Non-Proteolytic Aeroallergens from Mites, Cat and Dog Exert Adjuvant-Like Activation of Bronchial Epithelial Cells

Camilla Österlund^{a, b} Hans Grönlund^c Guro Gafvelin^c Anders Bucht^{a, b}

^aDepartment of Public Health and Clinical Medicine, Respiratory Medicine, Umeå University, and

^bSwedish Defence Research Agency, FOI CBRN Defence and Security, Umeå, and ^cDepartment of Medicine, Clinical Immunology and Allergy Unit, Karolinska Institute, Stockholm, Sweden

Key Words

Der p 2 • Der f 2 • Eur m 2 • Lep d 2 • Fel d 1 • Can f 2 •
Non-proteolytic • Epithelial cells

Abstract

Background: Exposure to seasonal or indoor allergens may cause sensitisation and development of allergic airway diseases. We have previously demonstrated that the non-proteolytic major house dust mite (HDM) allergen Der p 2 stimulates pro-inflammatory responses in bronchial epithelial cells. We aimed to determine if other clinically relevant non-proteolytic aeroallergens originating from HDMs, storage mites, cat, dog, birch and timothy also activate respiratory epithelial cells. **Methods:** Cultures of human bronchial epithelial cell line BEAS-2B, normal human bronchial epithelial cells and alveolar epithelial cell line A549 were exposed to recombinant (r)Der p 2, natural (n)Der f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1, rCan f 2, rBet v 1 or rPhl p 5a. A panel of secreted mediators and expression of cell adhesion receptors involved in recruitment, survival and adhesion of inflammatory cells in asthmatic airways was assessed. **Results:** The mite allergens rDer p 2, nDer f 2, rEur m 2 and rLep d 2 as well as the cat and dog allergens rFel d 1, nFel d 1 and rCan f 2

induced granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor, interleukin (IL)-6, IL-8, monocyte-chemotactic protein-1 and macrophage inflammatory protein-3 α secretion from bronchial epithelial cells as well as surface expression of intracellular adhesion molecule-1. The pollen allergens rBet v 1 and rPhl p 5a from birch and timothy did not activate the cells. None of the studied allergens affected the alveolar epithelial cells. **Conclusion:** These results show that both mite and structurally unrelated cat and dog allergens can activate respiratory epithelial cells by adjuvant-like protease-independent mechanisms.

Copyright © 2010 S. Karger AG, Basel

Introduction

Exposure to aeroallergens poses a major risk for sensitisation and development of perennial rhinitis and allergic asthma and can be caused by either intermittent or continued allergen exposure [1, 2]. Plant pollen is a major source of outdoor seasonal allergens, and exposure to these represents the former [3], while exposure to indoor allergens derived from house dust mites (HDMs), for ex-

ample, represent the latter [1]. There are great geographical variations, but in many parts of the world different species of HDMs, like *Dermatophagoides pteronyssinus*, *D. farinae* and *Euroglyphus maynei*, are among the most common sources of indoor aeroallergens [1, 4]. Allergens from storage mites like *Lepidoglyphus destructor*, known to cause occupational allergy, are also found in house dust, and sensitisation is especially high in regions with damp housing conditions [5–8]. Pets, such as cats and dogs, are another significant source of indoor allergens associated with continued exposure [1, 9, 10].

Specific recognition of an allergen by immunocompetent cells may induce hypersensitivity reactions. In addition, direct interaction of an allergen with the respiratory epithelium resulting in a local inflammatory response may affect the outcome of exposure and contribute to the pathogenesis of airway disease. Proteolytic allergens from HDMs are known to activate inflammatory responses in respiratory epithelial cells [11–13]. Several major aeroallergens, however, lack enzyme activity and there is limited knowledge regarding their potential effect on the respiratory epithelium. We have recently demonstrated that Der p 2, a major non-proteolytic allergen of *D. pteronyssinus*, activates bronchial epithelial cells and provokes secretion of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-6, IL-8, monocyte-chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-3 α and soluble intracellular adhesion molecule (sICAM)-1 via mechanisms dependent on nuclear factor (NF)- κ B and mitogen-activated protein kinases (MAPKs) [14]. Whether other non-proteolytic allergens have similar intrinsic adjuvant activity is not known.

