Immunity
Review

Biology of Lung Dendritic Cells at the Origin of Asthma

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DOI 10.1016/j.immuni.2009.08.008

Dendritic cells (DCs) initiate and maintain adaptive T helper 2 (Th2) cell responses to inhaled allergens in asthma. Various functions like antigen uptake, migration to the draining LNs, and induction of tolerance and adaptive immunity are not equally shared by all subsets of DCs, adding considerable complexity to understanding the immunology of allergic sensitization. Whereas the epithelium was initially considered solely as a physical barrier, it is now seen as a central player in controlling the function of lung DCs through release of Th2 cell-promoting cytokines. Although DCs are sufficient and necessary for induction of Th2 cell responses to many antigens, some allergens might require antigen presentation by basophils. Clinically relevant allergens, as well as environmental and genetic risk factors for allergy and asthma, often interfere directly or indirectly with the innate immune functions of airway epithelial cells, basophils, and DCs. This review summarizes the recent progress on our understanding how DCs control Th2 cell immunity in the lung.

Introduction: The Adaptive Immune Response in Allergic Asthma

In allergic asthma, genetically susceptible individuals mount a chronic Th2 cell-type of immune response to allergens like house-dust mites (HDMs), molds, plant pollen, and animal dander that is measured clinically by the presence of a serum IgE response or the presence of a positive skin-prick test. Bronchial biopsy studies in human asthmatics have revealed a predominant eosinophilic airway inflammation accompanied by an accumulation at the airway wall of helper T cells. In initial studies, it was found that $CD4^+$ T cells in human asthmatics as well as in mouse models of asthma secrete interleukin-4 (IL-4), IL-5, IL-13, and tumor-necrosis factor (TNF)—a phenotype consistent with inflammatory T helper 2 (Th2) cells (Robinson et al., 1992). Studies in mouse models with blocking antibodies or transgenic modeling have shown that individually, these cytokines can already explain many of the salient features of asthma such as IgE synthesis (IL-4 acting on B cells), airway eosinophilia (IL-5 acting on bone marrow progenitors), goblet cell metaplasia (IL-4 and IL-13 acting on epithelia), and bronchial hyperreactivity (BHR) (IL-13 acting on bronchial smooth muscle cells) (Wills-Karp et al., 1998). Increasingly, other Th cell subsets have been linked to disease pathogenesis. The presence of IL-9 in mouse and man is strongly associated with mast cell accumulation in the airways, as well as BHR and mucus cell metaplasia, although it remains to be established whether IL-9 derives from a dedicated Th9 cell (Longphre et al., 1999). Closely related to this Th9 cell subset, some patients with severe airway obstruction (often characterized by neutrophilic inflammation and steroid resistance) might have a predominant Th17 cell component (Traves and Donnelly, 2008; see article by Barrett and Austen, 2009, in this issue of *Immunity*). Similarly, in almost all patients with asthma, one can find a counterregulatory population of allergen-specific Treg cells that downregulate allergic inflammation through instruction by TGF-β and/or IL-10 (see article by Lloyd and Hawrylowicz, 2009, in this issue of *Immunity*).

Dendritic Cells Bridge Innate and Adaptive Immunity

Many other factors such as respiratory virus infection, exposure to airborne pollutants (tobacco smoke, diesel particles, and ozone), or physical stimuli (exercise and cold air) can either modify or exacerbate the disease (Cookson, 2004). Often these exacerbating factors interfere with the innate immune system or with the homeostasis of lung structural cells, so it is increasingly appreciated that asthma is more than a disorder of the adaptive immune response and is heavily influenced by pattern recognition by the innate immune cells such as bronchial epithelial cells, mast cells and basophils, natural killer (NK) cells, and NKT cells. Like in other epithelia and skin, the lung is equipped with an elaborate network of dendritic cells (DCs) that can be found throughout the conducting airways, lung interstitium, lung vasculature, pleura, and bronchial lymph nodes (for recent review see GeurtsvanKessel and Lambrecht, 2008). These cells perform a unique sentinel function in the pulmonary immune response in that they recognize inhaled antigens through expression of ancient pattern-recognition receptors such as Toll-like receptors, NOD-like receptors, and C-type lectin receptors that will recognize motifs on virtually any inhaled pathogen, allergen, or substance (Barrett et al., 2009). Additionally, lung DCs express numerous receptors for inflammatory mediators that are released upon damage (so-called damage-associated molecular patterns like uric acid, high mobility group box 1) to the tissues by pathogens, trauma, vascular damage, or necrosis. By exerting all these direct and indirect sensing mechanisms for danger in the airways, and at the same time expressing all the machinery to migrate to the regional lung-draining lymph nodes and process antigens on their way to the node (Vermaelen et al., 2001), DCs are at the nexus of innate and adaptive immunity in the lung. In this review, we will discuss recent progress in the

field of lung DC biology, with specific emphasis on how DCs get activated in response to allergens and contribute to allergic disease by programming and maintaining adaptive immunity. Most of the discussed data derive from studies in the mouse, and where possible, parallels to the situation in human asthma will be drawn.

