The Pollen Enigma: Modulation of the Allergic Immune Response by Non-Allergenic, Pollen-Derived Compounds

Stefanie Gilles[1], Heidrun Behrendt[1], Johannes Ring[2] and Claudia Traidl-Hoffmann[1,2,*]

[1]*Center of Allergy and Environment (ZAUM), Technische Universität and Helmholtz Center, Munich, Germany;* [2]*Department of Dermatology, Technische Universität Munich, Germany*

Abstract: The question what makes an allergen an allergen puzzled generations of researchers. Pollen grains of anemophilous plants are the most important allergen carriers in ambient air, and pollinosis is a highly prevalent multi-organ disease in civilized countries. In the past, research on the allergenicity of pollen has mainly focused on elucidating genetic predisposing factors and on defining certain structural characteristics of pollen derived allergens. Recently, studies extended to the analysis of non-allergenic, adjuvant mediators co-released from pollen. Besides active proteases and oxidases, extracts of pollen contain low molecular weight molecules like pollen-associated lipid mediators or adenosine exhibiting a potential to stimulate and modulate cultured human immune cells. This article reviews our current knowledge on non-allergenic, protein and non-protein compounds from pollen and their *in vitro* and *in vivo* effects on the allergic immune response. To ultimately judge the physiological relevance of these compounds, a systematic approach will be needed comparing their releasability, content and activity in different, allergenic and non-allergenic, pollen species. System biology such as proteome and metabolome analysis will be a useful future approach to better understand pollen biology.

Keywords: PALMs, pollen, metabolome, allergy, adjuvant factors.

INTRODUCTION

Allergy to pollen, colloquially referred to as "hay fever", has always been a scourge of humanity. Besides the well known respiratory symptoms, rhino-conjunctivitis and asthma, pollen allergy can manifest itself in various other organs such as skin (atopic eczema) and gastrointestinal tract (pollen-associated food allergy) and, in some cases, even cause life-threatening systemic conditions (anaphylaxis). Today, about one out of five Europeans suffer from allergic rhinitis [1], causing tremendous health care costs and a high socio-economic burden. Concurrently, anti-allergic drugs have become a more and more important market segment.

At a first glance, the history of research on pollen allergy reads like a fulminant story of success. The term hay fever was first introduced by the British physician John Bostock who mistakenly suspected the odor of fresh-mown hay as causative agent of seasonal rhinitis in peasants. In 1869, Charles Blackley detected that indeed not hay but pollen was the culprit. In 1921, Charles Prausnitz & Heinz Kuestner demonstrated, in a heroic self-experiment, that a serum factor was responsible for the cutaneous reaction to allergens. A few years later, this ominous serum factor, termed *atopic reagin*, was identified as a member of the immunoglobulin family [2]. With the identification of IgE molecule in 1966 [3], the puzzle of the allergic immune response seemed solved at last. According to the paradigm, allergens induce the differentiation of IL-4-secreting T helper type 2 (Th2) cells, which trigger the synthesis of IgE in B cells. IgE enters the circulation and can bind, with its Fc-tail, to the high-affinity receptor FcεRI on tissue-resident mast cells. When allergen binds to at least two different IgE molecules this will lead to crosslinking of FcεRI. This triggers the release of vasoactive, pro-inflammatory and chemotactic mediators such as histamine, mast cell proteases, leukotrienes and prostaglandins. The identification of the prime effector cells and molecules of the allergic immune response gave birth to symptomatic anti-allergic therapy based on anti-histamines, mast cell stabilizers and corticosteroids. The only causal therapy of allergic diseases is specific immunotherapy (SIT), which is highly successful for some allergens such as bee venom. Likewise, pollen-specific immunotherapy results in a marked reduction of symptoms and medication requirement (reviewed in [4]), but the treatment is time-consuming and expensive [5] and its efficacy usually decreases after a few years. Moreover, there are patients who do not respond to SIT. Thus, the challenge to develop more targeted therapy strategies for seasonal allergies remains.

*Address correspondence to this author at the Center of Allergy and Environment (ZAUM), Technische Universität and Helmholtz Zentrum München, Biedersteinerstr. 29, 80802 München, Germany; Tel: +49-89-41403472; Fax: +49-89-41403454; E-mail: traidl-hoffmann@lrz.tum.de

THE SENSITIZATION PUZZLE

Whereas mechanisms, cells and effector molecules of the allergic elicitation phase are well characterized, less is known about the allergic sensitization phase. According to a commonly accepted model, pollen grains deposited on the respiratory tract epithelium release certain proteins, the allergens, which are taken up by resident dendritic cells. As tissue sentinels, the antigen-loaded dendritic cells migrate to the regional lymph nodes to instruct specific T helper cells. In the case of pollen proteins, the default outcome of such interaction between DC and T cell is tolerance, consisting of the production of allergen-specific IgG_1 or IgG_4 antibodies and the differentiation of specific regulatory T cells (Tregs) [6]. In contrast, susceptible individuals, known as atopics, fail to mount this protective type of immune response. Instead, as yet unidentified signals trigger the differentiation of Th2 cells. Th2-derived IL-4 induces an immunoglobulin class switch in B cells, which eventually develop into long-lived, IgE-secreting plasma cells. The reason, however, why pollen proteins tend to trigger a Th2-driven immune response, remains enigmatic. Another question that continues to puzzle researchers is why some people acquire a robust regulatory response to pollen proteins, while others develop Th2-dominated, IgE-mediated allergy. The concept that only certain allergy-prone, *atopic,* individuals develop allergies is challenged by the fact that many people do not develop pollen allergies until late in life. Moreover, if atopy was simply a general tendency to develop allergies, one should wonder why some atopic individuals do not develop pollen-specific IgE while other environmental allergens, such as house-dust mite, do induce IgE in the same individual. Despite numerous attempts to find a clue to the mystery of allergenicity, the key questions remain unanswered to date. Sensitization to a protein seems to be a multi-factorial process depending on genetic and epigenetic factors, protein dose, exposure conditions, intrinsic char-

acteristics of proteins, and co-exposure to adjuvant factors derived from the allergen carrier [7].

