-13 for the IL-4A/IL-13RA1 signalling. IL-13 (and IL-4) signals through the Jak (signal transducer and activator of transcription) (STAT) pathway and specifically via STAT6. Activation of Jaks results in phosphorylation of the cytoplasmic tyrosine's in IL-4RA, leading to the recruitment of STAT6 to the receptor, followed by STAT6 phosphorylation and activation. This leads to its association with cytokine receptors, dimerization of phosphorylated STAT6 monomers, which then translocates to the nucleus, binds specific canonic DNA elements and initiates transcription of downstream genes. A feedback regulation could induce the production of the decoy receptor to dampen the IL-4A/IL-13RA1 signalling. *Abbreviation:* STAT, signal transducer and activator of transcription.

to play a very critical role. Specific blockade of IL-13 by systemic administration of a soluble form of IL-13RA2 chain, which binds only IL-13, to allergen-challenged mice completely abolishes AHR and mucus production without affecting eosinophil infiltration (Grunig et al., 1998; Wills-Karp et al., 1998). Furthermore, administration of IL-13 to naive mice recapitulates the entire phenotype (Grunig et al., 1998; Wills-Karp et al., 1998). The non-obligatory role of IL-4 and IL-5 in AHR has been identified in several studies in allergic models and gene-deficient mice (Foster et al., 1996; Grunig et al., 1998; Wills-Karp et al., 1998). An excellent insight came from the IL-13 $-/-$ mice where AHR failed to develop in spite of a vigorous Th2-biased eosinophilic pulmonary inflammation, which was restored upon recombinant IL-13 supplementation (Haczku et al., 2006; Madan et al., 2005). Similarly, adoptive transfer of antigen specific Th2 cells from IL-13 $-/-$ mice failed to elicit AHR despite high levels of IL-4 and IL-5 and airway inflammation (Walter et al., 2001). The mechanism of IL-13 induced AHR may be the result of its direct effects on airway smooth muscle cells which have been shown to express its receptor molecules (IL-4RA and IL-13RA1) (Heinzmann et al., 2000), and induction of mitogens that promote sub-epithelial fibrosis (Kuperman et al., 2002; Richter et al., 2001; Zhu et al., 1999). This may also explain its ability to increase mucus production leading to thickening of the airway wall and obstruction (Fallon et al., 2001; Walter et al., 2001; Zhu et al., 1999). Therefore IL-13 is the principal Th2 cytokine responsible for allergen-induced AHR.

The role of SP-D in the development of AHR is currently being dissected (Nayak et al., 2012; Zhang et al., 2003). Elevations in SP-D levels have been shown in sensitised and allergen challenged mice along with significant AHR (upon MCh inhalation)

and increased eosinophilia (Zhang et al., 2003). The kinetics of IL-13 elevation was found to be consistent with the pattern of AHR and eosinophil number. Upon SP-D administration of mice before allergen challenge, AHR and eosinophil numbers in BAL reduced in a dose-dependent manner, while the same therapy with a mutant SP-D molecule did not show any effect. Interestingly, C57BL/6J mice are relatively hypo-responsive and resistant to airway inflammation and AHR compared to other inbred strains of mice (Zhang et al., 1997, 2003). The C57BL/6J mice were shown to have higher basal SP-D levels than Balb/c mice after sensitisation and challenge. The elevated SP-D levels even during sensitisation could explain the resistance of C57BL/6J mice to develop AHR. Furthermore, SP-D $-/-$ mice have been shown to become more sensitive to allergenic challenge, showing elevated eosinophil count, and an increase in eosinophil peroxidase (EPO) activity when compared to wild-type mice (Madan et al., 2005). Moreover, it has also been reported that there is a significant infiltration in the peribronchiolar and perivascular regions of the lungs in SP-D $-/-$ mice, as well as increased observation of alveolar macrophages and lymphocytes (Madan et al., 2005).

2.2. IL-13 and SP-D association with goblet cell hyperplasia and mucus production

Allergic asthma is associated with a substantial increase in the mucus content of the airway epithelium (Gavett et al., 1997; Kuperman et al., 1998; Rankin et al., 1996; Zhu et al., 1999). Mucus hyperproduction in asthma results from a Th2-induced airway inflammation, significantly contributing to airway obstruction, wheezing and coughing (Cohn et al., 1999; James and Carroll, 1995).

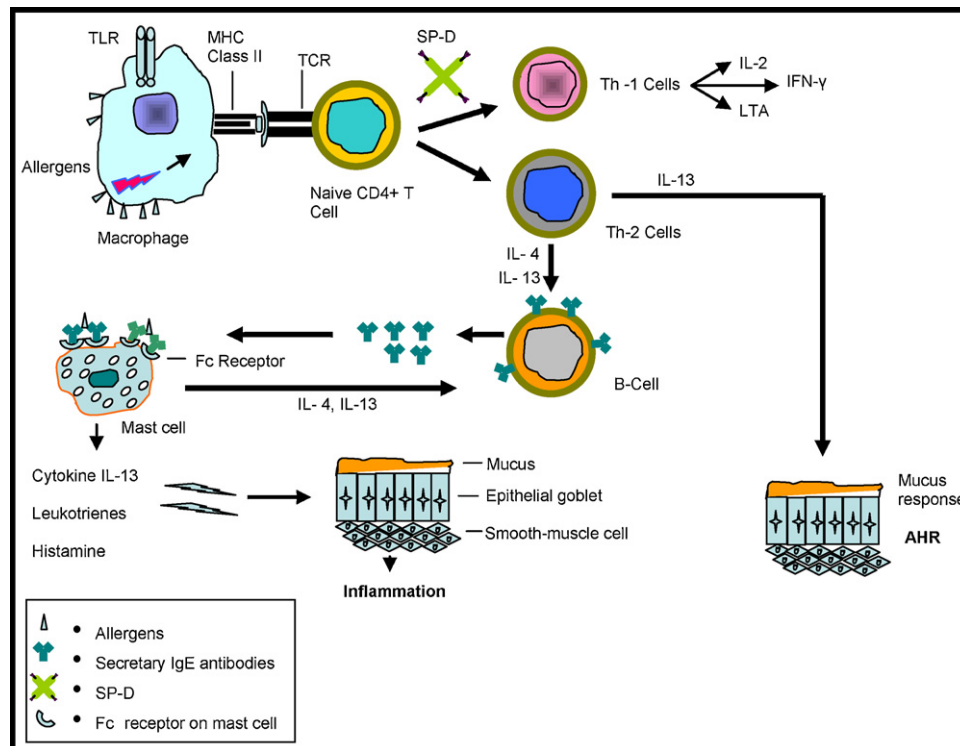


Fig. 3. Sensitisation and re-exposure to an allergen leads to inflammatory responses typical of asthma. The allergen is taken up and processed by antigen presenting cells (APCs) like macrophages and subsequently presented to the T cells. These changes trigger the production of cytokines like IL-4 and IL-13 by Th2 cells. IL-4 and IL-13 enhances class switching to IgE and DC's regulates the clonal expansion of IgE secreting plasma cells. The secreted IgE molecules bind to IgE specific Fc receptors on mast cells and a re-exposure to the allergen leads to cross linking of the bound IgE triggering the release of cytokines (IL-4 and IL-13), chemokines and pharmacologically active mediators e.g. leukotrienes, histamine (from mast cells causes smooth muscle contraction), increased vascular permeability vasodilatation and drives the inflammatory response leading to infiltration of eosinophils, lymphocytes, macrophages and epithelial cells into the lung. The cytokine profile is predominantly of the Th2 type characterized by high levels of IL-4 and IL-13. IL-13 itself can directly induce airways hyper responsiveness (AHR) and mucus production. Therefore, IL-13 plays an important role in initiating and generating the physiological abnormalities that come to play in the asthmatic diathesis. SP-D more known for its role in surfactant homeostasis and innate immunity has been shown to play a significantly protective role in asthma. SP-D has shown to shift the ongoing Th2 cytokine profile, typical in airway hyper-responsiveness and airway inflammation towards a protective Th1 response.