In this study, we examined if the group 2 mite allergens Der f 2, Eur m 2 and Lep d 2 can induce pro-inflammatory mediator release from bronchial epithelial cell line BEAS-2B, normal human bronchial epithelial (NHBE) cells and alveolar epithelial cell line A549. These allergens share approximately 87, 84 and 52% amino acid sequence identity with Der p 2, respectively [15, 16]. To further elucidate the structure-activity relationship of non-proteolytic allergen interactions with epithelial cells, we also investigated if the structurally dissimilar major allergens Fel d 1 from cat (*Felis domesticus*), Can f 2 from dog (*Canis familiaris*), Bet v 1 from birch (*Betula verrucosa*) and Phl p 5a from timothy (*Phleum pratense*) can induce similar responses. We employed the Bio-Plex multiple cytokine array in order to cover a broad repertoire of effector functions of lung epithelial cells to detect possible differences in the response to the different allergens.

Materials and Methods

Allergens

Recombinant (r)Der p 2, rLep d 2, rFel d 1 and rCan f 2 were expressed in *Escherichia coli* as described previously [17–20]. rEur m 2, affinity-purified natural (n)Der f 2, LoTox™ rBet v 1, LoTox rPhl p 5a™ and LoTox nFel d 1 were purchased from Indoor Biotechnologies Ltd. (Warminster, UK). The endotoxin content of the allergen preparations, determined using Limulus Amebocyte Lysate Endochrome assay (Charles River Endosafe, Charleston, S.C., USA), were 176 ng/mg (rDer p 2), 89.1 ng/mg (nDer f 2), 12.7 ng/mg (rEur m 2), 31 ng/mg (rLep d 2), 4.5 ng/mg (rFel d 1), 17 ng/mg (rCan f 2) and <0.5 ng/mg for the LoTox allergens.

Cell Cultures and Stimulation

The human bronchial epithelial cell line BEAS-2B, transformed with an adenovirus 12-SV40 virus hybrid (ATCC CRL-9609; American Type Culture Collection, Manassas, Va., USA) and NHBE cells (Clonetics, San Diego, Calif., USA) were grown in serum-free bronchial epithelial cell basal medium with supplements (complete medium) (BEGM; Cambrex, Verviers, Belgium) in tissue culture flasks coated with bovine plasma fibronectin, collagen and bovine serum albumin. The human type II alveolar epithelial cell line A549 (ATCC CCL-185) was cultured in RPMI 1640 medium (Gibco BRL, Paisley, UK) supplemented with 10% (v/v) fetal calf serum (HyClone; Perbio Science, Aalst, Belgium) and 50 μ g/ml gentamicin. All cells were maintained at 37°C in humidified atmosphere with 5% CO₂. For experiments cells were seeded in 24-well culture plates at 4×10^4 cells/well and allowed to attach overnight and then stimulated with the different allergens (10, 40 or 100 μ g/ml) for 24 h. All stimulations for the respective cell line were performed in complete medium.

Analysis of Released Mediators by ELISA

Concentrations of GM-CSF, IL-6, IL-8, MCP-1 and MIP-3 α in cell-free supernatants were measured in duplicates using DuoSet ELISA kits (R&D Systems, Abingdon, UK) according to the manufacturer's instructions.

Flow-Cytometric Analysis of ICAM-1 Expression

Cells were detached and surface expression of ICAM-1 was analysed by flow cytometry as previously described [21].

Bio-Plex Array

Concentrations of IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70), IL-13, IL-17, eotaxin, granulocyte colony-stimulating factor (G-CSF), GM-CSF, interferon (IFN)- γ , IP-10, MCP-1, MIP-1 α and tumor necrosis factor- α in cell-free supernatants were determined in singles using Bio-Plex assays and Luminex 100 suspension array system (Bio-Rad Laboratories, Hercules, Calif., USA) according to the manufacturer's instructions.

Statistical Analysis

Results are expressed as means $\pm$ standard deviation. Data were analysed by one-way-analysis of variance with Dunnett's post hoc test. Data were considered significant at $p < 0.05$. Calculations were performed using SPSS (SPSS Inc., Chicago, Ill., USA).

