

Review article

Dendritic cells and the regulation of the allergic immune response

Studies in mouse models of asthma have revealed a critical role for airway dendritic cells in the induction of Th2 sensitization to inhaled allergens. Under some conditions, subsets of dendritic cells can also induce tolerance or Th1 responses to the same allergens, depending on the context in which the antigen is seen. This article discusses various aspects of DC biology as it relates to allergic sensitization and also provides a summary of the recent evidence that dendritic cells function beyond sensitization.

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Asthma is an increasingly common disease that remains poorly understood and difficult to manage. Its incidence has doubled in westernized countries in the last two decades and worldwide costs are estimated to exceed those from tuberculosis and HIV/AIDS combined, necessitating a way to prevent this disorder. Asthma is a Th2 lymphocyte-mediated inflammatory airway disease characterized by airway eosinophilia, increased mucus production by goblet cells and structural remodeling of the airway wall. This leads to variable airway obstruction and to bronchial hyper-responsiveness (BHR) to nonspecific stimuli. In allergic asthma, the presence of high levels of allergen-specific IgE are a reflection of an aberrant Th2 immune response to common inhaled environmental allergens such as house dust mite (HDM) or pollen allergen (1). This process of Th2 sensitization to inhaled allergens occurs at a very young age and is influenced by genetic and environmental factors such as childhood infections and environmental exposure to microbial compounds (2–5). Naïve T cells need mature antigen presenting cells (APC) such as dendritic cells (DC) to proliferate and acquire Th2 effector function in response to antigen encounter (6). Studies in the late 1990s have clearly shown that DCs are crucial in determining the outcome of antigen encounter in the immune system and integrate signals derived from the antigen, its inflammatory context and the host environment into a signal that can be 'read' by naïve T cells in the lymphoid tissues (7). Over the last 10 years, it has become increasingly clear that airway DCs are crucial to the process of allergic Th2

sensitization, particularly through the use of mouse models of asthma. Transgenic and inducible knock out models have been developed to specifically study the role of DCs in the pulmonary allergic response. From these studies a model has emerged in which airway DCs are not only crucial for regulating the process of sensitization to inhaled antigens leading to allergy, but also for controlling established allergic inflammation. Interfering with DC function is a therapeutic concept for the future that could either prevent the onset of sensitization or treat established disease.

Airway DCs control the pulmonary immune response and determine tolerance or immunity to newly encountered antigens

Immature DCs are distributed throughout the lungs and are pivotal in controlling the immune response to inhaled antigen (Fig. 1). A network of airway DCs is located immediately above and beneath the basement membrane of respiratory epithelium of all species studied (8, 9). Fluorescently labeled macromolecules that do not cross the epithelial tight junction barrier are captured by airway DCs and taken to the T cell area of draining mediastinal lymph nodes (MLN) within 12 h (10, 11). DCs taking up antigen in the periphery are functionally immature and unable to stimulate T cells (12). As they arrive in the MLN T cell area carrying their antigenic cargo, DCs express an intermediate array of co-stimulatory

