

Therapeutic effect of surfactant protein D in allergic inflammation of mite-sensitized mice

C.-F. Liu*, Y.-L. Chen†, C.-C. Shieh†‡, C.-K. Yu†, K. B. M. Reid§ and J.-Y. Wang*‡

*Institutes of Basic Medicine and Biochemistry, †Department of Microbiology and Immunology and ‡Division of Allergy and Clinical Immunology, Department of Paediatrics, College of Medicine, National Cheng Kung University, Tainan, Taiwan and §MRC Immunochemistry Unit, Department of Biochemistry, University of Oxford, Oxford, UK

Summary

Background Surfactant protein D (SP-D) is involved in the innate immunity within the lung and may have important roles in modulating the inflammatory process of asthma.

Objective To examine the potential immunomodulating role of SP-D on the allergic response in mice, and its interaction with the alveolar macrophages (AMs) during allergic inflammation.

Methods A recombinant 60 kDa fragment of human SP-D (rfh SP-D), Surventa, and budesonide were administered, respectively, to Der p-sensitive BALB/c mice before or after allergen challenge (AC). Total and differential cell counts, levels of cytokines in bronchoalveolar lavage fluids (BALFs), and levels of Der p-specific IgE and IgG1 antibodies in sera, were assayed. The production of nitric oxide (NO), and inducible NO synthase (iNOS) expression, in AMs, were determined by ELISA and RT-PCR, respectively.

Results Instillation of rfh SP-D to sensitized mice 6 h after AC (therapeutic), but not 24 h before AC (preventive), markedly reduced infiltration of eosinophils, and also reduced levels of IL-4, IL-5, eotaxin, and TNF- α but elevated levels of IFN- γ in the BALF. These effects were comparable with those obtained with budesonide treatment, whereas Surventa did not have a suppressive effect, either before or after AC. There was significant inhibition of NO production in the rfh SP-D pre-treated AMs of allergen-sensitized mice, but not in naïve mice.

Conclusions These results indicate that rfh SP-D has a therapeutic effect on allergen-induced bronchial inflammation, and that this might be because of its inhibitory effect on NO and TNF- α production by AMs, and it thus prevents the development of T-helper type 2 cytokine response.

Keywords alveolar macrophage, asthma murine model, surfactant protein D

Submitted 19 April 2004; revised 6 December 2004; accepted 17 December 2004

Introduction

Allergic asthma is a form of chronic airway inflammation, provoked by a wide variety of stimuli, and which is characterized by the infiltration of eosinophils and T helper (Th) lymphocytes, accompanied by bronchial hyper-responsiveness that causes a reversible form of airway obstruction. During this process, several cytokines recruit activated immune and inflammatory cells to the lung [1]. The long-term effects of airway inflammation and airway remodelling are characterized by epithelial damage, microvascular leakage, mucus hypersecretion, and deposition of collagen beneath the basement membrane [2]. It is well known that the adaptive immune responses play essential roles in allergic asthma; however, the role of innate immunity in initiating allergic inflammation and sensitization remains to be clarified. The lung contains resident components of the innate immune

system that provide a first-line defense against infectious challenge [3, 4]. Among them, alveolar macrophages (AMs) are capable of directly interacting with inhaled allergens resulting in a rapid up-regulation of the mRNA for inducible NO synthase (iNOS), and subsequent release of NO and pro-inflammatory cytokines [5, 6]. Thus, AMs might play an important role in mediating the development of allergic sensitization and airway inflammation in asthma.

Surfactant-associated proteins A (SP-A) and D (SP-D) are members of the collectin family of proteins found in the alveolar lining layer of the lung [7, 8]. Both SP-A and SP-D have important roles in innate defense against inhaled pathogens [9–11]. Previously, we [12, 13] and Madan et al. [14] have demonstrated that SP-A and SP-D can bind to both mite (*Dermatophagoides pteronyssinus*, Der p), and fungus (*Aspergillus fumigatus*, Afu), allergens, and this appeared to inhibit the binding of allergen-specific IgE to these allergens, perhaps through competition or steric hindrance effects. Moreover, both of these surfactant proteins (SPs) were found to inhibit histamine release of basophils and allergen-induced lymphocyte proliferation, two essential events in the pathogenesis of asthma [13], indicating that SPs may be involved in

Correspondence: Jiu-Yao Wang, Division of Allergy and Clinical Immunology, Department of Pediatrics, College of Medicine, National Cheng Kung University, 138, Sheng-Li Road, Tainan 70428, Taiwan.
E-mail: a122@mail.ncku.edu.tw

the regulation of allergic sensitization and inflammatory processes that are frequently seen in asthmatic patients.

Given the potential roles of SPs in innate and adaptive immunity in various allergic responses, we assume that the immunomodulating role of SP-D on allergic inflammation may be through the down-regulation of the effects mediated by AMs when contact allergens. In the present study, the effects of administration of a recombinant 60 kDa fragment of SP-D (rfh SP-D), via an intratracheal route, were compared with synthetic corticosteroids, budesonide, used in treating asthma, and a commercial preparation of surfactant lipid, Survanta, in a murine model of pulmonary hypersensitivity to Der p allergens [15]. In this model, the mice showed the expected characteristics of a high level of specific IgE antibody, peripheral blood eosinophilia, pulmonary infiltration, and a Th type 2 (Th2) cytokine response. We examined whether rfh SP-D, when administered to these mice, could inhibit the allergen-induced production of NO, and iNOS, by AMs.

