

Interaction of human lung surfactant proteins A and D with mite (*Dermatophagoides pteronyssinus*) allergens

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(Accepted for publication 4 July 1996)

SUMMARY

Human lung surfactant proteins A (SP-A) and D (SP-D) are both collagenous C-type lectins which appear to mediate antimicrobial activity by binding to carbohydrates on micro-organisms and to receptors on phagocytic cells. Purified native SP-A and SP-D, isolated from human bronchoalveolar lavage fluid, were found to bind to whole mite extracts (*Dermatophagoides pteronyssinus*) and the purified allergen *Der p* I, in a carbohydrate-specific and calcium-dependent manner. Binding was inhibited by ethylenediamine tetra-acetic acid (EDTA) as well as by maltose in the case of SP-D, or mannose in the case of SP-A. A recombinant polypeptide, which trimerized to form the neck region and carbohydrate recognition domains of SP-D, also inhibited the binding of native SP-D to the whole mite extract and *Der p* I. Both SP-A and SP-D did not bind to deglycosylated whole mite extracts or to recombinant *Der p* proteins, which lacked carbohydrate residues. These results suggest that the ability of surfactant proteins to bind certain allergens is mediated through their carbohydrate-recognition domains (CRDs) interacting with carbohydrate residues on the allergens. Moreover, SP-A and SP-D were found to inhibit allergen-specific IgE binding to the mite extracts either via steric hindrance or competitive binding. It is therefore possible that SP-A and SP-D may be involved in the modulation of allergen sensitization and/or the development of allergic reactions.

Keywords surfactant protein A surfactant protein D mite allergen IgE

INTRODUCTION

Human surfactant protein A (SP-A) and D (SP-D) are synthesized and secreted into the lung airspaces by type II pneumocytes and Clara cells [1,2]. In addition to the regulatory function of these proteins in surfactant phospholipid metabolism, there is increasing evidence that they may also contribute to primary host-defence mechanisms in the lung [3]. The hydrophilic SP-A and SP-D can interact with lipopolysaccharides (LPS) from Gram-negative bacteria, facilitate opsonization, and enhance the respiratory burst of alveolar macrophages [4–7]. SP-A and SP-D along with several serum proteins, mannan-binding protein (MBP), bovine conglutinin and collectin-43, form the group of C-type lectins known as the collectins [8] which are characterized by having polypeptide chains composed of a short, cysteine-rich, N-terminal section followed by a collagen-like region, an α -helical section and a C-terminal carbohydrate recognition domain (CRD) [9]. The collagen-like regions form triple-helical structures and the α -helical sections also self-trimerize, thus yielding three-chain subunits which then oligomerize to give hexameric or tetrameric

molecules, having 18 or 12 CRDs, as found in SP-A and SP-D, respectively [9]. It is likely that this type of multivalent structure is important in the recognition of carbohydrate targets by the C-type lectin domains, and also in clearance of the target via interaction of the collectin–pathogen complex with collectin receptors on the cell surface [10].

The house dust mite (*Dermatophagoides pteronyssinus*, *Der p*) is a major source of environmental inhaled allergens [11]. *Der p* elicits an allergen-specific IgE antibody response in nearly 80% of allergic patients [12]. Based on IgE-binding studies, at least three major allergens (*Der p* I, *Der p* II and *Der p* III) and several minor allergens have been described [13–15]. Malhotra *et al.* [16] have reported that SP-A binds to water-extractable glycoproteins from pollens in a saturable and concentration-dependent manner, suggesting that the interaction of surfactant proteins with certain allergens may have some undefined roles in immediate type hypersensitivity.

In the present study, it has been demonstrated that human SP-A, SP-D and a recombinant fragment of SP-D (the α -helical neck region plus three CRDs), bind to carbohydrates on glycoproteins in whole mite extract and also to native *Der p* I; moreover, both SP-A and SP-D can inhibit allergen-specific IgE binding to mite allergens, either via steric hindrance or by a competitive inhibitory

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mechanism. These results suggest that hydrophilic surfactant proteins may play a role in the regulation of allergen sensitization or in the development of allergic reactions.

MATERIALS AND METHODS

Preparation of native human SP-A and SP-D, and a recombinant fragment of SP-D composed of the neck region and carbohydrate recognition domains (rSP-D(N/CRD))

Native human SP-A and SP-D were purified by affinity chromatography from bronchoalveolar lavage fluid obtained from alveolar proteinosis patients as previously described [17,18]. A polypeptide comprising the neck region and CRD (N/CRD) of human SP-D was expressed in *Escherichia coli* and isolated in the form of a self-associating homotrimer [19]. Purified SP-A, SP-D and rSP-D (N/CRD) were biotinylated by incubating 0.6 ml of 1 mg/solution of protein in 0.1 M NaHCO₃ with 34.2 µl of 10 mM N-hydroxyl succinimidobiotin (BNHS) (Pierce, Rockford, IL) in 0.1 M NaHCO₃ for 4 h at room temperature. Unreacted sulphydryl-NHS-biotin was removed by dialysis against 10 mM sodium phosphate, 150 mM NaCl, pH 7.4, and any insoluble material was removed by centrifugation in a microfuge prior to use.

