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Innate responses to pollen allergens

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Abstract

Purpose of review—The aim of the present review was to discuss the effects of pollen components on innate immune responses.

Recent findings—Pollens contain numerous factors that can stimulate an innate immune response. These include intrinsic factors in pollens such as nicotinamide adenine dinucleotide phosphate oxidases, proteases, aqueous pollen proteins, lipids, and antigens. Each component stimulates innate immune response in a different manner. Pollen nicotinamide adenine dinucleotide phosphate oxidases induce reactive oxygen species generation and recruit neutrophils that stimulate subsequent allergic inflammation. Pollen proteases damage epithelial barrier function and increase antigen uptake. Aqueous pollen extract proteins and pollen lipids modulate dendritic cell function and induce Th2 polarization. Clinical studies have shown that modulation of innate immune response to pollens with toll-like receptor 9- and toll-like receptor 4-stimulating conjugates is well tolerated and induces clear immunological effects, but is not very effective in suppressing primary clinical endpoints of allergic inflammation.

Summary—Additional research on innate immune pathways induced by pollen components is required to develop novel strategies that will mitigate the development of allergic inflammation.

Keywords

allergic inflammation; aqueous pollen extract; lipid; NADPH oxidase; reactive oxygen species

INTRODUCTION

Environmental pollens are well known as major causes of allergic disorders such as bronchial asthma, allergic rhinitis, and allergic conjunctivitis all over the world. There is a large body of literature describing the adaptive immune responses induced by pollens. Only recently, however, innate responses to pollens and their components have been described.

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Conflicts of interest

There are no specific conflicts of interest regarding the content of the present article.

Innate immune responses usually act against potential pathogens or endogenous molecules released by damaged cells that have conserved molecular patterns, known as pathogen-associated molecular patterns and damage-associated molecular patterns. Increasing evidence shows that various pollen components stimulate innate immune responses that induce allergic airway inflammation. In the present review, we summarize recent data describing the pollen-induced innate immune responses that contribute to allergic inflammation.

INTRINSIC POLLEN NADPH OXIDASES AND TWO-SIGNAL HYPOTHESIS OF POLLEN-INDUCED ALLERGIC INFLAMMATION

The contribution of oxidative stress to the pathogenesis of asthma and allergic diseases is an important area of research. Environmental factors such as ultrafine particles, diesel exhaust particles, smoking, and pollens can induce reactive oxygen species (ROS) such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH) [1]. Pollen allergen provocation induces ROS generation in human patients with bronchial asthma and seasonal allergic rhinitis [2–4]. ROS promotes allergic inflammation by changing dendritic cell function and modulating the Th1/Th2 balance [5–8].

Allergenic pollens have intrinsic nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [9]. In a landmark study, pollen NADPH oxidases from ragweed pollen extract (RWPE) were separated into NADPH oxidase-positive (RWPE^{NOX+}) and oxidase-negative fractions (RWPE^{NOX-}) by column chromatography. Intranasal challenge with RWPE^{NOX+}, but not RWPE^{NOX-}, induced significant neutrophil recruitment in naïve mice and mice lacking T cells, B cells, or mast cells [9]. RWPE^{NOX+} increased oxidized glutathione (GSSG) or 4-hydroxynonenal (4-HNE), which rapidly causes tyrosine phosphorylation of p38 mitogen-activated protein kinase and subsequent secretion of interleukin-8 [9]. In the experimental allergy model, RWPE^{NOX+}-induced oxidative stress significantly augmented the metaplasia of mucin-producing cells and the accumulation of inflammatory cells in the broncho-alveolar lavage and peribronchial areas [9]. Heat-inactivated RWPE, which lack NADPH oxidase activity, fail to induce allergic airway inflammation. Simultaneously administering an ROS generator such as xanthine and xanthine oxidase with heat-inactivated RWPE reconstitutes induction of allergic airway inflammation [9,10]. Similarly to the airway mucosa, exposure of RWPE^{NOX+} generates superoxide anion in the eye conjunctiva, and this oxidative insult promotes recruitment of inflammatory cells into the conjunctiva [10].

