In vivo depletion of lung CD11c$^+$ dendritic cells during allergen challenge abrogates the characteristic features of asthma

Leonie S. van Rijt,[1] Steffen Jung,[2] Alex KleinJan,[1] Nanda Vos,[1] Monique Willart,[1] Catherine Duez,[3] Henk C. Hoogsteden,[1] and Bart N. Lambrecht[1]

[1]Department of Pulmonary Medicine, Erasmus MC, 3015 GE Rotterdam, Netherlands
[2]Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel
[3]Institut National de la Santé et de la Recherche Médicale U416, Institut Pasteur de Lille, 59019 Lille, France

Although dendritic cells (DCs) play an important role in sensitization to inhaled allergens, their function in ongoing T helper (Th)2 cell–mediated eosinophilic airway inflammation underlying bronchial asthma is currently unknown. Here, we show in an ovalbumin (OVA)-driven murine asthma model that airway DCs acquire a mature phenotype and interact with CD4$^+$ T cells within sites of peribronchial and perivascular inflammation. To study whether DCs contributed to inflammation, we depleted DCs from the airways of CD11c-diphtheria toxin (DT) receptor transgenic mice during the OVA aerosol challenge. Airway administration of DT depleted CD11c$^+$ DCs and alveolar macrophages and abolished the characteristic features of asthma, including eosinophilic inflammation, goblet cell hyperplasia, and bronchial hyperreactivity. In the absence of CD11c$^+$ cells, endogenous or adoptively transferred CD4$^+$ Th2 cells did not produce interleukin (IL)-4, IL-5, and IL-13 in response to OVA aerosol. In CD11c-depleted mice, eosinophilic inflammation and Th2 cytokine secretion were restored by adoptive transfer of CD11c$^+$ DCs, but not alveolar macrophages. These findings identify lung DCs as key proinflammatory cells that are necessary and sufficient for Th2 cell stimulation during ongoing airway inflammation.

CORRESPONDENCE
Bart N. Lambrecht:
b.lambrecht@erasmusmc.nl

Abbreviations used: AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; DTR, diphtheria toxin receptor; i.t., intratracheal; MLN, mediastinal LN; nTg, nonTg; Tg, transgenic.

Allergic asthma is a chronic inflammatory disease of the airways associated with a predominant Th2 response to inhaled allergens leading to airway infiltration by eosinophils and mast cells, goblet cell hyperplasia, and bronchial hyperreactivity (1). Despite significant progress in our understanding of disease pathophysiology, prevalence continues to rise, particularly in Western societies. As effector Th2 lymphocytes are intimately involved in controlling the various aspects of the disease, elucidating the mechanisms by which these cells are activated in response to allergen challenge should lead to novel ways of treatment (2).

DCs are important antigen-presenting cells in the immune system, mainly recognized for their extraordinary capacity to induce primary immune responses (3, 4). Airway DCs form a network in the bronchial epithelium, capture inhaled antigen, and migrate to the mediastinal LNs (MLNs) where antigen is presented to recirculating naive CD4$^+$ and CD8$^+$ T cells (5–9). Not surprisingly, antigen presentation by airway DCs is at the basis of the Th2 sensitization process that occurs in patients with allergy and animals exposed to OVA antigen (2, 10–14). Despite these known functions of DCs on primary immune responses and allergic sensitization, it is less clear if airway DCs are also necessary for the presentation of allergen to resting memory Th2 and/or effector Th2 cells during a secondary immune response. In favor of DCs, it is known that the number and maturation state of lung DCs is elevated during secondary immune challenge with allergens and during chronic airway inflammation (15–17). This suggests that DCs are functionally involved in presenting allergens to T cells and, thus, control airway inflammation (10, 12, 18, 19). Arguing against a role for DCs is the notion that previously primed T cells have less need for costimulation in comparison with naive T cells, which led to the hypothesis that any peripheral APC expressing MHC II could stimulate effector Th2 cells (20–22).

To examine more clearly the need for DCs in the induction and maintenance of effector Th2 responses to allergen challenge, we decided to deplete these cells during OVA aerosol exposure of OVA-sensitized mice. Conditional depletion of DCs has recently become possible because we generated transgenic (Tg) mice in which the CD11c promotor element drives the expression of the monkey diphtheria toxin (DT) receptor (23). Murine cells are normally insensitive to DT as they lack the DT receptor. In CD11c-DTR mice, we were able to show that systemic administration of DT leads to a rapid decline in $CD11c^+$ DCs in the spleen, leading to a defect in $CD8^+$ T cell priming (23). Here, we adopted this strategy and administered DT locally to the airways, allowing the inducible, short-term ablation of lung $CD11c^+$ cells. Our data show that in the absence of $CD11c^+$ DCs, OVA allergen challenge in sensitized mice failed to elicit effector cytokine release by endogenous primed Th2 cells or adoptively transferred primed $CD4^+$ Th2 cells. Importantly, depletion of $CD11c^+$ cells abolished the cardinal features of asthma, but these features were restored by adoptive transfer of $CD11c^+$ DCs, but not alveolar macrophages. These studies indicate that lung DCs are necessary and sufficient antigen-presenting cells involved in the development and maintenance of asthmatic airway inflammation.