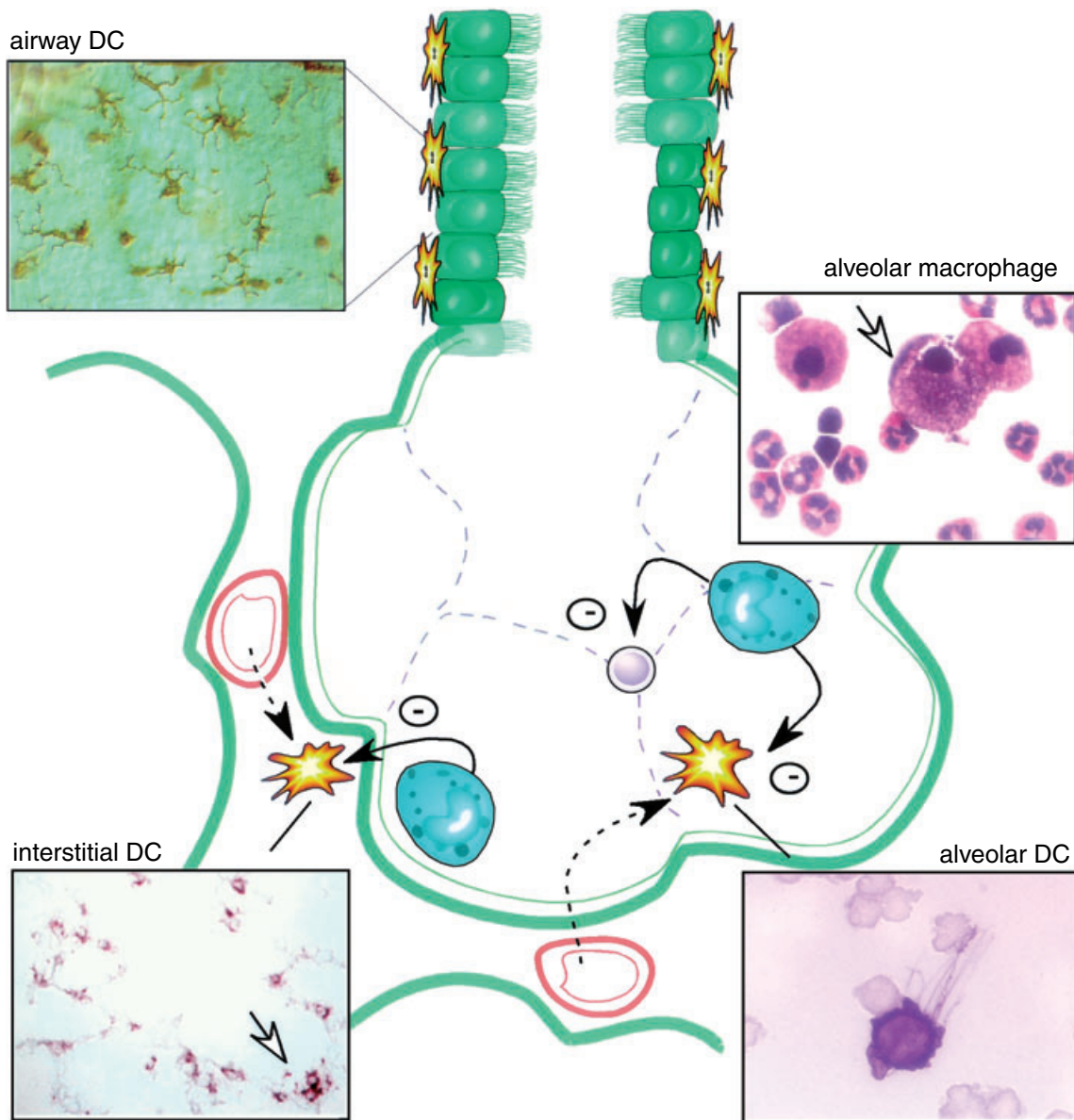


Figure 1. Distribution of dendritic cells in the lung. Airway DCs are located as a network immediately above and beneath the basement membrane, in between basal epithelial cells. Interstitial DCs (stained for CD11c) are composed of a B220⁺ Gr-1⁺ plasmacytoid DC subset and a B220⁻ myeloid DC subset. Their function can be suppressed by alveolar macrophages. Alveolar DCs can consistently be recovered by broncho-alveolar lavage in humans, rats and mice, particularly when inflammation is induced. The function of these cells can similarly be suppressed by alveolar macrophages. Alveolar macrophages can also directly suppress the function of T cells that are found in large numbers in the lung interstitium and alveolar compartment.

molecules (somewhere between that of naive and mature DCs) and high levels of MHC class II molecules capable of inducing proliferation of naive T cells (10). By developing a new model of adoptive transfer of antigen specific T cells obtained from naive TCR transgenic donors to nontransgenic recipients, we have been able to study the outcome of antigen presentation by DCs in the lung on the generation of primary T cell responses. Our studies have demonstrated that airway exposure to harmless endotoxin-free antigens leads to vigorous T cell prolifer-

ation *in vivo*, mainly in the superficial cervical and MLNs, but not in the nondraining lymph nodes or the lung itself (Fig. 3) (11, 13). Despite the massive induction of T cell proliferation, the functional outcome for the immune response is however tolerance (6, 13). The most probable explanation for this observation is that harmless antigens fail to fully activate the airway DC network, such that partially mature DCs induce an abortive proliferative response of unfit T cells that fail to reach a certain threshold required for their survival and differentiation

into effector T cells (14). After stimulation by immature DCs, T cells die 'by neglect' and therefore deletional tolerance is one plausible explanation for the occurrence of inhalational tolerance (13, 15). Others have shown that antigen presentation by partially mature airway DCs that express the co-stimulatory molecule ICOS-L and secrete the immunoregulatory cytokine IL-10 induce the formation of regulatory T cells (Treg) with a capacity to inhibit subsequent inflammatory effector responses (16, 17). We believe that deletional tolerance and tolerance mediated by Tregs are not mutually exclusive and operate together in maintaining immune homeostasis in the periphery.

Our most recent findings have demonstrated that a specific subset of pulmonary DCs, called plasmacytoid DCs are present in the lung and are crucial for maintaining tolerance to inhaled harmless antigens (Fig. 1) (18). These Gr-1⁺ B220⁺ lung pDCs also take antigens to the draining nodes, where they strongly influence the immune response induced by classical lung myeloid DCs. As others had suggested a tolerogenic potential for cultured pDCs *in vitro* (19), we hypothesized that these cells were important for maintaining tolerance to inhaled ovalbumin (OVA) in the lung. When we depleted pDCs using an antibody to Ly6C/G or a specific pDC depleting antibody 120G8, inhalation to otherwise inert OVA led to development of all cardinal features of asthma, such as airway eosinophilia, goblet cell hyperplasia, Th2 cytokine secretion, and IgE production. Conversely, when we transferred allergen-pulsed pDCs prior to sensitization to allergen in an immunogenic protocol, we could no longer induce asthma (18). How pDCs mediate tolerance is under investigation, but we have evidence to suggest that they suppress the generation of effector cells from proliferating naïve T cells, either by delivering a negative signal to T cells directly (through PDL-1 and/or tryptophan depletion) or by competing with myeloid DCs. We also demonstrated that lung pDCs exposed to antigen *in vivo* were able to induce the formation of regulatory T cells *ex vivo*. It will therefore be of great interest to study if pDCs are also implicated in inducing T reg cells *in vivo*.

