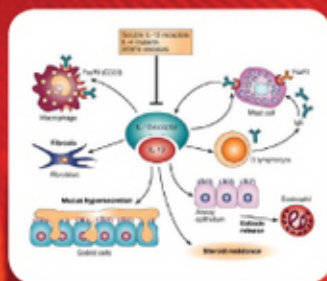


Inflammation and Allergy Drug Design

Edited by **Kenji Izuhara,**
Stephen Holgate, Marsha Wills-Karp



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Preface

Our knowledge and understanding of allergic diseases of the respiratory tract, such as asthma and rhinoconjunctivitis, has improved to a point where new therapies are being developed for patient benefit. Part of the problem in developing therapies has been our rather simplistic view of the allergic cascade and its components, as well as the over-reliance of the pharmaceutical and biotechnology industries on simple animal models of antigen sensitization and subsequent challenge to screen for active compounds. Asthma is a chronic relapsing disorder that varies its natural history over the life course and in which many environmental factors play a role beyond allergen exposure. This does not mean that enormous progress is not being made in these diseases, but that our ability to model the differing manifestations of asthma and associated disorders is limited. In addition, new technologies have led to the discovery of new targets in the airways that help link immunologic and inflammatory features to those of altered airway structure and function. Even within these immunologic mechanisms, we are gaining a wider appreciation of the role of the innate as well as the adaptive response and the importance of the formed airway elements such as epithelial cells, smooth muscle, nerves, and blood vessels in contributing to and supporting ongoing inflammation and tissue injury.

To give greater consideration to these issues in *Inflammation and Allergy Drug Design*, we have asked world leaders in their respective fields to provide us with the most up-to-date knowledge of

the biologic science that underpins the pathophysiology of asthma and related disorders. We have divided the book into three parts covering the cells involved, their cytokines, chemokines, and growth factors, and mediators. What is so different about this book is the authors' skills in embedding their field of interest in the concept of asthma being a chronic relapsing condition. While each chapter develops in some detail the cutting-edge developments in a particular field, it is also clear that important interconnections between the fields are providing a new framework for reviewing asthma, especially the interactions between environmental exposures and the subsequent development of different subphenotypes (stratified medicine).

We believe the book is state-of-the art in terms of providing the reader with a superb reference source and a global perspective of each field, which should be helpful for clinical and basic scientists interested in the mechanisms of these disorders, irrespective of their academic, clinical, or industrial affiliations. The three editors are indebted to the way the authors have responded to requests for chapters in their fields, covering the most exciting recent developments, and as a result the value of the book as a whole has greatly increased. The editors would also value readers' views on this publication and interactions that may flow from it.

*Kenji Izuhara, Stephen T. Holgate,
and Marsha Wills-Karp
April 2011*

Part I
**Cells contributing to the
pathogenesis of allergic diseases
in the respiratory tract**

Novel anti-inflammatory drugs based on targeting lung dendritic cells and airway epithelial cells

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General function of dendritic cells in the immune system: induction of immunity

Dendritic cells (DCs) were originally defined by their capacity both to efficiently process and present antigens and to prime naïve T cells [1]. Immature DCs are situated in the periphery at sites of antigen exposure. In the periphery, DCs are specialized in antigen recognition and uptake. Under homeostatic conditions and particularly upon recognition of pathogens, DCs migrate to the T-cell area of draining nodes, where they screen the repertoire of naïve T cells for antigen-specific T cells that can be directed against the pathogen. Upon cognate T-cell receptor (TCR)–major histocompatibility complex (MHC)–peptide interaction, DCs subsequently form more stable interactions, and optimally induce T-cell effector function by providing co-stimulatory molecules and T-cell stimulatory and survival cytokines. In homeostatic conditions, only harmless antigens or self antigens are presented to T cells. Owing to their lack of complete induction of co-stimulatory molecules and cytokines in DCs, these antigens induce only abortive T-cell proliferation and/or lead to a T-cell response in which regulatory T cells (Tregs) are induced. This system allows for dangerous antigens to be eliminated, while avoiding overt immune-mediated damage in response to harmless environmental antigens and self antigens.