The Increasing Complexity of Lung DC Subsets

Although lung DCs were originally described in the mouse as a single population of highly dendritic-shaped cells with high degree of expression of CD11c and MHCII (Sertl et al., 1986), it is now clear that at least five different subsets of DCs can be found in the lungs (see Figure 1). Most importantly, there is division of labor between these various lung DC subsets, making a closer distinction into subsets almost imperative if one is to understand the biology of lung DCs (GeurtsvanKessel and Lambrecht, 2008). The mouse lung is grossly divided into large conducting airways and lung interstitium containing alveloar septa and capillaries where gas exchange is taking place (Wikstrom and Stumbles, 2007). In steady-state conditions, the conducting airways of all species studied are lined with an intraepithelial highly dendritic network of MHCII^{hi}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 in addition has the propensity of extending dendrites into the airway lumen through formation of tight junctions with bronchial epithelial cells (GeurtsvanKessel et al., 2008; Jahnsen et al., 2006; Lambrecht et al., 1998; Sung et al., 2006). Immediately below, the lamina propria of the conducting airways contains MHCII^{hi}CD11c^{hi} cells that are highly expressing CD11b and are a rich source of proinflammatory chemokines (Sung et al., 2006; van Rijt et al., 2005). The CD11b^{+}CD103^{-} subset also expresses the SIRPα molecule, a binding partner to CD47 involved in DC migration (Raymond et al., 2009). A similar broad division into CD11b^{+} and CD11b^{-} can also be applied to lung interstitial DCs obtained by enzymatic digestion of peripheral distal lung (GeurtsvanKessel et al., 2008; von Garnier et al., 2005). Both CD11b^{+} and CD11b^{-} subsets express high amounts of CD11c, so they can best be denominated as conventional DCs (cDCs), to contrast this with another population of CD11c^{int} plasmacytoid DCs (pDCs) that express Siglec-H, and the bone-marrow stromal antigen-1, and some markers shared with granulocytes and B cells (Figure 1; De Heer et al., 2004; GeurtsvanKessel et al., 2008; Kool et al., 2009). The exact anatomical location of lung pDCs is unclear although they can be found to line alveolar septa in situ and have been recovered from digests of large conducting airways (De Heer et al., 2004; Wikstrom and Stumbles, 2007). The alveolar space also contains CD11c^{hi}MHCII^{hi} DCs and is easily accessible by bronchoalveolar lavage. At least in the rat and man, alveolar DCs are highly enriched in CD103^{+} subsets that resemble Langerhans cells in man. One caveat is, however, that lung alveolar macrophages express high amounts of CD11c while lacking CD11b, essentially confusing many analyses of lung DC biology if one does not use autofluorescence to identify macrophages (Vermaelen and Pauwels, 2004). Under inflammatory conditions, such as viral infection, allergen challenge, or 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 (GeurtsvanKessel et al., 2008; Hammad et al., 2009; Jakubzick et al., 2008a).

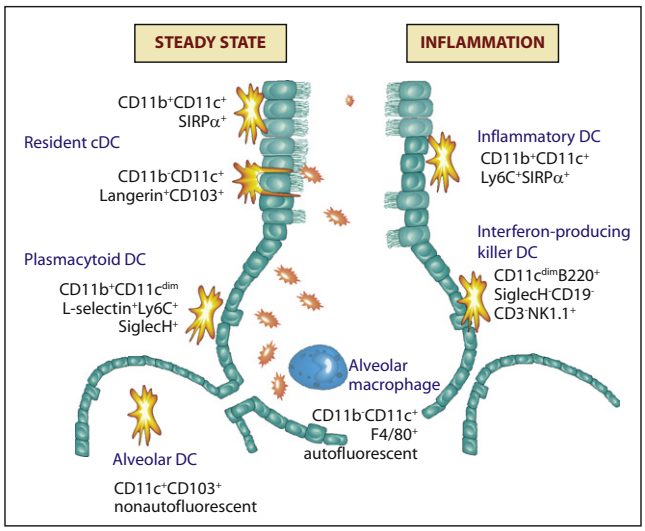

Figure 1. Lung Dendritic Cell Subsets

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 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 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.

Origin of Lung DC Subsets

Much progress has been made recently on the identification of the precise progenitor for DCs that reside in the central lymphoid organs. In a series of adoptive transfer studies, as well as Lysosyme (Lys)MCre-mediated lineage tracing of monocyte-derived cells, it was elegantly shown that the majority of lung-resident cDCs do not derive from the same Flt3^{+} progenitor that seeds the central lymphoid organs, but rather originate from circulating blood monocytes (Jakubzick et al., 2008a, 2008b; Varol et al., 2007). A population of Ly-6C^{hi}CCR2^{hi}CX$_3$CR1^{int} monocytes is characterized as the more classical monocytes that readily emigrate to sites of ongoing inflammation, whereas Ly-6C^{lo}CCR2^{lo} monocytes expressing high levels of the fractalkine receptor CX$_3$CR1 do not robustly emigrate to many tissues, but do migrate well to lung even in absence of inflammation (Landsman et al., 2007). It was suggested that under homeostatic conditions, CD103^{+}CD11b^{-} DCs preferentially derived from CCR2^{hi} monocytes, whereas CD11b^{hi} cDCs preferentially arise from CCR2^{lo} monocytes, thus lending proof to the concept that subsets of monocytes recruited to the same tissue undergo differential differentiation pathways within the DC lineage (Jakubzick et al., 2008b). The relative contribution of GM-CSF

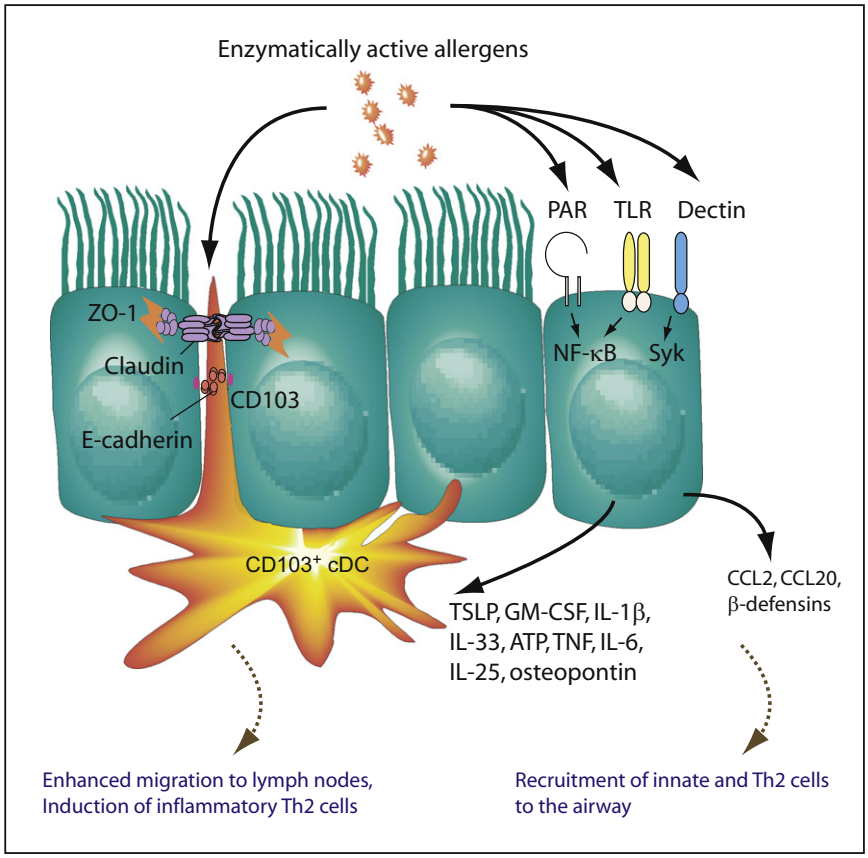

Figure 2. Interactions between Epithelial Cells and Dendritic Cells in the Airways

