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# Impact of Pollen on Human Health: More Than Allergen Carriers?

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## Key Words

Pollen · Phenology · Pollen-associated lipid mediators · Particles · Allergy

## Abstract

The transfer of pollen from floral anther to recipient stigma is the critical reproductive event among higher plants – this is the botanical view of pollen. Proteins and glycoproteins from pollen can function as allergens, environmental molecules interacting with the human immune system to elicit an allergic response in susceptible individuals – this is how allergists and immunologists see pollen grains. Between 10 and 25% of the population now have symptoms of hay fever or allergic asthma and the incidence has more than doubled in the past three decades while the reason(s) for this increment are only hypothetical, but there is a multitude of them. Despite our natural focus on this impact of pollen on human health, pollen have to be considered in a larger context. First of all, to evaluate the bioavailability of allergens from pollen, we have to understand their function and their influence factors. Furthermore, pollen grains are not only releasing proteins eliciting specific immune responses, but they also liberate bioactive lipid mediators and this much more rapidly. And last but not least, recent observations indicate, that pollen do not only

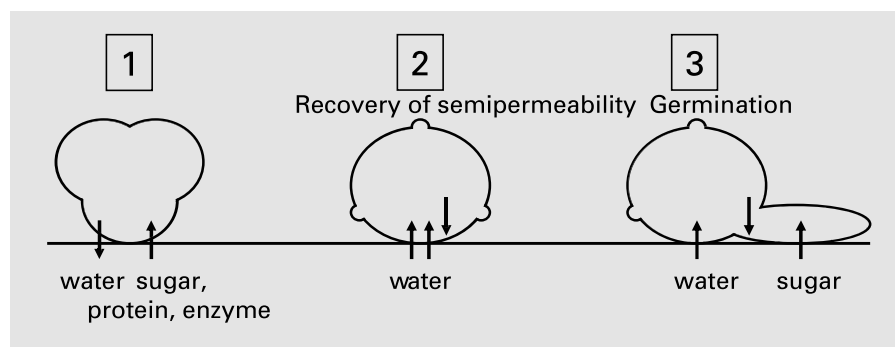
induce allergy and thus have a much broader impact on human health. This review is an attempt to favour this holistic view of pollen and their impact on human health.

## Introduction

Allergists and immunologists immediately and often exclusively connect pollen with the release of allergens and the development of allergic diseases. This is most unfortunate because, first of all, pollen grains primarily bear a natural mission. This natural mission is the unitary adaptive function to reach a receptive stigma and to deliver two haploid nuclei to the recipient ovary in order to transmit genetic information from the male parent to the offspring [1, 2]. The fact that pollen grains also induce allergic disease and provoke symptoms is unquestionably a consequence with which nature had not reckoned.

Under natural exposure conditions, the bioavailability of allergen depends on protein liberation from internal binding sites within the allergen carrier [3, 4]. Little is known about the physiological function of the allergenic proteins within the pollen grain even though some have been identified as defence proteins [5]. Thus, the release of these proteins – harmful for humans – depends, in part, on stress situations for the plant.

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**Fig. 1.** Hydration and transfer of organic molecules through cell membrane for pollen germination.

The majority of studies conducted to date concerning the elicitation and expression of allergic symptoms have concentrated primarily on individual allergens. However, the immune system of most individuals is not necessarily exposed to allergens in pure manner, but rather in particulate form [6, 7], either as pollen grains, their starch granules [8], or they may become associated with exogenous particulate material such as diesel exhaust particles [4, 9].

To better understand the effects of pollen and their components on human health, one has to consider pollen physiology in order to understand why specific proteins – which at the end are harmful for the patient – are released from pollen, as well as to realise which factors lead to an enhanced or reduced release of the allergenic protein. The idea of a ‘Bet v 1 knockout tree’ is a very interesting and fashionable one but, for sure, a typical line of thought by humans who would tend to overlook the fact that Bet v 1 is most likely a protein the plant uses for its defence.

The aim of this publication is to give the reader a detailed insight into the world of pollen grains as we know it today, as well as their physiology, their impact on human health and, last but not least, into the environmental factors that trigger flowering. Furthermore, it will serve to summarise our recent data on pollen associated lipid mediators released from pollen and their impact on the innate immune system.

In conclusion, this review aims to better understand the effect of pollen on human health and to favour a more holistic view thereof far and beyond the singular consequence of a specific protein.

### Physiology of Pollen Grains – Palynology

Pollen are multinucleate reproductive microgametophytes of plants. The anemophilous pollen, which are dispersed by winds and transported over wide areas, are

of particular allergological relevance. Pollen grains develop within anther sacs from specialised mural progenitor cells. They consist of nuclei, cytoplasm, a cell membrane and a cell wall. Pollen wall components reflect a dual origin: the pectocellulosic intine is secreted by the protoplast in which nuclear and metabolic components reside; the covering exine is derived from the anther sac cavity – the tapetum. The cytoplasm of pollen contains cytoplasmic organelles, Golgi apparatus, mitochondria and an endoplasmic reticulum. Unlike other plant cells, pollen grains do not contain chloroplasts, and are consequently not able to conduct photosynthesis. Thus, pollen cells, like animal cells, grow heterotrophically obtaining energy sources exogenously by absorbing sugars and amino acids from the stigma [2].

