

A recombinant fragment of human SP-D reduces allergic responses in mice sensitized to house dust mite allergens

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SUMMARY

C57Bl6 mice sensitized to *Dermatophagoides pteronyssinus* and challenged with *D. pteronyssinus* allergen extract given intranasally followed by treatment with intranasal applications of a 60-kDa truncated, trimeric recombinant form of human SP-D (rfhSP-D) showed a significant reduction in serum IgE, IgG1, peripheral blood eosinophilia and airway hyperresponsiveness compared to saline or bovine serum albumin-treated controls. Intracellular cytokine staining of lung and spleen homogenates showed increases in interleukin (IL)-12 production in lung tissue and normalization of IL-12 and interferon (IFN)- γ in spleen tissue. In previous studies we demonstrated the effectiveness of native SP-D and rfhSP-D in down-regulating allergic responses to allergens of *Aspergillus fumigatus*. The results reported here indicate that rfhSP-D can suppress the development of allergic symptoms in sensitized mice challenged with allergens of the common house dust mite.

Keywords airway hyperresponsiveness house dust mite IL-12 plethysmography SP-D

INTRODUCTION

The house dust mite, *Dermatophagoides pteronyssinus*, is one of the leading causes of allergic asthma in the industrialized world [1–3]. In some studies as many as 85% of asthmatics responded with a positive skin prick test to house dust mite allergen extract [4]. Many studies have shown the importance of interleukin (IL)-12 in inhibiting Th2 cytokine production and IgE both *in vitro* and *in vivo* [5–7], and administration of IL-12 by intraperitoneal injection in the mouse was shown to reduce *D. pteronyssinus*-specific IgE and airway eosinophilia [8]. However, there are toxicity and dosage problems with the direct administration of cytokines. Another approach is to use natural up-regulators to elevate endogenous IL-12 or interferon (IFN)- γ . Many microbial products, including heat-killed bacteria and CpG motifs, up-regulate Th1 cytokines, including IL-12 [9,10], but there is a concern that such microbial products could overstimulate the Th1 cytokine profile leading to Th1-mediated pathology such as autoimmunity [11].

Surfactant protein D (SP-D), like surfactant protein A (SP-A), is a member of the collectin family of proteins found in the alveolar lining layer of the lung. Both SP-A and SP-D have important roles in innate host defence against inhaled pathogens [12].

There is also mounting evidence that the surfactant proteins (in particular SP-D) are able to modulate the process of allergic inflammation to airborne allergens [13,14]. While the mechanism of their immunomodulatory effect remains to be clarified, it is probably due in part to the ability of the carbohydrate recognition domain (CRD) to recognize and bind to glycosylated residues of allergen molecules, such as those of the common house dust mite [14].

Both SP-D and SP-A exist as higher-order multimers of non-covalent trimers of polypeptides which consist of a cysteine-rich N-terminal region, a long collagen-like 'tail', an alpha-helical 'neck' region and a C-terminal CRD. The hydrophobic neck region is essential for trimerization of the whole molecule [15].

We have demonstrated previously that native human SP-D, as well as the truncated 60 kDa rfhSP-D trimer, consisting of the α -helical coiled coil neck region and CRD, has immunomodulatory properties in a mouse model of allergic hypersensitivity to allergen extracts of *Aspergillus fumigatus* [16,17]. These effects appear to be mediated through the CRD [18]. It was shown that treatment of *Afu* allergic mice with native human SP-D or rfhSP-D produced a significant reduction in IgE and peripheral blood eosinophilia. There was a concomitant decrease in IL-2, IL-4 and IL-5 and an increase in IFN- γ in spleen cell culture supernatants, which suggested that the truncated fragment of human surfactant protein D (rfhSP-D) was effective in down-regulating allergic hypersensitivity against fungal allergens, by promoting Th-1 responses.

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The aim of the present study was to investigate the effect of treatment with the same recombinant fragment of human SP-D in mice sensitized to and then challenged with allergens of the common house dust mite, *D. pteronyssinus*.

MATERIALS AND METHODS

Recombinant SP-D (rfhSP-D)

The 60 kDa trimeric fragment of human SP-D was expressed in *Escherichia coli* and purified as described elsewhere [16] and dissolved in endotoxin-free phosphate buffered saline (PBS). Endotoxin contamination was removed by passing over a column of polymyxin beads. Residual endotoxin levels were measured by the limulus amoebocyte lysate assay (BioWhittaker, UK) and only preparations containing less than 5 pg endotoxin/ μ g rfhSP-D were used.