Materials and methods

Reagents

Lyophilized house dust mite (*D. pteronyssinus*, Der p) was purchased from Allergon (Engelholm, Sweden). The crude mite preparation was extracted and prepared as previously described [15]. Lipopolysaccharide (LPS) (*Escherichia coli* 055:B5; Sigma-Aldrich, St Louis, MO, USA) was dissolved in pyrogenic-free isotonic saline, filtered through a 0.2 µm filter, and stored at -70°C before use. Budesonide, a topical glucocorticoid [16], and Survanta, which consists of cow lung extract containing 84% phospholipids; 4% triacylglycerol; 8% fatty acid, 7% cholesterol, sphingomyelin and other lipids, and lower than 1% hydrophobic SPs (SP-B and SP-C) [17], were purchased from Astrazeneca and GlaxoSmithKline pharmaceutical companies (Taipei, Taiwan), respectively.

Expression and purification of the recombinant 60 kDa fragment of human surfactant protein D

A recombinant homotrimer, composed of eight Gly-Xaa-Yaa repeats from the collagen region, the α -helical coiled-coil neck region, and carbohydrate recognition domain of human SP-D (rfh SP-D), was expressed in *E. coli* and purified as described elsewhere [18]. The rfh SP-D was also examined for its binding to simple sugars, phospholipids, and maltosyl-bovine serum albumin, as described previously [19]. The level of LPS in the rfh SP-D preparations was $<5\text{ pg}/\mu\text{g}$ of rfh SP-D (*Limulus* amebocyte lysate test; E-Toxate; Sigma-Aldrich).

Mice, immunization, and allergen challenge

Specific pathogen-free, 6–8-week-old BALB/c mice were housed in microisolator cages (Laboratory Products, Maywood, NJ, USA) and provided with sterile food and water *ad libitum*. All experimental animal care and treatment followed the guidelines set up by the National Science Council, Republic of China. Groups of six mice were subcutaneously immunized with 50 µL of an emulsion containing 40 µg of Der p in complete Freund's adjuvant (CFA; Difco Laboratories,

Detroit, MI, USA), at day 0 and at day 7, according to the method previously described [15]. Fourteen days later, mice were lightly anaesthetized with intraperitoneal injection of 60 mg/kg of sodium pentobarbital (Nembutal, Abbott Laboratories, North Chicago, IL, USA), and were intratracheally (i.t.) instilled with 50 µL of Der p (1 mg/mL) as the allergen challenge (AC). Controls included Der-p-sensitized mice challenged with saline and naïve (non-sensitized) mice immunized with CFA alone. To examine the effect of administration of rfh SP-D, budesonide, or Survanta (50 µL), each preparation was i.t. instilled to mice 24 h before (preventive), or 6 h after AC (therapeutic) as indicated.

Bronchoalveolar lavage

Mice were sacrificed using an overdose of sodium phenobarbital at various time periods after AC. Sera were collected and stored at -70°C until assay. Bronchoalveolar lavage (BAL) was performed and total, and differential, counts of BAL fluid (BALF) cells were determined as described previously [15, 20]. All samples were evaluated in a double-blind manner.

Allergen-specific immunoglobulin G1 and immunoglobulin E

Serum samples (1:5 dilution) were added in triplicate onto ELISA plates coated with Der p (20 µg/mL in 0.1 M NaHCO_3 , pH 8.3). After incubation overnight at 4°C , the plates were washed and incubated with biotinylated rat anti-mouse IgG1 (A85-1) or IgE monoclonal antibody (mAb) (2 µg/mL; BD PharMingen, Taipei, Taiwan) for 1 h, followed by washings and the addition of streptavidin-horseradish peroxidase conjugate (1:1000; BD PharMingen). The plates were washed and then developed with tetramethylbenzidine base microwell peroxidase substrate system (Kirkegaard & Perry Laboratories, Gaithersburg, MD, USA). Results are expressed as relative absorbance (A450 nm) after subtraction of background.

Enzyme-linked immunosorbent assay of cytokine levels

Concentrations of cytokines were measured by a sandwich ELISA technique using commercial mAb pairs, one of which was biotinylated (TNF- α , G281-2626 and MP6-XT3; IL-4, 11B11 and BVD6-24G2; IFN- γ , R4-6A2 and XMGI.2; BD PharMingen). The procedure was performed and developed as described above. Standards were run in parallel with recombinant cytokines. Eotaxin concentration was determined by use of a commercial ELISA assay kit (R & D Diagnostics, MN, USA). The detection limits were 7.5 pg/mL for IL-4, IL-5 and eotaxin, and 15 pg/mL for TNF- α and IFN- γ .

Stimulation of alveolar macrophages and determination of NO production

BAL cells from normal mice (2×10^5) were seeded on 96-well culture plates and incubated for 1 h at 37°C and 5% CO_2 . Residual non-adherent cells ($<5\%$) were removed, and samples of the adherent BAL macrophages ($>95\%$ viability) were cultured, in triplicate, in RPMI 1640 supplemented with 10% (v/v) fetal bovine serum, 20 mM L-glutamine, 10 mM

sodium pyruvate, 1% (w/v) non-essential amino acids, 100 U/mL penicillin, 100 µg/mL streptomycin, and 50 µM 2-mercaptoethanol. AMs were re-suspended at 1×10^6 cells/mL in culture medium, and the suspension cultured at 1 mL/well in a six-well plate (Falcon 3072; Becton Dickinson Labware, Franklin Lakes, NJ, USA). Different concentrations of LPS, rfh SP-D, and Der p extracts (as indicated in the figure legends) were then added to each well. Supernatants from these wells were collected and assayed after 0, 6, 12, 24, and 48 h incubation. Aliquots of culture medium from each well after addition of LPS and Der p extracts were assayed using Griess reagent for NO production as described elsewhere [15]. All samples were analysed in triplicate.