Dendritic cells (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 (ZO-1). In addition, the cells attach to airway epithelial cells via 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 and 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.

and Flt3L as homeostatic growth factors for lung DC progenitors has only partially been addressed, nor is it clear at present whether DCs at various anatomical compartments all depend on monocytic precursors or more committed Flt3$^+$ DC precursors. One report has studied the phenotype and distribution of lung DCs in Flt3L-deficient mice. Although these mice have a dramatic reduction in cDCs and pDCs in central lymphoid organs, there was virtually no reduction in conducting airway cDCs. Strikingly, however, lung DCs obtained by enzymatic digestion of peripheral lung were severly reduced (>95%) in these mice (Walzer et al., 2005). These data imply that peripheral lung DCs derive from a different Flt3-dependent progenitor than do mucosal cDCs. The small population of lung pDCs seems to derive from a Flt3$^+$ cDC progenitor, because administration of recombinant Flt3L to the lungs of mice leads to a large increase in these cells, in addition to a more immature cDC-like population (Kool et al., 2009; Shao et al., 2009).

Sentinel Function of Lung DCs Requires Instruction by Epithelial Cells

Seminal studies by Holt have revealed that conducting airway DCs have a rapid half-life of about 2 days, whereas more distal lung DCs have a slower turnover rate (Holt et al., 1994). By analogy with the gut mucosal immune system, where intestinal mucosa-derived DCs were found to migrate into afferent lymphatics even in the steady state, it was assumed that the conducting airway DC compartment was turning over so rapidly because of continuous sampling and migration of DCs to the mediastinal lymph nodes, even in the absence of overt insults to the lung. This was only an assumption and hard to reconcile with the fact that the gut is heavily colonized by microbes, whereas the (deeper) lung of unmanipulated animals is a relatively sterile environment. These studies were, however, supported by follow-up studies in which large fluorescent yet innocuous tracer molecules like FITC-dextran or FITC-OVA were seen to be taken by lung DCs to the mediastinal nodes in steady-state conditions, in a CCR7-dependent manner (De Heer et al., 2004; del Rio et al., 2007; Vermaelen et al., 2001). None of these studies have, however, eliminated completely the possibility that these findings were caused by manipulations in the mouse or that the fluorescent tracers were contaminated with low amounts of TLR agonists. In a very elegant study, Jakubzick et al. (2008a) have used a genetic tagging technique employing LysMCre crossed to ROSA26 stop-flox GFP mice to study lung DC migration behavior during true homeostasis in the lung. They essentially found that without some form of TLR stimulation, very little if any mediastinal LN DCs were derived from LysMCre-tagged monocyte-derived cDCs in the lung, essentially questioning the paradigm of steady-state sampling and migration by mucosal lung DCs (Jakubzick et al., 2008a).

It follows that most of the lung DC migration to the MLN 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 formation of tight junctions (see Figure 2). It is therefore possible that in the absence of any TLR 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 (Hammad and Lambrecht, 2008). By 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 (Hammad et al., 2009). A similar situation occurred in intestinal jejunum epithelium where TLR triggering of epithelial cells in response to invasive and noninvasive microbes also leads to a "periscope-up" function of mucosal DCs (Chieppa et al., 2006). This situation by which stromal cells instruct the functional behavior of DCs seems to be quite specific to the mucosal environment, as shown by the fact that Nolte et al. (2007) who used a similar radiation chimeric approach found no evidence for stromal instruction of splenic DCs to systemically administered antigens. Which epithelial signals program this scanning response is largely unknown, but one possibility is the regulated expression or display of chemokines along the extracellular matrix of epithelial cells, followed by the secretion of DC-activating cytokines (see below).

Allergic Sensitization Results from Activation of the Epithelial-DC Crosstalk

It is immediately clear from analysis of the common characteristics of clinically relevant allergens that most have the potential to modify epithelial barrier function or activate airway epithelial cells or innate and adaptive immune cells, like DCs and basophils (summarized in Hammad and Lambrecht, 2008). For example, house dust mite (*Dermatophagoides pteronyssinus*) fecal pellets contain many allergens (Derp1 to -9) that have either proteolytic activity or enhance TLR responsiveness, explaining why HDM acts as an allergen and a Th2 cell adjuvant. Derp1 allergen, a major allergen of the HDM, increases the permeability of the bronchial epithelium, as measured by a decrease in transepithelial electrical resistance by cleaving the tight junctional proteins claudin and occludin, thus gaining access to the DC network (Wan et al., 1999). 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 causing attraction of lung DCs (see Figure 2; Nathan et al., 2009). Along the same line, TLR4 signaling is also involved in recognition of HDM allergen (Phipps et al., 2009). In an elegant study, Trompette et al. recently demonstrated that Derp2 is a functional homolog of the adaptor MD-2 (also known as LY96), the LPS-binding component of the TLR-4 signaling complex in this way stabilizing TLR4 expression on bronchial epithelial cells (Trompette et al., 2009). In the same setting of TLR4 radiation chimerics, we have shown that it is mainly the epithelial TLR4-driven response that activates Th2 cell immunity to HDM allergen, through release of innate pro-Th2 cytokines, like GM-CSF, IL-33, TSLP, and IL-25 (see Figures 2 and 3; Hammad et al., 2009). 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 cell inducers (Hammad et al., 2009). GM-CSF promotes DC maturation and breaks inhalation tolerance, and previous studies demonstrated that HDM-driven asthma is neutralized by blocking GM-CSF (Cates et al., 2004). Interleukin-33 is made by epithelial cells, boosts Th2 cytokine production, and promotes goblet cell hyperplasia. It was recently shown to also promote Th2 cell differentiation by programming the function of DCs (Rank et al., 2009).

