

EDITORIAL

Surfactant protein D and asthma

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Introduction

Because of its structural uniqueness, specific localization and functional versatility, the lung collectin surfactant protein (SP)-D is an important major player of pulmonary immune processes. Recent publications indicated that collectins are potent modulators of the innate immune response in the lung and reports on human asthma and animal models suggested that they may be involved in allergen-induced airway changes. As this molecule is capable of inhibiting T cell activation and allergic inflammatory events, it is suggested that it may function as a local regulator of a T-helper type 2 (Th2) inflammation. Two reports in this issue of *Clinical and Experimental Allergy* provide further evidence for the importance of this molecule in allergic asthma. The first one by Koopmans et al. [1] shows that SP-D may be a valuable biomarker that increases in serum of patients following allergen exposure. The second paper by Schaub et al. [2] investigates the effects of the absence of this molecule in SP-D deficient mice during allergic airway inflammation.

The collectins

SP-D was initially described as one of several collagenous glycoproteins secreted by type II alveolar epithelial cells [3], the molecular weight of which is 43 kDa under denaturing and reducing conditions and is encoded by a single gene [4]. The term collectin indicates that these molecules have an NH₂-terminal collagen-like domain and a C-terminal lectin domain. The most studied human collectins are mannose-binding lectin (MBL), SP-A and SP-D. In humans the genes for SP-A, SP-D and MBL have been mapped to a cluster on the long arm of chromosome 10 [5]. These are large, oligomeric complex molecules composed of structurally similar carbohydrate recognition domains (CRDs) attached to collagenous regions. The shared CRD plays an essential role in decoding the biological information mediated by carbohydrates that are commonly present on the surface of microbial pathogens but not usually found on mammalian cells. This property allows the collectins to recognize non-self molecules and so participate in the innate immune defence. The quaternary structure of collectins is formed by higher order oligomers bound through the N-terminally located cysteines (hexameric 'flower bouquet' by SP-A and MBL, tetrameric 'cross' by SP-D). SP-D has a characteristic cruciform structure, which is assembled as tetramers of three identical polypeptides (Fig. 1). Of the collectins, SP-D has the largest and most extended collagen domain, which allows it to form an interlinked network with bound organisms. Like SP-

A and MBL, SP-D also binds various pathogens and stimulates their phagocytosis in addition to a potent ability to modulate immune and inflammatory cell function. The collagen tail of these collectins may serve as a ligand for collectin-receptors. SP-A, SP-D, MBL (and collectin-like C1q) can bind to calreticulin/CD91 (a 'collagenous tail C1q receptor' [6]). It is, however, not only the collagenous tail that can act as a receptor ligand. SP-A, for instance, has another receptor called SP-R210 to which it binds via its globular head while a 340-kDa glycoprotein (gp-340) was shown to bind both SP-A and SP-D in the presence of calcium but independently of the CRD region. That the binding direction of SP-A and SP-D to macrophages (globular head or collagen tail) determines whether their activity is pro- or anti-inflammatory was recently proposed [7].

Surfactant protein D is selectively induced during the acute asthmatic response

In the paper by Koopmans et al. in this issue of the Journal, serum levels of SP-D were significantly elevated in allergic asthmatic patients at baseline and following allergen challenge. Interestingly, while animal models in Balb/c [8], C57BL/6 [9], and 129J [10] mice and in rats [11] confirmed enhancement of SP-D production during asthmatic inflammation, previous studies on allergic asthma in humans have shown controversial results with description of both increases [12] and decreases [13] of SP-D levels in the lung.