GENETICS AND EPIGENETICS

There is clear evidence for a role of genetic predisposition for atopy [8]. Loci linked with susceptibility to allergies, eczema or asthma include members of the IL-4 gene cluster [9], the high-affinity IgE receptor [10], MHC class II [11], innate immune receptors [12], and genes involved in epidermal barrier function [13]. Particularly, development of an atopic phenotype can result from the interaction of allelic variants with specific environmental conditions. Examples for this are CD14, the co-receptor for bacterial lipopolysaccharide, and toll-like receptors (TLR)-2 and -4, genetic variants of which confer protection against asthma or respiratory allergies, depending on the living-conditions of the populations studied (rural versus urban life-style) [14],[15]. Epigenetic regulation is another proposed mechanism by which environmental factors can modulate the susceptibility to allergies. An example for this modified hygiene hypothesis is supplied by a recent murine study demonstrating that maternal exposure to farm-derived *Acinetobacter lwoffii* leads to decreased histone H4 deacetylation in the IFN-γ promoter of the offspring´s $CD4^+$ T cells, resulting in decreased susceptibility to experimental allergic asthma [16].

ALLERGEN DOSE AND ROUTE OF EXPOSURE

Most respiratory allergies are caused by pollen of anemophilous plants. The maximum daily exposure to pollen allergens can be roughly estimated based on the volume of inhaled air, pollen counts per volume of air, the volume of nasal lining fluid per day and the amount of allergen released from a given amount of pollen. For the birch pollen allergen Bet v 1, the approximated daily exposure is in the low nanogram range [17, 18]. As a general rule, exposure to low doses of proteins via the airways tends to induce IgE-mediated responses, while exposure of high doses induces tolerance [19]. This is also the principle behind allergen-specific immunotherapy.

ALLERGEN STRUCTURE

It is well conceivable that to act as an allergen, a protein has to possess IgE epitopes. Also, spatial clustering of IgE epitopes can play a role in determining allergenicity of a protein [20]. Recent advances in designing a hypoallergenic variant of the major birch pollen allergen Bet v 1 emphasize the importance of structural elements for the allergenicity of proteins [21].

Systematic *in silico* screenings of allergenic molecule databases have highlighted several characteristics linked to the allergenic potential of proteins [22-25]. “Typical” allergens cluster into only a few protein families and share certain structural and biochemical properties. In general, they are low molecular weight, hydrophilic proteins with a net negative charge, and many of them bear post-translational modifications such as glycosyl residues or disulfide bonds, possibly enhancing their stability *in vivo*. Some allergens form higher order complexes, which are thought to add to their IgE cross-linking potential. About 70% of all known allergens are enzymes. Furthermore, many allergens lack bacterial homologues [26], pointing out a role of co-evolution between host and bacteria in the decision-making process between tolerance and immune response. Finally, it has been proposed that cross-reactivity of environmental allergens with self-antigens may facilitate allergic sensitization by a molecular mimicry-related mechanism [27].

Structural features might be important in determining the outcome of an immune response. They cannot explain, however, why some individuals mount an IgE response to a given protein and others do not. Moreover, attempts to de novo predict allergenicity of proteins by computational models based on protein structure have been unsuccessful to date [28].

INTRINSIC ADJUVANT PROPERTIES OF ALLERGENS

Enzymatic function, especially protease activity, has been linked to allergenic potential of proteins. The most prominent allergens displaying intrinsic protease activity and immune-modulatory potential are the group I allergens of the house dust mite *Dermatophagoides pteronyssinus*, Der p 1, and of the storage mite *Dermatophagoides farina*, Der f 1. Both allergens belong to the family of papain-like cysteine proteases, and disruption of the protease activity of Der p 1 results in reduced allergenicity of mite extracts in an animal model of allergic sensitization [29, 30]. Der p 1 can degrade proteins involved in epithelial barrier integrity like protease inhibitors, tight-junction constituents and surfactant proteins [31, 32]. This might account for increased bioavailability of allergens in subepithelial layers and thus facilitate sensitization. Moreover, Der p 1 can cleave several surface immune receptors such as CD23 on B cells, CD25 on T cells and DC-SIGN (CD209) on dendritic cells. These activities of Der p 1 are thought to enhance IgE synthesis, to inhibit Th1 and Treg differentiation and to skew immune responses towards Th2 [33, 34].

The major allergen of birch pollen, Bet v 1, belongs to the pathogen related (PR)-10 family of proteins, and, due to its capacity to bind phospholipids it is capable to permeabilize cell membranes. This might facilitate allergen passage through the epithelium and uptake by antigen presenting cells [35]. Group I allergens from grass species (e.g. Phl p 1 of *Phleum pretense*, Lol p 1 of *Lolium perenne* and Zea m 1 of *Zea mays*) constitute a subgroup of the β-expansin family of proteins. These proteins, which display sequence homology to the Cathepsin family of cysteine proteases, catalyze the cell wall extension and cell wall loosening necessary for longitudinal plant cell growth. While Cathepsin B-like protease activity of Phl p 1 has been reported [36], this finding could not be confirmed by another study [37]. There is some evidence that Cyn d 1, a group I allergen of Bermuda grass cross-reactive to Phl p 1, exhibits protease activity [38]. A more recent study shows aspartic protease activity of a newly identified allergen from Japanese cedar pollen [39]. Group 13 allergens from grass pollen like Phl p 13 belong to the pectin-degrading polygalacturonases [40]. In summary, although there are examples of pollen allergens displaying enzymatic function, evidence for intrinsic adjuvant effects of pollen allergens is rare.