RESULTS

DCs acquire a mature phenotype and colocalize with T cells within peribronchial eosinophilic inflammation

We have reported previously in a mouse model of asthma that the number of $CD11b^+CD11c^+$ DCs increases up to 100-fold in the bronchoalveolar lavage fluid (BALF) after OVA challenge in OVA-sensitized mice, but have not studied the functional implications of this increase (17). First, we performed a detailed phenotypical analysis of DCs in the BALF, lung tissues, and MLNs (Fig. 1 A) of mice with or without eosinophilic airway inflammation (Fig. 1 A). In mice with airway inflammation, there was a consistent increase in expression of the costimulatory molecules CD80, ICAM-1, CD40, PDL-1, and PDL-2 on endogenous $CD11c^+$ $MHCII^+$ DCs in the BALF and lung tissue compartment compared with control mice. In the MLNs, the costimulatory molecules CD80, CD86, ICAM-1, and CD40 were consistently higher on DCs of allergic mice. To determine whether DCs interacted locally with $CD4^+$ T cells at the site of inflammation, we stained cryosections of lungs of OVA-sensitized and challenged mice for the presence of $CD11c^+$ DCs and $CD4^+$ T cells. Clusters of T cells and $CD11c^+$ DCs were seen in eosinophil-rich peribronchial and perivascular infiltrates (Fig. 1 B and Fig. 2 B) and in the surrounding parenchyma. Multiple large $CD11c^+$ cells were also present in the alveolar compartment, frequently surrounded by small $CD4^+$ cells (Fig. 1 C).

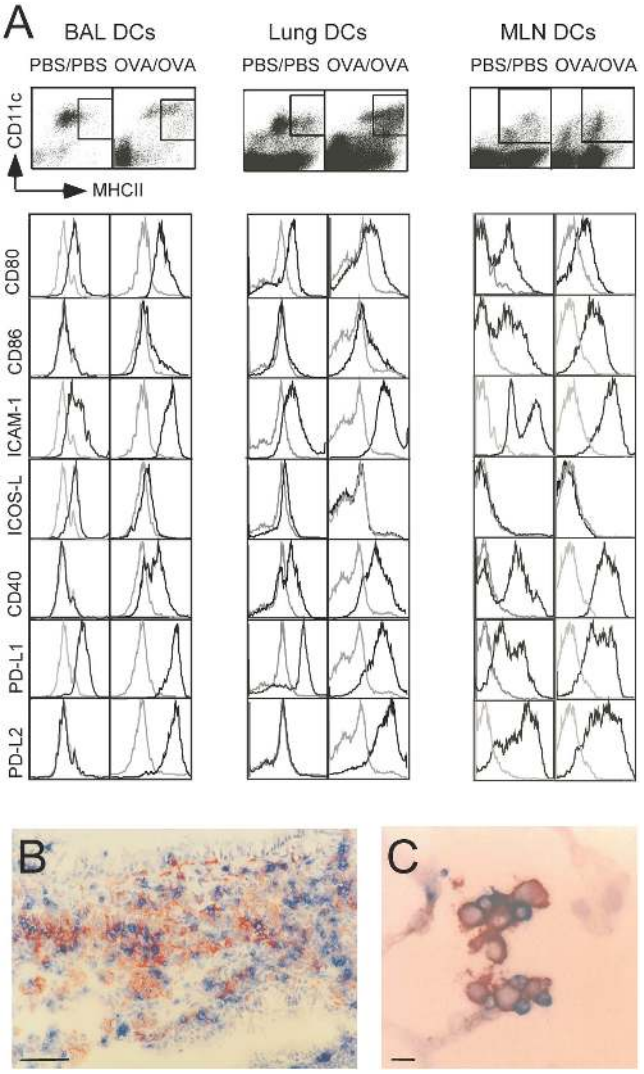

Figure 1. Phenotype of DCs in bronchoalveolar compartment, lung, and lung draining LNs in steady-state and during inflammation. (A) BALB/c mice were OVA sensitized and challenged (OVA/OVA), or as a control sham sensitized/challenged (PBS/PBS), to induce an eosinophilic airway inflammation. DCs were analyzed for the expression of molecules needed for T cell interaction. DCs were recognized as $CD11c^+$/$MHCII^+$ (top). Black lines indicate marker expression and gray lines indicate the isotype control staining. (B) 1 d after the last aerosol, peribronchial infiltrates were analyzed for the presence of $CD4^+$ T cells (blue) and $CD11c^+$ DCs (red) in frozen lung sections. Bar, 100 μm. (C) At a larger magnification, it is shown that DCs and T cells are interacting in the peribronchial infiltrates as well as in the lung parenchyma. Bar, 10 μm.