Mature pollen grains are dehydrated to some extent at the time of dispersal from the anthesis stage. Once grains are placed on the stigma or on artificial medium (or mucosal membranes), they swell due to water absorption – an almost passive mechanism [10, 11]. Consequently, turgor pressure inside the pollen increases, enabling the tube of the pollen cell to elongate (fig. 1). Hydrated grains can be seen to develop a pollen tube within a period, in some species, of as short as 90 seconds after contacting a suitable stigma [12]. The pollen-stigma interaction is influenced by the exsudate on wet stigmata and by the pollen coat. The importance of lipids in this context will be discussed later.

### Fatty Acids and Their Derivatives: Importance in Pollen and Plants

Trienoic fatty acids are invariably abundant in membranes of photosynthetic eukaryotes. Conservation of the high trienoic content of eukaryote membranes throughout evolution is evidence of their importance. McConn et al.

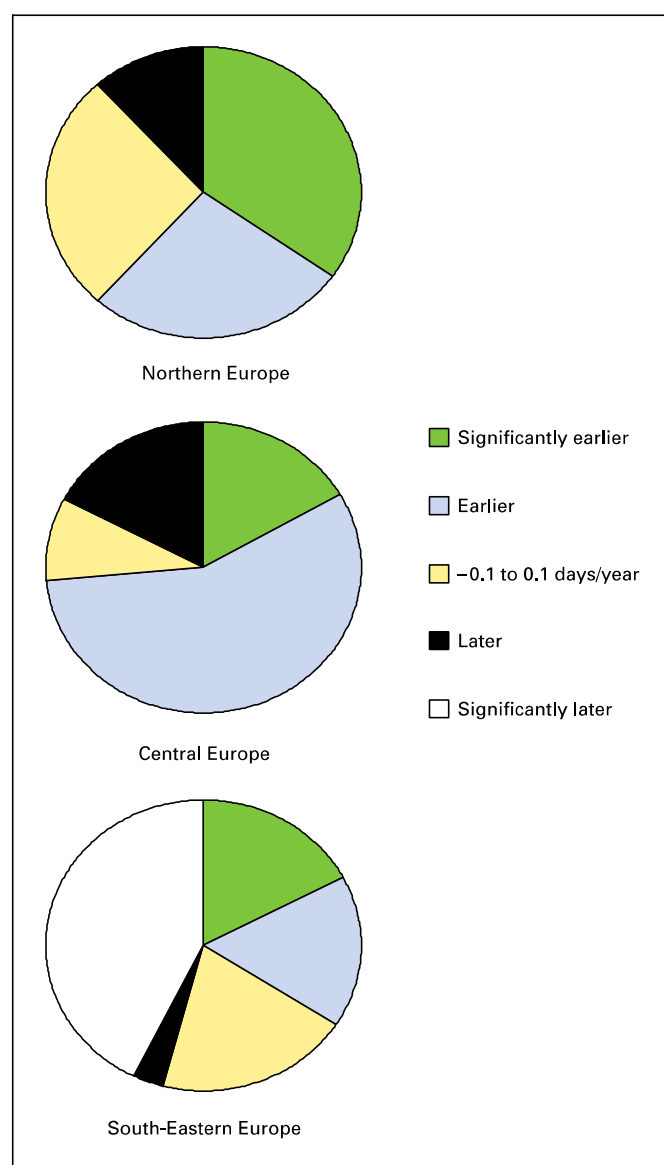
[13] described the critical requirement of trienoic fatty acids for pollen development. Using *Arabidopsis* mutant lines containing <0.1% trienoic acid, they observed the development of tricellular but unviable, male sterile pollen. Another essential requirement for unsaturated acids such as linolenic acid in the plant life cycle appears to be a substrate of the octadecanoid pathway, which produces signaling molecules such as jasmonoic acid [13].

In both dry- and wet-type stigmata, lipids, which form the major component of the exsudate and the pollen coat, are thought to be responsible for pollen hydration. In *Arabidopsis* mutants that are defective in long-chain lipids, the pollen grains fail to hydrate on the stigma [13]. The failure of these mutants to stimulate the release of water from the stigma to the pollen coat is interpreted as evidence that lipids in the pollen coat are involved in the cell-cell recognition required for hydration [14, 15]. Fertilisation of the ovules of flowering plants occurs when dehydrated pollen grains – on the receptive surface of the female (the stigma) – hydrate, germinate, and produce a pollen tube. These events occur in the lipid-rich environment formed by the pollen coat of the stigma exsudate [16, 17]. Pollen tubes navigate the route from the stigma to the ovule with great accuracy. Lipids seem to be one of the cues that guide them along this route. Wolters-Arts et al. [18] could show that unsaturated lipids are required for directional pollen tube growth. Furthermore, the effects of lipids on pollen hydration and tube growth are due to lipid or lipid fragments acting as signals for hydration or penetration [17].

### Phenology – the Timings of Flowering and Pollen Release Are Variable

In phenology, annually recurring events in plant and animal life are observed. These phenological phases include the start of flowering and rarely the full term or end of flowering. Flowering dates of allergy-inducing species can be related to pollen counts by sampling stations [19–21] and are utilised within pollen forecasting systems.

