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Pollen-induced asthma: a specific pheno-endotype of disease?

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Summary

Asthma is a heterogeneous syndrome with a significant social and economic impact. While the knowledge of pheno-endotypes has advanced in severe asthma, little attention has been paid to the phenotypes of mild-moderate asthma. Along this line, a systematic review of the current literature on pollen-induced asthma was carried out, targeting the question whether it can be considered a specific phenotype of disease, with a focus on the role of pollen and its interplay with asthma.

This article reports the first part of the review, which covered background information on the multiple atmospheric and environmental factors affecting pollen concentration, the molecular bases of pollen-induced allergenicity and the pathogenic effector circuits that sustain and amplify inflammatory signals in response to allergens in sensitized subjects.

IMPACT STATEMENT

Pollen-induced asthma could be considered a specific phenotype. Pollen allergenicity depends not only on genetic and environmental factors, but also on the immunostimulatory components of the pollen matrix, that contribute to airway disease and may represent a defining feature of allergic asthma.

Introduction

Currently, asthma is no longer considered a single disease but a complex and heterogeneous syndrome that includes variable clinical presentations (phenotypes) and specific pathophysiological mechanisms (endotypes) (1-6).

Asthma impacts over 300 million individuals of all ages worldwide, with a high count of disabilities, and premature deaths (7). According to the Global Burden of Disease (GBD), asthma is the second leading cause of death among chronic respiratory diseases, with 457.01 thousand deaths in 2017 (7, 8). Asthma is often associated with various comorbidities, such as allergic rhinitis, nasal polyps, gastroesophageal reflux disease, obstructive sleep apnea, and anxiety, leading to increased morbidity and seriously affecting patients' quality of life (7).

Prevalence data on asthma are important for the understanding of the clinical and economic burden of the disease. However, the estimation of the epidemiology of asthma at global level is challenging, because of the complex nature of the disease and the lack of universally accepted case definition and tests that are confirmatory for asthma (9-11). The results from a systematic analysis of the literature, including data extracted from the Global Burden of Diseases, Injuries and Risk Factors Study 2019, show considerable variation across countries in the estimation of asthma prevalence, ranging from 1.43 to 11.25% (10). Regarding incidence, an increase was observed globally over the 30-year period 1990-2019, which occurred especially in Africa countries, with the number rising from 6,487,957.18 (95 %UI: 4,578,735.08-8,736,387.55) to 7,604,488.39 (95% UI: 5,428,024.98-10,177,808.25) (7, 12). Mortality for asthma in adolescent and young adults has exhibited a consistent downward trend over a period of 30 years, which may be linked to improved asthma management. However, areas with lower socio-demographic index have higher age-standardized mortality rates for asthma and deserve attention and priority support for medical resources.

Allergic asthma, usually defined as asthma associated with sensitization to by otherwise harmless environmental substances, i.e. allergens (as pollen, fungal spores, animal hair, house dust mite), is the most common asthma phenotype (13, 14). It is estimated that up to 80% of childhood asthma and more than 50% of adult asthma cases may have an allergic component (15, 16). Molecular studies by Kaur *et al.* (2019) also found that T2 signature, with high sensitization to allergens, increased airway and blood eosinophils and good response to ICS, concerns a significant proportion of adult patients with asthma (6). The average age of onset of allergic asthma is younger than that of nonallergic asthma (13). Although the spectrum of allergic asthma may vary from mild to severe, studies have reported that allergic *versus* nonallergic asthma is less severe (13).

Allergens are triggers for asthma symptoms and can lead to increased morbidity. The majority of children with asthma in US

are found to be sensitive to at least one indoor allergen (mite, molds, cat, dog) (17). Exposure to airborne pollen grains is known to be associated with asthma exacerbations and hospital admissions, especially in sensitized individuals and in children (18, 19). A prospective cohort study demonstrated that the sensitization to specific aeroallergens differentially impacts the risk of developing asthma and rhinitis. Specifically, sensitization to perennial allergens, to dog in particular, was associated with higher asthma risk as compared to seasonal allergens. Poly-sensitization at all ages was greatly associated with increased asthma risk (17, 20). In the last decade, important knowledge milestones have been achieved in the description of the pheno-endotypes of severe asthma, while little attention has been so far paid to the phenotypes of mild-moderate asthma (21). Indeed, while current clinical guidelines underline the importance of phenotyping severe asthma, to target the appropriate therapy (*i.e.*, biologics), phenotyping mild-moderate asthma is not considered relevant, as the therapeutic approach recommended in these patients is considered to be independent of the phenotypes. In addition, the role of pollen, a major causal agent of respiratory allergy, in the complex interplay with asthma has not been completely elucidated. Along this line, the aim of our work is to investigate whether pollen-induced asthma (PIA) can be considered a specific phenotype in patients with mild-moderate asthma.

Materials and methods

A systematic review of the literature was conducted on Medline to identify English papers published up to March 31, 2024. Hand searching of references of interest was also performed within the selected studies. The search strategy included at least one keyword, in the title/abstract, for each of the following domains: pollen as a source of allergens (factors affecting pollen concentration, pollen size, immunologic mechanisms of response to airborne allergens), pollen-induced asthma (epidemiology, pollen-induced airway inflammation).