The increasing complexity of lung dendritic cell subsets

It is now clear that at least five different subsets of DCs can be found in the lungs (Figure 1.1). These subsets vary in origin, anatomical location, expression of cell surface markers and endocytic receptors, responsiveness to chemokines, and migratory behavior. Most importantly, there is division of labor between these various lung DC subsets, which makes a closer distinction between subsets almost imperative if one is to understand the biology of lung DCs [2]. The mouse lung is grossly divided into large conducting airways and lung interstitium, which contains alveolar septa and capillaries where gas exchange takes place [3]. The conducting airways of all species studied are lined with an intraepithelial, highly dendritic network of MHCII^{high} CD11c^{hi} cells that are mostly CD11b[−] and, at least in the mouse and rat, express langerin and the mucosal integrin CD103 ($\alpha_E\beta_7$), and have the propensity to extend dendrites into the airway lumen by forming tight junctions with bronchial epithelial cells [4]. Immediately below the epithelium, the lamina propria of the conducting airways contains MHCII^{high} CD11c^{high} cells that are mostly CD11b and are a rich source of proinflammatory chemokines [5]. A similar broad division into CD11b⁺ and CD11b[−] can also be applied to lung interstitial DCs [6,7]. As both CD11b⁺ and CD11b[−] subsets express high levels of CD11c, they are best

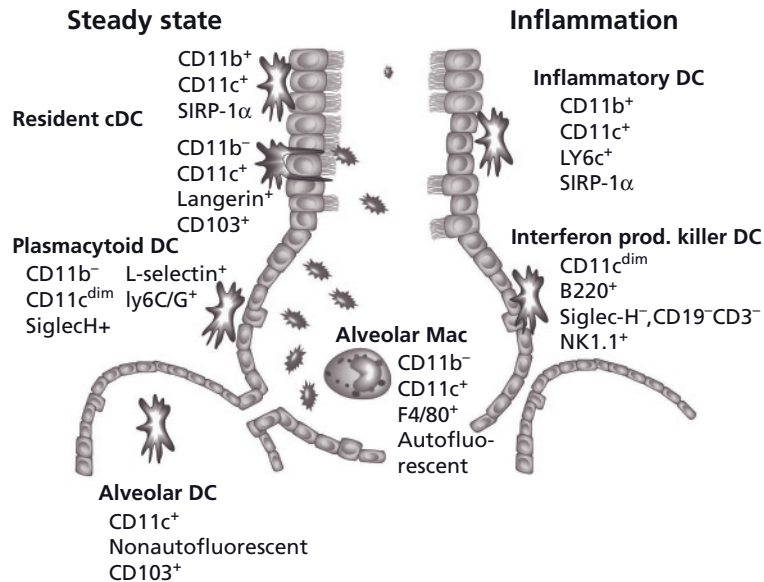


Figure 1.1 Lung dendritic cell (DC) subsets. In steady-state conditions (depicted on the left) conventional DCs (subdivided into CD11b⁺ and CD11b⁻ subsets) line the conducting airways. They can also be found back in the deeper interstitial compartments, obtained by enzymatic digestion of peripheral lung. Plasmacytoid DCs (pDCs) are also found in both compartments with a slight preference for the interstitial compartment. Finally, the alveolar space contains DCs that can be easily confused with alveolar macrophages if one does not take autofluorescence of

the latter into account. Under inflammatory conditions, there is recruitment of CD11b⁺ monocytes to the lungs and these rapidly become DCs. They can still express Ly6C as part of their monocytic descent. In viral infection as well as in some cancers there is also recruitment of interferon-producing killer DCs, a subset of natural killer (NK) cells that can be mistaken for pDCs in view of their intermediate expression of CD11c and expression of the B-cell marker B220. One way of discriminating these is via staining for NK1.1. cDC, conventional DC.

described as conventional DCs (cDCs) in order to differentiate them from another population of CD11c^{int} plasmacytoid DCs (pDCs) that express Siglec-H, the bone marrow stromal antigen 1, and the B-cell marker B220. Under inflammatory conditions, such as viral infection, allergen challenge, or lipopolysaccharide (LPS) administration, there is recruitment of additional subsets of CD11b⁺ monocyte-derived DCs that rapidly upregulate CD11c and retain expression of Ly6C as a remnant of their monocytic descent, and are easily confused with resident CD11b⁺ cDCs [7].

Function of lung dendritic cells: induction of tolerance in steady state and bridging innate and adaptive immunity

Airway DCs form a dense network in the lung that is ideally placed for sampling inhaled antigens by

forming tight junctions with airway epithelial cells and extending their dendrites into the airway lumen, analogous to the situation in the gut. Following antigen uptake across the airway epithelial barrier, DCs migrate to draining mediastinal lymph nodes (LNs) in order to stimulate naïve T cells [8,9]. As most allergens are immunologically inert proteins, the usual outcome of their inhalation is tolerance and thus inflammation does not develop upon chronic exposure [10]. This is best displayed in the model antigen ovalbumin (OVA). When given to the airways of naïve mice, it induces tolerance to a subsequent immunization with OVA in adjuvant, and effectively inhibits the development of airway inflammation—a feature of true immunologic tolerance [10]. It was therefore long enigmatic how sensitization to natural allergens occurred. An important discovery was the fact that most clinically impor-

tant allergens, such as the major house dust mite (HDM) allergen Der p 1, are proteolytic enzymes that can directly activate DCs or epithelial cells to break the process of tolerance and promote Th2 responses [11,12]. However, other allergens such as the experimental allergen OVA do not have any intrinsic activating properties. For these antigens, contaminating molecules or environmental exposures (respiratory viruses, air pollution) might initiate on DC activation [13]. Eisenbarth [14] showed that low-level Toll-like receptor (TLR) 4 agonists mixed with harmless OVA prime DCs to induce a Th2 response by inducing their full maturation, yet not their production of interleukin 12 (IL-12). This is clinically important information as most natural allergens such as HDM, cockroach, and animal dander contain endotoxin and undoubtedly other TLR agonists [15].

