

REVIEW

Dendritic cells in asthma: a function beyond sensitization

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Summary

Allergic asthma is one of the most common chronic diseases in western society, characterized by variable airway obstruction, mucus hypersecretion and infiltration of the airway wall with T-helper type 2 (Th2) cells, eosinophils and mast cells. If we are to devise new causal therapies for this disease, it is important to elucidate how Th2 cells are activated and respond to intrinsically harmless allergens. Dendritic cells (DCs) are the most important antigen-presenting cells in the lung and are mainly recognized for their exceptional potential to generate a primary immune response and sensitization to aeroallergens. Much less attention has been paid to the role of DCs in established inflammation. Based on functional studies in a murine model for asthma, in this review article, we propose that DCs are essential for generating allergen-specific effector Th2 responses in ongoing inflammation in sensitized mice. A better understanding of the role of DCs in the maintenance of the inflammatory response to allergens in asthma should lead to new therapeutic approaches intervening at the top of the inflammatory cascade.

Current views on asthma

Asthma is a chronic inflammatory disorder of the airways, characterized by the clinicopathologic symptoms of intermittent and reversible airway obstruction, enhanced mucus production, chronic eosinophilic inflammation and bronchial smooth muscle cell hypertrophy, eventually leading to impaired epithelial repair and airway remodelling. It is generally believed that asthma is controlled by T-helper type 2 (Th2) cells secreting IL-4, IL-5, IL-9, IL-10, IL-13 and granulocyte macrophage-colony stimulating factor (GM-CSF), whereas mast cells and eosinophils exert important effector functions. Under physiological conditions, activated Th2 cells will induce an immune response to fight extra-cellular or parasitic infections. In allergic asthma, IL-4 and IL-13 induce the expression of cell adhesion molecules on inflamed endothelium and epithelial production of chemokines, leading to the recruitment of inflammatory cells, stimulating the production of IgE by B cells and causing bronchial hyper-reactivity. IL-5 is important for the growth, differentiation and activation of tissue eosinophils. IL-9 is important for mast cell growth and activation. GM-CSF stimulates the growth of eosinophils and the activation antigen-presenting cells (APCs) [1, 2] (see Fig. 1). Moreover, cytokines produced by Th2 cells and inflammatory cells can affect the airway epithelium, subepithelial (myo-)fibroblasts and smooth muscle cells in the lungs, thus leading to structural abnormalities. As effector Th2 lymphocytes are intimately involved in controlling the various aspects of this

disease, elucidating the mechanisms by which these cells are activated is crucial for therapy development.

Dendritic cells in the sensitization to allergen

The role of dendritic cells (DCs) in allergic Th2 sensitization, believed to be one of the main risk factors for developing asthma, has received considerable attention in the last two decades. The results of many studies are elucidating step-by-step the mechanism by which DCs are able to direct immune responses to allergen.

Location of dendritic cells

First, the location of DCs enables them to have direct contact with incoming antigens. Many years ago, Holt has shown that a DC network is situated immediately above and beneath the basement membrane of the upper and lower airways (see Fig. 2), enabling these cells to sample the epithelium for inhaled antigens [3–5]. From this location, DCs are able to form long extensions throughout the epithelium to the airway lumen while maintaining the epithelial barrier function intact, as shown in the gut and lung [6, 7]. Mucosal DCs have an immature phenotype, meaning that they have exceptional allergen uptake and processing capability, but lack the power to stimulate naïve T cells [8]. With antigen uptake in the presence of a danger signal, DCs undergo a developmental programme called maturation, whereby they lose their capacity to take up antigen and acquire a phenotype of professional APCs expressing all the costimulatory molecules and chemokines to attract and stimulate naïve T cells. DCs transport the antigen from the mucosa to the draining lymph nodes of the lung, after degradation of the antigen in short immunogenic peptides and loading the peptide on major histocompatibility complex II (MHCII) molecules. DCs