During the last decades, a progressively earlier onset of spring activities such as the arrival of migrant birds, leaf unfolding and flowering of plants, has been observed [22–25]. These phenological trends in Europe and North America are mirrored by results from CO<sub>2</sub> records, Normalized Difference Vegetation Index (NDVI) satellite data and duration of ice cover and provide a relatively consistent image of the changes in the northern hemisphere with a clear lengthening of the growing season



**Fig. 2.** Frequency of linear trends (days/year) of flowering time series. Data are for long observational series (20 years and more) during the 1959–1996 period in the International Phenological Gardens in Europe. Northern Europe (n = 26), Central Europe (n = 121) and South-eastern Europe (n = 35 time series).

mainly due to the advance of spring. In Europe, observations on cloned trees and shrubs in the International Phenological Gardens revealed an average lengthening of the growing season by ~11 days and an advance of spring of 6.3 days in the last 30 years [26, 27]. Trends towards the earlier onset of flowering are apparent in the International Phenological Gardens of Northern and especially of Central Europe (fig. 2).

In general, higher spatial variability and regional differences of phenological changes are revealed. Advances are more pronounced in early spring than in mid and late spring [24, 28]. In some species, specific differences exist, e.g. annual species advance their flowering dates more than perennial species and entomophilous species more than anemophilous species [29].

Although a large number of environmental factors and physiological processes influence plant growth, the reproductive cycles in temperate zones are primarily controlled by temperature and day length. Temperature control of the timing of spring events includes chilling temperatures to break winter dormancy and subsequent increasing temperatures to induce bud burst. Modelling of phenophases and statistical analysis show that the air temperature of the preceding months and the related circulation patterns (e.g. the North Atlantic Oscillation Index) explain a high percentage of the inter-annual variability of leaf unfolding and flowering [27, 30].

Climate changes over the last few decades have resulted not only in significant alterations in the growing season, but also in the pollen seasons. Several studies have reported a premature start of the pollen season [31–33], often in accordance with the phenological changes observed [34]. A comprehensive analysis of the pollen data of the European Aeroallergen Network (1974–2001/2) revealed a generally earlier start and peak of pollen release, especially for early flowering species (hazel, alder, birch). The length of the pollination period was extended for late flowering species. However, the number of days with pollen count sometimes decreased. The strength of the pollen season, reflected by the peak value and the total annual pollen count, increased especially for grasses and weeds. Differences between urban/rural and coastal/inland sites were less pronounced. Almost no significant trends for olive trees were found [35, 36]. Thus, similar to the spatial and temporal variability in phenological changes, variations in the start of the pollination period differ among species [34, 37] and not all sites reveal equal pollen trends [37–39]. Consequently, there is a clear need for regional studies to be conducted.

Daily, seasonal, and inter-annual variations in the abundance of pollen through production, emission and transport is influenced by meteorological factors [32, 40–42]. However, the start of the pollen season, similar to the start of flowering, is particularly sensitive to the temperature of the previous months. Thus, temperature causing chilling and forcing are the most important variables to include in the different models of the start of the pollen season (temperature averages, growing degree days, physi-

ological-based modes) [33, 43–47]. Variations in the commencement of the grass pollen season show a close relationship to cumulative temperatures and to rainfall in May and June [40].

However, observed trends in grass pollen abundance may be more strongly associated with changes in land use and farming practices, such as seed-mix and silage production, than with weather conditions [48, 49]. Pollen counts have been shown to increase with higher seasonal temperatures (birch [32, 50], Japanese cedar [31]). However, the relationship between meteorological variables and specific pollen counts may vary from year to year [51].