On the basis of these and other studies, a two-signal hypothesis has been proposed for induction of pollen-induced allergic inflammation (Fig. 1): ‘signal 1’, consisting of innate signaling pathways activated by pollen-induced ROS, boosts ‘signal 2’, the classical pathway of antigen presentation to T cells that impart immunological specificity to the immune response. Support for the ‘two-signal hypothesis’ comes from several studies in addition to the landmark study discussed above [9]. One study [11] demonstrated that removal of signal 1 by prior administration of antioxidants such as ascorbic acid, *N*-acetylcysteine, or tocopherol coadministered with RWPE profoundly inhibits RWPE-induced allergic airway inflammation. Likewise, administration of the antioxidant lactoferrin, an

iron-binding protein, inhibits RWPE-induced intracellular ROS generation in epithelial cells and allergic airway inflammation in a mouse experimental model of asthma [12,13]. Finally, signal 1 is not just a laboratory phenomenon observed in cultured cells and mice because human studies have revealed that challenge with pollen extracts in patients with asthma induces early superoxide generation at the site of challenge [14,15] and recruits neutrophils to the airways [16].

Signal 1, as noted above, by itself is not sufficient to induce allergic inflammation [9,10], and requires a boost from additional signals to fully manifest the allergic inflammatory process. One mechanism of induction of allergic inflammation is recruitment of ROS-generating neutrophils [17^{••}]. Decreasing ROS generation from recruited neutrophils disrupting gp91phox NADPH oxidase also reduces allergic airway inflammation [17^{••}]. Another mechanism of augmentation of signal 1 is modulation of mitochondrial complexes and mitochondrial proteins by pollen NADPH oxidase. Thus, RWPE-induced mitochondrial dysfunction and mitochondrial ROS generation modulates allergic airway inflammation and airway hyperresponsiveness (AHR) by modulating signal 1 [18] (Fig. 1). Exposure to RWPE induces oxidative damage to ubiquinol-cytochrome *c* reductase core protein II in mitochondria, and increases H₂O₂ release from complex III [18]. Small interfering RNA suppression of ubiquinol-cytochrome *c* reductase core protein II enhances RWPE-induced allergic inflammation and mucin production in the airway [18]. Likewise, studies utilizing inhibitors of mitochondrial respiratory chain provide evidence supporting a role of mitochondrial respiratory chains in signal 1 and subsequent allergic inflammation induced by RWPE [18]. Thus, mitochondrial complex I inhibitor, complex II inhibitor, and inhibitor of the Qo site of complex III all suppress ROS generation in epithelial cells and histamine release from mast cells [18–20]. Furthermore, antimycin A, which inhibits the Qi site of complex III inhibitor, enhances production of mitochondrial ROS, and modulates allergic airway inflammation [18]. Other studies on experimental asthma have provided additional evidence of mitochondrial dysfunction, such as reduction of cytochrome *c* oxidase activity in lung mitochondria and reduction in the expression of subunit III of cytochrome *c* oxidase in bronchial epithelium in asthma [21]. Change and increase of mitochondria in the airway have been observed in patients with asthma [22,23]. Together, these observations support the hypothesis that mitochondria are associated with the pathogenesis of allergic asthma.

One report evaluating pollen NADPH oxidase in birch pollen extract suggested that these oxidases do not contribute to allergic sensitization, inflammation, and AHR [24]. The difference between the results of earlier published literature and this report could be because of the type of pollen antigen (RWPE in earlier studies vs. birch pollen extract) studied, the route of sensitization (intraperitoneal injection in earlier studies vs. intratracheal injection in the birch pollen study), or the technology utilized to destroy intrinsic pollen NADPH oxidases: 72°C for 30min in the earlier studies [9] to gently destroy enzyme activity vs. 95°C for 15 min in the birch pollen study [24], which can theoretically induce structural changes in proteins at a high temperature. To resolve this controversy, additional research is required to define the role of pollen NADPH oxidases from different pollens and elucidate their contribution to allergic sensitization and allergic inflammation. Ideally, these studies should utilize better technology than heat treatment to destroy the activity of this oxidase.