The research and selection of the studies were performed independently by five allergists, who collected and summarized the data from the studies. All the authors contributed to the definition of the research question and related keywords, and to the final selection of the studies to be included in the systematic review.

Results

Pollen as a source of allergens

Factors affecting pollen and allergen concentration

The concentration of pollens can be significantly affected by multiple atmospheric, environmental and botanical factors, thereby increasing the risk of respiratory symptoms and exacerbations in allergic pollen-driven asthma (18, 22-24).

Table I - Main factors affecting the concentration of pollen and allergens.

Atmospheric factors
Temperature
Humidity
UV radiation
Thunderstorms
Wind speed, distance, and direction (long-distance transport, air mass trajectories)
Pollution
Environmental and botanical factors
Soil contaminants
Microbiome
Tree biotic and abiotic stressors (<i>e.g.</i> , infections, other cultivated or native plants)
Urbanization and urban infrastructure topology
Tree urban planning (type and topology of trees)
Cultivar (plant variety that has been produced incultivation)
Land use

Most studies assessing the impact of pollen on respiratory health have used pollen count (number of airborne pollen grains) as a proxy for the concentration of airborne allergen. However, this may not reflect the true potential of allergens to exacerbate allergic respiratory symptoms, as subpollen particles (SPPs) carrying the allergens might come into play because of its size, small enough to reach the lower airways. The relationship between pollen count and pollen allergen levels (pollen potency, *i.e.*, amount of allergen per pollen) has been shown to be nonlinear, as the amount of allergen released from grains may vary significantly according to factors such as geographic location, time of the year, plant growth, weather conditions (25-27). Altogether, these observations explain why allergy symptoms are experienced even on days with low pollen counts and suggest that pollen count may not be a reliable proxy of allergen exposure (27, 28). Notably, *Fuente et al.* (2024) provided the first evidence that levels of airborne Phl p 5, an important grass pollen allergen, are more consistently associated with the occurrence of allergic and respiratory symptoms than pollen counts, after accounting for meteorological and environmental factors (27).

The main factors affecting the concentration of pollen and allergens are reported in **table I**.

Atmospheric factors

Temperature has been shown to be linked to an increase in sensitization frequency and allergic diseases. The emission of anthro-

pogenic carbon dioxide (CO₂) into the atmosphere and global warming can fertilize vegetation, enhancing the photosynthetic capacity and the growth of the plants, and are associated to an extended duration (mainly due to an earlier start) of pollen season and higher peak of pollen concentration (29, 30).

The effects of precipitation and humidity levels on pollen emission are complex (31, 32) and may depend on the specific pollen type (33). Heavy short-term precipitation significantly reduces atmospheric pollen concentrations, but, on the other hand, high humidity may induce hydration of pollen grains, sometimes followed by osmotic rupture, with generation of fragments of sub-micron diameter (0.5-2.5 µm) carrying allergens that can be dispersed by the wind into the atmosphere (31). However, the role of precipitation and humidity is rather complex to analyze, because there is not a standard definition of precipitation used across the studies and different scales of measuring precipitation are used (32). Under current climate change scenarios, heavy rainfall episodes, such as thunderstorms, cyclones and hurricanes, are expected to increase in intensity and frequency. Although mechanisms remain to be fully clarified, there is evidence in favor of a causal relationship between thunderstorms and epidemics of asthma attacks, including fatal and near-fatal (34). The most prominent hypotheses for “thunderstorm asthma” is that these events may concentrate aeroallergens at ground level to release respirable allergenic particles or other paucimicronic components after rupture of pollen grains by a combination of osmotic, mechanical, and electrical shock related to humidity, rainfall, wind gusts, and lightning strikes (34-37).

Wind speed and direction also play an important role in the process of lifting and transport of airborne pollen and allergens and in determining their load in the atmosphere (38, 39). The allergenic capacity of long-distance transport of pollen remains unclear. Pollen allergenicity could decrease or be lost altogether during flight in the higher layers of the atmosphere, where the action of factors such as air temperature, humidity and solar radiation on the pollen grains could impact on their ability to maintain allergenic potency (40). Air pollution may also aggravate the allergenicity of pollen (41-44) via different mechanisms: increase of pollen potency, damage of pollen surface with release of more allergens (45), change of its elemental composition, resulting in the release of more airborne SPPs. For instance, gaseous pollutants (nitrogen dioxide and ozone) have been shown to damage the pollen cell membranes in SPPs from plane tree pollen, leading to an increase in *Pla 3* allergen released into the atmosphere (46). When investigating these interactions between pollution and pollen, several variables should be considered, such as weather, urbanization, pollen species, type of pollutant, conditions of exposure, and individual susceptibility.

Environmental and botanical factors

Multiple atmospheric factors joint with environmental and botanical factors influence the concentration of allergens in pollen.