Preparation of whole mite extract, Der p I allergen and mite (Der p)-sensitive asthmatic sera

A purified, and biologically standardized, mite extract (*Dermatophagoides pteronyssinus*; *Der p*) was obtained in a freeze-dried form from Pharmacia AB (Uppsala, Sweden) and prepared for use as previously described [20]. Briefly, the mite powder (1 g) was defatted with ether (100 ml) for 48 h, then homogenized and stirred continuously in phosphate buffer saline (100 ml) for 36 h. After centrifugation (10 000 g), the supernatant was lyophilized and stored at -70°C. The protein content of the extract was determined by a BioRad protein assay kit (BioRad Lab., Richmond, CA), and the concentration of the extract adjusted to 1 mg/ml (100 000 biological units/ml). Native *Der p* I, the major allergen of the mite, and the recombinant non-glycosylated forms of *Der p* II and *Der p* V expressed in *E. coli*, were kindly provided by Dr K. Y. Chua (Graduate Institute of Immunology, College of Medicine, University of Taiwan, Taiwan). Sera were drawn and pooled from 10 asthmatic children who were sensitive to dust mite (*Der p*) allergen only, as judged by the skin-prick test and by radioallergosorbent assays (RAST, Pharmacia Diagnostics AB). Sera was also collected from normal subjects who were negative in skin-test and RAST assays. The negative *Der p* I and the glycoproteins in the whole mite extract were deglycosylated by enzymatic digestion with N-glycosidase F for 18 h at 37°C using the PG-Nase F kit (New England Biolabs, Beverly, MA).

Interaction of SP-A and SP-D with house dust mite extracts

Microtitre plates (Polysorp, Nunc, Kamstrup, Denmark) were coated with dilutions of mite extract, or *Der p* I, made in 100 µl coating buffer (100 mM Na₂CO₃, pH 9.3) at 4°C overnight. Non-specific sites were blocked with BSA (200 µl per well; 3 mg/ml) in loading buffer (10 mM Tris-HCl/150 mM NaCl, pH 7.4, 0.05% (w/v) Tween 20). After extensive washing with loading buffer, the plate was incubated with serial dilutions of biotinylated SP-A, SP-D or rSP-D (N/CRD) (100 µl; maximal amount 1 µg/well) in washing buffer containing 5 mM CaCl₂ for 3 h at room temperature. For inhibition studies, 100 µl samples (1 µg/well) of biotinylated proteins were added to mite-coated wells in the presence of 10 mM

EDTA, or 100 mM mannose (for SP-A), and 100 mM maltose (for SP-D). All incubation steps were carried out at room temperature for 3 h. After washing with the same buffer, bound biotinylated SP-A, SP-D, or rSP-D (N/CRD) were detected by the use of extravidine-alkaline phosphatase conjugate (1 : 5000) (Sigma) and the substrate *p*-nitrophenyl phosphate (PNPP: 1 mg/ml, Sigma). The plates were incubated for 30 min at 37°C after which the reaction was stopped by adding 1 N NaOH, and the colour development was read at 405 nm.

Fractionation of mite proteins which are recognized by SP-A and SP-D

Freeze-dried dust mite extract (1 mg) was resuspended in the starting buffer (1 ml, 20 mM Tris-HCl buffer, pH 7.4), and fractionated by ion-exchange chromatography on a Mono Q FPLC column (1 ml; Pharmacia, Milton Keynes, UK). After extensive washing, the bound proteins were eluted with a linear gradient (20 ml) (flow rate 1 ml/min) from zero to 0.5 M NaCl in 20 mM Tris-HCl buffer (pH 7.4). The eluted fractions were tested for SP-A- and SP-D-binding activities. The fractions that contained proteins to which SP-A or SP-D bound were pooled and dialysed against 20 mM Tris-HCl, 100 mM NaCl, pH 7.4 and 5 mM CaCl₂ buffer and fractionated by size-exclusion chromatography on a Superose-12 FPLC column (Pharmacia, Milton Keynes, UK). The fractions eluted from Superose-12 were tested for their ability to be bound by SP-A, SP-D and mite-specific IgE.

Ligand-blot analysis of mite extract proteins, using biotinylated SP-A, biotinylated SP-D or allergen-specific IgE antibody

As a first step towards examining the nature of the interaction of SP-A and SP-D with dust-mite extract, ligand-blot analysis was carried out on whole mite extract and also on the fractions of mite extract, collected from size-exclusion chromatography, which bound to SP-A or SP-D. Samples (whole mite extract, native *Der p* I and also fractions eluted from the Superose-12 FLPC column) were run on SDS-PAGE after reduction of disulphide bonds, and the transferred to a nitrocellulose membrane (Hybond C, Amersham International, Amersham, UK). The non-specific sites were blocked with bovine serum albumin (BSA) (3 mg/ml) in 10 mM Tris-HCl buffer (pH 7.4) containing 150 mM NaCl. The membrane was incubated with biotinylated SP-A or biotinylated SP-D (10 ml; 1 µg/ml) in incubation buffer (10 mM Tris-HCl/150 mM NaCl, pH 7.4, containing 0.1% (v/v) Tween and 5 mM CaCl₂), overnight at 4°C. After extensive washing with incubation buffer, the blots were incubated with extravidine-alkaline phosphatase conjugate (1 : 5000; Sigma) for 2 h at room temperature in the above buffer. After three washes with the same buffer (5 min per wash), nitro-blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP; Sigma) were used as substrates for the alkaline phosphatase reaction. To detect allergen-specific IgE binding, the membrane was incubated with a dilution of pooled sera (1 : 10 dilution in 10 mM Tris-HCl/150 mM NaCl, pH 7.4, containing 0.1% (v/v) Tween and 5 mM CaCl₂) from mite-sensitive asthmatic patients. After extensive washing, the membrane was incubated with a mouse monoclonal anti-human IgE conjugated with alkaline phosphatase (1 : 2500 dilution; Sigma) for 2 h at room temperature and colour development carried out as described above.