If the usual outcome of antigen encounter to harmless antigen is tolerance, how can sensitization to inhaled allergens ever occur? Two mechanisms that would lead to sensitization can be envisaged, and both can occur at the same time. First, sensitization is likely to occur when DCs reach full maturity during encounter with allergens. Secondly, suppression of the tolerogenic potential of pDCs would also lead to sensitization. Several epidemiological and mouse experimental data provide important clues that signals derived from the microbial world can influence sensitization through modulation of both mDCs and pDCs. When exposure to inhaled allergen is accompanied by an inflammatory stimulus, such as concomitant environmental LPS exposure or respiratory viral infection, fully mature DCs reach the draining lymph nodes and naive allergen-specific T cell proliferation leads

to 'fit' effector T cells, with a potential to orchestrate airway inflammation (15, 20, 21). Exposure to LPS and virus infection indeed recruits airway myeloid DCs into the lung and induces their full maturation upon arrival in the mediastinal nodes (22). There is also evidence that many allergens can induce maturation of DCs directly in the absence of microbial stimuli, and this would explain why sensitization to allergens is so common. Many allergens, such as Der p 1 derived from HDM directly induce DC maturation through their enzymatic activity (23). To prove the concept that mature myeloid DCs indeed are responsible for sensitization, we have injected allergen-pulsed mature BM-derived myeloid DCs into the trachea of naive mice or rats and observed the occurrence of sensitization and subsequent allergic airway inflammation upon rechallenge with the relevant allergen (24, 25). However, we failed to induce sensitization when cultured pDCs or immature myeloid DCs were injected into the lung (B. Lambrecht, unpublished data; 26, 27). These data unequivocally demonstrated that myeloid DCs were sufficient to cause sensitization to inhaled harmless antigens. Under conditions of intense immune stimulation by mature DCs, T cell proliferation was accompanied by differentiation into effector cells, in a process tightly linked to cell division cycle. After dividing first in the MLN, primed CD69^{lo} CD44^{hi} CCR5⁺ effector T cells that had undergone at least three divisions returned to the lungs as cytokine-producing effector T cells (Fig. 2) (13, 28). After 2–4 days, divided T cells also recirculated to the non-draining lymph nodes and the spleen, thus most probably representing the central memory T cells (T_{cm}) described *in vitro* (13, 29).

Enhancement of myeloid DC function is not the only possibility leading to sensitization. Modulation or deficiency of the intrinsic tolerogenic function of pDCs would be the other possibility. Two findings support this hypothesis. In one study, allergic children had significantly less circulating pDCs compared with nonallergic children (30). Secondly, lower respiratory tract viral infection modulates sensitization to allergens but also strongly interacts with pDC function (31). As part of their normal function as natural interferon- α producing cells, pDCs undergo functional maturation during viral infection and become immunogenic rather than tolerogenic cells (32). We are currently investigating if respiratory viral infections such as respiratory syncytial virus (para) influenza virus and metapneumovirus, known to influence allergic sensitization, do this by modulation of pDC function.

Dendritic cells and the hygiene hypothesis

As DCs express many pattern recognition receptors and communicate extensively with cells of the innate immune system, microbial stimuli accompanying a particular antigen can profoundly change the type of Th response being induced and could therefore also