Allergen extracts

Standardized *D. pteronyssinus* extract (Greer Laboratories, Lenoir, NC, USA) containing 10 000 allergy units (AU)/ml was diluted into sterile endotoxin-free PBS.

Sensitization

Female C57BL/6 mice were given 4-weekly i.p. injections of a mixture of allergen extract (63 AU *D. pteronyssinus*) with alum suspension (1 : 4 v/v Pierce Imject) in 100 μ l of sterile PBS.

Allergen challenge and treatment with rfhSP-D

Five days after the last i.p. injection, sensitized mice were anaesthetized with isoflurane and challenged with 50 AU of *D. pteronyssinus* extract in PBS given intranasally followed by intranasal doses of PBS or rfhSP-D or BSA, given within 1–2 h. This protocol of allergen challenge followed by treatment was repeated on a daily basis for 4–5 days as indicated.

Peripheral blood eosinophils

Blood was collected from the tail vein of mice for estimation of peripheral eosinophils. The total leucocytes were counted with an automatic cell counter and the proportion of eosinophils determined by differential counting of May–Grunwald–Giemsa-stained blood smears. Results are expressed as 10^6 cells/ml.

Serum IgE and *D. pteronyssinus*-specific IgG1

Attempts to measure *D. pteronyssinus*-specific IgE were unsuccessful, due probably to competitive blocking by *D. pteronyssinus*-specific IgG, which occurs at a much higher concentration than IgE and the low concentration of *D. pteronyssinus* proteins in the extract. Total serum IgE was measured by capture ELISA using a kit and following the manufacturer's instructions (BD PharMingen, Cowley, UK). Tail-vein blood serum was diluted serially to give values which were linear with respect to a purified mouse IgE standard. Results are expressed in μ g/ml. *D. pteronyssinus*-specific IgG1 was measured by first coating microwell plates with a 1/1000 dilution of the *D. pteronyssinus* extract diluted in sodium carbonate buffer overnight at 4°C, followed by blocking with 1% BSA (w/v) for 2 h. Serum was added at a dilution of 1/250–1/32 000 and incubated at 37°C for 2 h followed by washing and incubation with antimouse-IgG1-HRP conjugate (1/1000) for 1 h. Colour was developed by adding HRP substrate (Pierce & Warriner) and absorbance measured at 450 nm. Results

are expressed as relative absorbance (A450 nm) after subtraction of background.

Intracellular cytokine staining

After treatment, mice were sacrificed humanely by CO₂ asphyxiation and their lungs and spleens removed and homogenized in PBS. The homogenate was filtered and red blood cells lysed with ammonium chloride lysing reagent (BD Pharmingen) and fixed with 4% (v/v) paraformaldehyde for 20 min. The cells were washed with PBS supplemented with 3% (v/v) heat-inactivated fetal calf serum with 0.1% (w/v) sodium azide (FSB), resuspended in 10% (v/v) DMSO in FSB and stored at –80°C.

Cells were permeabilized with CytoPerm wash buffer (CPB, BD Biosciences, Cowley, UK) for 15 min at 4°C and aliquots of 10^6 cells were blocked by incubation for 30 min at 4°C with CPB supplemented with 50 μ g/ml rat IgG. Intracellular cytokines were stained with 1 μ g phycoerythrin (PE)-conjugated antimouse cytokine monoclonal antibody (BD Biosciences) incubated for 30 min at 4°C. The cells were washed with CPB followed by FSB and re-suspended in 0.5 ml FSB.

Flow cytometry was performed with a FACScan flow cytometer (Beckton Dickinson, Mountain View, CA, USA) using CellQuest software. Data were collected for 20 000 cells. The average FSC of cells was 100 in all cases. Stained cells (FSC > 100, FL2 > 100) were gated and the proportion of these cells staining intensely for PE (PE > 1000) was calculated. Results are expressed as the percentage of intensely stained cells after subtraction of background fluorescence for unstained cells incubated with rat IgG (% PE > 1000).