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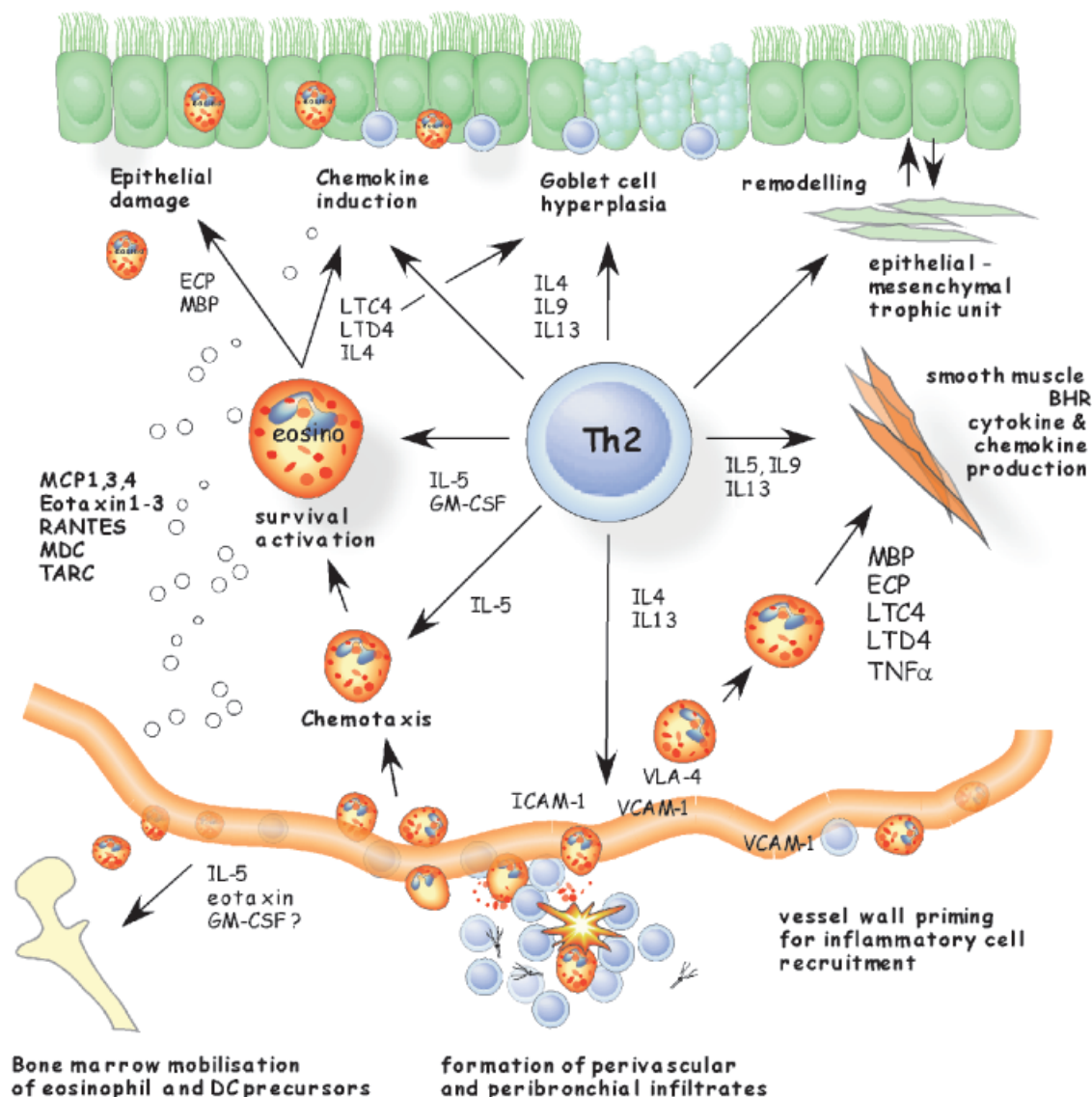


Fig. 1. Effector functions of T-helper type 2 (Th2) cells. Effector Th2 cells produce several cytokines and chemokines that exert their effects on eosinophils, airway smooth muscle, epithelium and endothelium, resulting in several pathologic characteristics of asthma.

migrate to the T cell-rich area of draining lymph nodes where naïve T lymphocytes continuously pass by [9, 10].

Upon encountering specific T cells, DCs undergo a stable interaction with these cells, ultimately leading to the formation of an immunological synapse and T cell activation, division and differentiation [11]. DCs can influence polarization of T cells by a specific expression and secretion cytokine pattern [12], depending on their lineage [13, 14], their maturation status and the consequent costimulatory molecule pattern [15] and the environment they are in [16]. The nature of an antigen can instruct the DC to influence the polarization of T cells by ligation of specific receptors during uptake [17, 18]. DCs are crucial for induction of effector T cells because an insufficient ligation will lead to anergic T cells or T cells that are not able to survive [19].

Link between innate and adaptive immunity

Secondly, this transport from the mucosa to the draining lymph nodes enables the (myeloid) DCs to establish a link

between the innate immune response and the adaptive immune response. There are many suggestions that DCs can establish this link between a non-specific innate immunity, based upon recognition of the type of pathogen, and specific adaptive immunity directed against a non-specific peptide of the allergen. The innate immune responses are based on preserved pathogen-associated molecular patterns (PAMPs) that identify the type of pathogen. It is therefore remarkable that DCs need to recognize a PAMP together with the antigen to prime CD4⁺ T cells efficiently [20].