From the above, it seems that the decision between tolerance or immunity (in the lungs) is controlled by the degree of maturity of the myeloid DCs (mDCs) that interact with naïve T cells, a process that is driven by signals from the innate immune system [16]. Indeed, it has been shown that immature mDCs induce abortive proliferation in responding T cells and induce Tregs [17]. Another level of complexity arose when it was shown that (respiratory) tolerance might be a function of a subset of pDCs [10]. The removal of pDCs from mice using depleting antibodies led to a break in inhalational tolerance to OVA and to the development of asthmatic inflammation [10].

Sentinel function of lung dendritic cells requires instruction by epithelial cells

Most of the lung DC migration to the mediastinal lymph node results from some form of insult to the lung, be it microbial, physical, or toxic in nature. Based on the anatomical distribution of even the most exposed DCs, it is immediately clear that DCs are basically always covered by a layer of epithelial cells that seals off the inhaled air by the formation of tight junctions (Figure 1.2). It is therefore possible that in the absence of any TLRs or other activating signals, the DCs do not extend dendrites across this epithelial barrier. We recently hypothesized that airway epithelial cells might be instructive in causing

DC sentinel behavior and activation in the lungs [18]. Using a series of radiation chimeric mice in which either radioresistant stromal cells or radiosensitive hematopoietic cells were deficient in the LPS receptor TLR4, we demonstrated that the initial dynamic scanning behavior of lung DCs as well as their directed migration to the mediastinal nodes in response to LPS inhalation was largely dependent on TLR4 signaling on epithelial cells [19].

It is immediately clear from analysis of the common characteristics of clinically relevant allergens that most have the potential to modify epithelial barrier function and to activate airway epithelial cells or innate and adaptive immune cells, like DCs and basophils (see Chapter 21). For example, HDM (*Dermatophagoides pteronyssinus*) fecal pellets contain many allergens (Der p 1 to 9) that have either proteolytic activity or enhance TLR responsiveness, explaining why HDM acts as an allergen and a Th2 adjuvant. Der p 1 increases the permeability of the bronchial epithelium, as measured by a decrease in transepithelial electrical resistance by cleaving the tight junction proteins claudin and occludin, thus increasing access to the DC network [20]. In addition to these proteolytic effects of HDM, β -glucan-rich motifs of HDM were able to trigger human bronchial epithelial cells, most likely via the dectin-1 receptor, and downstream Syk signaling to produce CCL20, a major chemokine, thus causing attraction of lung DCs (see Figures 1.2 and 1.3) [21]. Along the same line, TLR4 signaling is also involved in the recognition of the HDM allergen [22]. In an elegant study, Trompette *et al.* [23] recently demonstrated that Der p 2 is a functional homolog of the adaptor MD-2 (also known as LY96), the LPS-binding component of the TLR4 signaling complex, thus stabilizing TLR4 expression on bronchial epithelial cells. In the same setting of TLR4 radiation chimerics, we have shown that it is mainly the epithelial TLR4-driven response that activates Th2 immunity to the HDM allergen by releasing innate pro-Th2 cytokines, like granulocyte-macrophage colony-stimulating factor (GM-CSF), thymic stromal lymphopoietin (TSLP), IL-33, and IL-25 (Figures 1.2 and 1.3) [19]. The TLR C-type lectin, or proteolytic-mediated activation of epithelial cells by HDM can lead to release of these innate cytokines or other mediators that subsequently program DCs to become Th2 inducers [19].

