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Innate immunity in allergic disease

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Acknowledgement

The authors declare no conflicts of interest.

Summary: The innate immune system consists of multiple cell types that express germline-encoded pattern recognition receptors that recognize pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). Allergens are frequently found in forms and mixtures that contain PAMPs and DAMPs. The innate immune system is interposed between the external environment and the internal acquired immune system. It is also an integral part of the airways, gut, and skin. These tissues face continuous exposure to allergens, PAMPs, and DAMPs. Interaction of allergens with the innate immune system normally results in immune tolerance but, in the case of allergic disease, this interaction induces recurring and/or chronic inflammation as well as the loss of immunologic tolerance. Upon activation by allergens, the innate immune response commits the acquired immune response to a variety of outcomes mediated by distinct T-cell subsets, such as T-helper 2, regulatory T, or T-helper 17 cells. New studies highlighted in this review underscore the close relationship between allergens, the innate immune system, and the acquired immune system that promotes homeostasis versus allergic disease.

Keywords: innate immunity, allergy, asthma, atopic dermatitis, food allergy, inflammation

Introduction

For most people, daily exposure to allergens produces few, if any, clinical symptoms. However, in some individuals, exposure to an allergen breaks the natural tolerant state and triggers allergic inflammation plus an allergen-specific immune response that is maintained by host T and B lymphocytes. The innate immune system is interposed at the interface between allergens in the external environment and the internal acquired immune system (1). To understand the role of innate immunity in allergic disease, it is important to appreciate how innate immunity regulates acquired immune responses (2). Unlike the innate immune system, cells of the acquired immune system are able to generate a seemingly infinite set of allergen-specific receptor molecules by gene rearrangement and somatic hypermutation. These cells retain immunologic memory of the original allergen exposure, and upon allergen re-exposure, they are able to mount a memory immune response against the allergen. Innate immune receptors, termed pattern recognition receptors (PRRs), have evolved the capacity to detect a variety of molecular patterns on microbes,

allergens, and self-antigens (3, 4). The molecular patterns recognized by PRRs include pathogen-associated molecular patterns (PAMPs) (1, 5) and danger-associated molecular patterns (DAMPs) (6–8). DAMPs are molecules released by host cells that are injured or produced by host cells during inflammatory or immune responses.

Innate immune system molecules

All living organisms share in common a barrier presented to the external environment. The innate immune system acts principally within this barrier, where its major function is host defense against infection (1). Equally important is the role played by the innate immune system in the continual removal of massive amounts of environmental particles, microbial products, antigens, and allergens. Innate immune cells express surface and intracellular PRRs such as Toll-like receptors (TLRs) (9), nucleotide binding domain/leucine-rich repeat receptors (NLRs) (10–13), C-type lectin receptors (CLRs) (14–17), scavenger receptors (15, 16, 18, 19), and a variety of other receptor molecules that recognize DAMPs (20–22) (Table 1). Innate immune cells also bear receptors for soluble regulatory molecules including cytokines and chemokines (23, 24). The mucosal lining of epithelial barriers contains secreted PRRs that recognize PAMPs and DAMPs. Secreted PRRs include the complement proteins (25, 26), collectins (27–30), mucins (31, 32), pentraxins (33–35), ficolins (36, 37), and resolvins (38–40). Mucins, pentraxins, and ficolins serve to protect the airway epithelial barrier and are principally involved in the initial recognition and elimination of microbes and their products. Resolvins are involved in dampening inflammation and restoring tissue homeostasis.

Innate immune system cells

Innate immune cells include mast cells (MCs), granulocytes, mononuclear phagocytes, several types of 'innate' lymphocytes, and epithelial cells (ECs) (Table 2).

Granulocytes

MCs arise in the bone marrow, transit the blood, and take up residence in peripheral tissues, where they survive for months to years, proliferate in response to appropriate signals (41), and, in humans, are found associated with blood vessels and mucosal ECs (42). MCs express the high-affinity immunoglobulin E (IgE) receptor FcεR₁, and upon ligation, these cells release mediators of inflammation and the allergic response (42). Three classes of granulocytes arise in the bone marrow,

enter the bloodstream, and circulate throughout the body. Polymorphonuclear neutrophils (PMNs) are the most abundant circulating leukocytes in humans (43) and represent one of the first lines of defense against invading microbes, based on their ability to rapidly recognize, phagocytize, and kill microbes (44). Basophils are circulating granulocytes that leave the blood and enter tissues where they mature (45). Basophils also express FcεR₁, and upon activation, these cells also release mediators of inflammation and allergic responses (45, 46). Eosinophils are circulating granulocytes that migrate from the blood into mucosal sites. In humans, these cells reside in the *lamina propria* of the stomach, small intestine, colon, and airway (47). Eosinophils represent a key line of defense against microbial invasion at epithelial barriers. Upon activation, these cells produce a number of soluble mediators that direct inflammatory and allergic responses (48). Some studies suggest that basophils (49–52) and eosinophils (53–56) can serve as antigen-presenting cells (APCs) capable of fully activating naive T cells and driving T-helper 2 (Th2)-type T-cell responses. Recent studies have questioned the role of basophils as APCs in experimental models of Th2-mediated reactions (57–59).

Mononuclear phagocytes

Mononuclear phagocytes consist of peripheral blood monocytes, macrophages (MOs), and dendritic cells (DCs) (60). Bone marrow-derived monocytes leave the circulation and emigrate into tissues and sites of inflammation where they differentiate into mature MOs (61). MO ontogeny remains controversial, although data suggest that these cells may be self-renewing (62). Resident MOs not only maintain tissue homeostasis and dampen inflammation initiated by the clearance of allergens, but they are also fully functional APCs capable of activating naive T-cell responses. There are three types of DCs: conventional (c)DC, plasmacytoid (p)DC, and inflammatory DC (63). The wide variety of functionally different DC subsets form networks associated with mucosal epithelium throughout the body. These cells are considered 'professional' APCs, based on their capacity to trigger the differentiation of antigen-specific effector T cells from naive T cells, especially under conditions of low antigen abundance and cytokine 'help'.

Innate immune lymphocytes

There are several lymphocyte classes that participate in innate immune responses including γδT cells (64), natural killer (NK) and natural killer T (NKT) cells (65, 66), and invariant (i)NKT cells (67), although the role of iNKT cells in allergic diseases remains controversial (68, 69).