Results

Secretion of Inflammatory Mediators from BEAS-2B Cells by Non-Proteolytic Allergens from HDMs, Storage Mite, Cat and Dog, but Not Birch and Timothy

To determine if the allergens can induce a pro-inflammatory response in respiratory epithelial cells, secretion of mediators in 24 h cell culture supernatants from bronchial BEAS-2B and alveolar A549 cells were initially analysed by ELISA. After exposure with rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 or rCan f 2 levels of secreted GM-CSF, IL-6, IL-8, MCP-1 and MIP-3 α from BEAS-2B cells were increased, whereas rBet v 1 and rPhl p 5a did not affect the cells, not even after exposure at a high concentration of 100 μ g/ml (fig. 1). A549 cells, on the other hand, were not affected by any of the allergens (data not shown) and, therefore, this cell line was not used in subsequent experiments.

Induced Expression of ICAM-1 on BEAS-2B Cells by Non-Proteolytic Allergens from HDMs, Storage Mite, Cat and Dog, but Not Birch and Timothy

The effect of the allergens on surface expression of the adhesion molecule ICAM-1 on BEAS-2B cells was studied by flow cytometry after allergen exposure for 24 h. Untreated BEAS-2B cells constitutively express ICAM-1, but the median fluorescence intensity value was up-regulated by rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 and rCan f 2 (fig. 2). Neither rBet v 1 nor rPhl p 5a did induce an up-regulation (fig. 2), and since they did not seem to have any impact on the respiratory epithelial cells, they were excluded from subsequent experiments.

Comprehensive Analysis of Inflammatory Mediators Secreted from Bronchial Epithelial Cells by Non-Proteolytic Allergens from HDMs, Storage Mite, Cat and Dog

To further study the effect of the HDM, storage mites, cat and dog allergens and to detect differences in the responses, Bio-Plex assays were used to simultaneously determine the secreted amounts of 19 cytokines and chemokines in 24-hour cell culture supernatants. As previously detected with ELISA, increased secretion of IL-6, IL-8 and MCP-1 from BEAS-2B cells following exposure to rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 and rCan f 2 was also observed when analysed with the Bio-Plex system (fig. 3), whereas the GM-CSF secretion could not be confirmed. In addition, a significant increase in G-CSF secretion was observed after exposure to all of these allergens, while an increased secretion of oth-

er mediators included in the Bio-Plex array was consistently not observed.

The responses seen in the BEAS-2B cell line were next verified in primary human bronchial epithelial cells. Analysis by the Bio-Plex system demonstrated that the HDM, storage mites, cat and dog allergens induced up-regulated secretion of G-CSF and IL-6 from NHBE cells (fig. 4). IL-8 secretion was significantly increased after exposure for rFel d 1 and all mite allergens except rEur m 2. The levels of MCP-1 did not increase over the background level when stimulated with any of the allergens. Consistent with previous studies by us, background expression by unstimulated NHBE cells was generally higher than baseline expression by BEAS-2B cells [14, 22].

Discussion

We have previously demonstrated that the major HDM allergen Der p 2 of *D. pteronyssinus* can induce secretion of GM-CSF, IL-6, IL-8, MCP-1, MIP-3 α and sICAM-1 from bronchial epithelial cells, indicating that the respiratory epithelium may contribute to the pathogenesis of allergic asthma by mounting a pro-inflammatory response following direct interaction with aeroallergens [14]. Secretion of these cytokines suggests that the epithelial cells upon exposure to non-proteolytic allergens can contribute to the recruitment of neutrophils and eosinophils to the airways, enhanced co-stimulation of allergen-specific immune responses and remodelling of the airway epithelium. In the present study, we examined if other major non-proteolytic aeroallergens from HDM, storage mites, cat, dog, birch and timothy also induce similar responses in respiratory epithelial cells. Sensitisation to indoor allergens is strongly associated with asthma, whereas exclusive sensitisation to seasonal outdoor allergens is primarily associated with rhinitis. Hatzivlas-siou et al. [23] have recently shown that bronchial provocation with HDM or grass pollen extracts in asthmatic patients results in similar early asthmatic reaction with a decline in lung function, whereas the late asthmatic response was associated with HDM, but not grass pollen, provocation. We used the immortalised lung epithelial cell lines BEAS-2B and A549 to screen which of the allergens may induce a pro-inflammatory response following exposure, and thereafter we verified the findings in primary NHBE cells. We used the Bio-Plex cytokine array in order to simultaneously detect the levels of 19 mediators and cover a broad range of possible effector functions of lung epithelial cells.