Although lung collectins are known to increase in many forms of pulmonary injury there is currently a general controversy that surrounds the studies of changes to surfactant protein expression in the lung and in the circulation during inflammation. In experimental models, SP-D mRNA and immunoreactive protein can be induced both acutely after treatment with bacterial endotoxin or exposure to hyperoxia as well as chronically, by infecting mice and rats with various pathogens [14]. In human studies, acute pulmonary conditions such as adult respiratory distress syndrome are also characterized by prominent increases of SP-D [15]. Regulation of this lung collectin during chronic inflammatory changes, however, appears more complex. In certain inflammatory conditions, such as allergic bronchopulmonary aspergillosis (ABPA), hypersensitivity pneumonitis, chronic interstitial lung diseases, radiation-, amiodarone- and methotrexate- induced lung injury and idiopathic pulmonary fibrosis, SP-D was found to be increased. In contrast, in patients with cystic fibrosis or following lung transplantation, levels of this molecule were found generally low. Thus, acute pulmonary inflammation both in human

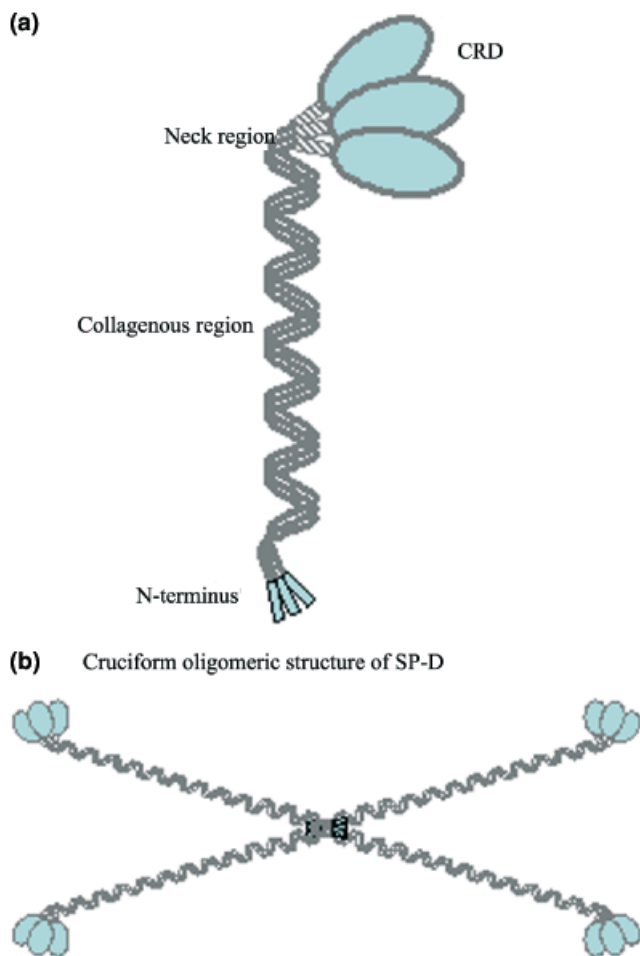


Fig. 1. The surfactant protein D (SP-D) structure. (a) Collectins are composed of structurally similar C-terminal carbohydrate recognition domains (CRDs) attached to a neck and an N-terminal collagenous regions. (b) The quaternary structure of collectins is formed by higher order oligomers bound through the N-terminally located cysteines (tetrameric 'cross').

studies and in animal models are characterized by increases of SP-D production. Chronic lung diseases, however, may be associated with either increased or decreased SP-D levels. The balance between synthesis/release, metabolism and/or clearance of this protein is intricately regulated and maintained under normal circumstances [16]. While increased SP-D levels could be a result of enhanced production and/or diminished clearance, reduced SP-D may be the result of enhanced metabolism. It is interesting to note for instance that SP-D was shown to be a target of numerous proteases present in the lung in cystic fibrosis and proteolytic cleavage of this molecule is thought to significantly contribute to impaired host defence characteristic of this disease [17, 18].