Although Th2 cells play an important role in mucus production, mucus could be induced in mice lacking IL-4 and IL-5 and corresponding inflammatory responses devoid of eosinophils or mast cells (Cohn et al., 1997, 1999; Rankin et al., 1996). IL-13, along with its receptor the IL-4A, seems to be the critical cytokine involved in this pathway through which mucus production is induced (Grunig et al., 1998; Whittaker et al., 2002). Allergen induced increase in airway mucus content can be reversed by blocking IL-13 (Wills-Karp et al., 1998) and has been shown to be abrogated in the IL-13 $-/-$ mice (Walter et al., 2001). Neutralizing IL-13 with soluble IL-13RA2-Fc chimeric protein abolishes goblet cell hyperplasia and mucus production (Taube et al., 2002). Furthermore, a partial blockade leads to only a modest decrease in mucus content suggesting the potent role of IL-13 (Whittaker et al., 2002). IL-13 has been shown to mediate mucus production by directly acting on airway epithelial cells, dependent on its downstream mediator Stat6 (Kuperman et al., 2002). IL-13 also induces expression of genes involved in mucus production (Muc5ac, Retnl, Tff2 and Clca3/Gob5) (Kuperman et al., 2002; Whittaker et al., 2002). Although other cytokines and inflammatory mediators have also been implicated in mucus production, IL-13 seems a central effector, perhaps integrating other mediators. Mucus is also an innate immune defence mechanism by which airway epithelium responds to pathogens and allergens. The Th2 inflammation in the airway stimulates epithelial cell hypertrophy and mucus metaplasia. The recombinant form of SP-D therapy in mice sensitised and challenged with OVA results in a significant decrease in goblet cell hyperplasia (Takeda et al., 2003). SP-D expression has also been

demonstrated in mucus cells of the rat gastric mucosa (Fisher and Mason, 1995) and on mucosal surfaces in numerous glands and organs (Madsen et al., 2000), where it may be involved in innate immunity. SP-D has been shown to be expressed in goblet cells of the airways, and the expression of SP-D strongly increases in sensitised and OVA-challenged mice in hyperplastic goblet cells (Kasper et al., 2002) (Fig. 3).

2.3. Inflammation and eosinophilia

The role of IL-13 has been documented in eosinophil production and regulation of IgE synthesis (Emson et al., 1998; Fallon et al., 2001; McKenzie et al., 1998; Webb et al., 2000). However, a controversy surrounding the effects of IL-13 is its ability to cause AHR independently of pulmonary eosinophilia and specific IgE, therefore also disassociating AHR from key characteristics of asthma (Wills-Karp et al., 1998). Mice lacking IL-13 have shown to have a full blown airway inflammation characterized by significant eosinophilia production and lymphocyte infiltration (Walter et al., 2001). However, intranasal administration of recombinant IL-13 has been shown to induce BAL eosinophilia and neutrophilia (Wills-Karp et al., 1998). In another study, neutralizing IL-13 did not abolish but led to a reduction (60%) in pulmonary eosinophils and lymphocytes (Taube et al., 2002). IL-13 directly promotes eosinophil survival, activation and recruitment (Luttmann et al., 1996; Pope et al., 2001). Therapeutic administration of a recombinant form of SP-D in a murine model of pulmonary allergy induced by allergens of *Aspergillus fumigatus* (Madan et al., 2001a)

Table 1
Immunomodulation by SP-D in asthma.

Functions of SP-D	References
Recognizes pathogen associated molecular patterns (PAMPs) and acts as an agglutinin, opsonin and immunomodulator thereby regulating proinflammatory responses induced by microbial components.	Madan et al. (1997) and Wang et al. (1996)
Contributes to immune homeostasis in the lung by recognizing and promoting removal of necrotic and apoptotic cells and enhances the uptake of these cells by alveolar macrophages.	Clark et al. (2002), Schagat et al. (2001) and Vandivier et al. (2002)
Decreases pollen induced IgE-dependent mast cell degranulation. Suppresses histamine release from basophils and mast cells together with antiproliferative effects on B and T lymphocytes	Madan et al. (1997), Wang et al. (1996) and Wright (2005)
Inhibits allergen- induced proliferation of PBMCs in asthmatic children sensitive to mite allergens	Wang et al. (1998)
Down regulates allergen specific (Afu)-IgE, (Afu)-IgG, peripheral and pulmonary eosinophilia and competes with Afu-IgE to block subsequent histamine release from sensitised basophils from ABPA patients	Madan et al. (1997, 2001a, 2005)
Inhibits eosinophilic response in sensitised mice challenged with allergen therefore limiting the inflammatory changes during airway inflammation; induced apoptosis in sensitised eosinophils.	Takeda et al. (2003) and Mahajan et al. (2008)
Exhibits immunomodulatory effects, inhibiting T cell proliferation and IL-2 production and also enhances chemotaxis of phagocytic cells.	Borron et al. (1998), Wang et al. (1998), and Madan et al. (2001a)
Induces Th1 cytokines and shifting the predominant Th2 response.	Takeda et al. (2003)
Acts as a potent antioxidant and as a free radical chain terminator inhibiting formation of lipid radicals.	Bridges et al. (2000)

and dust mite (Singh et al., 2003) have been shown to lower pulmonary and blood eosinophilia, specific IgE levels, and pulmonary infiltration.

On the other hand, SP-D $-/-$ mice exhibit a pronounced hyper-eosinophilia accompanied by an increased intrinsic Th2 cytokines including IL-13 (Madan et al., 2005). This hypereosinophilic phenotype can be reversed by exogenous administration of SP-D to SP-D $-/-$ mice. This clearly implicates the role of SP-D in regulating eosinophilia and modulating lung immune response in the allergic condition (Madan et al., 2005). In an ex vivo study, SP-D and a recombinant fragment, rhSP-D, showed a direct, dose, carbohydrate and calcium dependent binding to human eosinophils. rhSP-D resulted in a differential effect on eosinophils from allergic patients with increase in CD69 expression, oxidative burst and

apoptosis, while eosinophils from healthy controls were unaffected (Mahajan et al., 2008).

Similarly, SP-D has been shown to reduce the in vivo eosinophilic inflammation in mice sensitised and challenged with ovalbumin (Takeda et al., 2003). The eosinophil numbers in this study reduced by ~50% upon SP-D therapy. SP-D has been shown to induce production of IL-10, IL-12 and IFN- γ (Takeda et al., 2003), which are all capable of influencing eosinophilic airway inflammation and AHR. Therefore, one of the protective anti-inflammatory effects of SP-D appear to be the induction of Th1 cytokines and shifting the predominant Th2 response that is typical of airway inflammation (Madan et al., 2001a; Singh et al., 2003; Takeda et al., 2003). SP-D could also resolve inflammation by clearing apoptotic cells by alveolar macrophages (Vandivier et al., 2002). Similar observations have been made by Mahajan et al. where SP-D showed increased binding to apoptotic eosinophils and resulted in their increased phagocytosis by macrophages. SP-D may thus be involved in resolution of eosinophilic inflammation following allergen provocation (Mahajan et al., 2008).

MMP-2, -9 and -12 have been shown to be crucial in the infiltration of inflammatory cells like eosinophils (Kumagai et al., 1999). This has been supported by studies in murine models of antigen-induced airway inflammation (Cataldo et al., 2002; Corry et al., 2002; Kumagai et al., 1999). The over-expression of IL-13 also leads to an elevation of proteolytic enzymes like MMP-2, -9 and -12 in the lung (Zheng et al., 2000). Studies in IL-13 $-/-$ mice have also demonstrated the IL-13 dependent expression of MMP-12 in airway inflammation. SP-D has also been shown to modulate MMPs production contributing to inflammation (Cury et al., 1988; Wert et al., 2000a, 2000b). These effects of SP-D on MMP production are likely to be due to its interaction with alveolar macrophages, which have the capacity to secrete several members of MMPs (Campbell et al., 1991; Cury et al., 1988; Miyamura et al., 1994; Welgus et al., 1990). Therefore, common mediators like MMPs further suggest crosstalk between IL-13 and SP-D in inflammatory processes in the lung.