Inhibition by SP-A and SP-D of the binding of allergen-specific IgE antibody to mite extract

The ability of SP-A and SP-D to inhibit allergen-specific IgE

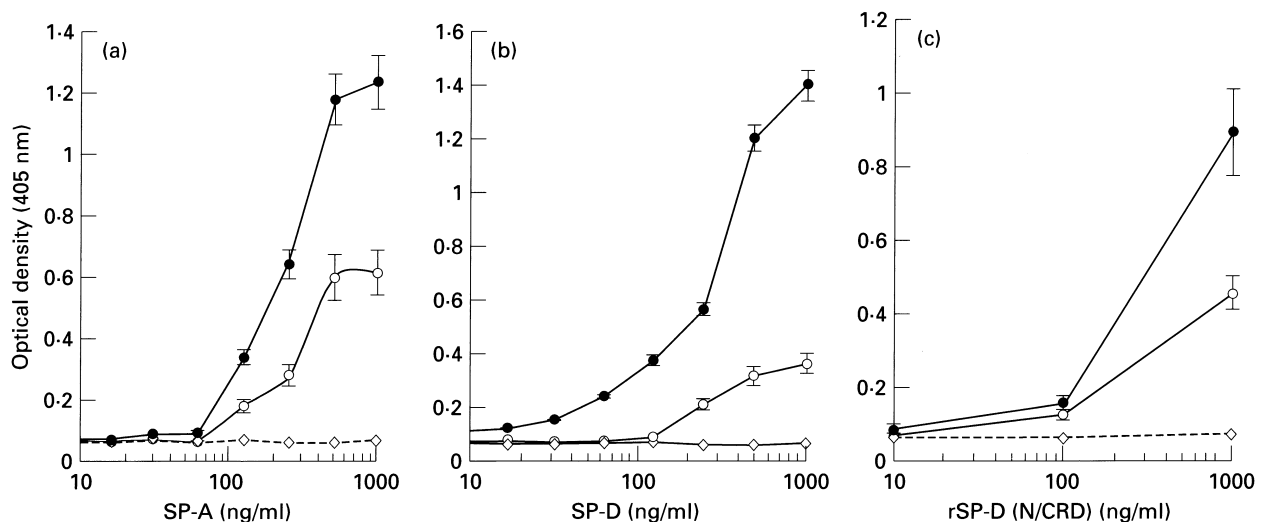


Fig. 1. Binding of lung surfactant protein A (SP-A), lung surfactant protein D (SP-D) and recombinant neck region and carbohydrate recognition domain of lung surfactant protein D (rSP-D (N/CRD)) to mite extract and *Dermatophagoides pteronyssinus* (*Der p* I). Serial dilutions of biotinylated SP-A (a), SP-D (b) and rSP-D (N/CRD) (c) were incubated with solid-phase-immobilized mite extract (●) or purified *Der p* I (○) as described in Materials and Methods. Binding to bovine serum albumin (BSA)-coated wells (□) was used as a control. Binding was estimated by reading the colour development at 405 nm after addition of extravidine-alkaline phosphatase and *p*-nitrophenyl phosphate. Results of separate experiments with the average and the range of triplicate determination points are shown. The inclusion of mannose (100 mM) or maltose (100 mM) in the assay buffer blocked the binding of SP-A, or SP-D, respectively. The inclusion of non-biotinylated SP-A, SP-D or rSP-D (N/CRD) blocked the binding of the corresponding biotinylated ligand by over 80% in each case.

binding to mite extracts or native *Der p* I were tested. Various concentrations of native human SP-A, SP-D and rSP-D (N/CRD) were incubated with pooled sera (diluted 1 : 1000 with buffer containing 5 mM CaCl_2), from 10 mite-sensitive asthmatic children, then added to microtitre wells coated with mite extract or native *Der p* I and left overnight at room temperature. After extensive washing with buffer, alkaline phosphatase-conjugated monoclonal rabbit anti-human IgE (Sigma) was added to the wells for 3 h at room temperature. Bound human IgE was detected, as described above, using a mouse monoclonal anti-human IgE. The percentage decrease in IgE-dependent binding was estimated from the reduction in the 405 nm measurements observed on addition of SP-A or SP-D.