POLLEN: MORE THAN ALLERGEN CARRIERS

When we are exposed to pollen, it is not only the allergenic proteins that we inhale. Rather, epithelia, phagocytes and antigen-presenting cells of the upper respiratory tract encounter biochemically complex particles like whole pollen grains or sub-micronic pollen particles [40, 41]. These particles, of course, are loaded with and release allergenic proteins. Along with the allergens, however, myriads of other bioactive compounds such as lipids, sugars, hormones and secondary metabolites are liberated from pollen.

Two recent studies strikingly showed that pollen exposure impacts on the local immune response not only in sensitized patients but also in healthy individuals. During the onset of birch pollen season, members of the caveolin family of protein-transporters are up-regulated in nasal epithelium of birch pollen-sensitized patients, accounting for an increased transport of birch pollen allergens through the epithelium [42]. In contrast, a fundamentally different change was observed in nasal epithelium of non-allergic subjects. Here, a successive up-regulation of factors involved in granulocyte chemotaxis and activation, such as serglycin, FMLP receptor-1, CXCL6, CXCL10, CXCR2, IL-8 and IL1-β occurred [43]. This suggests that in unsensitized individuals, pollen grains cause a non-specific, pro-inflammatory response, and it is conceivable that such a non-specific response precedes and even, eventually, paves the way for allergic sensitization.

The notion that pollen grains are more than just vehicles for allergens is relatively new but might supply valuable contributions to resolving the puzzle of pollen allergenicity. In the last part of this article we will therefore confine ourselves to reviewing current knowledge on non-allergenic, adjuvant substances from pollen and their effects on the human and murine immune system.

CHEWING THEIR WAY THROUGH THE TISSUE: POLLEN PROTEASES

Unlike the class I allergens from mite, most major allergens from pollen lack intrinsic protease activity. However, as early as in the 1990s, ragweed pollen extracts were identified as a source of trypsin- and chymotrypsin-like serine proteases shown to hydrolyze neuropeptides relevant bronchomotor tone, mucus production, micro vascular endothelial integrity and the function of immune cells [44]. Among the substrates of ragweed pollen proteases were atrial natriuretic peptide (ANP), vasoactive intestinal peptide (VIP), substance P and angiotensin 1 and -2 [45]. Similar results were obtained with aqueous extracts from various grass pollen species [38], as well as with pollen extracts from *Parietaria judaica*, a common cause of allergic rhinitis in Mediterranean countries [46]. The latter study also demonstrated that *P. judaica* pollen proteases induced the detachment of pulmonary epithelial cells by degrading the tight-junction protein occludin. Tight junction degradation via targeting of occludin, claudin-1 and ZO-1 was later shown for extracts of different pollen species such as Giant Ragweed, grass, birch and Easter Lily [47]. Japanese Cedar, a highly allergenic pollen species in Japan, was also shown to contain serine proteases [48]. In summary, all allergenic pollen species examined are a source of serine proteases with a broad substrate spectrum. A systematic comparison of protease activities in allergenic and non-allergenic pollen species could help to evaluate the relevance of these findings.

OXIDANT STRESS: NAPH OXIDASES

In 2005, two seminal studies were published describing that ragweed pollen extracts contain reduced nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidases [49, 50]. Not until several years later, a physiological role was attributed to extracellular, NADPH oxidase-generated reactive oxygen species in the process of pollen-tube growth [51].

In a pilot study by Instvan Boldogh and coworkers, pollen-derived NADPH oxidases were shown to induce oxidant stress in cultured airway epithelium and in lungs of ragweed pollen-challenged mice, leading to mucin production and eosinophil recruitment. Allergic airway inflammation to the major ragweed allergen Amb a 1 was boosted by addition of oxidant stress markers such as oxidized glutathione (GSSG) and 4-hydroxy-nonenal (4-HNE). In contrast, challenge with ragweed extracts stripped of NADPH oxidase activity resulted in reduced allergic lung inflammation [50]. A paper from the same group published only a few months later demonstrated adjuvant effects of ragweed pollen-derived NADPH oxidases in a murine model of allergic conjunctivitis [49]. Lactoferrin, an iron-binding protein that prevents the formation of other reactive oxygen species from NADPH oxidase-generated superoxide, was demonstrated to decrease allergic airway inflammation to ragweed pollen extract [52]. Intrapulmonary administration of antioxidants such as N-acetyl cysteine (NAC), ascorbic acid and tocopherol attenuated ragweed pollen-induced allergic airway inflammation [53]. A more recent study investigated the presence of NADPH oxidases in different allergenic pollen species. NADPH oxidase activity was demonstrated for all allergenic pollen species examined, with highest activity in ragweed pollen, followed by grasses, birch, Japanese cypress and Japanese cedar. In hydrated pollen, the enzymatic activity was restricted to the insoluble fraction, and hardly any activity was detectable in the aqueous supernatant. Localization of NADPH oxidases differed between pollen species. Notably, NADPH oxidase activity was detected in subpollen particles released from ragweed pollen [54], possibly accounting for the high asthma-inducing potential of this pollen species.