The recognition of PAMP acts as a third signal, in addition to the required signal 1 (T cell receptor and MHCII interaction) and signal 2 (costimulation by costimulatory molecules, like CD80 and CD86). Signal 3 relies on the ligation of pattern recognition receptors, such as Toll-like receptors (reviewed in reference [21]). Ligation of different Toll-like receptors by different pathogens may induce a 'tailor fit' response to the antigen [22]. In this way, DCs can establish a link between innate and adaptive immunity [23, 24]. The

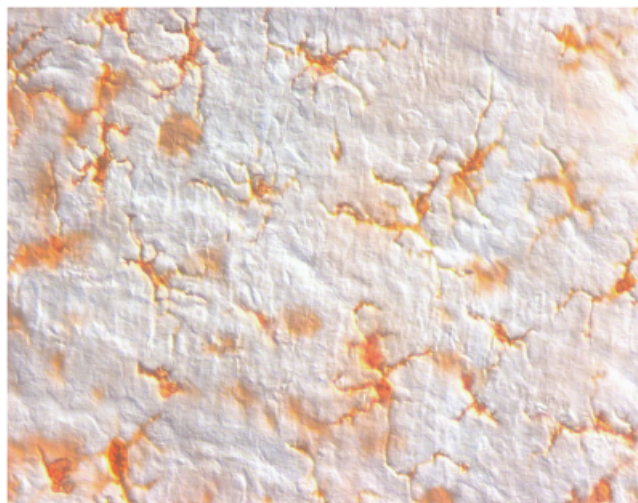


Fig. 2. Mucosal dendritic cell network visualized by major histocompatibility complex II staining on a murine tracheal wholemount. Trachea was taken from a naïve not-immunized mouse.

requirement of PAMP recognition to induce sensitization to inhaled allergen has been shown for lipopolysaccharide (LPS) in a murine asthma model. Mice were sensitized to inhaled ovalbumin (OVA) together with a low-dose exposure to LPS. Mice without TLR-4, the toll-like receptor for LPS, could not be sensitized to OVA. However, the dose of LPS appeared to be crucial for the induced response; in the case of a high dose of LPS, the response appeared to be T-helper type 1 (Th1) [20].

Role of dendritic cells in sensitization to allergen in humans

Although it has not been proven directly in humans that DCs are responsible for the Th2 sensitization process, some *in vitro* findings with human cells suggest that these cells are implicated. DCs of atopic and non-atopic individuals were compared for the manner in which they handled allergens. DCs from the peripheral blood of house dust mite (HDM)-allergic patients preferentially up-regulated CD86, IL-10 and programmed death ligand-1 (PDL-1) after incubation with Der p1, one of the major allergens in HDM, in contrast to DCs of healthy donors that up-regulated CD80 [18]. Up-regulation of CD86, PDL-1 and the enhanced secretion of IL-10 are all associated with the polarization to Th2 cells, as shown in the following experiments. In a murine model of asthma, administration of a CD86 antibody during allergen challenge could prevent the development of eosinophilic airway inflammation in already sensitized mice [25, 26]. Although in other experimental settings it has been shown that CD80 or both CD80 and CD86 were needed to induce Th2-mediated immune responses [27, 28], the other characteristics of DCs of asthmatic individuals could also favour the development of Th2 responses. Although IL-10 is often described to have an immunosuppressive effect on both Th1/Th2 cells [29], it can also favour the polarization to Th2 cells [30]. The presence of IL-10 at the site of T cell differentiation

diminishes the secretion of IL-12, thereby indirectly attenuating the differentiation of Th1 cells. Also, the up-regulation of PDL-1 can have pro-inflammatory effects. Although signalling through PD-1 is generally seen as an inhibitory signal [31], recent data do suggest that PDL-1 might also provide a costimulatory signal to T cells [32]. In a murine model for asthma, blocking PDL-1/PDL-2 using PD1-Fc had the potential to suppress eosinophilic airway inflammation (B. L. and H. Kuipers, unpublished work). Taking these results together, this could imply that DCs in atopic individuals favour the development of Th2 cells. In support of this theory, when DCs from atopics, after incubation with Der p 1, were cultured with naïve T cells a Th2 response was induced [18].

Do different subsets of dendritic cells lead to distinct T cell responses?

