



Contents lists available at ScienceDirect



Review

The dangerous liaison between pollens and pollution in respiratory allergy

Giovanna Schiavoni, BS^{*}; Gennaro D'Amato, MD[†]; Claudia Afferni, BS[‡]

^{*} Department of Hematology, Oncology and Molecular Medicine, Istituto Superiore di Sanità, Rome, Italy

[†] Division of Respiratory and Allergic Diseases, Department of Chest Diseases, High Speciality A. Cardarelli Hospital, Naples, Italy

[‡] Department of Infectious, Parasitic and Immune-mediated diseases, Istituto Superiore di Sanità, Rome, Italy

ARTICLE INFO

Article history:

Received for publication November 2, 2016.

Received in revised form December 6, 2016.

Accepted for publication December 19, 2016.

ABSTRACT

Objective: To recapitulate the more recent epidemiologic studies on the association of air pollution with respiratory allergic diseases prevalence and to discuss the main limitations of current approaches used to establish a link between pollinosis and pollution.

Data Sources: Through the use of PubMed, we conducted a broad literature review in the following areas: epidemiology of respiratory allergic diseases, effect of pollution and climate changes on pollen grains, and immunomodulatory properties of pollen substances.

Study Selection: Studies on short- and long-term exposure to air pollutants, such as gaseous and particulate materials, on allergic sensitization, and on exacerbation of asthma symptoms were considered.

Results: Trend in respiratory allergic disease prevalence has increased worldwide during the last 3 decades. Although recent epidemiologic studies on a possible association of this phenomenon with increasing pollution are controversial, botanic studies suggest a clear effect of several pollutants combined to climatic changes on the increased expression of allergenic proteins in several pollen grains. The current literature suggests the need for considering both pollen allergen and pollutant contents for epidemiologic evaluation of environmental determinants in respiratory allergies. We propose that a measure of allergenic potential of pollens, indicative of the increase in allergenicity of a polluted pollen, may be considered as a new risk indicator for respiratory health in urban areas.

Conclusion: Because public greens are located in strict proximity to the anthropogenic sources of pollution, the identification of novel more reliable parameters for risk assessment in respiratory allergic diseases is an essential need for public health management and primary prevention area.

© 2017 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Most chronic respiratory noncommunicable diseases (CRNCDs), particularly asthma and allergic rhinitis, may be partially preventable. Risk factors (RFs) have been identified and efficient preventive measures have been proposed. For example, tobacco smoking, outdoor and indoor air pollution, allergens exposure, environmental changes, occupational agents, and lifestyle are all preventable RFs for respiratory allergies. Despite the significant effect on morbidity and mortality, prevention in respiratory allergies and their RFs are especially underestimated by many governments. In fact, only 3% of European health expenditure is currently invested in prevention, whereas 97% is spent on treatment. In 2016 the

estimated annual cost of asthma treatment in Europe was more than €18 billion.¹ Allergic rhinitis therapy may even cost more (up to €100 billion), according to unpublished data from the Global Allergy and Asthma Network of Excellence investigators.

Several international organizations (World Health Organization, European Union), Italian institutions (Italian Ministry of Health, Italian Ministry of Environment), and scientific associations (European Respiratory Society, European Academy of Allergy and Clinical Immunology) have promoted the need to improve primary prevention measures for CRNCDs, which are cost-effective with respect to treatment.² In 2011, the European Respiratory Society launched a Respiratory Roadmap with the aim of providing health care policymakers with recommendations of the most urgent measures needed to reverse the increasing trend for the CRNCDs (www.ersroadmap.org). Two of 4 main interventions indicated by the roadmap fell in the areas of prevention and research. The primary prevention measures were to reduce urban air pollution, improve indoor air quality, and manage the consequences of natural events, such as climate changes. With regard to research, the

Reprints: Claudia Afferni, BS, Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy; E-mail: claudia.afferni@iss.it.

Disclosure: Authors have nothing to disclose.

Funding Sources: This work was supported by intramural funding of Istituto Superiore di Sanità, Rome, Italy.

<http://dx.doi.org/10.1016/j.anai.2016.12.019>

1081-1206/© 2017 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

roadmap identified specific targets to direct the funding resources, such as host defense response, innovative approaches to restore pulmonary function, and better translation into clinical practice. The present review is focused on the primary prevention area aiming to underline the importance of investigating the environmental determinants of respiratory allergic diseases and to suggest a novel approach for translating scientific data into preventive measures.

Environmental Determinants of Respiratory Allergic Diseases: Air Pollution and Pollens

Effects of Air Pollution on Respiratory Symptoms

Traffic-related air pollution (TRAP) in urban areas has a relevant effect on respiratory health, especially for infants and elderly people.³ It has become evident that urban individuals have more severe symptoms of respiratory allergies compared with those living in rural areas.⁴ Of interest, Fuertes et al⁵ found that the mean level of greenness within 500 m from the home address was positively associated with allergic rhinitis and eyes and nose symptoms in 10-year-old children from a German urban area, whereas a negative association was observed in children living in a rural area.

Air pollution has direct effects on respiratory symptoms of allergic individuals, causing an inflammatory response in the airways and an increased susceptibility to aeroallergens exposure.⁶ In addition, air pollution may have indirect effects by combining aeroallergens with particles, such as diesel exhaust,⁷ therefore modifying their features.⁸ Furthermore, air pollution and climate changes could induce an enhanced expression of allergenic and proinflammatory molecules in pollen grains attributable to adaptation of plants to abiotic stress.⁹

Several studies have analyzed the effects of short-term exposure to air pollutants on respiratory symptoms. A positive correlation was found between exposure to ozone, carbon monoxide, nitrogen dioxide, sulfur dioxide, and particulate matter (PM) of 10 μm or less (PM_{10}) and 2.5 or less ($\text{PM}_{2.5}$) with asthma-related hospitalizations.¹⁰ Moreover, several studies have found a significant association between long-term exposure to air pollutant with asthma incidence.¹¹ Bowatte et al¹² reported that TRAP exposure to levels well below the guidelines provided by the World Health Organization ($\text{PM}_{2.5}$, 10 $\mu\text{g}/\text{m}^3$ annual mean; nitric dioxide, 40 $\mu\text{g}/\text{m}^3$ annual mean) is associated with increased incidence of asthma and risk of sensitization to common allergens during childhood. Overall, these epidemiologic studies suggest that although short-term exposure to ozone, nitric dioxide, sulfur dioxide, $\text{PM}_{2.5}$, and TRAP preferentially exacerbate asthma symptoms, long-term exposure, especially to TRAP, is associated with asthma onset in children and adults.