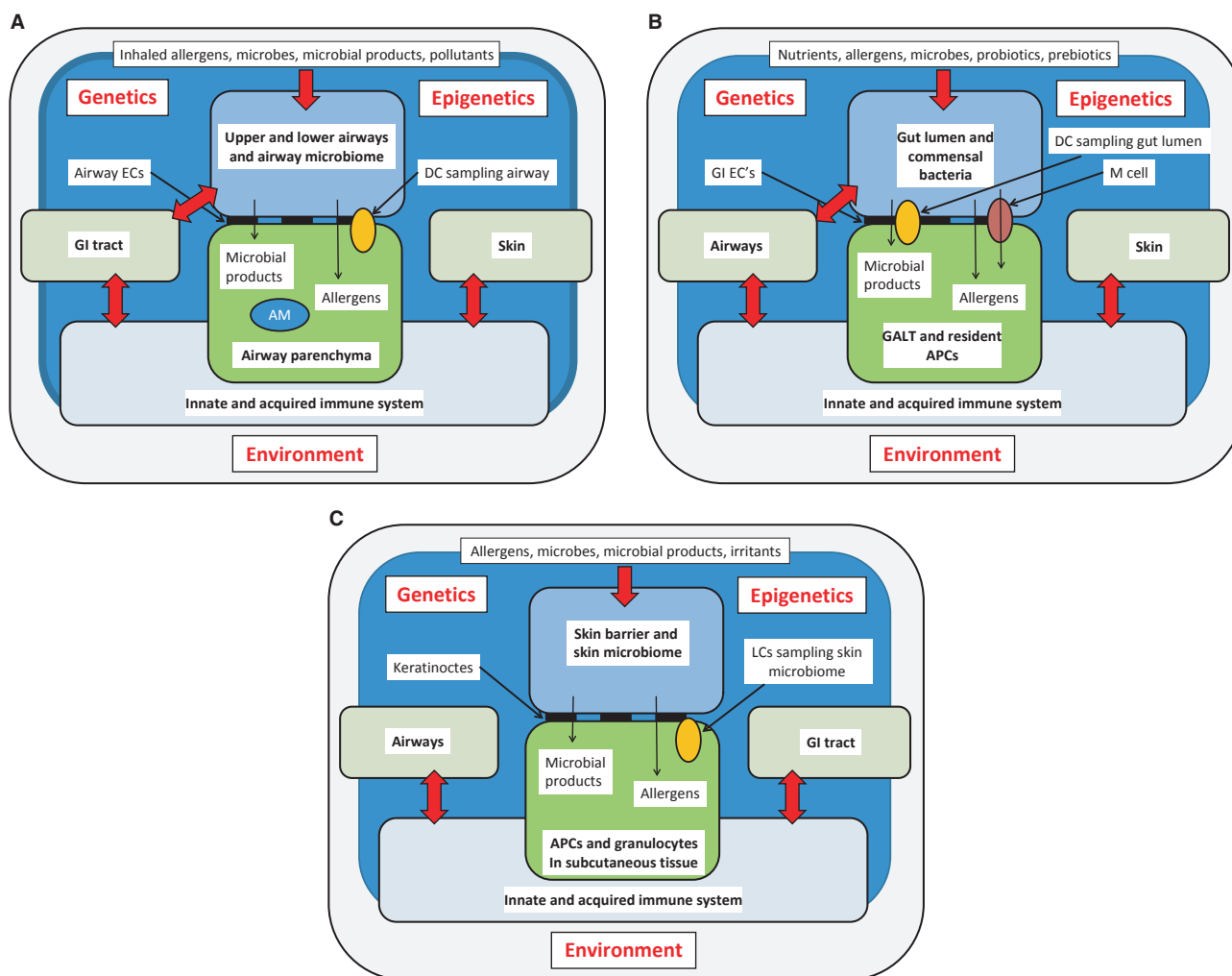


Fig. 1. Interactions of the mucosa, skin, and innate immune system in allergic disease. As an integral part of the (A) airways, (B) GI tract, and (C) skin, the innate immune system is interposed at the interface between allergens in the external environment and the internal acquired immune system. The primary function of the molecules and cells of the innate immune system at these anatomical sites is to maintain homeostasis by recognizing the continual exposure of allergens, microbes, microbial products, nutrients, probiotics, prebiotics, and pollutants. Genetic and epigenetic factors underlie basic differences in regulation of the innate immune system functions in diverse environments and individuals. Specialized cells serving as sentinels in the (A) lung include airway epithelial cells (ECs), dendritic cells (DC), and resident alveolar macrophages (AM). (B) In the GI tract, these cells include M cells, ECs, and DCs, and antigen-presenting cells (APCs) associated with specialized gut-associated lymphoid tissue (GALT). (C) In the skin, keratinocytes and specialized mononuclear phagocytes Langerhan's cells (LCs), DCs, granulocytes, and other APCs serve to maintain the skin barrier. There is a significant amount of cross-talk (large arrows) between the innate immune system and adaptive immune response between these disparate anatomical sites.

Epithelial cells

Non-immune cells can also support the function of immune cells and especially cells of the innate immune system. A single layer of ECs provide the basic cellular components and structure of human mucosal barriers (70–73). ECs express PRRs and receptors for soluble regulatory molecules. Through these receptor-mediated systems, ECs are activated and produce regulatory molecules that regulate the function of MCs, granulocytes, innate lymphocytes, and mononuclear phagocytes (74).

Function of the innate immune system in allergic disease

The innate immune system functions at the interface between the mucosal and skin barriers and the environment to non-specifically eliminate allergens, thereby restricting their ability to gain entry into the host. Innate immune cells located at mucosal barriers also 'sample' allergens via both cell surface-bound and secreted PRRs (Fig. 1). Overall and most importantly, these cells promote a state of immune tolerance that maintains tissue homeostasis. For most individuals, acquired immunity to an allergen also aids in the elimination of the

allergen, especially upon re-exposure, while still maintaining tolerance and homeostasis. For the purpose of this review, we purposefully make little distinction between the terms 'allergen' and 'antigen'. Not all antigens are allergens, and the latter are largely defined here as antigens that trigger the generation of IgE antibodies as part of the acquired immune response. These allergen-specific IgE antibodies go on to mediate many of the processes associated with an allergic reaction. From the perspective of the innate immune system, allergens and non-allergen antigens are recognized, processed, and presented to lymphocytes in very similar ways. Allergens generally have the ability to skew T-cell responses towards a Th2-type phenotype, a property that may be inherent in the allergen itself, or a consequence of an associated PAMP. Although only a subset of all possible antigens possess allergenic properties, the reasons that underlie these differences are not completely understood.

It is important to note that the innate immune system in the airways, skin, and gastrointestinal (GI) tract can 'communicate' across organ systems, and within tissues, it is highly integrated with the acquired immune system (Fig. 1). This integration belies older and more traditional notions about the sequential nature of innate and acquired immune responses, where the innate immune responses drive acquired immune response and then acquired immunity proceeds with little or no further input from the innate immune response.

Respiratory innate immunity and allergic disease

The innate immune system of the lungs has been recently reviewed (75). The primary function of innate immunity in the lungs is to maintain homeostasis and protect the host from respiratory pathogens. This is a daunting task, as the innate system samples over 10 000 l of air a day that contains microbes, microbial products, and a variety of allergens. Many of these inhaled substances are removed from the airways via the

Table 1. Innate immune system pattern recognition receptors (PRRs) in allergic disease

	References
PRRs	
Toll-like receptors (TLR)	9
Nucleotide binding domain/luciferase rich repeat receptors (NLR)	10–13
C-type lectin receptors (CLR)	14–17
Scavenger receptors	18
Adenosine receptors	21
Secreted PRRs	
Complement system proteins	25, 26
Collectins	27–30
Mucins	31, 32
Pentraxins	33–35
Ficolins	36, 37
Resolvins	38–40

mucociliary escalator. By sampling inhaled air, the innate system makes the host's first contact with an allergen (76). Following this initial contact, the innate system decides to quickly eliminate the allergen, initiate an inflammatory response, induce immune tolerance, induce an acquired immune response, or trigger a recall (memory) response if this is a re-exposure situation. Inhaled allergen particles that are >10 µm in size can be deposited in the upper airway, where they come into direct contact with mucus that overlays the continuous EC lining of the airways. This mucus layer contains a variety of secreted PRRs that target allergens for clearance and elimination by upper airway DCs associated with this EC barrier. If allergens escape removal by the mucociliary escalator and recognition by secreted PRRs at this liquid–EC interface, they next come in direct contact with ECs and lung DCs that line the upper airway. Both cells express cell surface-bound PRRs that recognize PAMPs in inhaled air and, in some cases, allergens themselves, triggering their endocytic removal and elimination. Particles that are <5 µm in size may gain access to the lower airways and alveoli. In the lower airways, allergens encounter secreted PRRs, the EC barrier, and lung DCs. In the alveoli, allergens may also encounter resident alveolar macrophages (AMs).

Lung PRRs play a central role in recognition of airway allergens

Secreted PRRs

Secreted PRRs in upper airway liquids primarily recognize microbial PAMPs, although their role in the direct recognition of allergens is only now coming to light. Collectins are a

Table 2. Innate immune cells in allergic disease

	References
Lung	
Dendritic cells	60, 88–90
Resident alveolar macrophages	60, 92–95
Epithelial cells	123–125
Granulocytes	
Mast cells	42, 139–143
Basophils	42, 45, 46
Eosinophils	42, 47, 48
Polymorphonuclear neutrophils	131, 138
NK, NKT, iNKT Cells	65, 69, 135–137, 144, 146
γδT cells	64, 147, 148
GI tract	
Dendritic cells	188–190
Oral cavity dendritic cells	202–206
Epithelial cells	116, 165–169, 188
M cells	182–185
Eosinophils	47
Skin	
Dendritic cells	246–250
Langerhans cells (skin)	104, 243–245
Keratinocytes	218, 221
Inflammatory mononuclear phagocytes	243

family of soluble proteins found in the mucosa and include surfactant proteins A and D (SP-A and SP-D), conglutinin, CL-43, and the mannan-binding lectin (MBL) (30). The collectin molecules contain a CLR domain that binds to carbohydrates and the complex facilitates phagocytic removal by DCs and MOs in the lungs (28). Although collectins can promote pro-inflammatory processes through their ability to mediate recognition of PAMPs by the innate system, some collectins such as SP-D also have anti-inflammatory properties that limit tissue damage (77). MBL activates complement resulting in the lysis and clearance of microbes. In contrast, SP-A and SP-D do not activate complement, although all collectins agglutinate microbes and promote phagocytic clearance.