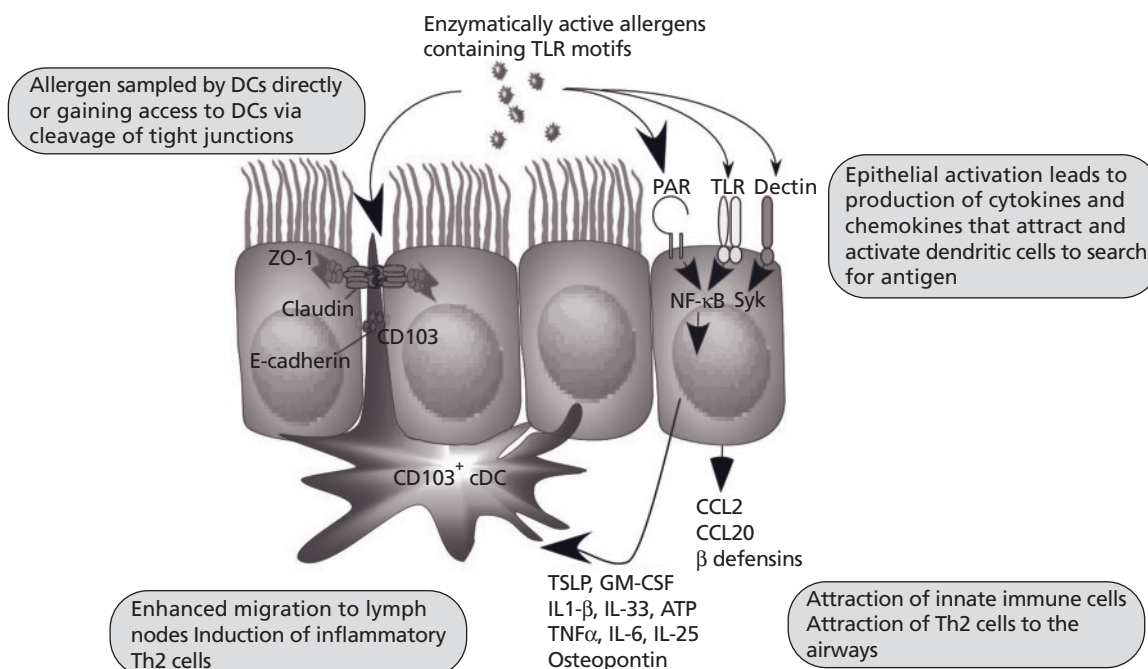


Figure 1.2 Interactions between epithelial cells and dendritic cells (DCs) in the airways. DCs sample the airway lumen by forming dendritic extensions in between epithelial cells. The cells form tight junctions with epithelial cells by expressing occludin and claudin family members as well as zona occludens 1. In addition, the cells attach to airway epithelial cells using E-cadherin and CD103 expressed by a subset of DCs that probes the airway lumen. Enzymatically active allergens can activate

protease-activated receptors (PARs) expressed by epithelial cells followed by nuclear factor-κB (NF-κB) activation and the production of chemokines and cytokines by epithelial cells that attract and activate DCs. Allergens often contain Toll-like receptor (TLR) agonists and C-type lectin agonists; triggering through these also induces NF-κB activation and DC activation either directly or indirectly via effects on epithelial cells that also express TLRs and C-type lectin receptors.

Induction of Th2 responses: collaboration between DCs and innate immune cells

In the field of lung immunology, several groups have shown that either endogenous lung DCs [10,24,25] or adoptively transferred bone marrow-derived DCs [26] are sufficient to induce Th2 responses to inhaled antigens. Studies by Eisenbarth's group [24] have elegantly shown that triggering TLR4 on lung-derived DCs by administering low doses of LPS promotes Th2 cell development through a myeloid differentiation primary response gene 88 (MyD88)-dependent pathway. There is also evidence to suggest that CD11c⁺ DCs are necessary for Th2 responses. The Th2-inducing adjuvant alum is used by many groups

to induce Th2 sensitization to inhaled OVA. However, these Th2 responses, as read out by induced T-cell proliferation, Th2 cytokine production, and IgG1 production, were eliminated when CD11c⁺ DCs were depleted when using diphtheria toxin treatment in CD11cDTR Tg mice [27,28]. Likewise, alum-exposed DCs clearly induced Th2 polarization from naïve TCR Tg T cells in a process requiring caspase-1 and IL-1β production. *In vitro* studies have also amply demonstrated that human DCs exposed to allergens like the HDM Der p 1 allergen [29] and pollen extracts (containing phytoprostanes and NADPH oxidases) [30] acquire Th2 polarizing capacity, even if IL-4 is not made by these exposed DCs. Several papers have recently demonstrated a crucial role for basophils in Th2 immunity [31–33], as they provide