Intratracheal (i.t.) injection of DT depletes $CD11c^+$ cells in CD11c-DTR Tg mice

The acquisition of a mature phenotype within the lung and the close interaction between DCs and $CD4^+$ T cells within areas of inflammation suggested that DCs were necessary for stimulating antigen-specific primed T cells. To investigate the functional role of this interaction, we used CD11c-DTR Tg BALB/c mice in which administration of DT allows a conditional ablation of $CD11c^+$ cells (23). In the mouse, CD11c is highly expressed on myeloid DCs, as well as on alveolar macrophages and some activated $CD8^+$ T cells. FACS analysis revealed that i.t. injection of 50 ng DT in naive CD11c-DTR Tg mice depleted

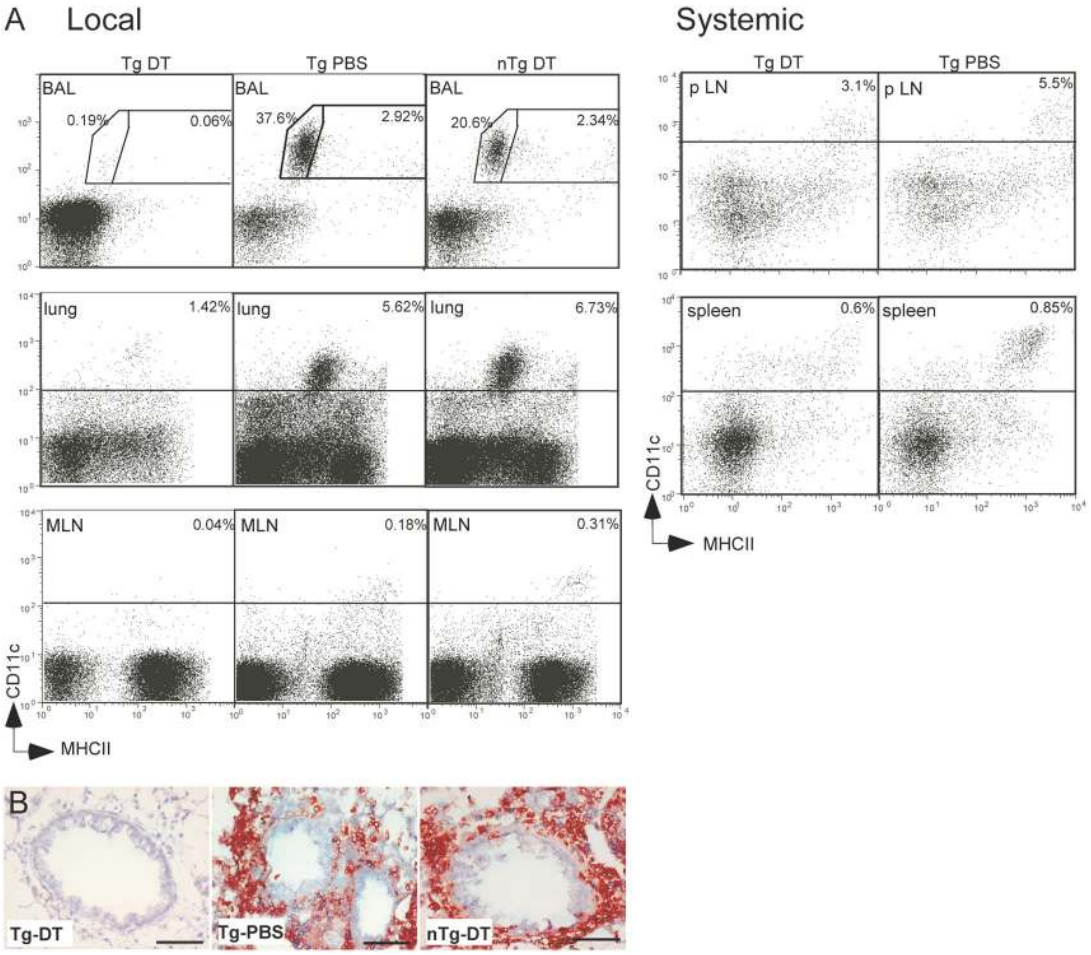

Figure 2. DT treatment depletes CD11c$^+$ cells in CD11c-DTR Tg mice. Naive CD11c-DTR Tg and nTg mice were i.t. injected with 50 ng DT (Tg DT and nTG DT) or only PBS (Tg PBS). (A, left) 48 h after instillation, BALF cells, lungs, and lung draining LNs were analyzed for the presence of CD11c$^+$MHCII$^+$ DCs by FACS. (right) Systemic effects of DT on peripheral axillary LNs and splenic DCs. (B) OVA-sensitized Tg and nTg mice were treated with DT or PBS and received three OVA aerosols. Lung sections stained for CD4$^+$ (blue) and CD11c$^+$ (red) cells showed that i.t. administration of 50 ng DT in Tg mice before challenge, inhibited the induction of peribronchial infiltrates containing CD4$^+$ and CD11c$^+$ cells (left), whereas in nTg mice (right) DT did not affect CD11c$^+$ DCs. As a control, Tg mice were treated with PBS (middle), showing a similar peribronchial infiltrate as in WT mice. The results shown represent two independent experiments. Bars, 100 μm.

local CD11c$^+$ MHCII$^+$ DCs in BALF, lung tissue, and MLNs (Fig. 2 A, left). However, in addition, the number of systemic DCs were affected, although not in the degree seen in the airways (Fig. 2 A, right). DT also depleted CD11c$^+$, MHCII^{int} highly autofluorescent alveolar macrophages in the BALF and lung tissue of naive mice (Fig. 2 A). Next, we determined in OVA-sensitized and -challenged mice that i.t. injection of 50 ng DT before challenge depleted CD11c$^+$ DCs in CD11c-DTR Tg mice, but not in nonTg (nTg) littermates. Control PBS-injected Tg mice had massive amounts of CD11c$^+$ cells within sites of inflammation (Fig. 2 B).