Detection of inducible NO synthase expression by semi-quantitative reverse transcription-polymerase chain reaction

The levels of iNOS transcription were analysed by semi-quantitative RT-PCR. Total RNA was extracted from pooled AMs samples using RNeasy mini kit (Qiagen, Hilden, Germany), followed by cDNA synthesis using StrataScript™-H-reverse transcriptase with oligo-dT primer in the presence of RNasin (Stratagen, La Jolla, CA, USA). The primers used for PCR amplification were as follows: iNOS sense primer, 5'-TCACTGGGACAGCACAGAAT-3'; iNOS antisense primer, 5'-TGTGTCTGCAGATGTGCTGA-3' (amplified fragment, 499 bp); β-actin sense primer, 5'-TGGAATCCTGTGGCATCCATGAAAC-3'; β-actin antisense primer, 5'-TAAACGCGAGCTCAGTAACAGTCCG-3' (amplified fragment, 348 bp) [15]. The PCR reagent contained the following: 1 µL cDNA, 0.2 µM primers, 1.5 mM MgCl₂, 200 mM of each dNTP, and 1 U of Taq DNA polymerase. Sterile water and 10 × PCR buffer were added to a total volume of 50 µL. The mixture was overlaid with 50 µL mineral oil. The generated cDNA was subjected to 30–40 cycles of PCR amplification on a DNA thermal Cycler (Hybaid Omnigene, Middlesex, UK). The PCR products were separated in a 1.9% (w/v) agarose gel and stained with ethidium bromide.

Statistical analyses

The measurement obtained for parameters of allergic mediators in AM culture supernatants, and nitrite formation values were analysed by one-way ANOVA test, followed by a *post hoc* comparison between groups. All the results were analysed using an unpaired *t*-test. Results are expressed as means ± SEM. A *P*-value < 0.05 was considered significant.

Results

The effect of exogenous surfactant phospholipids (Survanta), recombinant 60 kDa fragment of human surfactant protein, and budesonide on Der p-induced pulmonary inflammation in sensitized mice

We first tested whether exogenous Survanta, rfh SP-D, or budesonide could have a preventive or therapeutic effect on allergen-sensitive mice, when administered 24 h before, or 6 h after, AC. These two time-points were determined based on our previous reports [15, 20], which showed that intratracheal

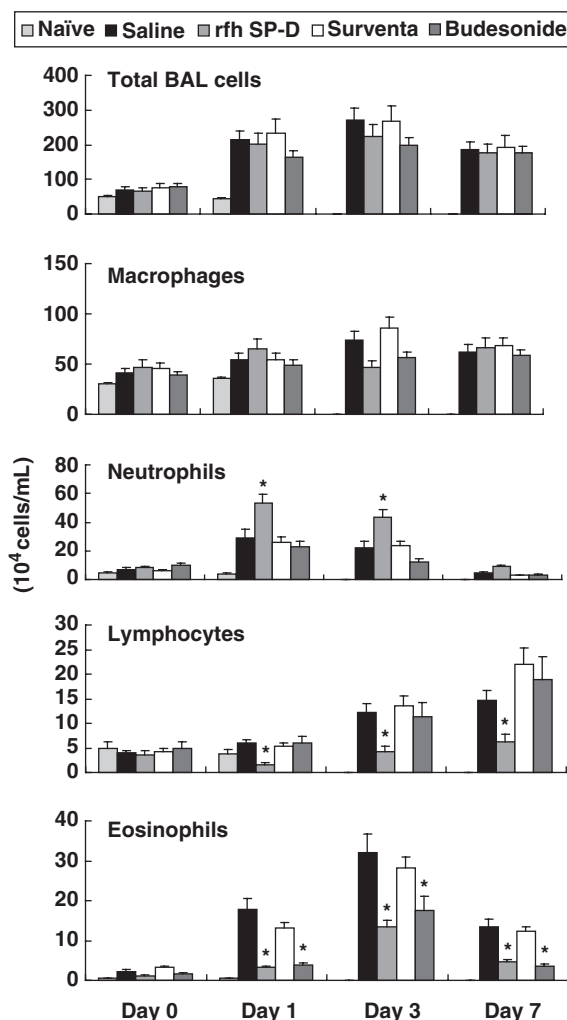


Fig. 1. The therapeutic effect of recombinant 60 kDa fragment of human surfactant protein D (rfh SP-D), Survanta and budesonide on Der p-induced airway inflammation in sensitized mice when administered 6 h after allergen challenge (AC). Groups ($n = 6$) of BALB/c mice were immunized with Der p (1 mg/mL) or saline (naïve) and were treated with saline, rfh SP-D, Survanta, or budesonide 6 h after AC. Total and differential counts of bronchoalveolar lavage fluid (BALF) cells were determined at days 1, 3, and 7 after AC. Data are the means ± SEM of six mice per group. * $P < 0.05$ compared with saline-treated mice.

challenge of crude extract of Der p to sensitized mice induced an extensive pulmonary inflammatory cells infiltrates mainly representing neutrophils, lymphocytes, and eosinophils, which lasted for approximately 7 days. Figure 1 showed that pre-treatment with rfh SP-D or budesonide 6 h after AC (therapeutic effect) could significantly reduce the infiltration of eosinophils from day 1 to day 7 as compared with untreated mice ($P < 0.001$). Moreover, rfh SP-D also decreased the numbers of lymphocytes ($P < 0.05$), but increased the numbers of neutrophils ($P < 0.05$) at days 1 and 3 after AC as compared with saline-treated controls. On the contrary, budesonide treatment decreased neutrophils counts at day 3 after AC ($P < 0.05$). Neither of these two agents was able to reduce the Der p-induced increase in macrophages and total cell counts of BALF in allergen-challenged mice. (Histological examination of the effect of treatment with rfh SP-D, budesonide, or Survanta on allergen-challenged sensitized mice is shown in the supplement.)

