

Dendritic Cells as Regulators of the Immune Response to Inhaled Allergen: Recent Findings in Animal Models of Asthma

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Key Words

Dendritic cells · Allergy · Animal models · Immunology · Asthma · Antigen-presenting cells

Abstract

Antigen-presenting dendritic cells are essential for the recognition and presentation of allergens to the cells of the immune system. Airway dendritic cells capture allergen in the mucosa and present it to naive T cells after migration into the draining lymph nodes. In this review article, we discuss the most recent findings from animal models of asthma, which highlight an essential role for these cells in the induction and maintenance of eosinophilic airway inflammation. This increasing knowledge might lead to the identification of new targets for the prevention and therapy of asthma.

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Animal Models of Asthma

Animal models of asthma have been used extensively in recent years to address the potential contribution of cells or mediators to the pathogenesis of eosinophilic airway inflammation, goblet cell hyperplasia, airway remodelling and bronchial hyperreactivity, which are typical of human asthma [1, 2]. Although incomplete in several aspects, these models have significantly contributed to

our understanding of how exactly this disease is regulated. To summarise years of work by various groups, it was shown that increased numbers of CD4⁺ T cells producing IL-4, IL-5 and IL-13 were found in mice and rats with eosinophilic airway inflammation induced by the model antigen ovalbumin (OVA) [3–5]. Moreover, adoptive transfer of OVA-specific CD4⁺ T helper 2 (Th2) cells had the capacity to transfer airway eosinophilia, goblet cell hyperplasia and bronchial hyperreactivity [6–8]. Most importantly, removal of CD4⁺ T cells by depleting monoclonal antibodies completely reversed existing OVA-induced eosinophilic airway inflammation, goblet cell hyperplasia and bronchial hyperreactivity [9, 10]. Additional experiments in cytokine knockout, transgenic and antibody-treated animals have provided evidence that the Th2 cytokines IL-4, IL-5, IL-6, IL-10 and IL-13 are functionally important downstream mediators that control the various histological and physiological features of the disease [1, 2, 11]. In many instances, the discoveries in animal models occur simultaneously with those in human asthma, but rather than showing an association between the disease and the presence of a cell or mediator, the use of animal models allows the demonstration of cause and effect, the IL-13 story being a nice example [5, 12, 13]. In this article, recent advances in the understanding of the role of antigen-presenting dendritic cells (DCs) in experimental asthma are reviewed and discussed against the background of a growing awareness that these cells are also important in human allergic disease.

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The Need for Antigen-Presenting Cells in the Initiation of T Cell Responses

The paradigm that CD4⁺ Th2 cells control asthma has largely ignored the fact that T cells need antigen-presenting cells (APCs) to become fully activated and respond to allergens. The recognition of allergen by APCs is in fact the first step of immune recognition that eventually leads to the formation of Th2 immunity to inhaled allergen and the development of asthma. During the development of sensitisation, APCs capture antigen and process it into small peptides of defined length for presentation on MHC molecules and presentation to the T cell receptor (TCR) or naive T cells. Moreover, APCs express co-stimulatory molecules such as CD80, CD86, ICAM-1 and heat stable antigen, among others, to provide a second signal for optimal induction of T cell activation, division and differentiation. In the absence of such co-stimulatory signals, the outcome of TCR ligation is anergy rather than immunity. Although a number of professional APCs, such as DCs, B cells and macrophages, have the capacity to capture and process foreign antigen for presentation to T cells, DCs are truly specialised for this and have no other effector function. DCs are bone marrow-derived cells that continuously patrol the mucosae, skin and internal organs of our body in search of foreign antigens [14]. Upon recognition of foreign antigens in peripheral tissues, these cells have the unique capacity to migrate to the T cell area of draining lymph nodes and report their antigenic cargo to naive T cells, at the same time providing essential co-stimulatory molecules and cytokines for inducing the primary immune response and for determining the type of immune response that will be induced to neutralise the antigen.

Lung DCs Induce the Primary Immune Response to Antigen Reaching the Airways

As an important role for DCs in controlling immunity throughout the body is now widely accepted, it is increasingly appreciated that the network of airway DCs is particularly important for inducing the primary immune response to inhaled allergen that eventually leads to sensitisation [15]. Landmark studies by Holt et al. [16] in rodents have demonstrated that the majority of cells expressing MHC class II in the lungs are airway and interstitial DCs. These two types of DCs demonstrate considerable heterogeneity [17]. Interstitial DCs are located in the alveolar walls and around lung venules and are fairly

immobile cells with a slow turnover. Functionally, it was shown that inhaled OVA was captured by these lung DCs, processed into an immunogenic form and could be presented to primed T cells *in vitro* following overnight culture in the absence of alveolar macrophages [17–19]. Indeed, the capacity of interstitial DCs to present antigen locally to T cells is strictly suppressed by secreted mediators derived from alveolar macrophages, which are found in close proximity to alveolar wall DCs [20, 21]. Therefore, and in the light of their non-migratory behaviour, it is unclear whether interstitial DCs contribute to the induction of primary immune responses to inhaled antigen or whether their main function is the amplification of memory T cell responses during ongoing inflammation [22, 23].

In contrast, airway DCs form a highly developed network of cells that reside in the lateral intercellular spaces formed by basal epithelial cells and are thus ideally positioned to capture antigen that is deposited in the conducting airways, the major site of antigen deposition in the lung [24, 25] (fig. 1). The number of DCs present within the airways rapidly increases following induction of non-specific inflammation [e.g. lipopolysaccharide (LPS), bacterial, viral inflammation] and airway DCs are recruited from the bloodstream by a variety of chemokines such as MIP-3 α , monocyte chemotactic protein (MCP)-1 and RANTES, increasing the likelihood that dangerous ‘inflammatory’ antigens will be recognised [26, 27]. Moreover, it was recently demonstrated by Vermaelen et al. [28] that in the absence of overt inflammation, specific airway migratory DCs capture fluorescently labelled dextran molecules *in situ* in the lung and rapidly transport them in small intracellular endosomes to the paracortical T cell area of draining thoracic lymph nodes, reaching these areas as quickly as 12 h after instillation. This rapid migration could be a reflection of the high rate of spontaneous turnover of airway DCs in areas of high microbial load, and is also observed in the gut [29]. If airway DCs continuously migrate to the draining lymph nodes, carrying antigen deposited in the airways in the absence of inflammation, the question then arises whether the same DCs can activate naive T cells to respond to the antigen.