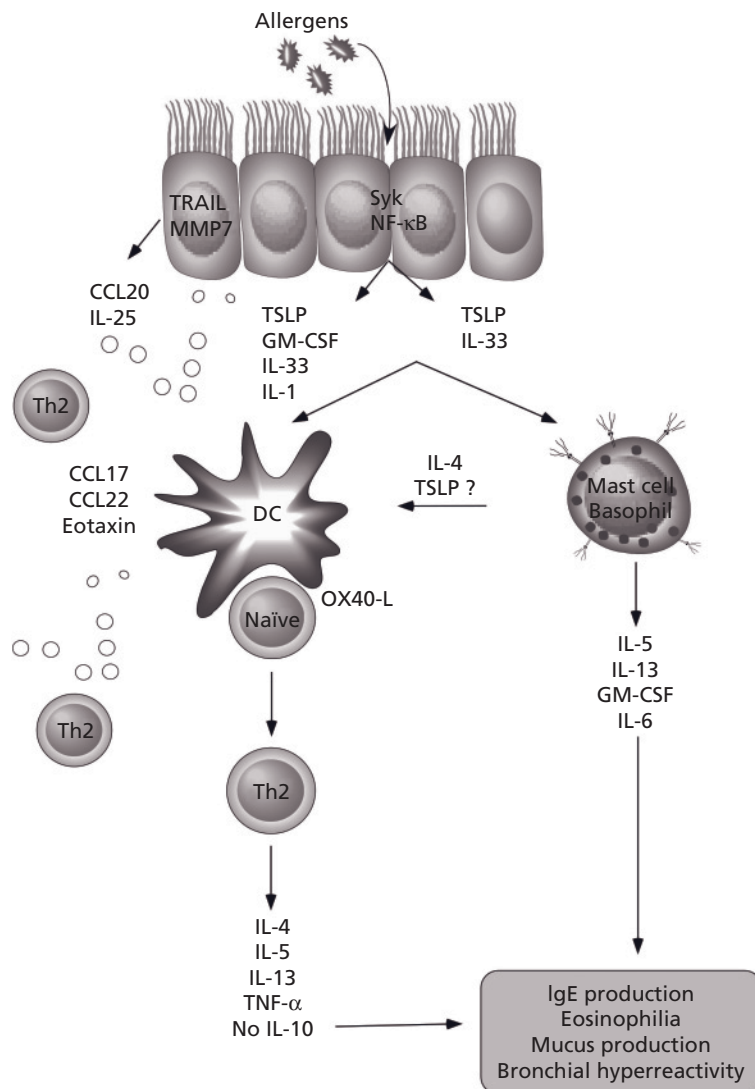


Figure 1.3 Early innate cytokine responses that promote allergic inflammation. Allergen triggering of protease-activated receptor 2 (PAR2) by C-type lectin receptors or by contaminating endotoxin acting on Toll-like receptors (TLRs) initiates the production of thymic stromal lymphopoietin (TSLP), granulocyte–macrophage colony-stimulating factor (GM-CSF), and interleukin 33 (IL-33) by airway epithelial cells. These cytokines are known as DC-activating cytokines. For example, TSLP induces immediate innate immune functions in DCs leading to chemokine-driven recruitment of Th2 cells and eosinophils to the airways, possibly providing a source for polarizing Th2 cell-associated cytokines. Epithelial cells produce CCL20 in a process involving tumor necrosis factor alpha-related apoptosis-inducing ligand (TRAIL) and IL-25 in a process requiring matrix metalloproteinase 7 (MMP7). The effects of CCL20 and IL-25 are to further attract innate immune cells and Th2 cells to the lungs.

TSLP and IL-33 stimulate the functions of mast cells and basophils. In mast cells, there is immediate release of the Th2 effector cytokines that can attract and activate eosinophils in a T-cell-independent way. Following innate immune induction, TSLP (and IL-33) trigger the maturation of DCs so that they migrate to the mediastinal lymph nodes and induce the polarization of inflammatory Th2 cells in an OX40L-dependent fashion. In contrast to most other triggers that induce DC maturation, TSLP-induced maturation is not accompanied by the production of IL-12, thereby explaining Th2 cell polarization. Mast cells and basophils can also serve an important role for providing an early source of IL-4 for Th2 development. Basophils are recruited to draining lymph nodes in a process requiring TSLP. Together with mediators released by mast cells and basophils, effector Th2 cells control the salient features of asthma.

an important source of IL-4 early during an innate response to parasite infection and proteolytic allergens like HDM or papain, and at the same time also serve as bona fide antigen-presenting cells (APCs) that provide peptide-major histocompatibility complex (MHC), co-stimulatory molecules, and instructive Th polarizing signals. We foresee a scenario by which resident lymph node basophils collaborate with migratory DCs, providing an early source of IL-4 to promote or sustain Th2 immunity (Figure 1.3). In this regard, eosinophils, mast cells, and natural killer T (NKT) cells might be similar innate helpers for Th2 immunity driven by DCs [34,35].