Conditional depletion of CD11c$^+$ cells in sensitized animals abolishes the cardinal features of asthma

DT-induced DC depletion allowed us to study the response to OVA challenge in OVA-sensitized mice in the presence or absence of DCs. CD11c-DTR Tg and wild-type nTg littermates were sensitized to OVA on day 0 by giving an i.t. injection of OVA-pulsed bone marrow–derived DCs. At day 10, both groups of mice were injected i.t with 50 ng DT and were subsequently challenged with three daily OVA aerosols during days 11–13. In nTg mice, this protocol induces eosinophilic airway inflammation only when OVA-pulsed DCs are used for immunization (reference 8 and unpublished data). Analysis of BALF cells of mice that were depleted of DCs (CD11c-DTR Tg mice treated with DT will be referred to as Tg DC$^-$) before challenge with OVA aerosols revealed a significantly lower number of total BALF cells and eosinophils compared with mice that were not DC depleted (nTg littermates treated with DT are referred to as nTg DC$^+$ and Tg animals treated with PBS are referred to as Tg DC$^+$; Fig. 3, A and B). In concordance, histological analysis

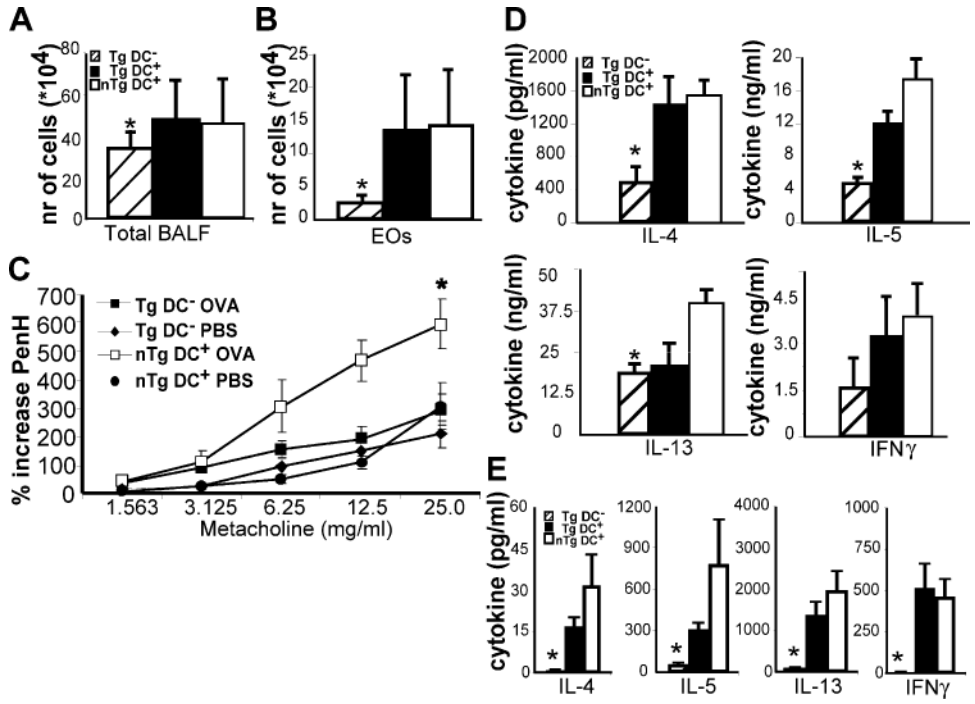

Figure 3. DC requirement for induction of secondary immune responses. Mice were OVA sensitized at day 0, received a DT i.t. injection at day 10, and received a daily 30-min aerosol at days 11–13. At day 14, mice were killed. (A) BALF cells were analyzed for total BALF numbers and (B) the number of eosinophils 1 d after the last aerosol. (C) AHR was measured in CD11c-DTR Tg mice and nTg littermates that were sensitized with OVA (alum) and challenged with three OVA aerosols or as a control with PBS aerosols. 24 h before the first OVA aerosol, all mice were treated with DT. Tg DC$^-$/OVA, OVA-challenged DC-depleted CD11c-DTR Tg; nTg DC$^+$/OVA, OVA-challenged nTg mice; Tg DC$^-$/PBS, PBS-challenged DC-depleted CD11c-DTR Tg; nTg DC$^+$/PBS, PBS-challenged nTg. (D) Th2 cytokine production by lung draining LNs in Tg DC$^-$, Tg DC$^+$, and nTg DC$^+$ mice. (E) A cohort of in vitro–obtained memory Th2 were adoptively transferred (i.v.) into Tg DC$^-$ and nTg DC$^+$, simultaneously with an i.t. injection of DT or as a control PBS (Tg DC$^+$). 24 h after the adoptive transfer, mice were challenged with OVA aerosols for 4 d. Lung draining LNs were restimulated ex vivo with OVA and IL-4, IL-5, IL-13, and IFNγ levels were determined in supernatant by ELISA (*, $P < 0.05$ vs. nTg DC$^+$). The results shown represent one out of five independent experiments and are expressed as means ± SEM.