In an attempt to demonstrate T cell priming by airway DCs, we injected bone marrow-derived DCs into the airways of naive mice and observed the generation of the primary immune response throughout the various compartments of the immune system [30]. The activation of naive T cells *in vivo* is difficult to detect because of the low frequency of high-affinity antigen-specific T cells. To circumvent this technical problem, naive T cell activation

Fig. 1. Tracheal whole-mount preparation of mouse trachea stained for MHC class II expression, revealing the extensive network of highly dendritic cells in the upper layers of the airway mucosa and epithelium. Whole mounts were stained with a rat-anti-mouse I-E monoclonal antibody followed by detection of peroxidase-coupled goat anti-rat. $\times 322$.

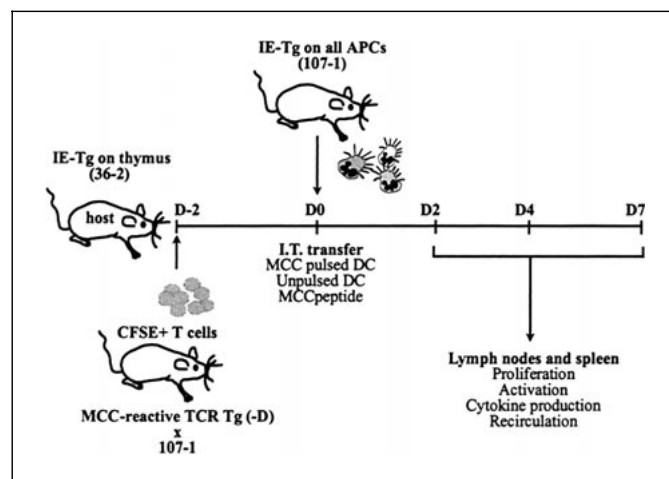
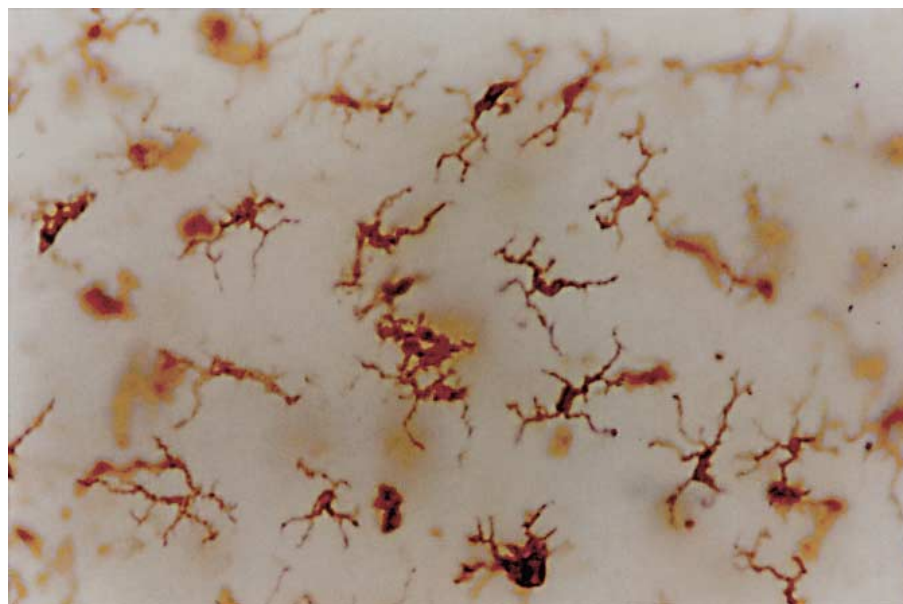


Fig. 2. An MHC-restricted model in which transferred I-E+ DCs are the only APCs capable of presenting the MCC peptide to adoptively transferred TCR-transgenic (Tg) T cells. Mice from the 107-1 line (in which the transgenic I-E α^d is expressed with a wild-type distribution on all APCs, paired with endogenous I-E β^b) served both as donors of I-E+ DCs for adoptive transfer and as parents of (-D TCR-transgenic $\times$ 107-1) F_1 T cell donors. Mice from the 36-2-transgenic host are tolerant of I-E+ cells as a result of thymic expression of the I-E α^d transgene, but express no I-E on peripheral APCs. On day -2, 25×10^6 CFSE-labelled MCC-reactive (107-1 $\times$ -D) responder T cells were injected intravenously into 36-2 hosts. On day 0, recipients were immunized by intratracheal injection of 10^6 MCC-pulsed or PBS-pulsed DCs. The response was followed in all accessible nodes and the spleen on days 2, 4 and 7 after immunization.

and division was studied in an adoptively transferred cohort of naive CD4+ moth cytochrome C (MCC) TCR-transgenic T cells that were labelled with the division-sensitive dye carboxy fluorescein succinimidyl ester (CFSE), allowing the number of cell divisions in vivo to be calculated [31]. The adoptive transfer system was set up in such a way that the injected MHC class II-positive DCs were the only cells that could present the MCC antigen to the MCC-reactive TCR-transgenic T cells, and so that the precursor frequency of naive antigen-specific T cells was kept at a low, but detectable frequency of around 2% of the total CD4+ T cell pool, thus avoiding the competition for antigen commonly observed when intact TCR-transgenic strains are used (fig. 2) [32]. As early as 12 h after intratracheal injection, DCs migrated into the draining mediastinal lymph nodes (MLN) and engaged interaction with naive T cells, as evidenced by the up-regulation of the early activation marker CD69 on almost 80% of naive antigen-specific CD4+ T cells within the MLN. Despite the widespread activation of antigen-specific T cells, only approximately 25% were actually recruited into cell division during the entire primary response. Forty-eight hours after injection, some antigen-specific T cells had already undergone 2 cell divisions and additional antigen-specific T cells were recruited from the bloodstream into the MLN, leading to an absolute increase in antigen-reactive T cells and swelling of the MLN (fig. 3a). Analysis of CFSE staining intensity on day 4 and 7 demonstrated that some progeny of activated cells continued dividing up to

6–7 times, while others seemed to be immobilised after only 1–3 divisions (fig. 3b). Despite the gross swelling and presence of IL-2 within the MLN, DCs were remarkably efficient in selecting and activating only those high-affinity T cells that were specific for antigen, as none of the changes observed in TCR-transgenic T cells were seen in non-specific T cells (fig. 3a, b). At the end of the primary response (day 7), a significantly higher frequency of antigen-reactive CD4⁺ T cells remained in the draining lymph node. This increase in antigen-specific T cells is in sharp contrast to the deletion of MCC-reactive T cells between day 3 and 7 that is observed in our model when the MCC antigen is delivered to the immune system in a tolerogenic fashion, e.g. through intranasal or intravenous administration of peptide without adjuvant [30, 32].