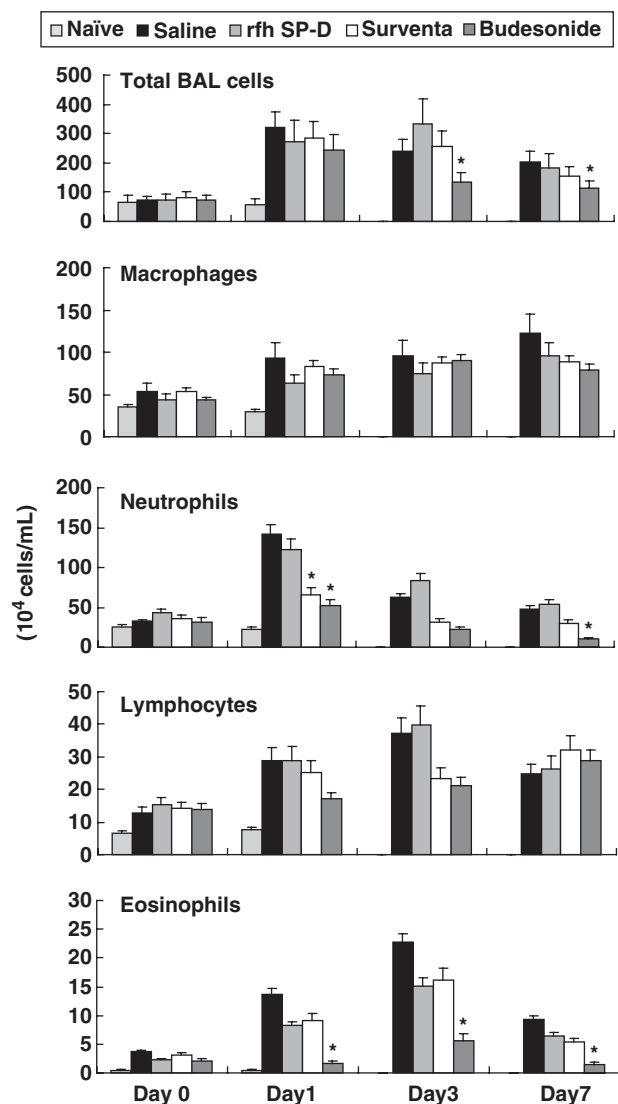


Fig. 2. The preventive effect of recombinant 60 kDa fragment of human surfactant protein D (rfh SP-D), Survanta, budesonide, or saline control in Der p-sensitized mice when administrated at 24 h before allergen challenge (AC). Groups ($n = 6$) of BALB/c mice were immunized with Der p (1 mg/mL) or saline (naïve) as described in Methods section, then sacrificed at days 1, 3, and 7 after AC. The preventive responses of above agents were evaluated by the same methods as in Fig. 1. Data are the means $\pm$ SEM of six mice per group. * $P < 0.05$ compared with saline-treated mice.

Survanta did not affect the cellular response when administrated 6 h after AC. However, Survanta significantly decreased the neutrophil counts from day 1 to day 3 after AC if it was given 24 h before AC (preventive effect) ($P < 0.05$), as shown in Fig. 2, otherwise, it had no effect on eosinophils infiltration. Pre-treatment of rfh SP-D before AC neither affected the cellular distributions nor total cell counts of BALF in Der p-sensitized mice. Budesonide, however, could significantly reduce the numbers of eosinophil (day 1 to day 7 AC) ($P < 0.05$), neutrophil (day 1 to day 7 AC) ($P < 0.05$), and total cells in BALF (day 3 to day 7 AC) ($P < 0.05$) as compared with saline-treated mice. Collectively, these results indicate that exogenous rfh SP-D can have a therapeutic effect in the suppression of allergen-induced inflammation and eosinophil infiltration, while Survanta, a mixture of

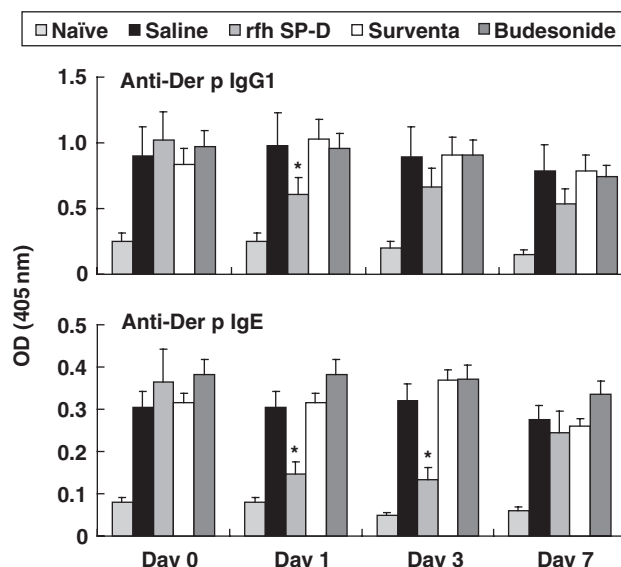


Fig. 3. Recombinant 60 kDa fragment of human surfactant protein D (rfh SP-D), but not Survanta or budesonide, decreases Der p-specific IgG and IgE antibodies of sensitized mice. Mice were treated as described in Fig. 1. Serum antibody concentrations were determined by ELISA. Each bar represents the mean $\pm$ SEM of six mice performed in triplicates. * $P < 0.05$ compared with the absorbance of saline-treated mice.