Endogenous mouse SP-D and SP-A in the lung

Endogenous levels of SP-A and SP-D were measured in the BAL of mice as described previously [16]. Briefly, immediately after sacrifice by CO₂ asphyxiation, bronchoalveolar lavage was performed with 3×1 ml PBS, which were pooled and the total volume BAL was noted for all samples. After centrifugation to remove cells, SP-D and SP-A were measured by ELISA using polyclonal antibodies raised previously against recombinant mouse SP-D and SP-A (provided by Dr P. Lawson). These antibodies were shown not to cross-react with human SP-D or SP-A and were specific for murine SP-D and SP-A. Results are expressed as μ g/ml of BAL fluid.

Lung histology

Immediately after treatment, the lungs of mice from each treatment group were fixed in 10% (v/v) neutral buffered formalin, embedded in paraffin, sectioned and stained with haematoxylin and eosin and the degree of peribronchial inflammation scored independently on a scale of 0–4 (corresponding to a score of normal to severe, respectively) by a veterinary histologist blinded to the experimental details, as described previously [16].

Whole body plethysmography

In this study, airway hyperresponsiveness was measured using unrestrained whole body plethysmography [19] with a four-chamber system (Buxco, Sharon, CT, USA). Mice were first challenged with intranasal antigen and allowed to recover for 2 h before being placed into the chambers and their breathing monitored for 10 min. When acclimatized, their baseline response was measured for 5 min. The mice were then subjected to 1 min of aerosolized PBS, followed by nebulized methacholine. Responses are

recorded for 5 min in every case with a short interval between to allow return to baseline Penh. Each group contained four to eight mice. Results are presented as the average percentage elevation in Penh above baseline after a challenge of methacholine.

Statistics

Results are the average for four to eight mice/group and error bars are $\pm$ s.e.m. Significance was determined by Student's two-tailed *t*-test. Significance was accepted for $P < 0.05$.

RESULTS

Treatment with rfhSP-D reduces serum IgE and

D. pteronyssinus-specific IgG1 response to allergen challenge

Sensitized mice had elevated serum IgE relative to non-sensitized mice, demonstrating that the sensitization protocol was effective (Fig. 1). Treatment with rfhSP-D of sensitized mice following allergen challenge for 5 days resulted in a significantly lower IgE response ($P < 0.01$, $n = 4-8$ mice/group). Treatment with PBS or the same dose of a control protein (BSA) did not lower the IgE. A similar reduction by treatment with rfhSP-D was measured on *D. pteronyssinus*-specific IgG1 (Fig. 2, $P < 0.05$, $n = 4-8$ mice/group). No *D. pteronyssinus*-specific IgG1 was detectable in non-sensitized animals.

Treatment with rfhSP-D decreases peripheral blood eosinophilia response to D. pteronyssinus allergen challenge

Having established that treatment with rfhSP-D had a specific effect on reducing serum IgE and IgG1, the effect of treatment on peripheral blood eosinophilia was investigated. Sensitized mice were challenged with *D. pteronyssinus* extract intranasally, followed by treatment with PBS or 10 μ g rfhSP-D on 4 successive days. Measurement of eosinophil counts in blood obtained from tail bleeding on the following day showed a 41% fall in eosinophil counts compared to mice treated with PBS, although this difference did not reach statistical significance (Fig. 3).

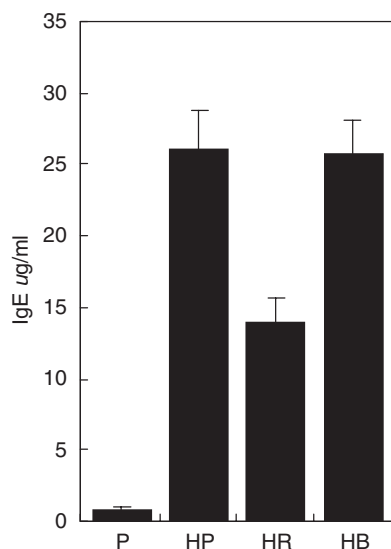


Fig. 1. Total serum IgE measured after treatment with five daily doses of PBS (HP), 10 μ g rfhSP-D (HR) or BSA (HB), given after Der p challenge. P = non-sensitized mice treated with PBS.