2.4. Emphysema, IL-13 and SP-D

Cigarette smoking is the most important risk factor for chronic obstructive pulmonary disease (COPD) and emphysema (Sandford et al., 2002). According to the Dutch hypothesis, the distinction between these two disorders is not absolute, with common factors contributing to both (Prescott et al., 1997; Sluiter et al., 1991). Tissue remodelling responses causing alveolar septal destruction and changes in lung compliance are characteristic of emphysema (Senior and Anthonisen, 1998). IL-13 and SP-D have both been implicated in the development of emphysema. IL-13 causes inflammatory cell infiltration and pronounced emphysema when inducibly expressed in murine lung, with lung volume enlargement and enhanced pulmonary compliance comparable to that seen in human COPD (Zheng et al., 2000). IL-13 mediated processes rely on proteases involved in airway remodelling and inflammation (Lanone et al., 2002). The overproduction of IL-13 in lung has been shown to cause emphysema (Kuperman et al., 2002; Zheng et al., 2000; Zhu et al., 1999) and regulate the expression of many genes like cathepsins (CTSB, CTSK, CTSL, CTSS and CTSZ), matrix metalloproteinases (MMP-2, -9, -12, -13 and -14), and tissue inhibitor of matrix metalloproteinases (TIMP1) (Kuperman et al., 2002; Lanone et al., 2002). IL-13 has also been shown to be a potent stimulator of pulmonary proteolysis. Elevated levels of MMPs have been noted in many patients that manifest features of asthma (Hoshino et al., 1999; Kelly and Locksley, 2000; Segura-Valdez et al., 2000; Selman et al., 2000; Suga et al., 2000; Zheng et al., 2000). SP-D levels have been shown to be decreased in many clinical conditions like cystic fibrosis (Postle et al., 1999) and in smokers (Honda et al., 1996),

contributing to COPD and emphysema. Cigarette smoke induces apoptosis of alveolar macrophages, which could be one mechanism of lung destruction leading to emphysema (Majo et al., 2001). SP-D $-/-$ mice have been shown to develop progressive pulmonary emphysema and fibrosis in association with chronic inflammation (Wert et al., 2000b). These abnormalities are associated with increased activity of oxidant and MMP (MMP-2 and MMP-9) production by alveolar macrophages contributing to inflammation and air space destruction leading to emphysema (Wert et al., 2000b). Conditional replacement of SP-D in the respiratory epithelium of SP-D $-/-$ mice does not reverse emphysema which is consistent with clinical observations (Zhang et al., 2002). SP-D is also a potent antioxidant due to its ability to act as a free radical chain terminator and inhibiting formation of lipid radicals (Bridges et al., 2000). In addition, it also contributes to regulation of MMP production (Trask et al., 2001; Wert et al., 2000b; Yoshida et al., 2001). The deficiency or reduction of SP-D in asthmatics and chronic smokers therefore could be a mechanism leading to the development of emphysema. Thus, SP-D preserves the anti-protease “shield” protecting the lung against injury and IL-13 removes this shield by promoting proteinases. However, the actual contributions of proteinases and whether or not IL-13 and SP-D regulate their levels in a concerted way need to be examined.

3. Is there a link between IL-13 and SP-D in asthma?

The first important link between IL-13 and SP-D was demonstrated in the SP-D $-/-$ mice (Madan et al., 2005), which develop type II cell hyperplasia, progressive accumulation of surfactant lipids and apoproteins in the alveolar space, foamy macrophages, and emphysema (Botas et al., 1998; Korfhagen et al., 1998b; Wert et al., 2000b). Interestingly, the IL-13 over-expressing mice also develop a similar phenotype comprising pulmonary type II cell hypertrophy, lipoproteinosis, inflammation, emphysema, fibrosis and AHR (Homer et al., 2002; Zhu et al., 1999). The IL-13 over-expressing mice show 70-fold increase in SP-D bronchoalveolar lavage (BAL) levels. Likewise IL-13 levels are up-regulated in the SP-D $-/-$ mice, which alleviates following SP-D treatment (Madan et al., 2005). A recent study with mice sensitised and challenged by *A. fumigatus*, antigens/allergens showed a direct regulation of SP-D by IL-13. Both SP-D mRNA and protein were induced directly in vitro by IL-13 and IL-4 treatment but not IFN- γ , suggesting a direct role of IL-4R mediated events. This regulatory role of IL-4 and IL-13 was also confirmed in the STAT-6-deficient mice as well as IL-4/IL-13 double $-/-$ mice, which did not induce an increase in SP-D on allergen challenge (Haczku et al., 2006). Therefore, SP-D seems to provide a negative regulatory control on IL-13. These findings suggest a mechanism of regulation of IL-13 by SP-D in lung allergy and inflammation.

A transcription factor, NFAT1 plays a central role in the regulation of Th2 and mast cells (Crabtree, 1999; Hogan et al., 2003; Rao et al., 1997; Solymar et al., 2002). The IL-13 promoter contains conserved motifs and potential binding sites for NFAT and NFAT1 has been shown to be involved in IL-13 transcription in mast cells (Monticelli et al., 2004). Interestingly, the NFAT1 $-/-$ mice show a similar phenotype like the SP-D $-/-$ mice, elaborating also increased levels of Th2 cytokines including IL-13 (Viola et al., 1998).

The protease-dependent acquired emphysema, developed in IL-13 transgenic mice, is pathophysiologically similar to that seen in SP-D $-/-$ mice (Botas et al., 1998; Wert et al., 2000b). The IL-13 transgenic mice show an elevation of MMP-2, MMP-9 and MMP-12 in the lung that is crucial in the infiltration of inflammatory cells like eosinophils. IL-13 has been reported to inhibit the production of pro-inflammatory mediators by monocytes and macrophages,

including reactive oxygen radicals, through a mechanism that probably involves NF- κ B (Ahn et al., 2007; Ahn and Aggarwal, 2005; Bao et al., 2009; Zhu et al., 1999). Alveolar macrophages derived from SP-D $-/-$ mice show increased expression of reactive oxygen radicals, hydrogen peroxide, MMP-9, MMP-12 and NF- κ B (Senft et al., 2005). Thus, it appears that the increased levels of IL-13 produced in SP-D $-/-$ mice regulate their increased oxidative state. These effects of SP-D on MMP production could also be due to its interaction with alveolar macrophages, which have the capacity to secrete several members of MMPs. SP-D mediated increased IFN- γ production may be another important mediator involved in the regulation of IL-13 (Haczku et al., 2006; Madan et al., 2010). IFN- γ is known to inhibit IL-13 induced goblet cell hyperplasia and airway eosinophilia (Ford et al., 2001). Therefore, the common mediators implicated in lung inflammation, fibrosis and emphysema further suggest cross-talk between IL-13 and SP-D pathways.

3.1. Transcriptional regulation of IL-13 and SP-D expression

The transcriptional regulation of Th2 genes like IL-13 constitutes another important facet of asthma since the Th1/Th2 differentiation of CD4 $^{+}$ T cells could control the balance between the Th1 and Th2 responses which is impaired/polarised in asthma (Djukanovic, 2000). Although there is a speculation of a co-regulation of IL-4, IL-13 and IL-5 due to their proximity at the chromosomal locus (McKenzie et al., 1993; Tindall et al., 2010), increasing evidence suggests a more prominent role of IL-13 in affecting asthma pathogenesis, and therefore, a non-overlapping mode of regulation. Nevertheless, there is still a lack of information on the transcriptional regulatory mechanisms of IL-13 that could lead to its dominance during the asthma phenotype than the other Th2 cytokines.