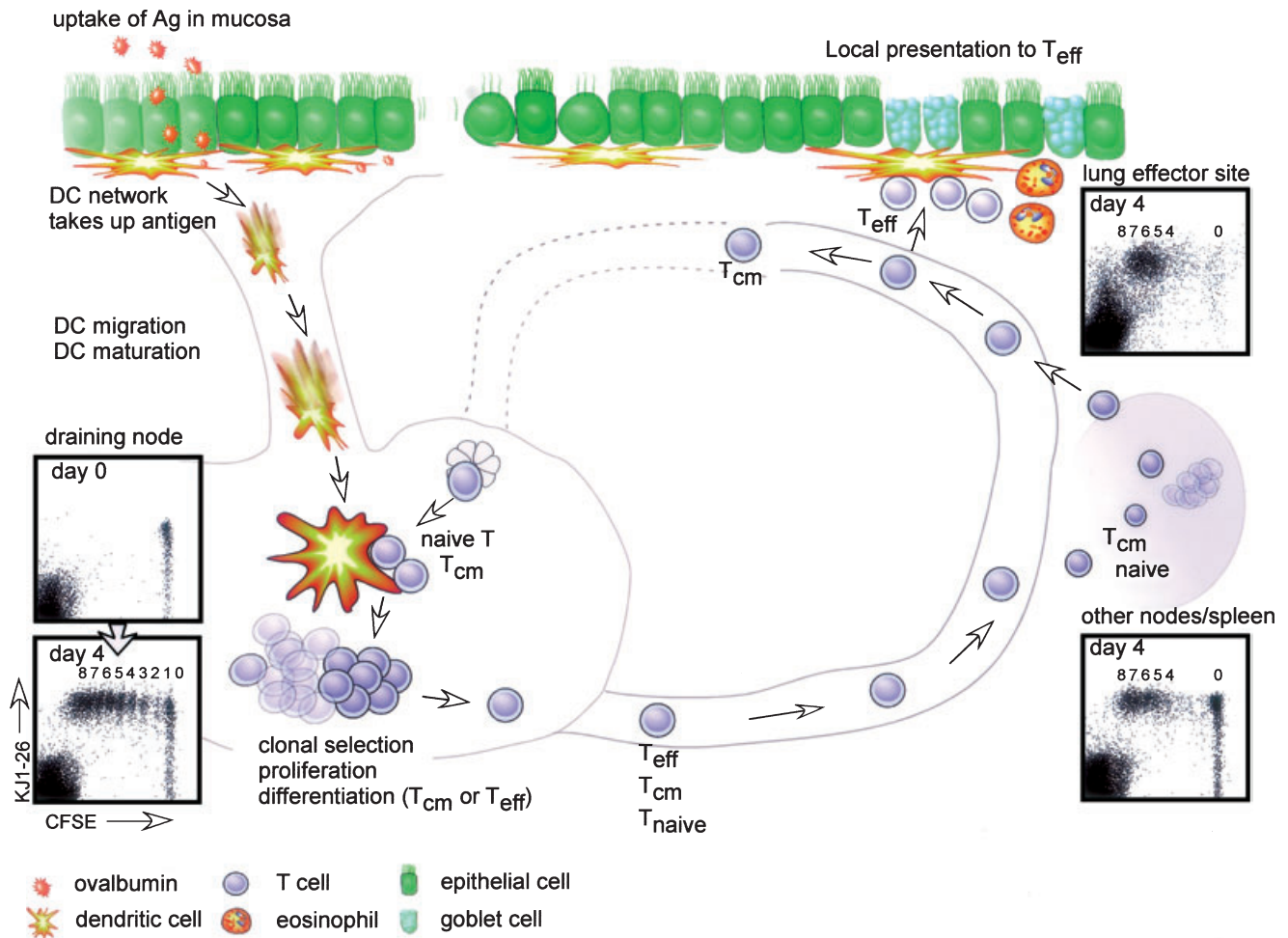


Figure 2. An integrated overview of DC and CD4 T cell migration during primary and secondary immune responses. Antigen is taken up by DCs across the mucosal impermeable barrier. Mucosal DCs continuously migrate from the lungs to the mediastinal lymph nodes (MLNs), at the same time undergoing functional maturation to stimulate naïve T cells. In the presence of inflammation, this process is amplified, enhancing the likelihood that pathogenic substances will be presented to recirculating naïve T cells or central memory T_{cm} cells. At the same time, DC maturation will be fully induced. Allergens can induce maturation of DCs in the absence of inflammation through the epithelial release of GM-CSF. When mature DCs arrive in the MLN, they select specific T cells from the polyclonal repertoire of cells that migrates through the high endothelial venules (HEVs) and T cell area. Within 4 days, this will lead to clonal expansion of antigen-specific T cells, with some cells undergoing eight cell divisions within this time. In the figure, the clonal expansion of carboxyfluorescein (CFSE) labeled OVA-specific naïve CD4 T cells, recognized by the clonotypic Ab KJ1-26 in response to immunization with OVA-pulsed DCs is illustrated. When a T cell has acquired a certain threshold number of divisions ($> 3-4$ divisions), it will downregulate expression of CD69 and leave the MLN, to become either a T_{cm} cell or an effector T cell. This is where migration pathways separate, and consequently the anatomical requirements for reactivation diverge. The T_{cm} cells will extravasate in other non-draining nodes and spleen, and will eventually accumulate in the spleen over time. Reactivation of these cells will therefore only occur in central lymphoid organs. By contrast, effector T cells will extravasate in peripheral sites of inflammation, including the lung when the original inflammation is still present. In contrast to naïve T cells that are excluded from lung tissues, these effector T cells can be stimulated by local airway DCs to exert their effector function. In this scenario, alternative antigen presenting cells might be eosinophils or even epithelial cells, expressing MHC molecules.

suppress sensitization (33). When full DC maturation is induced in the lung in the absence of strongly polarizing signals, the functional outcome is stable Th2-type immunity, as shown by the occurrence of Th2 recall responses (12, 24, 34, 35). However, very high doses of LPS or proliferative infection with influenza virus at the time of OVA exposure lead to stable Th1 immunity in

the lung (12, 15, 21). The known epidemiological association between conditions of poor hygiene and low socioeconomic status (associated with high level exposure to LPS, exposure to parasitic *Schistosoma* or mycobacterial infections) and reduced IgE levels and atopic diseases in children might be explained by modulation of DC function (3-5). Modulation of DC

function by these factors might thus lead to Th1 immunity or alternatively to tolerance mediated by Treg cells, both with a potential to reduce Th2 sensitization. This was demonstrated in a direct manner by showing that exposure of DCs *in vitro* with LPS, gram positive bacterial peptidoglycan or killed mycobacteria abolished the potential of DCs to induce Th2 sensitization to inhaled allergens in the lung (B. Lambrecht and H. Hammad, unpublished data; 28). LPS-exposed DCs induced Th1 immunity in the lung compartment in a process that was surprisingly independent of the Th1 polarizing cytokine IL-12 (28). In a large epidemiological observation, high level exposure to LPS in mattress covers was also associated with a reduced risk of atopic diseases, but surprisingly with lower levels of IL-12, suggesting that alternative explanations besides Th1 skewing can be found for the effect of LPS on sensitization (36). It will be interesting to study the effects of other microbial motifs on sensitization induced by DCs. Not all microbial motifs reduce sensitization through Th1 induction. It was demonstrated that prostaglandin D₂, produced by the parasite *Schistosoma mansoni* maintains airway DCs in an immature state leading to a strong reduction in Th2 sensitization (11, 27). Prostaglandin D₂, acting on the DP receptor and through prostaglandin J₂ metabolites on the PPAR- γ nuclear receptor expressed in DCs, leads to formation of Treg cells that suppress asthmatic inflammation in an IL-10 dependent fashion (26, 37). Given the fact that *Schistosoma* also contains other lipids such as a unique lysophosphatidylserine with immunomodulatory capacities on DCs there are multiple pathways by which *Schistosoma* might suppress asthmatic inflammation (38).

Dendritic cells are crucial players during established allergic airway inflammation

Recent evidence points out that DCs have a pathophysiological role in asthma beyond the sensitization phase (39). It is exceedingly difficult to prove that a cell has an important role in a complex multicellular disease process such as asthma, but animal models are ideally suited for this purpose. As a simplification, one can try to prove a causal relationship between a cell and a disease by satisfying the modified Koch's postulates (Fig. 3). These postulates form the intellectual basis for microbial science to prove a causal relationship between a microbe and a disease. The logic is simple yet powerful and has been adapted to cellular immunology to accommodate the availability of pharmacological antagonists and modern molecular techniques such as targeted gene deletion. In the current era, it can be said that a certain cell or mediator is involved in a particular disease when it is consistently found in higher numbers or concentrations in cases of the disease compared with healthy controls

(postulate 1), when administration of it causes features of the disease (postulate 2), and when removing it through antagonism or genetic knock-out eliminates features of the disease (postulate 3).

