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Allergen-encoded signals that control allergic responses

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Abstract

Purpose of review—The purpose is to review the important recent advances made in how innate immune cells, microbes, and the environment contribute to the expression of allergic disease, emphasizing the allergen-related signals that drive allergic responses.

Recent findings—The last few years have seen crucial advances in how innate immune cells such as innate lymphoid cells group 2 and airway epithelial cells and related molecular pathways through organismal proteinases and innate immune cytokines, such as thymic stromal lymphopoietin, IL-25, and IL-33 contribute to allergy and asthma. Simultaneously with these advances, important progress has been made in our understanding of how the environment, and especially pathogenic organisms, such as bacteria, viruses, helminths, and especially fungi derived from the natural and built environments, either promote or inhibit allergic inflammation and disease. Of specific interest are how lipopolysaccharide mediates its antiallergic effect through the ubiquitin modifying factor A20 and the antiallergic activity of both helminths and protozoa.

Summary—Innate immune cells and molecular pathways, often activated by allergen-derived proteinases acting on airway epithelium and macrophages as well as additional unknown factors, are essential to the expression of allergic inflammation and disease. These findings suggest numerous future research opportunities and new opportunities for therapeutic intervention in allergic disease.

Keywords

asthma; chronic rhinosinusitis; helminth; innate lymphoid cell; proteinase

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Conflicts of interest

There are no conflicts of interest.

INTRODUCTION

The fundamental causes of adult allergic diseases and especially asthma are now widely regarded as having an inflammatory basis. Studies over the past two decades have elucidated the intricate interplay between the innate and adaptive immune systems in allergic inflammation that comprise antigen presenting cells, T helper (T_H) 2 cells, IgE-producing B cells, eosinophils, mast cells and basophils, the cytokines IL-4, IL-5, and IL-13 and many other cells and molecules. Whereas the contributions of adaptive immune responses to allergic disease have been emphasized in the past, the last few years have seen crucial advances in how innate immune cells and molecular pathways contribute to allergy and asthma. Simultaneously with these advances, important progress has been made in our understanding of how the environment, and especially pathogenic organisms derived from the built environment, initiate allergic inflammation and disease. We review here the important recent advances made in these key areas, emphasizing the allergen-related signals that drive allergic responses.

KEY POINTS

- Chronic allergic diseases are increasingly recognized as beginning innately through the activation of AECs and group 2 innate lymphoid cells that secrete the cytokines TSLP, IL-25, IL-33, IL-4, and IL-13.
- Newly recognized innate immune receptors with potential to regulate allergic inflammation and disease include TLR4, PAR-2, and DP2.
- Diverse pathogens, including fungi and bacteria, can promote allergic inflammation through the release of proteinases and other factors.
- Proteinases mediate allergic inflammation and disease in part by cleaving the clotting factor fibrinogen, a fibrinogen cleavage product of which then induces innate allergic inflammation and antifungal immunity through TLR4.
- Environmental factors with antiallergic activity include lipopolysaccharide, which signals through TLR4 to activate the ubiquitin modifying factor A20 and parasites, such as helminths and the protozoan *T. gondii*.

INNATE ALLERGIC EFFECTOR CELLS

Airway epithelium

The airway epithelium together with phagocytic cells such as alveolar macrophages represent the first line of cellular defense against aeroallergens. Previously regarded as a mere barrier to allergen exposure, it is now clear that the airway epithelium, in addition to its physical barrier function, can recognize allergens through pattern recognition receptors and deploy sophisticated chemical and immunologic defenses against potentially hazardous allergens and pathogens including antimicrobial peptides, chemokines, and cytokines to recruit and activate other immune cells to initiate allergic inflammation. The 'default' airway response to innocuous aeroantigen signals is the development of tolerogenic, anti-