There was no migration of DCs towards any non-draining lymph nodes or spleen and, as a consequence, no division or up-regulation of CD69 in T cells beyond the first draining lymph node station on day 2 of the response (fig. 3a). In this way, we directly demonstrated the concept that the primary immune response was compartmentalised because of the restricted migration of DCs into lung-draining lymph nodes [28, 30, 33]. It was only between 72 and 92 h after injection that divided antigen-specific T cells emigrated from the draining MLN into the bloodstream and the peripheral tissues and spleen. The phenotype of these cells was remarkable in that they had divided at least 4 times and had universally high levels of the memory marker CD44 while totally lacking expression of the early activation marker CD69, a phenotype most consistent with activated effector T cells. Moreover, *in vitro* restimulation of these cells with specific antigen revealed primed cytokine production. Using this MHC-restricted system, we were thus able to demonstrate that myeloid DCs were potent inducers of CD4⁺ adaptive immunity in the lung following migration into the MLN and that the process of lymphocyte migration would ensure that an initially localised response became widespread after a few rounds of T cell division.

DCs Induce Sensitisation to Inhaled OVA Leading to Eosinophilic Airway Inflammation

Other investigators have used the intraperitoneal route of injection of OVA with or without alum adjuvant to systemically sensitise mice to OVA, leading to accumulation of OVA antigen in the spleen and MLN. In view of our knowledge that DCs could induce a primary CD4⁺ T cell response in the MLN (see above), and realising that the

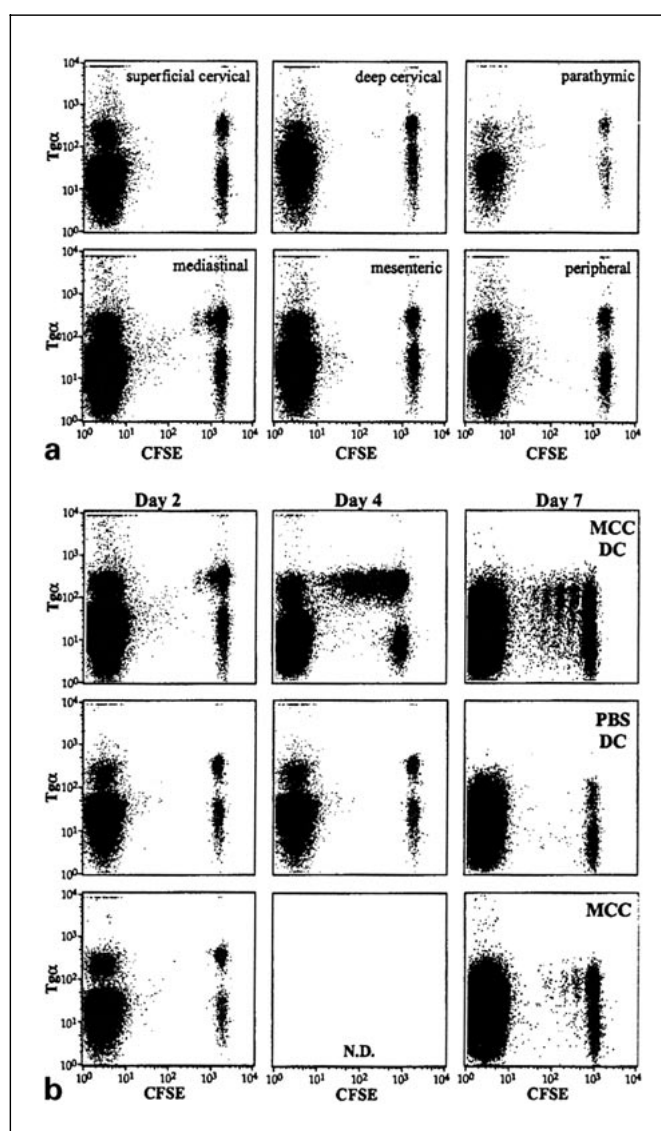


Fig. 3. Local response of CFSE-labelled T cells after intratracheal injection of I-E⁺ DCs. Lymph node T cells were gated for scatter characteristics, CD4 and exclusion of propidium iodide (PI). The cohort of adoptively transferred T cells can be readily identified by strong green fluorescence of CFSE (x axis). About 50% of CFSE-positive CD4⁺ cells express the transgene (Tg) encoded TCR α chain, recognized by the RR8 antibody (y axis). Cell division in Tg α ⁺ cells can be observed by sequential halving of CFSE fluorescence. **a** CFSE division profile of T cells in the various lymph nodes sampled 2 days after immunization with 10⁶ MCC-pulsed DCs. **b** Division profiles in draining MLN of the lung, taken 2, 4 and 7 days after immunization with MCC-pulsed DCs (upper panels), PBS-pulsed DCs (middle panels) or free MCC peptide (lower panels).