Effects of Air Pollution on Allergic Sensitization

Although the effects of air pollutants on asthma exacerbation have been well documented in epidemiologic studies, the effect of pollutants on the respiratory allergic sensitizations has been less investigated. Hansel et al¹³ conducted cross-sectional analyses on a population of children selected for the atopic phenotype. The authors found a significant association in the weighted road density with positivity to skin prick test and IgE specific to house dust mite, ryegrass, grass mix, cat dander, and *Alternaria*. Intriguingly, a recent meta-analysis study based on the aforementioned reports has argued that a positive association exists between the concentrations of particulate matter and allergen sensitization.¹⁴ However, the results of this meta-analysis outlined a trend, although not statistically significant, for children who grew up in the same location until 8 to 10 years of age to develop allergic sensitizations associated with particulate matters exposure ($\text{PM}_{2.5}$, PM_{10} , and PM coarse). The limits of this meta-analysis study were that clinical

data and PM exposure parameters were not rigorously measured for children who moved to other neighborhoods. Overall, we may hypothesize that other phenomena, besides PM levels, may contribute to the increase in allergic sensitizations. For example, the whole airborne allergenic contents and/or its allergenic potency may also be increased together with PM and, thus, differently contribute to allergic sensitization prevalence increase. In this regard, the contents of pollen allergens in PM_{10} have only rarely been analyzed.¹⁵ Plant debris, which represents 1% of total anthropogenic PM_{10} and is estimated to be composed by pollen grains, is equal to 6 $\text{kg}/\text{km}^2/\text{y}^{-1}$ in Europe.¹⁶ Another interesting study reported that an interquartile range increase of 17.6 grains/ m^3 for *Poaceae* pollen determined an adjusted relative risk of 1.54 (95% confidence interval, 1.33–1.79) for allergic sensitization.¹⁷

Air pollutants may enhance sensitization to aeroallergens by a direct effect on the respiratory mucosal environment. Two principal mechanisms of action have been proposed: the increased epithelial permeability attributable to oxidative injury and a direct adjuvant effect.¹⁸ In particular, studies on patients with asthma have suggested an increased susceptibility of airway epithelial cells to deleterious effects of air pollutants. In fact, ozone, nitric dioxide, and diesel exhaust particles were found to increase the permeability of bronchial epithelial layer attributable to oxidative and nitrosative stress and to inhibit the ciliary beat frequency, leading to delayed clearance of allergens and irritants inhaled. In turn, increased permeability of airways may favor the access of these agents to the submucosa, where they interact with resident cells, such as airway smooth muscle cells and fibroblasts.

Several in vitro studies conducted on human bronchial epithelial cells from patients with asthma suggest that air pollutants modulate various inflammatory parameters at the cellular level. For example, ozone increases the release of inflammatory mediators, including platelet-activating factor, interleukin (IL) 8, granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor alpha (TNF- α), and soluble intercellular adhesion molecule 1 (sICAM-1). Nitric dioxide enhances the release of leukotriene C4, GM-CSF, TNF- α , IL-8, regulated on activation normal T-cell expressed and secreted (RANTES), and sICAM-1. Diesel exhaust particles induces the production of IL-8, GM-CSF, and sICAM-1 from primary human bronchial epithelial cells. These inflammatory cytokines and chemokines attract into the airways and lung parenchyma mast cells, eosinophils, lymphocytes, and neutrophils, which in turn secrete additional soluble mediators that cause tissue damage.¹⁹ The increased permeability of airways may also favor the penetration of airborne allergens to the submucosa, where they may interact with resident dendritic cells and innate lymphoid cells and with recruited inflammatory mast cells, eosinophils, lymphocytes, and neutrophils.²⁰

Several studies have found a direct adjuvant effect of air pollutants. Studies on human B cells found that diesel exhaust particles and their derivative polyaromatic hydrocarbons can induce the synthesis of IgE in the presence of IL-4, suggesting that this pollutant may potentiate the sensitivity to common allergens.²¹ Another study on human airway epithelial cells found that diesel exhaust particles increased the expression of HLA-DR and CD86 molecules, essential to present allergens to T cells.²²

Effect of Air Pollution and Climate Change on Pollen Grains

Pollen allergens are found in respirable particles, including whole anemophylous pollen grains (10–30 μm mean diameter), pollen-derived starch cytoplasmic granules, pollen-derived debris (0.6–2.5 μm in diameter), and submicronic particles called orbicules (0.02–1 μm in diameter). Sch  ppi et al²³ first found the relevant contribution of particulate allergen carriers below 7.5 μm mean diameter to respiratory symptoms in allergic patients.