Several studies have shown that subclasses of DCs direct the development of distinct Th populations. In the mouse, a distinction can be made between lymphoid CD8 α^+ and myeloid CD8 α^- DCs. Administration of CD8 α^+ cells in mice leads to a Th1 differentiation by secretion of IL-12 and IFN- γ , while CD8 α^- cells lead to a Th2 response by the secretion of IL-10 and IL-4 [12, 14]. However, the functions of these subsets are somewhat flexible because CD8 α^- DCs are also capable of producing high amounts of IFN- γ when IL-4 coexists with IL-12 [33]. Although myeloid DCs are used in murine models for asthma because they preferentially induce Th2 sensitization to inhaled harmless antigens leading to asthmatic features [10, 20, 34], myeloid DCs are equally able to induce a Th1 response, under conditions of intense immune stimulation such as bacterial or viral infection [35, 36] or under environmental instruction by the presence of cytokines like IL-12 [33]. On the other hand, plasmacytoid DCs are not only able to induce Th1 responses, they can prevent Th2 sensitization and can even lead to tolerance [10, 20, 34]. The response induced by myeloid and plasmacytoid DCs is therefore not confined to one subset. Not only has the response they induce been proven to be flexible but also the origin of each subset is under discussion. Plasmacytoid DCs were thought of to be of lymphoid origin while myeloid DCs were derived from the bone marrow (BM). However, the observation that myeloid DCs were able to develop into plasmacytoid DCs under the influence of viral infections [37] illustrates the flexibility in this system.

In humans, myeloid DC and plasmacytoid DCs subsets were initially called, respectively, DC1 and DC2, referring to their capacity to preferentially induce the differentiation of naïve T cells into Th1 and Th2 effector cells, respectively, in contrast to their murine counterparts [38, 39]. The finding that asthmatic patients have more plasmacytoid DCs in peripheral blood compared with healthy individuals [40] could be related to the Th2-dominant phenotype in asthma. However, several studies described a plasticity of the two DC subsets in directing T cell responses [41, 42]. These studies suggest that not only their lineage but also the microenvironment of cytokines and/or inflammatory mediators and maturation status can influence the outcome of the immune response.

Are dendritic cells the most likely antigen-presenting cells in ongoing asthmatic inflammation too?

Despite these known functions of DCs on primary immune responses and allergic sensitization, it is less clear whether airway DCs are also required for the presentation of allergen to resting memory Th2 and/or effector Th2 cells during a secondary immune response. In favour of DCs, it is known that the number and maturation state of lung DCs are elevated during secondary immune challenge with allergens and during chronic airway inflammation [3, 43, 44]. This suggests that DCs are functionally involved in presenting allergens to T cells, and they thus control airway inflammation [4, 8, 45, 46]. Arguing against a role for DCs is the notion that previously primed T cells have less need for costimulation in comparison with naïve T cells, which led to the hypothesis that any peripheral APC-expressing MHCII, like macrophages, B cells and perhaps eosinophils, could stimulate effector Th2 cells [47–49].

Eosinophils as antigen-presenting cells?

Although eosinophils are considered to be effector cells, recent studies have reported their ability to activate primed Th2 cells through their expression of MHCII and even costimulatory molecules [50, 51]. Similar to DCs, eosinophils are able to migrate to the mediastinal nodes after intratracheal instillation, making them likely candidates for presenting antigen in the allergic asthmatic lungs. However, eosinophils sorted from bronchoalveolar lavage fluid of OVA-sensitized and challenged mice were not able to induce proliferation in allergen-specific naïve T cells *in vivo* or *in vitro* [52]. These findings suggest that eosinophils do not contribute to the induction of new effector Th2 cells but seem to amplify the inflammatory response by stimulating already primed T cells or by influencing DC antigen presentation indirectly through secretion of mediators and cytokines [53].

Macrophages as antigen-presenting cells?

Also, macrophages are not very likely to play a very important role in antigen presentation during secondary immune responses. Previous studies in mice in which alveolar macrophages were depleted using clodronate-filled liposomes have shown an actual enhancement of airway inflammation and T cell reactivity in the lung [54]. This is explained by the normally suppressive influence of alveolar macrophages on the activation of primed T cells and DCs in the lung [55].

Dendritic cells as antigen-presenting cells!

There are several intriguing findings that support a predominant role for DCs in secondary immune responses in an animal model of asthma. Upon allergen challenge to the lung, CD11c⁺ cells are strongly recruited to the lung, accumulate and acquire a mature phenotype expressing essential costimulatory molecules (CD80, CD40, intercellular adhesion molecule 1 (ICAM-1), PDL-1, PDL-2) when resident within the inflamed lung [44, 45, 56]. This accumulation of DCs in the lung was supported by an almost threefold expansion of myeloid CD31^{hi} Ly-6C^{neg} haematopoietic precursor cells in the BM. When these BM precursors were grown in GM-CSF,