Allergic airway inflammation is accompanied by an increase in airway DCs

We have shown that there is an 80-fold increase in the number of myeloid DCs in the airway mucosa and bronchoalveolar lavage fluid of mice and rats with experimentally induced asthma, supported by an increased production of precursors from the bone marrow (Fig. 3 top panels) (40–42). In contrast to DCs in naive animals, airway DCs in OVA-challenged mice had a mature phenotype, indicating that interaction with primed T cells could occur locally in the airways (Fig. 4). In support, Huh *et al.* clearly showed that airway DCs formed clusters with primed T cells in the airway mucosa, leading to local maturation of DC function (43). Julia *et al.* have also identified a subset of long lived CD11b⁺ CD11c⁺ F4/80⁺ cells in the lungs of mice with airway inflammation and this long lived population had a prolonged capacity for presenting antigen to Th2 cells *ex vivo* (44). The scenario in which DCs interact locally in the airways with primed Th2 cells is very likely given the fact that airway DCs produce chemokines (CCL17) that selectively attract memory CCR4⁺ Th2 cells to the lung (45). Local antigen presentation in the airway mucosa or submucosa is however not the only site of interaction between DCs and primed T cells. During antigen challenge of primed mice, there is increased migration of airway DCs to the MLN (41). The reason for this is unclear at present, but could involve the restimulation of resting central memory T_{cm} cells, inducing their proliferation and differentiation to new rounds of effector T cells that go back to the effector site and control allergic inflammation (Fig. 5) (13, 46). Moreover, even during secondary immune responses to inhaled allergen, there might be activation of naïve antigen-specific T cells.

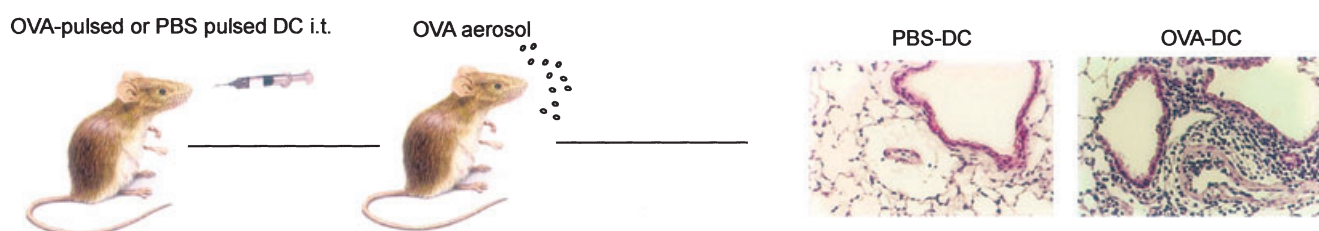
Administration of antigen-pulsed DCs induces and exacerbates asthmatic features

Airway DCs cause Th2 sensitization to inhaled allergen (see above). Not surprisingly, administration of OVA-pulsed myeloid DCs to the airways of naïve mice and rats induces sensitization to OVA, leading to a vigorous Th2 response and eosinophilic airway inflammation, goblet cell hyperplasia and bronchial hyper-reactivity after rechallenge of the airways with OVA aerosol (Fig. 3 middle panels) (24, 25, 47). This response depends on the provision of co-stimulation (CD80, CD86, T1/ST2) to T cells. OVA-pulsed DCs are also capable of inducing the full asthmatic phenotype when injected intratracheally in primed mice, in the absence of antigen aerosol. To our surprise, antigen presentation by DCs in sensitized mice

Koch I : number of DCs increased in mice with experimentally induced asthma



Koch II : administration of DCs to the airways of naïve mice induces features of asthma



Koch III : removing DCs from the airways of sensitized mice reduces features of asthma

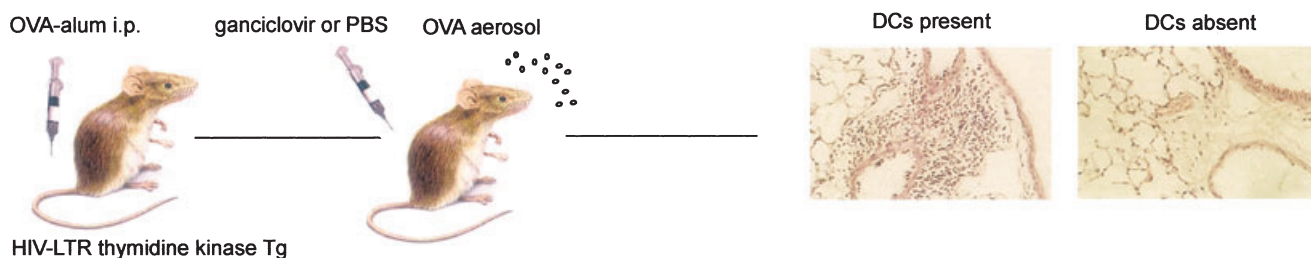


Figure 3. Koch's postulates applied to dendritic cells in experimental asthma. First it was shown that when challenged with ovalbumin (OVA) aerosol, OVA sensitized mice developed large increases in the number of $CD11c^{+} MHCII^{+}$ DCs in the airway mucosa (top panel), draining lymph nodes and bone marrow (not shown). When OVA pulsed DCs were given to naïve mice, they were able to induce sensitization to inhaled antigen, as exemplified by the occurrence of peribronchial and perivascular eosinophilic airway inflammation and goblet cell hyperplasia upon antigen challenge to the lung (middle panel). Finally when mice were first rendered allergic, removal of DCs using ganciclovir in HIV-LTR thymidine kinase transgenic mice during allergen challenge completely eliminated all features of airway inflammation (lower panel).