Thymic Stromal Lymphopoietin, a Unique DC-Instructive Signal

TSLP is a 140 amino acid IL-7-like 4-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) (Liu et al., 2007). TSLP can directly activate DCs to prime naive $CD4^+$ T cells to differentiate into proinflammatory Th2 cells that secrete IL4, IL-5, IL-13, and TNF-α, but not IL-10, and express the prostaglandin D_2 receptor CRTH2 (chemoattractant receptor-homologous molecule expressed on Th2 cells), a T cell phenotype that is also found in asthmatic airways (Ito et al., 2005). The way by which this polarization occurs has been studied in detail and involves the induction of the Th2 cell-prone costimulatory molecule OX40L and their production of the Th2 cell-attractive chemokines CCL17 and CCL22 by DCs (see Figure 2; Ito et al., 2005). In addition, TSLP might promote the production of Th2 cytokines by lymphocytes directly. 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 (Wang et al., 2007). Several reports showed that airway epithelial cells can produce IL-25 in response to an innate immune response to allergen, a process requiring epithelial cleaving of IL-25 by matrix metalloproteinase 7 (see Figure 2; Angkasekwinai et al., 2007; Goswami et al., 2009; Hammad et al., 2009). In addition to its effects on DCs, TSLP can also activate human mast cells to produce Th2 cell-associated effector cytokines in the absence of T cells or IgE crosslinking (Allakhverdi et al., 2007).

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 (Headley et al., 2009; Zhou et al., 2005). 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 (Al-Shami et al., 2005). 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. Strikingly, IL-13 was shown to induce expression of TSLP, pointing out to an important feedback-loop acting via DCs to enhance Th2 cell immunity (Miyata et al., 2009).

Direct Effects of Allergens on DCs

The indirect activation events through airway epithelial cells do not exclude direct effects of allergens on lung DCs. The proteolytic activity of Derp1 has long been known to activate human monocyte-derived cDCs and pDCs to promote Th2 cell responses through effects on polarizing cytokines, costimulatory molecules, and cell surface receptors (Charbonnier et al., 2003; Hammad et al., 2001, 2003b). In the mouse, Barrett et al. (2009) recently described a dectin-2-mediated recognition of HDM by bone-marrow-derived DCs that led to spleen tyrosine kinase (Syk)-dependent synthesis of cysteinyl leukotrienes, possibly explaining some of the proallergic effects of HDM.

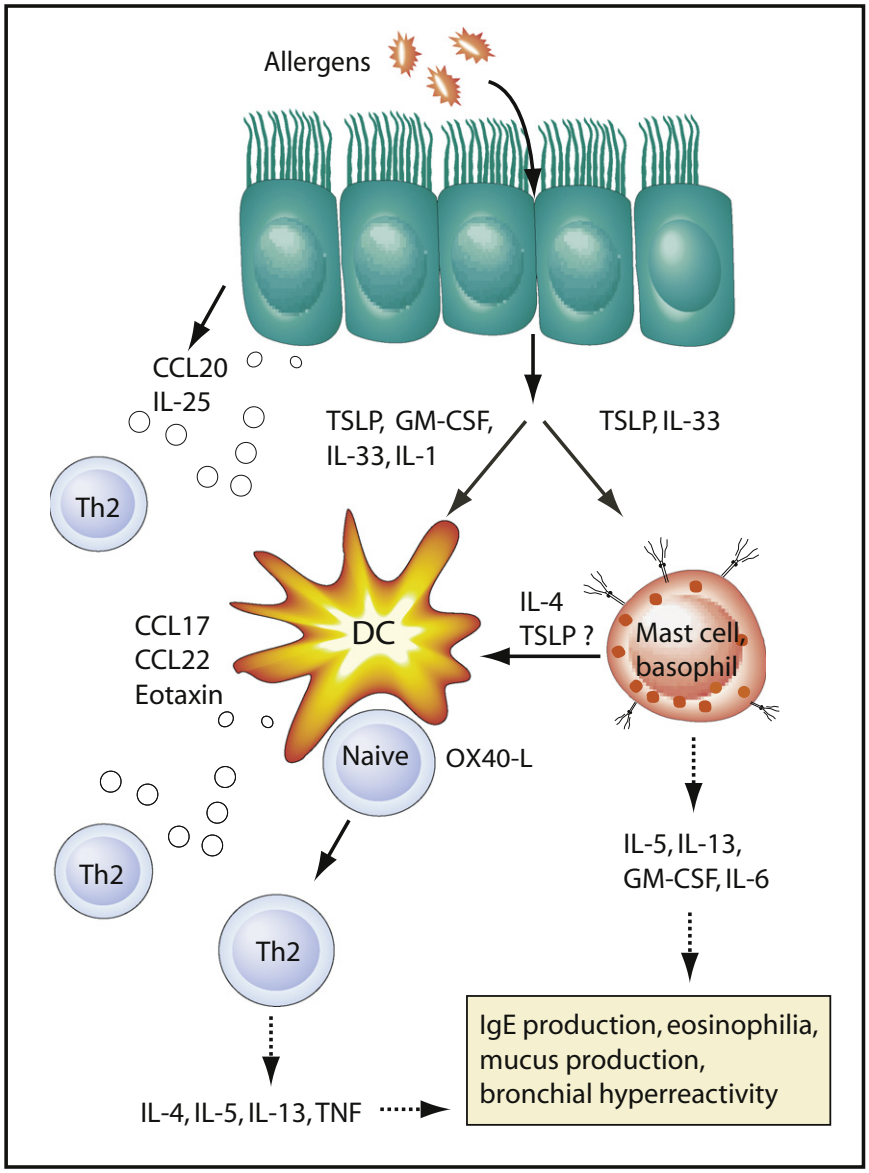

Figure 3. Early Innate Cytokine Responses that Promote Allergic Inflammation

Allergen stimulation of protease-activated receptor 2 (PAR2), C-type lectin receptors, or Toll-like receptors (TLRs) triggers the production of thymic stromal lymphopoeitin (TSLP), granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-33 by airway epithelial cells. TSLP induces immediate innate immune functions in DCs leading to chemokine-driven recruitment of Th2 cells and eosinophils to the airways. Epithelial cells produce CCL20 in a process involving TRAIL and IL-25 in a process requiring 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. TSLP and IL-33 also induce DCs to 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 interleukin-12 (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 cell 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.

Environmental Influence on Epithelium-DC Interactions

Although here we focused mainly on HDM as a relevant allergen, many other allergens have similar effects on either epithelial barrier, epithelial activation, or direct DC activation (summarized in Hammad and Lambrecht, 2008). Likewise, most known environmental risk factors that are associated with development of allergy like cigarette smoking, exposure to ambient particulate matter like diesel exhaust, exposure to high ozone concentrations, or exposure to RSV virus have the potential to interfere with the epithelial-DC crosstalk. Cigarette smoking enhances epithelial permeability and induces the production of TSLP by airway epithelial cells (reviewed in Robays et al., 2009; Monick et al., 2003), whereas the Th2 cell adjuvant effects of diesel depend on epithelial production of GM-CSF (Bleck et al., 2006). RSV infection leads to direct DC activation but also leads to persistent upregulation of the MD-2 molecule on epithelial cells, explaining why children might be hypersensitive to subsequent allergen exposure containing TLR4 agonists (Monick et al., 2003).