Table 1

Pollen Substances Upregulated by Pollution and Abiotic Stresses Endowed With Immunomodulatory Activity

Upregulated plant substances (Biochemical family)	Plant tissue	Plant substances already referenced as allergens	References mentioning the immunomodulatory activity	Abiotic stress or polluting substance
Actin-binding proteins (profilins)	Several different plant tissues	Approximately 203 plant allergens listed in the Allergome database: http://www.allergome.org/	Burastero et al ³⁶	Ozone
Allergen-bearing starch cytoplasmic and orbicules granules	<i>Phleum</i> pollen, Cupressaceae pollen, <i>Phaseolus</i> pollen		Pazmandi et al ²⁵	Ozone, nitric dioxide, sulfur dioxide, diesel exhaust material (DEM), traffic exhaust
Chitinases (class I and IV)	<i>Poa</i> pollen, <i>Ambrosia</i> pollen, <i>Helianthus</i> root, <i>Zea</i> root, <i>Pisum</i> root, <i>Vicia</i> root, <i>Brassica</i> flower bud, <i>Castanea</i> shelled nuts, <i>Hevea</i> natural latex, <i>Persea</i> fruit, <i>Musca</i> fruit, <i>Vitis</i> fruit	Hev b 6.01, Hev b 6.02, Hev b 11, Cas s 5, Cry j chitinase (CJP-4), Pers a 1, Mus a 1	Lee et al ³⁷	Cadmium, ozone, mercury, manganese, sodium chloride, drought, traffic exhaust
Expansins	<i>Cynodon</i> pollen, <i>Dactylis</i> pollen, <i>Lolium</i> pollen, <i>Phleum</i> pollen, <i>Oryza</i> pollen	Cyn d 1, Dac g 1, Lol p 1, Phl p1, Ori s 1	Roschman et al ³⁸	Ozone
Flavonoids	<i>Thuja</i> pollen, <i>Parietaria</i> pollen	<i>Parietaria judaica</i>	Sallusto et al ³⁹	Traffic exhaust
Glycans (as free polysaccharides and glycoconjugates)	Cupressaceae pollen, <i>Podocarpus</i> pollen, <i>Olea</i> pollen, <i>Artemisia</i> pollen, <i>Arachis</i> seed, <i>Actinidia</i> pollen		Royer et al ⁴⁰	Chromium
Isoflavone reductases	<i>Ole</i> pollen, <i>Betula</i> pollen, <i>Solanum</i> pollen, <i>Fraxinus</i> pollen, <i>Cryptomeria</i> pollen, <i>Pyrus</i> fruit, <i>Citrus</i> fruit, <i>Daucus</i> root, <i>Diospyros</i> fruit, <i>Litchi</i> fruit, <i>Hevea</i> natural latex	Bet v 6, Ole e 12, Fra e 12, Cry j IFR, Cit s IFR, Dau c 5, Dio k IFR, Lit c IFR Pyr e 5, Hev b IFR	Oseroff et al ⁴¹	UV-C, wound, microbial derived elicitors
Lipids and peroxidized lipids and lipoxigenases	Cupressaceae pollen, <i>Thuja</i> pollen, <i>Actinidia</i> pollen, <i>Lycopersicon</i> leaves, <i>Hordeum</i> pollen		Abos-Gracia et al ⁴² ; Plotz et al ⁴³ ; Russano et al ⁴⁴	Chromium, lead, osmotic stress
Lipid transfer proteins	<i>Ambrosia</i> pollen, <i>Betula</i> pollen, <i>Arachis</i> seed, <i>Corylus</i> seed, <i>Sesamum</i> seed, <i>Glycine</i> seed, <i>Prunus</i> fruit, <i>Bertholletia</i> seed	Amb a 6, Bet v 1, Par j1, Ara h 8, Mal d 1, Pru p 3, Ber e 1, Sin a 1, Ara h 10, Ara h 11, Ara h 14, Ara h 15, Cor a 12, Cor a 13, Ses i 4, Ses i 5, Gly m 30K, Gly m 5	Bublin et al ⁴⁵	Sulfur dioxide, traffic exhaust
NADPH oxidases	<i>Ambrosia</i> pollen, grasses pollen	Amb a 1	Csillag et al ⁴⁶ ; Bacsí et al ⁴⁷	Ozone, heavy metals, sodium chloride, drought
Oleosins	<i>Ambrosia</i> pollen, <i>Betula</i> pollen, <i>Arachis</i> seed, <i>Corylus</i> seed, <i>Sesamum</i> seed, <i>Glycine</i> seed, <i>Prunus</i> fruit, <i>Bertholletia</i> seed	Ara h 10, Ara h 11, Ara h 14, Ara h 15, Cor a 12, Cor a13, Ses i 4, Ses i 5	Bublin et al ⁴⁵	Sulfur dioxide, traffic exhaust
Pectatelyases	<i>Ambrosia</i> pollen, Cupressaceae pollen	Amb a 1, Cup a 1, Cr j 1, Jun o 1	Gabriele et al ⁴⁸	Ozone, carbon dioxide, traffic exhaust
Phospholipases A2	<i>Oryza</i> seeds and panicle, <i>Nicotiana</i> flowers, <i>Olea</i> pollen, <i>Citrus</i> pollen, <i>Triticum</i> pollen, <i>Arabidopsis</i> pollen, <i>Solanum</i> tuber, <i>Malus</i> pollen, <i>Hevea</i> natural latex	Heb v 7	Hanasaki et al ⁴⁹	Sodium chloride, drought, salicylic acid
Phytosteranes	<i>Betula</i> pollen and <i>Phleum</i> pollen		Tradl-Hoffmann et al ⁵⁰	Traffic exhaust
Proteases (serin-, cistein-, aspartic-protease)	Cupressaceae pollen, <i>Phleum</i> pollen, <i>Corylus</i> pollen, <i>Betula</i> pollen, <i>Ambrosia</i> pollen	CPA63	Tang et al ⁵¹	Ozone, heavy metals
RNAse	<i>Betula</i> pollen, <i>Secale</i> pollen	Bet v 1, Phl p 5, Lol p 5	Smole et al ⁵²	Ozone
Thaumantins (endo- β 1,3 glucanase) and thaumantin-like proteins	<i>Cupressus</i> pollen, <i>Juniperus</i> pollen, <i>Cryptomeria</i> pollen, <i>Olea</i> olive oil, <i>Triticum</i> wheat flour, <i>Malus</i> fruit, <i>Musa</i> fruit, <i>Prunus</i> fruit	Cup a 3, C s 3, Jun a 3, Jun r 3, Jun v 3, Cri j 3, Act d 2, Mal d 2, Mus a 4, Pru av 2, Pru p 2.0101, Pru p 2.0201, Tri a thaumantin-like protein, Ole e 13	Oseroff et al ⁴¹	Traffic exhaust, heat
Transglutaminase 2	<i>Corylus</i> pollen, <i>Malus</i> pollen		Iorio et al ⁵³	Heat, copper, acidic rain