POLLEN PROTEASES DAMAGE EPITHELIAL TIGHT JUNCTION PROTEIN

A considerable body of evidence has demonstrated that modulation of airway epithelial barrier function by pollens contributes to the pathogenesis of allergic disorders [25–27]. The loss of epithelial tight junction function could theoretically allow pollen allergens to pass into the airway and drive the sensitization and antigen responses [28]. Thus, the ability of some pollen proteases to change the integrity of airway epithelial cells is likely to facilitate transfer of pollen antigens to underlying dendritic cells, leading to sensitization and inflammation (Fig. 2). Thus, pollen diffusates from *Olea europaea*, *Dactylis glomerata*, *Cupressus sempervirens*, and *Pinus sylvestris* have proteases with serine or aminopeptidase activity that increase the transepithelial permeability [29]. Pollen diffusates from ragweed, white birch, and Kentucky blue grass have proteolytic activity that damages tight junctions in human airway epithelial cells [30]. Interestingly, timothy grass pollen extracts do not change the tight junction on human epithelial cells, suggesting that they are allergenic because of alternative factors [31]. Because barrier function in the airway epithelium is already impaired in patients with asthma [26,27], additional damage by pollen proteases is likely to further worsen this damage.

INNATE RESPONSES INDUCED BY AQUEOUS POLLEN EXTRACT PROTEINS AUGMENT TH2 CYTOKINE SECRETION

Increasing evidence has shown that aqueous pollen extract proteins (APEP) can modulate immune responses by activating hematopoietic cells. Birch aqueous pollen extract proteins (B-APEP) can facilitate CXCL12-mediated migration of dendritic cells [32], and stimulate Th2 chemokine CCL22 and CCL17 production, but not Th1 chemokine CXCL10 or CCL5 production [32]. B-APEP enhances lipopolysaccharide (LPS)-induced Th2 chemokine production, but suppresses LPS-induced Th1 chemokine production [32]. B-APEP also inhibits the maturation of 6-sulfo LacNAc1 dendritic cells (slanDCs) that stimulate Th1 cytokines [33]. By contrast, slanDCs exposed to low-molecular-weight nonprotein factors in aqueous birch pollen extracts fail to stimulate Th1 cell induction [33]. Like B-APEP, *Betula alba* APEP also suppresses LPS-induced interleukin-12 production from dendritic cells [34]. These results imply that APEPs modulate dendritic cell function and stimulate Th2 polarization.

Additional evidence for the role of APEP comes from in-vivo studies. *B. alba* APEPs modulate allergic inflammation in ovalbumin (OVA)-sensitized mice. Antigen-specific T cells from the mice challenged with OVA together with *B. alba* APEP demonstrate increased secretion of Th2 cytokines, but reduced interferon (IFN)- γ secretion, compared with antigen-specific T cells from the mice challenged with OVA alone [34]. In addition, *B. alba* APEP induces a shift in immune response toward Th2 differentiation, as demonstrated using OVA-specific DO11.10 T cells [34].