Treatment with rfhSP-D produces an elevation in IL-12 in the lung and reverses the decrease in IL-12 and IFN- γ measured in the spleen

To examine further the effect of rfhSP-D treatment, the cytokines IL-12 and IFN- γ were measured by intracellular cytokine staining of whole cell homogenates of lung and spleen after treatment with 4 daily doses of 10 μ g rfhSP-D given after challenge with 50 AU of *D. pteronyssinus* extract. Mice were re-challenged 4 days after treatment with *D. pteronyssinus* extract alone and cytokines measured the following day. Treatment with rfhSP-D produced a marked increase, although the difference was not statistically

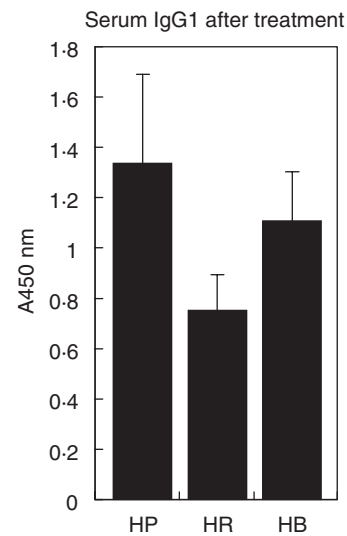


Fig. 2. *D. pteronyssinus*-specific IgG1 after treatment with five daily doses of PBS (HP), 10 μ g rfhSP-D (HR) or BSA (HB), given after allergen challenge.

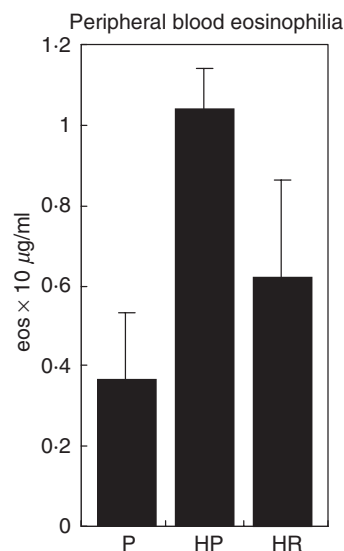


Fig. 3. Effect of treatment with four doses of PBS (HP), 10 μ g rfhSP-D (HR) or BSA (HB) on peripheral blood eosinophilia in allergen-challenged mice. P = non-sensitized mice treated with PBS.

significant, in IL-12 measured in the lungs (Fig. 4) of allergen-challenged mice, while treatment with PBS had no measurable effect when compared to non-sensitized mice treated with PBS. The level of IL-12 measured in the spleen was reduced significantly after allergen challenge ($P < 0.005$), but was elevated to levels comparable to non-sensitized mice after treatment with rfhSP-D (Fig. 5). Treatment with rfhSP-D but not PBS resulted in a significant elevation in IFN- γ levels measured in the spleen (Fig. 6, $P < 0.05$).

Effect of treatment with rfhSP-D on airway hyperresponsiveness

In order to find a dose of methacholine that would show a maximal difference between allergic and non-sensitized mice, mice were subjected to 1 min of nebulized methacholine over a range of increasing concentrations. This indicated that a concentration of 20 or 40 mg/ml would be appropriate to elicit a strong response. Sensitized mice responded with a significantly elevated Penh after allergen challenge (Fig. 7). Intranasal treatment of allergen-challenged sensitized mice with rfhSP-D at a concentration of 10 $\mu\text{g}/\text{ml}$ for 4 days resulted in a substantial and significant ($P < 0.001$) reduction in Penh when compared to treatment with PBS.

These mice were re-challenged with allergen alone given 8 days later and the Penh response measured after provocation with 40 mg/ml methacholine (Fig. 8). The rfhSP-D treated mice responded with 30% less airway hyperresponsiveness compared to mice treated with PBS, although the difference was not statistically significant.

Endogenous mouse SP-D and SP-A

Endogenous levels of SP-D measured in the BAL of allergic mice were elevated approximately 10-fold from a level of $0.53 \pm 0.43 \mu\text{g}/\text{ml}$ in BAL from normal mice treated with saline to $4.91 \pm 0.65 \mu\text{g}/\text{ml}$ in sensitized mice after challenge with mite allergen. Endogenous SP-A levels were $1.68 \pm 0.41 \mu\text{g}/\text{ml}$ in normal mice and were increased two- to threefold in sensitized mice

after allergen challenge. Treatment with five daily doses of 10 $\mu\text{g}/\text{ml}$ rfhSP-D (or control) did not produce any change in the level of endogenous SP-D or SP-A levels.