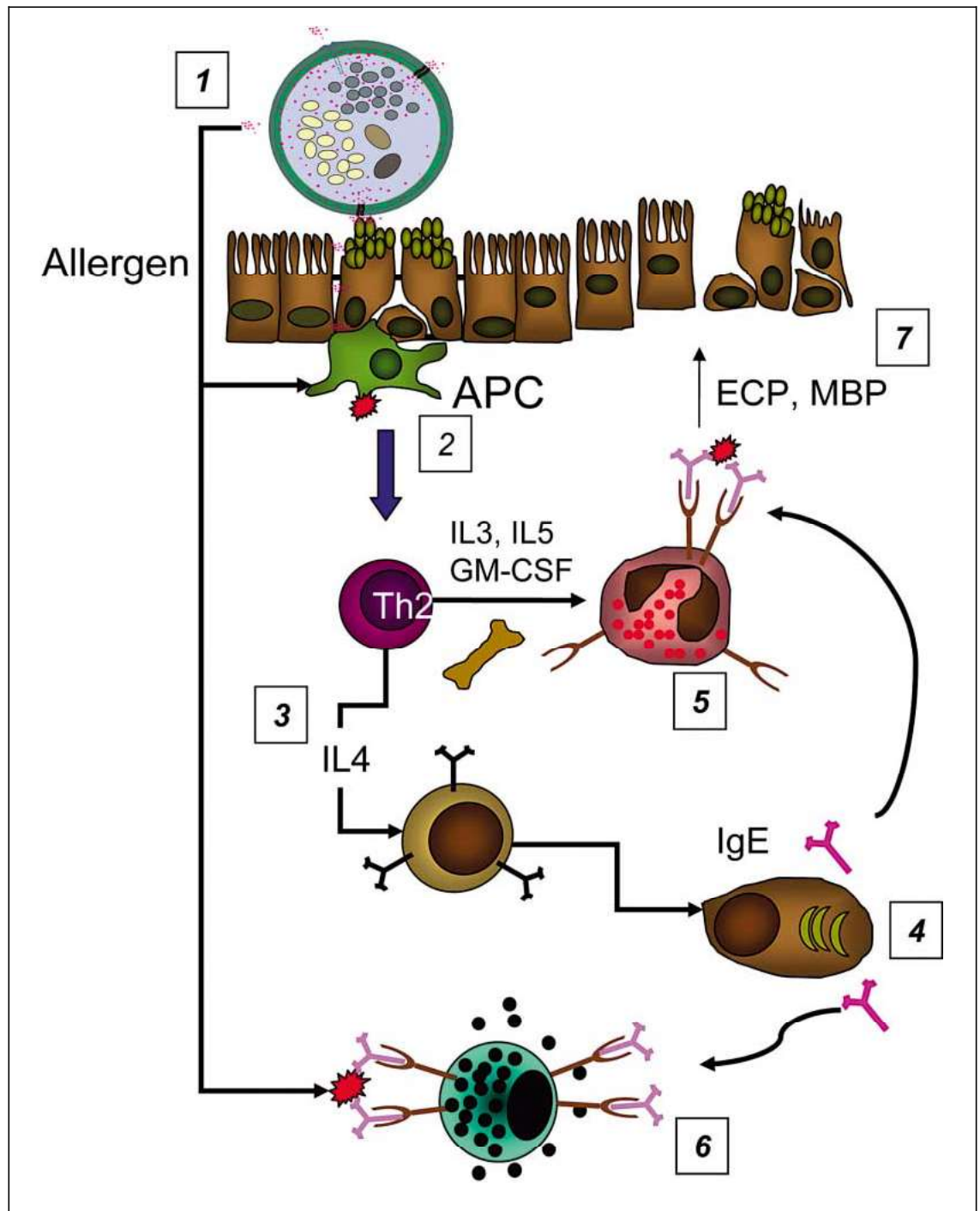
Higher ambient levels of CO<sub>2</sub> may also affect pollen production. Experimental research has shown that a doubling in CO<sub>2</sub> levels significantly stimulated and increased ragweed pollen production [52, 53].

It remains to be investigated to which degree variations in pollen production of observed trends and geographical differences in the prevalence of allergies and sensitisation to allergens are associated to the geographical and temporal variations in the production of pollen, i.e. allergen exposure, that have been observed during recent decades.

### **Pollen Grains as Allergen Carriers and Causative Agents of Allergic Diseases**

The importance of grass pollen in the form of ‘free granular matter’ as potent inducers of allergic reactions was established almost 130 years ago when Blackley [54] performed provocation tests and documented the relationship between pollen exposure and allergic symptoms. The biochemical nature of group 1 grass pollen allergens was analysed approximately 90 years later [55]. Today, it is generally accepted that specific aeroallergens released from pollen cause hypersensitivity and lead to allergic diseases (e.g. bronchial asthma, allergic rhinitis, and allergic conjunctivitis).

The pathophysiologic factors of allergic diseases involve many elements of systemic effector cell recruitment from the circulation, stimulation of bone marrow progenitors, systemic effector-cell priming and mediator release (fig. 3). These mechanisms are thought to be initialised by a specific protein – the allergen [56]. The current paradigm of allergy involves a dual-phase model implying that the first contact with the antigen leads to the formation of antigen-specific T-cells – predominantly Th2 – and the consecutive induction of IgE producing B-cells. A second



**Fig. 3.** Pathomechanisms of the allergic reactions. 1: The primary site of exposure to pollen grains is the epithelium of the upper respiratory tract, which is densely populated by immature dendritic cells [2]. Pollen grains are deposited on mucosal membranes and release the allergen in the aqueous phase. Since pollen allergens are water soluble and readily available they can be taken up by antigen presenting cells such as dendritic cells which migrate into the regional lymph nodes to present the antigen to naïve T-cells with an ensuing Th2 response in the case of type I allergens. The production of IL4 by Th2

cells, mast cells and further not identified cells lead to the isotype switching of B-cells with the resulting allergen-specific IgE production – in susceptible individuals [4]. During the second and every upcoming encounter with the allergen the cross linking of membrane bound (e.g. eosinophils, mast cells and basophils) leads to the release of proinflammatory substances resulting in the immediate allergic reactions [5, 6]. Repeated allergen challenge leads to airway hyper-reactivity [7].

encounter with the presented antigen results in an inflammatory reaction which, in turn, leads to the clinical manifestation of disease, i.e. rhinitis or asthma [56, 57].

Urban air pollution, especially caused by pollutants due to traffic emission, exhibits adjuvant activities in allergen-specific IgE production and skews cytokine production to a Th2-type pattern [58]. Furthermore, traffic-related pollution has been shown to increase prevalence rates of both sensitisation and symptoms of allergic rhinitis in atopic children [59]. Thus, allergies are among those few diseases in which environmental factors of both natural and anthropogenic origin have been identified as a cause of disease development as well as of elicitation and aggravation of symptoms.

Symptoms of rhinitis and asthma due to aeroallergens are major causes of morbidity, loss of productivity in sensitised individuals, and increasing healthcare costs [60]. According to the European Allergy White Paper [61] the prevalence of seasonal allergic rhinitis in Europe is approximately 15%. Current asthma prevalence rates vary from 2.5 to 10.0% and the prevalence of allergic atopic dermatitis ranges from 9 to 24%. These figures highlight the importance of ongoing research in the field of mechanisms and influence factors of allergic diseases.

