Allergo J Int
https://doi.org/10.1007/s40629-023-00262-9

What inhalant allergens can do and not do?—The cooperation of allergens and their source in Th2 polarization and allergic sensitization

Sophie Grosse-Kathoefer · Lorenz Aglas · Fatima Ferreira · Lisa Pointner

Received: 22 May 2023 / Accepted: 2 July 2023

Keywords Aeroallergens · Adjuvant pharmaceutics · Allergen source · IgE recognition · Molecular spreading

Abbreviations

AHR	Airway hyperresponsiveness
AIT	Allergen-specific immunotherapy
Alum	Aluminum hydroxide
APC	Antigen-presenting cell
AR	Allergic rhinitis
BALF	Bronchoalveolar lavage fluids
BM	Bone marrow-derived
BP	Birch pollen
BPE	Birch pollen extract
CCL20	Chemokine (C-C motif) ligand 20
DAMP	Damage-associated molecular patterns
DC	Dendritic cells
β-glucan	β-(1,3)-glucan
GP	Grass pollen
GPE	Grass pollen extract
HDM	House dust mites
HMW	High molecular weight
IFNγ	Interferon-γ
Ig	Immunoglobulin
IL	Interleukin
ILC2	Type 2 innate lymphoid cells
i.n.	Intranasal
i.p.	Intraperitoneal
LMW	Low molecular weight
LPS	Lipopolysaccharide
MD-2	Myeloid differentiation factor-2
MLN	Mediastinum lymph nodes
OVA	Ovalbumin
PALM	Pollen-associated lipid mediator
PAMP	Pathogen-associated molecular pattern
r	Recombinantly produced
RP	Ragweed pollen
RPE	Ragweed pollen extract
s.c.	Subcutaneous
TGF	Transforming growth factor
Th	T helper cells
Th2	Type 2 T helper cell
TLR	Toll-like receptor
TNFα	Tumor necrosis factor-α
wt	Wild-type
PPE1	PALM phytoprostane E1

S. Grosse-Kathoefer · L. Aglas · F. Ferreira · Dr. L. Pointner (✉)
Department of Biosciences and Medical Biology, University of Salzburg, Hellbrunner Str. 34, 5020 Salzburg, Austria
lisanaomi.pointner@plus.ac.at

Introduction

Inhalant allergens are the most common triggers of allergy-related respiratory symptoms [1] and represent a global health problem. Approximately 400 million people globally suffer from allergic rhinitis (AR) and about 150 million are affected by allergic asthma [2, 3]. Per definition, respiratory allergies to aeroallergens result in allergic symptoms upon inhalation. Sources of aeroallergens are not rare; they are ubiquitous in the environment and are transported by the air. Avoiding their contact is thus hardly possible. The primary indoor sources of allergens in central Europe are house dust mites (HDMs), molds, and animal dander from domestic cats and dogs [4], whereas for outdoor allergenic sources, grass pollen (GP) and birch pollen (BP) account for the highest sensitization rates, followed by mugwort and ragweed pollen (RP) [5, 6].

It is still unclear why the human immune system responds aberrantly to a small portion of normally harmless environmental proteins from an allergenic source. Such proteins are called allergens and defined by their property to be recognized by specific immunoglobulin (Ig)E antibodies (Table 1). IgE-me-

Published online: 28 July 2023

Table 1 Important terms and their definition

Term	Definition
Adjuvant	Usually, adjuvants are defined by their ability to induce an adaptive immune response (e.g., pro-inflammatory cytokines derived from activated innate immune cells). Herein, with "adjuvant activity" we refer to the activity of a component promoting the development of a Th2 polarization and allergen-specific IgE antibody production
Auto-adjuvant	Intrinsic activity of an allergen to stimulate the immune system towards a Th2 response and allergen-specific IgE antibody production (e.g., protease activity)
Inhalant allergen/ Aeroallergen	Proteins derived from inhalant allergen sources such as HDM and pollen with the property to be recognized by specific IgE antibodies
Initiator allergen	Allergens able to trigger and initiate a sensitization cascade to other allergens of the same or other sources of allergens
Matrix of an allergen	Context of an allergen source containing additional non-allergenic compounds, in which the allergen is delivered
Molecular spreading	Cascade of immunological events leading to the allergic sensitization to additional allergens derived from the same or other sources

HDM House dust mite, *IgE* Immunoglobulin E, *Th2* T helper class type 2 cell

diated allergic diseases are divided into two major phases: (i) the asymptomatic sensitization phase initiated upon the initial contact with the allergen source, resulting in a type 2 T helper cell (Th2) polarization and the production of allergen-specific IgEs in predisposed individuals, and (ii) the effector phase, in which subsequent allergen exposures lead to disease expression [7] via the release of pro-inflammatory mediators upon IgE-mediated degranulation of mast cells.

It seems apparent that major allergens must have unique features to become the specific target of IgEs, nonetheless, no properties were identified so far that are unique for allergens. What does then an inhalant protein require to become an allergen in the first place?

Non-allergenic components derived from allergenic sources that are co-delivered with the allergens during exposure were demonstrated to be able to provide an adjuvant activity, thus, shaping the adaptive immune response and allergic sensitization [8]. It is therefore imperative to consider the sensitizing potential of the entire allergen source, when defining allergens rather than relying only on the concept of IgE binding.

In this review, we will focus on major inhalant allergens, beginning with (i) the novel insights on allergen recognition, and (ii) the evolution of IgE and IgG repertoires to inhalant allergens. The discussion continues with the latest studies assessing (iii) the capacity of major aeroallergens to stimulate or not the immune system towards a Th2 response and IgE production, termed herein auto-adjuvant activity. Finally, (iv) in vivo models assessing the auto-adjuvant activity of the major aeroallergens in respect to their original sources, which contain co-delivered non-allergen compounds, will also be discussed (Fig. 1).

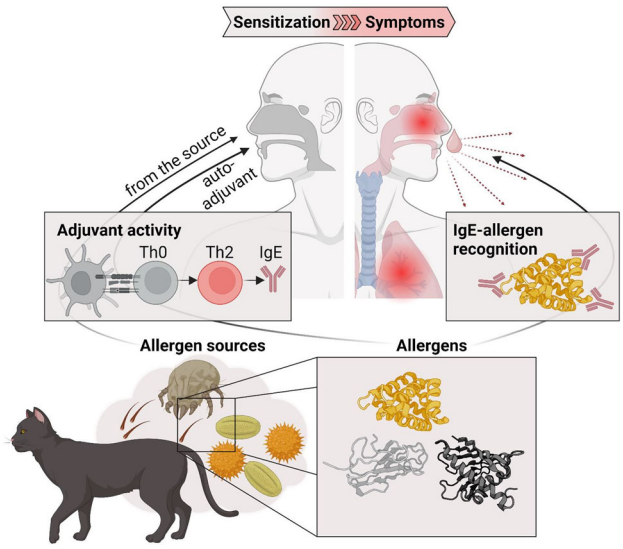

Fig. 1 The initiation of allergic sensitization to inhalant allergens, as characterized by Th2 polarization leading to the production of allergen-specific IgEs, relies on the adjuvant activity of either the allergen (auto-adjuvant activity) and/or bioactive compounds derived from the allergen source. The clinically most relevant allergen sources in Europe are dust mites, animal dander, and pollen. Specific aeroallergens, called "initiator allergens" leading to sensitization during the first decade of life trigger a sensitization cascade to other allergens of the same or other sources. This IgE-allergen recognition is essential for expression of allergic symptoms. *DC* dendritic cell, *Th2* T helper class 2, *Ig* Immunoglobulin