surfactant phospholipids, can only selectively prevent the recruitment of neutrophils, but not other cell types, when given before AC. To further clarify the therapeutic mechanism of exogenous rfh SP-D, all subsequent experiments reported here were performed in sensitized mice after AC.

Serum levels of anti-Der p immunoglobulin G1 and immunoglobulin E antibodies

As expected, a significant increase in the levels of Der p-specific IgG1 and IgE antibodies, an indicator of a Th2 immune response, was observed in the Der p-sensitized mice after challenge (Fig. 3). Pre-treatment of rfh SP-D, but not Survanta or budesonide, could significantly decrease the concentrations of Der p-specific IgG1 antibody on day 1, and IgE antibody levels started from day 1 to day 7 AC as compared with untreated mice (all $P < 0.05$).

The effect of exogenous surfactant phospholipids (Survanta), recombinant 60 kDa fragment of human surfactant protein D, and budesonide on cytokine concentrations in bronchoalveolar lavage fluid of sensitized mice

Administration of rfh SP-D significantly changed the cytokine profile of the Der p-sensitized and allergen-challenged mice as compared with that of saline-treated control mice (Fig. 4). There was a significant decrease in the levels of IL-4 (days 3 and 7) ($P < 0.05$), IL-5 (day 3) ($P < 0.05$), eotaxin (day 1 to day 7) ($P < 0.01$), and TNF- α (day 1 to day 3) ($P < 0.01$), and an increase in INF- γ concentrations in the BALF of rfh SP-D-treated mice as compared with those of saline-treated mice ($P < 0.05$). Survanta had no effect on cytokine concentrations. On the other hand, budesonide decreased IL-4 and IL-5 levels

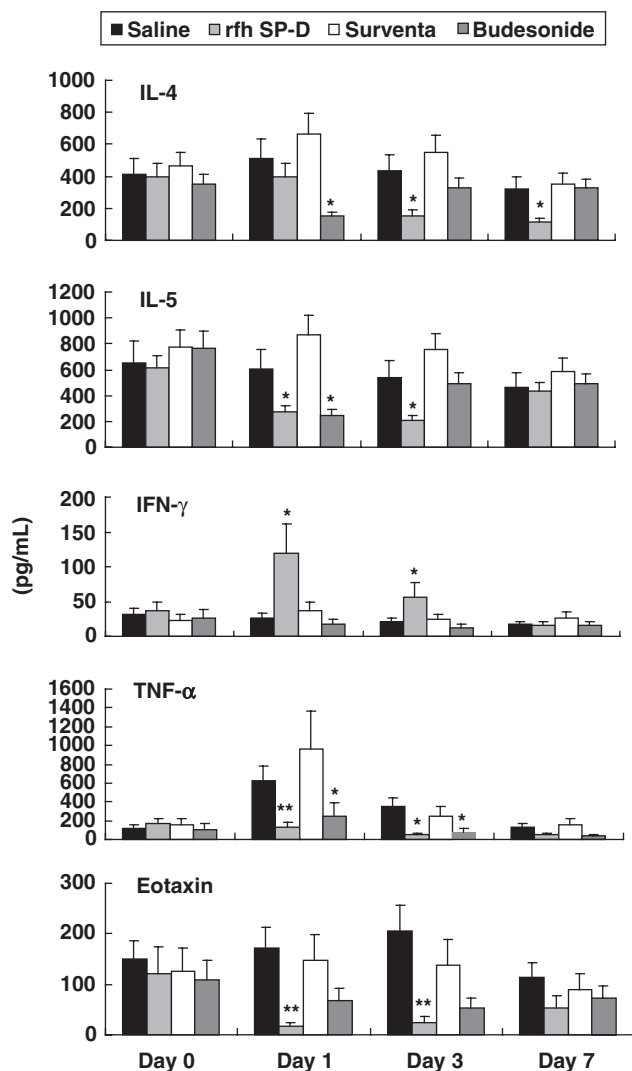


Fig. 4. Recombinant 60 kDa fragment of human surfactant protein D (rh SP-D) augments T-helper type 1 and diminishes T-helper type 2 cytokines of Der p-sensitized mice. Mice were treated as described in Fig. 1. Levels of IL-4, IL-5, IFN- γ , TNF- α , and eotaxin in the bronchoalveolar lavage fluid were measured on days 1, 3, and 7, following AC. Each bar represents the mean $\pm$ SEM of six mice performed in triplicates. * $P < 0.05$, ** $P < 0.01$ compared with the saline-treated mice.

only at day 1 of the AC, and decreased TNF- α from day 1 to day 3 AC.