Koopmans and colleagues observed acute increases of serum SP-D but not SP-A levels 24 h after allergen challenge of asthmatic patients. Such selective SP-D regulation was previously also reported in a murine model of acute asthmatic inflammation [8, 9] suggesting unique regulatory pathways of this lung collectin. Since regulators of surfactant protein expression include a variety of mediators and cytokines that may also play a important role in allergic inflammation, specific enhancement of SP-D production during the asth-

matic response is intriguing. Recent studies implied that Th2 cells may play a regulatory role in inducing collectin production during allergic inflammation. In a study by Haley et al. [10] increased SP-D immunostaining was detected in the airway epithelial cells after allergen sensitization and challenge that required T and B lymphocyte activation, because no changes were observed in mice lacking active lymphocytes. Significant positive correlations of bronchoalveolar lavage (BAL) SP-D levels with total serum IgE in other studies suggested the possibility of a common regulatory pathway and a role for IL-4 [8, 9]. Further confirmation for a Th2 cytokine-mediated pathway came from studies on mice overexpressing IL-4 [19] and IL-13 [20] as these animals had markedly elevated SP-D expression in the lung. The role of IL-4 was recently confirmed in a type-II epithelial cell culture system *in vitro* [21].

Koopmans et al. show here not only that baseline serum SP-D in allergic patients was significantly elevated in comparison with controls but also, that serum SP-D level appeared to be an independent predictor for the magnitude of the late asthmatic response and sputum eosinophilic cationic protein (ECP) concentration after allergen challenge. Serum SP-D levels have been previously shown to be sensitive markers of disease severity in a number of different lung diseases including ABPA [22], community-acquired pneumonia [23], interstitial lung diseases in patients with polymyositis [24], acute lung injury [25, 26], idiopathic pulmonary fibrosis [27] and hypersensitivity pneumonitis, just to mention a few recent studies. The present work by Koopman's and colleagues is important since it demonstrates that SP-D is now detectable relatively easily from the serum of asthmatic patients, and it could be useful as a biomarker, sensitive enough to predict the extent of airway inflammation.

Role of surfactant protein D in allergic airway inflammation

The paper published by Schaub et al. [2] in this issue demonstrates that SP-D-deficient mice are more susceptible to allergic sensitization than wild-type controls. Early evidence for the importance of collectins in immune responses of the lung comes from the observations that insufficient levels of MBL are associated with recurrent infections in children [28]. More recently, the significance of collectins has been confirmed by studies on surfactant protein A and D gene-deficient mice. These studies consistently reported increased susceptibility to certain pathogens, increased inflammatory response to pathogen challenge, chronic inflammation, and exaggerated early inflammation with influx of immune and inflammatory cells [29]. Schaub et al. found that following sensitization and challenge with ovalbumin (OVA) SP-D^{-/-} mice expressed higher BAL eosinophils and IL-13 concentrations and lower IFN- γ expression compared with wild-type mice [2]. The greater pro-inflammatory changes in the SP-D^{-/-} mice imply an inhibitory action of this molecule on airway inflammation. The mechanism of action of this lung collectin and its significance, however, remains to be elucidated.

There are several ways by which SP-D may affect allergic airway inflammation. Given the ability of the collectin-type molecules to bind specific carbohydrate sequences on the

surface of inhaled material, one way to interfere with allergen-induced changes in the lung is to bind and sequester the inhaled allergenic material. Many allergens contain glycoconjugates that makes it possible for collectins to recognize and bind inhaled allergenic particles [30]. It was shown for instance that SP-D can bind to whole mite extracts (*Dermatophagoides pteronyssinus*) and the purified allergen Der p I in a carbohydrate-specific and Ca^{++} -dependent manner [31]. It was also demonstrated that both collectins inhibited allergen-specific IgE binding to the mite extract [32]. Thus, lung collectins may facilitate clearance of allergenic particles by alveolar macrophages. The effects of SP-A- and SP-D- facilitated uptake of allergens by lung dendritic cells are not known. Although SP-D enhanced the antigen presentation of an OVA fusion protein expressed in *Escherichia coli* HB101 to OVA-specific major histocompatibility complex class II T cell hybridomas [33] it is unclear whether such mechanism would result in T cell activation *in vivo* and induce pro-inflammatory changes. There are other indications, however, that SP-D may directly modulate function of antigen presenting cells (APCs), in particular alveolar macrophages, and that these effects result in inhibition of allergic inflammation.

