Immune Polarization in Allergic Patients: Role of the Innate Immune System

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Abstract

Allergens come into contact with the immune system as components of a very diverse mixture. The most common sources are pollen grains, food, and waste. These sources contain a variety of immunomodulatory components that play a key role in the induction of allergic sensitization. The way allergen molecules bind to the cells of the immune system can determine the immune response. In order to better understand how allergic sensitization is triggered, we review the molecular mechanisms involved in the development of allergy and the role of immunomodulators in allergen recognition by innate cells.

Key words: Innate immune system. Allergy. Allergens.

Resumen

Los alérgenos entran en contacto con el sistema inmune como parte de una mezcla heterogénea de compuestos de muy diversa naturaleza. Las fuentes de alérgenos más comunes son los granos de polen, alimentos o restos de animales. Estas fuentes contienen una variedad de componentes inmunomoduladores que pueden desempeñar un papel importante en la inducción de la sensibilización alérgica. La unión de estas moléculas a las células inmunes pueden influir en el resultado inmunológico final. Actualmente, nuestro objetivo es revisar los mecanismos moleculares implicados en el desarrollo de la alergia, y el papel de estos inmunomoduladores en el reconocimiento de alérgenos por las células innatas, que es clave para entender cómo la sensibilización alérgica se desencadena.

Palabras clave: Sistema immune innato. Alergia. Alérgenos.

Introduction

The immune response results from the sequential activation of multiple components of the innate and the adaptive immune systems. These components are organized into 3 groups: *1*) physical barriers (skin and mucosal surfaces); *2*) the innate immune system, which responds to pathogen-associated molecular patterns and includes epithelial, endothelial, stromal, and phagocytic cells (dendritic cells [DCs], neutrophils, monocytes/macrophages, and eosinophils), as well as natural killer (NK) cells and the complement system; and *3*) the adaptive immune system, which is induced by DCs priming T and B lymphocytes to secrete specific cytokines that shape the immune response [1].

Innate Immune Signaling During the Allergic Response

The immune system is regulated to protect the host from exaggerated stimulatory signals, thus establishing a state of tolerance in healthy individuals. The imbalance arising between immunoregulatory mechanisms and effector mechanisms results in inflammation, allergy, and autoimmune disorders in genetically predisposed persons under specific environmental conditions. Although a variety of immunologic mechanisms are involved in the pathogenesis of allergy, most allergens trigger allergic manifestations through type I hypersensitivity reactions, which are characterized by IgE production [2].

Several immune cells are involved in the development of the innate immune response during allergic inflammation. Mast cells and basophils are responsible for anaphylaxis, asthma, and other allergic disorders [2]. Both cell types share numerous common features such as secretion of mediators (histamines, leukotrienes, and T_H2 cytokines), and expression of toll-like receptors (TLRs) and complement receptors. In allergic individuals, the high-affinity IgE receptor on these cells (also known as Fc epsilon receptor I) is almost fully saturated with IgE and essentially primed and ready to initiate IgE-mediated responses when it encounters a sufficient quantity of allergen [3,4].

Eosinophils are also relevant in the development of allergic responses. These cells usually accumulate in mucosal tissue during allergen-induced inflammation. The reaction is due to IL-5, the eosinophilic factor responsible for the proliferation and differentiation of these cells. It is secreted mainly by T_H2 cells. Additionally, granulocyte macrophage colony-stimulating factor (GM-CSF) produced by epithelial cells seems to have a role in the activation and recruitment of eosinophils in tissue [5].

Epithelial cells are the interface between the host and the environment and act as the first line of defense against microorganisms, gases, and allergens [6]. The activation of pattern recognition receptors on epithelial cells in the airways or in the gut leads to the release of cytokines (IL-25, IL-33, and thymic stromal lymphopoietin) that attract and activate innate and adaptive immune cells. These cytokines have been shown to function upstream of classic T_H2 cytokines, thus providing a molecular mechanism by which epithelial cells directly affect T_H2-induced immunity [7]. This is a key process in the recognition of inhaled/ingested allergens that activate the local network of DCs, which coordinates the subsequent immune response [8].

DCs provide an important bridge between the innate and the adaptive immune systems by processing antigens and presenting them to T cells. They are found in an immature state in tissue, but upon antigen uptake, they migrate to the draining lymph nodes where they present the antigens to T cells. This process induces the release of signature cytokines (IL-4, IL-12, and IFN-γ), which drive T-cell polarization during allergic inflammation [9-12].