was less dependent on provision of co-stimulatory molecules CD80, CD86 and B7RP-1 to primed T cells, as airway eosinophilia was still induced in the combined absence of these molecules on DCs (B. Lambrecht and L. van Rijt, unpublished data). Even more strikingly, eosinophilic airway inflammation could also be induced by repetitive injection of DCs not exposed to allergens. It is therefore tempting to speculate that intrinsic asthma is caused by DCs presenting some form of self-antigen, an area of active research in our laboratory. The exacerbation of Th2 responses *in vivo* also occurs in human Th2 cells. We reconstituted severe combined immunodeficient (SCID) mice with primed T and B cells from HDM-allergic asthmatic donors. Mice subsequently received Der p 1 pulsed DCs or unpulsed DCs intratracheally, followed by HDM aerosols. Only mice that received Der p 1 pulsed DCs developed peribronchial infiltrates of

human cells, human allergen specific IgE synthesis and airway eosinophilia (48). These combined experiments suggest that DCs not only induce an asthmatic phenotype in healthy mice, they also exacerbate it in already sensitized mice.

Removal of airway DCs from sensitized mice eliminates asthmatic features induced by antigen aerosol

Removing myeloid DCs from mice is made particularly difficult by the unavailability of depleting monoclonal Abs. The few mouse strains that genetically lack DCs also have profound deficiencies of T cells and abnormal anatomy of the immune system, and therefore cannot be properly sensitized to allergens. Inducible transgenic systems in which suicide genes are under the control of DC-specific promoters allow the conditional knock out

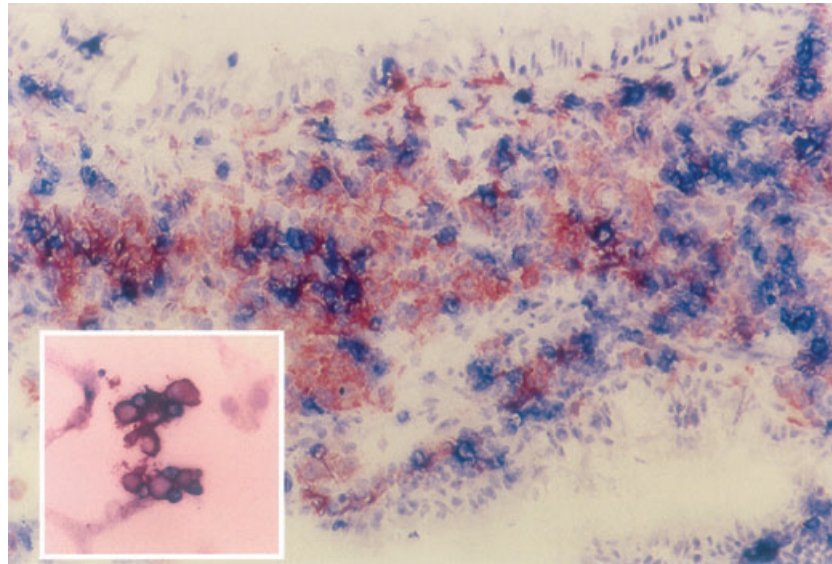


Figure 4. Interaction of CD11c⁺ DCs and CD4⁺ T cells in peribronchial inflammatory infiltrates. Balb/c mice were OVA sensitized and challenged to induce eosinophilic airway inflammation. One day after the last of three aerosol challenges, peribronchial infiltrates were analysed for the presence of CD4⁺ T cells (blue) and CD11c⁺ DCs (red) in frozen lung sections. At a larger magnification (insert), it is shown that DCs and T cells are interacting and form clusters *in situ* in the lung.

of DCs after sensitization to experimental antigens (49, 50). In HIV-LTR-thymidine kinase transgenic mice (TK-Tg), the HIV-LTR promotor drives high level expression of the TK suicide gene through relB transcription factors, allowing the selective depletion of myeloid DCs by ganciclovir treatment. Using these mice, we have shown that the systemic removal of myeloid DCs cured all the features of asthma when DCs were removed during the aerosol challenge period, accompanied by a decrease in Th2 effector cytokines (Fig. 3, lower panels) (9). We have recently extended these findings in CD11c-diphtheria toxin receptor transgenic mice, in which airway DCs can be deleted conditionally through airway administration of diphtheria toxin, without affecting systemic DCs. Again, selective removal of airway CD11c⁺ cells completely eliminated airway eosinophilia, goblet cell hyperplasia and bronchial hyper-reactivity to metacholine concomitant with decreased Th2 effector generation (B. Lambrecht and L. van Rijn, unpublished data). Even when DC depletion was initiated in the middle of an aerosol exposure period, when inflammation was full blown, the suppressing effect on inflammation was evident. As these experiments were carried out in already sensitized mice with a full Th2 bias, they are definite proof that DCs could be therapeutic targets for established asthma.