Subsequently, several authors identified relevant pollen allergens carried by airborne particles released by pollen grains of grass,²⁴ ragweed,²⁵ and cypress²⁶ under natural rain or on in vitro treatment with hypotonic buffer. The orbicules present on the exine also carry allergenic molecules and are released by hydration or pollution-induced rupture of pollen grains.²⁶

Increasing data indicate that pollen grains and air pollution mutually interact. Depending on the plant species and on the pollutant type and concentration, this interaction may alter the vitality, shape, size, physiologic features, and metabolism of the pollen grain.²⁷ Pollutants and climate changes are among the main plant stressors, especially in urban areas. One of these stress inducers is ozone, known to induce NADPH increase in ragweed

pollen, which in turn generates reactive oxygen species (ROS).²⁸ Plant ROS not only are an unwelcome by-product of metabolism but also serve as important regulators of plant development and growth, particularly during pollen germination and cell signaling on the stigma.²⁹ The pollen pectate lyases, including 2 major pollen allergens Amb a 1 and Cup a 1 (from *Ambrosia artemisiifolia* and *Cupressus arizonica* species, respectively), were also increased under ozone exposure.³⁰ Plants particularly rely on proteomic plasticity for self-remodeling during periods of developmental change, to endure varying environmental conditions, and to respond to biotic or abiotic stresses.³¹ A direct result of stress-induced cellular changes is the enhanced intracellular accumulation of toxic compounds, including ROS. Plants frequently respond to stress by

overexpressing different families of specific proteins, most of which belong to ROS-scavenging pathways (superoxide dismutase, ascorbate peroxidase, catalase, glutathione peroxidase, and peroxiredoxin). Comparative proteomics is currently the most powerful tool for the analysis of biochemical pathways and of complex responses of plants to environmental stimuli.³²

Climate changes seem also to have an effect on pollen allergenicity.¹⁸ Tashpulatov et al³³ found that temperature increase determine higher allergen contents in Birch pollen. In this regard, the urban environment is particularly sensitive to the global warming, owing to diffuse soil sealing that, in turn, causes land drying, particularly in the Mediterranean area. Thus, flowering plants growing in the urban areas develop mechanisms that protect flowers from severe dehydration. Recent data indicate that floral organs of *Arabidopsis thaliana* have more than 4,000 genes whose expression is modulated under severe drought and less than 2,000 genes modified under moderate drought conditions. *Ambrosia* pollen genes corresponding to allergenic proteins (Amb a 9, Amb a 10, Amb a 1.1, Amb a 1.2, Amb a 1.3) and the cystatin proteinase inhibitor (Amb a CPI) were found to be upregulated under drought condition.³⁴ Particular types of thunderstorms determine the formation and long-distance diffusion of small allergenic particles from pollen or mold (<10 μ m mean diameter), able to induce severe asthma symptoms (thunderstorm-related asthma).³⁵ The most relevant studies on immunomodulating activities of pollen substances that are enhanced in plants grown under abiotic stress are summarized in Table 1.

A Novel Concept of Pollen Allergenicity: Beyond the IgE-Binding Activity

Allergenicity Paradigm: The Underestimated Value of T_H2 Polarization

The physiopathologic mechanism of respiratory allergies is essentially supported by an altered immune response against apparently innocuous foreign proteins. Although it has been well established that this immune response is mediated by T_H2 lymphocytes, the mechanism of allergic sensitization, in particular to pollens, is still a subject of debate. It is accepted that the immune system can develop an adaptive immune response after sensing of pathogens through the so-called danger signals. These signals are normally provided by microbial toxins or other genetically conserved components called pathogen-associated molecular patterns that interact with germ line-encoded pattern recognition receptors, which are expressed by innate immune cells. Like microbial antigens, also allergens can engage innate pattern recognition receptors, leading to allergic immune responses classically indicated by T_H2 polarization.⁵⁴

T_H2 immune responses are strongly induced by multicellular parasites, such as helminths. It is likely that T_H2 -mediated responses may have evolved from the innate response as a mechanism to isolate and encapsulate macroparasites through the deposition of extracellular matrix proteins.⁵⁵ Parasitic pathogen-associated molecular patterns seem to have a critical role in driving the T_H2 type of immune response, and their modulatory activity is often mediated by multiple glycan moieties. For example, the $\alpha 1 \rightarrow 6$ fucosyl core motif is the active moiety of the helminths molecule $\kappa 5$ glycoprotein, a ligand of the mannose receptor.⁵⁶ Interestingly, this glycan moiety resembles a widely shared pollen allergen glyco-motif,⁵⁷ and some glycoprotein allergens have an immunomodulatory activity triggered by the carbohydrate moiety.^{40,58} Therefore, we might hypothesize that many of the immunomodulatory activities of pollen allergens may be triggered by such glycan motifs.