RESULTS

Human SP-A, SP-D and the recombinant fragment rSP-D (N/CRD) are able to bind mite allergens, through their CRDs in a calcium-dependent manner

The abilities of the purified SP-A, SP-D and rSP-D (N/CRD) to bind to dust-mite extract were tested in a solid-phase assay. As seen in Fig. 1, native SP-A and SP-D (isolated from human lung surfactant), and also rSP-D (N/CRD) were all found to bind to the dust-mite extract and the major allergen *Der p* I in a dose-dependent manner. The binding to whole mite extract and *Der p* I was inhibited by the presence of mannose in the case of SP-A or maltose in the case of SP-D and rSP-D (N/CRD) (Table 1). Moreover, the binding of biotinylated SP-A and biotinylated SP-D to mite extract was inhibited by unlabelled SP-A and rSP-D (N/CRD), respectively (data not shown). The calcium dependence of the binding was confirmed by replacing calcium chloride with

EDTA in the loading buffer, which also abolished the binding (Table 1). The SP-A and SP-D did not bind to the recombinant forms of the mite allergens *Der p* II and *Der p* V (Table 2), which is consistent with the fact that these polypeptides lack carbohydrate due to their expression in *E. coli*. Moreover, after enzymatic deglycosylation of whole-mite extract and native *Der p* I, both SP-A and SP-D showed no binding to these proteins in ELISA (Table 2) and dot-blot analysis (data not shown). These results suggest that the CRD regions of SP-A and SP-D interact with carbohydrate structures on native *Der p* I and glycoproteins within the mite extract.

Table 1. Binding of surfactant proteins to mite extract

Binding conditions	SP-A	SP-D	rSP-D (N/CRD)
CaCl_2	1.251 ± 0.080	1.433 ± 0.032	0.899 ± 0.022
Mannose/ CaCl_2	0.432 ± 0.120	ND	ND
Maltose/ CaCl_2	ND	0.254 ± 0.045	0.255 ± 0.024
EDTA	0.164 ± 0.031	0.067 ± 0.010	0.078 ± 0.012

The binding of fixed quantities (1 $\mu\text{g}/\text{ml}$) of lung surfactant protein A (SP-A), lung surfactant protein D (SP-D), recombinant neck region and carbohydrate recognition domain of lung surfactant protein D (rSP-D (N/CRD)) to immobilized mite extract (1 $\mu\text{g}/\text{well}$) were tested in the presence of 5 mM CaCl_2 , 100 mM mannose (SP-A) or maltose (SP-D) and 5 mM CaCl_2 or 5 mM ethylenediaminetetra-acetic acid (EDTA), as described in Materials and Methods. Binding is shown as colour development (A_{405} nm) in the enzyme-linked immunosorbent assay. Results are means $\pm$ s.d. of three determinations. ND, Not determined.

Table 2. Binding of surfactant proteins to various forms of allergens

Immobilized allergen	SP-A	SP-D	rSP-D (N/CRD)
Mite extract	1.251 ± 0.080	1.433 ± 0.032	0.899 ± 0.022
Native <i>Der p</i> I	0.625 ± 0.051	0.432 ± 0.022	0.435 ± 0.021
Recombinant <i>Der p</i> II	0.073 ± 0.022	0.061 ± 0.025	0.084 ± 0.012
Recombinant <i>Der p</i> V	0.068 ± 0.008	0.067 ± 0.005	0.073 ± 0.022
Deglycosylated mite extract	0.125 ± 0.018	0.113 ± 0.029	0.112 ± 0.017
Deglycosylated <i>Der p</i> I	0.098 ± 0.022	0.089 ± 0.032	0.086 ± 0.014

Binding in the presence of 5 mM CaCl₂ of a fixed amount (1 µg/ml) of lung surfactant protein A (SP-A), lung surfactant protein D (SP-D) or recombinant neck region and carbohydrate recognition domain of lung surfactant protein D (rSP-D (N/CRD)) to various preparations of mite allergens coated onto microtitre plates, as described in Materials and Methods. Binding is shown as colour development (A₄₀₅ nm) in enzyme-linked immunosorbent assay. Results are means ± s.d. of three determinations.

Many of the glycoproteins in mite extracts which are recognized by SP-A and SP-D are allergenic

To investigate further the number of proteins present in mite extracts which are recognized by SP-A and SP-D, the whole mite extract was fractionated on a column of Mono Q Sepharose by FPLC. The fractions eluted from the column were tested for SP-A and SP-D binding activity as described in Materials and Methods.

Several proteins, in the fractions that eluted between NaCl concentrations of 100 mM and 450 mM, bound to biotinylated human SP-A and SP-D (data not shown). These fractions, containing surfactant protein-binding activities, were pooled and then applied onto a Superose-12 gel-filtration column. The eluted fractions were collected and tested for the binding of SP-A, SP-D and mite-specific IgE at the same time. It can be seen that the fractions which were recognized by SP-A and SP-D lie within the range 150–20 kD, which also contains a number of proteins recognized by IgE from asthmatic patients' sera (Fig. 2). The mite-extract proteins were also examined by SDS-PAGE followed by ligand blotting involving detection by biotinylated SP-A or SP-D and by IgE antibody from asthmatic patients (Fig. 3). It can be seen that SP-A interacted with five major bands, with apparent mol. wts of 150, 125, 90, 55, 30 kD, as well as several minor proteins, while SP-D bound to material with apparent mol. wts of 200, 130, 60, 35 and 25 kD. Although SP-A and SP-D bind to different glycoproteins of differing apparent mol. wts in the mite extract, the majority of these proteins of mite extracts appear to be allergenic, since they were also recognized by allergen-specific IgE from mite-sensitive asthmatic patients.