Finally, NK cells are major producers of an array of proinflammatory and immunosuppressive cytokines such as IFN-γ, TNF-α, and IL-10. These cells include a recently identified lineage, the invariant NK T cells (iNKTs), which are characterized by the expression of a limited repertoire of T-cell receptors and the production of large amounts of cytokines (IFN-γ, IL-4, IL-5, and IL-13) [13-14]. Likewise, innate lymphoid cells (ILCs) are a recently discovered innate cell population, of which 3 subpopulations have been described, namely, ILC1 (predominantly expressing IFN-γ), ILC2 (expressing IL-5, IL-9, and IL-13), and ILC3 (expressing IL-22 and/or IL-17) [13]. ILC2s are T_H2 cytokine–producing cells that were initially identified in the gastrointestinal track, lung, nasal polyp tissue, and blood and are reported to have a potential role in allergic inflammation [14].

Factors Involved in Deviation of the Allergic Innate Immune Response

The main causes of allergy are genetic factors, environmental exposure, and the interaction between both [15]. Several epidemiological reports have indicated an association between the increasing incidence of allergic diseases in developed countries and improved health care and lifestyle conditions, which result in a substantial loss of early microbial stimulation [16], thus supporting the hygiene hypothesis [17]. According to this theory, increasingly hygienic living conditions, use of antibiotics, and sterile food preparation have resulted in the separation of the immune system from positive exposure to microbes early in life [18]. Consequently, immunoregulatory mechanisms remain underdeveloped, and an imbalance in immune homeostasis predisposes to T_H2 immune responses favoring allergic processes [19].

A critical event in the development of atopy seems to be the absence of a switch from the predominant T_H2 response observed in all newborns to an immune state characterized by the prevalence of regulatory and/or T_H1 responses. In allergic patients, fetal primed T_H2 immunity tends to persist until later in life and to boost T_H2 responses upon allergen exposure [20].

Epigenetic Modifications

The many genome-wide association studies conducted for asthma [21] and other allergic phenotypes [22] have identified hundreds of genes associated with allergic diseases. However, most only confer a small increased risk and do not correspond to the various phenotypes [23].

One possible explanation could be gene–environment interactions, which are thought to be mediated by epigenetic mechanisms. There are several examples of the association between environmental exposure and epigenetic modifications in allergic diseases. The best-known examples are probably those of exposure to a farm environment and smoking. A meta-analysis of 29 studies found a 25% lower prevalence of childhood asthma in patients exposed to a farm environment [24]; even in utero farm exposure is protective against hay fever, asthma, and eczema [25]. Likewise, levels of DNA methylation of several asthma-associated genes in the cord blood of newborns differ between children who have and have not been exposed to a farm environment [26]. Moreover, it has been reported that methylation of CD14 promoter in placenta is altered if the mother lives on a farm [27].

Maternal smoking during pregnancy is associated with allergic diseases [28,29]. Additionally, the risk of asthma is increased in children whose maternal grandmother smoked during their mother's fetal period [30].

Other examples of gene–environment interactions in allergic diseases include exposure to air pollution, which has been reported to worsen the severity of asthma [31], and the epigenetic modifications induced by rhinovirus infections in the nasal epithelial cells of asthma patients [32].

Recent research has revealed that several asthma- and allergy-related genes are epigenetically regulated; for example, *STAT6* and *FOXP3* are regulated by DNA methylation [33,34], and the transcription of *IL13* is regulated by histone acetylation [35].

It is evident that more research is needed to establish the association between allergy-related diseases and epigenetics.

Proteolytic Activity

The role of endogenous and exogenous proteases in airway inflammation and remodeling has been the subject of active research during recent decades. Direct cleavage of tight junctions and signaling of specific receptors (protease-activated receptors) have been reported to be involved in inflammatory processes [36].