Complement system proteins

Complement proteins, consisting of the classical and lectin/properdin (alternative) complement pathway proteins, are found in virtually all fluids of the body, including those in the lung (78–80). Activation of the properdin system results in the deposition of C3b complement fragments that facilitates allergen recognition, phagocytosis, and elimination by airway DCs and MOs. The complement system can also be activated by immunoglobulin bound to the surface of an allergen, by immunoglobulin complexes that form by binding molecules derived from allergens, or by MBL bound to the mannose-containing allergen. Innate immune cells within the alveoli bear complement receptors through which they phagocytize and remove complement-opsonized microbes and microbial products (26). DCs produce C1q and C3 complement proteins, which are essential to the full function of the complement system (81). Complement activation triggers pulmonary inflammation and allergen elimination, but complement products can regulate the function of lung DCs, which, in turn, control acquired immune responses. Recent studies show that complement fragments C3a and C5a play important regulatory roles in innate and acquired immune responses in asthma. APC activation via C3a favors Th2-mediated acquired immune response. In contrast, APC activation via C5a induces tolerogenic DC subsets that inhibit the development of Th2-type acquired immune responses (82–85). C3a and C5a levels are increased in the lungs of asthmatics and these complement products activate airway MCs and basophils to release mediators that contribute to airway hyper-responsiveness in asthma (82).

Examples of allergen–PRR interactions

Allergens themselves may be recognized by PRRs expressed on the surface of airway DCs and/or possibly by sub-muco-

sal eosinophils and basophils that, under at least experimental conditions, may be able to serve as APCs for the activation of allergen-specific naive T cells (46–56). Two examples of allergens that are also ligands for PAMPs are the peanut protein Ara h 1 and the house dust mite protein Der p 2. In the former case, Ara h 1 binds the PRR DC-SIGN, where it appears to act as a Th2 adjuvant by programming DC to skew naive T cells towards a Th2-type response (86). In the latter case, Der p 2 functions as a ligand for TLR4, presumably due to its structural homology to the mammalian TLR4 co-receptor MD-2 (87). MD-2 is a lipid-binding protein that is associated with the extracellular domain of TLR4 that also serves as the primary binding site for Gram-negative bacterial lipopolysaccharide (LPS), a glycolipid. The fact that other members of the MD-2-like lipid-binding family are allergens and that many major allergens are also thought to be lipid-binding proteins suggests that intrinsic adjuvant activity by such proteins and their accompanying lipid cargo may have some generality as a mechanism underlying the phenomenon of allergenicity.

Role of lung mononuclear phagocytes immune responses to allergens

Lung DC subsets and allergic disease

In addition to cDCs that reside within sub-mucosal epithelial barriers, pre-DCs, that include both pDCs and blood monocytes, are also associated with the lung parenchyma and blood vessels. Digests of human lung specimens show the presence of three DC populations associated with ECs, two cDC populations that express either blood dendritic cell antigen-1 (BDCA1) or -2 (BDCA2) and a pDC population that is BDCA2⁺ CD123⁺ (88). Throughout the respiratory mucosa and within alveoli, cDCs ‘snorkel’ through the epithelial tight junctions, sending their dendrites into the airway lumen where they come in contact with the external environment (89, 90). These dendrites recognize PAMPs and endocytose allergens in inhaled air (91).

Resident AMs and allergic disease

Within the alveolar lumen, resident AM comprise 80–95% of the alveolar luminal leukocyte population, whereas fewer than 0.1% of luminal cells are DCs (92). Resident AMs play a role in removing and eliminating allergens from the alveolar lumen. Resident AMs that have endocytosed antigens are capable of downregulating DC responses and thus serve to maintain alveolar homeostasis, which is likely to be their primary function (93). Early studies suggested that resident AMs do

not leave the alveoli and migrate to regional lymph nodes, and thus, do not play a significant role as in the activation of naive T cells (94). A more recent study shows that resident AMs are capable of migrating to regional lymph nodes following endocytosis (95). Other studies show that the tight binding of resident AMs to alveolar ECs induces ECs to secrete TGF- β , which subsequently induces resident AMs to produce cytokines that suppress DC function and maintain lung homeostasis, despite the continuous exposure to inhaled foreign substances (96). Through cellular contact between these innate cells in the alveoli, resident AMs regulate alveolar inflammation and lung fibrosis (96). Studies in rodents have shown that resident AMs suppress the APC function of DCs, and the removal of resident AMs is associated with an increase in DC APC function and in acquired immune responses (92, 93, 97). Thus, resident AMs represent a large MO population that, like DCs, also play a significant role as innate immune sentinels based on their ability to recognize, endocytose, and process a variety of inhaled substances including allergens (98, 99).

Activation of lung DCs in response to allergen

Lung DCs are principally 'immature' DCs, which efficiently recognize and endocytose inhaled allergens via PRR recognition of PAMPs. The PRR engagement triggers DC activation leading them to leave the lung and migrate to regional lymph nodes where they differentiate into mature DCs (100). DC maturation results in functional changes that 'polarize' the emigrant DC subset. The term 'polarization' is intended to indicate that the allergen-exposed DC subset acquires the ability to further differentiate into DCs capable of driving Th2-type, T regulatory (Treg), or other (Th1, Th17) acquired immune responses. The ability to leave the epithelial barrier and migrate to regional lymph nodes is mediated in part by the expression of matrix metalloproteinase-9 (MMP9). Mice lacking MMP9 fail to develop allergic airway inflammation (101). As immature DCs undergo their maturation program, they greatly decrease their endocytic ability, increase their processing of allergen-associated antigens, increase MHC expression as complexes with processed antigenic peptides (or as CD1d-associated lipid/glycolipid complexes), increase their expression of T-cell costimulatory molecules, and lose the ability to respond to endogenous signals such as pro-inflammatory cytokines and chemokines (102).

Allergen-activated DCs drive acquired immunity

The further differentiation of activated naive T cells into functional T-cell subsets is driven by the high levels and

patterns of APC-derived cytokines that induce a fully mature T-cell response. These patterns of cytokines then sustain T-cell activation and polarization (100). For example, IL-12, IL-13, and IL-27, type I interferons, and intercellular adhesion molecule engagement drive the polarization of naive T cell towards the Th1 phenotype. In contrast, CCL2, OX40 ligand, and IL-33 promote Th2 polarization, whereas IL-10 and TGF- β promote Treg development (100). For T-cell activation to occur after allergen exposure, the APC must encounter two simultaneous external signals, one signal derived from antigens expressed by the allergen that are processed inside the APC and a second signal derived from allergen-associated PAMPs (103, 104). If the APC endocytoses the allergen in the absence of a signal from a PAMP, the DC leaves the lung and migrates to the lymph node in an immature state. In the immature state, it expresses MHC class I-peptide, MHC class II-peptide, or CD1d-associated lipid/glycolipid surface complexes, but does not express costimulatory molecules or upregulate cytokine gene expression. Naive T cells activated by these immature APCs undergo a round of proliferation followed by apoptosis with the generation of antigen-specific Treg cells that mediate tolerance to allergen re-exposure (104). In contrast, allergen presentation by immature APCs concomitantly exposed to PAMPs induces these APCs to fully differentiate into mature DCs capable of fully activating naive T cells and polarizing them into various T-effector phenotypes (105, 106).