### **Interaction of Pollen Grains and Environmental Pollutants**

Evidence is accumulating that air pollution may contribute to the increase in pollen allergies and asthma in highly polluted areas [62]. However, no direct correlation was observed between pollen release and the emission peaks of NO<sub>x</sub>, SO<sub>2</sub> and atmospheric fine dust [63, 64]. Only a simultaneous increase and decrease of levels of pollen and of the secondary pollutant ozone was described by Gassner et al. [65]. Interestingly, morphological studies pointed to a direct interaction between pollen grains and pollutants showing that pollen are loaded with pollutants. Pollen grains absorb heavy metals, i.e. lead and cadmium, but also nitrate and sulphur [4, 66]. Furthermore, investigations of dust samples from highly polluted regions in Germany showed a significant degree of particle agglomeration on the surface of pollen grains [67–69]. Particle-absorbed organic substances mainly belong to the group of polycyclic hydrocarbons, phenols or aza-heterocyclic compounds [70] which have been shown to mediate pollen-particle interaction [63]. This interaction leads, firstly, to local pollen pre-activation at the site of contact, secondly, to modulation of allergen release, thirdly, to gener-

ation of allergenic aerosols within moist atmospheres and, finally, to absorption of pollen proteins to airborne particles [63].

The use of fluidised bed reactors facilitates the study of the influence of both gaseous and particulate air pollutants on pollen physiology, e.g. allergen release [71, 72]. In this experimental setting, it became evident that SO<sub>2</sub> induces a significant reduction in the release of Phl p 5, while NO<sub>2</sub> does not impair the bioavailability of the allergen. At the point in time at which grass pollen from *Dactylis glomerata* were incubated with aqueous extracts of airborne particulate matter, a significant dose- and time-dependent impact on the protein release was observed in a manner [71]. Interestingly, in vivo studies comparing allergen release from pollen gathered from rural and highly polluted areas, showed a significantly reduced allergen release, while the release of bioactive lipid mediators (see below) was higher [3].

### **Pollen Allergen Occurrence in PM<sub>2.5</sub> Particles of Ambient Air**

Although allergic reactions in easily accessible organs (skin, nose, eyes) can be explained by direct contact with intact pollen grains and subsequent elution of soluble allergens, it is unclear how pollen allergens access the deeper airways and elicit bronchial asthma. It is assumed that virtually all particles which become trapped in the upper airways have an aerodynamic diameter in excess of 10 µm. Pollen, therefore, which have a diameter *greater* than 10 µm, can be assumed not to play a significant role as potential triggers of allergic asthma. The importance of pollen grains in the induction of asthma gained impetus as a result of the 1987/1989 Melbourne [73, 74] and 1994 London [75] thunderstorm-associated asthma epidemics. The remarkable feature of allergic asthma in Melbourne was the positive correlation between seasonal (spring and early summer) asthma and the grass pollen count which had previously not been observed.

Anemophilous pollens, which are of allergological relevance because of wind pollination, have a diameter ranging from 10 to 100 µm with an average of 20–30 µm [76]. However, micronic particles ≤ 5 µm are required to trigger the asthmatic response [77, 78]. Until recently, the possible presence of pollen components in aerosols such as paucimicronic or smaller particles, was generally dismissed. One of the methodological keystones demonstrating allergenic activity on particles of small sizes was the sampling in different fractions according to the aerody-