Fig. 1. Release of inflammatory mediators from BEAS-2B cells following exposure to non-proteolytic allergens from HDMs and storage mite as well as from cat and dog dander. Cells were incubated with medium alone (white) or with 10 (light grey) and 40 $\mu\text{g/ml}$ (dark grey) rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 and rCan f 2 or with 10 and 100 $\mu\text{g/ml}$ (black) of rBet v1 and rPhl p 5a. The level of GM-CSF, IL-6, IL-8, MCP-1 and MIP-3 α (pg/ 10^4 cells) in 24-hour supernatants was assessed by ELISA. Results (means $\pm$ SD, $n \geq 3$) from 1 representative experiment of a minimum of 3 independent experiments are shown. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared with untreated cells.

Fig. 2. Expression of ICAM-1 on BEAS-2B cells following exposure to non-proteolytic allergens from HDMs and storage mite as well as from cat and dog dander. The expression of ICAM-1 (median fluorescence intensity, MFI, values) on the surface of BEAS-2B cells following a 24-hour incubation with medium alone (white) or with 10 (light grey) and 40 $\mu\text{g/ml}$ (dark grey) rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 and rCan f 2 or with 10 and 100 $\mu\text{g/ml}$ (black) of rBet v 1 and rPhl p 5a was assessed by flow cytometry. Results (means $\pm$ SD, $n \geq 3$) from 1 representative experiment of a minimum of 3 independent experiments are shown. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared with untreated cells.

Fig. 3. Release of inflammatory mediators from BEAS-2B cells following exposure to non-proteolytic allergens from HDMs and storage mite as well as from cat and dog dander. Cells were incubated with medium alone (white) or with 10 (grey) and 40 $\mu\text{g/ml}$ (black) rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 and rCan f 2. The level of mediators (pg/ 10^4 cells) in 24-hour superna-

nants was assessed using the Bio-Plex array. Results (means $\pm$ SD, $n \geq 4$) from 1 representative experiment of a minimum of 2 independent experiments are shown. Only cytokines and chemokines with a significant increased secretion are included in the figure. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$, compared with untreated cells.

Fig. 4. Release of inflammatory mediators from NHBE cells following exposure to non-proteolytic allergens from HDMs and storage mite as well as from cat and dog dander. Cells were incubated with medium alone (white) or with 10 (grey) and 40 µg/ml (black) rDer p 2, nDer f 2, rEur m 2, rLep d 2, rFel d 1, nFel d 1 and rCan f 2. The level of mediators (pg/10⁴ cells) in 24-hour supernatants was assessed using the Bio-Plex array. Results (means ± SD, n ≥ 4) from 1 representative experiment of a minimum of 2 independent experiments are shown. Only cytokines and chemokines with a significantly increased secretion are included in the figure. * p < 0.05; ** p < 0.01; *** p < 0.001, compared with untreated cells.

We demonstrated that group 2 mite allergens from the HDMs *D. farinae* and *E. maynei* as well as from the storage mite *L. destructor*, that is Der f 2, Eur m 2 and Lep d 2, respectively, induce increased secretion of G-CSF, GM-CSF, IL-6, IL-8, MCP-1 and MIP-3α, as well as surface expression of ICAM-1 in bronchial epithelial cells. These allergens are all structurally related with Der p 2 inducing a response similar to that of Der p 2. We also observed

that rCan f 2 from dog activates bronchial cells in a similar manner. Can f 2 is a member of the lipocalin protein family [10], to which several major animal allergens, especially from mammals like dog, mouse, rat, horse and cow belong [24]. The most potent allergen of cat dander, Fel d 1, is, however, an important exception, as it is an uteroglobin-like protein [25, 26]. Both natural and recombinant Fel d 1 also induced secretion of inflamma-

tory mediators from bronchial cells. The seasonal pollen allergens rBet v 1 from birch and rPhl p 5a from timothy, on the other hand, failed to activate both alveolar and bronchial cells, even at a very high concentration of 100 µg/ml. The structural properties that determine the inability of rBet v 1 and rPhl 5a remain to be defined. Both birch and grass extracts containing a mixture of different pollen allergens have been shown to induce secretion of pro-inflammatory cytokines from nasal and respiratory epithelial cells [27, 28], but in those studies the distinct allergens causing the effects were not identified.