Novel insights on allergen recognition: allergy initiation and spreading

The levels of specific serum IgE are sufficient to distinguish non-atopic individuals, from atopic, asymptomatic and allergic patients [9–11]. In a German population ($n = 104$), the evolution of the specificity of the IgE response was monitored using a multiplex microarray covering 35 allergens of 16 clinically relevant allergen sources in children [12]. During the first decade of life, aeroallergens represented the top 10 most prevalent IgE targets, indicating that the initial IgE responses in this cohort were mostly—if not exclusively—directed against inhalant allergens. The IgE prevalence occurred in a sequential order, of which Phl p 1 and Bet v 1 with 38% were the most prevalent ones among atopic children, followed by Fel d 1, Phl p 5 and the HDM allergens Der p 2 and Der p 1. Since these major allergens were observed to initiate a sensitization cascade to other allergens of the same or other sources, by a phenomenon termed "molecular spreading", they were termed "initiator allergens". The IgE profiles emerged not later than at the age of 5, remained stable over time and appeared to plateau when reaching 7–10 years [12–14]. By specifically addressing the molecular spreading as part of the same German Multicenter Allergy Study, in patients suffering either from GP- or HDM-related AR, sensitization always started either with Phl p 1

followed by Phl p 4 and 5, or Der p 1, 2 and 23, respectively, before extending to other *Phleum pratense* (Phl p 2 > Phl p 6 > Phl p 11 > Phl p 12 > Phl p 7) [14] or HDM allergens (Der p 5 > Der p 7 > Der p 4 > Der p 21 > Der p 11 > Der p 18 > Der p 14 > Der p 15) [13, 15]. Sensitization to the initiators, e.g., Phl p 1-, Der p 1- or Der p 23-specific IgE, at age 3–5 years was sufficient to predict the onset of AR and asthma at school age.

The underlying immunological mechanism of molecular spreading has not been further elucidated yet but most likely involves cellular events such as (i) the collateral Th2 priming, which is the induction of an antigen-specific Th2 response occurring due to a preprimed Th2 milieu [16], and (ii) the pump-priming effect, an IgE-dependent amplification loop favoring IgE production via secretion of interleukin-(IL-)4 and IL-13 [17]. Molecular spreading might explain the high incidences of polysensitization among allergic patients, estimated to range between 50 and 80% [18, 19]. Of note, the studies mentioned above describing the phenomenon of molecular spreading, refer to a birth cohort including only participants born in Germany in 1990, thus, limiting the translatability of the results to a global extent [12–14].

As sensitization profiles highly depend on genotype and allergen exposure and, accordingly, on the geographical location of exposed individuals, also the types of allergens and allergen sources involved in molecular spreading as well as their order differs greatly. In China, HDM is the most prevalent source of inhalant allergens in allergic patients during the first six years of life (approximately 25%), whereas cockroach sensitization takes over in patients aged 7 to 10 (>40%) and then remains the highest throughout lifetime [20]. Sensitization to other allergen sources such as dog dander and molds invariably stagnated around 5%. A longitudinal study monitoring the IgE response in Japanese children born just 10–15 years after the German cohort (in 2003–2005) revealed that at the age of 5 sensitizations to airborne allergens were most prevalent, despite having various types of allergens such as foods and insect venoms included in the multiplex screening [21]. The IgE responses were mostly directed against Japanese cedar Cry j 1, cat Fel d 1, dog Can f 1, as well as group 1 and 2 HDM allergens. The prevalence of IgE sensitization to these inhalant allergens further increased among 9-year-old individuals, although sensitizations to other allergens occurred at that age as well, for example, to Bet v 1, grass and weed pollen allergens. Specific molecular characteristics defining and making initiator allergens remain to be addressed, of which abundance and stability certainly play a contributing factor [22, 23]. If allergen-specific immunotherapy (AIT) against initiator allergens in children would prevent molecular spreading has not been addressed yet.

IgE and IgG repertoire to inhalant allergens

IgE responses towards inhalant allergens are often accompanied by an IgG response of the same specificity, although the evolution of IgG repertoire in general differs from IgE [12]. The IgG repertoire in individuals is primarily directed towards animal and vegetable food-borne molecules in the first decade of life, irrespective of an individual's atopic status.

Despite allergen-specific IgG antibodies representing one of the pillars of AIT efficacy by neutralizing allergen-binding to IgE, high subclinical levels of specific IgG4 are not considered to provide protection against allergy development [11]. This insufficiency in IgE-blocking raises the question how the IgG repertoire and binding affinity is modulated in the course of AIT. The majority of IgE and IgG directed against inhalant allergens are recognizing conformational epitopes [24, 25] and mapping thereof is a time- and labor-consuming endeavor and limited to complex methods such as computationally mapping, phage display arrays, inhibition studies, X-ray crystallography or nuclear magnetic resonance spectroscopy [26, 27].

An elegant indirect method was applied to characterize the IgE, IgG1, and IgG4 epitopes on Bet v 1 in 30 BP allergic patients, by grafting allergen surface patches onto a bacterial PR-10 homolog with high structural but low sequence similarity [24]. By using this low sIgE-binding protein as template for creating 13 chimeras—together covering the whole surface of Bet v 1—not only heterogenous patient-specific Ig-binding patterns were identified, but also binding differences between IgE and the IgG subclasses. While the IgGs were preferentially binding to certain surface patches, e.g., the IgG1 of 93% (28/30) of patients bound to a Bet v 1 patch containing a glycine-rich region called the p-loop, the IgEs tended to cover the whole allergen surface due to their polyclonal character. By grafting Bet v 1 surface patches onto the low-IgE binding PR-10 allergen from celery, Api g 1, the authors described that the IgE, IgG1, and IgG4 repertoire was hardly altered by AIT [28]. Nevertheless, the preference of IgG4 to recognize various Bet–Api chimeras appeared to expand over time. While Bet v 1-specific IgE levels remained rather constant throughout the study period, IgG1 and IgG4 increased after 1 or 6–18 months of treatment, respectively. The early increase of Bet v 1-specific IgG1 by BP AIT results in an IgG1-dominated blocking activity at the beginning of treatment, which is superseded by the blocking activity of IgG4 as AIT progresses [29]. Since also the blocking antibodies are mostly recognizing conformational epitopes, the question arises whether hypoallergenic fold-variants of major allergens, possessing an altered surface, are promising AIT vaccine candidates or would rather fail in inducing treatment efficacy to a similar extent as the wild-type (wt) molecules [30].

Table 2 In vivo reported auto-adjuvant activity of major inhalant allergens

Source	Allergen	Protein family	Size (kDa)	Reference
European house dust mite (*Dermatophagoides pteronyssinus*)	Der p 1	Papain-like cysteine protease	25	[44]
	Der p 2[a]	Group 2 mite allergen	15	[45]
Short ragweed (*Ambrosia artemisiifolia*)	Amb a 1	Pectate lyase	38	[49]
	Amb a 11[b]	Papain-like cysteine protease	28	[50]
Timothy grass (*Phleum pratense*)	Phl p 5	Group 5 grass pollen allergens	32	[53]

Information about the protein family and size (molecular weight assessed via SDS-PAGE) were extracted from Allergen.com
kDa kilodalton
[a]Auto-adjuvant activity only in presence of LPS by mimicking the TLR4 co-receptor MD-2
[b]Auto-adjuvant activity only shown in immunization model with additional adjuvant

The aeroallergen's intrinsic auto-adjuvant features

The abundance, solubility, size, posttranslational modification, stability, and ligand binding are common intrinsic characteristics of allergens (Table 2) and suggested to contribute to their allergenicity [22, 23, 31, 32]. However, the necessity of allergens to rely on individual intrinsic features to initiate Th2 polarization and allergic sensitization seem rather unlikely and has so far not sufficiently been studied.