Effects on alveolar macrophages

Schaub and colleagues showed that allergen-induced toll like receptor (TLR)4 expression on macrophages was increased in wild-type mice but this function was impaired in SP-D^{-/-} mice. The hypothesis that lung collectins may be responsible for maintenance of normal macrophage function has been previously confirmed many ways by studies on SP-A- and SP-D-deficient mice. Indeed macrophages in mice lacking these collectins have shown a wide array of functional impairment including increased susceptibility to bacterial, viral, fungal and endotoxin-induced lung inflammation [29]. Investigation of macrophage morphology, however, revealed normal cells in SP-A^{-/-} mice while SP-D^{-/-} mice had not only increased numbers of cells recovered from the BAL fluid but also a strikingly altered morphology [34], including significant enlargement, foamy inclusions and variable granulation. Macrophages from SP-D deficient mice also show increased activity including production of enzymes oxidative radicals and an increased susceptibility to apoptosis confirming that presence of SP-D in the lung is required in order to maintain normal macrophage function [34].

The role of SP-D in macrophage function has been also implicated during allergen-induced airway inflammation [35]. *Ex vivo* culture of alveolar macrophages with SP-D and allergen resulted in increased production of IL-12 and IFN- γ . Taken together these results and the inability of macrophages to express TLR4 in SP-D^{-/-} mice, reported here by Schaub et al. [2], suggest that inhibition of allergic airway inflammation by SP-D may occur at least partly by modulating the function of alveolar macrophages.

Inhibitory effects of surfactant protein D on T cells

Schaub et al. have also shown that lymphocytes that derived from SP-D^{-/-} mice had a greater response to mitogenic

stimulation than those from wild-type mice [2]. Unlike the early and intense investigation of the role of collectins in the innate immunity, the idea that these molecules may directly modulate the adaptive immune system has become attractive only recently. Pulmonary lymphocytes have long been known to be immunologically hyporesponsive to a variety of antigenic stimuli when compared with peripheral blood leucocytes. There is also evidence that the alveolar lining fluid contributes to the induction and maintenance of such cellular hyper-responsiveness *in vivo* and *in vitro*. Interestingly, in SP-D^{-/-} mice marked T cell activation was observed in the lungs, as reflected by an increased percentage of both CD4⁺ and CD8⁺ T cells expressing the activation markers CD69 and CD25 in the BAL. Pro-inflammatory cytokines were also consistently up-regulated in the lungs of SP-D knockout mice indicating that lack of local pulmonary production of SP-D leads to a state of persistent T cell activation, possibly in response to exogenous antigens [36].

In vitro studies using purified SP-D showed potent direct modulatory effects on lymphocytes but the mechanism of T cell susceptibility to inhibition by SP-D is unknown. SP-D is capable of inhibiting both mitogen- and antigen-induced T cell activation [32]. Although it blocks IL-2 production by T cells, an IL-2-independent inhibitory mechanism appears also involved in this inhibitory function [37]. Importantly, SP-D does not affect cell viability or apoptosis in mononuclear cells [37]. *In vivo* administration of SP-D down-regulated the eosinophilic airway inflammation in murine asthma models and shifted the cytokine profile towards a Th1 response [30].

In summary, quantitative assessment of SP-D in serum samples may be a valuable tool for diagnosing inflammatory changes in asthma and to monitor disease progression. SP-D appears to have a protective role in asthma because it has the capability to inhibit allergen-induced inflammatory changes at multiple levels affecting APCs, T cells and inflammatory cells. Presence of SP-D is essential in maintaining normal alveolar macrophage function and may be important in the elimination of inhaled allergens without eliciting a T-cell-mediated immune response. Although there is increasing evidence for the importance of this molecule, further studies are needed to extend our understanding of the complex role and regulation of this lung collectin in the pathogenesis of allergen-induced asthma.

Acknowledgements

This work was supported by ALA RG 144-N and R-01 AI055593.

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