Release of G-CSF, GM-CSF, IL-6, IL-8, MCP-1 and MIP-3α as well as expression of ICAM-1 by the bronchial epithelial cells upon allergen exposure indicate that the cells may potentiate inflammatory and allergic reactions by enhancing maturation, recruitment, activation and survival of different cells of the immune system, like eosinophils, neutrophils and dendritic cells [29–33]. The expression of these inflammatory mediators in concert plays an important role in the aggravation of allergic asthma to the severe acute form.

The results in this study confirm our previously reported data on Der p 2-induced activation of bronchial epithelial cells [14] and extend this intrinsic adjuvant-like property to other non-proteolytic mite allergens. Since Der f 2, Eur m 2 and Lep d 2 are structurally related with Der p 2 and therefore may activate cells via similar mechanisms, it was not unexpected that also these allergens activate bronchial epithelial cells. It has recently been reported that Der p 2 and possibly also other mite allergens exert adjuvant-like properties by activation of Toll-like receptor (TLR)-4 through functional mimicry of the TLR4 signalling complex protein MD-2 [34, 35]. On the other hand, Der p 2 has also been shown to activate airway smooth muscle by mechanisms involving TLR2 and MyD88 [36]. Neither the mite allergens, nor the cat, dog or pollen allergens affected the A549 cells, supporting the notion that A549 cells of unknown reasons might be generally unresponsive to non-proteolytic respiratory allergens.

Immortalised cell lines differ from primary cells cultures, which likely affect the responsiveness to allergen exposure. The observed differences in magnitude between the responses mounted by BEAS-2B as compared with NHBE cells might thus be explained by the immortalised phenotype of BEAS-2B. This is also consistent with results from previous studies by us [14, 22]. We observed some differences in responsiveness to recombinant and natural Fel d 1. In BEAS-2B cells, the magnitude of the response was consistently higher with the natural

allergen, although it should be noted that both allergens had a significant effect yielding a similar pattern of secreted mediators. In NHBE cells, the nFel d 1 induced a significant increase in only G-CSF, whereas the rFel d 1 induced both secretion of G-CSF as well as a statistically significant production of IL-6 and IL-8. Recombinant Fel d 1 differs from the natural allergen only in the linking of the two polypeptides via a peptide bond, the presence of a 6 His-tag and the lack of glycosylation [19]. It has been previously reported that these differences do not influence protein folding [26] or immunoreactivity [19], but it cannot be excluded that small differences in dimerisation between the recombinant and natural protein may influence the pro-inflammatory activity as previously reported by Kaiser et al. [37]. Furthermore, binding of natural ligands may influence biological activity of allergens.

The allergen preparations contain small amounts of lipopolysaccharide (LPS), which in theory might be able to activate respiratory epithelial cells. In our previous study with rDer p 2 [14], we therefore conducted extensive control experiments to rule out that the observed effect was due to LPS contamination in the allergen preparation. The BEAS-2B cells were not responsive to LPS at concentrations present in the allergen preparation and a relatively high dose of LPS was needed to achieve a cell response above background level. In addition, co-exposure of cells with rDer p 2 and polymyxin B, an LPS inhibitor, did not inhibit the activating effect of the allergen. Due to the small amount of LPS in the allergen preparations used in the present study and the lack of correlation between effects and endotoxin levels, it is highly unlikely that the responses observed with the allergens were caused by contaminating LPS. Moreover, natural Fel d 1 induced significant activation of cells, despite containing less than 0.5 ng LPS/mg allergen.

To our knowledge, this is the first study demonstrating direct activation of bronchial epithelial cells by dog (Can f 2) and cat (Fel d 1) allergens associated with asthmatic responses. These allergens are not structurally similar to the group 2 mite allergens, hence representing two additional structures capable of direct interaction with respiratory epithelial cells. This adjuvant-like effect may contribute to several aspects of asthma pathogenesis, such as recruitment of neutrophils and eosinophils to the airways, enhanced co-stimulation of allergen-specific immune responses and remodelling of the airway epithelium.