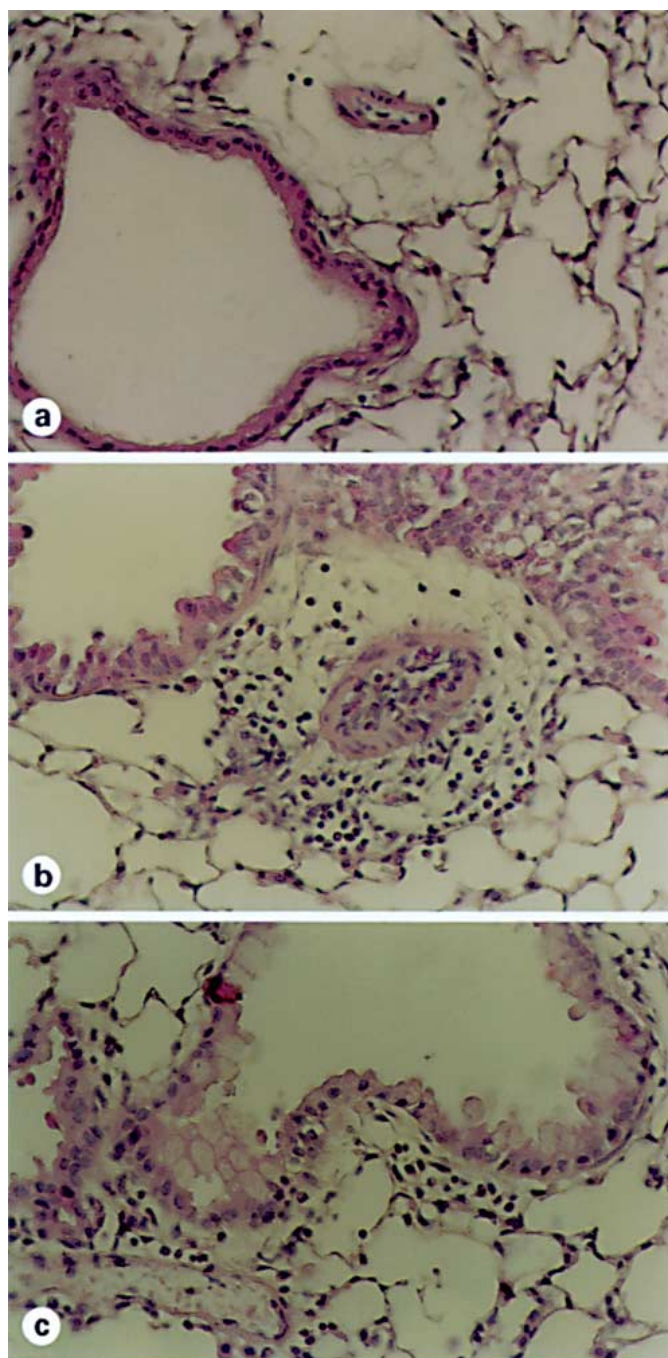


Fig. 4. Airway histology of mice immunized with PBS-pulsed DCs (**a**) or OVA-pulsed DCs (**b, c**) and subsequently exposed to OVA aerosol. **a** There are no peribronchial or perivascular changes in animals exposed to OVA when the animals were immunized with control PBS-DCs. $\times 350$. **b** Actively immunized mice demonstrate peribronchial and perivascular infiltrates that contain mononuclear cells, eosinophils and T cells. Some inflammatory cells adhere to the vessel wall. $\times 350$. **c** There is a marked increase in the number of goblet cells and the height of the airway epithelial cells in actively immunized mice.

intraperitoneal route of injection might not be the most relevant route for inducing sensitisation, we injected OVA-pulsed myeloid DCs into the airways of naive animals and studied the occurrence of sensitisation to inhaled OVA [30, 33, 34]. The proliferation data that could be obtained when naive OVA-TCR-transgenic T cells (DO11.10 mice) were CFSE labelled and injected into naive Balb/c mice, followed by immunisation with OVA-pulsed DCs, were identical to the data observed when MCC-transgenic T cells were transferred, followed by immunisation with MCC-pulsed DCs. This finding in itself was important, as the affinity of the DO11.10 TCR for IA^d-OVA_{323–339} is considerably weaker than that of the 5C.C7 TCR for IE^k-MCC_{87–103}, and illustrates that precursor frequency and TCR affinity are sensed by the immune system to generate a response of which the end result is both qualitatively and quantitatively very similar and determined predominantly by the characteristics and amount of the antigen [32]. To prove that OVA-pulsed DCs could induce the development of effector T cells that had the capacity to orchestrate airway inflammation, we challenged OVA-DC-immunised mice with an aerosol of OVA 14 days after primary immunisation [33]. In contrast to PBS-DC-immunised mice, OVA-DC-immunised mice developed peribronchial and perivascular infiltrates of eosinophils, T cells and mononuclear cells (fig. 4a, b). Goblet cell hyperplasia was also consistently observed in OVA-DC-immunised and -challenged mice (fig. 4c). The presence of goblet cell hyperplasia and eosinophils is dependent on IL-4 receptor signalling and IL-5, respectively, and was already a first indication that a CD4 Th2 response was being induced by DCs in the airways. When intracellular cytokine production was studied on individual CD4⁺ and CD8⁺ T cells within the bronchoalveolar lavage fluid (BALF) compartment, it was clear that CD4⁺ cells produced IL-4 and IL-5 but also IFN- γ , whereas CD8⁺ T cells exclusively produced IFN- γ (see below).

Although antigen presentation by adoptively transferred DCs in the lung clearly induced Th2-dependent sensitisation to inhaled OVA, one might argue that this is not a physiological system. Under conditions when endogenous DCs are exposed to inhaled FITC-OVA, it was clearly shown that airway-derived DCs take up FITC-OVA and transport it to the T cell area of draining lymph nodes, at the same time processing the OVA into an immunogenic form [28]. However, primary aerosolization with OVA and subsequent transport by DCs into the T cell area is normally a tolerogenic event that leads to down-regulation of the IgE response and eosinophilic airway inflammation. Although suppressor cells that express

the $\gamma\delta$ T cell receptor have been implicated in this antigen-specific down-regulation of the immune response, the precise mechanisms are unknown [35, 36]. Despite this, a number of studies have recently supported the concept that activation of endogenous DCs leads to sensitisation when mice are exposed to OVA aerosol. When the expression of the DC maturation cytokine GM-CSF was increased by adenoviral-mediated gene transfer into the airways, the numbers and activation status of airway DCs were greatly enhanced, rendering mice sensitive to primary OVA aerosol and leading to the abolition of inhalational tolerance and to development of Th2-dependent airway eosinophilia [37, 38]. Similarly, up-regulation of airway DC function was clearly associated with the augmentation of OVA sensitisation by influenza A virus infection in adult mice [39] and by exposure to the particulate air pollutant aerosol residual oil fly ash in neonatal mice [40]. We are currently investigating whether other known sensitisers, such as respiratory syncytial virus, mold-derived β -1,3-*D*-glucan, cigarette smoke or diesel particulate matter also affect the DC network of the lung [41, 42]. The fact that DCs in the airways induce Th2 responses to inhaled OVA immediately raises the question as to what factors determine this. DCs have been shown to induce both Th1 and Th2 types of responses upon transfer in vivo, depending on a variety of factors, of which the level of bioactive IL-12 p70 production seems to be the most important (fig. 5) [43]. IL-12 is essential for stimulating the innate immune system [e.g. natural killer (NK) cells] for IFN- γ production and for the development of a Th1 response, as evidenced by the lack of delayed type hypersensitivity (DTH) responses in IL-12-deficient mice [44]. One striking observation in murine asthma models was the identification of a gene locus that determines IL-12 production in susceptible and resistant mouse strains, by determining levels of the complement factor C5, a known stimulus for IL-12 production by APCs [45]. Similarly, there is a striking polymorphism between levels of soluble LPS receptor CD14 and protection against atopy in humans [46]. As for C5a, LPS is a known inducer of IL-12 production in DCs. Another possibility is that the environment of the lung favours the development of Th2 responses, by down-regulating the production of IL-12 in DCs [47]. Of the possible candidate factors involved in this process, IL-10 and prostaglandin E₂ are produced by airway epithelial cells and are known to down-regulate IL-12 production. In the presence of these factors, DCs induce Th2 responses [48, 49]. We have recently found that DCs obtained from IL-10-/- mice fail to induce Th2 responses in the airways [submit-