To this end, the inherent adjuvant features, which empower a protein with its full allergenic potential should be addressed and investigated [33, 34]. This so-called auto-adjuvant activity of an allergen is defined by its ability to induce a predominant Th2 polarization, thus, promoting the initiation of allergic sensitization with the production of allergen-specific IgEs independently of additional immune modulating factors [35, 36].

Among the reported features enabling allergens to stimulate the immune system, the most described include the activation of various pattern recognition receptors, such as protease-activated receptors and toll-like receptors (TLRs) [37, 38] expressed on innate immune cells and antigen-presenting cells (APCs). Via their enzymatic or proteolytic activity, allergens can damage epithelial tight-junctions, thus, facilitating allergen entry and the induction of damage signals, so-called damage-associated molecular patterns (DAMPs) that shape the consequent immune response [39–41]. The frequently described ability of allergens to bind small bioactive molecules, especially lipids from microbial or of intrinsic origin, is an important asset for the initiation of allergic immune responses [32, 42]. Nevertheless, the auto-adjuvant activity was only demonstrated for a few inhalant allergens resulting in in vivo allergic sensitization with allergen-specific IgE production.

House dust mite allergens

Just a few studies demonstrated a direct involvement of major allergens such as Der p 1 and Der p 2, in allergic sensitization, without additional adjuvant. The role of the proteolytic activity of Der p 1 is well studied [43]. More recently, in an intraperitoneal (i.p.) in vivo model the inhibition of Der p 1 in HDM extracts elicited a reduced eosinophil and neutrophil infiltration into bronchoalveolar lavage fluids (BALF) as well as decreased allergen-specific serum IgE and IgG1 compared to mice sensitized with extract in which Der p 1 was not inhibited [44]. This demonstrates the direct role of Der p 1 protease activity in the development of allergic airway inflammation. The structural homology to myeloid differentiation factor-2 (MD-2), the coreceptor of TLR4, enables the major allergen Der p 2 to bind lipopolysaccharides (LPS) and, thus, to induce TLR4 signaling. MD-2-deficient mice developed allergic asthma when sensitized and challenged with recombinantly produced (r)Der p 2 in the presence of LPS [45, 46]. However, since LPS is needed for these adjuvant activities of Der p 2, the whole context in which the allergens are delivered is necessary for sensitization to HDM allergens remains unclear. HDM extracts, prepared from whole mites, mite bodies or feces, are naturally contaminated not only with LPS but also with a large variety of microbial and fungal immunostimulatory substances [47]. Such contaminants in the extract are potential confounders in studies investigating the direct interactions of major allergens with the innate immune system [48].

Pollen allergens

Ragweed pollen allergens

The sensitizing potential of the major RP allergen Amb a 1 was reported in an in vivo sensitization model in which the purified isoform Amb a 1.01 induced a faster and stronger IgG1, IgG2a, and IgE response than the isoforms 1.02 and 1.03, also in the absence of aluminum hydroxide (alum) [49]. Another major RP allergen is the cysteine protease Amb a 11, accounting for sensitization rates of up to 69% among RP allergic patients [50]. The allergenicity of Amb a 11 was investigated in a murine i.p. sensitization model. Mice sensitized with rAmb a 11 adsorbed to alum displayed a strong Th2 allergic airway inflammation upon challenge with RP extract (RPE), including increased airway hyperresponsiveness (AHR), eosinophil and ILC2 infiltrates in BALF, as well as elevated Amb a 11-specific serum IgE and

IgG1. The involvement of the protease activity in the induction of airway inflammation and production of specific IgE was assessed by irreversibly inhibiting the enzyme prior to treatment. Immunization without additional adjuvant was not performed but would support the assessment of the auto-adjuvant activity of Amb a 11.

Grass pollen allergens

Although Phl p 1 is a major and initiator allergen of timothy grass (*Phleum pratense*) pollen, its proteolytic activity in natural and recombinant form is controversial in the literature. The enzymatic activity and additional functional characteristics of Phl p 1 was mainly investigated regarding its capacity to activate airway epithelial cells [51]. Phl p 1 purified from GP was tested for its contributing features to the development of allergic immune responses. Therefore, a detailed mRNA and protein analysis of stimulated human airway epithelial cells via the microarray approach was performed to compare the feature of Phl p 1 to the respective grass pollen extract (GPE) [52]. The findings showed that Phl p 1 can activate airway epithelial cells by modulating the expression of allergy-associated pro-inflammatory cytokines, such as IL-1A, IL-6, IL-8 and transforming growth factor (TGF)-β on mRNA and protein levels [51]. It should be noted that contamination by small immunomodulatory compounds in the natural Phl p 1 preparation used in this study cannot be ruled out. Thus, it remains unclear whether this activity is determinant for the capacity of Phl p 1 to induce Th2 polarization and IgE production by itself or relies on the contribution of other pollen matrix-derived factors.

More recently, we assessed the auto-adjuvant function of the major GP allergen Phl p 5 [53]. rPhl p 5, without any added adjuvant, induced an allergen-specific IgE response in a 44-days-long intradermal (i. d.) sensitization model accompanied by increased IL-4 secretion in restimulated splenocytes from rPhl p 5-sensitized mice compared to naïve mice. The intrinsic features and mechanisms underlying the auto-adjuvant activity of Phl p 5 should be further investigated.

The adjuvant activity of the matrix

Although inherent adjuvant features of allergens can favor the allergen's entry and endow it with immunostimulatory properties, there is no clear evidence for a consistent determinant of allergenicity. It therefore remains questionable to what extent most aeroallergens elicit an aberrant Th2 and IgE immune response on their own. Growing evidence challenges the allergen-centered view by presenting a novel concept taking into consideration the adjuvanticity of the allergenic source context.

According to this alternative allergenicity concept, allergens represent only a small portion of much more complex biologic sources carried by airborne particles [54], harboring a myriad of diverse bioactive molecules delivered alongside the allergens during exposure. Certain non-allergenic bioactive compounds derived from airborne allergen sources are well-described for their adjuvant properties and promoting Th2 polarization, allergic sensitization, and airway inflammation [34, 40].