Lung DC subsets play different roles in allergic disease

Airway DCs subsets play a central role in the pathogenesis of allergic airway inflammation and disease (71, 105–108). Clearance of a non-allergenic protein antigen by pDCs suppresses potential cDC-driven T-effector cell differentiation in response to that antigen, whereas proteins recognized as allergens typically trigger cytokine responses that promote Th2-type acquired immunity (109). For example, Th2 skewing of host responses to allergens by granulocyte macrophage colony-stimulating factor (GM-CSF) has been reported using animal models. The administration of GM-CSF, which drives the differentiation of cDCs, enhanced airway inflammation in allergen-sensitized mice (110). In GM-CSF-treated allergen-sensitized mice, the number of airway cDCs increases after allergen challenge. This results in the rapid emigration of bone marrow-derived monocytes from blood vessels to the lung EC barrier in a CCR2-dependent manner, where these cells differentiate into inflammatory DCs (111). In both humans and

mice, allergen challenge is accompanied by recruitment of new bone marrow cells into the inflamed airways and subsequent differentiation of these cells into additional inflammatory cDCs (112, 113). The production of CCR17 and CCL22 by inflammatory cDCs has been shown to attract CCR4⁺ Th2 T cells into the airways and thereby establish Th2-mediated lung pathology (114). The ability of APC-derived IL-33 to drive Th2-mediated lung pathology is most likely the result of selective expression of the IL-33 receptor, a heterodimer consisting of ST-2 and the IL-1 receptor accessory protein, on the surface of Th2 cells (115, 116).

Multiple cell types can activate allergen-specific T cells in the lung

Although the studies cited above emphasize the role of lung DC subsets in the pathogenesis of airway inflammation and allergic disease, there are several important unresolved issues. Fully activated lung DCs are a poor source of the IL-4 needed for the activation and differentiation of naive T cells. This limitation led to a search for other cellular sources of IL-4 that could potentially drive Th2-type responses. Several studies have shown that activated basophils secrete IL-4 and, upon allergen exposure, leave the airway and migrate to regional lymph nodes (49, 50, 52). More recent studies have shown that basophils are not essential to generate Th2-type responses to house dust mite or parasites (57–59). The capacity of eosinophils to serve as APCs that drive Th2-type allergic responses has been reported in animal models (56), but the relevance of this observation in human allergic airway disease remains to be demonstrated. We envisage that all these possible APCs can function as part of an integrated system within the airway. For example, pollen in the airway lumen may directly contact DCs that send dendrites through the EC barrier. The surface of pollen grains contains NADPH oxidases that can generate reactive oxygen species within the lung (117). Pollen-induced reactive oxygen species can drive DC differentiation, which fully activates them to serve as APCs for naive T cells (118). But, pollen can also bypass DCs that are sampling substances in the airway lumen. Pollen enzymes can disrupt EC tight junctions allowing pollen products to pass through the airway lumen–EC barrier interface and gain access to DC subsets, pDCs, and cDCs found in the sub-mucosa (119). Here, pollen products can also be recognized by sub-mucosal eosinophils or basophils (42). All these innate immune cells may be activated by the allergen, in the context of a PAMP, and migrate to regional lymph nodes where they can serve as APCs. As noted above, inhaled allergens can fail to

fully activate APCs in the absence of critical PAMPs. These immature APCs do not fully activate naive T cells, but rather they induce Treg cells. These allergen-specific Treg cells inhibit the innate and acquired immune systems' response to subsequent allergen re-exposures and thereby mediate a state of immune tolerance (inhalation tolerance) to many inhaled allergens (105, 120, 121). Allergen re-exposure stimulates these Treg cells to secrete IL-10 and TGF- β that suppresses the ability of APCs to fully activate naive T cells (120, 122). Inhalation tolerance to allergen is also mediated by pDCs resulting in the suppression of cDCs through the ability of pDCs to upregulate the production of Treg cells (120). Inhalation tolerance can then be broken by the exposure of airway cDCs to an allergen in the presence of a PAMP.

Airway ECs mediate the initial contact with allergen

There is a close relationship between populations of airway APCs and airway ECs, one that serves to maintain homeostasis, moderate innate and adaptive immune responses, and return the lung to its normal steady-state condition (123). Airway ECs express PRRs as well as receptors for cytokines produced during inflammation. Ligation of these receptors signals ECs to upregulate the expression of immunoregulatory proteins, including IL-6, IL-8, TNF- α , GM-CSF, IFN- γ , and chemokines. ECs respond to environmental stress, such as exposure to allergens and pollutants, by producing cytokines and chemokines that alter local APC function (124). Interest has focused on the production of thymic stromal lymphopoietin (TSLP) by respiratory ECs and other cells. TSLP has been shown to polarize APCs to drive Th2-mediated immune responses, although the role of TSLP in asthma and allergic rhinitis has yet to be fully characterized (123, 125). TSLP activates cDC to secrete CCL17 thereby facilitating Th2 cell recruitment into the inflamed airways (126). Human keratinocytes also produce TSLP after stimulation by ligands for TLR2, TLR3, TLR8, and TLR9, by the cytokines IL-4 and IL-13, and the pro-inflammatory cytokines TNF- α , IL-1 α , and IL-1 β (127). TSLP produced by these cells has been shown to be a powerful activator of immature DCs driving their maturation into polarized cDCs fully capable of activating naive T cells for proliferation and polarization towards the Th2 phenotype (128). TSLP-activated DCs also play a key role in the production of the allergen-specific Th2 central memory cells characteristic of chronic allergic airway disease (129). Not unexpectedly, *in situ* hybridization and immunohistochemistry have revealed that the lungs of human asthmatics express increased levels of TSLP compared with the lungs of healthy control subjects (130).

Role of additional innate immune cells in the lung's response to allergens

Polymorphonuclear neutrophils

There is an important emerging role for innate immune responses that precede DC activation. Recent studies have shown that, in the early phase of an immune response immature, DCs directly interact with PMNs (131). Exposure of PMNs to this inflammatory environment increases their lifespan and activates the expression of cytokine and chemokine genes that play a role in hematopoiesis, wound healing and tissue repair (132), and the activation of DCs (131). In turn, cytokine-activated human PMNs produce chemotactic factors, such as CCL19, CCL20, and β -defensins that attract immature DCs and pre-DCs. Human PMNs directly interact with monocyte-derived inflammatory DCs resulting in their upregulation of MHC class II surface molecules, the costimulatory molecules CD40 and CD86, and IL-12 production (133). Clearance of apoptotic PMNs by immature DCs leads to cross-presentation to T cells by DCs of antigens originally captured by the PMNs (134). In addition to PMNs, innate lymphocytes, such as $\gamma\delta$ T and iNKT cells, also mediate inflammatory and allergic responses. Recent studies show that IL-17 produced by $\gamma\delta$ T cells (135) and iNKT cells (136) promotes granulopoiesis and the increases in airway PMN numbers associated with human asthma (137, 138). Together, these studies have begun to define new roles for DC-PMN interactions in the induction of acquired immune responses and supplant older notions that PMNs play little, if any, role in acquired immunity.

Mast cells

Although MCs are well recognized for their role in early-phase responses associated with human allergic airway disease (42) and asthma (139), they are also important mediators of the innate immune system. MC proteases play a major role in mediating the adverse effects of MCs in allergic reactions, but studies show that MC protease-4 may actually counteract these harmful effects and preserve lung function in asthmatics (140–142). There is also evidence to support a role for MCs in antigen presentation. A recent study showed that antigen-bearing MC-derived exosomes are cleared by DCs, which in turn triggers DC antigen processing and induction of the signals needed to activate naive T cells to differentiate into effector T cells (143).

Innate immune lymphocytes

Other types of innate immune cells are also involved in regulating allergic responses and the pathogenesis of allergic

disease. Innate lymphocytes include the NK, NKT (68), and $\gamma\delta$ T cells (64). Type I classical iNKT cells (CD56⁺ CD3⁺) have invariant V α 14-J α 18 TCR α -chains and react to α -GalCer in a CD1d-dependent manner. Type II non-classical NKT cells are CD56⁺ CD3⁺, do not react to α -GalCer, and have diverse TCR α -chains but are also CD1d-dependent. Type III NK cells are CD56⁺ CD3⁻, CD1d-independent, do not respond to α -GalCer, and possess diverse TCR α -chains (68, 144). Some studies show increased numbers of iNKT cells in the lungs of subjects with severe or poorly controlled asthma (69), but this finding has been controversial (68). The role of iNKT cells in asthma pathogenesis remains unresolved. However, activated iNKT cells produce very high levels of a variety of regulatory cytokines (145). iNKT cells also trigger DC maturation (146) and may actually be directly involved in acquired (66) and memory (65) immune responses. In addition, animal models of allergic asthma suggest an important role for $\gamma\delta$ T cells in the production of IgE and IgG₁ antibodies (147, 148), although this finding needs to be fully confirmed in human asthma. Overall, the understanding the roles of innate lymphocytes in human allergic airway disease is an important new area for translational investigation.