Lung histology

H&E-stained lung sections were assessed independently for peribronchial inflammation using a scoring system as previously described [16] and overall showed a reduction in cellular inflammation in the *D. pteronyssinus*-sensitized and allergen-challenged

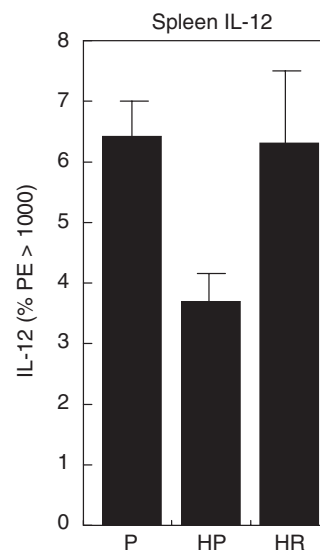


Fig. 5. IL-12 measured in the spleens taken 1 day after allergen re-challenge given 4 days after completion of treatment of allergen-challenged mice with four daily doses of PBS (HP) or 10 μg rfhSP-D (HR). P = non-sensitized mice treated with PBS.

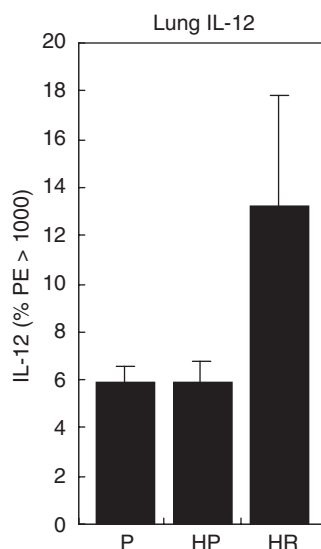


Fig. 4. IL-12 measured in the lungs 1 day after allergen re-challenge given 4 days after completion of treatment of allergen-challenged mice with four daily doses of PBS (HP) or 10 μg rfhSP-D (HR). P = non-sensitized mice treated with PBS.

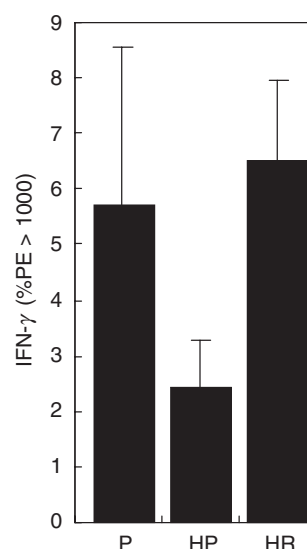


Fig. 6. IFN- γ measured in the spleens taken 1 day after allergen re-challenge given 4 days after completion of treatment of allergen-challenged mice with four daily doses of PBS (HP) or 10 μg rfhSP-D (HR). P = non-sensitized mice treated with PBS.

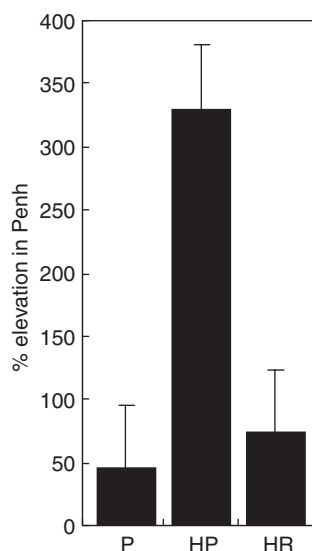


Fig. 7. Comparison of Penh response in groups challenged with 20 mg/ml methacholine after 4 days of treatment. P = non-sensitized mice treated with PBS. HP = allergen-challenged mice treated with PBS, HR = allergen-challenged mice treated with 10 µg rfhSP-D ($n = 4-8$ /group).

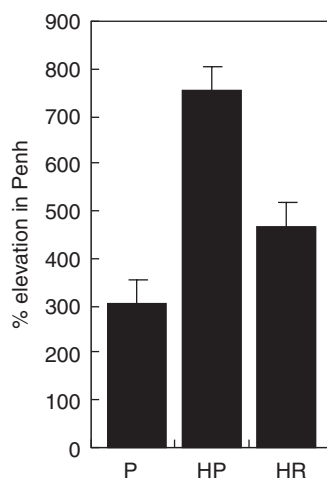


Fig. 8. Comparison of Penh response in groups challenged with 40 mg/ml methacholine on re-challenge with allergen given 4 days after the end of treatment. P = non-sensitized, treated with PBS. HP = allergen-challenged mice treated with PBS, HR = allergen-challenged mice treated with 10 µg rfhSP-D ($n = 4-8$ /group).

lung after treatment with rfhSP-D, but not after treatment with either PBS or BSA. Of the sensitized mice in this study six PBS-treated mice had scores of 2 or more, whereas four of the five mice in the rfhSP-D treatment group had lower scores than controls. The score for non-sensitized mice was 0.