Surfactant proteins can inhibit the binding of mite-specific IgE to mite allergens

The inhibition of the binding of mite-specific IgE to whole-mite extracts and to native *Der p* I by surfactant proteins was tested. The effect of SP-A and SP-D on the binding of allergen-specific IgE to whole-mite extracts is shown in Fig. 4. Both native SP-A and SP-D showed inhibition, in a dose-dependent manner, of the binding of

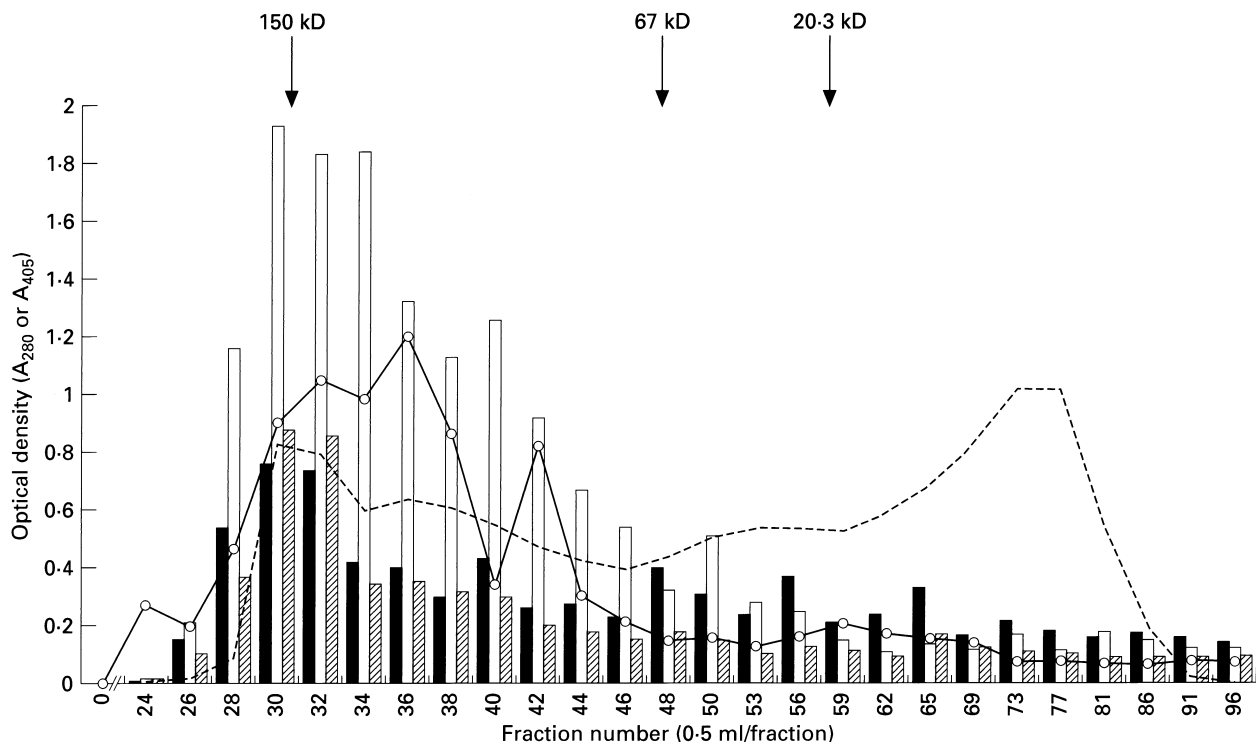


Fig. 2. The FPLC elution profile of mite extract proteins on Superose-12 after initial fractionation on Mono Q Sepharose. The lung surfactant protein A (SP-A) and lung surfactant protein D (SP-D) binding to fractions across the column is shown. Fractions containing the main SP-A and SP-D binding activity were collected and pooled. The eluted samples were also tested for their binding to mite-specific IgE (○). The