House dust mite matrix

The possible contribution of co-delivered bioactive compounds in HDM extracts to the onset and progression of the allergic disease is still largely unknown. In this regard, the natural polysaccharide chitin, part of the mite's exoskeleton and a well-known pathogen-associated molecular pattern (PAMP), was shown to stimulate TLR2 on murine peritoneal macrophages in vitro resulting in the production of IL-17 [55]. In vitro, chitin enhances IL-4 and IL-13 production in restimulated splenocytes of mice treated intranasally with ovalbumin (OVA) supplemented with chitin in comparison to OVA alone. In vivo, IL-5 and IL-13 levels in BALF were elevated upon treatment with chitin-spiked OVA in comparison to OVA only [56], which is in accordance with another study, where OVA challenge in mice sensitized with OVA plus chitin caused an eosinophil-rich pulmonary inflammatory response and IL-4, IL-5, and IL-13 production in the BALF [57]. Interestingly, IL-33 is required for inducing OVA-induced airway eosinophilia in the presence of chitin, while IL-25 and TSLP are not, as observed by a significant decrease in the number of eosinophils in BALFs and the production of IL-13, but not IL-4, in OVA-stimulated splenocytes from mice lacking IL-33 [56]. In mice, intranasal (i.n.) sensitization to HDM resulting in airway inflammation occurred also in an IL-33-dependent manner, suggesting a link between the potential adjuvant effect of chitin and the sensitizing potential of HDM [58]. Until now, there is no clear evidence that the auto-adjuvant activity of major HDM allergens such as Der p 1 is dependent of IL-33, even though Der p 1 was shown to regulate cytokine activity of IL-33 through cleavage of its sensor domain [59]. Using papain for sensitization with the same protease activity as Der p 1, papain-specific IgE and IgG1 in the serum and eosinophil number in the BALF was dramatically reduced in IL-33-deficient mice in comparison to wt mice, but studies investigating Der p 1 in this context are missing [60]. These results indicate that chitin can act as an adjuvant modulating allergic immune responses. However, to investigate the role of chitin in the sensitization process to HDM allergens, additional studies are needed, in which, for example, chitin is depleted or neutralized in HDM extracts prior to sensitization in appropriate mouse models.

β -(1,3)-glucan (β-glucan) is another PAMP, mainly found in the cell wall of fungi, but also of bacteria, plants, and in fecal pellets of HDM, that has been associated with allergic respiratory inflamma-

tory responses. Although β-glucan can interact with dectin-1, a C-type lectin receptor resulting in different immunostimulatory properties, its contribution in the development of allergic diseases remains unclear [61]. In vitro, the induction of chemokine (C-C motif) ligand 20 (CCL20), crucial for the recruitment of immature dendritic cells (DCs) to the lung in allergic airway inflammation, is dependent on dectin-1, since dectin-1 inhibition in epithelial cells and degradation of β-glucan via β-glucanase in HDM extracts resulted in a decreased level of CCL20 in culture supernatants of human epithelial cells. Interestingly, this effect was HDM-specific, since neither exposure to RPE, cockroach extract nor OVA to epithelial cells resulted in increased CCL20 levels [61]. In vivo, HDM-induced airway inflammation was reduced in dectin-1-deficient mice in comparison to wt mice [62]. Dectin-1 expressed on CD11b+ DCs was important for their migration into the mediastinum lymph nodes (MLNs), as the expression of chemokine receptors, including CCR7, was decreased in dectin-1-deficient DCs resulting in an increase in CD11b+ DCs in MLNs compared to wt mice. This was shown by DC migration assays with cells isolated from the lung and MLNs from mice treated with labeled HDM extracts. The adjuvant activity exhibited by highly purified β-glucan from *Saccharomyces cereviseae* was shown in vivo, as eosinophilic and Th2 responses (higher levels of IL-4, IL-5, IL-13, and IL-17) were exacerbated in BALFs collected from C57 BL/6 mice intratracheally sensitized with HDM extract as well as β-glucan in comparison to mice sensitized with HDM alone [63]. Similar results were obtained when sensitizing the mice to HDM solely through the airways or inhibiting dectin-1 with a dectin-1-blocking antibody in combination with HDM extract, which was associated with a decreased IL-33 production by epithelial cells and a reduction in IL-13+ ILC2 cells in BALFs after HDM exposure [64]. Remarkably, instead of β-glucan as the ligand of dectin-1, they identified Der p 10, a mite tropomyosin as a ligand via affinity purification followed by mass spectrometry analysis using HDM extracts and a recombinant extracellular domain human fusion dectin-1-Fc. Coimmunoprecipitation of dectin-1-Fc with other allergenic sources such as alder, peanut and shrimp showed that dectin-1 specifically binds to invertebrate tropomyosin from shrimp and mites, but not to plant-derived tropomyosin. Moreover, they found a single-nucleotide polymorphism associated with decreased dectin-1 expression in patients suffering from asthma or chronic rhinosinusitis.

Pollen matrix

Several immunostimulatory properties of specific pollen-derived compounds, such as lipids and other small molecules and their contribution to innate im-

Table 3 Bioactive compounds derived from the allergen sources reported to display immunostimulatory and/or adjuvant activities in the context of allergic sensitization

Source	Extrinsic compound/co-delivered bioactive molecule	Classification	Reported activity (interactions with the immune system or adjuvant function)	Reference
European house dust mite (*Dermatophagoides pteronyssinus*)	Chitin	Polysaccharide	Adjuvant effect in several mouse models using OVA for immunization	[48, 56, 57]
			Increased Th2-dominated inflammation	
	β-(1,3)-glucan	Polysaccharide	DC migration during allergic responses	[61–63]
Birch pollen (*Betula pendula*)	TLR4 agonist	Lipid	DC activation	[66]
	IL-4Rα agonist	HMW protein of aqueous pollen extract	Binding to human IL-4Rα	[67]
Mugwort pollen (*Artemisia vulgaris*)	LPS	Gram-negative bacteria-derived endotoxin	Presence of LPS essential for allergic sensitization and lung inflammation in mice	[80]
Birch pollen, short ragweed pollen (*Ambrosia artemisiifolia*), Timothy grass pollen (*Phleum pratense*)	PALM (e.g., E1 phytoprostane)	Lipids	Attraction and activation of human eosinophils and neutrophils in vitro	[22, 74, 77]
			Downregulation of Th1-associated cytokines (IL-12)	
			IL-6 release, murine mast cell chemotaxis and enhanced IgE-dependent degranulation	
			Enhanced IgE production	
	Adenosine	Metabolite	Inhibition of IL-12p70 production by DCs	[68, 70]
			Adenosine-mediated priming of Tregs by DCs derived from non-atopic donors treated with BPE	
			Human neutrophil and eosinophil migration towards RPE-stimulated supernatants of bronchial epithelial cells	
			Adenosine aggravated allergic lung inflammation in vivo	
	LMW (below 3 kDa) fraction of aqueous pollen extract	n. d.	Induction of expression of Th2-associated notch ligands on immature monocyte-derived DCs	[73, 74]
			Enhancement of IgE production	

TLR4 Toll-like receptor 4, *IL-4Rα* interleukin 4 receptor, *HMW* High molecular weight, *kDa* kilodalton, *LPS* Lipopolysaccharide, *PALM* pollen-associated lipid mediator, *LMW* low molecular weight, *n. d.* not determined

mune activation, Th2 polarization, and allergic airway inflammation have been described (Table 3; [32, 34]).