DISCUSSION

D. pteronyssinus is the most abundant species of house dust mite and is a major source of allergens that contribute to allergic asthma, characterized by high serum IgE and *D. pteronyssinus*-

specific IgG1, peripheral and lung eosinophilia and increased airway hyperresponsiveness. We have previously shown the effectiveness of a recombinant fragment of human SP-D in reducing these parameters in mice sensitized to fungal allergens [16,17], and the present study was carried out to test its effects in a mouse model of house dust mite allergy. The possibility that SP-A and SP-D may modulate airway responses to house dust mite allergens was first raised by Wang, who identified an interaction between the proteins and *D. pteronyssinus* extracts [14]. In a subsequent study he investigated the effect of native hSP-A, hSP-D and rfhSP-D on PHA and *D. pteronyssinus* extract-induced histamine release and proliferation of peripheral blood mononuclear cells (PBMC) obtained from asthmatic children [20]. Incubation with hSP-A, hSP-D and rfhSP-D reduced proliferation in both non-asthmatic age-matched control and stable asthmatic PBMCs by approximately 50%. The rfhSP-D showed a higher potency than either hSP-A or hSP-D and gave a 77.5% inhibition after only 6 h incubation at equivalent concentrations. The inhibitory effect was abolished by maltose confirming the involvement of the CRD in a carbohydrate-mediated interaction. It was suggested further that the antiproliferative effect is the result of a direct interaction between rfhSP-D and the CR3 integrin as incubation with rfhSP-D reduced FITC-anti-CD11b staining in PBMCs.

The results reported in the current study show that rfhSP-D is effective at reducing serum IgE, *D. pteronyssinus*-specific IgG1 and peripheral blood eosinophilia in a model in which allergic mice were challenged daily with allergen before treatment. This is a more severe test of the efficacy of rfhSP-D than in our previous model using *Afu* allergens [16], and mirrors a treatment scenario in which a patient might wish to take rfhSP-D therapeutically to reduce the symptoms of perennial allergy or allergic asthma to continuous exposure to house dust mite. Similarly, the effect of treatment with rfhSP-D on lung function was assessed by whole body plethysmography performed after a course of allergen challenge followed by treatment. There was a dramatic reduction in airway hyperresponsiveness, indicating a significant improvement in lung status, consistent with the biochemical, cellular and histological parameters. This effect lasted into the second week, with decreased AHR following re-challenge with allergen up to 8 days later (Fig. 8).

To investigate possible mechanisms, intracellular cytokines were assayed. There was an increase in IL-12 response, even after allergen challenge. IL-12 is a heterodimeric cytokine that is produced by macrophages and antigen-presenting cells and plays an important role in the regulation of both innate and adaptive immune responses. IL-12 stimulates the proliferation of Th1 lymphocytes while inhibiting proliferation of Th2 lymphocytes. Several studies have shown that IL-12 inhibits airway hyperresponsiveness [21,22], consistent with the present findings. IL-12 stimulates the production of IFN- γ by Th1 lymphocytes and natural killer cells and the present study also shows an increase in IFN- γ after rfhSP-D treatment. The two cytokines have a synergistic effect on reducing allergic hypersensitivity and reducing Th2 cytokines, including IL-4 [23]. IL-4 acts upon B cells, promoting class switching to IgG1 in mice (IgG4 in humans) and IgE [23,24] and IL-4 is directly implicated in the immunopathogenesis of asthma [25-27] and the development of airway hyperresponsiveness [28].

Consistent with the role of IL-12 in redirecting the immune response to Th1, it exerts a powerful regulatory effect on the

humoral immune response by promoting IgG2a, IgG2b and IgG3 by reducing IgG1 [29] and also suppressing IL-4-induced IgE production [30]. This effect is enhanced further by the up-regulation of IFN- γ by IL-12, which also suppresses IgE production. Kiniwa showed that administration of as little as 1 μ g/day of recombinant IL-12 inhibited *D. pteronyssinus*-specific IgE production by 88% and IgG1 production by 93% [30]. Other studies have demonstrated clearly a link between IgE and airway hyperresponsiveness in asthmatic patients [31].