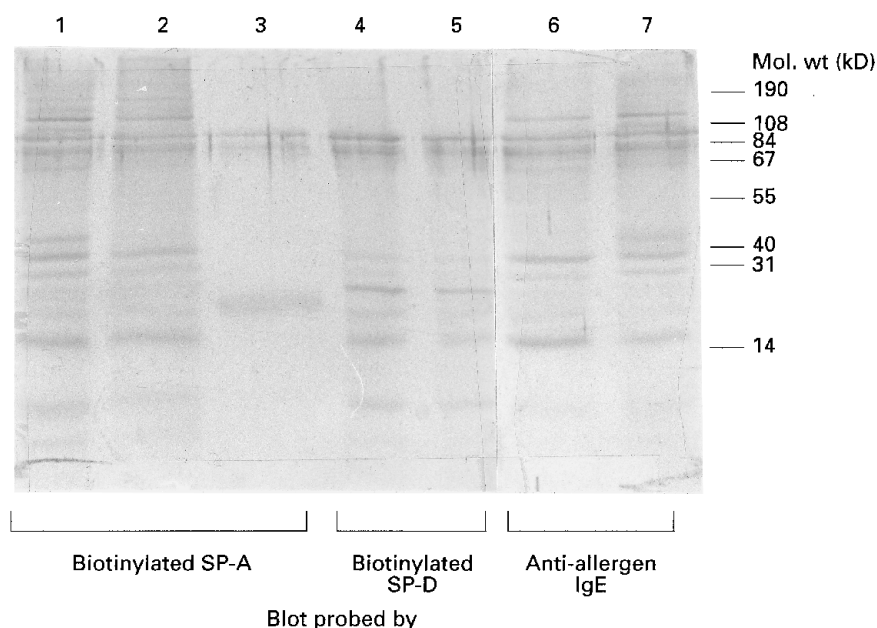


Fig. 3. Ligand-blot analysis of the surfactant protein-binding fractions of mite extract which were bound by lung surfactant A (SP-A) and lung surfactant protein D (SP-D). Samples were electrophoresed, after reduction of disulphide bonds, on a 10% (w/v) SDS-PAGE gel and then transferred to a nitrocellulose membrane. Whole-mite extract was loaded on lanes 1, 4 and 6. Samples from the Superose-12 column, over the regions which contained proteins which were bound by SP-A and SP-D, were loaded on lanes 2, 5 and 7. Purified native *Dermatophagoides pteronyssinus* (*Der p*) I was loaded on lane 3. Lanes 1 and 3 were probed with biotinylated SP-A, lanes 4 and 5 with biotinylated SP-D and lanes 6 and 7 with anti-allergen IgE (in the form of pooled sera from mite-sensitive asthmatic patients). The bound biotinylated SP-A, biotinylated SP-D and anti-allergen IgE were detected by use of extravidin-alkaline phosphatase conjugate (SP-A and SP-D) or a monoclonal anti-human IgE conjugated with alkaline phosphatase, as described in Materials and Methods.

allergens by IgE. The rSP-D (N/CRD) was also found to inhibit the binding of allergens by IgE, but to a lesser extent (50% inhibition) than that shown by native SP-D (75% inhibition) at the maximal concentration used (10 μ g/ml). Both SP-A and SP-D inhibited the binding of allergen-specific IgE to native *Der p* I (results not shown).

DISCUSSION

The respiratory tract is continuously exposed to micro-organisms and allergenic particles present in inhaled air. There is increasing evidence that the hydrophilic surfactant proteins SP-A and SP-D play a first-line defence role in the alveolar airspace and interact with alveolar macrophages or phagocytic cells during the recognition and clearance of pathogens responsible for pulmonary inflammation [3,6,8,21–23]. With respect to allergic diseases, the mechanisms for sensitization and provocation by airborne allergens such as dust mites and pollen grains are largely undefined [24]. It is not known whether surfactant proteins participate in the allergic inflammatory processes that are frequently seen in asthmatic patients.

To date, there has been no comprehensive analysis of surfactant protein and phospholipid compositions in broncho-alveolar lavage samples from asthmatic subjects. There is one recent study [25] that describes a decreased concentration of SP-A relative to phospholipid in broncho-alveolar lavage from asthmatic patients compared with normal controls. Other evidence for a possible involvement of surfactant in asthma is based on animal studies. Previous studies, in ovalbumin sensitized guinea-pigs exposed to aerosolized allergen,

have shown that the reduction of surfactant content promoted the cellular inflammatory response [26], while pretreatment with natural surfactant was found to reduce bronchial obstruction following allergen challenge and to decrease the extent of the impaired lung function significantly [27]. Using the same animal model, Liu *et al.* [28] have also measured the surface tension of lavage samples following allergen challenge. Over the 30 min of the experiment, there was a dramatic increase of minimal surface tension without any change in the lavage phospholipid composition of the lavage, and the infiltration of plasma proteins, in response to the airway-allergen challenge, caused a rapid inhibition of the surface activity of broncho-alveolar lavage surfactant. These results suggested that a rapid inactivation of surfactant within the distal non-cartilaginous airways as an early event contributed to the severity of impaired lung function in acute asthma. Prior administration of exogenous surfactant diminished the problems associated with allergen-induced responses, which included effects on lung function, cellular infiltration and histopathology [27,29]. Finally, there has been one anecdotal report of the administration of exogenous surfactant (1 ml, 10 mg/ml) as a therapy in acute asthma [30] which demonstrated improved parameters of lung function in 11 out of 12 subjects. It was suggested that pulmonary surfactant is important in the stability of the small airways and may be involved in the pathogenesis of bronchial obstruction of patients with bronchial asthma.

Recently, Malhotra *et al.* [16] reported that SP-A bound to pollen grains from *Populus nigra italica*, *Poa pratensis*, *Secale cereale*, and *Ambrosia elatior* in a saturable and concentration-dependent manner. The interaction of SP-A with pollen grains was