of Tg DC$^-$ mice did not show peribronchial and perivascular inflammatory infiltrates as seen in nTg DC$^+$ mice or Tg DC$^+$ mice (unpublished data). To measure bronchial hyperresponsiveness, Tg DC$^-$ and nTg DC$^+$ mice were exposed to increasing doses of metacholine. After OVA challenge, nTg DC$^+$ mice had an increase in PenH over baseline after exposure to 6.25, 12.5, and 25 mg/ml metacholine compared with nTg DC$^+$ mice challenged with PBS aerosol challenge, illustrating the induction of airway hyperresponsiveness (AHR) by OVA challenge in OVA-sensitized mice. In contrast, after OVA challenge, Tg DC$^-$ mice had an AHR curve that followed the curve seen in Tg DC$^-$ and nTg DC$^+$ mice that were OVA sensitized but were challenged with PBS (Fig. 3 C).

Next, we determined whether the absence of these cardinal features of asthma in CD11c$^+$ cell–depleted mice was associated with an inability to generate effector function in primed Th2 cells. In vitro restimulation of lung draining LN cells with OVA showed that DC depletion before allergen challenge greatly diminished IL-4, IL-5, and IL-13 production, whereas IFNγ production was unaffected (Fig. 3 D). Supporting an essential role for DCs in eliciting effector cytokine release from primed Th2 cells, we performed adoptive transfer experiments in which previously primed in vitro–generated OVA-specific Th2 cells from D011.10 mice were injected intravenously into naive Tg DC$^-$ and Tg DC$^+$ and nTg DC$^+$ mice. As described previously (24), OVA aerosol challenge leads to Th2 cytokine production by these adoptively transferred Th2 cells in mice with DCs (Tg DC$^+$ and nTg DC$^+$). However cytokine production was impaired in recipient mice (Tg DC$^-$) that were depleted of DCs (Fig. 3 E).

DC reconstitution restores eosinophilic airway inflammation in DC-depleted mice

Because CD11c is also expressed by alveolar macrophages and weakly by activated CD8$^+$ T cells and eosinophils, we performed adoptive transfer reconstitution experiments after depletion with DT to test our hypothesis that DCs are indeed the cells required for the induction of the secondary immune response. Tg DC$^-$ mice received at the time of OVA aerosol challenge i.t. 2×10^6 unpulsed DCs, i.t. 2×10^5 alveolar

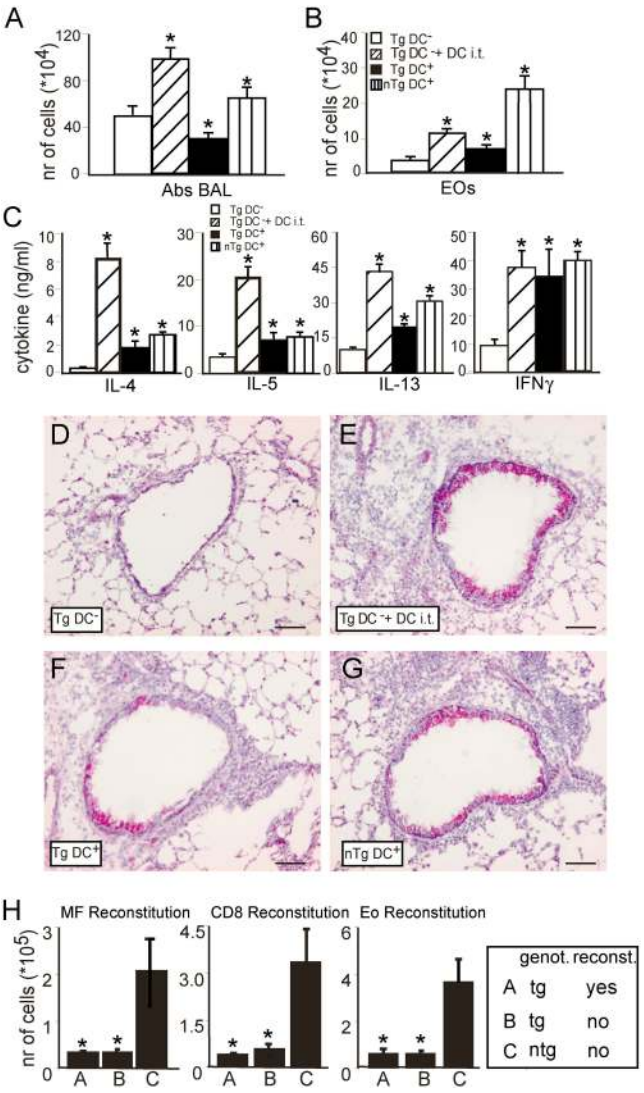

Figure 4. DC reconstitution restored eosinophilic airway inflammation. CD11c-DTR Tg and nTg mice were OVA sensitized at day 0, received i.t. DT (Tg DC$^-$, Tg DC$^-$ + DC i.t. and nTg DC$^+$) or PBS (Tg DC$^+$) at day 10 and were challenged with OVA aerosols at days 11–13. Mice were reconstituted with unpulsed 2×10^6 DCs i.t. immediately after OVA challenge. BALF was analyzed for (A) total BALF numbers and (B) the number of eosinophils at day 14. (C) Th2 cytokine production by MLN after ex vivo restimulation with OVA was measured in supernatant by ELISA (*, $P < 0.05$ vs. Tg DC$^-$). (D–G) Histological sections of Tg DC$^-$, Tg DC$^-$ + DC i.t., Tg DC$^+$, and nTg DC$^+$ mice stained with PAS reagent. Bars, 100 μm. (H) Number of eosinophils in BALF after reconstitution with macrophages, CD8$^+$ cells or eosinophils (*, $P < 0.05$ vs. C). The results shown represent one out of two independent experiments and are expressed as means ± SEM.