ted for publication]. Conversely, models of intraperitoneal OVA immunisation have shown that exogenous administration of recombinant IL-12 reduces Th2 sensitisation and subsequent airway eosinophilia in an IFN- γ -dependent manner [50]. Moreover, factors known to increase the levels of IL-12 within APCs, such as unmethylated CpG motifs within bacterial DNA and heat-killed *Listeria monocytogenes* micro-organisms are able to inhibit sensitisation to inhaled OVA [51, 52]. Although bacterial LPS is a clear inducer of IL-12 production in DCs, it is presently unclear whether its concomitant exposure with OVA reduces sensitisation, some investigators showing a reduction in Th2 responses to inhaled OVA and others showing an enhancement [53, 54]. An explanation for this discrepancy could be that LPS-induced production of IL-12 critically depends on the time point after stimulation, early time points favouring high IL-12 production, while later in the response, IL-12 production is shut down [55]. Our most recent data in mice using adoptive transfer of LPS-exposed DCs seem to support the idea that LPS can actually enhance Th2 responses to OVA by paralyzing IL-12 production in DCs [Kuipers et al., unpubl. results]. Thus, it seems that levels of IL-12 during the interaction of DCs and naive T cells are critical for determining Th polarisation.

Despite the fact that absence of IL-12 might predispose to Th2 development, the prototypic Th2 cytokine IL-4 is essential for Th2 development, as evidenced by strongly reduced Th2 responses and airway eosinophilia in anti-IL4 antibody-treated mice, IL-4-deficient mice, IL-4 receptor α chain (IL-4R α)-deficient mice or mice deficient in the critical downstream signal transducer and activator of transcription (STAT) 6 [13, 56–59]. Closer analysis of these various knockout mouse strains has, however, suggested that there may be IL-4R- and STAT6-dependent, but IL-4-independent mechanisms to generate IL-5-producing CD4+ T cells, IL-13 being a likely candidate for Th2 development in the absence of IL-4 [5, 11, 13, 60, 61]. It is interesting to observe that the importance of IL-13 in inducing Th2 development might be related to the route of immunisation and the genetic background of the mouse strain. In this regard, sensitisation of C57B1/6 mice to OVA via the skin (where the network of Langerhans cells is operative) seems to depend exclusively on IL-13, whereas sensitisation via the airways depends on IL-4 [62].

The important role of IL-4 and/or IL-13 in the development of Th2 sensitisation immediately raises the question as to what cell type initially produces these cytokines. Although DCs do not produce IL-4, some recent reports

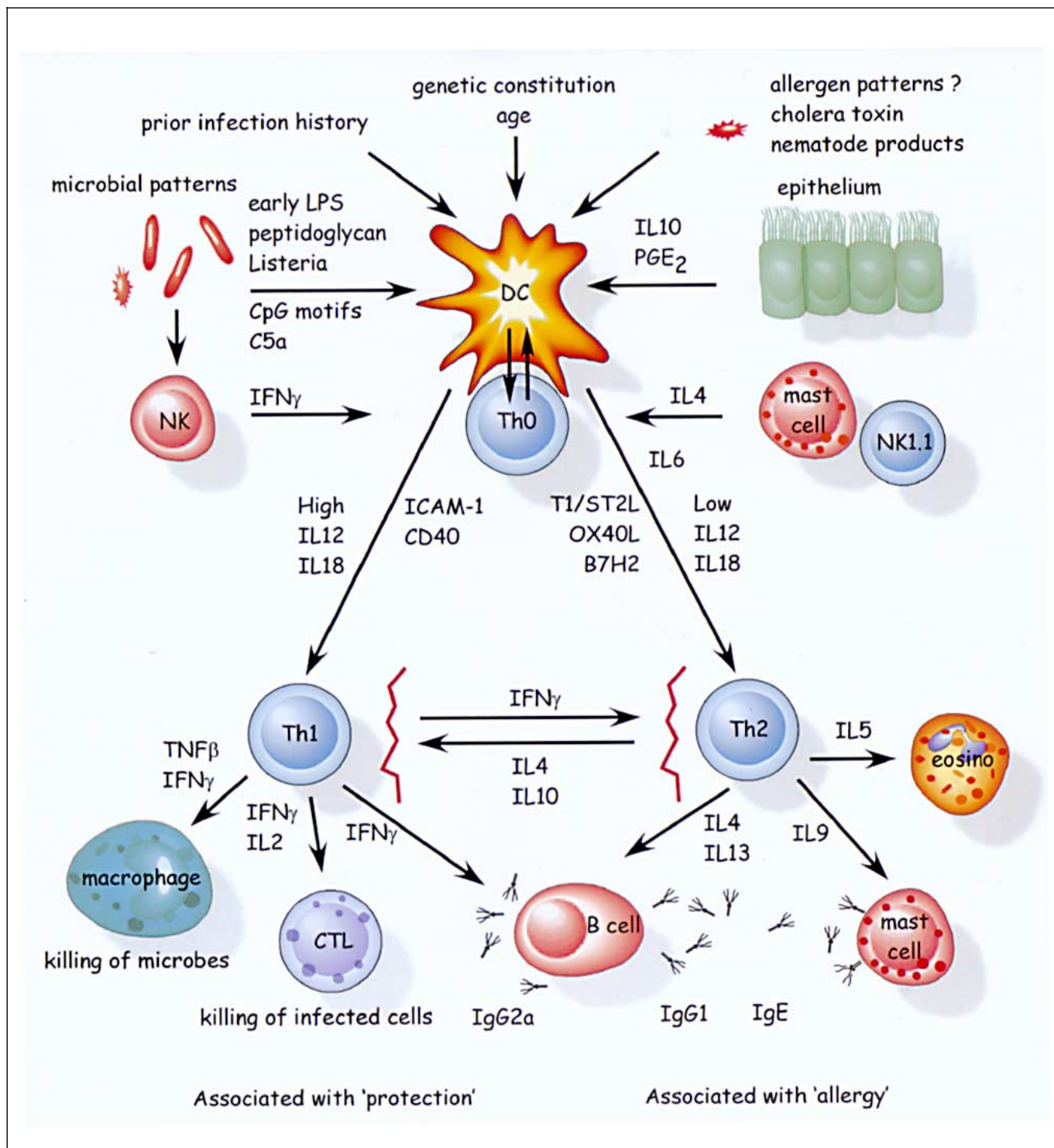


Fig. 5. Differentiation of Th cells induced by DCs.