Birch pollen matrix

A typical example in which specific pollen-derived compounds were shown to be essential for Th2 polarization and allergic airway inflammation is BP and its major allergen, Bet v 1. Although it accounts for approximately 95% of the sensitization rate in BP-sensitized individuals, in vivo findings performed in IL-4 reporter mice showed the inability of rBet v 1 to induce Th2 polarization after i.d. immunization without adjuvant [65]. In contrast, an aqueous birch pollen extract (BPE) efficiently increased the percentage of IL-4+CD4+ cells in the inguinal skin-draining lymph nodes even when Bet v 1 was depleted from the extract. These data clearly supported the notion that compounds within the pollen matrix can empower Bet v 1 with its full Th2-polarizing capacity. In vitro, activation of murine bone marrow-derived (BM)DCs and human monocyte-derived DCs by BPE was demonstrated to be TLR4-dependent. The identification of potential BP-derived ligands was recently investigated, suggesting the involvement of TLR4 agonist(s) other than the well-known endotoxin LPS, as lipid- (but not protein-) depleted BPE fraction partially lost the ability to activate BMDC in vitro, evaluated by the upregulation of co-stimulatory markers and cytokine secretion [66]. Our research group also identified an IL-4Rα ligand of protein nature contained in the high molecular weight (HMW) fraction of BPE but not of RPE and GPE. The HMW BPE fractions had an enriched capacity to bind to the recombinant human IL-4Rα via ELISA compared to the total BPE and induced IL-4 expression in CD4+ T cells in IL-4 reporter mice to the same extent as BPE. The characterization of the putative IL-4 mimic is currently under investigation [67].

Ragweed pollen matrix

In contrast to the previously mentioned auto-adjuvant activity of the major short RP allergen Amb a 1 [49], another study showed that Amb a 1 is not able to reproduce the full sensitization potential of RPE [68]. In this in vivo allergic airway inflammation model via i.n. instillation, Amb a 1 was screened regarding its pro-inflammatory and sensitizing potential, without additional adjuvant, and compared to a whole RPE. Eleven instillations of RPE elicited neutrophil, eosinophil, and lymphocyte infiltration in the BALF, Amb a 1-specific serum IgG1, increased AHR and Th2-associated cytokine secretion in restimulated splenocytes compared to non-sensitized mice. Mice immunized with Amb a 1 alone or in combination with a low molecular weight (LMW) fraction, containing components below 3 kDa, previously suggested to display adjuvant properties [69], showed significantly less eosinophil and lymphocyte lung infiltration, and no Amb a 1-specific IgG1 serum. Locally, mRNA expression levels of IL-4, IL-5, IL-13, and IL-10, but not of interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα), were detected in digested lung tissues of RPE-treated mice, whereas Amb a 1 and Amb a 1 plus LMW fraction did not modulate these parameters. This study highlights the contribution of yet unknown RP-derived factors beside the major allergen to induce a full allergic airway inflammation. However, convincing data supporting a pro-sensitizing function for the RP matrix is still lacking since measurements of allergen-specific IgE were not performed. The divergent results between the studies can likely be explained by the immunization routes, i.n. instillation [68], and subcutaneous (s.c.) injections [49], which influence the immunogenicity of Amb a 1, determined by its capacity to induce specific IgG1 and IgG2a.

Adenosine, a metabolite and immunomodulator contained in pollen, was shown to have opposing functions depending on the stage of the allergic disease development in an i.n. mouse model using adenosine depleted RPE. Lack of adenosine led to an enhanced secretion of Th2-associaed cytokines compared to the whole RPE after i.n. instillation of naïve mice, suggesting a protective effect of the metabolite, while adenosine rather displayed a pro-inflammatory effect in the effector phase [70].

Grass pollen matrix

By comparing the effect of a complete timothy GPE to that of Phl p 1 on airway epithelial cells [51], the allergen induced a lower regulation of cadherin-1 gene, an important adhesion molecule for epithelial cells, compared to that induced by the whole extract. This implies that also for GP additional non-allergenic factors of the matrix, here proteases, are involved in the destruction of epithelial barriers, thus facilitating allergen entry.

In the same study demonstrating the auto-adjuvant capacity of rPhl p 5 in a s.c. in vivo model [53], the allergen was unable to induce IL-4+CD4+ cells in a 5-day adjuvant-free i.d. reporter mouse model, whereas GPE induced strong IL-4 expression in CD4+ lymphocytes. These data further highlight the crucial role of the accompanying components within the allergenic source in the orchestration of allergic sensitization via their interaction with immune cells and initiation of a Th2-biased response.

Eicosanoid-like molecules found in the low molecular weight fractions of aqueous pollen extracts including timothy grass, birch, and ragweed pollen, called pollen-associated lipid mediators (PALMs), were reported to act as adjuvants by favoring the polarization of Th2 responses in different ways [71]. PALMs can influence the function of DCs by dictating the Th1/Th2 balance in vitro via inhibition of LPS-induced IL-12p70 secretion [72], or by up-regulating the expression of Th2-associated notch ligands on the cell surface [73]. The LMW fraction of RPE aug-

mented the production of IgE antibodies by murine B cells stimulated in vitro with anti-CD40 and IL-4 to the same extent as total RPE, as well as the PALM phytoprostane E1 (PPE1) detected in the LMW fraction [74]. Increased IgE production induced by RPE was therefore suggested to rely on non-allergenic LMW compounds; however, in the study no ragweed-specific IgE could be detected. Moreover, PALMs can attract and activate innate immune cells such as eosinophils and neutrophils in vitro implying the involvement of these non-allergenic factors in the development of the allergic disease [75–77]. Nonetheless, studies investigating the role of specific PALMs in the establishment of allergen-specific Th2 and IgE responses will be essential to confirm their relevance in allergen-induced sensitization.

Pollen microbiome

Allergenic sources, including pollen, are naturally contaminated with microbes. The pollen microbiome represents an important part of the extrinsic composition of allergenic sources and was frequently considered as an important adjuvant for the initiation of allergic airway inflammation and sensitization [34]. In this sense, the role of TLR4 signaling and LPS in allergic diseases [78] and the concept of the hygiene hypothesis [79] are largely discussed. In vivo, the capacity of mugwort pollen (*Artemisia vulgaris*) to induce allergic sensitization in an i.n. model without added adjuvant was suggested to rely on the presence of endotoxin in the aqueous extract [80]. The extract with higher LPS level triggered stronger eosinophil, neutrophil, and lymphocytes BALF infiltration, increased lung hyperresponsiveness upon methacholine challenge, and increased mugwort pollen-specific IgG1 antibodies compared to mice immunized with PBS or with low LPS-containing extract.

Concluding remarks

Major inhalant allergens have been the focus of allergy research for decades, which is justified as they represent the IgE targets resulting in allergic symptoms, and important initiators of allergic sensitization. This prompted us to dissect whether these allergens possess their own (auto-)adjuvant activity or rely on the adjuvant role of certain non-allergenic compounds co-delivered with the allergen source. So far, only a few inhalant allergens (Der p 1, Amb a 1, and Phl p 5) were described in the context of an auto-adjuvant activity in vivo. Especially the cooperative assessment of the Th2-polarzing adjuvant function of major allergens and their sources is essential. In this regard, physiologically relevant in vivo models are indispensable to assess the complex molecular and cellular pathways leading to adaptive immune responses. More research is needed to better understand the synergy between allergens, their sources, and the immune network that determine the development of allergic diseases: a framework that will certainly help to further answer the question: “What an inhalant allergen can do and not do?”

Funding The research was supported by Austrian Science Funds (Projects P34207 and I5312), by the University of Salzburg priority program Allergy-Cancer-BioNano Research Center, and by the Doctoral School Program Biomolecules of the University of Salzburg.

Author Contribution Each author has participated in writing the manuscript. LA prepared the figure using Biorender (Agreement number: CD25CUBWQ8). SGK and LP prepared the tables. All authors read and approved the final manuscript.

Funding Open access funding provided by Paris Lodron University of Salzburg.