Increasing evidence indicates that the microbial composition of pollen (pollen microbiome) may affect its allergenicity (47, 48), as suggested by the observation that significantly higher amounts of major endotoxins synthesized by bacteria occur in high allergenic pollen in contrast to low allergenic pollen (48).

Pollen release and allergenicity may be also affected by soil pollutants and contaminants, such as cadmium (49) and indirectly by factors that influence plant growth and development, such as biotic stressors (living organisms like virus, bacteria, fungi and insects) and abiotic stressors (pollution, heat, cold, drought, salinity, high UV light, wounding, hypoxia) (50, 51).

Other environmental factors to be considered are land use (agriculture, pasture, plant varieties produced in cultivation by selective breeding), urbanization and urban infrastructure topology. Urban areas, where vegetation coverage is limited, may become “islands” of higher temperatures relative to outlying suburban or rural area (“urban heat island effect”), with possible impact on plant growth and pollen emission (52, 53). This may have implications in epidemiological studies, as large temperature differences between the pollen monitoring station and the study area could result in differences in pollen count and allergen content. In summary, pollen exposure and allergenicity are influenced by multiple specific and nonspecific environmental stressors (pollen exposome) and their consequences at organ and cell level are considered to play a role in the development, progression and exacerbation of pollen-induced asthma (28).

An important question concerns pollen threshold used in warning systems, that are intended to inform people of the risk of developing allergy symptoms. There is no consensus about which pollen concentrations provoke allergy symptoms (54). First of all, pollen traps are usually installed on roofs at a height of 15–20 m, but the pollen concentrations may differ from ground level, where exposure mainly occurs (55), and where it is highly variable both locally and spatially (56). Secondly, the clinical threshold of pollen is very variable as well. In fact, the relation between pollen/allergen exposure and symptom development is complex, and the dose threshold above which symptoms are experienced is influenced by factors such as individual sensitivity, sensitization, allergen content of pollen, age, geographical areas (54, 57).

Pollen and the airways: a matter of size

Experimental models aimed at predicting the relationship between aerosol particle size and lung penetration show that large particles, with aerodynamic diameters > 6 mm, mainly deposit at the oropharyngeal, whereas smaller particles penetrate the bronchiolar tree (58, 59).

Factors influencing pollen deposition in the airways

The deposition of pollens in the airways can be significantly affected by multiple factors (28, 31, 32). Besides the factors affecting pollen and allergen concentration reported in **table I**, pollen-specific characteristics such as size and morphology may also play a role.

Intact pollen grains are typically between 22 mm (birch) and 100 mm (corn) in size, thus too large to reach the lower airways where asthmatic reaction occur. For instance, grass pollen is present in the atmosphere both as whole grains (approx. 20 to 55 µm in diameter) and as smaller size fractions (< 2.5 µm) (60); ragweed pollen has a geometric diameter ranging between 16 and 27 µm (61), Parietaria pollen between 16–18 µm (62, 63). The question how the pollen grains may affect the respiratory system (the “size paradox”) and the processes by which pollen allergens become airborne particles of respirable size have been investigated. As previously reported, during heavy precipitation or periods of high humidity pollen grains are hydrated and may undergo osmotic rupturing into SPPs that can penetrate deeper into the lung (28, 31). These data are supported by recent studies based on the measurement of chemical and biological markers demonstrating a significant increase in the SPPs with diameters 0.25–2.5 µm during thunderstorms and rain events in the pollen season, with peak concentrations occurring during convective thunderstorms with strong downdrafts, high rates of rainfall, electrical ions, and lightning (64, 65). Importantly, SPPs derived from pollen after osmotic shock have been shown to retain allergenicity (37). The main allergens of *Parietaria Judaica* (Par j 2), olive tree (Ole e 1) and grass pollen (Phl p 2 and Phl p 5) are detectable in SPPs and all of them are consistently associated with the epidemic of thunderstorm asthma (37). The impact of pollen morphology on its deposition in the airways has also been investigated. High-resolution imaging techniques have revealed pollen grain is commonly found in round, ellipsoidal, triangular, disc or bean-shape, with a smooth to spiky texture. Wind-pollinated plants produce lots of lightweight, smooth pollen, whereas the pollen of insect-pollinated plants is heavy and sticky. Experimental studies by Hassan (2011) have investigated the effect of size and surface morphology of pollen-shape carriers on drug delivery performance. The results might be extrapolated to the actual pollen morphology and showed that, at low flow rates, sparse surface asperity was associated to a significant improvement in the delivery of the drug fine particle fraction (the dispersed drug powder with diameter ≤ 5 µm) as compared to pollen-shape carriers with dense surface asperity (66). In the study by Inthavong *et al.* (2021), pollen particles exhibited higher drag coefficients (*i.e.*, resistance in a fluid environment, such as air or water) and lower particle density compared to aerodynamic equivalent spheres, suggesting that pollen has greater mobility in its aerodynamic flight and greater potential to penetrate the nasal cavity (67).