The IL-13 gene structure includes the conserved consensus sequences for binding GATA, NFAT and AP-1 (McKenzie et al., 1993). Among the transcription factors that regulate the expression of IL-13, the calcium regulated transcription factor- NFAT and GATA families mainly control its production along with other Th2 cytokines such as IL-4 and IL-5 (Pahl et al., 2002). Among the four family members of NFAT (NFAT1, NFAT2, NFAT3 and NFAT4), NFAT1 has been shown to play a predominant role in IL-13 gene transcription in mast cells (Moller et al., 1998; Monticelli et al., 2004). Interestingly, both NFAT1 and NFAT2 can drive transcription of IL-13 gene in Th2 cells (Monticelli et al., 2004). Therefore, the expression of IL-13 seems to be regulated differently in different cell types owing perhaps to the presence of cell type specific co-activators. In the same study, NFAT1 was also shown to synergize with the GATA transcription factors to activate transcription from the IL-13 promoter (Monticelli et al., 2004). IL-13 has been shown to bind GATA-3 which enhances the expression of IL-13 gene expression in CD8 $^{+}$ T cells (Barnes, 2008; Fuschiotti, 2011). An IL-4/IL-13 intergenic regulatory region has also been characterized that could be involved in their co-regulation and GATA-3 with STAT-6 seemed to induce chromatin remodelling in this region (Takemoto and Kamogawa, 2000). One study reported a distinct transcriptional regulation of IL-13 from IL-4 in Th2 cells by GATA3 and showed that IL-13 is not always co expressed with the other Th2 cytokines (Kitamura et al., 2005). GATA-3 is a Th2-specific transcription factor that functions as a Th2 lineage determinant (Hattori et al., 1996; Ting et al., 1996). GATA-3 could therefore be an important link between IL-13 and SP-D expression since GATA-3 also represses the opposing Th1 specific genes like IFN- γ (Ferber et al., 1999; Ouyang et al., 1998; Zheng and Flavell, 1997). One speculation for regulation of IL-13 is its high affinity receptor IL-13R α 2 which has been considered to function as a decoy receptor (Chiaromonte et al., 2003; Daines and Hershey, 2002; Yasunaga

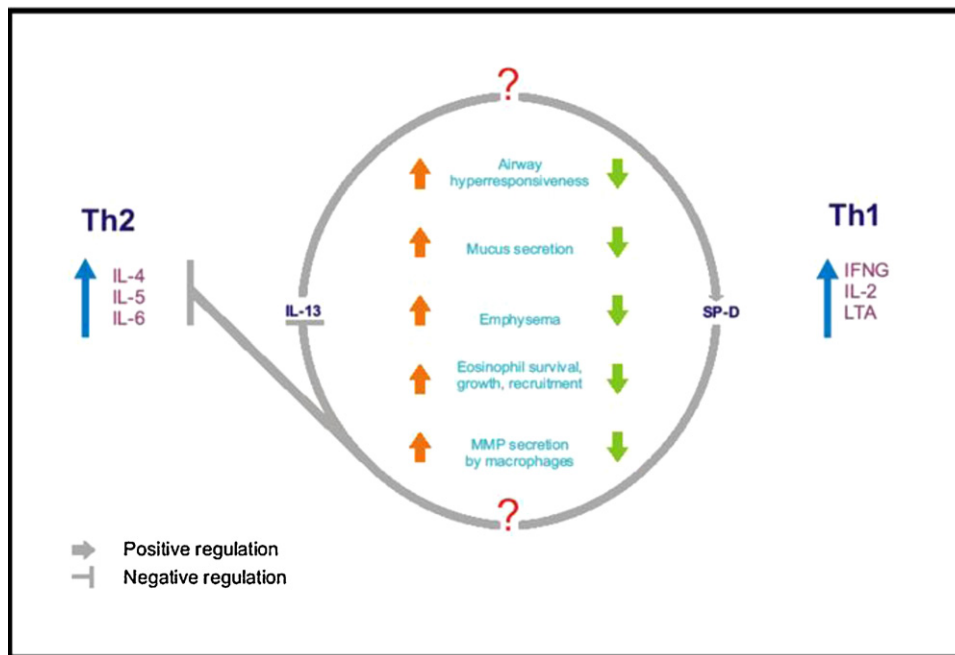


Fig. 4. Shown in the figure above are asthma related outcomes regulated by IL-13 and SP-D. IL-13, produced by T cells, eosinophils and mast cells represents a Th2 response, which is a hallmark of allergic asthma. SP-D however, favours a Th1 response and reduces these symptoms by down regulating the Th2 cytokines and shifting the Th2 response towards a protective Th1 response. Therefore, SP-D negatively regulates IL-13. However, upon IL-13 over expression, SP-D levels increase as a counter-regulatory phenomenon. The pathways and other mediators that directly link the two are as yet unknown.

et al., 2003). This receptor has been shown to be up regulated during Th2 mediated response (Chiaramonte et al., 2003). However, in a Th1 skewed response, the receptor is inhibited. This could be an efficient mechanism to quench the bioavailability of IL-13 for further Th2 mediated downstream signalling.

Although an increased Th2 immune response is evidently associated with asthma, a decreased Th1 immune response also favours the outcome of the pathogenesis. The regulation of SP-D that alters the response towards Th1 is equally important in understanding the regulation of the Th2/Th1 balance (Fig. 4).

SP-D has been regarded to be constitutively expressed. However its expression is regulated in asthma, a higher expression positively correlating with the resolution of the disease phenotype (Crouch, 2000). Similar to IL-13, the transcriptional regulation of SP-D is not well defined. The SP-D promoter region has revealed the presence of conserved motifs for C/EBPs, NFAT, AP-1, STATs and TTF-1 (Dave et al., 2004). SP-D has also been considered to be regulated by SP-1 like transcription factors. The AP-1 family members like JunB and JunD have been shown to enhance whereas c-Jun and c-Fos inhibit its transcription (He et al., 2000). FoxA1, Fox2 transcription factors and CCAAT enhancer binding protein- α , β and δ (C/EBP- α , β and δ) have also been shown to be involved in regulating the transcription of SP-D (He et al., 2000). A calcineurin/NFAT pathway in respiratory epithelial cells of the lung which also assembles NFAT, AP-1 and TTF-1 on a proximal promoter in the 5' region of SP-D was recently identified to regulate transcription of this gene.

4. Conclusions

Although the importance of IL-13 in mediating the effector phase of asthma and of SP-D in pulmonary host defence and more recently in the control of lung inflammation has been well described, the mechanisms that integrate this system and bring about the effects remain unclear. Recent finding and insights hint towards a connection of both SP-D and IL-13 processes in the development of airway disease and the likelihood of sharing and

mediating common pathways. SP-D seems to provide a negative regulatory control of IL-13 and vice versa. It remains to be seen whether the effects of IL-13 and SP-D are direct effects on each other or indirect events induced by common mediators. Since SP-D overturns the Th2 shift towards a Th1 type, which could further regulate and control the IL-13 levels. These new observations linking IL-13 and SP-D phenotypes assume great importance since there is a general therapeutic interest in modulating IL-13 expression given its central role in AHR and airway remodelling. Interestingly, corticosteroids, widely used for the management of asthma, inhibit IL-13 gene expression while up-regulating SP-D production. With increasing reports of IL-13 and SP-D being produced by different cells and their counter regulatory actions on each other, a combination of factors integrating the two systems is likely that allows cell specific and/or temporal regulation of both genes. Thus, common factors or mediators could be a means of sensing and efficiently regulating opposing events mediated by IL-13 and SP-D in asthma.

References

- Ahn, J.H., Kim, C.H., Kim, Y.H., Kim, S.J., Lee, S.Y., Kim, Y.K., Kim, K.H., Moon, H.S., Song, J.S., Park, S.H., Kwon, S.S., 2007. Inflammatory and remodeling events in asthma with chronic exposure to house dust mites: a murine model. *Journal of Korean Medical Science* 22, 1026–1033.
- Ahn, K.S., Aggarwal, B.B., 2005. Transcription factor NF-kappaB: a sensor for smoke and stress signals. *Annals of the New York Academy of Sciences* 1056, 218–233.
- Akbari, O., Stock, P., Meyer, E., Kronenberg, M., Sidobre, S., Nakayama, T., Taniguchi, M., Grusby, M.J., DeKruyff, R.H., Umetsu, D.T., 2003. Essential role of NKT cells producing IL-4 and IL-13 in the development of allergen-induced airway hyper-reactivity. *Nature Medicine* 9, 582–588.
- Bao, Z., Guan, S., Cheng, C., Wu, S., Wong, S.H., Kemeny, D.M., Leung, B.P., Wong, W.S., 2009. A novel anti-inflammatory role for andrographolide in asthma via inhibition of the nuclear factor-kappaB pathway. *American Journal of Respiratory and Critical Care Medicine* 179, 657–665.
- Barnes, P.J., 2008. Role of GATA-3 in allergic diseases. *Current Molecular Medicine* 8, 330–344.
- Borron, P.J., Crouch, E.C., Lewis, J.F., Wright, J.R., Possmayer, F., Fraher, L.J., 1998. Recombinant rat surfactant-associated protein D inhibits human T lymphocyte proliferation and IL-2 production. *Journal of Immunology* 161, 4599–4603.
- Borron, P.J., Mostaghel, E.A., Doyle, C., Walsh, E.S., McHeyzer-Williams, M.G., Wright, J.R., 2002. Pulmonary surfactant proteins A and D directly suppress CD3+/CD4+