GI innate immunity and allergic disease

Derived from a common embryonic lineage with the respiratory system, the GI tract is a single polarized epithelial layer that continually encounters PAMPs as well as beneficial and harmful substances present in the lumen. Like the respiratory tract, the upper and lower human GI tract is in constant contact with the external environment. Continual bacterial colonization is a major feature the GI tract (149, 150), except perhaps in the fetal intestine (151). GI tract colonization is a dynamic process that changes over the life of the host and is highly influenced by ingested foods and microbes (152). In a healthy adult human, it is estimated that the GI tract harbors over 10¹⁴ bacteria. It should not be surprising that the presence of these commensal bacteria can influence the innate and acquired immune response (153–155). The bacteria that colonize the GI tract also display a complexity of strains and regional distributions (156). To maintain homeostasis, the GI tract must also permit the proper absorption of nutrients, support a microenvironment for the growth of commensal bacteria, and respond appropriately to pathogenic agents and inflammatory stimuli. Here, we focus on the roles of innate and acquired immunity in the pathogenesis of IgE-mediated food allergy (157–161).

Food allergy affects about 4% of the US adult population and is a significant health problem in developed countries.

Severe food-allergic reactions are a leading cause for anaphylaxis treatment in emergency departments in the USA (162). Ingestion of food by a healthy individual leads to state of immune tolerance in the GI tract that also prevents a damaging immune response upon subsequent exposures to the food (163). Oral tolerance to food is an active process that relies on a complex series of interactions between components of the innate immune and the acquired immune systems. Conventional GI tract ECs, specialized ECs termed microfold cells or M cells, and GI tract DCs play important roles in the maintenance of oral tolerance to food allergens (164, 165).

Animals raised under germ-free conditions from birth and lack intestinal microflora are likely to develop food intolerance. The underlying mechanism driving this food intolerance is believed to result from inadequate TLR4 stimulation of the GI tract ECs (166). In experimental animals, the dose and frequency of food allergen exposure contributes to the induction of food tolerance (167). In humans, prenatal exposure to some foods in the mother's diet has been shown to result in allergic sensitization demonstrated by the presence of food allergen-specific IgE in the blood of newborns (162), although the impact of such sensitization on the later development of clinical food allergy has not been demonstrated.

Gut ECs mediate the initial contact with allergen

GI tract ECs are believed to play a role in tolerance to food allergens by MHC class I-restricted and CD8⁺ T cell-dependent mechanisms (165). By comparison, MHC class II-restricted antigen presentation occurs on the basolateral sides following antigen transport through the GI tract ECs, where these antigens are presented to T cells in the *lamina propria* (168). For the full activation of naive T cells, the presentation of food allergens by GI tract ECs also requires the concomitant expression of appropriate costimulatory molecules. Thus, it is unclear whether GI tract ECs can serve as professional APCs (168). However, the possibility remains that food allergens presented to naive T cells by GI tract ECs does not fully activate them for the expression of acquired immunity but rather serves to drive immune oral tolerance and maintenance of GI tract homeostasis.

GI tract ECs are also capable of releasing numerous cytokines and chemokines that regulate both innate and acquired immune responses. TSLP is constitutively expressed by GI tract ECs, where it likely functions to promote normal GI tract homeostasis, normal barrier function, and tolerance to commensal bacteria (116, 169). Infection of GI tract ECs with viruses, pathogenic bacteria, and exposure to microbial PAMPs increases EC expression and production of TSLP. Overexpres-

sion of TSLP in the lung appears to affect only the local lung microenvironment, yet skin and gut seem to be different as overexpression of TSLP at these tissue sites appears to have systemic effects (169). The cellular targets of TSLP include DCs, lymphocytes, and granulocytes. TSLP serves to activate DCs for the downstream activation of naive CD4⁺ T cells that, in turn, promote Th2-type allergic responses (170). Using a mouse model of food anaphylaxis, a recent study showed that TSLP was required to develop food allergy but was not required for primary food allergen sensitization, nor was it required for the induction of immune tolerance to the food allergens (171). We emphasize caution in evaluating data from experimental animal models of food allergy that require the use of cholera toxin as an adjuvant, a manipulation that may complicate the interpretation of such findings, and their relevance to human food-allergic responses.

Although GI tract ECs create a formidable physical barrier to the environment of the gut lumen, the polarized ECs lining of the GI tract do not always prevent luminal antigens from entering the tissues. For example, GI tract ECs are constantly being shed at villus tips of the intestine. Food allergens could cross through breaks in the inter-epithelial tight junctions at these locations. GI tract EC tight junction complexes are dynamic and can be modified by physiological, pharmacological, and pathophysiological stimuli, which thereby serve to compromise the barrier (172). Once past the EC barrier, food allergens are likely to directly contact MOs, DCs, MCs, and T cells within the gut *lamina propria*. The development of food allergy in animal models has been correlated with a decrease in GI tract EC barrier integrity (173, 174). This observation suggests that food antigens are able to bypass EC tight junctions and may be presented to T cells by various *lamina propria* APCs, as opposed to presentation by tolerogenic GI tract ECs. Thus, the development of food allergy may be mediated by these *lamina propria* APCs that can fully activate naive CD4⁺ T cells, rather than on antigen-presenting GI tract ECs.

Mammalian GI tract ECs express the low-affinity IgE receptor CD23 (FCεRI) (175–177). A recent study provides evidence that GI tract ECs also express the high-affinity IgE receptor (FCεRII) (178). It is still unclear whether both isoforms of CD23 (CD23a and CD23b) are expressed by GI tract ECs, but it is clear that the endocytosis and transport of IgE–allergen complexes is mediated by a CD23a-dependent pathway (179). IgE–allergen complexes remain intact as they are transported from the luminal surface of the GI tract EC to the basolateral surface, where these complexes can ultimately activate sub-epithelial effector cells (180). These IgE–antigen immune complexes also trigger chemokine release by GI tract

ECs leading to DC recruitment (181). Together, the data suggest that, in addition to their primary function of food and nutrient absorption, GI tract ECs also serve as sentinels for the maintenance of oral tolerance to food allergens and, under certain conditions, may play a role in the induction of IgE-mediated food allergy.

Specialized GI tract ECs: the M cells

The crypts, which overlay secondary lymphoid organs termed Peyer's patches, contain specialized ECs known as M cells. M cells overlaying these regions of the GI tract lack the microvilli that are characteristics of GI tract ECs and also lack the ability to secrete mucins and digestive enzymes. Studies suggest that M cells are responsible for transporting antigens from the GI tract lumen to the Peyer's patch (182). Although a substantial amount of work has been performed using M cells derived from several animal species, human M cells have been less well characterized. It is generally accepted that M cells share a common precursor with GI tract enterocytes (182). M cells also express a diverse set of PRRs including TLRs, NLRs, CLRs, scavenger receptors, and a variety of other receptor molecules that recognize PAMPs and DAMPs. One study demonstrated that M-cell PRRs and their associated signaling molecules are more highly expressed in these cells compared with other GI tract ECs suggesting that M-cell subsets may be more responsive to PAMPs and DAMPs (183). Although originally thought of as being predominantly associated with intestinal crypts and Peyer's patches, M cells have also been found in the intestinal villi. These villous M cells appear to share all the same features of the traditional Peyer's patch-associated M cells, but in mouse models they appear to function independently of these secondary lymphoid organs (184).

M cells are capable of the trans-epithelial molecular transport of macromolecules, antibodies, and intact microbes (185). Although it was originally believed that M cells play a role in antigen processing and basolateral antigen presentation, recent evidence suggests that M cells may not be the key effector cell for antigen presentation and that successful induction of immune responses is dependent upon GI tract CD11c^{high} cDCs (186, 187). Studies in a mouse model shows that GI tract cDCs likely play a principal role as professional APCs; however, when high levels of antigen are used, antigen-specific T-cell proliferation is observed in DC-depleted mice suggesting that M cells may also serve as APCs under some conditions (186). We envisage that, in the human GI tract, ECs, M cells, and DCs work in concert to maintain immune oral tolerance to food allergens and maintain gut homeostasis.