Site of inhaled pollen airway deposition

As the SPPs are several times smaller than intact pollen grains, they can evade filtration by the nasopharynx and penetrate deeper into the airways, provoking respiratory symptoms.

The association between grass pollen exposure and early markers of asthma exacerbations, such as lung function changes and

increase in airway inflammation, is limited, yet results from available studies suggest the evidence of a correlation (68). In a community-based cohort of 936 adult participants, increasing grass pollen concentrations were significantly associated to changes in FEF25%-75% and FEV₁/FVC ratio, measured 2-3 days after exposure, but not in FEV₁, suggesting that the greatest impact might be on medium-sized to small airways (69). Modifications in lung function parameters (FEV₁ and FVC) following pollen exposure have been reported also in children and in pollen sensitized adolescents (68).

The study by Nassikas *et al.* (2024) on a large cohort of 490 adolescents exposed to high concentrations of pollen reported a significant increase in airway inflammation (assessed by the measurement of FeNO levels), even in the absence of allergic sensitization and asthma (70).

The results from study on 85 asthmatic patients suggest that there are differences between house dust mite (HDM) mono-sensitized subjects and weed pollen mono-sensitized subjects, not only in airway wall thickness, but also the indices of small airway obstruction, reflecting airway remodeling (71). The results need to be confirmed on a larger population of patients.

Altogether, increasing evidence suggests that a large proportion of allergens is associated with particles of respirable size, either fragments of pollen, soluble allergen adsorbed to air pollutants of various origin or part of the dehiscing anther releases at the time of pollen shedding. These particles are small enough to deposit in the peripheral airways and induce inflammation and respiratory symptoms in predisposed subjects. Limited evidence shows effects on lung function parameters, reflecting a deposition on medium to small airways.

Mechanisms of innate and adaptive immune response to aeroallergen

The concept of the pollen matrix in allergic sensitization

Allergic asthma may involve various types of hypersensitivity reactions to allergens (antibody-mediated, immune cell-mediated, tissue-driven or linked to metabolic mechanisms), resulting in the development of symptoms (72). Classically, the mechanisms of allergies are associated with the type 1, IgE-dependent immune response, characterized by involvement of T helper 2 (T₂) cells and production of cytokines including IL-4, IL-5, and IL-13. However, recent evidence shows endotypes of allergic diseases related to T₁ or T₃-driven activation pathways (72).

Up to now, 987 different allergens have been officially described, of which 195 are registered as plant-derived airborne allergens (<https://www.allergen.org>).

The key question is why only some environmental proteins cause allergic sensitization and others do not. The molecular bases of allergenicity, *i.e.*, the capacity of certain molecules to induce type 2 inflammation and specific IgE antibodies, are not fully under-

stood. Results from epidemiological and experimental studies support the notion that allergic sensitization is not only dependent on the genetics of the host and environmental factors, but also on intrinsic features of the allergenic source itself, specifically the composition of the pollen matrix (73-80).

The intrinsic and extrinsic compartment of the pollen matrix

Pollen allergens are embedded in a complex and heterogeneous matrix composed of a various bioactive molecule that are co-delivered during the allergic sensitization. The pollen matrix can be divided into two compartments, an intrinsic part consisting of compounds inherent to the pollen (proteins, metabolites, lipids, carbohydrates) and an extrinsic fraction, that includes viruses, aerosols and particles from air pollutants and a pollen-linked microbiome (73, 81-87). Together these components of the matrix provide a specific context for the allergen and are determinant of T₂ sensitization (**figure 1**).

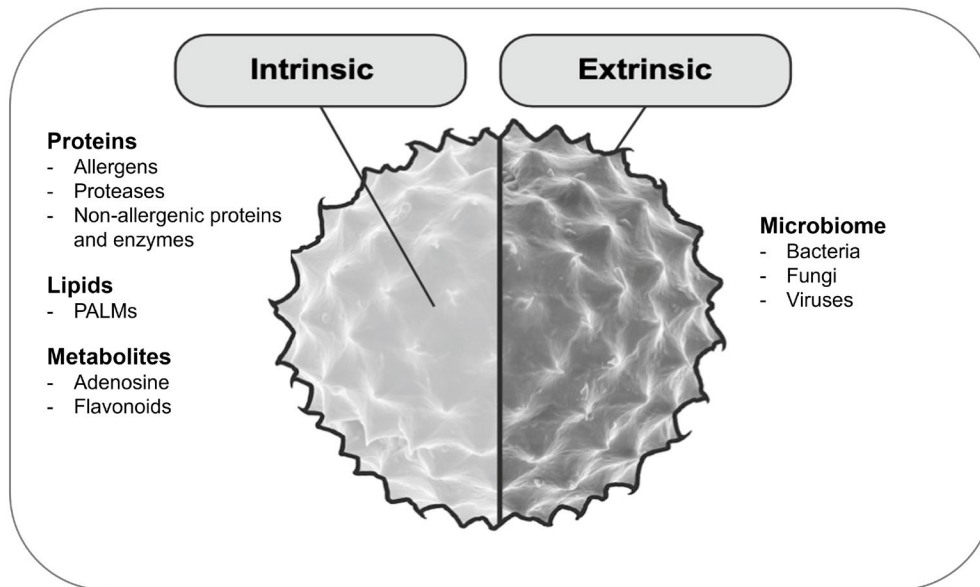
Specifically, the initiation of allergic sensitization to pollen is likely to occur via distinct molecular mechanisms, involving pollen species-specific immune adjuvants that may contribute to the generation of a pro-inflammatory microenvironment to favor T₂ polarization. Indeed, experimental studies have shown that several purified allergens were lacking inherent sensitizing potential, supporting the role of pollen-derived components as key players in the initiation of the inflammatory allergic response in predisposed subjects (73, 74, 82, 88).