Epithelial-DC interactions might also be necessary for preventing overt Th2 cell responses. It has been demonstrated that asthmatics have a reduced innate immune response to rhinovirus infection, a common trigger for asthma exacerbation, reflected by reduced production of type I and III interferon from bronchial epithelial cells (Contoli et al., 2006). It was recently shown in an in vitro study that monocyte-derived DCs that were differentiated in the presence of a bronchial epithelial cell line were instructed to produce IL-12, IL-6, IL-10, and TNF-α, thus promoting Th1 cell responses, away from potentially harmful Th2 cell responses through epithelial-derived type I interferon production (Rate et al., 2009). Collectively, these findings suggest that epithelial cells modulate local DC differentiation to optimize antimicrobial defenses in the airways and in the process downmodulate capacity for expression of potentially damaging Th2 cell immunity. A defective innate immune response of epithelial cells might therefore be at the heart of allergic sensitization.

Th2 Cell Responses: Are DCs Really Redundant?

Several recent papers have demonstrated a crucial role for basophils in Th2 cell immunity (Perrigoue et al., 2009; Sokol et al., 2009; Yoshimoto et al., 2009). These papers have reinforced the idea that basophils are 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 APCs that provide peptide-MHC, costimulatory

molecules, and instructive Th cell polarizing signals. The results from the experimental systems in which DCs were either eliminated with CD11c-DTR or antigen presentation was restricted to CD11c-positive cells led the authors to conclude that DCs are neither necessary nor sufficient for Th2 cell immunity. This statement stems from a long-held paradox in DC biology that DCs do not produce a Th2 cell-polarizing instructive cytokine, yet can induce Th2 cell responses. This is in contrast to Th1 cell immune responses, where DCs are a well-known source of instructive IL-12 acting downstream via STAT-4 to instruct and stabilize Th1 cell development (Macatonia et al., 1995). For Th2 cell polarization, IL-4 was the first and foremost polarization factor. Lung or other DCs were never found to produce IL-4, so it was long assumed that Th2 cell development might occur by default, when DCs were expressing peptide-MHC and costimulatory molecules in the absence of polarizing IL-12 secretion (Stumbles et al., 1998). In addition to this default pathway of Th2 cell development, others have proposed that the secretion of IL-6 by (lung) DCs was a clear instructive signal to Th2 cell development (Constant et al., 2002; Dodge et al., 2003; Krishnamoorthy et al., 2008). Alternatively, cell surface instructive signals like the Notch ligand jagged-2 (Krishnamoorthy et al., 2008), c-Kit (Krishnamoorthy et al., 2008), and OX40L (Ito et al., 2005) were shown to mediate DC-driven Th2 cell development.

In the field of lung immunology, several groups have shown that either endogenous lung DCs (De Heer et al., 2004; Eisenbarth et al., 2002; Krishnamoorthy et al., 2008; Lewkowich et al., 2008) or adoptively transferred bone-marrow-derived DCs (Lambrecht et al., 2000; Matsubara et al., 2006; Piggott et al., 2005) are *sufficient* to induce Th2 cell responses to inhaled antigens. This is a direct effect and not due to transfer of injected antigen from injected DCs to resident APCs like basophils, as shown by the fact that the injection of DCs from MHCII-deficient mice failed to induce Th2 cell priming in a wild-type host (Kuipers et al., 2009). This property stems mainly from the SIRPα^+CD11b$^+$ subset of lung DCs or adoptively transferred DCs (Raymond et al., 2009). Studies by Kim Bottomly's group have elegantly shown that triggering of TLR4 on lung-derived DCs by low doses of LPS promotes Th2 cell development through a myeloid differentiation primary-response gene 88 (MyD88)-dependent pathway (Eisenbarth et al., 2002; Piggott et al., 2005).

There is also evidence that CD11c$^+$ DCs are *necessary* for Th2 cell responses. The Th2 cell-inducing adjuvant alum is used by many groups to induce Th2 cell sensitization to inhaled ovalbumin. However, these Th2 cell responses, as read out by induced T cell proliferation, Th2 cell cytokine production, and IgG1 production, were eliminated when CD11c$^+$ DCs were depleted by diptheria toxin treatment of CD11cDTR Tg mice (Kool et al., 2008a, 2008b). Likewise, alum-exposed DCs clearly induced Th2 cell polarization from naive TCR Tg T cells in a process requiring caspase-1 and IL-1β production (Sokolovska et al., 2007). In vitro studies have also amply demonstrated that human DCs exposed to allergens like the HDM Derp1 allergen (Hammad et al., 2001) and pollen extracts (containing phytoprostanes and NADPH oxidases) (Gilles et al., 2009) acquire Th2 cell-polarizing capacity, even if IL-4 is not made by these exposed DCs. One report identified BDCA-3 (thrombomodulin; CD141)-positive blood DCs as the predominant inducers of Th2 cell responses when exposed to HDM allergen (Yerkovich et al., 2009).

All these findings suggest that DCs can be *sufficient* and *necessary* for Th2 cell immunity to allergens, at odds with the recent reports on basophils being the only Th2 cell inducers. The discrepancy between these studies might be in the precise model antigen (papain as a model proteolytic allergen versus alum-complexed antigen) or organism used, the route of administration (gut versus skin versus lung), the use of splenic DCs versus bone-marrow DCs for comparison with basophils, and the use of specific DC or basophil depletion strategies. All three papers used a depleting antibody against the high-affinity IgE receptor (MAR-1) expressed on mast cells and basophils, and concluded that in the absence of these cells, Th2 cell immunity failed to develop. However, in our experiments employing 10-color flow cytometry to simultaneously look at basophils and DCs, FcεRI is also expressed on a subset of CD11c^{hi} DCs after allergen exposure (unpublished observations) and others have reported this receptor similarly to be induced by virus infection on lung DCs (Grayson et al., 2007). Therefore, it is also possible that some of the effects of "specific" basophil depletion were affecting DC subsets. In our view, it also remains to be conclusively shown whether there are sufficient basophils at the right time and the right place to induce strong and lasting Th2 cell immunity to inhaled allergens relevant for asthma. 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 cell immunity (see Figure 2). In this regard, eosinophils, mast cells, and NKT cells might be similar innate helpers for Th2 cell immunity driven by DCs.