Role of GI tract phagocytic cells recognition and immune responses

GI tract DCs

Studies show that GI tract DCs are needed to maintain the balance between immune tolerance and immune responses that defines gut homeostasis (188, 189). As observed in the lung, different subsets of DCs are also found associated within the GI tract *lamina propria*, Peyer's patches, lymphoid follicles, and the draining lymph nodes. This suggests that distinct functional responses are mediated by different subsets DCs in each micro-environment (190). Direct access of DCs to the food antigens is normally prevented by EC tight junctions, although transport of luminal IgA-antigen complexes can stimulate *lamina propria* DCs to secrete IL-10 and TGF- β , and polarize naive T cells to become Treg cells (191). In the mouse, the majority of cDCs found in the *lamina propria* are CD11b⁺, CD8 α ⁻, and MHC II^{int/hi} (192). Human DCs do not express CD8 α , and the human counterpart to this cell has only recently been described. Thus, its role in the GI tract is presently unknown (193).

It should be noted that the human GI tract EC barrier is awash in LPS. TLR4-dependent activation of *lamina propria* DCs by gut lumen-derived LPS upregulates the expression of MHC II and CD80, CD86, and CD40 costimulatory molecules (192). Whether gut lumen-derived LPS acts as aTh2-skewing adjuvant for food allergens has not yet been proven. *Lamina propria* DCs extend dendrites through the GI tract EC barrier into the lumen of the gut without disrupting EC barrier integrity. These dendrites continuously sample material in the gut primarily by recognition of PAMPs (194, 195). Extension of these dendrites through the EC barrier is dependent upon a direct interaction with ECs and the expression of the chemokine receptor CX3CR1 on the DCs (196). However, these CX3CR1⁺ DCs may not be the cells that ultimately deliver processed food antigens to naive T cells in regional lymphoid tissues. Although CXCR1⁺ DCs are able to capture luminal antigens, they are less effective at stimulating T-cell responses compared with CXCR1⁻ DCs. Also, CXCR1⁺ DCs do not appear to migrate to lymph organs (197). In comparison, CX3CR1⁻ DCs migrate to secondary lymph organs and appear to be required to affect Treg development from naive T cells (197, 198). This would suggest some form of communication among the different DC subsets as part of the processes of antigen capture and oral tolerance induction (199). A recent study shows that β -catenin signaling in *lamina propria* DCs mediates the expression of anti-inflammatory retinoic acid-metabolizing enzymes, IL-10 and TGF- β , the induction of Tregs, and suppression of T effector cells thereby driving

lamina propria DCs into a tolerogenic state that limits GI inflammation (200).

GI tract eosinophils and MCs

In addition to GI tract ECs, M cells, and DCs, GI tract eosinophils and MCs may play a role in connecting the innate immune response to the acquired immune response. Recent studies show the capacity of eosinophils to potentially serve as professional APCs (53–56). Intact eosinophil granules with functioning membrane receptors are found in mouse tissues, even when viable eosinophils are not present. These granules release preformed cytokines and cytotoxic proteins that could reduce EC barrier integrity (201). It remains to be determined if there is a correlation between GI tract eosinophils and allergic sensitization to foods or the development of food allergy.

The unique oral mucosal environment

As part of the GI tract, anatomy of the oral mucosal epithelium is more similar to the skin rather than the remainder of the GI tract (202, 203). In contrast to the lower GI tract, the oral epithelium consists of keratinized ECs that are tightly attached to each other via desmosomes and arranged in distinct layers: the epithelium, the lamina propria, and the sub-mucosa (202). Until recently, the oral mucosa was thought to be immunologically inert, but attempts at sublingual and oral immunotherapy have challenged this earlier notion (202, 204). A number of immune cell types including T-effector cells, Tregs, DCs, and CD14⁺ Langerhans cells (LCs) are found in the oral mucosa (202, 205, 206). Furthermore, a recent report has identified a previously uncharacterized LC subset in the oral cavity of mice (207). Although these LCs appear to have a novel regulatory role that potentiates the CD8⁺ T-cell responses, it remains to be confirmed whether these cells are present in the oral cavity of humans. Despite these early attempts to understand the immunology of the oral cavity, there is a significant gap in our knowledge and understanding about the oral mucosal immune system. Additional research in this area is needed to aid in the design of effective sublingual and oral immunotherapies.

Skin innate immunity and allergic disease

Like the EC barrier in the GI tract and the respiratory tract, the skin serves as a protective physical barrier to the external environment. In contrast to the lower GI tract and respiratory tracts, the skin is covered by a thick layer of stratified epithelium, the epidermis, composed predominantly of keratino-

cytes. The development, maintenance, physiology, and barrier function of normal epidermis has been reviewed (208, 209). LCs are interspersed throughout the epidermis where they mediate immune surveillance of substances in the external environment that come in contact with the skin. Below the dermis is a vascularized layer that contains numerous cell types including fibroblasts, lymphocytes, MOs, DCs, and other innate immune cells. One important function of these innate immune skin cells may be to maintain the balance between the host and the skin microbiome. In a recent study, normal human skin was shown to serve as the permanent home for a variety of microbial species. Grice et al. (210) examined 20 skin sites characteristically associated with dermatological disorders and found them to be colonized by distinct populations of commensal organisms. Perhaps like commensal microbes found in the GI tract, these microbes may serve a beneficial role and prevent pathogenic microbes from occupying these unique microenvironments (211).

Atopic dermatitis (AD) is characterized by erythematous skin lesions, and lichenification in flexural areas of the body with intense pruritus and cutaneous hypersensitivity (212). AD begins in childhood and is considered to be mediated by allergen-specific IgE (213). Examination of lesional skin from AD subjects has demonstrated increased numbers of eosinophils, activated memory T cells, LCs, and MOs (212). AD is widely believed to represent a Th2-type disease characterized by overexpression of cytokines such as IL-4 and IL-13. AD lesions are also characterized by a decrease in innate immune cell signaling, decreased production of antimicrobial peptides, and deficiencies in barrier proteins (214). As compared with normal control subjects, AD subjects are observed to have more frequent viral and bacterial infections with increased colonization with *Staphylococcus aureus* (215). Non-lesional skin from AD patients has been reported also to be affected as demonstrated by a greater sensitivity to irritants and increased dryness and the presence of sparse perivascular T-cell infiltrates (212).

Disruption to the skin barrier and its contribution to AD

Genetic causes

The physical barrier function of the skin is maintained by the maturation, death, and desquamation of keratinocytes and by several major structural proteins that mediate this continuous process (216). Dysregulation of this maturation process has identified an important role for the protein filaggrin. Filaggrin aggregates keratin filaments into keratin fibrils that are then

proteolytically degraded into polycarboxylic acids within the decaying keratinocytes where they act as osmolytes to draw water into the cell (217, 218). First reported in 2006, loss-of-function mutations in the *filaggrin* gene were shown to be strongly associated with AD (219). It has also been shown *in vitro* that the Th2 cytokines IL-4 and IL-13 can decrease *filaggrin* expression in both normal and AD skin samples (220). By affecting a loss in barrier integrity, it was hypothesized that allergens would better able to penetrate the skin and be phagocytized by LCs, processed and presented to naive CD4⁺ T cells for the expression of acquired immunity in the skin (221). Although clearly relevant to AD, *filaggrin* is not the only keratinocyte-derived protein that can affect the formation of a functional skin barrier. A recent report has identified the tight junction protein claudin-1 as a potential molecule contributing to barrier dysfunction (222). Nevertheless, defects in barrier function cannot be the sole cause of AD as several keratin-associated defects have been found to not be associated with AD (221).