have demonstrated that human immature DCs can produce IL-13 [63]. Our recent studies have, however, demonstrated that murine DCs fail to induce Th-2-dependent airway eosinophilia when transferred into IL-4-deficient C57B1/6 mice [33]. Alternatively, the source of initial IL-4 production could be found within cells of the innate immune system. A population of NK1.1+ cells (NK cells or NK T cells), is known to produce large amounts of this cytokine *in vivo* [64]. However, sensitised β_2 -microglobulin-deficient or CD1d-deficient mice, which lack functional NK1.1 T cells, have normal Th2-dependent eosinophilic responses to inhaled OVA [64–66]. Despite this, depleting NK1.1 antibodies given before sensitisation abolish the Th2 response to OVA, suggesting that NK cells may be important [64]. It has been shown that DCs can directly interact with NK cells, but the relevance of this interaction for initial IL-4 production and allergic sensitisation remains to be determined [67]. Alternatively, T cells expressing the $\gamma\delta$ TCR could be an early source of initial IL-4, as mice lacking these cells have deficient Th2 responses to OVA [68, 69]. The most likely source of initial IL-4, however, is the naive T cell itself. Under conditions where IL-12 production is low, naive T cells polarize towards IL-4 production in the absence of exogenous IL-4 [55, 70, 71]. Exposure to inhaled allergens occurs at low concentrations, generating a low MHC peptide density on the DC. A number of experimental systems suggest that low antigen dose and low avidity interactions between T cells and DCs favour the development of a Th2 response [72]. Also, chronic exposure to low levels of allergen might lead to low numbers of DCs continuously reaching the draining lymph nodes. Sustained TCR activation at low stimulator DC/responder T cell ratios independently favours the development of a Th2 response [73, 74].

It has been suggested that the particular expression of co-stimulatory molecules on APCs is critical for Th2 differentiation from naive T cells [75]. DCs in the lung express low levels of CD80, CD86, CD40 and ICAM-1 but rapidly up-regulate the expression of CD86, CD40 and ICAM-1 following migration into the draining lymph nodes [28]. Airway DC-induced Th2 responses in the lung fail to develop in the absence of CD28, the receptor for CD80 and CD86 expressed on T cells [33]. Models of intraperitoneal sensitization to OVA have highlighted that administration of CTLA4-Ig or blocking antibodies to either CD80 and/or CD86 can inhibit airway eosinophilia when given during primary immunisation [76–78]. Although CD86 preferentially stimulates Th2 and CD80 stimulates Th1 formation in some disease models, the situation is far from clear in the murine asthma model,

CD80 and CD86 having redundant roles [79, 80]. It will also be interesting to study whether the newly described B7-related protein B7-H2 [one of the human inducible co-stimulator (ICOS) ligands] and the Th2-associated TNF receptor family member OX40L have direct effects on Th polarization induced by DCs in the lung [81–85]. We have recently found that a ligand for the newly described Th2-associated type I IL-1 receptor family member T1/ST2 is expressed on DCs and critically contributes to Th2 induction in the lung [33, 86].

Because of their inability to migrate into the draining lymph nodes and their low-level expression of co-stimulatory molecules, B cells and macrophages do not seem to play an important role in the sensitization process to inhaled antigen. This is supported by the finding of a normal development of Th2 responses and airway eosinophilia in B cell-deficient mice [87]. Macrophages were unable to induce sensitisation to inhaled OVA when adoptively transferred into naive animals [34]. Moreover, both alveolar and interstitial macrophages seem to actively suppress the activation and proliferation of naive T cells in the lung by their direct inhibitory effects via nitric oxide and TGF- β on T cell proliferation and DC activation [18, 88]. This suppressive effect can, however, be overruled by enhanced expression of GM-CSF [21, 37]. Alveolar macrophage elimination *in vivo* is associated with an increase in the primary pulmonary immune response to inhaled antigen in mice [89].

Role of APCs during Challenge to Inhaled Antigen in Sensitised Mice

In addition to their contribution to the primary immune response leading to sensitisation, DCs are likely to contribute to the chronic secondary Th2 immune response that occurs in the airways of asthmatics. Bronchial biopsies have revealed increased numbers of CD1a+ MHC class II+ DCs in the epithelium and in the subepithelial lamina propria of non-smoking atopic asthmatics compared with non-atopic controls [90, 91]. We observed a 60-fold increase in the number of BALF DCs in OVA-sensitised and -exposed rats with eosinophilic airway inflammation [92]. The kinetics of allergen-induced increase of DCs closely resembled those of Th2 cells and eosinophils, suggesting that they were attracted by the same mechanisms. Allergic airway inflammation is indeed accompanied by the local release of chemokines and mediators such as RANTES, eotaxin, MIP-1 α , MCP-1, MCP-3, MCP-4 and platelet-activating factor, which have

been shown to attract both inflammatory cells and DCs in vitro and in vivo [27, 93]. Another possibility is the presence of a DC differentiation signal acting on monocytes after transendothelial migration into the airways [94, 95]. Hammad et al. [96] demonstrated that human peripheral blood mononuclear cells of house dust mite-allergic donors that were injected into severe combined immunodeficiency mice (humanised SCID model) developed into DCs after inhalation exposure to allergen. The classical differentiation signals for monocytes to become DC precursors in vitro are GM-CSF in combination with IL-4 [97]. These cytokines are likely to contribute to the differentiation of lung DCs in vivo, as mice expressing recombinant GM-CSF in the airways have greatly increased numbers of DCs [37], and as human airway DCs are found in close proximity to IL-4- and GM-CSF-producing cell types. Finally, allergen challenge in OVA-sensitised rats and mice induces an increase in the earliest precursor for DCs in the bone marrow, leading to an increased yield of DCs after culture in GM-CSF and an increase in circulating DCs [van Rijn et al., unpubl. results] [92]. These studies suggest that the recruitment of DCs to the airways is accompanied by increased production of DCs in the bone marrow.