- cell function: evidence for two shared mechanisms. *Journal of Immunology* 169, 5844–5850.
- Botas, C., Poulain, F., Akiyama, J., Brown, C., Allen, L., Goerke, J., Clements, J., Carlson, E., Gillespie, A.M., Epstein, C., Hawgood, S., 1998. Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D. *Proceedings of the National Academy of Sciences of the United States of America* 95, 11869–11874.
- Bridges, J.P., Davis, H.W., Damodarasamy, M., Kuroki, Y., Howles, G., Hui, D.Y., McCormack, F.X., 2000. Pulmonary surfactant proteins A and D are potent endogenous inhibitors of lipid peroxidation and oxidative cellular injury. *Journal of Biological Chemistry* 275, 38848–38855.
- Brinker, K.G., Garner, H., Wright, J.R., 2003. Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 284, L232–L241.
- Campbell, E.J., Cury, J.D., Shapiro, S.D., Goldberg, G.I., Welgus, H.G., 1991. Neutral proteinases of human mononuclear phagocytes. Cellular differentiation markedly alters cell phenotype for serine proteinases, metalloproteinases, and tissue inhibitor of metalloproteinases. *Journal of Immunology* 146, 1286–1293.
- Cataldo, D.D., Bettiol, J., Noel, A., Bartsch, P., Foidart, J.M., Louis, R., 2002. Matrix metalloproteinase-9, but not tissue inhibitor of matrix metalloproteinase-1, increases in the sputum from allergic asthmatic patients after allergen challenge. *Chest* 122, 1553–1559.
- Chiaramonte, M.G., Mentink-Kane, M., Jacobson, B.A., Cheever, A.W., Whitters, M.J., Goad, M.E., Wong, A., Collins, M., Donaldson, D.D., Grusby, M.J., Wynn, T.A., 2003. Regulation and function of the interleukin 13 receptor alpha 2 during a T helper cell type 2-dominant immune response. *Journal of Experimental Medicine* 197, 687–701.
- Clark, H., Palaniyand, N., Strong, P., Edmondson, J., Hawgood, S., Reid, K.B., 2002. Surfactant protein D reduces alveolar macrophage apoptosis in vivo. *Journal of Immunology* 169, 2892–2899.
- Cohn, L., Elias, J.A., Chupp, G.L., 2004. Asthma: mechanisms of disease persistence and progression. *Annual Review of Immunology* 22, 789–815.
- Cohn, L., Homer, R.J., MacLeod, H., Mohrs, M., Brombacher, F., Bottomly, K., 1999. Th2-induced airway mucus production is dependent on IL-4R alpha, but not on eosinophils. *Journal of Immunology* 162, 6178–6183.
- Cohn, L., Homer, R.J., Marinov, A., Rankin, J., Bottomly, K., 1997. Induction of airway mucus production by T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus production. *Journal of Experimental Medicine* 186, 1737–1747.
- Corry, D.B., Rishi, K., Kanellis, J., Kiss, A., Song, L.Z., Xu, J., Feng, L., Werb, Z., Kheradmand, F., 2002. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. *Nature Immunology* 3, 347–353.
- Crabtree, G.R., 1999. Generic signals and specific outcomes: signaling through Ca²⁺, calcineurin, and NF-AT. *Cell* 96, 611–614.
- Crouch, E.C., 2000. Surfactant protein-D and pulmonary host defense. *Respiratory Research* 1, 93–108.
- Cury, J.D., Campbell, E.J., Lazarus, C.J., Albin, R.J., Welgus, H.G., 1988. Selective up-regulation of human alveolar macrophage collagenase production by lipopolysaccharide and comparison to collagenase production by fibroblasts. *Journal of Immunology* 141, 4306–4312.
- Daines, M.O., Hershey, G.K., 2002. A novel mechanism by which interferon-gamma can regulate interleukin (IL)-13 responses. Evidence for intracellular stores of IL-13 receptor alpha-2 and their rapid mobilization by interferon-gamma. *Journal of Biological Chemistry* 277, 10387–10393.
- Dave, V., Childs, T., Whitsett, J.A., 2004. Nuclear factor of activated T cells regulates transcription of the surfactant protein D gene (Sftpd) via direct interaction with thyroid transcription factor-1 in lung epithelial cells. *Journal of Biological Chemistry* 279, 34578–34588.
- Djukanovic, R., 2000. The role of co-stimulation in airway inflammation. *Clinical and Experimental Allergy* 30 (Suppl 1), 46–50.
- Doucet, C., Brouty-Boye, D., Pottin-Clemenceau, C., Jasmin, C., Canonica, G.W., Azzarone, B., 1998. IL-4 and IL-13 specifically increase adhesion molecule and inflammatory cytokine expression in human lung fibroblasts. *International Immunology* 10, 1421–1433.
- Elias, J.A., Lee, C.G., 2011. IL-13 in asthma. The successful integration of lessons from mice and humans. *American Journal of Respiratory and Critical Care Medicine* 183, 957–958.
- Elias, J.A., Lee, C.G., Zheng, T., Shim, Y., Zhu, Z., 2003. Interleukin-13 and leukotrienes: an intersection of pathogenetic schema. *American Journal of Respiratory Cell and Molecular Biology* 28, 401–404.
- Emsen, C.L., Bell, S.E., Jones, A., Wisden, W., McKenzie, A.N., 1998. Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. *Journal of Experimental Medicine* 188, 399–404.
- Fallon, P.G., Emson, C.L., Smith, P., McKenzie, A.N., 2001. IL-13 overexpression predisposes to anaphylaxis following antigen sensitization. *Journal of Immunology* 166, 2712–2716.
- Ferber, I.A., Lee, H.J., Zonin, F., Heath, V., Mui, A., Arai, N., O'Garra, A., 1999. GATA-3 significantly downregulates IFN-gamma production from developing Th1 cells in addition to inducing IL-4 and IL-5 levels. *Clinical Immunology* 91, 134–144.
- Fisher, J.H., Mason, R., 1995. Expression of pulmonary surfactant protein D in rat gastric mucosa. *American Journal of Respiratory Cell and Molecular Biology* 12, 13–18.
- Forbes, L.R., Haczku, A., 2010. SP-D and regulation of the pulmonary innate immune system in allergic airway changes. *Clinical and Experimental Allergy* 40, 547–562.
- Ford, J.G., Rennick, D., Donaldson, D.D., Venkayya, R., McArthur, C., Hansell, E., Kurup, V.P., Warnock, M., Grunig, G., 2001. IL-13 and IFN-gamma: interactions in lung inflammation. *Journal of Immunology* 167, 1769–1777.
- Fortin, M., Wagner, J.G., Brault, J., Harkema, J.R., Renzi, P.M., Paquet, L., Ferrari, N., 2010. Spatial and temporal expression of CCR3 and the common beta chain of the IL-3, IL-5 and GM-CSF receptor in the nasal epithelium and lymphoid tissues in a rat model of allergic rhinitis. *Cytokine* 52, 194–202.
- Foster, P.S., Hogan, S.P., Ramsay, A.J., Matthaei, K.I., Young, I.G., 1996. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *Journal of Experimental Medicine* 183, 195–201.
- Fuschiotti, P., 2011. CD8+ T cells in systemic sclerosis. *Immunologic Research* 50, 188–194.
- Gavett, S.H., O'Hearn, D.J., Karp, C.L., Patel, E.A., Schofield, B.H., Finkelman, F.D., Wills-Karp, M., 1997. Interleukin-4 receptor blockade prevents airway responses induced by antigen challenge in mice. *American Journal of Physiology* 272, L253–L261.
- Grunig, G., Warnock, M., Wakil, A.E., Venkayya, R., Brombacher, F., Rennick, D.M., Sheppard, D., Mohrs, M., Donaldson, D.D., Locksley, R.M., Corry, D.B., 1998. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 282, 2261–2263.
- Haczku, A., Cao, Y., Vass, G., Kierstein, S., Nath, P., Atochina-Vasserman, E.N., Scanlon, S.T., Li, L., Griswold, D.E., Chung, K.F., Poulain, F.R., Hawgood, S., Beers, M.F., Crouch, E.C., 2006. IL-4 and IL-13 form a negative feedback circuit with surfactant protein-D in the allergic airway response. *Journal of Immunology* 176, 3557–3565.
- Hattori, N., Kawamoto, H., Fujimoto, S., Kuno, K., Katsura, Y., 1996. Involvement of transcription factors TCF-1 and GATA-3 in the initiation of the earliest step of T cell development in the thymus. *Journal of Experimental Medicine* 184, 1137–1147.
- He, Y., Crouch, E.C., Rust, K., Spaite, E., Brody, S.L., 2000. Proximal promoter of the surfactant protein D gene: regulatory roles of AP-1, forkhead box, and GT box binding proteins. *Journal of Biological Chemistry* 275, 31051–31060.
- Heinzmann, A., Mao, X.Q., Akaiwa, M., Kreomer, R.T., Gao, P.S., Ohshima, K., Umeshita, R., Abe, Y., Braun, S., Yamashita, T., Roberts, M.H., Sugimoto, R., Arima, K., Arinobu, Y., Yu, B., Kruse, S., Enomoto, T., Dake, Y., Kawai, M., Shimazu, S., Sasaki, S., Adra, C.N., Kitaichi, M., Inoue, H., Yamauchi, K., Tomichi, N., Kurimoto, F., Hamasaki, N., Hopkin, J.M., Izuhara, K., Shirakawa, T., Deichmann, K.A., 2000. Genetic variants of IL-13 signalling and human asthma and atopy. *Human Molecular Genetics* 9, 549–559.
- Hogan, P.G., Chen, L., Nardone, J., Rao, A., 2003. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes and Development* 17, 2205–2232.
- Hohlfield, J.M., Erpenbeck, V.J., Krug, N., 2002. Surfactant proteins SP-A and SP-D as modulators of the allergic inflammation in asthma. *Pathobiology* 70, 287–292.
- Homer, R.J., Zheng, T., Chupp, G., He, S., Zhu, Z., Chen, Q., Ma, B., Hite, R.D., Gobran, L.L., Rooney, S.A., Elias, J.A., 2002. Pulmonary type II cell hypertrophy and pulmonary lipoproteinosis are features of chronic IL-13 exposure. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 283, L52–L59.
- Honda, Y., Takahashi, H., Kuroki, Y., Akino, T., Abe, S., 1996. Decreased contents of surfactant proteins A and D in BAL fluids of healthy smokers. *Chest* 109, 1006–1009.
- Hoshino, M., Takahashi, M., Takai, Y., Sim, J., 1999. Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma. *Journal of Allergy and Clinical Immunology* 104, 356–363.
- Howard, T.D., Koppelman, G.H., Xu, J., Zheng, S.L., Postma, D.S., Meyers, D.A., Bleecker, E.R., 2002. Gene-gene interaction in asthma: IL4RA and IL13 in a Dutch population with asthma. *American Journal of Human Genetics* 70, 230–236.
- Howard, T.D., Whittaker, P.A., Zaiman, A.L., Koppelman, G.H., Xu, J., Hanley, M.T., Meyers, D.A., Postma, D.S., Bleecker, E.R., 2001. Identification and association of polymorphisms in the interleukin-13 gene with asthma and atopy in a Dutch population. *American Journal of Respiratory Cell and Molecular Biology* 25, 377–384.
- James, A., Carroll, N., 1995. Theoretic effects of mucus gland discharge on airway resistance in asthma. *Chest* 107, 1105.
- Kasper, M., Sims, G., Koslowski, R., Kuss, H., Thuemmler, M., Fehrenbach, H., Auten, R.L., 2002. Increased surfactant protein D in rat airway goblet and Clara cells during ovalbumin-induced allergic airway inflammation. *Clinical and Experimental Allergy* 32, 1251–1258.
- Kawakami, K., Taguchi, J., Murata, T., Puri, R.K., 2001. The interleukin-13 receptor alpha2 chain: an essential component for binding and internalization but not for interleukin-13-induced signal transduction through the STAT6 pathway. *Blood* 97, 2673–2679.
- Kelly, B.L., Locksley, R.M., 2000. Coordinate regulation of the IL-4, IL-13, and IL-5 cytokine cluster in Th2 clones revealed by allelic expression patterns. *Journal of Immunology* 165, 2982–2986.
- Kishor, U., Madan, T., Sarma, P.U., Singh, M., Urban, B.C., Reid, K.B., 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.
- Kishore, U., Greenhough, T.J., Waters, P., Shrive, A.K., Ghai, R., Kamran, M.F., Bernal, A.L., Reid, K.B., Madan, T., Chakraborty, T., 2006. Surfactant proteins SP-A and SP-D: structure, function and receptors. *Molecular Immunology* 43, 1293–1315.
- Kitamura, N., Kaminuma, O., Mori, A., Hashimoto, T., Kitamura, F., Miyagishi, M., Taira, K., Miyatake, S., 2005. Correlation between mRNA expression of Th1/Th2