Pollen grains are rich in lipids displaying immunomodulatory effects (74). For instance, in sensitized individuals, but not in healthy controls, cypress pollen-derived phospholipids were shown to be presented to T cells by major histocompatibility complex (MHC)-related molecules on dendritic cells, an interaction causing T cell proliferation and secretion of IL-4 (playing a key role in the initiation of sensitization) and IFN γ (73, 83, 84, 89). Further evidence comes from human studies with olive pollen and *in vitro* murine models with birch pollen, showing that pollen lipids activate invariant natural killer T cells by upregulating CD1d expression on dendritic cells (90, 91).

Regarding the extrinsic compartment of the matrix, the pollen microbiota, whose composition is variable and specific for each pollen species (47, 92), seems to play a role in allergenic inflammation. In fact, besides intrinsic pollen-derived lipids, microbial lipids constitute a source of immunomodulators and act as strong adjuvant of the sensitization process (83, 87).

The influence of plant viral infection on the sensitizing potential of pollen is still largely unknown. A pilot study on a small sample (n = 15) of subjects with a history of seasonal allergic rhino-conjunctivitis enrolled outside the pollen season observed that virus-induced modifications in components of grass pollen have the potential to alter its allergenic potency, as assessed by skin testing (85). The results suggest that virus infection of grasses deserves consideration as a factor in pollen-induced allergic disease.

Figure 1 - The composition of the pollen matrix influencing the sensitizing potential of allergenic pollen source (adapted from ref. 73).



Additionally, air pollutants, such as diesel exhaust particles, ozone, carbon dioxide and nitrogen oxides, may influence the composition of the pollen matrix as well as of the pollen microbiota, displaying an assistive role in the development of the allergic inflammation (41-43, 93, 94). In this regard, a correlation between exposure to atmospheric pollutants and the content of allergens and immunostimulatory compounds in pollen was reported (95, 96).

The role of epithelium in the initiation of the sensitization process

Increasing evidence suggests that an epithelial dysfunction, coupled with inherent properties of environmental allergens, can be responsible for the inflammatory response (97, 98).

Epithelial cells are endowed with a series of specialized pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) and protease activated receptors (PARs), which are required to provide first defense mechanisms towards pathogens. In atopic individuals, upon encounter with the epithelium, the pollen releases allergens and various matrix bioactive molecules that cause the disruption of the epithelial tight junctions, enabling the transportation of allergens across the membrane (81, 99-101), the activation of PRRs, the release of epithelial cytokines, like thymic stromal lymphopoietin (TSLP), IL-25, IL-33, and various pro-inflammatory cytokines (IL-8, IL-1, IL-6, TNF α). In turn, all these molecules activate the dendritic cell network and other innate immune cells, such as basophils and type 2 innate lymphoid cells, that drive pollen-induced T2 inflammation (88, 102-105). In this context, evidence is emerging on the epithelial cytokine TSLP as a critical player in the development and progression of

allergy and asthma (106). TSLP is positioned at the early phase of the inflammatory cascade, therefore, its inhibition could simultaneously suppress multiple pathways of inflammation. In allergic asthma, TSLP promotes the differentiation of T2 lymphocytes secreting T2 cytokines targeting B cells, eosinophils, mast cells and airway smooth muscle cells (106). The pollen-induced secretion of TSLP and the associated type 2 inflammation were shown to be dependent on TLR4 and myeloid differentiation primary response 88 (MyD88), and probably linked to oxidative stress (107-109). In this respect, stimulation of epithelial cells with pollen extracts from short ragweed, birch, timothy grass and mountain cedar caused elevation in the levels of reactive oxygen species (ROS) (110-113). In addition to TSLP, a TLR4/MyD88-dependency was also observed for pollen-induced IL-33-mediated T2 responses for IL-25, which has the potential to initiate and activate type 2 innate lymphoid cells and T2 cells (73).

Once activated by pro-inflammatory cytokines, dendritic cells instruct T2 polarization through three types of signals to naïve T cells: 1) antigen-derived peptides presented via MHC-II, 2) expression of co-stimulatory molecules and 3) secretion of pro-inflammatory cytokines and chemokines (114). In addition, activated dendritic cells secrete chemokines (CCL17, CCL22 and CXCL13) and chemokine receptors enable them to migrate to the lymph nodes, where they prime naïve T cells to become antigen-specific T2 cells (115-119).