Conflict of interest S. Grosse-Kathoefer, L. Aglas, F. Ferreira and L. Pointner declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Passali D, Cingi C, Staffa P, Passali F, Muluk NB, Bellussi ML. The international study of the allergic rhinitis survey: outcomes from 4 geographical regions. Asia Pac Allergy. 2018;8:e7. https://doi.org/10.5415/apallergy.2018.8.e7.
2. Pawankar C, Holgate L. WAO white book on allergy: update 2013. World Allergy Organization; 2013.
3. Pakkasela J, Ilmarinen P, Honkamäki J, Tuomisto LE, Andersén H, Piirilä P, et al. Age-specific incidence of allergic and non-allergic asthma. BMC Pulm Med. 2020;20:9. https://doi.org/10.1186/s12890-019-1040-2.
4. Heinzerling LM, Burbach GJ, Edenharter G, Bachert C, Bindslev-Jensen C, Bonini S, et al. GA(2)LEN skin test study I: GA(2)LEN harmonization of skin prick testing: novel sensitization patterns for inhalant allergens in Europe. Allergy. 2009;64:1498–506. https://doi.org/10.1111/j.1398-9995.2009.02093.x.
5. Panzner P, Vachová M, Vítovcová P, Brodská P, Vlas T. A comprehensive analysis of middle-European molecular sensitization profiles to pollen allergens. Int Arch Allergy Immunol. 2014;164:74–82. https://doi.org/10.1159/000362760.
6. Burbach GJ, Heinzerling LM, Röhnelt C, Bergmann K-C, Behrendt H, Zuberbier T. Ragweed sensitization in Europe—GA(2)LEN study suggests increasing prevalence. Allergy. 2009;64:664–5. https://doi.org/10.1111/j.1398-9995.2009.01975.x.

7. Chen X, Corry DB, Li E. Mechanisms of allergy and adult asthma. Curr Opin Allergy Clin Immunol. 2020;20:36–42. https://doi.org/10.1097/ACI.0000000000000601.
8. Albrecht M, Dittrich A-M. Cytokines in sensitization to aeroallergens. Allergol Sel. 2018;2:94–100. https://doi.org/10.5414/ALX1480E.
9. Corsico AG, de Amici M, Ronzoni V, Giunta V, Mennitti MC, Viscardi A, et al. Allergen-specific immunoglobulin E and allergic rhinitis severity. Allergy Rhinol (Providence). 2017;8:1–4. https://doi.org/10.2500/ar.2017.8.0187.
10. Nur Husna SM, Md Shukri N, Tan H-TT, Mohd Ashari NS, Wong KK. Higher wheal sizes of dermatophagoides farinae sensitization exhibit worse nasal symptoms in allergic rhinitis patients. Front Med (Lausanne). 2022;9:843432. https://doi.org/10.3389/fmed.2022.843432.
11. Xu Q, Jiang Q, Yang L, Li W, Huang N, Yang Y, et al. IgE and IgG4 repertoire in asymptomatic HDM-sensitized and HDM-induced allergic rhinitis patients. Int Arch Allergy Immunol. 2021;182:1200–11. https://doi.org/10.1159/000517824.
12. Huang X, Tsilochristou O, Perna S, Hofmaier S, Cappella A, Bauer C-P, et al. Evolution of the IgE and IgG repertoire to a comprehensive array of allergen molecules in the first decade of life. Allergy. 2018;73:421–30. https://doi.org/10.1111/all.13269.
13. Posa D, Perna S, Resch Y, Lupinek C, Panetta V, Hofmaier S, et al. Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life. J Allergy Clin Immunol. 2017;139:541–549.e8. https://doi.org/10.1016/j.jaci.2016.08.014.
14. Hatzler L, Panetta V, Lau S, Wagner P, Bergmann RL, Illi S, et al. Molecular spreading and predictive value of preclinical IgE response to Phleum pratense in children with hay fever. J Allergy Clin Immunol. 2012;130:894–901.e5. https://doi.org/10.1016/j.jaci.2012.05.053.
15. Posa D, Hofmaier S, Arasi S, Matricardi PM. Natural evolution of IgE responses to mite allergens and relationship to progression of allergic disease: a review. Curr Allergy Asthma Rep. 2017;17:28. https://doi.org/10.1007/s11882-017-0697-y.
16. Eisenbarth SC, Zhadkevich A, Ranney P, Herrick CA, Bottomly K. IL-4-dependent Th2 collateral priming to inhaled antigens independent of Toll-like receptor 4 and myeloid differentiation factor 88. J Immunol. 2004;172:4527–34. https://doi.org/10.4049/jimmunol.172.7.4527.
17. Gould HJ, Sutton BJ. IgE in allergy and asthma today. Nat Rev Immunol. 2008;8:205–17. https://doi.org/10.1038/nri2273.
18. Beutner C, Forkel S, Gupta S, Fuchs T, Schön MP, Geier J, Buhl T. Sex- and age-dependent changes in polysensitization to common aeroallergens over 20 years. J Asthma Allergy. 2020;13:725–30. https://doi.org/10.2147/JAA.S280771.
19. Migueres M, Dávila I, Frati F, Azpeitia A, Jeanpetit Y, Lhéritier-Barrand M, et al. Types of sensitization to aeroallergens: definitions, prevalences and impact on the diagnosis and treatment of allergic respiratory disease. Clin Transl Allergy. 2014;4:16. https://doi.org/10.1186/2045-7022-4-16.
20. Luo W, Chen H, Wu Z, Hu H, Tang W, Chen H, et al. A new trend in sensitization to cockroach allergen: A cross-sectional study of indoor allergens and food allergens in the inland region of Southwest China. Asian Pac J Allergy Immunol. 2020; https://doi.org/10.12932/AP-281019-0678.
21. Yamamoto-Hanada K, Borres MP, Åberg MK, Yang L, Fukuie T, Narita M, et al. IgE responses to multiple allergen components among school-aged children in a general population birth cohort in Tokyo. World Allergy Organ J. 2020;13:100105. https://doi.org/10.1016/j.waojou.2020.100105.
22. Soh WT, Aglas L, Mueller GA, Gilles S, Weiss R, Scheiblhofer S, et al. Multiple roles of Bet v 1 ligands in allergen stabilization and modulation of endosomal protease activity. Allergy. 2019;74:2382–93. https://doi.org/10.1111/all.13948.
23. Foo ACY, Mueller GA. Abundance and stability as common properties of allergens. Front Allergy. 2021;2:769728. https://doi.org/10.3389/falgy.2021.769728.
24. Schmalz S, Mayr V, Shosherova A, Gepp B, Ackerbauer D, Sturm G, et al. Isotype-specific binding patterns of serum antibodies to multiple conformational epitopes of Bet v 1. J Allergy Clin Immunol. 2022;149:1786–1794.e12. https://doi.org/10.1016/j.jaci.2021.10.026.
25. Figo DD, Cordeiro Macedo PR, Gadermaier G, Remuzgo C, Castro FFM, Kalil J, et al. IgE and IgG4 epitopes of dermatophagoides and blomia allergens before and after sublingual immunotherapy. Int J Mol Sci. 2023; https://doi.org/10.3390/ijms24044173.
26. Valente AP, Manzano-Rendeiro M. Mapping conformational epitopes by NMR spectroscopy. Curr Opin Virol. 2021;49:1–6. https://doi.org/10.1016/j.coviro.2021.04.001.
27. Breiteneder H. Mapping of conformational IgE epitopes of food allergens. Allergy. 2018;73:2107–9. https://doi.org/10.1111/all.13592.
28. Gepp B, Lengger N, Möbs C, Pfützner W, Radauer C, Bohle B, Breiteneder H. Monitoring the epitope recognition profiles of IgE, IgG1, and IgG4 during birch pollen immunotherapy. J Allergy Clin Immunol. 2016;137:1600–1603.e1. https://doi.org/10.1016/j.jaci.2015.10.022.
29. Strobl MR, Demir H, Sánchez Acosta G, Drescher A, Kitzmüller C, Möbs C, et al. The role of IgG1 and IgG4 as dominant IgE-blocking antibodies shifts during allergen immunotherapy. J Allergy Clin Immunol. 2023; https://doi.org/10.1016/j.jaci.2023.01.005.
30. Aglas L, Bethanis A, Chrusciel P, Stolz F, Gruen M, Jaakkola U-M, et al. In vivo induction of functional inhibitory IgG antibodies by a hypoallergenic bet v 1 variant. Front Immunol. 2020;11:2118. https://doi.org/10.3389/fimmu.2020.02118.
31. Halim A, Carlsson MC, Madsen CB, Brand S, Møller SR, Olsen CE, et al. Glycoproteomic analysis of seven major allergenic proteins reveals novel post-translational modifications. Mol Cell Proteomics. 2015;14:191–204. https://doi.org/10.1074/mcp.M114.042614.
32. Bublin M, Eiwegger T, Breiteneder H. Do lipids influence the allergic sensitization process? J Allergy Clin Immunol. 2014;134:521–9. https://doi.org/10.1016/j.jaci.2014.04.015.
33. Gandhi VD, Vliagoftis H. Airway epithelium interactions with aeroallergens: role of secreted cytokines and chemokines in innate immunity. Front Immunol. 2015;6:147. https://doi.org/10.3389/fimmu.2015.00147.
34. Pointner L, Bethanis A, Thaler M, Traidl-Hoffmann C, Gilles S, Ferreira F, Aglas L. Initiating pollen sensitization—Complex source, complex mechanisms. Clin Transl Allergy. 2020;10:36. https://doi.org/10.1186/s13601-020-00341-y.
35. 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. J Allergy Clin Immunol. 2008;121:847–852.e7. https://doi.org/10.1016/j.jaci.2008.01.025.
36. Kuehn A, Hilger C. Animal allergens: common protein characteristics featuring their allergenicity. Front Immunol. 2015;6:40. https://doi.org/10.3389/fimmu.2015.00040.