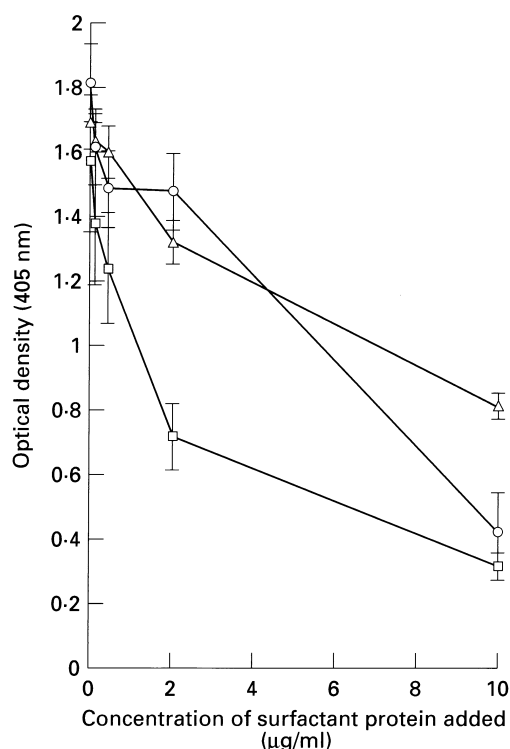


Fig. 4. Inhibition of mite-specific IgE binding to allergens by lung surfactant protein A (SP-A) and lung surfactant protein D (SP-D). Various concentrations of native human SP-A (□), SP-D (○) and recombinant neck region and carbohydrate recognition domain of lung surfactant protein D (rSP-D (N/CRD)) (△) were mixed with pooled sera (diluted 1 : 1000 with loading buffer containing 5 mM CaCl₂), from 10 mite-sensitive asthmatic children, then added to mite-coated microtitre wells (1 µg/ml) to see if there was inhibition of the binding of mite-specific IgE to the mite extracts. The procedure for measuring the binding of mite-specific IgE is described in Materials and Methods. The decrease in IgE binding was calculated from the reduction in 405 nm measurements observed on addition of SP-A or SP-D.

shown to take place via water-extractable glycoproteins and, since it was calcium dependent and inhibited by mannose, appeared to be mediated by the CRD of SP-A. These results indicate that, in the presence of SP-A, pollen grains could potentially be presented to different cell types via SP-A interacting with the C1q receptor [31]. The present study indicates that both human SP-A and SP-D can bind to the whole mite extract and to *Der p* I, one of the major allergen proteins in mite extracts. The binding is calcium-dependent, and inhibitable by sugars, which suggests that specific binding takes place by the CRDs regions of SP-A and SP-D. This view is supported by the finding that the trimeric recombinant fragment (N/CRD) of the SP-D polypeptide binds to allergen extracts, and also by the fact that SP-A and SP-D do not bind to recombinant forms of the allergen polypeptides, such as *Der p* II and *Der p* V which were expressed in *E. coli* in non-glycosylated forms. Furthermore, the studies on the enzymatically deglycosylated whole-mite extract, and deglycosylated native *Der p* I, indicate that the binding of allergens by surfactant proteins take place through their CRD regions which recognize the carbohydrate residues on the allergens. It is now well established that SP-A and SP-D recognize and bind to the ordered arrays of carbohydrate structures displayed on surface glycoproteins of viruses and bacteria [8]. Normally, SP-A and SP-D do not interact strongly

with carbohydrate structures on soluble, eukaryotic-derived glycoproteins since multivalent binding does not take place. However, in airborne particles containing denatured and degraded dust-mite glycoproteins it appears that the carbohydrate structures on these glycoproteins may be arranged in such a manner that they mimic the ordered arrays seen on the surfaces of bacteria and viruses and are therefore readily recognized and tightly bound by SP-A and SP-D.

Both SP-A and SP-D are capable of blocking the binding of allergen-specific IgE to whole mite extracts. The precise nature of inhibition of IgE binding to allergens by human SP-A and SP-D is unknown, i.e. whether surfactant proteins and IgE share the same binding site on the mite allergens or if SP-A and SP-D exert a steric hindrance effect, thus preventing subsequent binding of IgE. The results suggest that surfactant proteins SP-A and SP-D may be involved in the modulation of allergen sensitization and/or the development of allergic reactions, and these possibilities are worthy of further investigation.

REFERENCES

- Haller EM, Shelley MR, Montgomery MR, Balis JU. Immunochemical localization of lysosome and surfactant protein A in rat type II cells and extracellular surfactant forms. *J Histochem Cytochem* 1992; **40**:1491–1500.
- Voorhout WF, Veenendall T, Kuroki Y, Ogasawara Y, van Golde LMG, Geuze HJ. Immunochemical localization of surfactant protein D (SP-D) in type II cells, Clara cells and alveolar macrophages of rat lung. *J Histochem Cytochem* 1992; **40**:1589–97.
- Pison U, Max M, Neuendank S, Weissbach S, Pietschmann S. Host defence capacities of pulmonary surfactant: evidence for 'non-surfactant' functions of the surfactant system. *Eur J Clin Invest* 1994; **24**:586–99.
- Lim BL, Wang JY, Holmskov U, Hoppe HJ, Reid KB. Expression of the carbohydrate recognition domain of lung surfactant protein D and demonstration of the binding to lipopolysaccharides of Gram-negative bacteria. *Biochem Biophys Res Commun* 1994; **202**:1674–80.
- van Iwaarden JF, Pikaar JC, Storm J, Brouwer E, Verhoef J, Oostings RS, van Golde LMG, van Strijp JAG. Binding of surfactant protein A to the lipid A moiety of bacterial lipopolysaccharides. *Biochem J* 1994; **303**:407–11.
- Wright JR, Youmans DC. Pulmonary surfactant protein A stimulates chemotaxis of alveolar macrophage. *Am J Physiol* 1993; **264**:L338–L344.
- Weissbach S, Neuendank A, Pettersson M, Schaberg T, Pison U. Surfactant protein A modulates of reactive oxygen species from alveolar macrophages. *Am J Physiol* 1994; **268**:L660–L666.
- Holmskov U, Malhotra R, Sim RB, Jensenius JC. Collectins: collagenous C-type lectins of the innate immune defense system. *Immunol Today* 1994; **15**:67–74.
- Hoppe HJ, Reid KBM. Collectins-soluble proteins containing collagenous regions and lectin domains—and their roles in innate immunity. *Prot Sci* 1994; **3**:1143–58.
- Riddihough G. First-line defence. *Nature* 1994; **372**:114.
- Baldo BA, Donovan GR. House dust mite (*Dermatophagoides* spp.) allergens: research direction and priorities in the 1990s. *Monogr Allergy* 1990; **28**:1338–58.
- Chapman MD, Platts-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980; **125**:587–92.
- Chua KY, Stewart GA, Thomas WR, Simpson RJ, Dilworth RJ, Plozza TM, Turner KJ. Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p* I. Homology with cysteine protease. *J Exp Med* 1988; **167**:175–82.
- Lake FR, Ward LD, Simpson RJ, Thompson PJ, Stewart GA. House