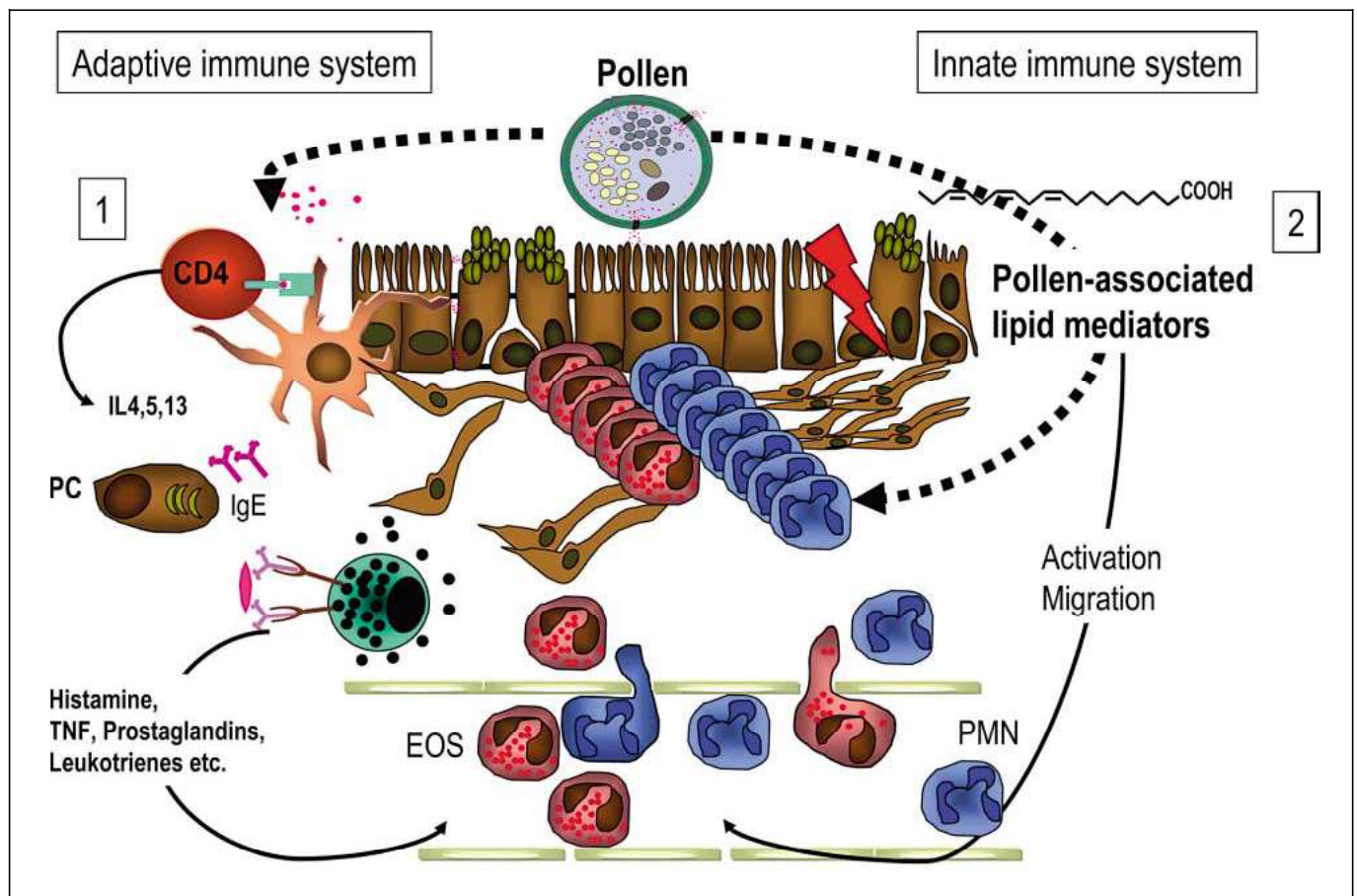
nanic sizes of particles. By the 1980s, the existence of allergen-containing micronic particles had been demonstrated for ragweed [6] and grass pollen allergens [7]. Most data describe microaerosols during periods of source plant anthesis [79] but reports of extraseasonal occurrence also exist [80]. These particles were shown to contain allergens and were submicronic and thus of respirable size ( $\leq 5 \mu\text{m}$ ). Concerning the origin of these respirable, allergen-containing particles, many hypotheses were advanced. One such theory was that pollen fragments are produced by physical degradation in the environment [4]. Another possible source for them is the anther lining in ragweed and grasses which is coated with orbicules (also known as Ubisch bodies [81]), small spherical particles  $0.02 \mu\text{m}$  in diameter, that, like the pollen grain wall, are made of the biopolymer sporopollenin, which could be released into the environment. However, it is worth noting that orbicules are absent from some clinically important *Artemisia* and *Ambrosia* species [82]. Grote et al. [83] suggested that pollen – after hydration – shows expulsion of cytoplasm, namely subcellular, allergen-bearing particles of respirable size. The first description of the emanation of grass pollen allergens as respirable aerosols directly from the flower was provided from Taylor in 2002 [84]. Sch  ppi et al. [8] suggested that pollen grains germinate after light rainfall, producing pollen tubes. Subsequently, each pollen tube releases about 400 starch granules coated with allergen molecules, which can then become dispersed as respirable particles in the atmosphere [8]. Starch granules range in size from  $0.6$  to  $2.5 \mu\text{m}$ , making them easily respirable. Indeed, inhalation of starch granules by asthmatics has been shown to elicit significant bronchoconstriction [85].

Further possible causes of pollen induced asthma include allergen-containing aerosols that bind with physical particulate matter in the environment of various submicronic sizes [9, 86]. Whatever their origin, allergens have been shown to be present in the environment in respirable particles of as small as  $0.1 \mu\text{m}$  [87]. Therefore, in addition to pollen counts, we now need allergen load analyses of aerobiological samples such as immunocytochemical detection of Bermuda grass pollen in the atmosphere, as pioneered by Schumacher et al. [88]. In addition, immunodetection will reveal both pollen-based and micronic particle-based allergens, giving a precise environmental estimate and assisting sufferers in their allergen avoidance programs.