inflammatory immune responses that are coordinated by IL-10-secreting T-regulatory cells (Tregs). The central paradox of allergen sensitization is that tolerance against innocuous inhaled allergens breaks down with the emergence of robust allergic, especially IgE, responses in response to incompletely defined allergen signals. To explore further the mechanisms underlying the breakdown of tolerance, Ather *et al.* used transgenic mice capable of expressing constitutively active IkappaB kinase beta in airway epithelium in an ovalbumin (OVA)-induced inhalation tolerance model. They found that soluble mediators including IL-1 and IL-4 generated in response to airway epithelial-dependent nuclear factor kappa B (NF-κB) activation determined the activation of pulmonary dendritic cells, innate lymphoid cells (ILCs) and CD4⁺ T cells to orchestrate the breaking of inhalational tolerance and the initiation of allergic antigen sensitization and asthma-like disease [1[■]]. The master inflammatory transcription factor, NF-κB, has also been found to induce airway mucus hypersecretion and the induction of the Mucin gene 5AC, oligomeric mucus/gel-forming gene, a key pathophysiological feature in allergic asthma, concomitant with another transcription factor, signal transducer and activator of transcription 6, and is negatively regulated by RhoA/Rho kinase inhibitor, fasudil [2[■]]. In addition, NF-κB activation in airway epithelial cells (AECs) has been reported to mediate allergen-induced neutrophilic airway inflammation, modulated by glutathione S-transferase M1, a phase II enzyme and regulator of inflammatory signaling in AECs [3[■]]. Together, these studies demonstrate that NF-κB is a critical factor operating in epithelial cells that broadly influences the expression of allergic airway inflammation and disease.

Airway epithelial cells express many pattern recognition receptors, including toll-like receptors, as well as proteinase-activating receptors, C-type lectin receptors and Nod-like receptors for allergen/pathogen recognition [4–7]. However, little is known about the signals released by allergens that result in the activation of these receptors. Recent report from Choi *et al.* [8[■]] identified a Toll-like receptor 4 (TLR4)-associated phospholipase D1 (PLD1) signaling cascade that was crucial for house dust mites (HDM)-induced production of IL-13. The finding not only sheds light on the mechanism of HDM-mediated allergic inflammation but also suggests PLD1 as a novel therapeutic target. Another intriguing, but controversial, immune receptor expressed by AECs is proteinase-activated receptor 2 (PAR2). Recent study indicated that challenge with cockroach allergen extract, known to activate PAR2 by the proteinases in this mixture, led to upregulation of dual oxidases (DUOX)-2/reactive oxygen species signaling-induced airway reactivity, inflammation, oxidative stress and apoptosis that could be significantly inhibited by administration of ENMD-1068, a small molecule antagonist of protease activated receptor 2 (PAR-2) in allergic mice. This suggests that AECs are critical for regulation of airway reactivity and allergic inflammation via oxidative stress and apoptosis [9[■]]. Another study [10[■]] using asthmatic patient samples has identified a new receptor, D prostanoid receptor 2 (DP2), on AECs and that its activation drives epithelial differentiation, suggesting its potential role in airway remodeling in asthma. It remains unclear what allergen-derived signal is responsible for activation of DP2.

Upon allergen exposure, the airway epithelium releases cytokines such as IL-1, IL-25, IL-33, thymic stromal lymphopoietin (TSLP), granulocyte macrophage colony stimulating factor, as well as endogenous danger signals such as high-mobility group box 1, uric acid, and ATP to recruit and activate antigen presenting cells and other immune cells, including

group 2 ILCs (ILC2) [4,7,11]. How these factors influence innate allergic immune responses remains poorly understood. IL-25, a member of the IL-17 cytokine family that is secreted by the airway epithelium, has recently been reported to activate dendritic cells through upregulation of costimulatory molecules CD80 and CD86 that then induce the development of T_H2 cells in a murine model of OVA-induced allergic asthma [12]. Epithelial expression of IL-25, but not IL-33 or TSLP, has also been identified to be an important marker for T_H2 high, corticosteroid-responsive asthma, providing a potential tool in predicting responses to asthma therapy [13].