- dust mite-derived amylase: allergenicity and physiochemical characterization. *J Allergy Clin Immunol* 1991; **87**:1035–42.
- 15 Lind P, Lowenstein H. Characterization of asthma-associated allergens. *Clin Immuno Allergy* 1988; **2**:68–89.
- 16 Malhotra R, Haurum J, Thiel S, Jensenius JC, Sim RB. Pollen grains bind to lung alveolar type II cells (A459) via lung surfactant protein A (SP-A). *Biosci Rep* 1993; **13**:79–90.
- 17 Haurum JS, Thiel S, Haagsman HP, Laursen SB, Larsen B, Jensenius JC. Studies on the carbohydrate-binding characteristics of human pulmonary surfactant associated protein A and comparison with other collectins: mannan-binding protein and conglutinin. *Biochem J* 1993; **293**:74–80.
- 18 Lu J, Willis AC, Reid KBM. Purification, characterization, and cDNA cloning of human lung surfactant protein D. *Biochem J* 1992; **284**:795–802.
- 19 Kishore U, Wang JY, Hoppe HJ, Reid KBM. 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* 1996; **318**: 505–11.
- 20 Wang JY, Lei HY, Hsieh KH. The effect of immunotherapy on interleukin 1 and tumor necrosis factor production on monocytes in asthmatic children. *J Asthma* 1992; **29**:193–201.
- 21 Geerstma MF, Nibbering PH, Haagsman HP, Daha MR, van Furth R. Binding of surfactant protein A to C1q receptors mediates phagocytosis of *Staphylococcus aureus* by monocytes. *Am J Physiol* 1994; **26**:L578–L584.
- 22 Hartshorn K, Crouch E, White MR, Eggleton P, Tauber AI, Chang D, Sastry K. Evidence for a protective role of pulmonary surfactant protein D against influenza A viruses. *J Clin Invest* 1994; **94**:311–19.
- 23 Hoppe HJ, Reid KBM. Trimeric C-type lectin domains in host defence. *Structure* 1994; **2**:1129–33.
- 24 MacMenamin C, Holt PG. The natural immune response to inhaled soluble protein antigen involve major histocompatibility complex (MHC) class I-restricted CD8⁺ T cell-mediated but MHC class II-restricted CD4⁺ T-cell dependent immune deviation resulting in selective suppression of immunoglobulin E production. *J Exp Med* 1993; **178**:889–99.
- 25 van de Graaf EA, Jansen HM, Lutter R, Alberts C, Kobesen J, de Vries IJ, Out TA. Surfactant protein A in bronchoalveolar lavage fluid. *J Lab Clin Med* 1992; **120**:252–63.
- 26 Richman PS, Batchner S, Catnzaro A. Pulmonary surfactant suppresses the immune lung injury response to inhaled antigen in guinea-pigs. *J Lab Clin Med* 1990; **116**:18–26.
- 27 Becher G. Lung surfactant prevent allergic bronchial constriction in ovalbumin-sensitized guinea-pigs. *Biochemica Biophys Acta* 1985; **44**:K57–K63.
- 28 Liu M, Wang L, Enhorning G. Surfactant dysfunction develops when the immunized guinea-pig is challenged with ovalbumin aerosol. *Clin Exp Allergy* 1995; **25**:1053–60.
- 29 Liu M, Wang L, Li E, Enhorning G. Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea-pigs. *Clin Exp Allergy* 1996; **26**:270–5.
- 30 Kurashima K, Ogawa H, Ohka T, Fujimura M, Mtsuda T, Kobayashi T. A pilot study of surfactant inhalation in the treatment of asthma attack. *Jpn J Allergol* 1991; **40**:160–3.
- 31 Malhotra R, Thiel S, Reid KBM, Sim RB. Human leukocyte C1q receptor binds other soluble proteins with collagen domains. *J Exp Med* 1990; **172**:955–9.