References

- Platts-Mills T, Vervloet D, Thomas W, Aalberse R, Chapman M: Indoor allergens and asthma: report of the third international workshop. *J Allergy Clin Immunol* 1997; 100:S2–S24.
- Custovic A, Taggart S, Francis H, Chapman M, Woodcock A: Exposure to house dust mite allergens and the clinical activity of asthma. *J Allergy Clin Immunol* 1996;98:64–72.
- D'Amato G, Cecchi L, Bonini S, Nunes C, Annesi-Maesano I, Behrendt H, Liccardi G, Popov T, van Cauwenberge P: Allergenic pollen and pollen allergy in Europe. *Allergy* 2007;62:976–990.
- Zock J, Heinrich J, Jarvis D, Verlato G, Norbäck D, Plana E, Sunyer J, Chinn S, Olivieri M, Soon A, Villani S, Ponzio M, Dahlman-Hoglund A, Svanes C, Luczynska C: Distribution and determinants of house dust mite allergens in europe: the European Community Respiratory Health Survey II. *J Allergy Clin Immunol* 2006;118:682–690.
- Macan J, Kanceljak B, Plavec D, Milković-Kraus S: Differences in mite fauna between the continental and Mediterranean climates of Croatia: microscopy and dustscreen test findings. *Allergy* 2003;58:780–783.
- Vidal C, Boquete O, Gude F, Rey J, Meijide L, Fernández-Merino M, González-Quintela A: High prevalence of storage mite sensitization in a general adult population. *Allergy* 2004;59:401–405.
- Boquete M, Iraola V, Fernández-Caldas E, Arenas Villaroel L, Carballada F, González de la Cuesta C, López-Rico M, Núñez Orjales R, Parra G, Soto-Mera M, Varela S, Vidal C: House dust mite species and allergen levels in Galicia, Spain: a cross-sectional, multicenter, comparative study. *J Invest Allergol Clin Immunol* 2006;16:169–176.
- Iversen M, Dahl R: Allergy to storage mites in asthmatic patients and its relation to damp housing conditions. *Allergy* 1990;45:81–85.
- Roost H, Künzli N, Schindler C, Jarvis D, Chinn S, Perruchoud A, Ackermann-Liebrich U, Burney P, Wüthrich B: Role of current and childhood exposure to cat and atopic sensitization. European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1999;104:941–947.
- Konieczny A, Morgenstern J, Bizinkauskas C, Lilley C, Brauer A, Bond J, Aalberse R, Wallner B, Kasaian M: The major dog allergens, Can f 1 and Can f 2, are salivary lipocalin proteins: cloning and immunological characterization of the recombinant forms. *Immunology* 1997;92:577–586.
- Mori L, Kleimberg J, Mancini C, Bellini A, Marini M, Mattoli S: Bronchial epithelial cells of atopic patients with asthma lack the ability to inactivate allergens. *Biochem Biophys Res Commun* 1995;217:817–824.
- King C, Brennan S, Thompson P, Stewart G: Dust mite proteolytic allergens induce cytokine release from cultured airway epithelium. *J Immunol* 1998;161:3645–3651.
- Sun G, Stacey M, Schmidt M, Mori L, Mattoli S: Interaction of mite allergens Der p3 and Der p9 with protease-activated receptor-2 expressed by lung epithelial cells. *J Immunol* 2001;167:1014–1021.
- Österlund C, Grönlund H, Polovic N, Sundström S, Gafvelin G, Bucht A: The non-proteolytic house dust mite allergen Der p 2 induce NF- κ B and MAPK dependent activation of bronchial epithelial cells. *Clin Exp Allergy* 2009;39:1199–1208.
- Smith W, Mills K, Hazell L, Hart B, Thomas W: Molecular analysis of the group 1 and 2 allergens from the house dust mite, *Euroglyphus maynei*. *Int Arch Allergy Immunol* 1999;118:15–22.
- Schmidt M, Olsson S, van der Ploeg I, van Hage-Hamsten M: cDNA analysis of the mite allergen Lep d 1 identifies two different isoallergens and variants. *FEBS Lett* 1995; 370:11–14.
- Johansson L, Chen C, Thorell J, Fredriksson A, Stone-Elander S, Gafvelin G, Arnér E: Exploiting the 21st amino acid-purifying and labeling proteins by selenolate targeting. *Nat Methods* 2004;1:61–66.
- Olsson S, van Hage-Hamsten M, Whitley P, Johansson E, Hoffman D, Gafvelin G, Schmidt M: Expression of two isoforms of Lep d 2, the major allergen of lepidoglyphus destructor, in both prokaryotic and eukaryotic systems. *Clin Exp Allergy* 1998;28:984–991.