Certain pollen allergens with function of proteolytic enzymes are endowed with potent T_H2 polarizing activity. The best known

Table 2
Simulation of a Risk Assessment Concerning the Effect of Several Environmental Determinants on Respiratory Allergic Diseases

Environmental determinants		Risk determinants				Risk indicators	Health effect
Rain level per day	Presence of allergenic plant species in public and private green	Air pollution by vehicular traffic	Air pollution by anthropogenic oxidizing (ozone and carbon dioxide)	Heavy metals present in PM, soil, and water	Global warming and heat waves	Droughts by soil sealing	
X	X	X	X	X	X	X	Shifting of the cytokines pattern toward an inflammatory T_H2 -type pattern
X	X	X					Increasing prevalence of respiratory allergic diseases; expansion of the allergenic specificities recognized by patients' IgE; increasing spectrum of the allergenic cross-reactivities
							Increasing prevalence of respiratory allergic diseases and symptoms worsening
X	X	X	X	X	X	X	Increasing prevalence of respiratory allergic diseases and symptoms worsening
							Increasing prevalence of respiratory allergic diseases and symptoms worsening
							Increasing prevalence of respiratory allergic diseases; increasing number of days with symptoms

Abbreviations: APP, allergenic potential of pollen; PM, particulate matter. X indicates the presence of the indicated environmental determinant.

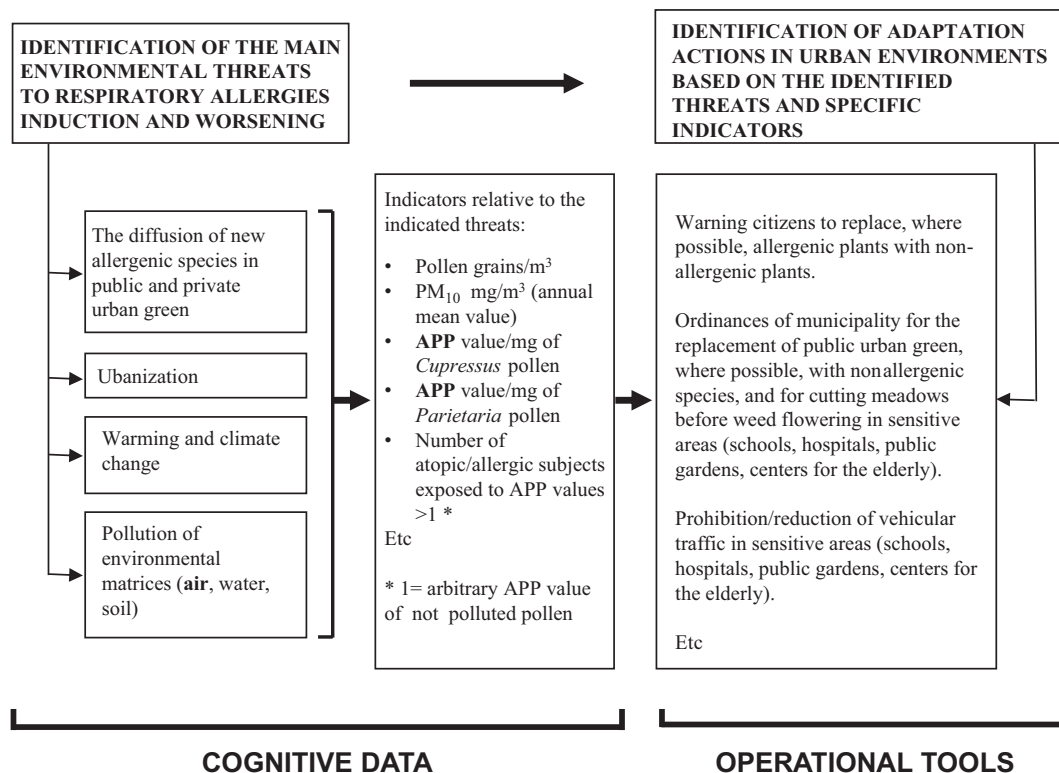


Figure 1. Model of information flow showing how the allergenic potential of pollens (APP) measure for *Cupressus* or *Parietaria* pollens (cognitive data) could be used to monitor the effectiveness of actions (operational tools). PM₁₀ indicates particulate matter of 10 μ m or less.

examples are found in pollens from Japanese cedar (*Cryptomeria japonica*), Japanese cypress (*Chamaecyparis obtusa*), Rocky Mountain juniper (*Juniperus scopulorum*), short ragweed (*Ambrosia artemisiifolia*), Kentucky blue grass (*Poa pratensis*), rye grass (*Lolium perenne*), Bermuda grass (*Cynodon dactylon*), and white birch (*Betula alba*).⁵⁹ Because approximately 70% of natural allergens are enzymes, it may be envisaged that the enzymatic activity could be somehow relevant to the immunomodulatory activity of allergens. For example, NADPH oxidase, found in pollens from 38 allergenic species, is able to induce local oxidative stress by directly increasing ROS in airway epithelium,⁶⁰ dendritic cells,⁴⁶ and mast cells,⁶¹ which, in turn, generate an intracellular danger signal and/or histamine/serotonin secretion. Moreover, pollen-mediated oxidative stress was also proven to be clinically relevant for immediate hypersensitivity reactions and late-phase inflammation in the allergic conjunctivitis.⁶²

Immunomodulating activities have also been ascribed to bioactive pollen-associated lipid mediators (eg, oxylipins) identified in birch, grasses, ragweed, Rocky Mountain juniper, Japanese cedar, and Japanese cypress^{63,64} and structural phosphatidylethanolamine and phosphatidylcholine from both Cypress and *Olea* pollen.⁴⁴ Importantly, the pollen grain is an active site for triacylglycerol biosynthesis and gathers large amounts of oil bodies in the cytoplasm. Because most of the above mentioned substances are not IgE-binding molecules, we propose that the concept of pollen allergenicity should be reconsidered. In fact, because pollen grains and derived particles are complex particulate structures, their effect on respiratory health should be evaluated not only on the basis of the IgE binding properties but also for their TH₂-polarizing potential.