The recombinant fragment of human surfactant protein D modulates nitric oxide production of alveolar macrophages stimulated by Der p or lipopolysaccharide

We have previously reported that house dust mite allergen, Der f, could augment the production of pro-inflammatory mediators by AMs, which was related to the development of allergic sensitization and airway inflammation [15]. To further explore the anti-inflammatory effect of rh SP-D on the allergen-challenge-sensitized mice, we examined whether rh SP-D might interfere with the activation of AMs. AMs collected from sensitized or naïve BALB/c mice were treated with LPS, Der P, or rh SP-D. The maximal NO production of AMs as measured by the formation of nitrate was observed

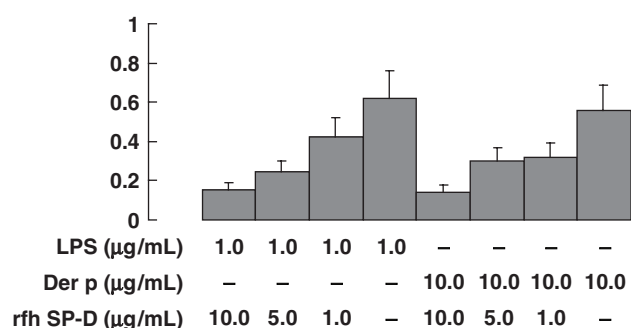


Fig. 5. Recombinant 60 kDa fragment of human surfactant protein D (rh SP-D) suppresses lipopolysaccharide- (LPS-) or Der p-induced inducible NO synthase (iNOS) expression of alveolar macrophages (AMs). RT-PCR was performed of equivalent aliquots of total RNA extracted from LPS- and Der p-stimulated AMs, pre-treated with various concentrations of rh SP-D (12 h incubation), and analysed for iNOS mRNA expression. The panel shows the optical density of iNOS mRNA in each condition plotted as the ratio to individual β -actin mRNA. Each bar represents the mean $\pm$ SEM of six mice performed in triplicates.

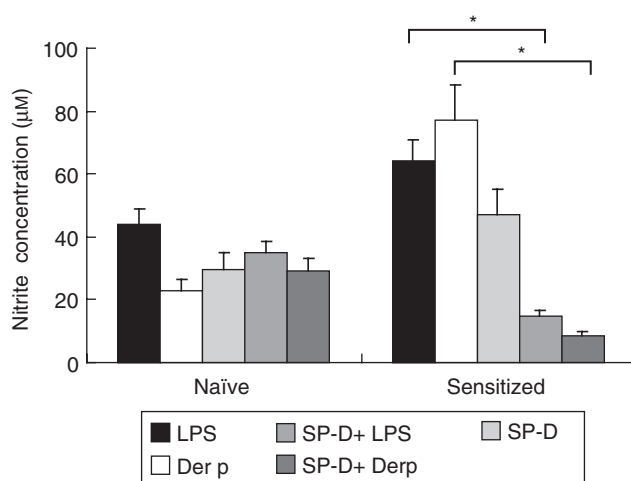


Fig. 6. Concentration of nitrate in the supernatants of lipopolysaccharide- (LPS-), Der p-, recombinant 60 kDa fragment of human surfactant protein D- (rh SP-D-) treated alveolar macrophages (AMs), which were collected from naïve and sensitized mice compared with the concentration of nitrite in the same AMs after pre-treatment of these AMs with rh SP-D. Data are the means $\pm$ SEM of six mice per group. Each value is the mean of triplicates from each mouse ($n = 6$). * $P < 0.05$ compared with the mean values of LPS- or Der p-treated mice, respectively.

when incubated with 1 $\mu\text{g/mL}$ of LPS, 10 $\mu\text{g/mL}$ of Der p, and 10 $\mu\text{g/mL}$ of SP-D for 24 h, respectively (data not shown). This result was consistent with the maximal expression of the iNOS transcript at 12 h after incubation with the above stimulants (Fig. 5). Furthermore, rh SP-D suppressed the LPS- or Der p-induced increase in iNOS expression in a dose-dependent manner.

When AMs were first incubated with rh SP-D for 12 h, then extensively washed by medium, followed by LPS- or Der p-stimulation for another 24 h, we found that there was significant inhibition (more than 70% of original level) of NO production in comparison with the rh SP-D pre-treated AMs and non-treated AMs from allergen-sensitized mice (Fig. 6). In contrast, there was no difference of NO production in naïve mice whether their AMs were pre-incubated with rh SP-D or not. In another experiment, we found that there was no inhibition of NO production from sensitized AM when rh

SP-D and LPS, or rfh SP-D and Der p, were added at the same time in the culture medium as compared with those treated with LPS or Der p alone, respectively (data not shown).

Discussion

In this study, we have shown that a direct administration of the rfh SP-D to the airway can suppress allergen-induced inflammation and sensitization in a murine model of asthma; rfh SP-D not only decreased eosinophil infiltration and inflammatory cytokines production, but also shifted the cytokine profiles from a Th type 2 predominant allergic status to a Th1 non-allergic status. Our results were agreeable with those of Madan et al. [14, 21] and Strong et al. [22]. They reported a similar protective effect of human SP-A, SP-D, and the recombinant truncated 60 kDa fragment of human SP-D (rfh SP-D) on pulmonary hypersensitivity in a model of allergic bronchopulmonary aspergillosis and Der p-sensitized mice, respectively. However, the timing of rfh SP-D administration was essential, as it exerted no effect on allergen-induced inflammation when given before allergen provocation. In contrast, the effect of Survanta was quite different to that of SP. Survanta decreased neutrophils infiltration, only when given before AC, but not the total cells and eosinophils in the BALF of sensitized mice, suggesting that SPs and phospholipids have different targets or roles in the maintenance of lung immunity. The different effects of rfh SP-D with respect to the timing of its administration, suggests that rfh SP-D has potential as a therapeutic in allergen-induced inflammation, but not as a preventive agent before allergen provocation. Although the mechanism for this difference is not clear, it may involve the interaction of SPs with antigen-presenting cells at the initial phase and their effects on subsequent humoral- or cellular-mediated immunity in the allergic inflammation [13].

