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New insights into an old story
Pollen ROS also play a role in hay fever

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Reactive oxygen species (ROS) can exhibit negative and benign traits. In plants, ROS levels increase markedly during periods of environmental stress and defense against pathogen attack. ROS form naturally as a by-product of normal oxygen metabolism, and evenly play an essential role in cell growth. The short ROS lifespan makes them ideal molecules to act in cell signaling, a role they share in both plants and animals. A particular plant organism, the pollen grain, may closely interact with human mucosa and an allergic inflammatory response often results. Pollen grain ROS represent a first, crucial signal which primes and magnifies a cascade of events in the allergic response.

ROS is a collective term that includes oxygen radicals (e.g., superoxide, hydroxyle, hydroperoxyle), and certain non radicals (e.g., H_2O_2, ozone, singlet oxygen) that are oxidising agents and/or are easily converted into radicals. These agents are continuously produced in plants and other aerobic organisms as a result of partial O_2 reduction during a number of normal metabolic processes, such as respiration, photosynthesis and photorespiration.[1-4]

The ROS pool size depends on the relative rates of ROS generation and destruction, and on the life time of the main ROS species. Plants possess a complex battery of ROS scavenging enzymes (e.g., superoxide dismutase, ascorbate peroxidase, glutathione peroxidase and catalase), and low molecular non-enzymatic antioxidants (e.g., ascorbate and glutathione, but also tocopherol, flavonoids, alkaloids and carotenoids), often confined to particular compartments, that play a key role in ROS cellular control levels.[5,6] Excess ROS is harmful to many plant cell constituents, including lipids, proteins and nucleic acids, and in some case can induce plant cell death.[7,8]

Several biochemical and genetic studies have emphasized the key role of ROS as PCD (apoptosis) triggers in plant cell response to specific stimuli.[9,10] PCD controlled by ROS occurs during developmental processes, including aleurone cell death and leaf senescence, hypersensitive response, allelopathic plant-plant interactions, and various forms of abiotic stress.[6,11,12] External adverse conditions (high light, drought, low and high temperature and mechanical stress) disrupt intracellular ROS homeostasis.[6] Interestingly, ROS/redox signaling networks in the chloroplast and mitochondria play essential roles in plant acclimation to abiotic stress via the regulation of important biological pathways, such as gene expression, energy metabolism and protein phosphorylation.[13]

Until recently, ROS were considered unwelcome by-products of metabolism. However, information continues to emerge on the range of ROS forms, and has demonstrated the molecules serve as important plant development and growth regulators, which operate in strict association with hormonal signaling pathways.[14] The varied ROS forms exhibit different molecular properties, their levels can change rapidly, and their production may be limited to specific cellular locations. Mittler and colleagues[15] reported that ROS signaling should be considered a "ROS wave", which is a dynamic process occurring within cells, among cells over long distances, and among different organelles.

The "ROS wave" originated by pollen grains is the main topic of the present mini-review. It plays substantial role in allergic reactions that occur during the flowering season of specific wind-pollinated plants.

ROS on the Life of Pollen

Pollen is the small male gametophyte of flowering plants. ROS play several crucial roles in the second phase of the short life cycle of pollen; when the pollen tube is produced to confer the male gametes to the female gametophyte. ROS serve as wall loosening agents at the onset of pollen germination, i.e., during tube organization and emergence. The microfibrillar network requires loosening for the pollen intine to bulge outward, allowing pollen tube protrusion. This process is analogous to cell wall relaxation during extension growth of vegetative plant cells, although more spatially confined. Speranza and colleagues reported very early ROS production following pollen grain re-activation, ROS localization at the germination apertures, strict ROS production requirement to enable germination and early presence of ROS-producing or ROS-scavenging activities in kiwifruit, which suggests a close association of ROS with pollen tube emergence.[16] Similar results were demonstrated in tobacco pollen.[17] It is clear the role of ROS extends far beyond pollen tube emergence. ROS generation accompanies pollen tube elongation, and is intimately involved in the tip-growth process, either (i) by accumulation at the pollen tube tip, and correlation with Ca^{2+} permeable channel activation or (ii) interaction with another key factor and radical