Other APCs are of less importance in asthma

These data indicate that DCs are essential APCs during both the initiation and maintenance of eosinophilic airway inflammation. Does this eliminate a role for other APCs

during asthmatic inflammation? Alveolar macrophages are scavenger cells that ingest particulate antigen and suppress adaptive immunity by producing mediators that inhibit T cell and DC activation (51). When alveolar macrophages were depleted during exposure to harmless antigens, both the primary immune response and the secondary response during challenge were greatly enhanced (52). Finally, when adoptively transferred to naive animals, we and others showed that alveolar macrophages cannot prime for eosinophilic airway inflammation, but rather induce protective Th1 responses following activation with IFN- γ (25, 53). Although B cells can serve as APCs during cognate interaction with Th2 cells, sensitized B-cell-deficient mice can develop all the cardinal features of asthma (54). Epithelial cells have also been attributed antigen presenting capacity for primed T cells *in vitro* and epithelial cells in asthmatics can express MHC class II and co-stimulatory molecules. Perhaps the best alternative candidate APC in allergic airways would be the eosinophil. Numerous studies have demonstrated that eosinophils can express MHC class II and co-stimulatory molecules (54). Similar to DCs, these cells accumulate in areas of allergic airway inflammation and in the MLNs (55). Administration of eosinophils to sensitized mice amplifies Th2 responses through unclear mechanisms (55, 56). We have recently addressed the antigen presenting capacity of eosinophils. Eosinophils sorted from OVA-challenged lung were unable to induce division of naive OVA-specific TCR-transgenic T cells *in vitro* and after intratracheal injection *in vivo* (57). Therefore, eosinophils might have an amplifying role in antigen presentation, but seem to be unable to induce Th2 responses.

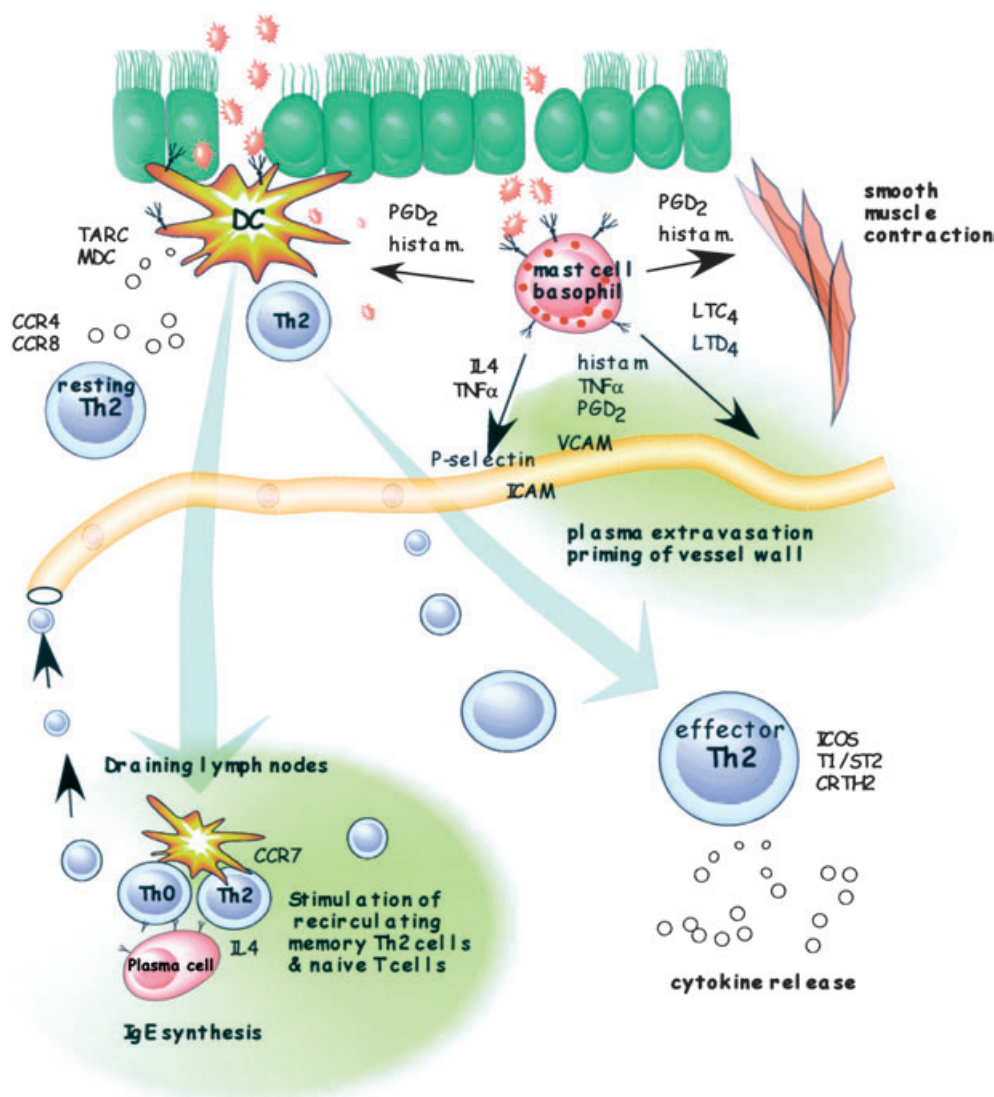


Figure 5. Role of airway DCs during ongoing inflammation. The first cells that recognize allergen are DCs, epithelial cells and mast cells. These cells can all bind allergen-specific IgE either through FcεRI or CD23, possibly enhancing recognition of the allergen. The allergens induce the release of prostaglandins, histamine, chemokines, cytokines, neuropeptides and complement-breakdown products, which attract circulating DCs to the mucosa and influence DC function. In the case of house dust mite allergens, the epithelium releases GM-CSF, leading to local activation of DC function. At the same time, the Der p 1 allergen activates DCs to produce CCL17 and CCL22 leading to the local attraction of Th2 cells expressing CCR4 and possibly CCR8. The attracted Th2 cells can directly exert their effector function when activated by APCs, but fail to proliferate locally. The allergen also induces the migration of allergen loaded DCs to the draining lymph nodes by increased expression of the CCR7 receptor, which is necessary for homing to the T cell area. In these area, DCs attract recirculating resting central memory Th2 cells and possibly some naive allergen-specific T cells, inducing their proliferation and further differentiation to Th2 cells. Effector Th2 cells are generated that are biased to migrate to the inflamed lung tissue, where they collaborate with locally activated Th2 cells to orchestrate the eosinophilic inflammation.