DCs and the Maintenance of Ongoing Allergic Inflammation

Not only do DCs have a role in the primary immune response to inhaled allergens, they also have a crucial role during the effector phase of asthma (Lambrecht and Hammad, 2003). The number of CD11b$^+$ inflammatory DCs is increased in the conducting airways and lung interstitium of sensitized and challenged mice during the acute phase of the response, where they are seen to form clusters with activated T cells, at areas of nerve endings (Beaty et al., 2007; Veres et al., 2009). Because the chemokine receptor CCR6 was first cloned from human lung DCs, and its ligands CCL20 (previously known as MIP-3α) and epithelial β-defensin are produced by airway epithelial cells (Nathan et al., 2009), it was long assumed that CCR6 was the predominant chemoattractant of immature DCs to the airways. Recent studies on mice with mixed bone-marrow chimeras have, however, revealed that CCR2 and not CCR5 or CCR6 is the predominant receptor for attracting Th2-cell-inducing DCs to the lungs of mice after allergen exposure (Robays et al., 2007). The model is most consistent with recruitment of CCR2$^+$ monocytes to the lungs, followed by rapid differentiation to monocyte-derived inflammatory DCs, akin to the situation of alum-induced inflammatory DCs. Not surprisingly, HDM challenge leads to rapid upregulation of the CCR2 ligand CCL-2 (MCP-1) in epithelial cells, although also CCL20 production has been found (Hammad et al., 2009; Nathan et al., 2009). In asthmatics, allergen challenge leads to an accumulation of myeloid DCs and possibly pDCs in the airways, concomitantly with a reduction in circulating CD11c$^+$ DCs, indicating that these cells are recruited from the bloodstream in response to allergen challenge (Jahnsen et al., 2001).

A functional role for inflammatory DCs in the secondary immune response is mostly supported by the fact that their depletion at the time of allergen challenge abrogated all the characteristic features of asthma, such as eosinophilic inflammation, goblet cell metaplasia, and bronchial hyperreactivity (Lambrecht et al., 1998; van Rijt et al., 2005). The defect was restored by the intratracheal administration of bone-marrow-derived $CD11b^+$ myeloid DCs, which most closely resemble the monocyte-derived inflammatory DCs that are recruited upon allergen challenge, and also express SIRPα involved in trafficking to the mediastinal nodes (Raymond et al., 2009; Van Rijt et al., 2004). In humanized SCID mice, allergen-pulsed monocyte-derived DCs also exacerbated allergic inflammation when administered to the lungs of sensitized mice (Hammad et al., 2002). A similar critical role for DCs has also been found in animal models of allergic rhinitis as well as human nasal biopsies from perennial and seasonal allergic rhinitic patients (KleinJan et al., 2006). Together, these experiments demonstrated that inflammatory type DCs are necessary and sufficient for secondary Th2 cell responses to allergen.

Function of Lung DCs in Effector Th2 Cell Responses

After allergen challenge, DCs upregulate the expression of several costimulatory molecules that might be involved in T cell activation or in the differentiation of regulatory T cells (Huh et al., 2003; Van Rijt et al., 2004, 2005). Elegant experiments by Harris et al. (2002) have demonstrated that division of memory Th2 cells occurs mostly in the draining mediastinal nodes during allergen challenge, and this probably explains the need for a migratory APC that takes the inhaled antigen to the nodes. Moreover, in allergen-challenged mice, DCs might also be the most prominent source of the Th2-cell-attracting inflammatory chemokines CCL17 and CCL22, acting on the chemokine receptor CCR4 to attract Th2 cells to the lungs (see Figure 3; Beaty et al., 2007). Two recent studies have indeed demonstrated that this is the case. It was long known that STAT6 deficiency abrogated Th2-cell-selective chemokine production in mouse models of asthma. However, the critical cellular source of STAT6-mediated chemokine production had not been defined. Medoff et al. (2009) demonstrated that STAT6 in bone-marrow-derived myeloid cells was sufficient for the production of CCL17, CCL22, CCL11, and CCL24 and for Th2 lymphocyte and eosinophil recruitment into the allergic airway. In contrast, STAT6 in airway-lining cells did not mediate chemokine production or support cellular recruitment. Selective depletion of $CD11b^+$ myeloid cells (most likely $CD11b^+$ inflammatory DCs) in the lung with CD11b-DTR mice identified these cells as the critical cellular source for the chemokines CCL17 and CCL22 (Medoff et al., 2009). In a second study, we have used a humanized SCID model of asthma in which peripheral blood mononuclear cells from HDM allergic donors were reconstituted, followed by HDM challenge to the lungs. In these mice, human DCs were a predominant source of CCL17 and CCL22 attracting human $CCR4^+$ $CRTH2^+$ Th2 cells to the lungs of mice, excluding any contribution from epithelial chemokines, because these were of murine origin and did not crossreact with the human CCR4 (Perros et al., 2009).

The formation of allergen-specific IgE can lead to IgE-facilitated allergen presentation via DCs. The presence of specific IgE bound to the high-affinity receptor for IgE (FcεRI) on DCs can lead to a 1000-fold lowering of the threshold for allergen recognition and in this way IgE might sustain Th2 cell responses via effects on DCs, as observed in clinical studies in which serum IgE was neutralized with anti-IgE monoclonal antibodies (omalizumab) (Maurer et al., 1998). Although this mechanism has been mainly described in human DCs, demonstration of the FcεRI receptor in lung DCs propose a similar mechanism in the mouse (Grayson et al., 2007).

Many of the Th2 cell effector pathways of allergic inflammation maintain the DC network in an activated state, and often epithelial cells are an important intermediate cell type. Allergen challenge or adoptive transfer of Th2 cells leads to enhanced production of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in epithelial cells. Expression of TRAIL subsequently leads to enhanced production of CCL20, in this way attracting inflammatory $CCR6^+$ DCs and Th2 cells and perpetuating allergic inflammation (see Figure 3; Weckmann et al., 2007). Prototypical Th2 cytokines like IL-4 and IL-13 also feed back on the function of the airway DCs and epithelial cells to directly or indirectly (via DC-activating cytokines like TSLP) stimulate DCs in a STAT-6-dependent way (Medoff et al., 2009; Miyata et al., 2009; Webb et al., 2007). These feedback loops might be important in understanding the chronicity of allergic inflammation.