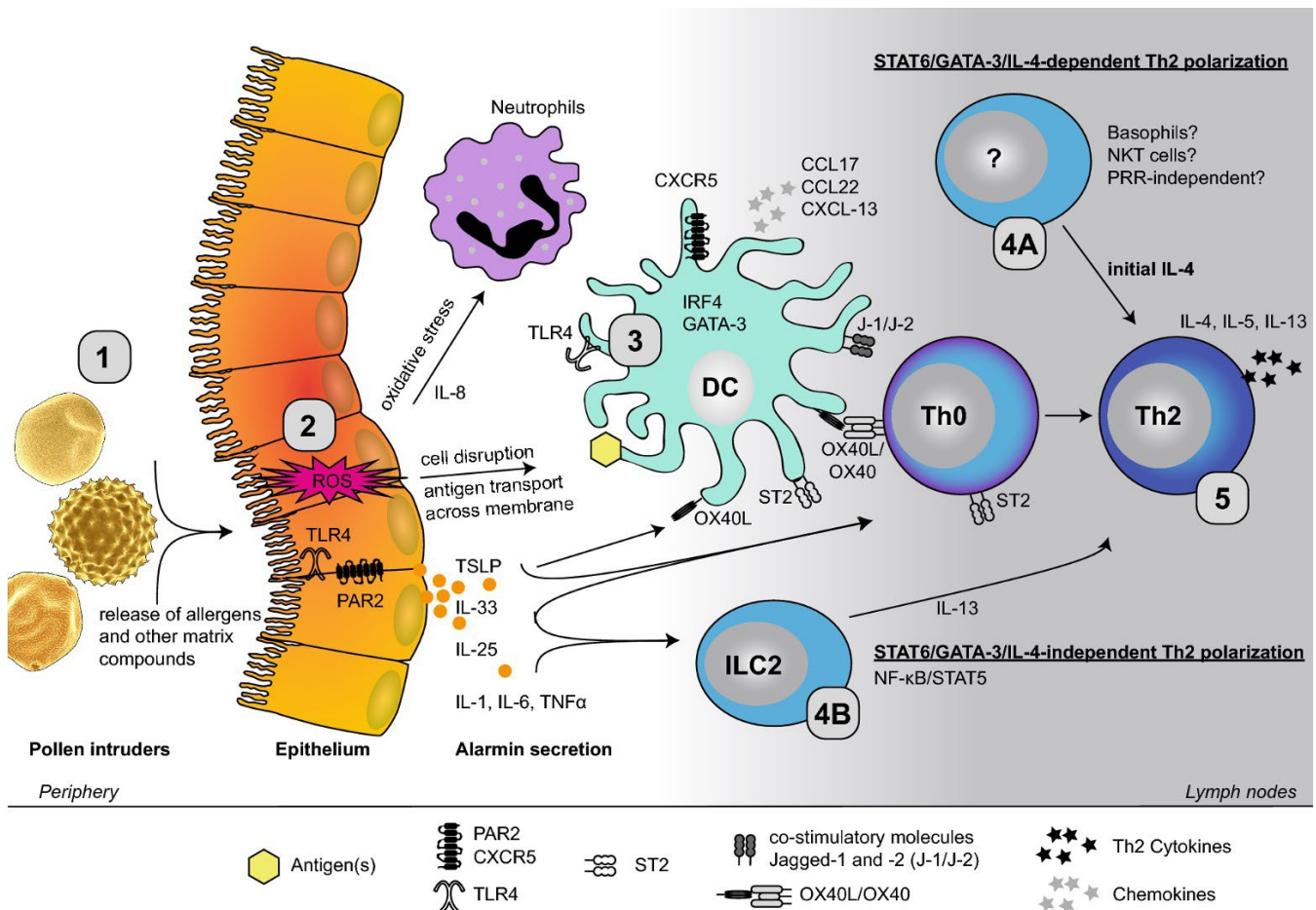
For efficient T2 priming IL-4 seems to be important. Basophils, mast cells and NKT cells were shown to produce IL-4 (120) and

once generated, T2 cells themselves represent the most important source of IL-4.

An overview of the initiation process of allergic sensitization is shown in **figure 2**.

In summary, the mechanisms involved in pollen-induced activation of the innate immune system and T2 polarization are complex and not fully understood. It seems that different allergenic pollen sources interact with distinct innate receptors and signal-

Figure 2 - Pollen-induced activation of the innate immune system and T2 polarization (reproduced from ref. 73). Upon encounter with the epithelium the pollen hydrates and releases its content including allergens and various other bioactive molecules (1). At the epithelium (2), this immunogenic cocktail causes the disruption of the epithelial membrane, activates PRRs such as TLR4 and PAR2, triggers the release of alarmins (TSLP, IL-25 and IL-33), and induces oxidative stress and secretion of IL-8 and other pro-inflammatory cytokines (IL-1, IL-6 and TNF α). In turn, DCs are activated (upregulation of surface markers including OX40L and notch ligands), migrate to the lymph nodes (expression of CXCR5), where they present processed antigens via MHC-II to naïve T cells (3). Th2 polarization occurs either STAT6/GATA-3/IL-4-dependent (4A) or -independent via the NF- κ B/STAT5 pathway and the contribution of ILC2s (4B). The origin of initial IL-4 for Th2 polarization is still a matter of discussion; proposed candidate are basophils and NKT cells. Once a Th2 immune response has been initiated, a class-switch of B cells to antigen-specific IgE-producing plasma cells occurs resulting in the sensitization of susceptible individuals to pollen allergens (5).