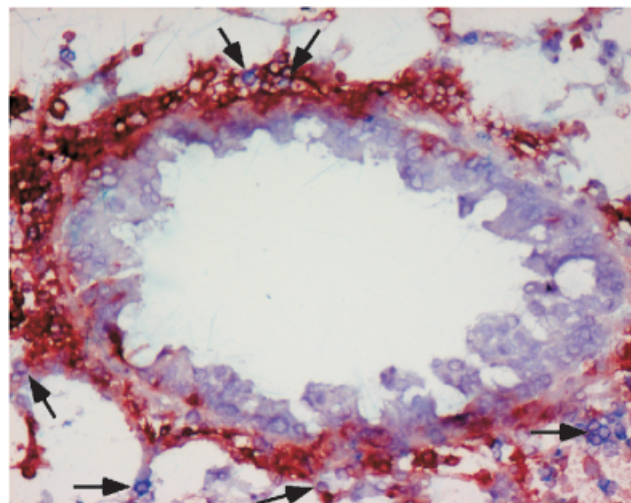


Fig. 3. Staining of CD4⁺ T cells (membrane-bound blue; see arrows) and CD11c⁺ dendritic cells (DCs) (red) in the lung of ovalbumin-sensitized and challenged mice shows co-localization of CD4⁺ T cells and CD11c⁺ DCs within peribronchial sites of inflammation. Goblet cell hyperplasia is seen.

they are differentiated into MHCII⁺ CD11c⁺ DC, but when grown in IL-5 they generated eosinophils, illustrating their pluripotent character. As these cells express the CCR3 eotaxin receptor, a serum rise in eotaxin might be the signal that increases the output of DCs and eosinophils at times when they are needed in the airways [44, 57].

In the same murine asthma model, CD11c⁺ DCs accumulated within sites of eosinophilic airway inflammation and clustered with CD4⁺ T cells in the airway submucosa and the perivascular space at areas of intense eosinophilic inflammation, forming multiple contacts with CD4⁺ T cells (see Fig. 3 [56]). The consequences of this interaction with T cells have been studied elegantly by Huh et al. in a rat model of asthma. After a single OVA aerosol challenge in OVA-sensitized rats, airway DCs accumulated in the airway mucosa and submucosa and formed contacts with primed T cells, in turn inducing the up-regulation of costimulatory molecules on otherwise immature airway DCs [8]. These authors hypothesized that contact with primed T cells turns immature mucosal DCs from an antigen uptake to an antigen-presenting mode, leading to local antigen presentation to mucosal T cells. This is indeed supported by the phenotype of mucosal DCs in allergen-induced airway inflammation, which have up-regulated costimulatory molecules, while lung DCs in the parenchyma retained their original phenotype.

Direct proof for a functional role for dendritic cells in stimulating effector T-helper type 2 responses

As intense local interactions between primed CD4⁺ T cells and locally matured DCs are a feature of eosinophilic airway inflammation across species, we studied whether effector CD4⁺ T cells functionally depend on DCs to exert their effector functions and in this way orchestrate airway inflammation. In human studies, it is almost impossible to study the functional role of DCs because it is not possible to

deplete DCs and study the subsequent inflammatory response to inhaled allergen. We addressed this question by using a novel system of conditional depletion of CD11c⁺ cells expressing a diphtheria toxin (DT) receptor transgene under the control of the CD11c promoter [58]. Systemic administration of DT to these mice leads to a rapid reduction in splenic and lymph node CD11c⁺ DCs, and concomitantly to an absence of the primary cytotoxic T cell responses to *Listeria* infection or experimental administration of protein antigens [4]. We adopted this strategy and administered the DT locally via intratracheal injection, leading to a loss of CD11c⁺ cells in the BAL fluid and lung tissues (DCs and macrophages) and lung-draining mediastinal lymph nodes (DCs). Using this unique system, we conditionally depleted CD11c⁺ cells locally from the lung immediately prior to OVA challenge in OVA-sensitized mice and observed that the cardinal features of asthma such as eosinophilic airway inflammation, goblet cell hyperplasia and bronchial hyper-reactivity to metacholine failed to develop [56]. Similarly, asthmatic features were also absent when DCs were depleted from already fully inflamed airways. As these features critically depend on CD4⁺ T cells and their secreted products, the most likely explanation was that effector function was not induced in the absence of CD11c⁺ cells. In support of this theory, we observed that in the absence of CD11c⁺ cells, *ex vivo* isolated CD4⁺ cells produced greatly diminished amounts of IL-4, IL-5 and IL-13. We then adoptively transferred *in vitro* primed CD4⁺ Th2 cells from DO11.10 T Cell Receptor (TCR) transgenic T cells in CD11c⁺-depleted mice. Previous data have shown that such *in vitro*-differentiated Th2 cells mimic *in vivo* generated memory T cells and can be used to transfer Th2 reactivity to OVA aerosol passively [59]. However, when Th2 cells were adoptively transferred in mice that were depleted of CD11c⁺ cells, no Th2 effector cytokines IL-4, IL-5 and IL-13 were produced by the T cells in the draining lymph nodes of the lung, whereas they readily did in the presence of lung CD11c⁺ DCs. All the salient features of asthma were restored by adoptive transfer of wild-type BM-derived CD11c⁺ myeloid DCs, but not by transfer of CD11c⁺ wild-type alveolar macrophages. These data of local depletion of CD11c⁺ DCs immediately prior to antigen challenge to the lung support our previous work in which we systemically depleted all myeloid DCs from the lungs, lymph nodes, BM and spleen of human immunodeficiency virus-leukotriene receptor promoter-driven thymidine kinase transgenic mice prior to allergen challenge [4].