POLLEN-DERIVED LIPIDS STIMULATE INNATE AND ALLERGIC RESPONSES

Investigators have profiled the pollen lipid from allergenic plant species by gas chromatography–mass spectroscopy and identified more than 106 lipid molecular species including fatty acids, *n*-alkanes, fatty alcohols, and sterols [35^{***}]. Among these lipids, several lipid compounds stimulate dendritic cells and induce differential cytokine production patterns [35^{***}]. Pollen-derived E1-phytoprostanes (PPE1) in *B. alba* L. aqueous pollen extracts activate dendritic cells, leading to Th2 skewing [36]. PPE1 inhibit LPS-induced secretion of interleukin-12 p70 and interleukin-6 from dendritic cells and slanDCs [33,34]. They modulate dendritic cell function via peroxisome proliferator-activated receptor- γ -dependent nuclear factor- κ B activation. These events lead to suppression of interleukin-12 production from dendritic cells [37]. PPE1 inhibits LPS-induced increase in CCL5, CXCL10, and CCL22 expression in dendritic cells. These dendritic cells attract more Th2 cells [32]. The total lipid fractions from *O. europaea* pollen can activate dendritic cells and stimulate invariant natural killer T cells [38]. Thus, pollen lipid affects the function of a variety of cells by modulating dendritic cell function.

Increasing evidence suggests that pollen lipids can also directly stimulate other hematopoietic cells. The cypress pollen lipid components, phosphatidylcholine and phosphatidylethanolamine, are recognized by T cells and cause Th2-dominant response associated with increased Th2 cytokine production [39]. Gamma delta T cells from allergic patients recognize pollen-derived phosphatidylethanolamine in a CD1d-dependent manner, leading to secretion of Th1 and Th2 cytokine and enhancement of immunoglobulin E (IgE) production [40]. Natural killer T cells stimulated with lipid compounds from several pollens induce secretion of distinct patterns of cytokine production [35^{***}]. The lipid mediators in aqueous pollen extracts from *Phleum pratense* and *B. alba* pollen recruit and activate eosinophils [41]. Likewise, aqueous pollen extracts and lipid mediator from *P. pratense* L. and *B. alba* L. pollen induce migration and activation of polymorphonuclear granulocytes [42]. These studies indicate that pollen lipids can activate a variety of cells and stimulate allergic inflammation.

INNATE IMMUNE RESPONSES INDUCED BY POLLEN EXTRACTS

Airway and corneal epithelium play critical roles in host mucosal immunity, and provide the first line of defense system against constant environmental insults. Prior studies [16,43,44] have shown that challenge with allergenic extracts induce an early wave of neutrophil recruitment. Microarray analysis has revealed that Phl p 1, a major timothy grass pollen allergen, increases the expression of proinflammatory cytokine in airway epithelial cells [45]. Interleukin-8 is a major neutrophil chemoattract chemokine. Phl p 1 induces interleukin-8 secretion from human airway epithelial cells independently of its protease activity [46]. Likewise, timothy grass pollen extracts induce interleukin-8 secretion in bronchial epithelial cells from healthy volunteers and patients with severe asthma [31]. Microarray analysis of the innate responses induced by timothy grass pollen extract induces a similar inflammatory cytokine expression, including interleukin-8 secretion from airway epithelial cells [47]. Likewise, *Parietaria judaica* pollen extract can directly stimulate

interleukin-8 secretion from human lung microvascular endothelial cells [48]. We have demonstrated that toll-like receptor (TLR)4 is essential for pollen allergenic extracts to induce nuclear factor- κ B and CXCR signaling-linked neutrophil recruitment [49^{***}]. Suppression of these pollen extract-induced innate responses by disrupting TLR4 or treating mice with a CXCR2 inhibitor also inhibits induction of allergic airway inflammation [49^{***}]. These data suggest that TLR4 mediates the innate response of the airway epithelium by inducing CXCR chemokines that recruit neutrophils and drive allergic inflammation (Fig. 3). How neutrophils contribute to the pathogenesis of asthma is still unclear. A variety of factors in neutrophils, such as matrix metalloproteinase 9 [50], neutrophil elastase [51,52], α -defensins [53,54], transforming growth factor- β 1 [55], and ROS [56], however, can mediate AHR, allergic inflammation, and airway remodeling.