The increased presence of DCs in the airways of atopic asthmatics and allergen-exposed animals suggests that DCs make a critical contribution to the disease pathogenesis. However, one needs to take into account that asthma is controlled by memory/effector CD4⁺ T cells. These cells have been shown in vitro to be less dependent on co-stimulation and can in theory respond to any APC, such as B cells, macrophages and even eosinophils and epithelial cells. To address the contribution of DCs to eosinophilic airway inflammation in sensitised animals, we used transgenic mice in which the suicide gene thymidine kinase is preferentially expressed in cells of the DC lineage [98]. Using this transgenic strain, treatment with the antiviral drug ganciclovir selectively depleted airway DCs, but not macrophages, B cells or T cells from the airways of OVA-sensitised mice [28, 98]. In sensitised animals treated with ganciclovir, there was a complete disappearance of eosinophilic airway inflammation and goblet cell hyperplasia induced by OVA (fig. 6). These findings strongly suggest that memory/effector CD4⁺ Th2 cells were not properly activated in the absence of DCs. Indeed, the levels of the Th2 cytokines IL-4 and IL-5 in the airways were suppressed in animals lacking DCs. Moreover, there was no allergen-induced boosting of OVA-specific IgE levels in the absence of DCs.

Studies on depletion of other APCs during the secondary challenge to inhaled OVA have suggested that elimination of macrophages using clodronate-filled liposomes is associated with an enhancement of Th2 responses and airway inflammation [99]. Similarly, B cells do not seem to contribute significantly to the secondary immune response and the development of airway eosinophilia, although they might contribute to plasma extravasation during the early-phase response to OVA aerosol and to bronchial hyperreactivity [87, 100, 101]. Recently, it was also shown that airway eosinophils express MHC class II and the co-stimulatory molecules CD80 and CD86, effectively contributing to the stimulation of effector Th2 cells in the lungs and lymph nodes of sensitised mice [80, 87, 102]. However, sensitised IL-5-deficient and anti-IL-5-treated mice that lack eosinophils in the airways demonstrate normal Th2 responses in the draining lymph nodes [103]. Therefore, eosinophils might have an enhancing role in antigen presentation, but do not seem to be essential for mounting the Th2 effector response.

What could be the reason that DCs are such critical APCs for eosinophilic airway inflammation? One possibility is that memory T cells in vivo are more dependent on co-stimulation than appreciated from in vitro data, and therefore rely on professional APCs that can provide these stimuli. Indeed, allergen-induced IL-4 and IL-13 production in bronchial explants and in peripheral blood cells from asthmatics is dependent upon delivery of co-stimulation via the CD80/CD86 pathway [for a review see ref. 104]. Similarly, animal models have shown that eosinophilic airway inflammation in sensitised mice can be suppressed by blocking the CD80/CD86-CD28 pathway during challenge [33, 76–78, 80]. In this regard, it was recently shown that the CD28-related molecule ICOS and the T1/ST2 molecule, both expressed on Th2 cells, are critical for Th2 effector function in the lung [83, 86]. Alternatively, DCs may be critical for the recognition of allergen via specific IgE bound to the high-affinity or low-affinity (CD23) receptor for IgE. Indeed, CD1a⁺ airway DCs express the α chain of the high-affinity IgE receptor [91]. When allergen is recognised via the high-affinity IgE receptor on DCs, it is very efficiently targeted to the MHC class II-rich endocytic compartment, the site of peptide loading onto MHC class II [105]. It has been suggested that the presence of IgE on DCs lowers the threshold for allergen recognition, boosting the secondary immune response by efficiently stimulating memory Th2 cells. In this regard, an interesting study by Coyle et al. [106] demonstrated that memory Th2 lymphocytes of sensitised mice failed to produce Th2 cytokines upon recognition of