## Pollen Interactions with the Immune System

The respiratory tract contains a number of cell types including epithelial cells, dendritic cells, alveolar macrophages and granulocytes such as neutrophils and eosinophils, all of which are capable of interacting with inhaled particles or allergen-bound particles. Thus, by virtue of their anatomical location, all of these cells have the potential to play a significant role in initiating and regulating airway inflammation following exposure to pollen derived material. Siegel and Shermann [89], in the early seventies, were the first – to our knowledge – to describe pollen interactions with cells of the immune system [89]. In the experimental model of guinea pigs, they showed in vivo and in vitro cross-talk of macrophages, neutrophils and eosinophils with ragweed pollen in terms of adherence and morphological markers for phagocytosis. Lymphocytes, platelets and erythrocytes did not adhere to pollen. The proportion of pollen grains with adhering leucocytes and the number of leucocytes adhering to each pollen grain were dose dependently increased in the presence of serum. Lindberg et al. [90] also described the interaction between PMN and serum-loaded pollen grains leading to the formation of so-called ‘pollen-rosettes’ and ‘frustrated phagocytosis’ [90]. Kay and his colleagues [91, 92] were able to identify this granulocyte/pollen binding protein as serum transferrin following progressive purification [91, 92]. They hypothesised that the widespread extracellular distribution of transferrin may be relevant to the role of this protein in the removal of organic matter, including pollen grains. However, neither the receptor for transferrin (in this interaction) nor the detailed mechanism of granulocyte/pollen interaction has been clarified to date. Our group investigated the outcome of granulocytes/pollen interactions in serum free conditions. We were able to show that pollen grains (birch and grass) adhere to neutrophils [93] and eosinophils [94] leading to the release of granule proteins such as myeloperoxidase and eosinophilic cationic protein, respectively. Concerning the interaction of respirable pollen particles and cells of the respiratory tracts, Currie et al. [95] demonstrated that rat alveolar macrophages bind and phagocytose allergen-containing pollen starch granules via C-type lectin and integrin receptors. Pollen starch granules were also strongly bound by human monocyte-derived dendritic cells, underlining the potential for antigenic sampling of allergen within the pollen starch granules by dendritic cells of the airway lining. The interaction between pollen starch granules and rat alveolar macrophages or human dendritic cells highlights the importance of resident air-





**Fig. 4.** Impact of pollen associated lipid mediators on the allergic cascade. Pollen release the allergen (1) leading to the priming of naïve T-cells and the consecutive allergic cascade (fig. 3). Apart from the allergen pollen also releases bioactive lipid mediators (2) which were shown to activate neutrophils and eosinophils in vitro in terms of

calcium flux, upregulation of  $\beta 2$ -integrins and induction of directed migration. These lipid mediators most probably derive from LA and LeA which are abundantly present in pollen grains. Effects of pollen derived lipid mediators on other cells involved in the allergic cascade are most likely and are currently being investigated.

way cells in the early recognition of allergenic material. This is particularly relevant since such interaction seems to occur in an IgE-independent manner. Firstly, this indicates that, along with the adaptive immune system, innate mechanisms may also contribute to recognition of allergens within the respiratory tract and, secondly, that these mechanisms may occur in allergic and non-allergic patients.

#### Does Pollen Have an Impact on Other Diseases?

Air pollution is mostly regarded as anthropogenic pollution in terms of traffic- or industrial-related smog including  $\text{SO}_2$ , NO and volatile organic compounds. Many studies have investigated the association of daily

variations of this kind of air pollution and cardiovascular and respiratory deaths [96]. Brunekreef et al. [97] expanded upon this view by considering pollen as a form of biogenic air pollution. Most interestingly, they found – in a time-series study in the Netherlands – a strong association between the day-to-day variation in pollen concentrations and that of deaths due to cardiovascular disease, chronic obstructive pulmonary disease and pneumonia. The size of this association was comparable to that observed in mortality on ‘high pollution’ days, which typically shows 5–10% [96]. The association between air pollution and the number of daily deaths was hypothesised to be related to the inflammatory potential of very small particles [98]. One possibility might be that particles of biological origin may have similar effects – and pollen grains

were repeatedly shown to release paucimicronic particles. Another possibility might be that pollen grains release pro-inflammatory substances (see below) contributing to this effect. Of all the aforementioned ideas one thing becomes concretely clear: airborne pollen concentrations seem to have far more effects on human health than previously thought. This effect goes far and beyond the sole induction of allergy.

### **Pollen – Positive Effect on Human Health?**

Apart from its life-threatening effects, pollen also appear to exert positive effects. Bee pollen, that is, floral pollen collected by the honey bee for its protein content, has been used as nutrient-rich health food for many centuries [99]. In addition, different authors state that pollen contains antioxidants such as flavonoids [100, 101], which are mainly found in the exine and are released in moist surroundings [101, 102]. Indeed, pollen extract shows scavenging effects in vitro [103]. Nagai et al. [103] showed that the positive impact of honey and royal jelly on health can be attributed to the antioxidative properties of the pollen. However, to our knowledge, in vivo data are scarce and controlled studies concerning the positive effects of pollen are lacking.

### **Secretion of Proinflammatory Eicosanoid-Like Substances Precedes Allergen Release from Pollen Grains**

As discussed above in detail, pollen releases an exudate containing proteins and lipids upon contact with the aqueous phases. These are most likely significant contributors to the interaction of the stigma with the pollen. Interestingly, a part of these substances exhibits strong cross-reactivity with leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), as measured by ELISA [104].

The release of the 'LTB<sub>4</sub>-like' and 'PGE<sub>2</sub>-like' substances takes place in an aqueous environment without contact with the organism or its inflammatory cells. The release is temperature, pH and time dependent and peaks after 5 min at a pH of 7.4 and 37°C. Interestingly, the lipid mediators – measured by cross-reactivity with LTB<sub>4</sub> – reach the maximum release much faster than, for example, Bet v 1.