Thymic stromal lymphopoietin (TSLP) is considered a key epithelium-derived cytokine for allergic airway inflammation and remodeling [57–59]. Short ragweed pollens can trigger allergic conjunctivitis through TLR4-dependent TSLP secretion in sensitized mice [57]. Challenge of sensitized mice with *Cupressus arizonica* pollen allergen, nCup a 1, increases the expression of TSLP in the lungs of sensitized mice [60]. Likewise, interleukin-33 secretion is higher in airway epithelial cells from allergic patients, and can facilitate induction of allergic inflammation and airway contraction [61–64]. Inhaled cypress pollen nCup a 1 induces secretion of interleukin-33 and increases ST2 expression on dendritic cells migrating to the lung. These dendritic cells skew Th2 polarization through CD4 Th cells [60].

Dendritic cells are crucial cells that connect innate immunity to adaptive immunity, and contribute to the pathogenesis of allergic disorders by regulating Th1/Th2 polarization. Because dendritic cells are resident in the surface of airway epithelium and underlying lamina propria, pollens most likely stimulate dendritic cells directly. RWPE activates dendritic cells through oxidative stress and NF-E2-related factor 2-mediated signaling [65]. These activated dendritic cells suppress secretion of interleukin-12 and interleukin-18 [65]. Likewise, Cupressaceae pollens suppress LPS-induced interleukin-12 secretion from dendritic cells [66]. Pollen grains induce secretion of proinflammatory cytokines from dendritic cells independently of TLR4 [66]. Uptake and presentation of pollen allergens Bet v 1 and Phl p 1 by dendritic cells can activate autologous naïve T lymphocytes [67]. Taken together, these studies indicated that pollens modulate the function of dendritic cells and skew their immune response toward Th2 by suppressing Th1 cytokine secretion.

INNATE IMMUNE RECEPTORS MODULATE POLLEN-INDUCED INFLAMMATION

TLR4 is an innate immune receptor for a variety of pathogen-associated molecular patterns and damage-associated molecular patterns. The role of TLR4 in the mechanism of asthma and allergy has been investigated. TLR4 gene variants have been associated with sensitization and bronchial asthma [68–71]. Atopic children have impaired LPS-induced TLR4 signaling [72]. TLR4 signaling suppresses allergic airway inflammation and AHR via upregulation of nitric oxide synthase 2 activity [73]. LPS–TLR4 signaling modifies the Th2 response depending on the dose of LPS [74,75]. With regard to antigens, house dust mite

allergen signals via TLR4 signaling in airway structural cells and induces Th2 skewing in murine asthma model [76]. Der p 2, which is a major component of house dust mite, shows structural homology with MD2 and enhances allergic inflammation by facilitating TLR4 signaling, even at low LPS conditions [77]. The relation between TLR4 and pollens, however, is not well understood. Our preliminary studies have suggested that RWPE can induce TLR4-dependent effects on airways such as induction of ROS, CXCL1/2 secretion, and neutrophil recruitment [49^{***}]. RWPE-induced neutrophilic inflammation can itself promote allergic airway inflammation by further increasing ROS levels in airways [49^{***}]. Likewise, challenge with short ragweed pollen in sensitized mice facilitates the allergic conjunctivitis through TLR4 pathway [57]. TLR4 enhance the birch pollen extract-induced allergic inflammation, but is not required for the AHR [24]. By contrast, coadministration of protollin, a mucosal adjuvant composed of TLR2 and TLR4 ligands, with birch pollen allergen extract suppressed allergic inflammation through the induction of inducible costimulatory molecule-expressing CD4 T cells [78]. One report indicated that pollen-induced TLR4 signaling could be due to LPS because contamination of Ryegrass with LPS increased Th1 and Th2 effector T cells, and decreased induction of CD4⁺ Foxp3 high regulatory T-cells [79]. Par37 (37-amino acid peptide) in the major allergen of the *Parietaria* pollen Par j 1 contains an LPS-binding region and inhibits LPS-induced interleukin-6, tumor necrosis factor (TNF)- α , and IFN- γ production [80]. Thus, carefully constructed studies are required to investigate and define the contribution of TLR4 to pollen-induced innate and allergic immune response.