IL-33, a member of the IL-1 cytokine family and another major epithelial-derived cytokine, has been suggested to play critical roles in allergic airway disease pathogenesis, but the exact role of this cytokine in allergic disease is controversial. Kaur *et al.* [14[•]] have recently found that IL-33 can directly promote mast cell activation and airway smooth muscle (ASM) wound repair and drive airway hyper-responsiveness via upregulation of mast cell-derived IL-13, whereas another group has found that IL-33 exacerbates allergic bronchoconstriction by increasing synthesis, storage, and secretion of serotonin from mast cell [15[•]]. Contrary to its role in promoting allergic bronchoconstriction through mast cells, Morita *et al.* [16^{••}] using mast cell-deficient c-Kit (W-sh/W-sh) mice have shown that the IL-33-Mast Cell-IL-2 axis suppresses papain-induced allergic inflammation by increasing Tregs. In contrast to its roles in mast cell-mediated responses, IL-33 has also been found to significantly enhance TSLP-DC-driven or tumor necrosis factor (ligand) superfamily, member 4 (OX40L)-driven T_H2 responses by promoting production of IL-5, IL-9, and IL-13 from CRTH2⁺ CD4⁺ T_H2 memory cells [17]. Together, these studies demonstrate the conflicting regulatory functions of IL-33 on allergic inflammation depending on the source of this multifaceted cytokine.

Underlying the secretion of innate allergy-associated cytokines and other early allergic inflammatory events is the unifying biochemical concept that organismal proteinases derived from fungi, dust mites, plant pollens, and other organisms including bacteria drive these responses (Fig. 1). Florsheim *et al.* [18^{••}] recently showed that subtilisin, a bacterial-derived proteinase that is widely used in laundry detergents and has long been linked to human allergic disease, induces airway epithelial TSLP and IL-33 in mice.

Group 2 innate lymphoid cells

Allergen activation of airway epithelium leads to the recruitment and activation of other innate immune cells. Perhaps the cell of greatest current interest to allergic disease is ILC2. The groundbreaking discovery of ILC2s was made simultaneously in 2010 by three independent research groups [19–21]. ILC2 share many similar features with adaptive immune T_H2 cells in terms of their ability to secrete IL-4, IL-5, and IL-13 in response to epithelial cell-derived cytokines, such as IL-33 and IL-25 and the dependency on the transcription factors GATA sequence binding protein 3 and retinoic acid receptor, alpha for their development and function [22–24]. However, unlike T_H2 cells, ILC2s are lineage negative, that is, they carry no typical markers of previously well described immune cells (e.g. CD4, CD8, NK1.1, etc.). Although ILC2s have been shown to mediate the immune pathology of allergy and asthma independently of adaptive immunity, we are just beginning to understand the basis of their function, mechanism and interaction with other immune cell

types. What is clear thus far, however, is that proteinases derived from fungi can mediate their recruitment or expansion in lung [25].

MICROBES AND ALLERGIC DISEASE

Viruses, bacteria, fungi, helminths, and protozoa all exhibit protease activity and can each contribute other unique signals that contribute to allergic and asthmatic phenotypes, either positively or negatively. Recent advances have helped to elucidate the specific mechanisms of these microbial influences.

Bacteria

Bacteria have been implicated in both the origin of allergic airway disease and the worsening of existing allergic symptoms. It has been recently demonstrated in a murine model of allergic airway disease using OVA that early-life *Chlamydia* infection results in a T_H2 skewing of the airway immune response with increased eosinophilia, *Chlamydia*-specific IgE, and IL-4, 5, 10, and 13 compared with the T_H1 response seen in mice infected as adults. Furthermore, chronic *Chlamydia* infection resulted from neonatal exposure and persisted into adulthood [26]. Another atypical bacterium, *Mycoplasma pneumoniae*, has been shown to exacerbate existing asthma in a mouse model. Community acquired respiratory distress syndrome toxin produced by *Mycoplasma* increased eosinophilic inflammation, T_H2-associated cytokines, and airway hyperreactivity in mice compared with controls [27]. Whether these observed proallergic effects are specific to atypical bacteria is not yet entirely clear. In a related development, preexisting allergic lung inflammation induced by OVA sensitization in mice has been found to reduce lung bacterial burdens and reduce mortality in response to *Klebsiella pneumoniae*. This effect was dependent on neutrophils and chemokine (C-C motif) ligand 8 despite OVA sensitization causing a reduction in neutrophilia and T_H17 cytokines [28]. Bacteria may also have an additive role in steroid-resistant asthma. It has been recently shown that lipopolysaccharide derived from gram-negative bacteria when used in combination with β -glucans and HDM allergens may promote neutrophilic and corticosteroid-resistant inflammation involving Dectin-1 and TLR4 [29].