37. Alvarez-Simon D, Ait Yahia S, de Nadai P, Audousset C, Chamaillard M, Boneca IG, Tsicopoulos A. NOD-like receptors in asthma. Front Immunol. 2022;13:928886. https://doi.org/10.3389/fimmu.2022.928886.
38. Ma CH, He Q, Zhou LF. Toll-like receptors link atopic march and hygiene hypothesis. Chung Hua Chieh Ho Ho Hu Hsi Tsa Chih. 2022;45:803–8. https://doi.org/10.3760/cma.j.cn112147-20211206-00858.
39. Zhang J, Chen J, Robinson C. Cellular and molecular events in the airway epithelium defining the interaction between house dust mite group 1 allergens and innate defences. Int J Mol Sci. 2018; https://doi.org/10.3390/ijms19113549.
40. Abu Khweek A, Kim E, Joldrichsen MR, Amer AO, Boyaka PN. Insights into mucosal innate immune responses in house dust mite-mediated allergic asthma. Front Immunol. 2020;11:534501. https://doi.org/10.3389/fimmu.2020.534501.
41. Jacquet A, Robinson C. Proteolytic, lipidergic and polysaccharide molecular recognition shape innate responses to house dust mite allergens. Allergy. 2020;75:33–53. https://doi.org/10.1111/all.13940.
42. Scheurer S, Toda M, Vieths S. What makes an allergen? Clin Exp Allergy. 2015;45:1150–61. https://doi.org/10.1111/cea.12571.
43. Reithofer M, Jahn-Schmid B. Allergens with protease activity from house dust mites. Int J Mol Sci. 2017; https://doi.org/10.3390/ijms18071368.
44. Zhang J, Chen J, Zuo J, Newton GK, Stewart MR, Perrior TR, et al. Allergen delivery inhibitors: Characterisation of potent and selective inhibitors of der p 1 and their attenuation of airway responses to house dust mite allergens. Int J Mol Sci. 2018; https://doi.org/10.3390/ijms19103166.
45. Trompette A, Divanovic S, Visintin A, Blanchard C, Hegde RS, Madan R, et al. Allergenicity resulting from functional mimicry of a Toll-like receptor complex protein. Nature. 2009;457:585–8. https://doi.org/10.1038/nature07548.
46. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. Nat Med. 2009;15:410–6. https://doi.org/10.1038/nm.1946.
47. Klimov P, Molva V, Nesvorna M, Pekar S, Shcherbachenko E, Erban T, Hubert J. Dynamics of the microbial community during growth of the house dust mite dermatophagoides farinae in culture. FEMS Microbiol Ecol. 2019; https://doi.org/10.1093/femsec/fiz153.
48. Jacquet A. Characterization of innate immune responses to house dust mite allergens: pitfalls and limitations. Front Allergy. 2021;2:662378. https://doi.org/10.3389/falgy.2021.662378.
49. Wolf M, Twaroch TE, Huber S, Reithofer M, Steiner M, Aglas L, et al. Amb a 1 isoforms: Unequal siblings with distinct immunological features. Allergy. 2017;72:1874–82. https://doi.org/10.1111/all.13196.
50. Groeme R, Airouche S, Kopečný D, Jaekel J, Savko M, Berjont N, et al. Structural and functional characterization of the major allergen Amb a 11 from short ragweed pollen. J Biol Chem. 2016;291:13076–87. https://doi.org/10.1074/jbc.M115.702001.
51. Röschmann K, Farhat K, König P, Suck R, Ulmer AJ, Petersen A. Timothy grass pollen major allergen Phl p 1 activates respiratory epithelial cells by a non-protease mechanism. Clin Exp Allergy. 2009;39:1358–69. https://doi.org/10.1111/j.1365-2222.2009.03291.x.
52. Röschmann KIL, van Kuijen A-M, Luiten S, Jonker MJ, Breit TM, Fokkens WJ, et al. Purified Timothy grass pollen major allergen Phl p 1 may contribute to the modulation of allergic responses through a pleiotropic induction of cytokines and chemokines from airway epithelial cells. Clin Exp Immunol. 2012;167:413–21. https://doi.org/10.1111/j.1365-2249.2011.04522.x.
53. Araujo GR, Aglas L, Vaz ER, Machado Y, Huber S, Himly M, et al. TGFβ1 mimetic peptide modulates immune response to grass pollen allergens in mice. Allergy. 2020;75:882–91. https://doi.org/10.1111/all.14108.
54. Dramburg S, Hilger C, Santos A, de las Vecillas L, editors. Molecular allergology user's guide 2.0. The European Academy of Allergy and Clinical Immunology (EAACI); 2022.
55. Da Silva CA, Hartl D, Liu W, Lee CG, Elias JA. TLR-2 and IL-17A in chitin-induced macrophage activation and acute inflammation. J Immunol. 2008;181:4279–86. https://doi.org/10.4049/jimmunol.181.6.4279.
56. Arae K, Morita H, Unno H, Motomura K, Toyama S, Okada N, et al. Chitin promotes antigen-specific Th2 cell-mediated murine asthma through induction of IL-33-mediated IL-1β production by DCs. Sci Rep. 2018; https://doi.org/10.1038/s41598-018-30259-2.
57. Da Silva CA, Pochard P, Lee CG, Elias JA. Chitin particles are multifaceted immune adjuvants. Am J Respir Crit Care Med. 2010;182:1482–91. https://doi.org/10.1164/rccm.200912-1877OC.
58. Chu DK, Llop-Guevara A, Walker TD, Flader K, Goncharova S, Boudreau JE, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. J Allergy Clin Immunol. 2013;131:187–200.e8. https://doi.org/10.1016/j.jaci.2012.08.002.
59. Cayrol C, Duval A, Schmitt P, Roga S, Camus M, Stella A, et al. Environmental allergens induce allergic inflammation through proteolytic maturation of IL-33. Nat Immunol. 2018;19:375–85. https://doi.org/10.1038/s41590-018-0067-5.
60. Kamijo S, Takeda H, Tokura T, Suzuki M, Inui K, Hara M, et al. IL-33-mediated innate response and adaptive immune cells contribute to maximum responses of protease allergen-induced allergic airway inflammation. J Immunol. 2013;190:4489–99. https://doi.org/10.4049/jimmunol.1201212.
61. Nathan AT, Peterson EA, Chakir J, Wills-Karp M. Innate immune responses of airway epithelium to house dust mite are mediated through beta-glucan-dependent pathways. J Allergy Clin Immunol. 2009;123:612–8. https://doi.org/10.1016/j.jaci.2008.12.006.
62. Ito T, Hirose K, Norimoto A, Tamachi T, Yokota M, Saku A, et al. Dectin-1 plays an important role in house dust mite-induced allergic airway inflammation through the activation of CD11b+ dendritic cells. J Immunol. 2017;198:61–70. https://doi.org/10.4049/jimmunol.1502393.
63. Hadebe S, Kirstein F, Fierens K, Redelinghuys P, Murray GI, Williams DL, et al. β-Glucan exacerbates allergic airway responses to house dust mite allergen. Respir Res. 2016;17:35. https://doi.org/10.1186/s12931-016-0352-5.
64. Gour N, Lajoie S, Smole U, White M, Hu D, Goddard P, et al. Dysregulated invertebrate tropomyosin-dectin-1 interaction confers susceptibility to allergic diseases. Sci Immunol. 2018; https://doi.org/10.1126/sciimmunol.aam9841.
65. Aglas L, Gilles S, Bauer R, Huber S, Araujo GR, Mueller G, et al. Context matters: TH2 polarization resulting from pollen composition and not from protein-intrinsic allergenicity. J Allergy Clin Immunol. 2018;142:984–987.e6. https://doi.org/10.1016/j.jaci.2018.05.004.