Pollen Allergenicity Assessment

The relevance of the immunomodulatory activity of pollen-associated substances (Table 1) to allergic sensitization suggests

the measure of pollen allergenicity as a parameter for risk assessment of air emissions and climate changes. Currently, the measure of whole pollen allergenicity is provided only for the environmental risk assessment of genetically modified plants cultivation. The European Food Safety Authority expert opinions (www.efsa.europa.eu) suggest evaluating on a case-by-case basis the need of using animal models to determine the allergenic potential of genetically modified pollens. Several mouse models of respiratory and food allergy have been developed during the last 10 years.⁶⁵

On the basis of the extensive experience on preclinical mouse models of allergic disorders, we propose that validated end points should be established to measure the allergenic potential of a given polluted pollen with respect to the reference pollen preparation. Such end points establish a score value that may be called allergenic potential of pollen (APP), indicative of the increase in allergenicity of a polluted pollen with respect to the nonpolluted one. In support of the importance of determining the allergenicity of pollens, a recent study⁶⁶ indicates that the simple count of pollen grains not always correlate with the prevalence of pollen sensitization or with allergic respiratory symptoms. Instead, the authors of this study suggest determining the whole airborne allergen contents rather than the number of pollen grains. Because the environmental pressure factors do not just quantitatively change the allergenic molecules' contents but mostly determine an increase in the whole allergenic potential of pollen particles, we suggest that this latter parameter, namely APP, be evaluated. The APP is a score value of a pollen that takes account of validated pathologic and immunologic end points (ie, inflammation score, specific IgE, mucous production) within a well-established in vivo model of respiratory allergy. According to the simulation given in Table 2, the column of risk indicators could be implemented with the addition of the APP measure, which may help predict the increase of prevalence and exacerbation of symptoms in allergic respiratory diseases (health effect column). In this context, we propose a model of

information flow (Fig 1) that connects the cognitive data with the policies for adaptation of urban life to climate changes and pollution. As an example, in this model the APP measure for *Cupressus* or *Parietaria* pollens (cognitive data) could be used to monitor the efficacy of actions (operational tools) aimed at modifying the environmental threats and adaptation behaviors.

Conclusions

Despite a positive trend in the prevalence of allergic sensitization in urban places, the factors that account for this phenomenon have not been established so far. However, accumulating evidence indicates that pollutants and climate change act as plant stressors, modifying the expression of plant molecules endowed with immunogenic properties, such as those present in pollens. In this view, a new concept of pollen allergenicity that takes into consideration stress-induced increase in the allergenic potential of pollen particles should be considered as a new reliable environmental determinant in respiratory allergies epidemiology. Thus, it would be useful to validate the APP measure by means of mouse models, for example, by determining a range of scores for some known pathological end points, such as lung cellular infiltration, mucous production, and airway hyperreactivity. In perspective, a recently developed in vitro technology named lung-on-chip⁶⁷ may represent a valuable approach, alternative to animal models, to evaluate the allergenicity of pollens and other sensitizers by closely mimicking the in vivo scenario.