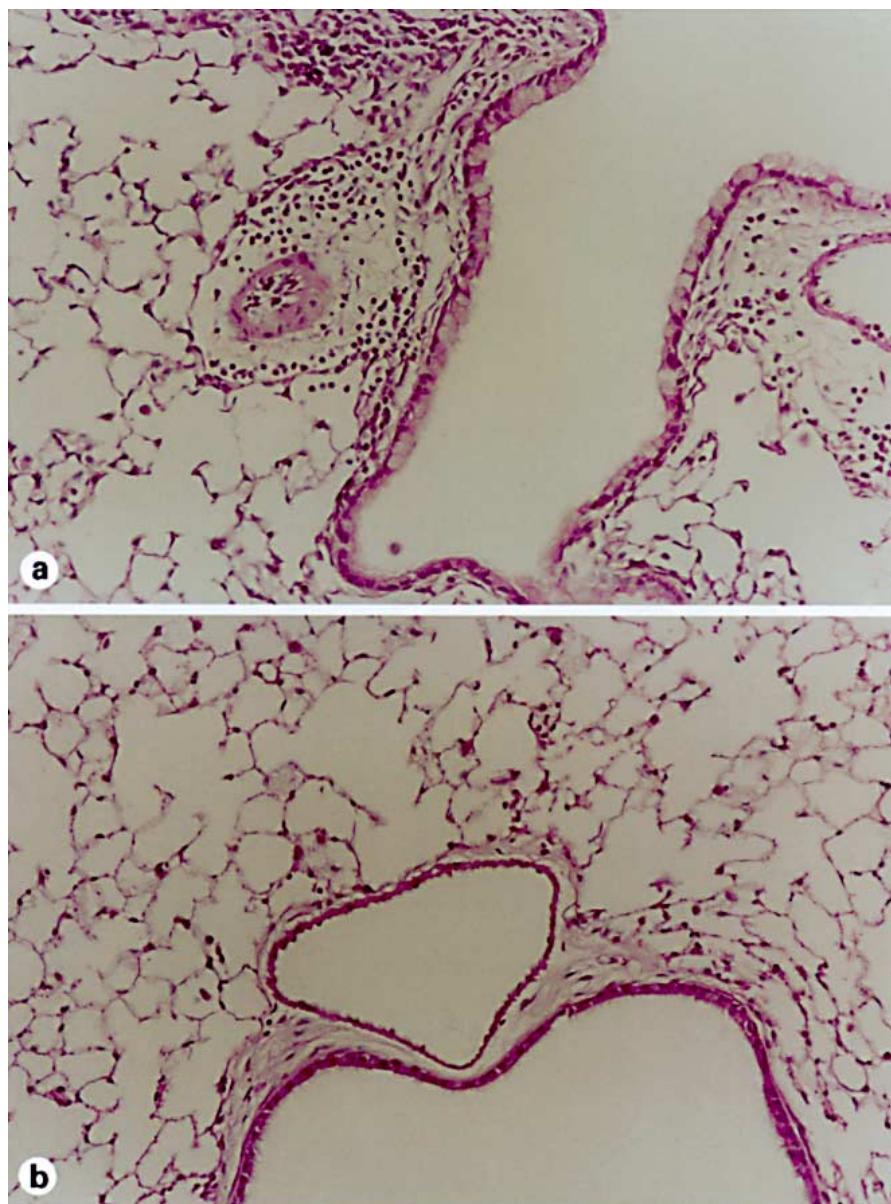


Fig. 6. Effect of depletion of airway DCs during secondary exposure to inhaled OVA. Mice were sensitized to OVA on day 0 by intraperitoneal injection of OVA in alum at a time point when DC numbers were normal. From day 16 to 20, mice were daily exposed to OVA aerosol for 30 min. **a** Response of wild-type C57B1/6 animals treated with ganciclovir and having normal numbers of DCs. There is peribronchial and perivascular inflammation and goblet cell hyperplasia. **b** Response of HIV-LTR thymidine kinase-transgenic mice treated for 6 days with ganciclovir to deplete airway DCs. The inflammation induced by OVA aerosol is completely suppressed.

inhaled allergen when allergen-specific IgE was captured by a non-anaphylactogenic anti-IgE antibody. The phenotype of CD23-deficient mice suggests that the low-affinity IgE receptor CD23 is not the most critical receptor for IgE targeting and Th2 cytokine enhancement, although other inhibitory effects of CD23 on IgE synthesis and allergic inflammation might obscure the precise role of this receptor *in vivo* [107].

Finally, DCs could be critical for maintaining a chronic localised immune response in the airways. It is tempting to speculate that the presence of chronic inflam-

mation and airway structural changes leads to local maturation of DCs and to antigen presentation occurring in the airways. The cytokines TNF- α , GM-CSF and IL-4 are expressed in asthmatic epithelium and have the potential to up-regulate the co-stimulatory capacity of DCs. Mast cells in asthmatic airways express CD40L and produce cytokines that can activate DCs and prolong their survival in tissues [108, 109]. The process of airway remodelling leads to the deposition of extracellular matrix components (collagens, fibronectin, heparan sulphate) that have the capacity to enhance DC differentiation and co-stimu-

latory function [110, 111]. In addition to their potential to activate T cells in the airways, myeloid DCs produce chemokines that preferentially attract recently activated Th2 cells. In this regard, monocyte-derived DCs have been shown to produce the CC chemokines monocyte-derived chemokine and thymus and activation-regulated chemokine, which attract recently activated Th2 cells expressing CCR4 [112–114]. Activation of DCs induces the mRNA for MCP-4 and I-309, the ligands for CCR3 and CCR8 receptors, also expressed on Th2 cells [113, 115]. In this way, DCs ensure that the recently activated effector cells are attracted and retained in the airways and do not migrate back to the draining lymph nodes [116]. Moreover, it has been shown that chronic antigen presentation by DCs leads to neo-formation of organised lymphoid structures within peripheral tissues, such as the pancreas [117]. These organised follicles contain high endothelial venules, specialised for the extravasation of lymphocytes, and are the sites of local immunoglobulin production. It is speculative that repetitive presentation of antigen to Th2 cells by DCs in the airways would also lead to formation of these aggregates and to local production of IgE. At least in rodents, repetitive inhalation of allergen in sensitised animals leads to the formation of organised lymphoid tissues in the airways [118]. Switching to IgE production in B cells can be enhanced locally by Th2 cells and mast cells producing IL-4 and expressing CD40L [119]. Additionally, DCs might directly interact with B cells in the airways to enhance immunoglobulin production.

Applicability to Human Asthma

From the above discussion, it seems likely that human DCs are also implicated in Th2-driven eosinophilic airway inflammation in genetically predisposed individuals. In humans, an extensive network of MHC class II-positive DCs is present in the nose and large conducting airways of the lung [120]. In atopic asthmatics, the density of this network is increased, suggesting that DC function is upregulated during allergic inflammation [90, 121]. Moreover, the DCs express the α chain of the high-affinity IgE receptor, suggesting that they may capture low thresholds of specific allergen from inspired air [91]. The function of DCs of patients with or without atopic diseases has been compared only in vitro. It was shown that immature monocyte-derived dendritic cells (mo-DCs) from atopic asthmatics have an enhanced allostimulatory function and higher levels of CD11b and HLA-DR compared with those from non-atopic patients, although others have

refuted this idea [122, 123]. When mo-DCs were pulsed with relevant allergen (Der p 1 and 2, Bet v 1) and cultured with autologous naive or memory T cells, they induced both Th1 and Th2 cytokines – but predominantly Th2 when atopic donors were used – despite similar IL-12 production [123–125]. The further addition of recombinant IL-12 induced a separate IFN- γ -producing subset without decreasing the IL-5-producing subset, whereas addition of anti-IL-12 reduced bulk IFN- γ production without affecting IL-4 production. These data confirm that DCs can efficiently stimulate human allergen-specific memory Th2 cells to produce Th2 cytokines and maybe contribute to ongoing allergic inflammation [15, 98]. Consistent with this idea, Hammad et al. [96] [unpubl. results] were able to show in a humanised SCID mouse model that human mo-DCs pulsed with Der p 1 significantly enhanced the cellular infiltration of human allergen-specific T cells in the lungs of challenged mice and boosted the formation of allergen-specific IgE. Finally, removal of airway DCs using inhaled corticosteroids in atopic asthmatics is associated with a reduction in symptoms and improvements of lung function, although numerous other mechanisms of steroid action might contribute to this effect as well.

Conclusion

Recent advances in animal models of allergy have identified DCs as the principal APCs involved in the induction and maintenance of Th2 allergic responses. These models are now slowly being validated in human asthma. It can be foreseen that in the near future, studies on the functional role of these cells in humans will lead to an increased insight into the pathogenesis of allergic diseases and hopefully to better strategies for preventing and treating these conditions.

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