CHEMOTAXIS AND IMMUNE MODULATION: POLLEN-ASSOCIATED LIPID MEDIATORS

When analyzing supernatants of cells stimulated with aqueous birch pollen extracts on commercial LTB_4 and PGE_2 ELISAs, the pollen extracts showed cross-reactivity. This chance finding lead to the discovery of the pollen-associated lipid mediators (PALMs), bioactive lipids structurally and functionally homologous to mammalian eicosanoids. The release of PALMs from hydrated birch pollen grains is a rapid process completed within 30 minutes and even precedes the release of allergens [55], arguing for a true co-exposure of human tissues to pollen allergens and PALMs. PALMs are not restricted to aqueous birch pollen extracts but were identified in diffusates of different allergenic pollen species such as grasses, ragweed, rocky mountain juniper, Japanese cedar and Japanese cypress [56].

According to structural criteria, PALMs can be divided into two groups. The first group consists of the LTB_4-like PALMs, monohydroxylated derivatives of linoleic and α-linolenic acid. Members of this group are 13-hydroxy-octadecadienoic acid and 13-hydroxy-octadecatrienoic acid. The second group of PALMs is the plant isoprostanes (phytoprostanes), which are formed in a non-enzymatic, oxygen radical catalyzed reaction from α-Linolenic acid [57]. In aqueous birch pollen extracts, phytoprostanes of the isoforms A_1/B_1-, E_1 and F_1 have been identified, the E_1-phytoprostanes being the most abundant isoform [58]. F_1-phytoprostanes accumulate in plant tissues in response to oxidative damage [59], suggesting that phytoprostanes might generally be involved in stress response mechanisms. In pollen, long chain unsaturated lipids are known to be involved in signaling during pollen-stigma interaction [60]. The role of phytoprostanes in this process, however, is not clear. Whatever their physiological functions may be, both types of PALMs act in multiple ways on cells of the human immune system. First, PALMs of the LTB_4-like class were shown to induce chemotaxis and activation of human neutrophils and eosinophils [61, 62]. This finding was followed by the discovery that pollen-derived E_1-phytoprostanes inhibit the production of IL-12 p70 in human dendritic cells, licensing them to induce a Th2-skewed response in naïve T cells [58]. This was the first report demonstrating immune modulation by pollen-derived, non-allergenic molecules. In a follow-up study, the inhibition of the dendritic cell IL-12 response by E_1-phytoprostanes was shown to occur via blocking of NFκB nuclear translocation and by a mechanism involving the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) [63]. Eventually, however, evidence accumulated for the presence of other immune modulatory substances in aqueous birch pollen extracts (APE). The first hint was supplied by a study on the effects of APE in a murine sensitization model. Mice were sensitized to the allergen ovalbumin (OVA) by intraperitonal injection and subsequently challenged by intranasal instillation of OVA, OVA plus APE or OVA plus E_1-phytoprostanes (PPE_1). The T helper cells isolated from draining lymph nodes of OVA/APE-challenged mice showed a Th2-skewed cytokine profile, while T cells from OVA/PPE_1-challenged mice failed to produce either, Th1 and Th2 cytokines [64]. The second line of evidence came from a human *in vitro* study on the modulation of chemokine receptors on monocyte-derived dendritic cells by APE [65]. This study showed that dendritic cells exposed to APE acquire migratory properties resembling that of dendritic cells exposed to PGE_2, an effect that depended on adenylyl cyclase and intracellular cyclic AMP induction. However, in contrast to APE, PPE_1 failed to induce a cyclic AMP signal in dendritic cells. The third hint that modulation of dendritic cell function by APE cannot solely be attributed to PPE_1 came from investigating the effects of APE on maturation, cytokine production and T cell differentiating potential of primary dendritic cells from human

peripheral blood. Whereas an APE fraction depleted of proteins potently inhibited the LPS-induced maturation of this blood dendritic cell subset, E_1- and F_1-phytoprostanes did not [66]. In a more recent study, Metz and coworkers demonstrated that a non-allergen containing fraction of birch APE, when injected intradermally into the ears of mice, induced a local ear-swelling response. This response was accompanied by extensive degranulation of resident mast cells. Moreover, this allergen-independent inflammation was shown to be dependent on mast cells as it was not inducible in mast-cell deficient $Kit^{W/W\text{-}v}$ mice and could be reconstituted by injection of bone-marrow-derived mast cells. These pro-inflammatory effects of non-protein components from pollen might account for seasonal allergy-like symptoms described by some patients with low or non-detectable levels of specific IgE [67]. Acknowledging the fact that the immune systems of mice and humans differ significantly in some respects, the observation of adjuvant or aggravating effects of APE in both systems – human *in vitro* and murine *in vivo* –, emphasizes their potential importance. However, only controlled human trials will be able to ultimately answer the question of clinical relevance.