C-type lectin dendritic cell-specific intercellular adhesion molecule 1-grabbing nonintegrin (dendritic cell-SIGN) (CD209) is known as antigen uptake receptor. Bermuda grass pollen allergens BG60 and Cyn d 1 were recognized by both dendritic cell-SIGN and dendritic cell-SIGN receptor [81]. BG60 led to the secretion of TNF- α through dendritic cell-SIGN [81]. Alveolar macrophages from rat bind and phagocytose pollen starch granules through C-type lectin and integrin receptors and result in an upregulation in inducible nitric oxide synthase mRNA and the production of nitric oxide [82].

THERAPEUTIC EFFICACY OF STRATEGIES THAT MODULATE INNATE IMMUNE RESPONSES TO POLLENS

Recently, allergen immunotherapy has been the standard treatment for the patients with pollen allergy with poor control in spite of conventional pharmacotherapy and avoidance of allergens. The problems of allergen immunotherapy are low levels of efficacy and the prolonged duration required to demonstrate efficacy. To address these problems, the innate immune response of DNA has been utilized to modulate response of pollens. TLR9-stimulating unmethylated CpG motifs in immunostimulatory DNA sequences stimulate Th1 innate immune responses [83]. Amb a 1-immunostimulatory phosphorothioate oligonucleotide conjugate (AIC) is well tolerated [84]. Early studies [83,85] demonstrated that AIC reduced the clinical symptoms during the second ragweed season in the patients with allergic rhinitis sensitized to ragweed. Furthermore, AIC suppressed the seasonal increase of Amb a 1-specific IgE antibody [83], generation of interleukin-4 mRNA-positive cells, and recruitment of eosinophils. By contrast, it increased IFN- γ mRNA-positive cells in

nasal mucosa after allergen challenge [85]. Despite these earlier reports indicating benefit of AIC, two large multicenter studies, TOLAMBA and Dynavax Allergic Rhinitis TOLAMBA Trial (DARTT), failed to demonstrate significant benefit of immunostimulatory sequence (ISS) conjugates in the primary efficacy endpoint [86].

Another TLR-stimulating strategy that has been explored is tyrosine-absorbed specific allergoids enhanced with the TLR4-stimulating adjuvant monophosphoryl lipid A to potentially boost an immune response in patients with allergic rhinitis [87]. The study demonstrated strong stimulation of allergen-specific immunoglobulin G (IgG)1 and IgG4. A large study [86] of over 1000 patients of allergoid-monophosphoryl lipid A, however, demonstrated a slight benefit of only about 13% over the placebo arm.

In addition to stimulating TLR9 or TLR4 by ISS DNA or allergoid-monophosphoryl lipid A, several other strategies are being developed to modulate innate immune response to pollens. Oral immunotherapy utilizing Cry j1 conjugated with galactomannan in Japanese cedar pollinosis that demonstrated its safety, clinical efficacy, and ability to stimulate allergen-specific serum IgG4 and interleukin-10 secretion from peripheral blood mononuclear cells [88]. Conjugation of Bet v 1 to the crystalline surface layer proteins derived from Gram-positive eubacteria changed the production of interleukin-12 from T cells [89]. Finally, a completely different approach from these chemically induced conjugates is the creation of chimeric proteins by DNA shuffling of tree pollen allergens; these conjugates demonstrated low allergenicity and high immunogenicity [90]. Future studies will have to evaluate the efficacy of these new strategies.

Because TLR9 and TLR4 conjugate strategies have not been satisfactory in humans, novel technologies are being developed to monitor the events *in vivo* following exposure to pollens to better understand the events *in vivo*. Superparamagnetic iron oxide nanoparticles conjugated to Phl p5a and an optical probe (Alexa647) can interact with IgE antibodies and are suitable for monitoring by magnetic resonance imaging and flow cytometry [91]. Likewise, DNA aptamers CJ2-06 against the Cry j 2 can be useful for biosensing applications [92].