Endogenous DAMPs Contribute to DC Activation in Asthma

In addition to this adaptive feedback loop, DCs express a plethora of receptors for endogenous DAMPs that are released at sites of ongoing inflammation. As only a few examples, DCs express receptors (protease-activated receptors [PARs] that are activated by proteolytic proteins like tryptase and thrombin) (Hammad et al., 2001). Shortly after insult to the vascular compartment or after pathogen entry in the mucosa, complement activation occurs, and not surprisingly, lung DCs can sense this "acute alert" through expression of the C5a and C3a anaphylatoxin receptors (Kohl et al., 2006). They also express neuropeptide receptors that can respond to neurotransmitters released in response to axon reflexes or efferent neural responses, supported by the fact that lung DCs synapse with unmyelinated nerve endings in and beneath the airway mucosa and produce neurotransmitters (Veres et al., 2009). Lung DCs express receptors for prostaglandins and these acutely released inflammatory mediators can profoundly impact on the migration and maturation of the cell (Hammad et al., 2007; Idzko et al., 2007a). Endogenously released metabolites like extracellular 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 (Idzko et al., 2007b; Willart and Lambrecht, 2009). 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 cell responses (Yang et al., 2008). 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 (Willart and Lambrecht, 2009).

Regulatory Lung DC Subsets

The character of the gut immune response is heavily influenced by the continuous presence of commensal flora and continuous

intake of food antigens, to which an active regulatory response has evolved. Likewise, the lung immune response has evolved to protect the delicate gas-exchange apparatus from overt inflammatory reactions to inhaled harmless antigens, yet allow induction of proper adaptive and innate immunity. It is increasingly clear that again DCs have a crucial role in setting this fine regulatory balance. Despite the fact that most inhaled antigens are transported to the lymph node by lung-derived DCs, the usual outcome after the inhalation of harmless protein antigen is the induction of tolerance. When the model antigen ovalbumin (OVA) is given into the airways of naive mice, it renders the mice tolerant to a subsequent immunization with OVA in alum adjuvant and effectively inhibits the development of allergic airway inflammation (De Heer et al., 2004; Ostroukhova et al., 2004). This process resembles high-dose oral tolerance and is accompanied by a considerable degree of T cell division that ultimately leads to deletional tolerance, implying that at least the antigen is presented by lung-derived DCs to T cells in conjunction with costimulatory molecules (Akbari et al., 2002; De Heer et al., 2004; Vermaelen et al., 2001). Studies with FLT3L-deficient mice have concluded that it is mainly the conducting airway DCs (deriving from the monocytic precursors) that mediate inhalation tolerance (Walzer et al., 2005). Alternatively, partially mature DCs could stimulate the induction of IL-10- and/or transforming growth factor-β (TGF-β)-producing regulatory T cells (see review by Lloyd and Hawrylowicz, 2009, in this issue), in an IL-10 and/or inducible T cell costimulator ligand (ICOSL)-dependent manner (Akbari et al., 2001, 2002; Ostroukhova et al., 2004). Recently, the formation of regulatory DC subsets was shown to be promoted by lung stromal cells that instructed DCreg development via secretion of TGF-β (Li et al., 2008).

Once Treg cells are induced, they control respiratory immunity by interfering with the function of resident or freshly recruited cDCs. Mice lacking the transcription factor Runx3, involved in downstream TGF-β signaling, spontaneously develop asthma features. In the lungs of these mice, there is a strong increase in the number of DCs, displaying a mature phenotype with increased expression of MHC II, OX40-Ligand, and CCR7 (Fainaru et al., 2005) and demonstrating an increased immunostimulatory capacity. Moreover, *Runx3*$^{-/-}$ DCs are able to mount inflammatory responses to otherwise harmless inhaled antigens. In mice normally resistant to HDM-induced asthma and BHR (C3H mice), Treg cell depletion via the CD25-depleting Ab similarly led to increased numbers of pulmonary CD11b^{+} DCs with elevated expression of MHCII, CD80, and CD86 and an increased capacity to stimulate T cell proliferation and Th2 cytokine production. In normally susceptible A-J mice, Treg cells did not suppress inflammation and BHR. These data suggest therefore that resistance to allergen-driven BHR is mediated in part by CD4^{+}CD25^{+} Treg cell suppression of DC activation and that the absence of this regulatory pathway contributes to susceptibility (Lewkowich et al., 2005). In a chronic model of antigen exposure in the rat, CD4^{+}Foxp3^{+} Treg cells were likewise shown to suppress BHR via suppression of airway DC function (Strickland et al., 2006).

Plasmacytoid DCs Suppress Allergic Responses

A series of experiments have suggested that pDCs play an important role in inhalation tolerance and homeostasis in the respiratory tract. After inhalation of OVA, mediastinal pDCs internalize OVA, but in contrast to lung-derived cDCs, they do not present the antigen in an immunogenic manner to CD4^{+} T cells (De Heer et al., 2004; Oriss et al., 2005). However, functional evidence that these cells do influence pulmonary adaptive immunity came from experiments in which pDCs were depleted via antibodies. In pDC-depleted animals, inhalation tolerance was abolished, whereas after adoptive transfer of FMS-related tyrosine kinase 3 (FLT3L)-cultured bone-marrow-derived pDCs, tolerance was induced (De Heer et al., 2004). Similarly, the Th2 cell-like immunopathology induced by respiratory syncitial virus was exacerbated when pDCs were depleted (Smit et al., 2006). Comparative phenotypic and functional analysis of pulmonary DC populations in mice susceptible (A-J) or resistant (C3H) to experimental asthma revealed that susceptibility to BHR is associated with preferential CD11b^{+} cDC allergen uptake and production of Th17 cell-skewing cytokines (IL-6, IL-23), whereas resistance is associated with increased allergen uptake by pDCs (Lewkowich et al., 2008).