Fungi

Fungi are frequently associated with allergic disease and extracts from fungi are a common method of inducing asthma in animal models. In the past 2 years, significant progress into understanding the mechanism of fungi-mediated allergic disease has been made. A protease allergen extracted from the fungus *Aspergillus fumigatus* was shown to penetrate airway epithelium and submucosa to trigger airway hyperresponsiveness by degrading the extracellular matrix of the ASM [30]. Furthermore, inhaled fungal proteinase has been found to cleave fibrinogen in the airway to provoke allergic disease and antifungal immune responses. These responses were mediated in part by AECs and macrophages and were dependent on TLR4 [25]. An additional study adds significantly to the growing body of literature indicating that severe allergic airway diseases, including steroid-refractory chronic rhinosinusitis (CRS), allergic fungal rhinosinusitis, and severe asthma, are fungal infectious diseases. Porter *et al.* [31] demonstrated in a cohort of patients undergoing endoscopic sinus surgery that fungi are culturable from the sinus lavage fluid obtained from the majority of

patients with allergic airway disease (CRS with nasal polyps, many also having asthma), but in less than 20% of patients without CRS undergoing the identical procedure [31]. They also showed that the majority of patients with allergic airway disease demonstrate fungus-specific T_H2 responses, whereas no patient without allergic disease demonstrated similar responses. These findings together suggest that CRS with or without asthma is because of the noninvasive growth of fungi along the airway mucosal surface (airway mycosis) and that clearance of such fungi might be palliative in these conditions. This is further supported by studies demonstrating that many live fungi are alone sufficient to reproduce all salient features of asthma in mice [32].

Viruses

Airway infection with viruses, especially human rhinovirus (HRV) has long been recognized as a major asthma-exacerbating factor. HRV is believed to cause additional inflammation that may worsen a patient's existing asthma symptoms. The mechanism by which rhinovirus infection leads to exacerbation has become more clear through several recent studies. Using an HRV-infection model, IL-33 and other T_H2 -associated cytokines were elevated following infection of the asthmatic airway and correlated with exacerbation severity [33[•]]. Airway epithelial-derived IL-25 has also been implicated in HRV-induced asthma exacerbation. Human asthmatic bronchial epithelial cells produced more IL-25 *in vitro* compared with controls prior to and following rhinovirus infection. In the same study [34^{••}], mice in which the IL-25 receptor was inhibited showed decreased T_H2 inflammation and airway cellularity.

Macrophage polarity is another crucial element of asthma exacerbation during rhinovirus infection. In an OVA murine model of allergic airway disease, IL-4R knockout mice lacking IL-4/IL-13 signaling responded to rhinovirus infection by producing type 1 cytokines and IL-17A from M1-type macrophages along with primarily neutrophilic inflammation. In wild-type mice, rhinovirus infection of asthmatic airways resulted in additional alternatively-activated (M2) macrophage cytokines and neutrophilic and eosinophilic airway inflammation [35].

The types and timing of interferon production may also be an important factor in virus-induced worsening of asthmatic symptoms. In asthmatic human viral infection, one report found no significant difference between airway cellularity in patients who experienced an exacerbation and those who did not. However, they did observe that individuals with reduced IFN- γ expression following infection resolution experienced more severe asthma symptoms and a higher risk for exacerbation [36[•]].

Helminths/protozoa

Helminths and protozoa interact in complex ways with the immune system, resulting in either enhanced or suppressed allergic inflammation and disease. For example, infection of mice with the gut parasite *Heligmosomoides polygus* strongly inhibited T_H2 and innate lymphoid cell-dependent allergic inflammation induced by fungal extract and OVA challenge. Heat killing of the nematodes prior to administration abrogated this suppressive response, indicating that inhibition of type 2 immunity is likely an active, self-protective mechanism employed by helminths during infection [37]. On the other hand, helminths may

induce additional IgE against other allergens similar to helminth antigens. In another study [38[•]], serum from individuals with filarial infections was more likely to have IgE specific to cockroach and dust mite antigens that were structurally similar to helminth-derived antigens. The mechanism by which the single-celled protozoan parasite *Toxoplasma gondii* inhibits allergic airway inflammation has recently been further characterized. *T. gondii* was found to acutely induce a strong T_H1 response and reduce lung inflammation in allergic mice. This effect appeared to involve increased generation of FoxP3+ Tregs [39[•]].