macrophages, i.t. 10^6 eosinophils, or i.v. 7.5×10^6 CD8$^+$ T cells. Reconstitution with unpulsed DCs in Tg DC$^-$ mice increased total BALF cells (Fig. 4 A) and was able to restore the absolute number of eosinophils in BALF (Fig. 4 B) as well as Th2 cytokine production in lung draining LNs (Fig. 4 C) and inflammatory peribronchiolar and perivascular infiltrates (Fig. 4 E) to a similar level seen in Tg DC$^+$ (Fig. 4 F) and nTg DC$^+$ (Fig. 4 G). Th2 cytokine production was the highest in the group receiving adoptive transfer of unpulsed DCs. In contrast, adoptive transfers with macrophages, CD8$^+$, or eosinophils were unable to restore the response to OVA challenge in Tg DC$^-$, as shown by the lack of recruitment of eosinophils to the BALF compartment (Fig. 4 H).

DC depletion during ongoing Th2 inflammation diminishes cardinal features of asthma

Next, we determined the role of lung DCs during an ongoing inflammation in which effector Th2 cells are already present in the lung and eosinophilia is established. CD11c-DTR Tg or nTg mice were sensitized to OVA and challenged at days 10–12 with daily OVA aerosol (8). On day 13, when both groups had an eosinophilic airway inflammation, DT was given intratracheally to both groups to generate nTg DC$^+$ and Tg DC$^-$ mice. Mice were subsequently challenged for another three times at days 14–16 by daily OVA aerosol. One additional group of CD11c-DTR Tg mice received the same treatment but was challenged with PBS aerosols at days 14–16 after DC depletion (Tg DC$^-$/PBS) to determine the resolution of airway inflammation in the absence of allergen challenge. Analysis of Tg DC$^-$/OVA mice revealed a lower number of total BALF cells and eosinophils compared with nTg DC$^+$/OVA mice (Fig. 5, A and B; 0.18×10^6 vs. 10^6 eosinophils). In Tg DC$^-$/OVA mice, BALF cellularity and eosinophilia was similar to that seen in Tg DC$^-$/PBS mice, suggesting that OVA was not presented by other CD11c$^-$ APCs. In support, IL-4, IL-5, and IL-13 cytokine production by lung draining LNs of Tg DC$^-$/OVA mice was decreased compared with nTg DC$^+$/OVA littermates. Also, Tg DC$^-$/PBS-challenged mice produced lower, although not significantly, levels of IL-4, IL-5, and IL-13 (Fig. 5 C). Histological analysis revealed significantly ($P \leq 0.032$) less inflammatory peribronchial and perivascular infiltrates and goblet cell hyperplasia in Tg DC$^-$/OVA mice (Fig. 5 D) and DC$^-$/PBS (Fig. 5 E) compared with nTg DC$^+$/OVA mice (Fig. 5 F). Tg DC$^-$/OVA mice had a mean inflammatory area ratio of 0.49 compared with 0.77 in nTg DC$^+$ mice ($P < 0.001$). In Tg DC$^-$ mice in which PBS aerosol was given during the last 3 d, inflammation was least prominent (area ratio of 0.13; $P < 0.0001$ compared with Tg DC$^+$/OVA).

Adoptive transfer of DCs is sufficient to induce all asthmatic features in sensitized mice

Next, we investigated whether DCs have an intrinsic capacity to induce a secondary immune response in sensitized animals, without any form of adjuvant or aerosol exposure. Mice were sensitized by i.t. injection of 10^6 OVA-pulsed DCs (OVA-DCs). On days 10 and 12, mice were challenged i.t. with OVA-DCs (DC/DC group). As a positive control