FRIEND OR FOE: POLLEN-DERIVED ADENOSINE

Non-allergic individuals, as well as pollen allergic patients who underwent successful immunotherapy, react to pollen allergens predominantly by producing IgG_1 and IgG_4 antibodies [68, 69]. This "healthy" response to allergen is presumably orchestrated by allergen-specific T helper cells secreting IFN-γ and IL-10 and resembles a Th1/Treg response [70, 71]. In our own studies, we consistently observed a profound Th1-inhibitory effect of aqueous pollen extracts (APE). One class of substances, the above-mentioned E_1-phytoprostanes, was identified as mediator of such effects. However, some of the immune modulatory effects of APE could not be explained by the presence of the E_1-phyotprostanes. Consequently, a screening was set up to identify other substances with the potential to modulate the T helper cell-priming capacity of dendritic cells. First, a protein-free, low molecular weight fraction of APE was gained by ultra-filtration. Ultra high-resolution mass spectrometry performed with the extracts revealed over 12,000 discrete mass signals that were translated into 900 annotated compounds, representing the birch pollen metabolome. Subsequent pathway analysis of the annotated compounds confirmed that the low molecular weight fraction of APE contained numerous linoleic and linolenic acid derivatives, among them the previously described phytoprostanes. Additionally, various constituents of the purine and pyrimidine metabolism were discovered, among them the nucleoside adenosine. Since adenosine is a molecule known to modulate dendritic cell function and is implicated in the differentiation and function of Tregs, we sought to further analyze this finding. The presence of adenosine in aqueous pollen extracts of different pollen species was confirmed by ultra performance liquid chromatography and mass spectrometry, and concentrations of adenosine in different birch pollen extracts were found to range from 0.5-10µM. Pollen derived adenosine was then shown to contribute to the inhibition of the IL12 response of dendritic cells to LPS. Furthermore, dendritic cells exposed to pollen-derived adenosine had a compromised capacity to prime Th1 and Th2 responses in naïve $CD4^+$ T cells. Instead, they induced the regulatory cytokine IL10 in the co-cultured T cells and promoted the differentiation of $CD4^+CD25^+Foxp3^+$ regulatory T cells. This suggested that pollen-derived adenosine, rather than signaling danger to the immune system, represents a tolerogenic molecule. Most intriguingly, dendritic cells cultured from monocytes of pollen-allergic donors were compromised in their response to pollen-derived adenosine: they induced lower numbers of Tregs and lower levels of IL10 in the same naïve $CD4^+$ T cells than dendritic cells cultured from healthy donor monocytes [72]. In the past, several independent studies had demonstrated that pollen-allergic patients fail to develop a functional, specific Treg response to pollen allergens during the pollen season [73-75]. The discovery that sensing of tolerogenic signals like adenosine is compromised in dendritic cells of allergics might be a clue to this failure of tolerance induction. Notably, aqueous extracts of pine pollen, a species hardly inducing allergies, contained high concentrations of adenosine, while no adenosine was measurable in an extract prepared from commercial ragweed pollen. In the future, systematic studies will be needed which compare molecules of the adenosine-signaling pathway in myeloid cells of healthy and atopic individuals. Apart from this, adenosine receptors are also expressed on basophils and mast cells. On maturing dendritic cells, adenosine mediates its anti-inflammatory effects via receptors of the isoforms A2a and A2b [76, 77]. In mast cells and basophils, adenosine has been shown to differentially modulate mediator release, depending on adenosine concentration and receptor isoform expression [78-80]. Consequently, pollen-derived adenosine might turn out to modulate not only allergic sensitization but also the allergic elicitation phase.

THE FUTURE: POLLEN SYSTEM BIOLOGY

In recent years, the recognition of the fact that pollen grains are more than just allergen carriers has lead to the discovery of several immune stimulatory and immune modulatory compounds such as proteases, NADPH oxidases, PALMs and adenosine. While all of these molecules have been identified in various different allergenic pollen species, none of the studies investigated these compounds in non-allergenic pollen or supplied comprehensive comparisons between allergenic and non-allergenic pollen species. Systematic comparative studies on non-allergenic, adjuvant substances in different pollens might help to deduce the relevance of these substances for pollen allergenicity. A promising future approach will be to collect proteome and metabolome data of different pollen species and use these data to perform differential pathway analyses in order to identify molecular patterns relevant to allergenicity. Comparing the molecular signatures of pollen from different, allergenic and less allergenic, species will identify potentially relevant compounds for further *in vitro* screenings and ultimate clinical trials. The challenge of such an approach, of course, will be to obtain not only qualitative but also quantitative metabolomic data. If successful, it will enable us to specifically target the relevant receptors in susceptible individuals, paving the way for new anti-allergic medication or improved extracts for SIT. It might even lead to the development of prophylactic drugs that can be topically applied during pollen season to prevent sensitization in not yet atopic individuals. Apart from this, a system biology approach to tackle pollen biology will further our understanding of how environmental factors, e.g. traffic-related air pollution, ozone content and soil heavy metal composition, influence the allergenicity of pollen.

ACKNOWLEDGEMENT

This work was supported in part by the CK-Care – Christine Kühne - Center for Allergy Research and Education and DFG Grant TR467/5-1.

REFERENCES

[1] Bauchau V, Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. The European respiratory journal : official J Eur Society for Clinical Respiratory Physiol 2004; 24(5): 758-64.

[2] Coca AF. The Skin as a Shock Tissue. Bulletin of the New York Academy of Medicine 1929; 5(3): 223-31.

[3] Ishizaka K, Ishizaka T, Hornbrook MM. Physicochemical properties of reaginic antibody. V. Correlation of reaginic activity wth gamma-E-globulin antibody. J Immunol 1966; 97(6): 840-53.

[4] Calderon MA, Casale TB, Togias A, Bousquet J, Durham SR, Demoly P. Allergen-specific immunotherapy for respiratory allergies: from meta-analysis to registration and beyond. J allergy clinical Immunol 2011; 127(1): 30-8.

[5] Lockey RF, Hankin CS. Health economics of allergen-specific immunotherapy in the United States. J allergy clinical Immunol 2011; 127(1): 39-43.

[6] Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy. J allergy and clinical Immunol 2011; 127(1): 18-27; quiz 8-9.

[7] Traidl-Hoffmann C, Jakob T, Behrendt H. Determinants of allergenicity. J Allergy Clin Immunol 2009; 123: 558-66.