- cytokines and their specific transcription factors in human helper T-cell clones. *Immunology and Cell Biology* 83, 536–541.
- Korfhagen, T.R., LeVine, A.M., Whitsett, J.A., 1998a. Surfactant protein A (SP-A) gene targeted mice. *Biochimica et Biophysica Acta* 1408, 296–302.
- Korfhagen, T.R., Sheffelyevich, V., Burhans, M.S., Bruno, M.D., Ross, G.F., Wert, S.E., Stahlman, M.T., Jobe, A.H., Ikegami, M., Whitsett, J.A., Fisher, J.H., 1998b. Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo. *Journal of Biological Chemistry* 273, 28438–28443.
- Kumagai, K., Ohno, I., Okada, S., Ohkawara, Y., Suzuki, K., Shinya, T., Nagase, H., Iwata, K., Shirato, K., 1999. Inhibition of matrix metalloproteinases prevents allergen-induced airway inflammation in a murine model of asthma. *Journal of Immunology* 162, 4212–4219.
- Kuperman, D., Schofield, B., Wills-Karp, M., Grusby, M.J., 1998. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. *Journal of Experimental Medicine* 187, 939–948.
- Kuperman, D.A., Huang, X., Koth, L.L., Chang, G.H., Dolganov, G.M., Zhu, Z., Elias, J.A., Sheppard, D., Erle, D.J., 2002. Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nature Medicine* 8, 885–889.
- Lanone, S., Zheng, T., Zhu, Z., Liu, W., Lee, C.G., Ma, B., Chen, Q., Homer, R.J., Wang, J., Rabach, L.A., Rabach, M.E., Shipley, J.M., Shapiro, S.D., Senior, R.M., Elias, J.A., 2002. Overlapping and enzyme-specific contributions of matrix metalloproteinases-9 and -12 in IL-13-induced inflammation and remodeling. *Journal of Clinical Investigation* 110, 463–474.
- Lu, J., Wiedemann, H., Timpl, R., Reid, K.B., 1993. Similarity in structure between C1q and the collectins as judged by electron microscopy. *Behring Institute Mitteilungen*, 6–16.
- Luttmann, W., Knoechel, B., Foerster, M., Matthys, H., Virchow Jr., J.C., Kroegel, C., 1996. Activation of human eosinophils by IL-13. Induction of CD69 surface antigen, its relationship to messenger RNA expression, and promotion of cellular viability. *Journal of Immunology* 157, 1678–1683.
- Madan, T., Kishore, U., Shah, A., Eggleston, P., Strong, P., Wang, J.Y., Aggarwal, S.S., Sarma, P.U., Reid, K.B., 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. *Clinical and Experimental Immunology* 110, 241–249.
- Madan, T., Kishore, U., Singh, M., Strong, P., Clark, H., Hussain, E.M., Reid, K.B., Sarma, P.U., 2001a. Surfactant proteins A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigens and allergens. *Journal of Clinical Investigation* 107, 467–475.
- Madan, T., Kishore, U., Singh, M., Strong, P., Hussain, E.M., Reid, K.B., Sarma, P.U., 2001b. Protective role of lung surfactant protein D in a murine model of invasive pulmonary aspergillosis. *Infection and Immunity* 69, 2728–2731.
- Madan, T., Reid, K.B., Singh, M., Sarma, P.U., Kishore, U., 2005. Susceptibility of mice genetically deficient in the surfactant protein (SP)-A or SP-D gene to pulmonary hypersensitivity induced by antigens and allergens of *Aspergillus fumigatus*. *Journal of Immunology* 174, 6943–6954.
- Madan, T., Reid, K.B., Clark, H., Singh, M., Nayak, A., Sarma, P.U., Hawgood, S., Kishore, U., 2010. Susceptibility of mice genetically deficient in SP-A or SP-D gene to invasive pulmonary aspergillosis. *Molecular Immunology* 47, 1923–1930.
- Madsen, J., Kliem, A., Tornøe, I., Skjold, K., Koch, C., Holmskov, U., 2000. Localization of lung surfactant protein D on mucosal surfaces in human tissues. *Journal of Immunology* 164, 5866–5870.
- Mahajan, L., Madan, T., Kamal, N., Singh, V.K., Sim, R.B., Telang, S.D., Ramchand, C.N., Waters, P., Kishore, U., Sarma, P.U., 2008. Recombinant surfactant protein-D selectively increases apoptosis in eosinophils of allergic asthmatics and enhances uptake of apoptotic eosinophils by macrophages. *International Immunology* 20, 993–1007.
- Majo, J., Ghezzi, H., Cosio, M.G., 2001. Lymphocyte population and apoptosis in the lungs of smokers and their relation to emphysema. *European Respiratory Journal* 17, 946–953.
- Malherbe, D.C., Erpenbeck, V.J., Abraham, S.N., Crouch, E.C., Hohlfeld, J.M., Wright, J.R., 2005. Surfactant protein D decreases pollen-induced IgE-dependent mast cell degranulation. *American Journal of Physiology. Lung Cellular and Molecular Physiology* 289, L856–L866.
- McKenzie, A.N., Li, X., Largaespada, D.A., Sato, A., Kaneda, A., Zurawski, S.M., Doyle, E.L., Milatovich, A., Francke, U., Copeland, N.G., et al., 1993. Structural comparison and chromosomal localization of the human and mouse IL-13 genes. *Journal of Immunology* 150, 5436–5444.
- McKenzie, G.J., Emson, C.L., Bell, S.E., Anderson, S., Fallon, P., Zurawski, G., Murray, R., Grencis, R., McKenzie, A.N., 1998. Impaired development of Th2 cells in IL-13-deficient mice. *Immunity* 9, 423–432.
- Miyamura, K., Leigh, L.E., Lu, J., Hopkin, J., Lopez Bernal, A., Reid, K.B., 1994. Surfactant protein D binding to alveolar macrophages. *Biochemical Journal* 300 (Pt 1), 237–242.
- Moller, A., Henz, B.M., Grutzkau, A., Lippert, U., Aragane, Y., Schwarz, T., Kruger-Krasagakes, S., 1998. Comparative cytokine gene expression: regulation and release by human mast cells. *Immunology* 93, 289–295.
- Monticelli, S., Solymar, D.C., Rao, A., 2004. Role of NFAT proteins in IL13 gene transcription in mast cells. *Journal of Biological Chemistry* 279, 36210–36218.
- Nayak, A., Dodagatta-Marri, E., Tsolaki, A.G., Kishore, U., 2012. An insight into the diverse roles of surfactant proteins, SP-A and SP-D in innate and adaptive immunity. *Frontiers in Immunology* 3, 131.
- Ouyang, W., Ranganath, S.H., Weindel, K., Bhattacharya, D., Murphy, T.L., Sha, W.C., Murphy, K.M., 1998. Inhibition of Th1 development mediated by GATA-3 through an IL-4-independent mechanism. *Immunity* 9, 745–755.