In addition to protecting against air-borne pathogens, studies using SP-A- or SP-D-deficient mice have provide clues to support roles for the pulmonary SPs as immunomodulators that regulate the cellular recruitment and activation arms of the inflammatory response [23–25]. Moreover, recent evidences suggesting that SP-A and SP-D may have protective roles against airway inflammation and asthma. Asthmatics show abnormal levels of SP-A and SP-D in their bronchiolar, and alveolar, lavage as compared with those seen in normal controls [26, 27]. In the murine models of asthma, involving sensitization by allergens from mites, fungi, or by ovalbumin, there are significant changes seen in SPs levels in lavage fluids during the height of allergen-induced bronchial inflammation [20, 21, 28].

In this study, we have extended our investigation to the exogenous administration of rfh SP-D to mite-sensitized mice and compared the effects seen with those obtained using surfactant phospholipids and topical corticosteroids. It was found that rfh SP-D was not only effective as a therapeutic of bronchial inflammation as comparable with budesonide, but also has a systemic effect by decreasing allergen-specific IgE production, which was not seen as administration of budesonide or Survanta. Exogenous Survanta has been

administrated in asthmatic animal models [29] as well as in patients with various lung inflammation diseases, including asthma [30–32], with some beneficial effect. However, as judged by this study, the Survanta-induced effect is somewhat limited and would be very expensive in practical use. In contrast, the immunomodulation effects exerted by SPs may be mediated through the direct inhibition of T lymphocyte proliferation and IL-2 production [33], or indirectly, via accessory cells such as dendritic cells or AMs [34, 35].

It is interesting to note that the effect of the recombinant fragment of SP-D, on NO production by AMs, was dependent on the activation state of the AMs. Only AMs derived from Der p-sensitized mice, but not naïve mice, responded to the inhibitory action of rfh SP-D after *in vitro* stimulation with Der p or LPS. A similar observation has been reported by Stamme et al. [36], which showed that the effect of SPs on the production of reactive nitrogen species by AMs was dependent on the stimulus and the activation state of the cells. Alternately, Gardai et al. [37] suggested that lung collectins might have a dual function in different states of pulmonary conditions. Whether either of these two mechanisms is also involved in the modulation of inflammation in the allergic lung is intriguing, and merits further investigation.

In conclusion, previous studies and our present investigation suggest that SPs exert different effects, on the production of reactive nitrogen species by AMs, which depend upon the stimulus and activation state of the immune cells. These results help to provide some of the explanation for the therapeutic effect, rather than preventive effect, which is mediated by administration of rfh SP-D in allergen-induced bronchial inflammation.

Acknowledgements

This work was supported by grants NSC 91-2314-B-006-055 and NSC 92-2314-B-006-077 from the National Science Council, Taiwan, Republic of China.

References

- 1 Broide DH. Molecular and cellular mechanisms of allergic disease. *J Allergy Clin Immunol* 2001; 108:S65–71.
- 2 Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma: from bronchoconstriction to airway inflammation and remodeling. *Am J Respir Crit Care Med* 2000; 161:1720–45.
- 3 Reis E, Sousa C, Sher A, Kaye P. The role of dendritic cells in the induction and regulation of immunity to microbial infection. *Curr Opin Immunol* 1999; 11:392–9.
- 4 Rescigno M, Borrow P. The host-pathogen interaction: new themes and dendritic cell biology. *Cell* 2001; 106:267–70.
- 5 Tylor-Robinson AW, Liew FY, Severn A et al. Regulation of the immune response by nitric oxide differentially produced by T helper type 1 and T helper type 2 cells. *Eur J Immunol* 1994; 24:980–7.
- 6 Currie AJ, Stewart GA, McWilliam AS. Alveolar macrophages bind and phagocytose allergen-containing pollen starch granules via C-type lectin and integrin receptors: implications for airway inflammatory disease. *J Immunol* 2000; 164:878–86.
- 7 Eggleton P, Reid KBM. Lung surfactant proteins involved in innate immunity. *Curr Opin Immunol* 1999; 11:28–33.