ENVIRONMENTAL FACTORS

Human-built occupational and domestic environments (the built environment) generate unique exposures that have been linked to allergic disease. In an updated review of the literature, Kanchongkittiphon *et al.*, revised prior conclusions regarding the importance of indoor exposures and asthma exacerbations. They found that indoor dampness may have a direct causal relationship with asthma exacerbations in both children and adults. More tempered evidence suggests that indoor culturable fungi, nitrogen dioxide, rodents, feathered bedding, mites, cockroaches, and dogs are all likely contributory factors in asthma exacerbations [40[•]]. The concept that fungal exposure, especially to fungal proteinases, is an important factor in increased asthma severity continues to receive support from diverse studies. One such study found that adults with asthma living in homes with high levels of mold had decreased forced expiratory volume in 1 s % (FEV1%). These FEV1% values also correlated with the patient's own assessment of their symptom severity [41[•]]. In another metaanalysis, certain fungal species (*Cladosporium*, *Alternaria*, *Aspergillus*, and *Penicillium*) were not only found to be in higher concentrations in asthmatic patient's homes, but also associated with more exacerbations [42[•]]. Another recent finding is a possible mechanism for how farm exposure to dust and lipopolysaccharide protects individuals from allergic disease. In a murine model, long-term exposure to low-dose lipopolysaccharide suppressed type 2 immunity via a reduction in epithelial cell cytokines. This effect was dependent on A20, a ubiquitin-modifying enzyme that inhibits NF-κB. Furthermore, it was demonstrated that humans with a mutation in the A20 gene were at increased risk of allergy and asthma despite being raised on a farm [43^{••}]. Occupational exposures represent a wide range of asthma-causing allergens. In a recent review of reported occupational allergens, Baur *et al.* found that the most prominent associations were among people who worked with laboratory animals. A moderate effect was found for those who are exposed to enzymes from *Aspergillus*, enzymes from *Bacillus subtilis*, mites, proteinases, food products, latex, farms, rodents, sea life, dyes, and certain chemicals such as toluene diisocyanates and platinum salts [44].

CONCLUSION

Studies over the recent past emphasize that all classes of microorganisms, including bacteria, viruses, fungi, and different parasites can all participate in the expression of allergic disease, whether stimulatory or inhibitory (Fig. 1). The mechanisms by which these pathogens influence allergic inflammation and disease are diverse, but recent research has reemphasized the central role that proteinases play in allergic disease pathogenesis. Two major mechanisms by which proteinases participate in allergic disease have been

emphasized: first, an airway epithelial program that is modified by PAR-2 and PDL1 that promotes secretion of the cytokines TSLP, IL-33, and IL-25 and activates the transcription factor NF- κ B. This cytokine and transcription factor module promotes the development of ILC2 that in turn promote the development of T_H2 cells. It appears that both ILC2 and T_H2 cells work obligatorily together to promote severe allergic inflammation and allergic disease. Second, proteinases cleave the clotting factor fibrinogen, creating fibrinogen cleavage products, one of which acts as a novel ligand for TLR4 to promote the development of allergic upper (chronic sinusitis) and lower (asthma) respiratory tract disease. Intriguingly, while it has been known for some time that fungi, parasites, and viruses release allergenic proteinases, more recent studies show how bacterial enzymes such as subtilisin can cause allergic disease. In addition to the extraordinary discovery of ILCs themselves, recent studies have shed light on how these cells arise and promote allergic disease through transcription factors (BCL11b) and costimulatory molecules (OX40L). Among the factors that suppress allergic inflammation and disease are certain helminth and protozoan factors, currently poorly understood, that promote T_H1 cells and Tregs and the epithelial cell ubiquitin modifying enzyme A20 (Fig. 1).

Much more is known about the early immune events that are triggered by allergens than about the allergen-derived signals that initiate allergic inflammation. Exogenous proteinases that are almost ubiquitously found in allergens are clearly essential; other emerging factors include β -glucans and the biochemical properties of pollens as will be discussed in a separate article in this issue. Future studies are likely to explore further the molecular and cellular paradigms considered here and will be interesting to follow for their potential therapeutic implications.

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FIGURE 1.

Summary of recent advances in understanding the pathogenesis of allergen-induced allergic inflammation and disease. See text for details.