66. Pointner L, Kraiem A, Thaler M, Richter F, Wenger M, Bethanis A, et al. Birch pollen induces toll-like receptor 4-dependent dendritic cell activation favoring T cell responses. Front Allergy. 2021;2:680937. https://doi.org/10.3389/falgy.2021.680937.
67. Pointner L, Adamkova V, Bethanis A, Gerkhardt S, Moelter L, Traidl-Hoffmann C, et al. Can birch pollen directly influence the IL-4/IL-4R interaction to modulate Th2 responses? Allergy. 2023; https://doi.org/10.1111/all.15673.
68. Wimmer M, Alessandrini F, Gilles S, Frank U, Oeder S, Hauser M, et al. Pollen-derived adenosine is a necessary cofactor for ragweed allergy. Allergy. 2015;70:944–54. https://doi.org/10.1111/all.12642.
69. Gilles-Stein S, Beck I, Chaker A, Bas M, McIntyre M, Cifuentes L, et al. Pollen derived low molecular compounds enhance the human allergen specific immune response in vivo. Clin Exp Allergy. 2016;46:1355–65. https://doi.org/10.1111/cea.12739.
70. Gilles S, Fekete A, Zhang X, Beck I, Blume C, Ring J, et al. Pollen metabolome analysis reveals adenosine as a major regulator of dendritic cell-primed T(H) cell responses. J Allergy Clin Immunol. 2011;127(9):454–461.e1. https://doi.org/10.1016/j.jaci.2010.12.1082.
71. Gilles S, Mariani V, Bryce M, Mueller MJ, Ring J, Behrendt H, et al. Pollen allergens do not come alone: pollen associated lipid mediators (PALMS) shift the human immune systems towards a T(H)2-dominated response. Allergy Asthma Clin Immunol. 2009;5:3. https://doi.org/10.1186/1710-1492-5-3.
72. Traidl-Hoffmann C, Mariani V, Hochrein H, Karg K, Wagner H, Ring J, et al. Pollen-associated phytoprostanes inhibit dendritic cell interleukin-12 production and augment T helper type 2 cell polarization. J Exp Med. 2005;201:627–36. https://doi.org/10.1084/jem.20041065.
73. Gilles S, Beck I, Lange S, Ring J, Behrendt H, Traidl-Hoffmann C. Non-allergenic factors from pollen modulate T helper cell instructing notch ligands on dendritic cells. World Allergy Organ J. 2015;8:2. https://doi.org/10.1186/s40413-014-0054-8.
74. Oeder S, Alessandrini F, Wirz OF, Braun A, Wimmer M, Frank U, et al. Pollen-derived nonallergenic substances enhance Th2-induced IgE production in B cells. Allergy. 2015;70:1450–60. https://doi.org/10.1111/all.12707.
75. Behrendt H, Kasche A, Traidl C, Plötz S, Huss-Marp J, Risse U, et al. Pollen grains contain and release not only allergens, but also eicosanoid-like substances with neutrophil chemotactic activity: a new step in the initiation of allergie sensitization? In: Ring J, Behrendt H, editors. New trends in allergy V. Berlin, Heidelberg: Springer; 2002. pp. 3–8. https://doi.org/10.1007/978-3-642-55994-5_1.
76. Traidl-Hoffmann C, Kasche A, Jakob T, Huger M, Plötz S, Feussner I, et al. Lipid mediators from pollen act as chemoattractants and activators of polymorphonuclear granulocytes. J Allergy Clin Immunol. 2002;109:831–8. https://doi.org/10.1067/mai.2002.124655.
77. Plötz SG, Traidl-Hoffmann C, Feussner I, Kasche A, Feser A, Ring J, et al. Chemotaxis and activation of human peripheral blood eosinophils induced by pollen-associated lipid mediators. J Allergy Clin Immunol. 2004;113:1152–60. https://doi.org/10.1016/j.jaci.2004.03.011.
78. Kumar S, Adhikari A. Dose-dependent immunomodulating effects of endotoxin in allergic airway inflammation. Innate Immun. 2017;23:249–57. https://doi.org/10.1177/1753425917690443.
79. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis. Nat Immunol. 2017;18:1076–83. https://doi.org/10.1038/ni.3829.
80. Oteros J, Bartusel E, Alessandrini F, Núñez A, Moreno DA, Behrendt H, et al. Artemisia pollen is the main vector for airborne endotoxin. J Allergy Clin Immunol. 2019;143:369–377.e5. https://doi.org/10.1016/j.jaci.2018.05.040.