Dendritic cells in established allergic airway inflammation

Not only do DCs play a role in the primary immune response to inhaled allergens, they are also crucial for the outcome of the effector phase in asthma. The number of mDCs is increased in the airways of sensitized and challenged mice during the acute phase of the response [36]. The mechanisms for this enhanced recruitment are that DC precursors, most likely at the monocyte stage of development, are attracted from the bone marrow via the bloodstream to the lung in a CCR2-dependent way [37]. However, during the chronic phase of the pulmonary response, induced by prolonged exposure to a large number of aerosols, respiratory tolerance develops through unclear mechanisms. During this regulatory phase, the number of mDCs in the lungs steadily decreased, and this was associated with a reduction of bronchial hyperreactivity (BHR). Inflammation, however, reappeared when mDCs were given [38]. The role of mDCs in the secondary immune response was further supported by the fact that their depletion at the time of allergen challenge abrogated all the features of asthma, including airway inflammation, goblet cell hyperplasia, and bronchial hyperresponsiveness [9,39]. antigenin the defect was restored by intratracheal injection of CD11b⁺ inflammatory mDCs, but not by other APCs such as macrophages. It therefore seems that inflammatory mDCs are both necessary and sufficient for secondary immune responses to allergens.

In humans, allergen challenge leads to an accumulation of myeloid, but not plasmacytoid DCs to the airways of asthmatics, concomitantly with a reduc-

tion in circulating CD11c⁺ cells, showing that these cells are recruited from the bloodstream in response to allergen challenge [40,41]. In stable asthma, the number of CD1a⁺ DCs is increased in the airway epithelium and lamina propria, and these numbers are reduced by treatment with inhaled corticosteroids [42]. Based on the above argumentation in mice studies of asthma, it is very likely that part of the efficacy of inhaled steroids might be due to their effects in dampening airway DC function.

Novel targets for anti-inflammatory disease based on knowledge of DC-epithelial biology

Blocking innate pro-Th2 instructive cytokines

Thymic stromal lymphopoietin, a unique dendritic cell-instructive signal

Thymic stromal lymphopoietin is a 140 amino acid IL-7-like four-helix bundle cytokine that has potent DC-modulating capacities by binding its receptor complex, composed of the IL-7 receptor (IL-7R) and the TSLP receptor (TSLPR) [43]. TSLP can directly activate DCs to prime naïve CD4⁺ T cells to differentiate into proinflammatory Th2 cells that secrete IL-4, IL-5, IL-13, and TNF- α , but not IL-10, and express the prostaglandin D₂ receptor CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), a T-cell phenotype that is also found in asthmatic airways [44]. This pathway involves the induction of the Th2-prone co-stimulatory molecule OX40L and the production of the Th2-attractive chemokines CCL17 and CCL22 by DCs [44] (Figure 1.3). In addition to its effects on DCs, TSLP can also activate human mast cells to produce Th2-associated effector cytokines in the absence of T cells or IgE cross-linking [45] (Figure 1.3).

The most convincing evidence for a role for TSLP in DC-driven Th2 cell development came from studies in mice that conditionally overexpressed TSLP in the lungs. These mice mounted a vigorous DC-driven primary Th2 cell response to environmental antigens in the airways [46]. By contrast, *Tlspr*^{-/-} mice fail to develop an antigen-specific Th2 cell inflammatory response in the airways unless they are supplemented with wild-type CD4⁺ T cells [47]. Taken together, these data suggest that TSLP produced by the lung epithelium might represent a crucial factor that can

initiate allergic responses at the epithelial-cell surface. Therefore, it will be very important to study how the production of TSLP by epithelial cells and other inflammatory cells is regulated.

IL-25, IL-33, and GM-CSF

The polarization of Th2 cells induced by TSLP-matured DCs is further enhanced by IL-25, which is produced by epithelial cells, basophils, and eosinophils [48]. Several reports showed that airway epithelial cells can produce IL-25 in response to an innate immune response to allergens, a process requiring epithelial cleaving of IL-25 by matrix metalloproteinase 7 (Figure 1.3) [19,49]. GM-CSF is released by bronchial epithelial cells in response to HDM exposure, as well as a number of environmental sensitizers like diesel exhaust particles and cigarette smoke. GM-CSF promotes DC maturation and breaks inhalation tolerance, and previous studies demonstrated that HDM-driven asthma is neutralized by blocking GM-CSF [50]. IL-33 is made by epithelial cells, boosts Th2-cytokine production, and promotes goblet cell hyperplasia. It was recently shown to also promote Th2 differentiation by programming the function of DCs [51]. Obviously, these cytokines could be high on the list for targeting inflammation in asthma, either individually or simultaneously, by blocking the innate receptors like TLR4, C-type lectin receptors, or protease-activated receptors that induce them [19,21,52].