These data indicate that DCs are essential for the development and maintenance of secondary immune responses but are they also sufficient? This question was answered by the following experiment. Mice were sensitized by an intratracheal injection with antigen-pulsed DCs to induce a primary immune response. To induce a secondary immune response in sensitized mice, mice are often challenged with an allergen aerosol or allergen in the presence of adjuvantia. In this experiment, we administered these sensitized mice DCs intratracheally as a challenge but without any of the classically used challenges. These mice developed a similar eosinophilic airway inflammation, Th2 cytokine synthesis and bronchial hyper-reactivity as observed after a regularly used aerosol challenge [56, 60]. The development of an eosinophilic airway inflammation by challenging sensitized

with only DCs, together with the results of the depletion studies, support the hypothesis that DCs are key inflammatory cells that function beyond sensitization.

Why are dendritic cells so essential for T-helper type 2-mediated airway inflammation?

Based on studies on the functional interaction between mucosal T cells and DCs [8] and based upon our current findings, it is clear that effector Th2 responses *in vivo* in the lung continuously depend on antigen-presenting DCs.

Role of costimulation during secondary immune responses by T-helper type 2 cells

One possible explanation would be that effector T cells *in vivo* remain dependent on costimulation in contrast to their *in vitro* counterparts [47, 61]. Numerous models of asthma have demonstrated that blocking the interaction of costimulatory molecules of the B7 superfamily (CD80, CD86, ICOSL) or tumour necrosis factor (TNF)-R family (OX40L) can reduce features of asthma, even when given during challenge in sensitized mice and even when given together with *in vitro* primed Th2 cells [62–66]. As lung DCs are the predominant cell type expressing CD80/CD86 family members at times of allergen challenge, an absence of DCs might have the same effect as blocking these costimulatory molecules [8, 45, 56]. However, challenge with DCs derived from the BM of CD80/86 double knockout mice in sensitized mice induced similarly strong airway inflammation similar to the one observed after challenge with wild-type DCs. It is likely that other costimulatory molecules besides CD80/CD86 are involved in activating T cells in secondary immune responses. Several molecules on the surface of the DC, like B7RP-1 (also known as ICOSL), OX40L, 4-1BBL, CD40 and ICAM-1, have been reported to have costimulatory capacity, and may be responsible for the induced reaction in the absence of CD80/CD86 [67]; a likely candidate in this context is B7RP-1 (ICOSL), induced on mature DCs in lung-draining lymph nodes [68]. Blockade of ICOS–ICOSL using an ICOS–Ig fusion protein was able to completely suppress asthmatic features induced by adoptive transfer of OVA-specific Th2 cells to the airways of mice [62]. However, blocking of ICOS–ICOSL interaction with an anti-ICOS antibody during CD80/86^{−/−} DC challenge in sensitized mice did not reduce airway eosinophilia significantly, although there was a trend towards reduced airway eosinophilia [60]. One explanation for the poor efficacy of ICOS in this model in contrast to previous models could be the recent observation that DCs down-regulate the expression of B7RP-1 during eosinophilic airway inflammation [45, 56], and therefore the ICOS–ICOSL interaction between T cells and DCs would not play as important a role in this DC-driven model as observed in Th2-driven models. An alternative explanation could be that blockade of ICOS–ICOSL interaction is preventing the induction of regulatory T cells and therefore aggravating the immune response [68]. Strikingly, we also observed an increase in the B7 family members PDL-1 and PDL-2, ligands of the inhibitory PD-1 receptor, on DCs within eosinophilic inflammation [56]. As mentioned earlier in this review, PD-1