[8] Cookson W. The immunogenetics of asthma and eczema: a new focus on the epithelium. Nat Rev Immunol 2004; 4(12): 978-88.

[9] Kabesch M, Schedel M, Carr D, *et al*. IL-4/IL-13 pathway genetics strongly influence serum IgE levels and childhood asthma. The J allergy and clinical Immunol 2006; 117(2): 269-74.

[10] Weidinger S, Gieger C, Rodriguez E, *et al*. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. PLoS genetics 2008; 4(8): e1000166.

[11] Lara-Marquez ML, Yunis JJ, Layrisse Z, *et al*. Immunogenetics of atopic asthma: association of DRB1*1101 DQA1*0501 DQB1*0301 haplotype with Dermatophagoides spp.-sensitive asthma in a sample of the Venezuelan population. Clin Exp Allergy 1999; 29(1): 60-71.

[12] Yang IA, Fong KM, Holgate ST, Holloway JW. The role of Toll-like receptors and related receptors of the innate immune system in asthma. Current opinion allergy clinical Immunol 2006; 6(1): 23-8.

[13] Weidinger S, O'Sullivan M, Illig T, *et al*. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. The J allergy and clinical Immunol 2008; 121(5): 1203-9 e1.

[14] Tesse R, Pandey RC, Kabesch M. Genetic variations in toll-like receptor pathway genes influence asthma and atopy. Allergy 2011; 66(3): 307-16.

[15] Eder W, Klimecki W, Yu L, *et al*. Toll-like receptor 2 as a major gene for asthma in children of European farmers. The J allergy and clinical Immunol 2004; 113(3): 482-8.

[16] Brand S, Teich R, Dicke T, *et al*. Epigenetic regulation in murine offspring as a novel mechanism for transmaternal asthma protection induced by microbes. The J allergy and clinical Immunol 2011.

[17] Buters JT, Kasche A, Weichenmeier I, *et al*. Year-to-year variation in release of Bet v 1 allergen from birch pollen: evidence for geographical differences between West and South Germany. International archives of allergy and Immunol 2008; 145(2): 122-30.

[18] Buters JT, Weichenmeier I, Ochs S, *et al*. The allergen Bet v 1 in fractions of ambient air deviates from birch pollen counts. Allergy 2010; 65(7): 850-8.

[19] Blaser K. Allergen dose dependent cytokine production regulates specific IgE and IgG antibody production. Advances in experimental medicine and biology 1996; 409: 295-303.

[20] Flicker S, Steinberger P, Ball T, *et al*. Spatial clustering of the IgE epitopes on the major timothy grass pollen allergen Phl p 1: importance for allergenic activity. The J allergy and clinical Immunol 2006; 117(6): 1336-43.

[21] Vrtala S, Fohr M, Campana R, Baumgartner C, Valent P, Valenta R. Genetic engineering of trimers of hypoallergenic fragments of the major birch pollen allergen, Bet v 1, for allergy vaccination. Vaccine 2011; 29(11): 2140-8.

[22] Aalberse RC. Structural biology of allergens. The J allergy and clinical Immunol 2000; 106(2): 228-38.

[23] Furmonaviciene R, Sutton BJ, Glaser F, *et al*. An attempt to define allergen-specific molecular surface features: a bioinformatic approach. Bioinformatics 2005; 21(23): 4201-4.

[24] Radauer C, Breiteneder H. Pollen allergens are restricted to few protein families and show distinct patterns of species distribution. The J allergy and clinical Immunol 2006; 117(1): 141-7.

[25] Radauer C, Bublin M, Wagner S, Mari A, Breiteneder H. Allergens are distributed into few protein families and possess a restricted number of biochemical functions. The J allergy and clinical Immunol 2008; 121(4): 847-52 e7.

[26] Emanuelsson C, Spangfort MD. Allergens as eukaryotic proteins lacking bacterial homologues. Molecular Immunol 2007; 44(12): 3256-60.

[27] Zeller S, Glaser AG, Vilhelmsson M, Rhyner C, Crameri R. Immunoglobulin-E-mediated reactivity to self antigens: a controversial issue. International archives of allergy and Immunol 2008; 145(2): 87-93.

[28] Aalberse RC, Stadler BM. In silico predictability of allergenicity: from amino acid sequence via 3-D structure to allergenicity. Molecular nutrition & food research 2006; 50(7): 625-7.

[29] Gough L, Schulz O, Sewell HF, Shakib F. The cysteine protease activity of the major dust mite allergen Der p 1 selectively enhances the immunoglobulin E antibody response. The J experimental medicine 1999; 190(12): 1897-902.

[30] Kikuchi Y, Takai T, Kuhara T, *et al*. Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses. J Immunol 2006; 177(3): 1609-17.

[31] Wan H, Winton HL, Soeller C, *et al*. Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. J clinical Investigat 1999; 104(1): 123-33.

[32] Deb R, Shakib F, Reid K, Clark H. Major house dust mite allergens Dermatophagoides pteronyssinus 1 and Dermatophagoides farinae 1 degrade and inactivate lung surfactant proteins A and D. J Biolog Chem 2007; 282(51): 36808-19.

[33] Shakib F, Schulz O, Sewell H. A mite subversive: cleavage of CD23 and CD25 by Der p 1 enhances allergenicity. Immunol today 1998; 19(7): 313-6.

[34] Furmonaviciene R, Ghaemmaghami AM, Boyd SE, *et al*. The protease allergen Der p 1 cleaves cell surface DC-SIGN and DC-SIGNR: experimental analysis of in silico substrate identification and implications in allergic responses. Clin Exp Allergy 2007; 37(2): 231-42.