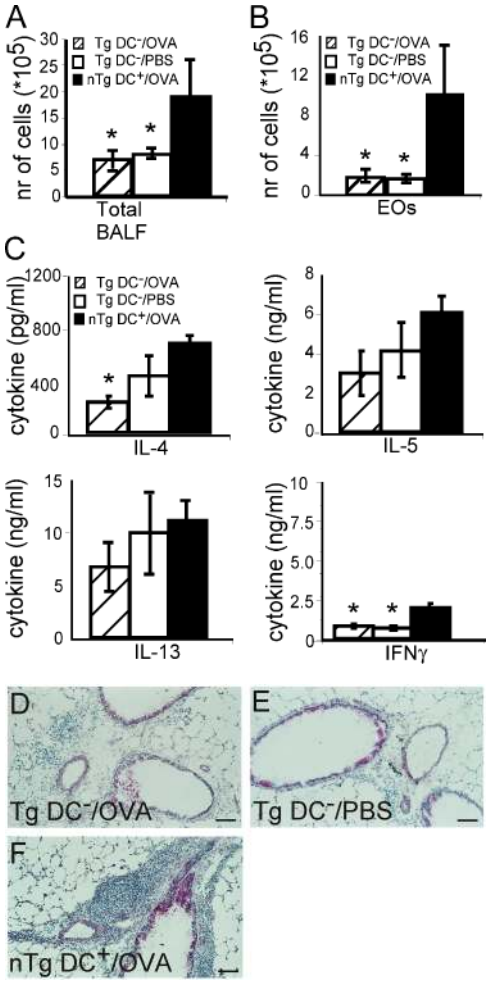

Figure 5. DC requirement for maintaining eosinophilic airway inflammation. CD11c-DTR Tg (Tg DC$^-$/OVA) and nTg mice (nTg DC$^+$/OVA) were OVA sensitized at day 0, received a daily 30-min aerosol at days 11–13. At day 14, mice received DT, and at days 15–17 mice were challenged daily with a 30-min OVA. One group of CD11c-DTR Tg mice (Tg DC$^-$/PBS) received the same treatment but was challenged with PBS aerosol after DC depletion. BALF was analyzed for (A) total BALF numbers and (B) the number of eosinophils. (C) Th2 cytokine production by MLN after ex vivo restimulation with OVA was measured in supernatant by ELISA. (D–F) Histological sections of Tg DC$^-$/OVA, Tg DC$^-$/PBS, and nTg DC$^+$/OVA mice stained with PAS reagent (*, $P < 0.05$ vs. nTg DC$^+$ /OVA). The results shown represent one out of three independent experiments and are expressed as means ± SEM. Bars, 100 μm.

for the efficiency of sensitization, some sensitized mice received four daily OVA (DC/OVA group) or PBS (DC/PBS group) aerosols for 30 min, starting at day 10. On day 14, total recovery of BALF cells and BALF eosinophilia in both DC/DC mice and DC/OVA mice were identical and >20 times higher than in DC/PBS mice (Fig. 6, A and B). These data were supported by similar levels of Th2 cytokines seen in DC/DC mice compared with DC/OVA mice (Fig. 6 C). Histological analysis revealed intense peribronchial and perivascular eosinophilic infiltrates and goblet cell hyperplasia, of similar intensity in DC/DC and DC/OVA mice (Fig. 6, D and E; $P = 0.2$). As expected, DC/PBS mice did not develop eosinophilic airway inflammation (Fig. 6 F; $P = 0.008$ vs. DC/OVA and $P = 0.016$ vs. DC/DC). Quantitative histological analysis (area ratio) resulted in similar findings as obtained with ranking by investigators: DC/PBS, 0.05 versus DC/OVA, 0.65 ($P < 0.0001$) or DC/DC, 0.59 ($P < 0.0001$). DC/DC and DC/OVA mice both had a significant increase in PenH after metacholine inhalation compared with DC/PBS mice, indicative of induction of bronchial hyperreactivity (Fig. 6 G; $P < 0.05$). These findings did not occur in NOD/SCID mice, suggesting that DCs required interaction with T cells to generate inflammation and were not proinflammatory per se (unpublished data).

DISCUSSION

Although it is generally accepted that effector Th2 cells and their secreted products are important in controlling the cardinal features of asthma, little is known about their activation in response to allergen inhalation (2). DCs are believed to be the most important APCs in the lung in steady-state (2, 3, 12) and are mainly known for their potential to induce the activation of naive T cells in the draining MLNs leading to antimicrobial, antitumoral, and allergic T cell responses (9, 25, 26). However, from the perspective of T cell–mediated diseases such as atopic asthma, an important question is whether lung DCs are also responsible for the reactivation of allergen-specific primed T cells at times of allergen challenge and, therefore, would constitute a target for therapeutic intervention. Evidence is accumulating that DCs are indeed responsible for the reactivation of these primed Th2 cells upon allergen challenge (for review see reference 12). Relevant allergen challenge to the airways of allergic asthmatics leads to the recruitment of circulating DCs to the airways and stable asthmatics have higher numbers of airway lining DCs (15, 16). Similarly, OVA allergen challenge in OVA-sensitized rodents increased the number of myeloid DCs in the airway mucosa, interstitial, and bronchoalveolar compartment, at a time point that CD4$^+$ T cells were also accumulating in the lungs (10, 17, 27).

These data suggest in an indirect way that airway DCs might be involved in the functional restimulation of previously primed Th2 cells. In this work, we have directly addressed this issue by studying the role of CD11c$^+$ lung DCs in a well-established mouse model for asthma induced by inhalation of OVA in OVA-sensitized mice. First, we confirmed that, upon allergen challenge to the lung, CD11c$^+$ cells acquired a mature phenotype expressing essential costimulatory molecules (CD80, CD40, ICAM-1, PDL-1, and PDL-2) when resident within the inflamed lung. 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$^+$