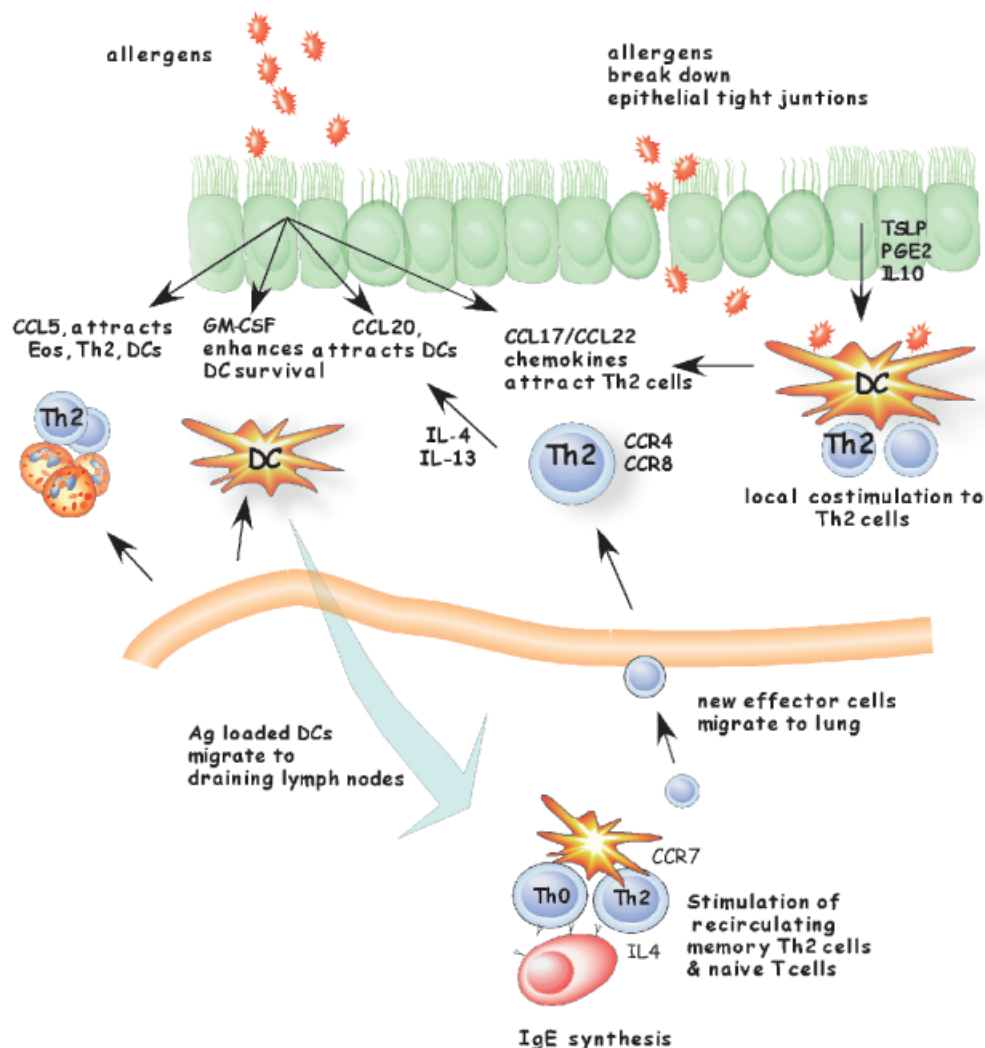


Fig. 4. Interaction between epithelial cells and dendritic cells (DCs) during established inflammation. Allergens stimulate epithelial cells to release chemokines and growth factors for DCs, T-helper type 2 (Th2) cells and eosinophils. The recruited DCs are also stimulated by allergen and produce even more chemokines for Th2 cells. Locally attracted Th2 cells interact with DCs in the airways, leading to local DC maturation and T cell costimulation of effector cytokine production. These activated Th2 cells eventually control the inflammatory process by activating eosinophils and mast cells and by feeding back on the epithelium and DCs.

is generally seen as an inhibitory signal but recent data suggest that PDL-1 might also provide a costimulatory signal to T cells [32].

Another costimulatory pathway able to compensate for the lack of CD80/86/B7RP-1 costimulation on DCs would be OX40L. OX40 (CD134), a member of the TNFR family, is a major regulator of anti-apoptotic proteins such as Bcl-xL and Bcl-2, and strongly promotes the survival of antigen-activated primary CD4 T cells. In addition, OX40 is preferentially expressed by memory Th2 cells. Blocking of OX40–OX40L interaction impaired all features of asthma induced by adoptive transfer of OVA-specific Th2 cells [69]. The requirement of CD40 and CD40L interaction has been investigated in several murine models for asthma with different protocols. It appeared that sensitized CD40^{-/-} and CD154^{-/-} (CD40L^{-/-}) mice develop, respectively, less eosinophilia and airway hyper-reactivity in response to an allergen challenge when compared with wild-types [70, 71]. However, an inevitable disadvantage of using transgenic mice

to study secondary immune responses is also that during sensitization, CD40–CD40L interaction is absent. Therefore, it is possible that the final consequence of asthma development in mice is determined by the sensitization phase because mice need to be sensitized before they can develop the effector phase. In another model where they could circumvent sensitization by using the OVA TCR transgene in CD40-deficient mice to obtain sufficient OVA-specific T cells without sensitization, the results were completely opposite. Mice without CD40 developed more eosinophilia and a higher AHR compared with CD40^{+/+} OVA TCR animals [72]. These results suggest that during sensitization, CD40/CD40L can act as a pro-inflammatory signal while having a protective role during the effector phase. In support of this theory, it has been described that CD40 ligation of DCs can release immature DCs from suppression of regulatory T cells [73]. However, CD40 is expressed by several cells like B cells, DCs, a subset of lymphocytes and monocytes. The role of CD40/CD40L interaction between DCs and T cells during a

secondary immune response in allergic airway inflammation still has to be elucidated further.