Human respiratory viruses have been associated with an increased risk of asthma [37,38]. Respiratory syncytial virus

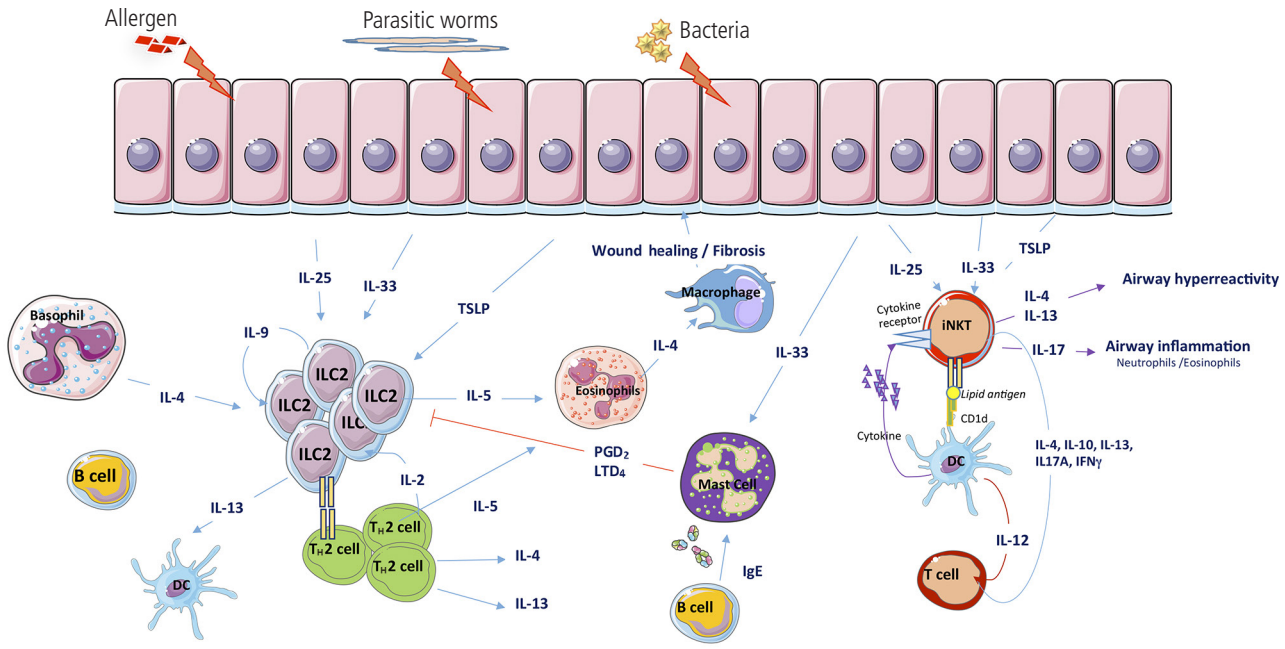

Figure. Interactions between innate immune cells in allergic diseases. TSLP indicates thymic stromal lymphopoietin; ILC, innate lymphoid cell; PG, prostaglandin; LT, leukotriene.

was recently shown to upregulate matrix metalloproteinase 10, thus suggesting a role for endogenous proteases in the onset of asthma [39,40]. Protease-activated receptors are upregulated in other inflammatory diseases, such as chronic rhinosinusitis [41].

Since the discovery of the cysteine protease activity of the major allergen Der p 1 [42], several mite allergens have been identified as proteases. Mite allergy is the most prevalent allergic disease worldwide. Several publications report that mite proteases mediate inflammatory mechanisms [36,37,43]; however, allergen exposure is caused by mite feces, an extremely effective platform where proteases interact with innate proinflammatory signaling [44]. In addition to mites, fungal and insect allergens, such as those of *Alternaria* [45,46], *Aspergillus* [47], and cockroach [48], have been reported to induce protease-mediated allergic inflammation. All of these allergens seem to drive allergic sensitization in areas with low exposure to mite allergen.

First described a decade ago [49], IL-33 exerts its function through the specific ST2 receptor that is present in T_H2 cells. IL-33 exerts a dual function, namely, an anti-inflammatory function when released from apoptotic cells, as it is inactivated by caspases, and a proinflammatory function when released from necrotic cells. iNKTs produce IL-4 in the presence of IL-33 and α-galactosylceramide [50], and ILC2s, which also express ST2, produce T_H2 cytokines in response to IL-33 [51]. In a T_H2-skewed immune system, IL-33 will thus promote allergic sensitization. Both mites [52] and *Alternaria* [53,54] have been reported to induce IL-33 production.