References

- [1] European Respiratory Society. *European Lung White Book: The First Comprehensive Survey on Respiratory Health in Europe*. Lausanne, Switzerland: European Respiratory Society Journals Ltd; 2003.
- [2] Zube-Erbier T, Lotvall J, Simoons-Sch, Subramanian SV, Church MK. Economic burden of inadequate management of allergic diseases in the European Union: a GA(2) LEN review. *Allergy*. 2014;69:1275–1279.
- [3] Laumbach RJ, Kipen HM. Respiratory health effects of air pollution: update on biomass smoke and traffic pollution. *J Allergy Clin Immunol*. 2012;129:3–11.
- [4] D'Amato G, Cecchi L, D'Amato M, Liccardi G. Urban air pollution and climate change as environmental risk factors of respiratory allergy: an update. *J Invest Allergol Clin Immunol*. 2010;20:95–102.
- [5] Fuertes E, Markevych I, von Berg A, et al. Greenness and allergies: evidence of differential associations in two areas in Germany. *J Epidemiol Community Health*. 2014;68:787–790.
- [6] Provost EB, Chaumont A, Kicinski M, et al. Serum levels of club cell secretory protein (Clara) and short- and long-term exposure to particulate air pollution in adolescents. *Environ Int*. 2014;68:66–70.
- [7] Chehregani A, Kouhkan F. Diesel exhaust particles and allergenicity of pollen grains of *Lilium martagon*. *Ecotoxicol Environ Saf*. 2008;69:568–573.
- [8] Chehregani A, Majde A, Moin M, Gholami M, Ali Shariatzadeh M, Nassiri H. Increasing allergy potency of *Zinnia* pollen grains in polluted areas. *Ecotoxicol Environ Saf*. 2004;58:267–272.
- [9] Bowles DJ. Defense-related proteins in higher plants. *Annu Rev Biochem*. 1990;59:873–907.
- [10] Zheng XY, Ding H, Jiang LN, et al. Association between air pollutants and asthma emergency room visits and hospital admissions in time series studies: a systematic review and meta-analysis. *PLoS One*. 2015;10:e0138146.
- [11] Anderson HR, Favarato G, Atkinson R. Long-term exposure to air pollution and the incidence of asthma: meta-analysis of cohort studies. *Air Qual Atmosphere Health*. 2013;6:47–56.
- [12] Bowatte G, Lodge C, Lowe AJ, et al. The influence of childhood traffic-related air pollution exposure on asthma, allergy and sensitization: a systematic review and a meta-analysis of birth cohort studies. *Allergy*. 2015;70:245–256.
- [13] Hansell AL, Rose N, Cowie CT, et al. Weighted road density and allergic disease in children at high risk of developing asthma. *PLoS One*. 2014;9:e89878.
- [14] Gruzdeva O, Gehring U, Aalberse R, et al. Meta-analysis of air pollution exposure association with allergic sensitization in European birth cohorts. *J Allergy Clin Immunol*. 2014;133:767–776.e767.
- [15] Buters J, Prank M, Sofiev M, et al. Variation of the group 5 grass pollen allergen content of airborne pollen in relation to geographic location and time in season. *J Allergy Clin Immunol*. 2015;136:87–95.e86.
- [16] Winiwarter W, Bauer H, Caseiro A, Puxbaum H. Quantifying emissions of primary biological aerosol particle mass in Europe. *Atmos Environ*. 2009;43:1403–1409.
- [17] Huynh BT, Tual S, Turbelin C, et al. Short-term effects of airborne pollens on asthma attacks as seen by general practitioners in the Greater Paris area, 2003–2007. *Prim Care Respir J*. 2010;19:254–259.
- [18] D'Amato G, Holgate ST, Pawankar R, et al. Meteorological conditions, climate change, new emerging factors, and asthma and related allergic disorders: a statement of the World Allergy Organization. *World Allergy Organ J*. 2015;8:25.
- [19] Carlsten C, Blomberg A, Pui M, et al. Diesel exhaust augments allergen-induced lower airway inflammation in allergic individuals: a controlled human exposure study. *Thorax*. 2016;71:35–44.
- [20] Lambrecht BN, Hammad H. Allergens and the airway epithelium response: gateway to allergic sensitization. *J Allergy Clin Immunol*. 2014;134:499–507.
- [21] Diaz-Sanchez D. The role of diesel exhaust particles and their associated polyaromatic hydrocarbons in the induction of allergic airway disease. *Allergy*. 1997;52(38 suppl):52–56.
- [22] Takizawa R, Pawankar R, Yamagishi S, Takenaka H, Yagi T. Increased expression of HLA-DR and CD86 in nasal epithelial cells in allergic rhinitis: antigen presentation to T cells and up-regulation by diesel exhaust particles. *Clin Exp Allergy*. 2007;37:420–433.
- [23] Schappi GF, Suphioglu C, Taylor PE, Knox RB. Concentrations of the major birch tree allergen Bet v 1 in pollen and respirable fine particles in the atmosphere. *J Allergy Clin Immunol*. 1997;100:656–661.
- [24] Abou Chakra O, Rogerieux F, Poncet P, et al. Ability of pollen cytoplasmic granules to induce biased allergic responses in a rat model. *Int Arch Allergy Immunol*. 2011;154:128–136.
- [25] Pazmandi K, Kumar BV, Szabo K, et al. Ragweed subpollen particles of respirable size activate human dendritic cells. *PLoS One*. 2012;7:e52085.
- [26] Shahali Y, Pourpak Z, Moin M, Mari A, Majd A. Instability of the structure and allergenic protein content in Arizona cypress pollen. *Allergy*. 2009;64:1773–1779.
- [27] Malayeri BE, Noori M, Jafari M. Using the pollen viability and morphology for fluoride pollution biomonitoring. *Biol Trace Elem Res*. 2012;147:315–319.
- [28] Pasqualini S, Tedeschini E, Frenguelli G, et al. Ozone affects pollen viability and NAD(P)H oxidase release from *Ambrosia artemisiifolia* pollen. *Environ Pollut*. 2011;159:2823–2830.
- [29] Hiscock SJ, Bright J, McInnis SM, Desikan R, Hancock JT. Signaling on the stigma: potential new roles for ROS and NO in plant cell signaling. *Plant Signal Behav*. 2007;2:23–24.
- [30] Kanter U, Heller W, Durner J, et al. Molecular and immunological characterization of ragweed (*Ambrosia artemisiifolia* L.) pollen after exposure of the plants to elevated ozone over a whole growing season. *PLoS One*. 2013;8:e61518.
- [31] Ghiani A, Aina R, Asero R, Bellotto E, Citterio S. Ragweed pollen collected along high-traffic roads shows a higher allergenicity than pollen sampled in vegetated areas. *Allergy*. 2012;67:887–894.
- [32] Bryce M, Drews O, Schenk MF, et al. Impact of urbanization on the proteome of birch pollen and its chemotactic activity on human granulocytes. *Int Arch Allergy Immunol*. 2010;151:46–55.
- [33] Tashpulatov AS, Clement P, Akimcheva SA, et al. A model system to study the environment-dependent expression of the Bet v 1a gene encoding the major birch pollen allergen. *Int Arch Allergy Immunol*. 2004;134:1–9.
- [34] El Kelish A, Zhao F, Heller W, et al. Ragweed (*Ambrosia artemisiifolia*) pollen allergenicity: SuperSAGE transcriptomic analysis upon elevated CO₂ and drought stress. *BMC Plant Biol*. 2014;14:176.
- [35] D'Amato G, Vitale C, D'Amato M, et al. Thunderstorm related asthma: what happens and why. *Clin Exp Allergy*. 2016;46:390–396.
- [36] Burastero SE, Paolucci C, Breda D, et al. T-cell receptor-mediated cross-allergenicity. *Int Arch Allergy Immunol*. 2004;135:296–305.
- [37] Lee MF, Lin SJ, Wang NM, Wu HJ, Chen YH. Plant chitinase III Zm 1 stimulates multiple cytokines, most predominantly interleukin-13, from peripheral blood mononuclear cells of latex-fruit allergic patients. *Ann Allergy Asthma Immunol*. 2012;108:113–116.
- [38] Roschmann KI, van Kuijen AM, Luiten S, et al. Comparison of Timothy grass pollen extract- and single major allergen-induced gene expression and mediator release in airway epithelial cells: a meta-analysis. *Clin Exp Allergy*. 2012;42:1479–1490.
- [39] Sallusto F, Poupot R, Clergue M, DePalma R, Fournie JJ. A flavonoid sulfate antigen activates human alpha-beta CD8+ Th2 lymphocytes in pollen allergy. *Eur J Immunol*. 2000;30:964–968.
- [40] Royer PJ, Emara M, Yang C, et al. The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. *J Immunol*. 2010;185:1522–1531.
- [41] Oseroff C, Sidney J, Vita R, et al. T cell responses to known allergen proteins are differentially polarized and account for a variable fraction of total response to allergen extracts. *J Immunol*. 2012;189:1800–1811.
- [42] Abos-Gracia B, del Moral MG, Lopez-Relano J, et al. *Olea europaea* pollen lipids activate invariant natural killer T cells by upregulating CD1d expression on dendritic cells. *J Allergy Clin Immunol*. 2013;131:1393–1399.
- [43] Plotz SG, Traidl-Hoffmann C, Feussner I, et al. Chemotaxis and activation of human peripheral blood eosinophils induced by pollen-associated lipid mediators. *J Allergy Clin Immunol*. 2004;113:1152–1160.
- [44] Russano AM, Aaga E, Corazzi L, et al. Recognition of pollen-derived phosphatidyl-ethanolamine by human CD1d-restricted gamma delta T cells. *J Allergy Clin Immunol*. 2006;117:1178–1184.
- [45] Bublin M, Eiwegger T, Breiteneder H. Do lipids influence the allergic sensitization process? *J Allergy Clin Immunol*. 2014;134:521–529.
- [46] Csillag A, Boldogh I, Pazmandi K, et al. Pollen-induced oxidative stress influences both innate and adaptive immune responses via altering dendritic cell functions. *J Immunol*. 2010;184:2377–2385.