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molecule, i.e., the reactive nitrogen species NO.[18] NO, which is produced in the pollen tube sub-apical zone, served as a negative regulator of pollen tube growth in *Lilium longiflorum*.[19] In addition, it is hypothesized that ROS and NO are involved in a sort of molecular "courtship" between male and female cells during the progamic phase of plant fertilization, since pollen-derived NOs counteract abundant ROS/H_2O_2 produced by stigmatic papillae, thus enabling pollen access to female tissues.[20] In fact, when mating is impossible due to self-incompatibility for gametophytic systems based on the cytotoxic enzyme S-RNase, which specifically inhibits self-pollen tube elongation, ROS disruption at the pollen tube tip is an expressed, primary target of S-RNase. In self-incompatible pear pollen, ROS formation was arrested in the mitochondria and cell wall, which was subsequently followed by Ca^{2+} current termination, depolymerization of the actin cytoskeleton and degradation of nuclear DNA.[21]

Pollen Allergens and Inflammation Response: Classic and Recent Views

Several highly expressed proteins in pollen (often glycoproteins) behave as allergens for humans. Profilins, Calcium Binding Proteins (CBPs), β-espansins, Lipid Transfer Proteins (LTPs), Pathogenesis Related Proteins (PRs), and a few enzymatic proteins are the main allergen families of pollen grains and the profilins and CBPs are most common.[22] Several of the allergenic pollen proteins appear to have such important roles, that attempts to engineer plants with low levels of these allergens would fully compromise pollen function. For example, the first allergen purified in a clinically important pollen species such as ragweed, *Ambrosia artemisiifolia*, that is, Amb a 1, is a major allergen, which indicates that more than 90% of ragweed-sensitive subject have antibodies against it. *Amb a 1* has 45–48% homology with a pollen-specific gene expressing pectate lyases, which is required for cell wall breakdown during pollen tube growth through the style.[23]

When pollen grains enter the upper respiratory tract and land on the mucosa, allergenic (and non-allergenic) proteins are released upon rehydration. In some cases, primarily in grasses, the allergens are carried by microscopic (≤ 5.0 μm) sub-pollen particles which may be derived from pollen grain bursting in the atmosphere during rainfall. The particles are subsequently able to directly reach the lower respiratory tract without further assistance. Mast cells serve as one of the most important mediators in the pathogenesis of respiratory allergies, as well as in other chronic inflammatory diseases. The commonly accepted view is that allergens trigger immunoblobulin E (IgE) antibody production from B-lymphocytes, and the IgE molecules bind to corresponding receptors on the surface of mast cells. The specific IgE antibodies are subsequently bound and cross-linked by allergens onto the surface of mast cells; this triggers exocytotic release of cytoplasmic granules, which contain an array of preformed and newly synthesized mediators involved in the allergic inflammatory response (e.g., histamine, proteases, prostaglandins, leukotriene and cytokines) specifically from mast cells.[24]

However, at least two pollen species were shown to induce allergenicity not solely through IgE-mediated mast cell degranulation: the oxidative microenvironment to which mast cells are exposed can produce their activation independently from adaptive immunity. Chodczek and colleagues proposed an alternative hypothesis from ragweed pollen experiments, a species unquestionably inducing severe allergy symptoms worldwide.[25] Mast cells exposed to ragweed pollen extracts experienced mitochondrial dysfunction, resulting in increased ROS production; ROS, in turn, induced histamine and serotonin secretion from the mast cells.[25] Mountain cedar pollen, *Juniperus ashei*, another species which causes severe allergic reactions throughout the world, provided similar evidence. In fact, *J. ashei* pollen directly induced mediator release via IgE-independent mechanisms, with a 2- to 8-fold increase in mast cell ROS levels. Antioxidants inhibited ROS generation and serotonin release. Furthermore, pollen enhanced the mediator release induced by IgE-cross-linking.[26] A similar mast cell degranulation case in intracellular ROS generation concomitance, through a non-IgE-dependent pathway, was observed following treatment with the environmental pollutant sodium sulfite, which induced bronchoconstriction within minutes of exposure.[27]

ROS are indeed well documented to be associated with mast cell-dependent inflammatory conditions. Several studies have demonstrated intracellular ROS generation by a variety of agents known to induce degranulation. The NADPH oxidase system is commonly considered the major source of oxidative stress during acute or chronic inflammation. The NADPH oxidases have an oxidase activity that can produce $\bullet O_2^-$ ions, which are converted into H_2O_2 through the activity of superoxide dismutase. Endogenously produced ROS critically contribute to airway injury and disease pathogenesis.[24,28]