Production of T-helper type 2-attractive chemokines

Besides their capacity to provide costimulation, an alternative characteristic that makes DCs so important during secondary immune responses could be that lung DCs are essential for the recruitment of Th2 cells by producing Th2-selective chemokines. It was indeed shown that lung CD11c⁺ DCs within sites of eosinophilic airway inflammation and human monocyte-derived DCs exposed to the house dust mite allergen Der p 1 produce high amounts of the Th2-selective chemokine TARC (CCL17), acting on CCR4⁺ Th2 cells (see Fig. 4) [74, 75]. Therefore, elimination of the inflammation by depleting lung DCs might lead to an 'unfavourable' chemokine gradient, failing to attract primed Th2 cells to the lung.

Role of dendritic cells in diseases other than asthma

Our findings that DCs are essential for mounting secondary T lymphocyte effector responses might prove to be important for explaining other T cell-mediated diseases such as diabetes, multiple sclerosis, rheumatoid arthritis and colitis. It was recently shown that mature OX40L⁺ DCs form extensive clusters with pathogenic T cells in the gut mucosa in the CD45RB^{hi} transfer model of colitis in SCID mice [76]. Similarly, in T cell-mediated peri-insulinitis of pre-diabetic NOD mice, DCs are prominently present and cluster with pathogenic T cells [77]. Although not proven yet, it is very likely that in light of our data, DCs are essential for activation of pathogenic T cells in these diseases as well.

A different role for dendritic cells in the chronically inflamed and remodelled lung?

Most murine asthma models mimic the reversible allergen-induced airway inflammation but are generally based on an acute airway inflammation. It is clear from pathological investigations in human asthmatics that structural remodelling exists in the airway, even at a very early stage of disease [78]. Remodelling is a dynamic process that involves the laying down of extracellular matrix structures, which are also broken down by the action of tissue-degrading enzymes. How exactly collagen or extracellular disposition of matrix degradation products might influence DC function in remodelled airways is currently mainly unknown. Collagen type 1 has been described to up-regulate allostimulatory capacity and cytokine production by DCs in a murine system, and is able to induce maturation in human DCs [79, 80]. Ligation of integrin receptors on DCs by extracellular matrix components can also lead to alterations in DC trafficking, thereby altering their function [80]. Alternatively, some matrix degradation products such as heparan sulphate have been shown to induce DC maturation [81]. Structural remodelling of the airways could recruit more DCs, which acquire a mature phenotype in this way. Moreover, asthmatic epithelium might release factors such as GM-CSF, Thymic Stromal Lymphopoietin (TSLP) or chemokines that pro-

foundly influence DC survival and/or function as shown in *in vitro* cultures of asthmatic epithelium [82, 83] (Fig. 4). Considering the finding by Julia et al. [84], that a certain subtype of antigen-bearing cells can remain in the lungs for months, this might be part of the explanation regarding why some allergic asthmatic reactions become chronic, despite avoidance of allergens.

Challenging the main opinion that subepithelial deposition of collagen, leading to pseudo-thickening of the basement membrane [85], might contribute to the deterioration of asthma symptoms, we also speculate that thickening of the basement membrane might actually be beneficial to the host, as it shields off the incoming allergens, as the basement membrane is less permeable to incoming allergens and less allergen would be able to reach the DC network. In support of this theory, it is remarkable that asthmatics are not more susceptible to viral infections compared with healthy subjects while the epithelial barrier is often disrupted [86]. This would indicate that asthmatics are protected in another way, perhaps by a thickened membrane. Although intellectually attractive, this theory remains to be investigated.

Finally, many inflammatory cell types such as mast cells, basophils and eosinophils are recruited to the airways in chronic asthma. These cells release many mediators such as cytokines, neuropeptides, enzymes and lipid mediators that may also profoundly influence DC function and in this way might perpetuate ongoing inflammation [46]. As only one example, it is known that histamine and PGD₂, both released by mast cells upon cross-linking, reduce the potential of DCs to produce bioactive IL-12, and contribute to Th2 polarization in this way [87, 88].

Conclusion

Although further work is required to identify the role of the DC during the secondary immune response in the remodelled lung, we propose that DCs have an antigen-presenting function that reaches beyond sensitization. DCs therefore constitute a novel target for the development of anti-asthma therapy aimed at the origin of the inflammatory cascade.

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