CCL17; CCL22 chemokine (C-C motif): ligand 17 and 22; CCR7: C-C chemokine receptor type 7; CD80; CD86 and CD40: cluster of differentiation 80; 86 and 40; CXCL-13: C-X-C motif chemokine 13; CXCR5: C-X-C chemokine receptor type 5; DCs: dendritic cells; GATA-3: GATA binding protein 3; IL-: Interleukin; ILC2: type 2 innate lymphoid cells; IRF4: interferon regulatory factor 4; NF- κ B: nuclear factor "kappa-light-chain-enhancer" of activated B cells; NKT: natural killer T; OX40L: OX40 ligand; PARs: protease activated receptors; PRRs: pattern recognition receptors; ROS: reactive oxygen species; ST2: IL-33 receptor; STAT5, STAT6: signal transducer and activator of transcription 5 and 6; Th: T helper cells; TLR4: toll-like receptor 4; TNF α : Tumor necrosis factor alpha; TSLP: thymic stromal lymphopoietin.

ing pathways, that are also influenced by genetic polymorphisms affecting epithelial pattern recognition, barrier function, and cytokine production. Altogether, the data suggest that allergic sensitization to pollen most likely results from particular combinations of pollen-specific signals rather than from a common determinant of allergenicity.

Pollen-induced airway inflammation: specific features on allergic asthma

Experimental evidence suggests that allergen-specific T2 cells and their cytokines orchestrate allergic airway inflammation, induce mucus production from airway epithelium and promote airway hyper-responsiveness (121-123).

Along this line, studies on a human model of allergen-induced asthma exacerbation have been conducted aimed at exploring differences between allergic asthmatics and allergic non-asthmatic controls in the airway response to allergen, that could provide fundamental insights into asthma pathogenesis and possibly identify novel therapeutic targets (124, 125).

Cho *et al.* (2016) showed that both groups developed prominent allergic airway type 2 inflammation in response to allergen. However, allergic asthmatic subjects compared to allergic non-asthmatic controls had markedly higher levels of innate type 2 receptors on allergen-specific CD4⁺ T cells recruited into the airways and increased levels of type 2 cytokines, total mucin as well as airway baseline smooth muscle mass (124).

Further research by Alladina *et al.* (2023) showed that transcriptional profile of airway epithelial cells upon allergen challenge with allergens was markedly altered in allergic asthmatics subjects as compared to allergic non-asthmatic controls (125). Specifically, in asthmatic subjects a subset of epithelial cells – goblet and suprabasal quiescent goblet cells as well as basal cells – displayed the greatest response to allergen, with upregulation of genes involved in type 2 inflammatory cell recruitment and signaling, mucus metaplasia, and genes that promote extracellular matrix degradation and connective tissue regeneration. In contrast, in allergic non-asthmatic subjects the basal and suprabasal cells were able to promote an injury-repair response to allergen challenge, with increased expression of alarmins (IL33 and HMGB1) and neutrophil chemoattractants. Collectively, these results identify airway basal and secretory cells as highly dynamic cells during allergic inflammation and reveal mechanisms by which they may drive asthma pathogenesis.

IL9-expressing pathogenic T2 cells, that amplify type 2 inflammation and promote the expression of profibrotic mediators and pathologic airway remodeling, have also been shown to be highly specific to asthmatic airways and were only observed after allergen challenge (125).

Additionally, airways of allergic asthmatics, after allergen challenge, were uniquely enriched for conventional type 2 dendritic cells (that express *CD1C*) and *CCR2*-expressing monocyte-derived cells, with up-regulation of genes that sustain type 2 inflammation

and promote airway remodeling. In contrast, airways of allergic non-asthmatic subjects were enriched for macrophage-like monocyte cells (MCs), characterized by production of factors modulating endocytic clearance, cell differentiation and survival, and expression of trophic factors promoting angiogenesis and tissue repair, as shown in animal models (126, 127). This finding suggests that these populations play an important role in the resolution of inflammation and protection against airway remodeling, as opposed to IL-4/IL-13 signaling via STAT6 in the airways of asthmatics, that may prevent or arrest macrophage differentiation and direct a pathogenic monocyte cell phenotype characterized by up-regulation of genes involved in inflammatory signaling, antigen presentation, and pathologic airway remodeling. Cellular crosstalk between airway epithelial and immune cells is also critical to the initiation and resolution phases of allergic inflammation (128-130). Cellular communication pathways in allergic controls were characterized by growth factor signaling and injury-repair response to allergen, whereas asthmatics were dominated by basal cell-Th2-mononuclear phagocyte interactions that may sustain and amplify type 2 signals, leading to failure to engage antioxidant response, loss of growth factor signaling, increase in mediators of airway remodeling.