Pollen Allergens do Not Work Alone: Pollen ROS Act First

We now know that the allergic response involves IgE-dependent or ROS-mediated mast cell activation; these are molecules produced by the recipient host in reaction to foreign pollen grains. But, pollen itself plays a double active role in allergic inflammation. Boldogh and colleagues provided evidence that another pollen component was responsible for the allergic response, in addition to allergenic proteins, namely NADPH oxidase.[29] Pollen NADPH oxidase induces local oxidative stress, by directly increasing ROS levels in airway epithelium, or by increasing the amount of oxidized glutathione, malondialdehyde and other oxidative stress markers in the airway lining fluid. Therefore, two distinct signals are combined in the 2-signal ROS-antigen model proposed by Boldogh and colleagues,[29] and both are required to orchestrate the development of robust allergic lung inflammation. Signals generated by intrinsic pollen NADPH oxidase (signal 1), that is, oxidative stress represent an innate response which occurs within minutes of exposure and is independent on the following recruitment of inflammatory cells. It boosts allergic inflammation induced by pollen antigens (signal 2), that is, the adaptive immune response.

Therefore, effective inflammatory factors are definitely of two distinct types, and packed together into the small male gametophyte of higher plants. ROS elicited from an allergic response are not exclusively derived from host immune cells, as pollen ROS provide an acute "danger signal," which occurs first. Subsequently, pollen proteins that lack NADPH oxidase activity (i.e., Amb a 1) are unable to deliver signal 1, and are consequently unable to individually induce a robust allergic inflammation. A new dimension was therefore added to the existing paradigm of allergic inflammation initiation.[29,30] In addition to airway allergic inflammation, ROS generated by NADPH oxidases of hydrated short ragweed pollen intensified immediate allergic reactions and recruitment of inflammatory cells in murine conjunctivitis.[31]

Pollen extracts from 38 other allergenic species (weeds, trees and grasses) were shown to exhibit intrinsic NADPH oxidase activity.[29] Subsequently, Wang and colleagues described NADPH oxidase activity and localization in several allergenic pollen species.[32] Differences in intensity and localization based on plant families were characterized in the study. Microscopic analyses of grass (Poaceae) and birch (Betulaceae) pollen revealed that pollen NADPH oxidase activity was located in the cytoplasm, plasma membrane, or the outer wall. However, Cupressaceae/Taxodiaceae pollen lacked activity in the outer wall, which ruptured and sloughed off upon hydration, and NADPH oxidase occurred at the inner wall or cytoplasm. Interestingly, studies in ragweed showed NADPH oxidase was localized in the small sub-pollen particles released during pollen grain rehydration.[17] Furthermore, a 38 kDa protein band with 100% homology to Amb a 1 was detected in ragweed pollen sub-particles.[33] Similarly, birch pollen was found to rupture in rainwater, releasing Bet v 1-containing allergen particles.[34]

More to the Story

Several major and minor factors may converge and compose a multi-faceted, complex role in pollen grains that induce an allergic response. Proteases, prostaglandin E_2-(PGs) and leukotriene (LK) B_4-like substances released from several allergenic pollen grains have also been hypothesized to be in someway involved in the pathogenesis of allergic diseases. By releasing these components, pollen grains have the potential to form an optimal microenvironment that initiate sensitization or exacerbation of an allergic response.[35,36] PGs and LKs are arachidonic acid-derived eicosanoids, which are a class of lipid mediators.[37] These bioactive lipids exhibit regulatory functions in a wide range of physiological and pathological systems. Eicosanoid production is considerably increased during inflammation. In mammalian cells, eicosanoid biosynthesis is usually initiated by the activation of phospholipase A_2 (PLA_2) and release of arachidonic acid from membrane phospholipids.[38]

PLA_2s are enzymes that hydrolyse ester bonds of membrane phospholipids. The enzymes are released at low levels in normal airways, and show a tendency to increase during respiratory allergies as a result of local secretion. Mast cells, following IgE-mediated activation, are a primary source of extracellular PLA_2s during allergic reactions.[39] PLA_2 is also a transglutaminase (TGase) substrate, including plant TGases.[25] TGases are an enzyme class widely distributed in animals, plants and unicellular organisms. The enzymes are responsible for post-translational protein modification, which act as calcium-dependent protein cross-linkers. A substantial activation in mammalian PLA_2 using a recombinant plant TGase was proven. In addition, *Corylus avellana* (Fagaceae) pollen extracts containing TGase effectively increased PLA_2 activity by > 3-fold, and even > 4-fold when pollen was subjected to environmentally stressful conditions (humidity, temperature, acid rain and copper pollution). Concomitantly, also TGase activity was substantially increased under environmental stress.[40] TGases are suggested to play a central role in the onset of mammalian pathologies and associated autoimmune inflammation.[41] In airway inflammation observed in toluene diisocyanate (TDI)-induced occupational asthma, TDI increased TGase activity, which was mediated by TDI-induced ROS production.[42] Therefore, a relationship between TGase and ROS in the inflammation response seems plausible. However, increases in the former activity appeared a secondary effect, and subordinate to heightened ROS.