Blocking endogenous DAMPs that contribute to DC activation in asthma

Dendritic cells express a plethora of receptors for endogenous danger-associated molecular patterns (DAMPs; Figure 1.4) that are released at sites of ongoing inflammation. For example, DCs express receptors (protease activated receptors [PARs]) that are activated by proteolytic proteins like tryptase and thrombin [29]. Shortly after insult to the vascular compartment or after pathogen entry in the mucosa, complement activation occurs. Lung DCs can sense this “acute alert” through expression of the C5a and C3a anaphylatoxin receptors [53]. DCs also express neuropeptide receptors, which can respond to the neurotransmitters that are released in response to axon reflexes or efferent neural responses, this is supported by the fact that lung DCs synapse

with unmyelinated nerve endings in and beneath the airway mucosa and produce neurotransmitters [54]. Lung DCs express receptors for prostaglandins and these acutely released inflammatory mediators can profoundly impact on the migration and maturation of the cell [55,56]. Endogenously released metabolites like extracellular adenosine triphosphate (ATP) trigger purinergic receptors on lung DCs, and in this way relay information about allergen-induced platelet aggregation or metabolic cell stress to the cells of the immune system through widely expressed purinergic receptors [57,58]. Eosinophil and mast cell degranulation can lead to the release of eosinophil-derived neurotoxin (EDN) and histamine that can feed back on DCs and promote further Th2 responses [59]. Clearly, much more effort is required before we can fully grasp the importance of these inflammatory mediators and DAMPs in explaining the chronicity of asthma [58]. These endogenous DAMPs are obvious targets for intervention, and blocking their production or neutralizing their effects has proven to be successful in intervening in mouse models of asthma.

Direct blocking of dendritic cell function

If DCs are so crucial in mounting and maintaining immune responses to inhaled allergens, then interfering directly with their function could constitute a novel form of treatment for allergic diseases. A strategy to eliminate DCs from the airways is probably not a valuable option, as local depletion of airway DCs was recently shown to lead to severe exacerbation of respiratory viral infections like influenza, whereby the virus failed to be cleared from the lungs and led to severe systemic illness [7]. Therefore, we are favoring the idea of targeting the fine-tuning mechanisms whereby DCs maintain allergic inflammation. Recently, several new molecules have been identified that may alter DC function in allergic inflammation and therefore could be possible therapeutic targets. Many of these compounds were first discovered by their potential to interfere with DC-driven Th2 cell sensitization. The sphingosine 1-phosphate receptor antagonist FTY720 is currently used in clinical trials for multiple sclerosis and transplant rejection. When given locally in the lungs of mice with established inflammation, it strongly reduced inflammation by suppressing the T-cell stimulatory capacity and migratory behavior of lung DCs without causing

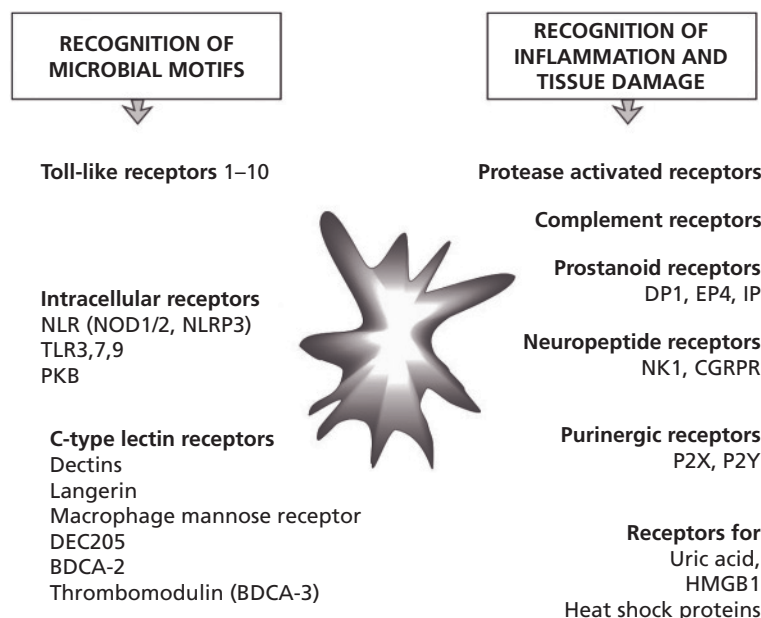


Figure 1.4 Dendritic cells (DCs) express extracellular and intracellular receptors that recognize pathogen-associated molecular patterns (PAMPs) that are found inside microbial motifs, as well as a wide variety of C-type lectin receptors that discriminate glycosylation patterns on self versus non-self proteins. What is less emphasized in the literature is that they also express receptors that recognize an ongoing inflammation response. Although PAMP receptors are mainly triggered by microbial motifs, it is possible that they are also activated by self ligands such as heat shock proteins. Tryptase, which is released by mast cells, and thrombin, which is released during the blood coagulation process, can trigger protease-activated receptors (PARs). Complement activation is an early innate immune reaction in response to allergen inhalation and can also lead to alterations in DC function. Inflammation often leads to the production of prostaglandins that can either stimulate (through the type 4 prostaglandin