[35] Mogensen JE, Ferreras M, Wimmer R, Petersen SV, Enghild JJ, Otzen DE. The major allergen from birch tree pollen, Bet v 1, binds and permeabilizes membranes. Biochemistry 2007; 46(11): 3356-65.

[36] Grobe K, Poppelmann M, Becker WM, Petersen A. Properties of group I allergens from grass pollen and their relation to cathepsin B, a member of the C1 family of cysteine proteinases. Eur J biochemistry / FEBS 2002; 269(8): 2083-92.

[37] Li LC, Cosgrove DJ. Grass group I pollen allergens (beta-expansins) lack proteinase activity and do not cause wall loosening via proteolysis. Eur J biochemistry / FEBS 2001; 268(15): 4217-26.

[38] Raftery MJ, Saldanha RG, Geczy CL, Kumar RK. Mass spectrometric analysis of electrophoretically separated allergens and proteases in grass pollen diffusates. Respiratory research 2003; 4: 10.

[39] Ibrahim AR, Kawamoto S, Aki T, *et al*. Molecular cloning and immunochemical characterization of a novel major Japanese cedar pollen allergen belonging to the aspartic protease family. International archives of allergy and Immunol 2010; 152(3): 207-18.

[40] Swoboda I, Grote M, Verdino P, *et al*. Molecular characterization of polygalacturonases as grass pollen-specific marker allergens: expulsion from pollen via submicronic respirable particles. J Immunol 2004; 172(10): 6490-500.

[41] Taylor PE, Flagan RC, Valenta R, Glovsky MM. Release of allergens as respirable aerosols: A link between grass pollen and asthma. J allergy Clinical Immunol 2002; 109(1): 51-6.

[42] Joenvaara S, Mattila P, Renkonen J, *et al*. Caveolar transport through nasal epithelium of birch pollen allergen Bet v 1 in allergic patients. The J allergy and clinical Immunol 2009; 124(1): 135-42 e1-21.

[43] Mattila P, Renkonen J, Toppila-Salmi S, *et al*. Time-series nasal epithelial transcriptomics during natural pollen exposure in healthy subjects and allergic patients. Allergy 2010; 65(2): 175-83.

[44] Bagarozzi DA, Jr., Pike R, Potempa J, Travis J. Purification and characterization of a novel endopeptidase in ragweed (Ambrosia artemisiifolia) pollen. The J biological chemistry 1996; 271(42): 26227-32.

[45] Bagarozzi DA, Jr., Travis J. Ragweed pollen proteolytic enzymes: possible roles in allergies and asthma. Phytochemistry 1998; 47(4): 593-8.

[46] Cortes L, Carvalho AL, Todo-Bom A, Faro C, Pires E, Verissimo P. Purification of a novel aminopeptidase from the pollen of Parietaria judaica that alters epithelial integrity and degrades neuropeptides. J allergy clinical Immunol 2006; 118(4): 878-84.

[47] Runswick S, Mitchell T, Davies P, Robinson C, Garrod DR. Pollen proteolytic enzymes degrade tight junctions. Respirology 2007; 12(6): 834-42.

[48] Gunawan H, Takai T, Ikeda S, Okumura K, Ogawa H. Protease activity of allergenic pollen of cedar, cypress, juniper, birch and

ragweed. Allergology international : official J Japanese Society Allergology 2008; 57(1): 83-91.

[49] Bacsi A, Dharajiya N, Choudhury BK, Sur S, Boldogh I. Effect of pollen-mediated oxidative stress on immediate hypersensitivity reactions and late-phase inflammation in allergic conjunctivitis. J allergy and clinical Immunol 2005; 116(4): 836-43.

[50] Boldogh I, Bacsi A, Choudhury BK, *et al*. ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflammation. The J clinical investigation 2005; 115(8): 2169-79.

[51] Potocky M, Jones MA, Bezvoda R, Smirnoff N, Zarsky V. Reactive oxygen species produced by NADPH oxidase are involved in pollen tube growth. The New phytologist 2007; 174(4): 742-51.

[52] Kruzel ML, Bacsi A, Choudhury B, Sur S, Boldogh I. Lactoferrin decreases pollen antigen-induced allergic airway inflammation in a murine model of asthma. Immunol 2006; 119(2): 159-66.

[53] Dharajiya N, Choudhury BK, Bacsi A, Boldogh I, Alam R, Sur S. Inhibiting pollen reduced nicotinamide adenine dinucleotide phosphate oxidase-induced signal by intrapulmonary administration of antioxidants blocks allergic airway inflammation. The J allergy and clinical Immunol 2007; 119(3): 646-53.

[54] Wang XL, Takai T, Kamijo S, Gunawan H, Ogawa H, Okumura K. NADPH oxidase activity in allergenic pollen grains of different plant species. Biochemical and biophysical research communications 2009; 387(3): 430-4.

[55] Behrendt H, Kasche A, Ebner von Eschenbach C, Risse U, Huss-Marp J, Ring J. Secretion of proinflammatory eicosanoid-like substances precedes allergen release from pollen grains in the initiation of allergic sensitization. International archives of allergy and Immunol 2001; 124(1-3): 121-5.

[56] Gunawan H, Takai T, Kamijo S, *et al*. Characterization of proteases, proteins, and eicosanoid-like substances in soluble extracts from allergenic pollen grains. International archives of allergy and Immunol 2008; 147(4): 276-88.

[57] Thoma I, Krischke M, Loeffler C, Mueller MJ. The isoprostanoid pathway in plants. Chem Phys Lipids 2004; 128(1-2): 135-48.