These findings show that early intervention strategies in viral infections [55,56], blockade of cystein protease activity [57], and mite-specific immunotherapy in sensitized subjects [58] could constitute novel approaches for the prevention of asthma.

House Dust Mite Major Group 1 Allergens as a Model

The protease activity of house dust mite major group 1 allergens (Der f 1 and Der p 1), two of the most important allergens worldwide, has been shown to cause barrier dysfunction, induce production of proinflammatory cytokines by epithelial cells and keratinocytes, cleave various molecules, modulate functions of several cell types, and induce T_H2 responses [57].

The repeated exposure of airway mucosa to cysteine protease allergens such as Der p 1 and Der f 1 results in lung eosinophilia and serum IgE/IgG1 production in a manner dependent on the protease activity of the allergen, primarily with the cooperation of adaptive immune cells and IL-33–responsive innate cells [59]. Der p 1 can efficiently activate the *Drosophila* innate immune system in terms of both epithelial and systemic responses. These responses depend on the activation of the transcription factor NF-κB. In addition, Der p 1 cleaved the ectodomain of peptidoglycan recognition protein LC, thus activating the innate immune response.

Furthermore, interaction of Der p 1 with respiratory epithelium caused rapid secretion of CCL20 and thymic stromal lymphopoietin, thus inducing recruitment of immature DCs to the lung. This response was dependent on the presence of β-glycan structures in Der p 1 [60,61]. Accordingly, the deglycosylated preparation of Der p 1 exhibited minimal uptake

by DCs [61]. The recognition of this allergen would then be mediated by specific glycan receptors such as the DC-specific intercellular adhesion molecule-3-grabbing nonintegrin [62]. The carbohydrate moieties on these allergens played a vital role in recognition by innate immune cells, resulting in downstream deleterious T_H2 cell activation and IgE production [63].

Lipid Ligands

Allergens are never delivered to the immune system in a pure form but are contained in allergen sources such as pollen grains, food matrices, or fecal particles. In order to assess the presence of lipid immunomodulators in pollen allergens, Bashir et al [64] performed a lipidomic analysis of pollen from 22 species and established a database of lipid antigens as new candidate molecules involved in allergy. Cypress pollen contains phosphatidylcholine and phosphatidylethanolamine on the surface of its grains. This finding seems to be relevant for the activation of DCs by CD1 molecules and the subsequent stimulation of T-cell proliferation in patients with cypress pollen allergy [64].

Moreover, a recent study of the general ability of olive pollen lipids to activate DCs and stimulate iNKT cells in healthy individuals showed that polar lipids (eg diacylglycerols, free fatty acids, and triacylglycerols) isolated from olive pollen grains upregulated CD1d on DCs and iNKT cells in coculture experiments [65].

Taken together, these results show that lipids play a role in the early stages of allergic sensitization by interacting with several components of the innate immune system, such as those involving TLRs or CD1d.

ML-Domain Lipid-Binding Proteins

Allergenic proteins commonly have lipid-binding properties, implying that the intrinsic biological function contributes to allergenic activity. The group 2 house dust mite allergens (Der f 2 and Der p 2) are homologs of the ML-domain protein MD-2, the lipopolysaccharide-binding component of the TLR 4 signalling complex, for innate immune signalling [66]. HEK293 cells transfected with Der p 2 did not require MD-2 to bind and induce a TLR4-mediated response [66], although induction was weaker. However, IL-8 production was increased 30-fold when Der p 2 was transfected into MD-2 cells, possibly owing to an additive effect. Der p 2 can also stimulate proinflammatory cytokine secretion from epithelial cells [66,67].

Der p 2 local inflammatory responses in the airway epithelium are primarily mediated by airway smooth muscle cells. The response includes activation of the MyD88 signalling pathway via TLR2, downstream signalling of NF-κB activation, and production of high levels of proinflammatory cytokines [68,69].

Lipocalins

The lipocalin family is complex and includes both animal and bacterial allergens [70,71], although its presence in the plant kingdom cannot be ruled out [72].

Lipocalins have a characteristic fold enclosing a hydrophobic ligand-binding site [73,74]. Ligands such as retinoic acid and 18-carbon chain fatty acids bind within this cavity [75-77], while longer chain and complex lipids, especially with polar groups, must be extruded from the cavity. Lipocalins can also bind directly to cellular receptors such as type 1 and 2 lipocalin receptors and TLRs [78,79], thus mediating their endocytosis.