- 8 Holmskov U, Thiel S, Jensenius C. Collectins and ficolins: humoral lectins of the innate immune defense. *Annu Rev Immunol* 2003; 21:547–78.
- 9 McCormack FX, Whitsett JA. The pulmonary collectins, SP-A and SP-D, orchestrate innate immunity in the lung. *J Clin Invest* 2002; 109:707–12.
- 10 Lim BL, Wang JY, Holmskov U, Hoppe H, Reid KBM. 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.
- 11 Harshon KL, White MR, Shepherd V, Reid K, Jensenius JC, Crouch EC. Mechanisms of anti-influenza activity of surfactant protein A and D: comparison with serum collectins. *Am J Physiol Lung Cell Mol Physiol* 1997; 273:L1156–6.
- 12 Wang JY, Kishore U, Lim BL, Strong P, Reid KBM. Interaction of human surfactant proteins A and D with mite (*Dermatophagoid ptyrosinissus*) allergens. *Clin Exp Immunol* 1996; 106:367–73.
- 13 Wang JY, Shieh CC, You PF, Lei HY, Reid KBM. The inhibitory effect of pulmonary surfactant protein A and D on allergen-induced lymphocyte proliferation and histamine released in asthmatic children. *Am J Respir Crit Care Med* 1998; 158:510–8.
- 14 Madan T, Kishore U, Eggleton P et al. Lung surfactant proteins A and D can inhibit specific IgE binding to the allergens of *Aspergillus fumigatus* and block allergen-induced histamine release from human basophils. *Clin Exp Immunol* 1997; 110:241–9.
- 15 Chen CC, Lee CT, Liu YC, Wang JY, Lei HY, Yu CK. House dust mite *Dermatophagoides farinae* augments proinflammatory mediators production and accessory function of alveolar macrophages: implications for allergic sensitization and inflammation. *J Immunol* 2003; 170:528–36.
- 16 Elul-Micallef R, Hansson E, Johansson SA. Budesonide: a new corticosteroids in bronchial asthma. *Eur J Respir Dis* 1980; 61: 167–73.
- 17 Fujiwara T, Maeta H, Chida S, Morita T, Watabe Y, Abe T. Artificial surfactant therapy in hyaline-membrane disease. *Lancet* 1980; 1:55–9.
- 18 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.
- 19 Shrive AK, Tharia HA, Strong P et al. High-resolution structural insights into ligand binding and immune cell recognition by human lung surfactant protein D. *J Mol Biol* 2003; 331:509–23.
- 20 Wang JY, Shieh CC, Yu CK, Lei HY. Allergen-induced bronchial inflammation is associated with decreased levels of surfactant proteins A and D in a murine model of asthma. *Clin Exp Allergy* 2001; 31:652–62.
- 21 Madan T, Kishore U, Sing M et al. Surfactant protein A and D protect mice against pulmonary hypersensitivity induced by *Aspergillus fumigatus* antigen and allergens. *J Clin Invest* 2001; 107:467–75.
- 22 Strong P, Reid KBM, Clark H. Intranasal delivery of a truncated recombinant human SP-D is effective at down-regulating allergic hypersensitivity in mice sensitized to allergens of *Aspergillus fumigatus*. *Clin Exp Immunol* 2002; 130:19–24.
- 23 Korfhagen TR, Bruno MD, Ross GF et al. Altered surfactant function and structure in SP-A gene targeted mice. *Proc Natl Acad Sci USA* 1996; 93:9594–9.
- 24 Botas C, Poulain F, Akiyama J et al. Altered surfactant homeostasis and alveolar type II cells morphology in mice lacking surfactant protein D. *Proc Natl Acad Sci USA* 1998; 95:11869–74.
- 25 LeVine AM, Whitsett JA, Gwozda JA et al. Distinct effects of surfactant protein A or D deficiency during bacterial infection on the lung. *J Immunol* 2000; 165:3934–40.
- 26 van de Graaf EA, Jansen HM, Lutter R et al. Surfactant protein A in bronchoalveolar lavage fluid. *J Lab Clin Med* 1992; 120:252–63.
- 27 Cheng G, Ueda T, Numao T. Increased levels of surfactant protein A and D in bronchoalveolar lavage fluids in patients with bronchial asthma. *Eur Respir J* 2000; 16:831–5.
- 28 Haley KJ, Ciota A, Conteras JP, Boothby MR, Perkins DL, Finn PW. Alterations in lung collectins in an adaptive immune response. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L573–84.
- 29 Liu M, Wang L, Li E, Enhorn G. Pulmonary surfactant given prophylactically alleviates an asthma attack in guinea-pigs. *Clin Exp Allergy* 1996; 26:270–5.
- 30 Anzueto A, Jubran A, Ohar JA et al. Effects of aerosolized surfactant in patients with stable chronic bronchitis. A prospective randomized controlled trial. *JAMA* 1997; 278:1426–31.
- 31 Kurashima K, Ogawa H, Ohka T, Fujimura M, Matsuda T, Kobayashi T. A pilot study of surfactant inhalation in the treatment of asthmatic attack. *Aerugi (Jpn J Allergol)* 1991; 40:160–3.
- 32 Oetomo SB, Dorrepaal C, Bos H et al. Surfactant nebulization does not alter airflow obstruction and bronchial responsiveness to histamine in asthmatic children. *Am J Respir Crit Care Med* 1996; 153:1148–52.
- 33 Borron PJ, Crouch EC, Lewis JF et al. Surfactant associated protein A inhibits human lymphocyte proliferation and IL-2 production. *J Immunol* 1998; 161:4599–603.
- 34 Miyamura K, Leigh LE, Lu J, Hopkin J, Lopez Bernal A, Reid KB. Surfactant protein D binding to alveolar macrophages. *Biochem J* 1994; 300:237–42.
- 35 Clark H, Palaniyar N, Strong P, Edmondson J, Hawgood S, Reid KB. Surfactant protein D reduces alveolar macrophages apoptosis in vivo. *J Immunol* 2002; 169:2892–9.
- 36 Stamme C, Walsh E, Wright JR. Surfactant protein A differentially regulates IFN-gamma- and LPS-induced nitrite production by rat alveolar macrophages. *Am J Respir Cell Mol Biol* 2000; 23: 772–9.
- 37 Gardai SJ, Xiao YQ, Dickinson M et al. By binding SIRP alpha or calreticulin/CD91, lung collectins act as dual function surveillance molecules to suppress or enhance inflammation. *Cell* 2003; 115: 13–23.