A role for DCs in human asthma

By combining data obtained from human biopsy studies and intervention studies with data obtained from humanized SCID models of asthma, we propose that DCs also contribute significantly to allergic diseases in human. First, it has been shown by us and others that CD1a⁺ DCs accumulate in the lamina propria and epithelium of

steroid-naïve asthmatic patients, and that allergen challenge increases the number of myeloid DCs even further (58–61). Increased numbers of DCs have also been found in related allergic diseases such as allergic rhinitis and atopic dermatitis, also characterized by eosinophilic inflammation (62). Next, we have shown that the intra-tracheal administration of human Der p 1-pulsed myeloid DCs leads to a boosting of human allergic responses in

reconstituted huSCID mice (see above) (48). As for mouse DCs in allergen-challenged airways, human monocyte-derived DCs from HDM-sensitive asthmatics stimulated with Der p 1 preferentially produced the Th2 selective chemokines CCL17 and CCL22, to attract CCR4⁺ Th2 cells preferentially (63). This is of physiological significance as the *in vivo* use of CCR4 receptor blocking antibodies reduced airway inflammation in the hu-SCID model of asthma, by reducing the DC-Th2 interaction (B. Lambrecht and H. Hammad, unpublished data). Finally, intervention studies with inhaled steroids in patients with asthma or allergic rhinitis have shown a correlation between the reduction in number and function of airway DCs and clinical efficacy and this might partly explain why these drugs are so effective (58, 60, 62).

Directions for future research

These data in mice, humans and huSCID mice show that DCs have a key role in the pathogenesis of the pulmonary allergic response during sensitization and during established disease. This places the DC in the forefront of a complex multicellular disease process, in which aberrant T cell responses, genetic influences on allergen recognition, structural changes to the airway wall and inherent epithelial defects all play an important role (Fig. 6). Important questions that remain unanswered in human asthma are the influence of epithelial cells, fibroblasts and inflammatory cells and their products on the function of airway DCs in established inflammation. Allergen challenge leads to local activation of the nervous system and to the release of neuropeptides (substance P, neurokinin A, calcitonin gene related peptide) that can influence DC function (64, 65), but the functional implications need to be worked out better. After recognition of allergen through specific IgE, mast cells release histamine, PGD₂ and neutral proteases. We have recently shown that PGD₂ slows the migration of endogenous airway DCs to the draining lymph nodes, which could lead to DC accumulation in allergen challenged lungs (11, 27). We are currently studying the consequences of mast cell activation on DC behavior *in vivo*. Bronchial epithelial cells are at the interface of the external and internal environments and react to allergens, viruses and pollutants by releasing chemokines that attract immature DCs to the lung, and by producing GM-CSF or other cytokines that induce DC maturation (66, 67). It will therefore be interesting to study if and how epithelial cells obtained from asthmatics or healthy subjects interact differently with DCs and how this would feed back on the epithelial-mesenchymal trophic unit. It will be important to study whether DCs are also involved in the differential tissue expression of allergic diseases such as atopic dermatitis, allergic rhinitis or asthma in patients with sensitization to common allergens. In this context, the residence of DCs among different neighbors, such as

keratinocytes or bronchial epithelial cells may profoundly influence the function and survival of DCs. One important aspect that needs study is the influence of the structural remodeling that occurs in chronic asthma on the function of DCs. If these structural changes could enhance DC function, they might provide an answer to the question of why some allergic asthma is progressive, despite allergen avoidance. It could also turn out that DCs become progressively less important as structural abnormalities of the airways are more pronounced. Finally, it is tempting to speculate that even nonatopic asthma is driven by airway DCs that stimulate Th2 cells either autonomously in response to self-antigen (e.g. cytokeratin 18) or in response to some hitherto unrecognized environmental antigen.

Implications for drug development

One way in which this knowledge could be applied is the design of new vaccines to prevent sensitization to inhaled antigen. If we know more precisely how DCs react to both allergens and microbial patterns, this information could be exploited into design of more efficient desensitization protocols or could unravel which microbial motifs offer protection to allergy without causing pathology. Alternatively, therapies targeted at amplifying the intrinsic tolerogenic capacity of lung plasmacytoid DCs may limit the development of new sensitization.

Therapies aimed at interfering with airway DC function in established asthma are the ultimate goal. Conclusive evidence that reduction in the number and activity of DCs would be beneficial in ongoing asthma in humans is lacking at present and awaits the development of DC-selective drugs for human use. As DCs are crucial for the immune response to microorganisms and tumors, and control of autoimmunity, therapies should be aimed at suppressing DCs locally in the lung and therefore use respirable formulations. Currently, only inhaled steroids reduce the number and functional activity of airway DCs (58). Despite the reduction in DCs, there is no increased risk of bacterial or viral lung infection in patients taking these drugs, which illustrates the feasibility of targeting DCs without inducing local immunodeficiency.

Conclusion

We propose a role for airway DCs not only in the sensitization to inhaled allergens, but also in the maintenance of eosinophilic airway inflammation. Although this review has mainly focused on the role of DCs in asthma, many of the concepts are also applicable to allergic rhinitis and atopic dermatitis. Dendritic cells are extremely potent regulators of the immune response to allergens and therefore obvious targets in the fight against the ever increasing allergic diseases.

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