[58] Traidl-Hoffmann C, Mariani V, Hochrein H, *et al*. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. The J experimental medicine 2005; 201(4): 627-36.

[59] Imbusch R, Mueller MJ. Analysis of oxidative stress and wound-inducible dinor isoprostanes F(1) (phytoprostanes F(1)) in plants. Plant Physiol 2000; 124(3): 1293-304.

[60] Pruitt RE, Vielle-Calzada JP, Ploense SE, Grossniklaus U, Lolle SJ. FIDDLEHEAD, a gene required to suppress epidermal cell interactions in Arabidopsis, encodes a putative lipid biosynthetic enzyme. Proceedings of the National Academy of Sciences of the United States of America 2000; 97(3): 1311-6.

[61] Traidl-Hoffmann C, Kasche A, Jakob T, *et al*. Lipid mediators from pollen act as chemoattractants and activators of polymorphonuclear granulocytes. The J allergy and clinical Immunol 2002; 109(5): 831-8.

[62] Plotz SG, Traidl-Hoffmann C, Feussner I, *et al*. Chemotaxis and activation of human peripheral blood eosinophils induced by pollen-associated lipid mediators. The J allergy and clinical Immunol 2004; 113(6): 1152-60.

[63] Gilles S, Mariani V, Bryce M, *et al*. Pollen-derived E1-phytoprostanes signal via PPAR-gamma and NF-kappaB-dependent mechanisms. J Immunol 2009; 182(11): 6653-8.

[64] Gutermuth J, Bewersdorff M, Traidl-Hoffmann C, *et al*. Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanes on primary immune responses *in vivo*. The J allergy and clinical Immunol 2007; 120(2): 293-9.

[65] Mariani V, Gilles S, Jakob T, *et al*. Immunomodulatory mediators from pollen enhance the migratory capacity of dendritic cells and license them for Th2 attraction. J Immunol 2007; 178(12): 7623-31.

[66] Gilles S, Jacoby D, Blume C, *et al*. Pollen-derived low-molecular weight factors inhibit 6-sulfo LacNAc+ dendritic cells' capacity to induce T-helper type 1 responses. Clin Exp Allergy 2010; 40(2): 269-78.

[67] Metz M, Gilles S, Geldmacher A, Behrendt H, Traidl-Hoffmann C, Maurer M. Evidence for non-allergic mast cell activation in pollen-associated inflammation. J Invest Dermatol 2011; 131(4): 987-90.

[68] Francis JN, James LK, Paraskevopoulos G, *et al*. Grass pollen immunotherapy: IL-10 induction and suppression of late responses precedes IgG4 inhibitory antibody activity. The J allergy and clinical Immunol 2008; 121(5): 1120-5 e2.

[69] James LK, Shamji MH, Walker SM, *et al*. Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. The J allergy and clinical Immunol 2011; 127(2): 509-16 e1-5.

[70] Van Overtvelt L, Wambre E, Maillere B, *et al*. Assessment of Bet v 1-specific CD4+ T cell responses in allergic and nonallergic individuals using MHC class II peptide tetramers. J Immunol 2008; 180(7): 4514-22.

[71] Wambre E, Bonvalet M, Bodo VB, *et al*. Distinct characteristics of seasonal (Bet v 1) vs. perennial (Der p 1/Der p 2) allergen-specific CD4(+) T cell responses. Clin Exp Allergy 2011; 41(2): 192-203.

[72] Gilles S, Fekete A, Zhang X, *et al*. Pollen metabolome analysis reveals adenosine as a major regulator of dendritic cell-primed T(H) cell responses. The J allergy and clinical Immunol 2011; 127(2): 454-61 e1-9.

[73] Mittag D, Scholzen A, Varese N, *et al*. The effector T cell response to ryegrass pollen is counterregulated by simultaneous induction of regulatory T cells. J Immunol 2010; 184(9): 4708-16.

[74] Grindebacke H, Wing K, Andersson AC, Suri-Payer E, Rak S, Rudin A. Defective suppression of Th2 cytokines by CD4CD25 regulatory T cells in birch allergics during birch pollen season. Clin Exp Allergy 2004; 34(9): 1364-72.

[75] Thunberg S, Akdis M, Akdis CA, *et al*. Immune regulation by CD4+CD25+ T cells and interleukin-10 in birch pollen-allergic patients and non-allergic controls. Clin Exp Allergy 2007; 37(8): 1127-36.

[76] Novitskiy SV, Ryzhov S, Zaynagetdinov R, *et al*. Adenosine receptors in regulation of dendritic cell differentiation and function. Blood 2008; 112(5): 1822-31.

[77] Panther E, Idzko M, Herouy Y, *et al*. Expression and function of adenosine receptors in human dendritic cells. Faseb J 2001; 15(11): 1963-70.

[78] Peachell PT, Columbo M, Kagey-Sobotka A, Lichtenstein LM, Marone G. Adenosine potentiates mediator release from human lung mast cells. Am Rev Respir Dis 1988; 138(5): 1143-51.

[79] Yip K, Lau H, Wise H. Reciprocal modulation of anti-IgE induced histamine release from human mast cells by A(1) and A(2B) adenosine receptors. Br J Pharmacol 2011; 164(2b): 807-19.

[80] Nunomura S, Gon Y, Yoshimaru T, Kashiwakura J, Kawakami T, Ra C. FcepsilonRI beta-chain ITAM amplifies PI3K-signaling to ensure synergistic degranulation response via FcepsilonRI and adenosine receptors. Eur J Immunol 2010; 40(4): 1205-17.