CONCLUSION

The innate immune responses to APEPs, pollen-derived proteases, lipids, and NADPH oxidases are complex, and lead to allergic inflammation. An important area of research has been to understand how these rapid innate immune responses induced by pollen components stimulate allergic inflammation. These components recruit neutrophils, modulate dendritic cell functions, and induce Th2 polarization and allergic inflammation. Some clinical studies have shown that modulation of innate immune response to pollens with TLR9- and TLR4-stimulating conjugates is well tolerated and has clear immunological effects, but is not very effective in primary clinical endpoints. Further investigations are required to clarify the innate immune mechanisms induced by pollens and its components. Future studies should evaluate modulating TLR4/MD2 function with a reagent other than allergoid-monophosphoryl lipid A. Another approach could be inhibiting CXCR2-mediated recruitment of activated neutrophils that drive allergic airway inflammation [49⁹³].

Because innate immune responses to pollens occur rapidly within minutes, clarification of these early mechanistic events is likely to lead to development of strategies and drugs that will prevent initiation of allergic sensitization in naïve patients, and block induction of allergic inflammation in sensitized patients at the start of the inflammatory process. This approach will be fundamentally different from strategies that directionally force immune response such as pollen-ISS or lipid A conjugates, or inhibit later established allergic events such as blocking Th2 cytokines or their receptors.

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KEY POINTS

- Pollens have aqueous pollen extract proteins, proteases, lipids, and NADPH oxidases that stimulate innate immune responses.
- These innate immune responses recruit neutrophils, modulate dendritic cell functions, induce Th2 polarization, and stimulate allergic inflammation.
- A two-signal hypothesis has been proposed for induction of pollen-induced allergic inflammation: ‘signal 1’, consisting of innate signaling pathways activated by pollen-induced reactive oxygen species (ROS), boosts ‘signal 2’, the classical pathway of antigen presentation to T cells that impart immunological specificity to the immune response.
- Multicenter clinical studies have shown that modulation of innate immune response to pollens by conjugating pollen proteins with DNA or lipids can stimulate TLR9 and TLR4 and induce clear immunological effects, and is well tolerated in humans, but is not very effective in primary clinical endpoints.
- To address these somewhat disappointing results with the use of pollen conjugates, a careful study of the early innate signaling events induced by pollens is required, and is likely to provide vital clues for the development of novel treatment and prevention strategies for pollen-mediated allergic diseases.

FIGURE 1.

Two-signal hypothesis of pollen-induced allergic inflammation. ROS generated by the pollen NADPH oxidases causes GSSG or 4-HNE production and DNA damage. GSSG, 4-HNE, and DNA damage induce the production of interleukin-8, a proinflammatory cytokine that attracts and activates neutrophils. Activated neutrophils also generate ROS. Pollen NADPH oxidase-mediated oxidative stress induces mitochondrial ROS generation. These ROS mediated by innate signaling pathways ‘signal 1’ promote the allergic inflammation ‘signal 2’, the classical allergic inflammation pathway. APC, antigen-presenting cell; 4-HNE, 4-hydroxynonenal; GSSG, oxidized glutathione; ROS, reactive oxygen species; SOD, superoxide dismutase.

FIGURE 2.

Proteases in pollens degenerate the epithelial tight and adherence junction. Pollen protease increases the permeability of the airway epithelium by disruption of tight and adherence junction. Pollen allergens can go through airway epithelium easily because of the loss of epithelial barrier function. JAM, junctional adhesion molecules; ZO-1, zonula occludens-1.

FIGURE 3.

Neutrophil recruitment by pollens is important for induction of allergic inflammation. Pollen allergen activates nuclear factor- κ B, leading to the production of CXCL8 in airway epithelium. Activated neutrophils recruited by CXCL-8 facilitate subsequent allergic inflammation by releasing MMP-9, neutrophil elastase, α -defensins, TGF- β 1, and ROS. 4-HNE, 4-hydroxynonenal; GSSG, oxidized glutathione; MMP-9, matrix metalloproteinase 9; ROS, reactive oxygen species; TGF- β 1, transforming growth factor beta 1.