The salivary lipocalins Equ c 1 [80], Fel d 4 [81], and Can f 6 [82] and the structurally related major urinary proteins, such as mouse Mus m 1 [83], induce IgE responses. They can bind small volatile compounds such as odorants, pheromones, and sex steroids [84], thus enabling them to enhance TLR4-dependent lipopolysaccharide signalling [85].

Another example of lipocalin-binding lipids is the major mammalian allergen β-lactoglobulin, milk lipocalin, which can interact with membrane phospholipids, thus disrupting structure and exposing hydrophobic residues [86,87].

Nonspecific Lipid Transfer Proteins

nsLTPs are important allergens for fruits (Pru p 3), vegetables and nuts (Cas s 8), and aeroallergens, such as Par j 1 from *Parietaria judaica* pollen and Ole e 7 from *Olea europaea* [88,89]. They have a tunnel-like cavity that can accommodate phospholipids, glycolipids, with and fatty acids [90,91]. nsLTPs can transfer phospholipids and glycolipids between plant organelles [92] and present antimicrobial activity resulting from their interaction with and permeabilization of biological membranes [93]. Ligands transported by allergenic LTPs can contribute to the activation of innate immune cells, although there is little experimental evidence of this [94].

Tordesillas et al [95] showed that the peach nsLTP Pru p 3 can cross the monolayer formed by Caco-2 epithelial cells through the endocytic pathway by means of lipid rafts and caveolar endocytosis. The authors also reported that the lower transport rate of a hypoallergenic peach nsLTP was associated with significantly lower expression of T_H2-related cytokines compared with Pru p 3.

Par j 1 has been reported to function as an activator of the immune system by inducing IFN-γ production by NK cells exclusively in healthy individuals [96].

2S albumins

2S albumins, which are defined based on their sedimentation coefficient, constitute a major group of seed storage proteins that are widely distributed in both monocotyledonous and dicotyledonous plants. They are embedded in the protein bodies of developing seeds and are utilized by the plant as a source of nutrients (amino acids and carbon skeletons) during germination and seedling growth [97].

2S albumins adopt a common and compact 3-dimensional structural scaffold comprising a bundle of 5 α-helices in different regions and a C-terminal loop folded in a right-handed super helix stabilized by 4 conserved disulfide bonds [98]. In general, 2S albumins can bind lipids, as has been demonstrated for Sin a 1 from yellow mustard and various 2S albumin isoforms from sunflower seeds [99]. The 2S albumin from Brazil nut, Ber e 1, has a potential lipid-binding hydrophobic cavity of the same size as that of nsLTPs [100,101]. Ber e 1 was

not able to induce production of IgE or IgG in mice, although it was able to induce production of both antibodies when administered with sterol-rich or polar lipid fractions isolated from Brazil nuts. The response comprised a cooperative effect between Ber e 1 and β-sitosterol and glycolipid-rich fractions and was partially dependent on iNKT cells [101].

Bet v 1-like protein

Bet v 1 from the pathogenesis-related (PR) 10 family is the major allergen of birch pollen. PR-10 proteins are also the principal allergens for other *Fagales* trees, such as Cor a 1 and Aln g 1 from hazel and alder and food allergens such as Mal d 1 from apple. The PR-10 protein fold creates a large hydrophobic pocket that can carry or store many ligands (including flavonoids) and fatty acids [102]. The natural ligand for the main isoform of Bet v 1 is now known to be the flavonoid glycoside quercetin-3-O-sophoroside [102], which requires both the glycan and the lipid fractions for binding. Curiously, different isoforms of Bet v 1 can be found in pollen and share 95% amino acid sequence identity. By contrast, not all show the same IgE-binding capacity, and the difference lies in the ligands they can bind.

Concluding Remarks

Despite much research on allergen characterization and the interaction of allergens with the immune system, we are still far from fully understanding how allergic responses are triggered and what defines the final outcome of this response. In fact, as shown in this review, many of the factors involved in allergy are either intrinsic to the allergen and its properties (proteolytic activity, ligand binding) or associated with the host environment (epigenetic modifications, new types of immune cells). These factors can skew the immune system towards an allergic response. Extensive research on innate immune responses during allergic sensitization will help us to characterize the "decision-making process" that takes place when the immune system encounters an allergenic antigen.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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