The reduction in peripheral blood eosinophilia observed in the present study is consistent with an up-regulation of IL-12 and IFN- γ . Sur *et al.* showed that administration of IL-12 decreased allergen-induced lung eosinophilia in a murine model of ragweed allergy [32]. Similar effects were found when as little as 0.1 μ g/day was given to *D. pteronyssinus*-sensitized mice [8]. Treatment with IL-12 resulted in an increase in IFN- γ and a decrease in IL-5 in splenocytes from allergic mice after stimulation with *D. pteronyssinus* extract. Additionally, IL-12 up-regulation correlates with a reduction in airway hyperresponsiveness [19,33].

While these cytokine changes are consistent with the reduction in allergic responses in treated mice, the precise mechanism by which rfhSP-D produces these effects on immune cells remains to be established. The up-regulation of IL-12 suggests an interaction with macrophages and dendritic cells, which are the sole producers of this cytokine. SP-D is known to bind directly to alveolar macrophages [34,35]. An interaction of SP-D with dendritic cells and involvement of SP-D in antigen presentation has been demonstrated recently [36]. SP-D bound preferentially to immature rather than mature dendritic cells in a calcium-dependent carbohydrate-mediated manner and it was shown that this interaction enhanced antigen presentation. Recent studies from our group have shown an interaction between rfhSP-D and dendritic cells. The recombinant fragment binds preferentially to immature dendritic cells and interestingly, the expression of gp-340, a putative receptor for SP-D, was higher on immature rather than mature dendritic cells [37]. We have also shown recently that rfhSP-D reduces lung inflammation by promoting the clearance of apoptotic alveolar macrophages in the lung [38] and the possibility that this mechanism is also involved in promoting removal of apoptotic eosinophils in the allergic lung is intriguing, and merits further investigation. The obvious question of why the addition of exogenous rfhSP-D should produce an effect over and above that resulting from endogenous SP-D is puzzling. However, it has been observed by our group and others that SP-D levels are markedly up-regulated in the lung during *A. fumigatus*-induced allergic inflammation [16,39] and it is possible that the addition of exogenous SP-D augments this protective effect. Treatment with rfhSP-D did not increase endogenous SP-D or SP-A expression further but exogenous rfhSP-D provides an additional pool detectable in lung lavage after intranasal administration [16]. The current study is consistent in showing up-regulation of SP-A and SP-D in BAL fluid after challenge with house dust mite allergen in sensitized mice. Again the SP-D response was more dramatic than that of SP-A, increasing 10-fold compared to 2-fold increases in SP-A levels. At first sight these results appear to conflict with those of Wang *et al.*, who reported decreased levels of SP-A and SP-D in a murine model of asthma [40]. This is not likely to be due to the different strain of mice employed in the studies (BALB/c versus C57Bl/6 used in this study), as it has been shown that SP-A and SP-D were consistently up-regulated in both BALB/c and C57Bl/6 strains in a murine model of asthma after ovalbumin challenge

[13] and after challenge with *Afu* allergens [16,39]. Similarly, SP-D expression was markedly up-regulated in a rat model of ovalbumin sensitivity [41]. In Wang's study collectin levels were, however, increased at 24 h after challenge with house dust mite allergens and immunohistochemistry showed increased expression of collectins in areas of eosinophilic or monocytic infiltration for up to 3 days after challenge [40]. These findings are consistent with other studies reported and with the current study, in which collectin levels were assessed within 6 h of an intranasal allergen challenge. In clinical studies, both low levels of SP-A [42] and high collectin levels have been found in asthma sufferers [43]. An explanation for this variability may be that measured levels of collectins in the airways of patients are also related to the timing of sampling in relation to allergen challenge.

In conclusion, we have demonstrated that there are increased levels of both SP-A and SP-D after challenge with allergens of *D. pteronyssinus* during allergen-induced lung inflammation in mice. Treatment with a recombinant fragment of human SP-D down-regulated airway hyperresponsiveness, serum eosinophilia, *D. pteronyssinus*-specific IgG1 and total serum IgE after allergen challenge and was accompanied by increased expression of IL-12 and IFN- γ . The results provide further support for the developing hypothesis that the lung collectins, especially lung surfactant protein D, have an important protective role in the response of the lung to allergens. The efficacy of a recombinant fragment of human SP-D in down-regulating allergic responses in this murine model and in our previous work using a model of allergic hypersensitivity to fungal allergens suggests that recombinant collectins may have therapeutic potential in modulating pulmonary allergic responses.

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