Prostaglandins and leukotrienes are metabolites of arachidonic acid (not present in birch pollen) [105]. Other unsaturated fatty acids, like linoleic acid and linolenic

acid, are major components of membrane fatty acids in plants, as already discussed in detail. By HPLC and GC analysis, we identified high amounts of linoleic acid (LeA) and linolenic acid (LA) and its octadecanoid metabolites (9-, 12-, 13-, 16-HOTE, 9-, 13-HODE) in lipid extracts (hexan-isopropanol extracts from whole pollen) from birch and grass pollen (295 nmol/g LeA, 288 nmol/g LA vs. 3,800 nmol/g LeA, 821 LA). Notably, LeA and LA as well as their auto-oxidative products were found in aqueous pollen extracts, whereas lipoxygenase mediated enzymatic products of LeA and LA were minor in birch and even absent in grass. Interestingly, a distinctive and reproducible pattern of monohydroxylated products was observed for both grass and birch pollen extracts. Whether definable lipids function in the variable and complex reproductive process at stigmatic surfaces remains to be determined. However, their very rapid release makes them ideal candidates in these pollen-stigma negotiations. Concerning their effects on human health, the cross-reactivity to potent human lipid mediators known to effect the human innate and adaptive immune system prompted us to further investigate the effects of aqueous pollen extracts and their constituents.

### **Effects of Pollen-Associated Lipid Mediators on the Innate Immune System**

Recently, oxidized derivatives of LeA have been identified in a variety of human cells and their metabolites have been shown to exhibit a number of biological functions including modulation of cell proliferation, apoptosis and inflammation [106, 107]. In order to delineate the biological activity of the observed pollen derived lipid mediators we investigated their effects on human granulocytes – neutrophils and eosinophils. Eosinophils are generally accepted as a cell population playing an important role in the allergic reaction [108]. On the contrary, the precise role of PMN in the pathophysiology of allergic diseases is still a matter of debate. However, there is general consensus that PMN contribute to the manifestation of allergic inflammation. As cells of the innate immune system PMN play an important role in a number of allergic diseases [109–111]. In asthma, PMN are among the first cell type to enter the lung following allergen challenge [112], and in allergic rhinitis local inflammation is associated with an accumulation of PMN in the nasal tissue [113, 114].

In a most recent study we could demonstrate, that aqueous pollen extracts activate and recruit PMN in vitro

[115]. The observed effects are independent of the donor sensitisation status (i.e. can be induced in PMN from allergic as well as non-allergic individuals) and cannot be triggered by stimulation with allergen alone. In addition, we demonstrated that lipid extracts from pollen and RP-HPLC-purified extracts containing primarily the mono-hydroxylated products of the LA and LeA induce similar effects in PMN. Thus, pollen-derived lipid mediators are most likely responsible for the chemotactic and stimulating activity of aqueous pollen extracts on PMN. Nevertheless, aqueous pollen extracts contain considerable amounts of minor and major allergens [104]. Thus, one may argue that the observed effects were induced by allergen specific mechanisms. However, the occurrence of chemotaxis in both allergic and non-allergic individuals makes an IgE mediated mechanism unlikely. Additionally, we performed migration assays with recombinant Phl p 5, the major grass pollen allergen from *Phleum pratense* L., in concentration comparable to those found in APE. Here, no PMN migration was observed, suggesting that the APE induced migration was indeed mediated via an allergen-independent mechanism. Furthermore, we also demonstrated, the LTB<sub>4</sub>-receptor was involved in the induction of migration because the migration was blocked by the LTB<sub>4</sub>-receptor antagonist LY293111. Investigations are ongoing to characterise the substance(s) responsible for the chemotactic activity and to perform ligation studies of the LTB<sub>4</sub>-receptor.

In addition, pollen derived lipid mediators induced an upregulation of  $\beta$ 2-integrin (CD11b) suggesting these substances act not only chemotactically but also facilitate the transmigration of PMN into the tissue since  $\beta$ 2-integrins are critically involved in the interaction with the endothelium during cell recruitment in inflamed tissue.

In conclusion, bioactive lipid mediators not only show structural resemblance to leukotrienes (crossreactivity in the LTB<sub>4</sub>-ELISA) but also functional similarities (induction of migration and activation of PMN). This prompted us to name these substances *pollotrienes*. Whether the prostaglandin-like substances correspond to the phyto-prostanoids described by Müller [105] and his group is currently under investigation.

## Conclusion

The dispersion of replicate units such as pollen grains in massive abundance assures the success of wind pollination as well as its human health effects. The chemical complexity of pollen, including a host of diverse allergens

and bioactive lipid mediators, reflects the demands of the complex reproductive mission that it subserves. The research on effects of pollen on human health has to take into account this complexity. First of all, it is imperative that we gain a more precise idea about the physiologic role of substances released from pollen, which are harmful for humans. Thus, it is also apparent that we should not only focus on the effects on the singular protein on the immune system but rather to investigate the effect of the whole causative agent – the pollen grain. Our observations on bioactive lipid mediators suggest that rapid release of these substances from pollen during contact with mucous or respiratory membranes may act as allergen-independent pro-inflammatory factors contributing to initiation, manifestation or aggravation of allergic inflammation. Increasing our understanding on the nature of the effects of pollen derived lipid mediators on the immune system of allergic patients may pave the way for new therapies based on inhibition of such interactions or subsequent mediator release.

Furthermore, studies indicating that pollen also affects the cardiovascular and pulmonary system strongly suggest that we have to consider various pollen effects on human health.

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