Both in vitro and in vivo studies have clearly shown that pDCs stimulate the induction of regulatory T cells, possibly in an ICOSL-dependent manner (De Heer et al., 2004; Ito et al., 2007). Once induced, regulatory T cells could also induce the production of the tryptophan catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) through reverse signaling in pDCs (Grohmann et al., 2007). However, this scenario is less likely in the lung because IDO is mainly involved in promoting Th2 cell immunity by DCs in the lung (Xu et al., 2008). Recently, several studies have more carefully studied how pDCs mediate anti-inflammatory effects in the lung. The anaphylatoxin C5a has been previously implicated in protection from allergic sensitization in the lungs, by setting the balance in favor of pDCs over cDCs (Kohl et al., 2006). In a follow-up study by the same group, C5aR targeting regulated the frequency of pulmonary plasmacytoid DCs expressing costimulatory molecules B7-H1 and B7-DC. Ex vivo blockade of B7-H1 (PD-L1) and B7-DC (PD-L2) increased Th2 cytokine production from T cells of wild-type but not of C5aR-targeted mice, suggesting a protective role for C5a through regulation of B7 molecule expression on plasmacytoid DCs (Zhang et al., 2009). Along the same lines, adoptive transfer of cultured pDCs to lungs of mice, or in vivo expansion of these cells through Flt3L administration suppressed the salient features of asthma when given during secondary challenge. Adoptive transfer of PD-L1-deficient (*Cd274*$^{-/-}$) pDCs failed to transfer this regulatory effect, whereas transfer of *Ido1*$^{-/-}$, *Icosl*$^{-/-}$ pDCs readily did, even in the presence of blocking IFNα antibodies (Kool et al., 2009). It therefore seems that PD-L1 has a predominant effect in dampening lung immune responses by pDCs, although it remains to be established how this affects possible Treg cell induction. The cytokine osteopontin is produced in higher amounts in the airways of asthmatics compared with nonasthmatic controls. It is most likely that osteopontin promotes Th2 cell sensitization by altering the balance between the numbers of tolerogenic and immunogenic lung-derived DCs, although the mechanism involved remains unclear at present (Xanthou et al., 2007).

Lung DCs and IgA Responses

Secretory IgA is a crucial component of first-line immune mechanisms at mucosal surfaces and has many anti-inflammatory

functions that might suppress allergy. IgA responses in the gut have been shown to be imprinted in Peyer's patches by DCs that program B cells to class switch to IgA synthesis. Peyer's patch DCs program B cells to class switch to IgA synthesis and home to the lamina propria of the gut via TGF-β and retinoic acid metabolites (Mora et al., 2006). Although there is only preliminary evidence for an involvement of retinoic acid in lung immune responses (Goswami et al., 2009), lung DCs were shown to promote IgA switching in B cells in a TGF-β-dependent manner (Naito et al., 2008). By employing the mucosal adjuvant cholera toxin B (CTB) subunit to the lungs, we were able to induce IgA responses to coadministered OVA antigen via effects on lung DCs. Adoptive transfer of CTB-exposed DCs induced IgA responses that dampened allergic inflammation and BHR, in a process dependent on secreted IgA, because all protective effects were abolished in the *Pigr*$^{-/-}$ mice, that cannot secrete IgA or IgM into their epithelial lumina (Smits et al., 2009). The expression of PIGR is under the control of Th2 cytokines, leukotriens, and allergen challenge, so this is a pathway of immunoregulation in the lung that clearly deserves more study.

Dendritic Cell Targeting to Treat Allergic Disease

If DCs are so crucial in mounting and maintaining immune responses to inhaled allergen, then interfering 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 viable option, because local depletion of airway DCs was recently shown to lead to severe exacerbation of respiratory virus infection like influenza, whereby the virus failed to be cleared from the lungs and led to severe systemic illness (Geurts-vanKessel et al., 2008). Therefore, we are favoring the idea of targeting the fine-tuning mechanisms whereby DCs maintain allergic inflammation. Recently, several unique 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 the commonly observed lymphopaenia when the drug is given orally (Idzko et al., 2006). FTY720 inhibited the potential of DCs to form stable synapses with naive 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 cell induction by airway DCs (Barrett et al., 2009; Krishnamoorthy et al., 2008; Matsubara et al., 2006), and downstream of this pathway, the signaling intermediate PI3Kδ might similarly be a good drug target.

The number and activation status of lung CD11b^{+} DCs during secondary challenge seems crucial for controlling allergic inflammation, so studying the factors that control recruitment, survival, or egress of DCs from the lung during allergic inflammation will be important, because this might reveal new therapeutic targets (Robays et al., 2007). Eicosanoid lipid mediators such as prostaglandins and leukotrienes can also influence the migration of lung DCs (Hammad et al., 2007). Selective agonists of particular receptors for members of the prostaglandin family might also suppress DC function. Prostaglandin D_2 has pleiotropic effects in the immune system, because of 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 (Hammad et al., 2003a). 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 its respective receptor with small-molecule inhibitors or blocking antibodies might prove very useful. Downstream of these, blockade of CCR4 or its ligands might prevent DC-driven recruitment of Th2 cells.

Disease Modification

Stimulation of the immunoregulatory properties of DCs might reset the balance of the allergic immune response in favor of the development of regulatory T cells 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, both compounds impacting on DC function and stimulating Treg cell differentation (see review by Lloyd and Hawrylowicz, 2009, this issue). 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 nose of patients with allergic asthma and allergic rhinitis (Hammad and Lambrecht, 2006). 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 (Grohmann et al., 2007). Prostaglandins or their metabolites might have the same effect. In the presence of the DP1 agonist BW245C, DCs induced the formation of induced forkhead box P3 (FOXP3)$^{+}$ regulatory T cells from FOXP3^{-} antigen-specific T cells, in a process requiring cAMP and protein kinase A (Hammad et al., 2007). A very similar mechanism was described for inhaled iloprost, a prostacyclin analog that acts on the I prostanoid (IP) receptor expressed by lung DCs (Idzko et al., 2007a; Zhou et al., 2007). Downstream metabolites of prostaglandins include agonists of the PPARγ family. Pharmacological PPARγ 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 (Hammad et al., 2004). Finally, the stimulation of the IgA-inducing capacities of lung DCs might be an appropriate strategy that could have prolonged effects in allergic disease, akin to the effects of desensitization immunotherapy (Smits et al., 2009).

Concluding Remarks

Epidemiological studies on the risk factors and genetics of asthma, as well as mouse models of the disease, have revealed a crucial interplay between airway epithelial cells and DCs. In

a reductionist view, the reason why certain antigens are "allergens" and the reason why the exposure to certain environmental factors promote Th2 cell responses can almost all be brought back to a fundamental interference with the way the antigen is sampled by airway DCs across the epithelial barrier or the way some allergens are able to activate epithelial cells or directly activate DCs. In the future it will be important to study in greater detail how genetic polymorphisms regulating epithelial barrier function and epithelial and DC activation promote allergic sensitization.

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