A role for endogenous lipids in barrier integrity

The role of lipids in AD pathogenesis has drawn attention as lipids, especially ceramides, are needed for the proper hydration and integrity of the epidermis (223). During the process of desquamation, keratinocytes and lamellar bodies are extruded from the cells and form an extracellular matrix helping to prevent water loss and penetration of the skin barrier by environmental insults (224). Arachidonic acid metabolites such as leukotrienes and prostanoids are well-documented lipid mediators associated with inflammatory disorders (225, 226). Produced by a variety of cells including MCs, eosinophils, and MOs, leukotrienes and prostanoids can also be released by protease-activated keratinocytes (227). Due to the limited specificity of pharmaceutical pharmacological inhibitors, conflicting results have been reported for the role of leukotrienes in the pathophysiology of AD (228). Although studies in mice have implicated prostanoids in the pathogenesis of AD, the clinical relevance of these findings remains to be determined (225, 229). Recently, a family of lipid receptors for phosphatidylserine [the T-cell membrane, immunoglobulin, and mucin receptors (TIMs)] has been implicated in modulating innate and acquired immunity (230). How these receptors might function in AD remains to be determined. It should be noted that not all lipid mediators are pro-inflammatory, and multiple anti-inflammatory lipids have been identified that may play a role in limiting the severity of AD (231). A better understanding of

the role of skin-derived lipids in mediating communication between keratinocytes skin-associated immune cells is needed.

The contribution of vitamin D to AD

The epidermis is an important source of vitamin D. UV light triggers the cleavage of 7-dihydrocholesterol into previtamin D, which is rapidly converted to vitamin D₃ through spontaneous isomerization (232). Vitamin D₃ receptors are found on cells of the innate and acquired immune system (233, 234). Several studies have shown that decreased levels of serum vitamin D₃ correlates with the development and pathogenesis of AD and food allergy (235, 236). Furthermore, human IgE synthesis is downregulated by vitamin D₃ (237).

Following stimulation with IL-4, keratinocytes express cell-surface CD23, the low-affinity IgE receptor, which can be released upon ligation with IgE as soluble CD23 (sCD23) (238, 239). The presence of sCD23 is associated with downregulation of IgE synthesis, and as such, keratinocytes may suppress IgE synthesis via their production of sCD23 (240). Keratinocytes also express innate immune PRRs, including TLRs, NLRs, and CD14. Engagement of these PRRs results in the release of antimicrobial peptides, TSLP, and other cytokines (214), and topical application of vitamin D₃ has been shown to stimulate antimicrobial peptide synthesis and TSLP production (241, 242).

Roles for skin DC subsets in the pathology of AD

There are three subsets of human skin DCs, LCs, dermal cDCs, and pDCs (243). Langerhans cells are a unique subset of DCs and are defined by their expression of the C-type lectin receptor CD207 (langerin). In the epidermis, LCs are in intimate contact with keratinocytes. Upon exposure to and uptake of antigenic substances, these cells leave the skin and emigrate to regional lymph nodes where they activate naïve CD4⁺ T cells. Based on this almost continuous process of antigen removal, LCs are believed to induce and maintain tolerance to cutaneous antigens (104). Significant debate remains about the functional role of LCs in humans. Although many early studies in mouse models were used to define the mechanistic basis for immune tolerance mediated by LCs, more recent studies have identified additional Langerhans-like DC subsets in mice that do not have human counterparts, making comparison of mouse models with human AD problematic (244, 245).

In AD subjects, the number of circulating pDCs is elevated compared with levels in normal control subjects (246). Plasmacytoid DC numbers in the lesional skin of AD subjects are

reduced compared with other skin disorders such as psoriasis or contact dermatitis (247). This decrease in AD patient skin pDCs has been proposed as a contributing factor for the increased susceptibility to viral infections observed in AD subjects (247). The relative lack of pDCs could result in less local production of type I interferons, antiviral cytokine expressed in large quantities by activated pDCs, resulting in increased virus survival and replication. It has been reported that CD207 is a potential viral receptor for human immunodeficiency virus-1 (248). If CD207 could serve as a receptor for other viral pathogens, the increase in CD207 expression on LCs could provide another possible mechanism for increased viral susceptibility. Considerable phenotypic and functional differences have been reported for LCs and skin pDCs between humans and mice, and thus, extrapolations of animal model data to human models should be viewed with caution (249).

Blood monocytes emigrate into the inflamed skin of AD subjects where they differentiate into inflammatory DCs that are characteristically found in increased numbers in the dermal layer of the skin in AD subjects (250). It appears that the functional response of these inflammatory DCs is dependent upon the type of disease, for example AD versus psoriasis. Skin-associated cDCs in AD subjects have been shown to produce a different array of cytokines and chemokines as compared to cDCs associated with psoriasis (251).

Studies of skin biopsies obtained from AD subjects have demonstrated the presence of cDCs, pDCs, and LCs, all of which express the high-affinity surface receptor for IgE (FcεRI). Engagement of FcεRI on LCs promotes the release of the chemokines CCL2, CCL17, and CCL22 that may enhance allergen presentation to T cells (252). By comparison, stimulation of FcεRI on cDCs results in the production of IL-12 and IL-18 (253). For some investigators, these disparate results help explain the apparent change in the immune profile in the lesions of AD subjects reported during their acute (Th2-type) and chronic (Th1-type) episodes (254). However, this switch from Th2- to Th1-type phenotypes has not been reported by all investigators (255).

Knowledge gaps and future considerations

Although much research over the past few decades has focused on how immune responses cause clinical features of allergic disease, we believe that factors such as environmental exposures, gene–environment interactions, microbiomes of the airway, skin, and GI tracts, the hygiene hypothesis, and immune homeostasis represent new research areas that could

have a significant impact on the primary prevention of allergic diseases and on the development of therapeutic strategies to impede the progression of allergic sensitization to clinical disease.

Host–environment interaction in allergic disease

Several studies have demonstrated that environmental exposures, especially during prenatal life, can influence an individual's susceptibility to asthma and other allergic diseases (256–259). Although a relationship between the environment and allergic diseases has long been recognized, one early attempt to explain this relationship was the hygiene hypothesis. This hypothesis posits that a relatively germ-free lifestyle alters immune system homeostasis in a way that favors the development of allergic disease. This idea is supported by studies showing an association between births by cesarean section or frequent antibiotic use increases the risk for developing asthma in childhood. A variety of environmental factors can increase (cigarette smoke) or decrease (farm living, pet ownership) the risk of developing an allergic disease. We envisage that some of these environmental factors may lead to epigenetic modifications and provide a likely link between the environment and genome. How strong of a causal link exists between environmental exposures and allergic disease remains to be determined.

As originally conceived, the hygiene hypothesis does not fully explain the decades-long increase in allergic diseases observed in developed countries nor the apparent protection against asthma provided by farm living and pet ownership. The hygiene hypothesis predicts that the incidence of immune-based diseases mediated by Th1 responses, as observed in psoriasis, should concomitantly decrease as the incidence of Th2-mediated diseases such as asthma, food allergy, and AD increase in developed countries. Epidemiological studies have shown that this is not the case (260). A more refined hypothesis should take into account not simply whether an individual is routinely exposed to microbes but also the types of microbes to which an individual is exposed. Timing and chronicity of exposure to microbes are likely to be important factors. Exposure to live microbes versus microbial products from dead organisms can also drive different outcomes. This latter concept is important given that measurements of LPS are often used as a surrogate marker for microbial exposure but these measurements do not distinguish between exposures to live versus dead bacteria or exposure to other bacterial products such as peptidoglycan or bacterial DNA.

The host microbiome and allergic disease

New findings about commensal bacteria and the impact of the human diet on bacterial metabolism have shown that bacterial metabolites, in particular short-chain fatty acids (SCFAs), can have a significant effect on the development of allergic and inflammatory diseases (261). This connection between diet and microbial ecology in the GI tract provides a new perspective on the hygiene hypothesis. Diet has a significant impact of the spectrum of microbes that colonize the GI tract. Certain GI tract bacteria and their metabolites are required for the regulation of immune responses. Perturbations in the microbial ecosystem could result in a lack of immunoregulation, the outgrowth of more pathogenic bacteria, and the promotion of inflammatory diseases. This concept is illustrated by the phenotype of the MyD88-deficient non-obese diabetic (NOD) mouse (262). MyD88 is an adapter protein that is necessary for intracellular signaling by most TLR proteins. MyD88-deficient NOD mice are protected from the development of type-1 diabetes, but surprisingly, this protection is lost when the mice are housed under germ-free conditions and lack gut microbes. The absence of MyD88 leads to an over-representation of *Bacteroidetes* bacteria, which are major producers of SCFA, suggesting that *Bacteroidetes*-derived products/metabolites possess immunomodulatory activity that suppresses the development of type-1 diabetes. Another example of the link between the microbiome and inflammatory diseases comes from studies showing that gut microbial ecology is altered in people with asthma and allergies compared with non-atopic controls (263). Infants in whom atopy did and did not develop had significant differences in their GI tract microbiomes, and these differences preceded the development of atopy. Taken together, these findings underscore the importance of the microbiome and how the human diet can impact GI tract microbial ecology to promote the development of inflammatory and atopic diseases. Future studies are needed to determine how prospective changes in the human diet and its effect on the GI tract microbiome can impact on the prevention and/or treatment of allergic diseases.