In summary, allergen challenge leads to increased eosinophilia and type 2 cytokine levels in the airways of both allergic asthmatic and allergic non-asthmatic subjects, but the effector pathways elicited by T2 inflammation are distinct. The airway epithelium of asthmatic subjects is highly dynamic, with basal and secretory epithelial cells up-regulating the genes involved in matrix degradation, mucus metaplasia, and remodeling, while failing to induce the epithelial injury-repair and antioxidant processes observed in non-asthmatic controls, that are possibly protective against pathologic remodeling.

How pollen interacts with the respiratory mucosa remains largely unknown due to a lack of representative model systems. In this respect, Van Cleemput *et al.* (2019) demonstrated that pollen proteases of three plants, Kentucky bluegrass, white birch and hazel, selectively destroy the integrity and anchorage of columnar respiratory epithelial cells, but not of basal cells, in both *ex vivo* respiratory mucosal explants and *in vitro* primary equine respiratory epithelial cells (131). Interestingly, Blume *et al.* (2013) analysed the effect of grass pollen exposure on differentiated human primary bronchial epithelial cells derived from severe asthmatic donors and non-asthmatic controls. The results show a differential response in terms of inflammation mediator release, without any difference in physical barrier properties (132).

Discussion and conclusions

Asthma is a global problem and a significant social and economic burden. Although specific epidemiological data on pollen-induced asthma are scarce, overall allergic asthma, which is the most com-

mon phenotype, is costly for the healthcare systems, with large additional societal costs due to lost work productivity.

Clinical manifestations are intimately linked with the release of plant pollen into the environment. The factors that influence pollen concentration and potency are multiple, region- and species-specific, difficult to identify, quantify and predict in terms of type of effect, as it is increasingly clear that they all have independent and joint effects on respiratory health. The variability of pollen and allergen concentration is often overlooked in clinical studies, even in randomized controlled trials, suggesting that allergic-type asthma is not always properly investigated and introducing a possible bias in studies on allergic populations.

In the future, temperature and precipitation are projected to increase, all factors that will potentially augment pollen emission and allergenicity, with negative impact on respiratory health. Also, urbanization will further increase in the next decades, with negative consequences on the health and survival of urban trees, leading to loss of biodiversity. In this context, tree urban planning and the integration of green infrastructure may mitigate the impact of urban development.

The molecular bases of allergenicity are not fully understood. There is evidence that allergic sensitization depends not only on the genetics of the individuals and the environmental factors, but also on species-specific immunostimulatory components of the pollen matrix that may contribute to the generation of a pro-inflammatory microenvironment to favor T2 polarization. Future investigation will contribute to elucidate the pathogenic effects of pollen in the airway.

Importantly, in allergic asthmatics, as compared to allergic non-asthmatics, the pathogenic effector circuits sustain and amplify T2 signals in response to allergens, while the circuits facilitating the resolution of inflammation and tissue repair are inhibited: therefore, tissue reprogramming in response to T2 inflammation could drive structural airway disease and may represent a defining feature of allergic asthma.

The observation that many allergic individuals develop asthma over time (133), suggests that the pathogenic mechanisms leading to asthma may be incremental. Thus, a key question is whether a pharmacologic intervention may slow down or at least partially revert the cellular pathways driving airway remodeling.

Inhaled glucocorticoids reduce airway inflammation and some aspects of remodeling, as proliferation of lung fibroblasts, metaplasia of goblet cells and thickening of subepithelial basal membrane (134, 135), but currently there are no drugs or other interventions available that can definitely reverse this process (134). *In vivo* animal models of allergen-induced airway inflammation, using sensitized rats exposed to repeated allergen challenge, showed established structural alterations of the airways could not be reversed by the treatment with inhaled corticosteroid administered post challenge, but concomitant treatment could partly prevent these changes (136). In addition, glucocorticoid could inhibit *in vitro* the differ-

entiation of human lung fibroblasts to contractile myofibroblasts, that are involved in the development of the inflammatory cascade. The effect of reversion to the normal phenotype occurs both at the very early and also at a mild stage of the differentiation process (137). The clinical relevance of these findings is not known, since no animal model of allergic airways disease encompasses all features of the human disease, and results cannot be easily translated to the clinic; however, the data support the hypothesis that early intervention with inhaled glucocorticoids could at least in part prevent or slow down airway remodeling in asthma.

Advances in the understanding of the molecular circuits underlying airway structural changes and remodeling in response to allergens as well as repair mechanisms may facilitate the development of novel and more effective therapeutic approaches.

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Contributions

All authors equally contributed to the manuscript draft and critical revision for important intellectual content, to data collection, analysis and interpretation. All authors read and approved the final version of the manuscript.

Conflict of interests

The authors declare that they have no conflict of interests.

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