- [47] Bacsı A, Choudhury BK, Dharajiya N, Sur S, Boldogh I. Subpollen particles: carriers of allergenic proteins and oxidases. *J Allergy Clin Immunol*. 2006;118:844–850.
- [48] Gabriele L, Schiavoni G, Mattei F, et al. Novel allergic asthma model demonstrates ST2-dependent dendritic cell targeting by cypress pollen. *J Allergy Clin Immunol*. 2013;132:686–695 e687.
- [49] Hanasaki K. Mammalian phospholipase A2: phospholipase A2 receptor. *Biol Pharm Bull*. 2004;27:1165–1167.
- [50] Traidl-Hoffmann C, Kasche A, Jakob T, et al. Lipid mediators from pollen act as chemoattractants and activators of polymorphonuclear granulocytes. *J Allergy Clin Immunol*. 2002;109:831–838.
- [51] Tang H, Cao W, Kasturi SP, et al. The T helper type 2 response to cysteine proteases requires dendritic cell-basophil cooperation via ROS-mediated signaling. *Nat Immunol*. 2010;11:608–617.
- [52] Smole U, Radauer C, Lengger N, et al. The major birch pollen allergen Bet v 1 induces different responses in dendritic cells of birch pollen allergic and healthy individuals. *PLoS One*. 2015;10:e0117904.
- [53] Iorio RA, Di Sandro A, Paris R, et al. Simulated environmental criticalities affect transglutaminase of *Malus* and *Corylus* pollens having different allergenic potential. *Amino Acids*. 2012;42:1007–1024.
- [54] Paul WE, Zhu J. How are T(H)2-type immune responses initiated and amplified? *Nat Rev Immunol*. 2010;10:225–235.
- [55] Allen JE, Maizels RM. Diversity and dialogue in immunity to helminths. *Nat Rev Immunol*. 2011;11:375–388.
- [56] Meevissen MH, Driessen NN, Smits HH, et al. Specific glycan elements determine differential binding of individual egg glycoproteins of the human parasite *Schistosoma mansoni* by host C-type lectin receptors. *Int J Parasitol*. 2012;42:269–277.
- [57] Afferni C, Iacovacci P, Barletta B, et al. Role of carbohydrate moieties in IgE binding to allergenic components of *Cupressus arizonica* pollen extract. *Clin Exp Allergy*. 1999;29:1087–1094.
- [58] Shreffler WG, Castro RR, Kucuk ZY, et al. The major glycoprotein allergen from *Arachis hypogaea*, Ara h 1, is a ligand of dendritic cell-specific ICAM-grabbing nonintegrin and acts as a Th2 adjuvant in vitro. *J Immunol*. 2006;177:3677–3685.
- [59] Gunawan H, Takai T, Ikeda S, Okumura K, Ogawa H. Protease activity of allergenic pollen of cedar, cypress, juniper, birch and ragweed. *Allergol Int*. 2008;57:83–91.
- [60] Wang XL, Takai T, Kamijo S, Gunawan H, Ogawa H, Okumura K. NADPH oxidase activity in allergenic pollen grains of different plant species. *Biochem Biophys Res Commun*. 2009;387:430–434.
- [61] Endo S, Hochman DJ, Midoro-Horiuti T, Goldblum RM, Brooks EG. Mountain cedar pollen induces IgE-independent mast cell degranulation, IL-4 production, and intracellular reactive oxygen species generation. *Cell Immunol*. 2011;271:488–495.
- [62] Bacsı A, Dharajiya N, Choudhury BK, Sur S, Boldogh I. Effect of pollen-mediated oxidative stress on immediate hypersensitivity reactions and late-phase inflammation in allergic conjunctivitis. *J Allergy Clin Immunol*. 2005;116:836–843.
- [63] Mariani V, Gilles S, Jakob T, et al. Immunomodulatory mediators from pollen enhance the migratory capacity of dendritic cells and license them for Th2 attraction. *J Immunol*. 2007;178:7623–7631.
- [64] Gilles S, Mariani V, Bryce M, et al. Pollen-derived E1-phytoprostanes signal via PPAR-gamma and NF-kappaB-dependent mechanisms. *J Immunol*. 2009;182:6653–6658.
- [65] Shin YS, Takeda K, Gelfand EW. Understanding asthma using animal models. *Allergy Asthma Immunol Res*. 2009;1:10–18.
- [66] Buters JTM. Release of Bet v 1 from birch pollen from 5 European countries: results from the HIALINE study. *Atmos Environ*. 2012;55:496–505.
- [67] Harrington H, Cato P, Salazar F, et al. Immunocompetent 3D model of human upper airway for disease modeling and in vitro drug evaluation. *Mol Pharm*. 2014;11:2082–2091.