Exposure to microbes occurs via two major pathways, transient exposure through inhalation, skin contact, or ingestion, and constant microbial carriage in the form of the host microbiome. In the former case, infection with respiratory pathogens in infancy has been linked to the later development of asthma (149, 150). In the latter case, the GI tract and skin support permanent colonization with distinct microbiota. Recent studies have challenged the long-standing concept that the lung is a sterile microenvironment by demonstrating the

lung is continually colonized, and it is noteworthy that the variety of colonizing microbes in the lower airways differs in healthy people and asthmatics (264, 265). It remains to be seen whether an imbalance in the host's microbiomes, compared with some homeostatic norm, is a cause or a consequence of immune dysregulation that is associated with allergic disease.

A healthy skin barrier prevents the unwanted entry of microbes, microbial products, and allergens into the body. Defects in the this barrier can expose the immune system to these substances and lead to allergic sensitization, a risk factor for the subsequent development of allergic disease. The skin possesses its own microbiome that varies across different parts of the body and as a consequence of disease (210). There is a strong association between AD and over-colonization of the skin by *S. aureus*. Whether this change in the skin microbiome is a cause or consequence of AD remains to be determined. It would not be surprising if skin bacteria are the source of PAMPs that gain entry through a defective skin barrier and skew the immune response towards a Th2-type and pro-allergenic phenotype. Such a phenotype is probably the result of specific innate immune responses mediated initially by skin LCs and possibly by other APCs in the epidermis and dermis that program naive T cells to differentiate into Th2-type or Th17 cells. Interestingly, skin barrier defects can be selective. Not all defects in the skin barrier lead to an increased risk of allergic disease and even children with genetic polymorphisms in the flaggrin gene can still outgrow their AD in spite of this permanent skin barrier defect. Some diseases associated with a defective skin barrier are characterized by either a Th1-type immune response (as observed in psoriasis) or by a Th2-type immune response (as observed in AD). The mechanisms that control this polarization of the immune response in each case remain unclear.

Genetics of the host–environment–microbiome interaction

Underlying the impact on environmental exposures and composition of the host microbiomes on the development and severity of allergic diseases is the contribution of genetic variability. It is not surprising that gene polymorphisms that affect host defense, PRR function, cytokine and cytokine receptors, and barrier function can impact on an individual's risk of developing an allergic disease. Many gene polymorphisms have been associated with these diseases (266), although the impact of individual gene polymorphisms on disease risk and severity is generally modest. Nevertheless, such associations are useful in identifying components of signaling and regulatory pathways that collectively determine the overall disease risk and severity.

Therapeutic strategies focused on the innate immune system

An in-depth understanding of the role of innate immunity in allergic diseases is absolutely necessary for the development of new immunotherapeutics. The success of immunotherapy, defined as the induction of long-lasting immune tolerance to one or more allergens, depends on several factors. These include the therapeutic itself (extracts, purified proteins, recombinant proteins, peptides), dose (for both up-dosing and maintenance dosing), route of administration (subcutaneous, sublingual, oral, epicutaneous), duration of therapy, and the presence/absence of PAMPs and/or adjuvants. Nearly, all these factors depend on some component of the innate immune system, whether it is the PRR that encounters allergenic substance (or purified allergen plus adjuvant), the identity and function of the APC that triggers differentiation the naive T cell, the nature of the allergen-specific IgE-bearing cell that is responsible for early-phase adverse systemic reactions to the immunotherapeutic, or the anatomical nature of the site at which the immunotherapeutic is delivered. Learning how to control innate immune responses to trigger the various possible tolerogenic mechanisms (T effector cell anergy or deletion, Treg-mediated suppression of T-effector responses, or Th skewing to restore a homeostatic Th1/Th2 balance) remains a paramount goal.

One could argue that the best possible therapeutic approach would be to prospectively block the development of allergic sensitization and disease. Both allergic sensitization (priming, as measured by the presence of allergen-specific IgE antibodies) and the atopic march begin before or shortly after birth. Early environmental exposure to allergens, microbes, microbial products, and pollutants, genetic polymorphisms, and epigenetic changes all contribute to the overall risk of developing an allergic disease. The relative contribution of each of these factors to overall risk is not known. These factors are likely to vary widely among diverse populations in different parts of the world. One could also speculate that there could be multiple distinct mechanisms that maintain immune homeostasis and protection from the development of allergic diseases in these diverse populations given different exposures, genetic backgrounds, diets, and microbiota.

Homeostasis is an ongoing process that resists allergic disease

Recent studies have begun to reveal the complex roles that host mechanisms play in maintaining homeostasis and protecting against allergic sensitization. Multiple families of

host proteins, including the resolvins (39, 40, 267), pentraxins (35), ficolins (37), and collectins (28, 30), recognize large groups of microbial molecules resulting in their elimination from the host while also downregulating inflammatory responses, thereby helping to maintain homeostasis. Autophagy regulates the removal of self-antigens by signaling mechanisms that maintain self-tolerance but autophagy also plays a role in the processing of ingested allergens into pathways that trigger MHC class I-restricted acquired immune responses (268). Apoptosis results in the daily clearance of large numbers of dying host cells and triggers anti-inflammatory response mediated by the production of TGF- β , IL-10, and PGE₂ (269–271). However, clearance of apoptotic cells may result in cross-presentation of allergens, leading to the generation of allergen-specific lymphocytes.

Immune homeostasis in the airway, skin, and GI tract is mediated by a very active and continual regulatory mechanism that operates in the face of continual exposure to allergens at these anatomical sites. There is a key gap in studies linking the initial interaction of allergen with the innate immune system to downstream readouts, such as IgE production. This opinion is based on the observation that serum levels of IgE does not necessarily correspond to the ability of an allergen to elicit a severe reaction upon allergen re-exposure (162). Many current studies focus only on the acquired immune response as surrogate readout when in fact an IgE-mediated immune response to an allergen actually represents a failure of the homeostatic response. Future studies should begin to define the roles of these homeostatic host processes and their ability to operate in the face of continual exposure to allergens to fully define pathways susceptible to immunotherapeutic intervention. Therapies that target downstream pathways far removed from these important initial steps may ultimately be far less efficacious than therapies that attempt to restore immune homeostasis.

Future needs

Lastly, there are specific areas where more research is needed to better understand the roles of innate immunity in the pathogenesis of allergic diseases, to aid in the development of improved immunotherapeutics, and to facilitate the development of primary prevention strategies: use single-cell technologies to isolate and interrogate individual immune cells; identification of additional immune cell subsets, and develop a better understanding of their 'plasticity' (their ability to change their phenotype in response to specific signals); development of non-invasive or minimally invasive, methods to interrogate cells and tissues especially when these cells and

tissues need to be obtained from infants and children; use of systems biology approaches to reveal how regulatory and effector mechanisms interact in a complex network influenced by genotype, the microbiome, and environmental exposures; determination of how the anatomy of microenvironments affects allergic disease pathogenesis and, conversely, the efficacy of immunotherapeutics; study of the interaction between classical immune cells and 'non-immune' cells that regulate immune responses in the airway, GI tract, and skin; use of functional studies to take advantage of a large existing body of genome-wide association studies to identify key mechanisms

that underlie the mechanisms of allergic disease pathogenesis. In conclusion, the innate immune system plays an important role in the protection from allergic diseases, and when homeostatic norms are disrupted, the development of such diseases. A great deal of progress has been made in recent years to identify the cells, genes, and molecules involved in both allergic pathogenesis and immune homeostasis. Future studies are needed to better understand the complex connections between the immune system and environmental exposures, genetic polymorphisms, human diet, as well as the microbiomes of the airway, skin, and GI tract.

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