- Pahl, A., Zhang, M., Kuss, H., Szelenyi, I., Brune, K., 2002. Regulation of IL-13 synthesis in human lymphocytes: implications for asthma therapy. *British Journal of Pharmacology* 135, 1915–1926.
- Pope, S.M., Brandt, E.B., Mishra, A., Hogan, S.P., Zimmermann, N., Matthaei, K.I., Foster, P.S., Rothenberg, M.E., 2001. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. *Journal of Allergy and Clinical Immunology* 108, 594–601.
- Postle, A.D., Mander, A., Reid, K.B., Wang, J.Y., Wright, S.M., Moustaki, M., Warner, J.O., 1999. Deficient hydrophilic lung surfactant proteins A and D with normal surfactant phospholipid molecular species in cystic fibrosis. *American Journal of Respiratory Cell and Molecular Biology* 20, 90–98.
- Prescott, E., Osler, M., Andersen, P.K., Bjerg, A., Hein, H.O., Borch-Johnsen, K., Lange, P., Schnohr, P., Vestbo, J., 1997. Has risk associated with smoking increased? Results from the Copenhagen Center for Prospective Population Studies. *International Journal of Epidemiology* 26, 1214–1218.
- Prueringer, R.J., Hunninghake, G.W., 1992. Inflammation and airway reactivity in asthma. *American Journal of Medicine* 92, 32S–38S.
- Rankin, J.A., Picarella, D.E., Geba, G.P., Temann, U.A., Prasad, B., DiCosmo, B., Tarallo, A., Stripp, B., Whitsett, J., Flavell, R.A., 1996. Phenotypic and physiologic characterization of transgenic mice expressing interleukin 4 in the lung: lymphocytic and eosinophilic inflammation without airway hyperreactivity. *Proceedings of the National Academy of Sciences of the United States of America* 93, 7821–7825.
- Rao, A., Luo, C., Hogan, P.G., 1997. Transcription factors of the NFAT family: regulation and function. *Annual Review of Immunology* 15, 707–747.
- Renaud, J.C., 2001. New insights into the role of cytokines in asthma. *Journal of Clinical Pathology* 54, 577–589.
- Richter, A., Puddicombe, S.M., Lordan, J.L., Bucchieri, F., Wilson, S.J., Djukanovic, R., Dent, G., Holgate, S.T., Davies, D.E., 2001. The contribution of interleukin (IL)-4 and IL-13 to the epithelial-mesenchymal trophic unit in asthma. *American Journal of Respiratory Cell and Molecular Biology* 25, 385–391.
- Sandford, A.J., Joos, L., Pare, P.D., 2002. Genetic risk factors for chronic obstructive pulmonary disease. *Current Opinion in Pulmonary Medicine* 8, 87–94.
- Schagat, T.L., Wofford, J.A., Wright, J.R., 2001. Surfactant protein A enhances alveolar macrophage phagocytosis of apoptotic neutrophils. *Journal of Immunology* 166, 2727–2733.
- Schmid-Grendelmeier, P., Altmann, F., Fischer, B., Bizer, C., Straumann, A., Menz, G., Blaser, K., Wuthrich, B., Simon, H.U., 2002. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *Journal of Immunology* 169, 1021–1027.
- Segura-Valdez, L., Pardo, A., Gaxiola, M., Uhal, B.D., Becerril, C., Selman, M., 2000. Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. *Chest* 117, 684–694.
- Selman, M., Ruiz, V., Cabrera, S., Segura, L., Ramirez, R., Barrios, R., Pardo, A., 2000. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? *American Journal of Physiology. Lung Cellular and Molecular Physiology* 279, L562–L574.
- Senft, A.P., Korfhagen, T.R., Whitsett, J.A., Shapiro, S.D., LeVine, A.M., 2005. Surfactant protein-D regulates soluble CD14 through matrix metalloproteinase-12. *Journal of Immunology* 174, 4953–4959.
- Senior, R.M., Anthonisen, N.R., 1998. Chronic obstructive pulmonary disease (COPD). *American Journal of Respiratory and Critical Care Medicine* 157, S139–S147.
- Singh, M., Madan, T., Waters, P., Parida, S.K., Sarma, P.U., 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. *Immunology Letters* 86, 299–307.
- Sluiter, H.J., Koeter, G.H., de Monchy, J.G., Postma, D.S., de Vries, K., Orie, N.G., 1991. The Dutch hypothesis (chronic non-specific lung disease) revisited. *European Respiratory Journal* 4, 479–489.
- Solymar, D.C., Agarwal, S., Bassing, C.H., Alt, F.W., Rao, A., 2002. A 3' enhancer in the IL-4 gene regulates cytokine production by Th2 cells and mast cells. *Immunity* 17, 41–50.
- Suga, M., Iyonaga, K., Okamoto, T., Gushima, Y., Miyakawa, H., Akaike, T., Ando, M., 2000. Characteristic elevation of matrix metalloproteinase activity in idiopathic interstitial pneumonias. *American Journal of Respiratory and Critical Care Medicine* 162, 1949–1956.
- 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., Gelfand, E.W., 2003. Surfactant protein D regulates airway function and allergic inflammation through modulation of macrophage function. *American Journal of Respiratory and Critical Care Medicine* 168, 783–789.
- Takemoto, N., Kamogawa, Y., Jun Lee, H., Kurata, H., Arai, K.I., O'Garra, A., Arai, N., Miyatake, S., 2000. Cutting edge: chromatin remodeling at the IL-4/IL-13 intergenic regulatory region for Th2-specific cytokine gene cluster. *Journal of Immunology* 165, 6687–6691.
- Taube, C., Duez, C., Cui, Z.H., Takeda, K., Rha, Y.H., Park, J.W., Balhorn, A., Donaldson, D.D., Dakhama, A., Gelfand, E.W., 2002. The role of IL-13 in established allergic airway disease. *Journal of Immunology* 169, 6482–6489.
- Taussig, L.M., Wright, A.L., Morgan, W.J., Harrison, H.R., Ray, C.G., 1989. The Tucson Children's Respiratory Study. I. Design and implementation of a prospective study of acute and chronic respiratory illness in children. *American Journal of Epidemiology* 129, 1219–1231.
- Tindall, E.A., Severi, G., Hoang, H.N., Ma, C.S., Fernandez, P., Southey, M.C., English, D.R., Hopper, J.L., Heyns, C.F., Tangye, S.G., Giles, G.G., Hayes, V.M., 2010. Comprehensive analysis of the cytokine-rich chromosome 5q31.1 region