- Grönlund H, Bergman T, Sandström K, Alvelius G, Reininger R, Verdino P, Hauswirth A, Liderot K, Valent P, Spitzauer S, Keller W, Valenta R, van Hage-Hamsten M: Formation of disulfide bonds and homodimers of the major cat allergen Fel d 1 equivalent to the natural allergen by expression in *Escherichia coli*. *J Biol Chem* 2003;278: 40144–40151.
- Madhurantakam C, Nilsson O, Jönsson K, Grönlund H, Achour A: Production, crystallization and preliminary x-ray diffraction analysis of the allergen Can f 2 from *Canis familiaris*. *Acta Crystallogr Sect F Struct Biol Cryst Commun* 2009;65:467–471.
- Österlund C, Lilliehöök B, Ekstrand-Hammarström B, Sandström T, Bucht A: The nitrogen mustard melphalan activates mitogen-activated phosphorylated kinases (MAPK), nuclear factor- κ B and inflammatory response in lung epithelial cells. *J Appl Toxicol* 2005;25:328–337.
- Ekstrand-Hammarström B, Österlund C, Lilliehöök B, Bucht A: Vitamin E downmodulates mitogen-activated protein kinases, nuclear factor- κ B and inflammatory responses in lung epithelial cells. *Clin Exp Immunol* 2007;147:359–369.
- Hatzivlassiou M, Grainge C, Kehagia V, Lau L, Howarth P: The allergen specificity of the late asthmatic reaction. *Allergy* 2010;65: 355–358.
- Virtanen T, Zeiler T, Mäntylä R: Important animal allergens are lipocalin proteins: why are they allergenic? *Int Arch Allergy Immunol* 1999;120:247–258.
- Morgenstern J, Griffith I, Brauer A, Rogers B, Bond J, Chapman M, Kuo M: Amino acid sequence of Fel d 1, the major allergen of the domestic cat: protein sequence analysis and cDNA cloning. *Proc Natl Acad Sci USA* 1991; 88:9690–9694.
- Kaiser L, Grönlund H, Sandalova T, Ljunggren H, van Hage-Hamsten M, Achour A, Schneider G: The crystal structure of the major cat allergen Fel d 1, a member of the secretoglobulin family. *J Biol Chem* 2003;278: 37730–37735.
- Tomee J, van Weissenbruch R, de Monchy J, Kauffman H: Interactions between inhalant allergen extracts and airway epithelial cells: effect on cytokine production and cell detachment. *J Allergy Clin Immunol* 1998;102: 75–85.
- Salib R, Lau L, Howarth P: The novel use of the human nasal epithelial cell line RPMI 2650 as an in vitro model to study the influence of allergens and cytokines on transforming growth factor- β gene expression and protein release. *Clin Exp Allergy* 2005; 35:811–819.
- Lampinen M, Carlson M, Håkansson L, Venge P: Cytokine-regulated accumulation of eosinophils in inflammatory disease. *Allergy* 2004;59:793–805.
- Conti P, DiGioacchino M: MCP-1 and RANTES are mediators of acute and chronic inflammation. *Allergy Asthma Proc* 2001;22: 133–137.
- Gangur V, Oppenheim J: Are chemokines essential or secondary participants in allergic responses? *Ann Allergy Asthma Immunol* 2000;84:569–579, quiz 579–581.
- Rose CJ, Sung S, Fu S: Significant involvement of CCL2 (MCP-1) in inflammatory disorders of the lung. *Microcirculation* 2003;10: 273–288.
- Dieu M, Vanbervliet B, Vicari A, Bridon J, Oldham E, Ait-Yahia S, Brière F, Zlotnik A, Lebecque S, Caux C: Selective recruitment of immature and mature dendritic cells by distinct chemokines expressed in different anatomic sites. *J Exp Med* 1998;188:373–386.
- Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde R, Madan R, Thorne P, Wills-Karp M, Gioannini T, Weiss J, Karp C: Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. *Nature* 2009;457:585–588.
- Hammad H, Chieppa M, Perros F, Willart M, Germain R, Lambrecht B: House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med* 2009;15:410–416.
- Chiou Y, Lin C: Der p2 activates airway smooth muscle cells in a TLR2/MyD88-dependent manner to induce an inflammatory response. *J Cell Physiol* 2009;220:311–318.
- Kaiser L, Velickovic T, Badia-Martinez D, Adedoyin J, Thunberg S, Hallén D, Berndt K, Grönlund H, Gafvelin G, van Hage M, Achour A: Structural characterization of the tetrameric form of the major cat allergen Fel d 1. *J Mol Biol* 2007;370:714–727.