E_2 receptor, EP4) DC activation or dampen it (through the type 1 prostaglandin D_2 [DP1] and prostaglandin I_2 receptor [IP]). As airway DCs “live” in close proximity to unmyelinated nerve endings, the various neuropeptides that are released during neurogenic inflammation can also activate DCs by triggering neurokinin 1 (NK1) and the calcitonin gene-related protein receptor (CGRPR). Necrotic cell death leads to the release of damage-associated molecular patterns (DAMPs). Extracellular adenosine triphosphate (ATP) triggers a broad family of purinergic P2X and P2Y receptors. Uric acid is recognized by the NALP3 (NACHT-, LRR-, and pyrin-domain-containing protein) receptor. The chromatin-binding protein high-mobility group box 1 protein (HMGB1) is released by necrotic cells and triggers the receptor for advanced glycation end products (RAGE). (BDCA, blood dendritic cell antigen; TLR, Toll-like receptor; NLR, NOD-like receptor).

lymphopenia, which is caused when the drug is given orally [60]. FTY720 inhibited the potential of DCs to form stable synapses with naïve antigen-specific T cells as well as effector Th2 cells, providing a possible explanation as to how these drugs might work to inhibit allergic inflammation. The drugable spleen tyrosine kinase (Syk) pathway has been shown to be crucial for Th2 induction by airway DCs [61], and downstream of this pathway the signaling intermediate PI3K δ might similarly be very drugable.

As the number and activation status of lung CD11b⁺ DCs during secondary challenge seems crucial for controlling allergic inflammation, studying the factors that control recruitment, survival, or egress of DCs from the lung during allergic inflammation will be important, as this might reveal new therapeutic targets [37]. Eicosanoid lipid mediators, such as prostaglandins and leukotrienes, can also influence the migration of lung DCs [56]. Selective agonists of particular receptors for members of the prostaglandin family

might also suppress DC function. Prostaglandin D₂ has pleiotropic effects in the immune system owing to its activity on the DP1 and CRTH2 (also known as DP2) receptors, which are widely expressed on immune cells. The DP1 agonist BW245C strongly suppressed the spontaneous migration of lung DCs to the mediastinal lymph node [62]. More importantly, BW245C suppressed airway inflammation and bronchial hyperreactivity when given to allergic mice by inhibiting the maturation of lung DCs. More detailed information on the interactions between DCs, epithelial cells, basophils, and other inflammatory cells will undoubtedly lead to the discovery of more potentially interesting drugs. In this regard, blocking the interaction of TSLP and GM-CSF with their respective receptors with small molecule inhibitors or blocking antibodies might prove very useful. Downstream of these, blocking CCR4 or its ligands might prevent DC-driven recruitment of Th2 cells.

Disease modification based on interfering with DC function

Stimulation of the immunoregulatory properties of DCs might reset the balance of the allergic immune response in favor of the development of Tregs, and could lead to a more long-lasting effect on the natural course of allergic disease. One way of achieving this would be by using a combination of steroids and vitamin D analogs to impact DC function and stimulate Treg differentiation. Steroids are currently the cornerstone of anti-inflammatory treatment in allergic disease. Inhaled steroids reduce (but do not eliminate) the number and modulate the function of DCs in the lungs and noses of individuals with allergic asthma and allergic rhinitis [63]. Steroids also induce the activation of the IDO enzyme in pDCs in a glucocorticoid-induced TNF receptor-related protein ligand (GITRL)-dependent way, thereby broadly suppressing inflammatory responses [64]. Prostaglandins or their metabolites might have the same effect. In the presence of the DP1 agonist BW245C, DCs induced the formation of forkhead box P3 positive (FOXP3⁺) Tregs from FOXP3⁻ antigen-specific T cells in a process requiring cyclic adenosine monophosphate (cAMP) and protein kinase A [56]. A very similar mechanism was described for inhaled iloprost, a prostacyclin analog that acts on the I prostanoid (IP) receptor expressed by lung DCs [55,65]. Downstream metabo-

lites of prostaglandins include agonists of the peroxisome proliferator-activated receptor γ (PPAR γ) family. Pharmacologic PPAR γ agonists like the antidiabetic drug rosiglitazone were able to modify lung DC function and stimulate the formation of IL-10-producing T cells, thus suppressing features of asthma [66]. Finally, the stimulation of the IgA-inducing capacities of lung DCs might be a possible strategy that could have prolonged effects in allergic disease akin to the effects of desensitization immunotherapy [67].

Concluding remarks

It is now clear that DCs and epithelial cells play crucial roles in the initiation and maintenance of allergic airway inflammation. Interfering with their function, either directly or indirectly via disruption of intercellular communication, promises to provide novel therapeutics for this disease.

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