- suggests a role for IL-4 gene variants in prostate cancer risk. *Carcinogenesis* 31, 1748–1754.
- Ting, C.N., Olson, M.C., Barton, K.P., Leiden, J.M., 1996. Transcription factor GATA-3 is required for development of the T-cell lineage. *Nature* 384, 474–478.
- Trask, B.C., Malone, M.J., Lum, E.H., Welgus, H.G., Crouch, E.C., Shapiro, S.D., 2001. Induction of macrophage matrix metalloproteinase biosynthesis by surfactant protein D. *Journal of Biological Chemistry* 276, 37846–37852.
- 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., 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. *Journal of Immunology* 169, 3978–3986.
- Viola, J.P., Kiani, A., Bozza, P.T., Rao, A., 1998. Regulation of allergic inflammation and eosinophil recruitment in mice lacking the transcription factor NFAT1: role of interleukin-4 (IL-4) and IL-5. *Blood* 91, 2223–2230.
- von Mutius, E., Weiland, S.K., Fritzsche, C., Duhme, H., Keil, U., 1998. Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. *Lancet* 351, 862–866.
- Walter, D.M., McIntire, J.J., Berry, G., McKenzie, A.N., Donaldson, D.D., DeKruyff, R.H., Umetsu, D.T., 2001. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *Journal of Immunology* 167, 4668–4675.
- Wang, J.Y., Kishore, U., Lim, B.L., Strong, P., Reid, K.B., 1996. Interaction of human lung surfactant proteins A and D with mite (*Dermatophagoides pteronyssinus*) allergens. *Clinical and Experimental Immunology* 106, 367–373.
- Wang, J.Y., Shieh, C.C., You, P.F., Lei, H.Y., Reid, K.B., 1998. Inhibitory effect of pulmonary surfactant proteins A and D on allergen-induced lymphocyte proliferation and histamine release in children with asthma. *American Journal of Respiratory and Critical Care Medicine* 158, 510–518.
- Webb, D.C., McKenzie, A.N., Koskinen, A.M., Yang, M., Mattes, J., Foster, P.S., 2000. Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyperreactivity. *Journal of Immunology* 165, 108–113.
- Weinberger, S.E., 1993a. Recent advances in pulmonary medicine (1). *New England Journal of Medicine* 328, 1389–1397.
- Weinberger, S.E., 1993b. Recent advances in pulmonary medicine (2). *New England Journal of Medicine* 328, 1462–1470.
- Welgus, H.G., Campbell, E.J., Cury, J.D., Eisen, A.Z., Senior, R.M., Wilhelm, S.M., Goldberg, G.L., 1990. Neutral metalloproteinases produced by human mononuclear phagocytes. Enzyme profile, regulation, and expression during cellular development. *Journal of Clinical Investigation* 86, 1496–1502.
- Wert, S., Jones, T., Korfhausen, T., Fisher, J., Whitsett, J., 2000a. Spontaneous emphysema in surfactant protein D gene-targeted mice. *Chest* 117, 248S.
- Wert, S.E., Yoshida, M., LeVine, A.M., Ikegami, M., Jones, T., Ross, G.F., Fisher, J.H., Korfhausen, T.R., Whitsett, J.A., 2000b. Increased metalloproteinase activity, oxidant production, and emphysema in surfactant protein D gene-inactivated mice. *Proceedings of the National Academy of Sciences of the United States of America* 97, 5972–5977.
- White, M.V., 1993. Nasal cholinergic hyperresponsiveness in atopic subjects studied out of season. *Journal of Allergy and Clinical Immunology* 92, 278–287.
- Whittaker, L., Niu, N., Temann, U.A., Stoddard, A., Flavell, R.A., Ray, A., Homer, R.J., Cohn, L., 2002. Interleukin-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4T cells and interleukin-9. *American Journal of Respiratory Cell and Molecular Biology* 27, 593–602.
- Wills-Karp, M., Luyimbazi, J., Xu, X., Schofield, B., Neben, T.Y., Karp, C.L., Donaldson, D.D., 1998. Interleukin-13: central mediator of allergic asthma. *Science* 282, 2258–2261.
- Wright, J.R., 2005. Immunoregulatory functions of surfactant proteins. *Nature Reviews Immunology* 5, 58–68.
- Yasunaga, S., Yuyama, N., Arima, K., Tanaka, H., Toda, S., Maeda, M., Matsui, K., Goda, C., Yang, Q., Sugita, Y., Nagai, H., Izuhara, K., 2003. The negative-feedback regulation of the IL-13 signal by the IL-13 receptor alpha2 chain in bronchial epithelial cells. *Cytokine* 24, 293–303.
- Yoshida, M., Korfhausen, T.R., Whitsett, J.A., 2001. Surfactant protein D regulates NF-kappa B and matrix metalloproteinase production in alveolar macrophages via oxidant-sensitive pathways. *Journal of Immunology* 166, 7514–7519.
- Zhang, F., Pao, W., Umphress, S.M., Jakowlew, S.B., Meyer, A.M., Dwyer-Nield, L.D., Nielsen, L.D., Takeda, K., Gelfand, E.W., Fisher, J.H., Zhang, L., Malkinson, A.M., Mason, R.J., 2003. Serum levels of surfactant protein D are increased in mice with lung tumors. *Cancer Research* 63, 5889–5894.
- Zhang, L., Ikegami, M., Dey, C.R., Korfhausen, T.R., Whitsett, J.A., 2002. Reversibility of pulmonary abnormalities by conditional replacement of surfactant protein D (SP-D) in vivo. *Journal of Biological Chemistry* 277, 38709–38713.
- Zhang, Y., Lamm, W.J., Albert, R.K., Chi, E.Y., Henderson Jr., W.R., Lewis, D.B., 1997. Influence of the route of allergen administration and genetic background on the murine allergic pulmonary response. *American Journal of Respiratory and Critical Care Medicine* 155, 661–669.
- Zheng, T., Zhu, Z., Wang, Z., Homer, R.J., Ma, B., Riese Jr., R.J., Chapman Jr., H.A., Shapiro, S.D., Elias, J.A., 2000. Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema. *Journal of Clinical Investigation* 106, 1081–1093.
- Zheng, W., Flavell, R.A., 1997. The transcription factor GATA-3 is necessary and sufficient for Th2 cytokine gene expression in CD4T cells. *Cell* 89, 587–596.
- Zhu, Z., Homer, R.J., Wang, Z., Chen, Q., Geba, G.P., Wang, J., Zhang, Y., Elias, J.A., 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *Journal of Clinical Investigation* 103, 779–788.