Air Pollution Influences the Allergic Response

Boldogh and colleagues[29] propose a signaling model where pollen NADPH oxidase and allergens represent two distinct danger signals arriving to the mucosa; signals arrive from the same "invading" organism, and are responsible for a distinct segment of the allergic inflammation response. These events occur within a framework of increasing environmental factors, which can affect signals 1 and 2. Studies have detected a positive association between air pollution and pollen sensitization among 6-y-old children, and in general, urbanisation increases allergic susceptibility.[43-45] The role of traffic-related air pollution in sensitization development is supported by experimental evidence obtained in humans, animals and in vitro test systems; it was shown that diesel exhaust particles enhanced responses to allergens and elicited inflammatory reactions in the airways at relatively low concentrations and short exposure durations.[46] Ozone, diesel exhaust particles (DEP) and cigarette smoke can generate oxidative stress in the airways and participate in the worsening of disease symptoms.[31,47,48]

Recent research indicated ozone pollution affected signal 1 in the allergy response. Significant induction of the ROS-generating enzyme NADPH oxidase was produced in ragweed pollen extracts following 7 d of ozone exposure. This could be the result of ozone pollen damage due to decreased pollen viability, which may occur in vivo if pollen is exposed to ozone during atmospheric travel. However, Amb a 1 allergen content and expression were not affected. The effects of ozone on allergenic protein content remain equivocal.[49] For example, studies in *Betula* showed major birch allergens were not expressed differentially in pollen samples from urban or rural areas. Pollen from polluted areas exhibited increased allergenicity, which emphasizes the fact that allergens do not work alone in inducing the allergic response.[50]

In regards to signal 2, a few days exposure to DEP resulted in elevated novel proteins expression in a non-allergenic pollen, e.g.,

Lilium martagon. A new band absent in untreated pollen grains was detected in DEP-treated pollen and strongly reacted with anti-IgE.[51] Therefore, not only are DEP capable of promoting allergen release, but also induce new allergenic protein formation.

Hayfever: Its History

"Allergy" has Greek roots, and is derived from the words "allos" and "ergos", literally meaning "reaction to the other." It was first introduced in 1906 by the Austrian pediatrician Clemens Freiherr von Pirquet (1874–1929) describing a state of altered immunological reactivity. The allergic response to pollen, commonly called hay fever (or pollinosis) is the most widely recognized form of allergy. "Hay fever" is an older term than "allergy," since its first introduction by John Bostock (1773–1846). Bostock provided a through description of the complex of clinical symptoms in 1819, which are the hallmarks of hay fever. Initially, hay fever was considered an idiosyncratic response to a wide range of stimuli. Only later, in 1831, did John Elliotson (1791–1868) provide the first definitive suggestion that hay fever was associated with grass flowers harvested in meadows, and "probably" with its pollen. Charles Harrison Blackley (1874–1929) experimented on numerous grass pollen; he performed the first skin tests by applying pollen through a small break in the skin, and found that pollen from the great majority of these plants produced hay fever symptoms, both in catarrhal and asthmatic forms.

It has been suggested that allergy became the archetypal disease of modern civilization and emerged as a global public health and socio-economic problem during the twentieth century.[52] Allergies attract public and media interests or fears. Just "Pollen" is the title of a science-fiction tale describing the invasion of Manchester, England, by a deadly pollen strain. An enormous cloud of pollen infiltrated the city and people literally sneezed to death.[53] Furthermore, metaphorical meanings became invested with the term "allergy," where allergies were a subject interlaced with political and cultural undertones. In the late nineteenth century, hay fever was regarded as an aristocratic affection. It was indicative of social, racial and intellectual superiority, with the educated classes forming a considerable proportion of the sufferers.[52] Due to past views of reported allergy sufferes, one of the authors here is wondering if she should be flattered by her own pollinosis.

Conclusions

At the cellular and tissue levels, the human immune system actively rejects foreign invaders. Flowering plants do not possess an adaptive immune system, however during the reproductive phase, exhibit a detailed system which actively rejects self-pollen (meaning pollen sharing the same allelic profile of the incompatibility S-gene with the pistil). If we anthropomorphise plants in our discussion, plants and humans exhibit opposite views about the concepts of "self" and "foreign." Therefore, it is no surprise any close interaction between humans and pollen grains often results in a literally "burning debate"; the two opponents providing their own ROS to inflame the condition.

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