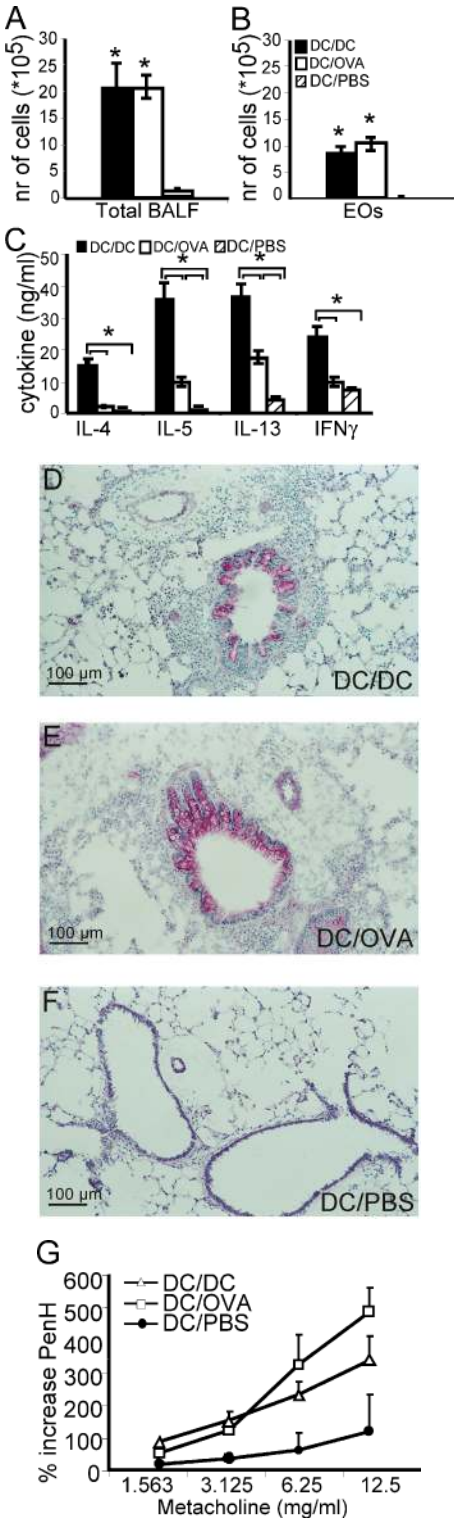

Figure 6. Adoptive transfer of DCs is sufficient to induce all asthmatic features in sensitized mice. Mice were OVA DC sensitized at day 0, received at days 10 and 12 an i.t. injection of OVA DCs (black bars), or received at days 10–13 a daily OVA aerosol (white bars) or PBS aerosols (diagonally striped bars). (A and B) At day 14, total BALF cells numbers and eosinophil numbers were determined by FACS (*, $P < 0.05$ vs. DC/PBS). (C) Th2 cytokine production by MLNs was determined after ex vivo restimulation with OVA. IL-4, IL-5, IL-13, and IFN-γ were measured in supernatant by ELISA. (D–F) Histological lung sections of mice challenged with OVA DCs (DC/DC), OVA aerosols (DC/OVA), and PBS aerosols (DC/PBS). (G) Airway responsiveness was increased in DC/DC and DC/OVA compared with DC/PBS mice. The results represent one out of two independent experiments and are expressed as means ± SEM.

T cells (28). 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 (10). 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 (10). Strikingly, we also observed an increase in the B7 family members PDL-1 and PDL-2, ligands of the inhibitory PD-1 receptor. Although signaling through PD-1 is generally seen as an inhibitory signal, recent data suggest that PDL-1 might also provide a costimulatory signal to T cells (28). In support, blocking PDL-1/PDL-2 using PD1-Fc has the potential to suppress eosinophilic airway inflammation (unpublished data).

As intense local interactions between primed $CD4^+$ T cells and locally matured DCs are a feature of eosinophilic airway inflammation across species, next we studied whether effector $CD4^+$ T cells functionally depend on DCs to exert their effector functions and, in this way, orchestrate airway inflammation. We addressed this question by using a novel system of conditional depletion of $CD11c^+$ cells expressing a DT receptor transgene under the control of the CD11c promoter (23). Systemic administration of DT to these mice leads to the rapid reduction in splenic and LN $CD11c^+$ DCs, and concomitantly to an absence of the primary cytotoxic T cell responses to *Listeria* infection or experimental administration of protein antigens (19). Here, we adopted this strategy and administered the DT locally via i.t. injection, leading to a loss of $CD11c^+$ cells in the BALF and lung tissues (DCs and macrophages) and draining MLNs (DCs). We cannot explain at present by what mechanism the $CD11c^+$ DCs were depleted from the MLNs, but we speculate that they were reduced because of the loss of import of freshly migrating DCs from the mucosa, as these DCs have a very short half-life in the mucosa and continuously migrate to the nodes even in noninflammatory conditions (7, 29, 30). Alternatively, DT might have reached the MLNs directly from the bronchial lumen via the afferent lymphatics or via the bloodstream, causing local damage to resident LN DCs. In support of the latter, we observed partial depletion of systemic DCs; however, not to the degree seen in the airways.

Using this unique system, we conditionally depleted $CD11c^+$ cells locally from the lung immediately before OVA challenge in OVA-sensitized mice and saw that the

cardinal features of asthma such as eosinophilic airway inflammation, goblet cell hyperplasia, and bronchial hyperreactivity to metacholine failed to develop. 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 saw 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 adoptively transferred in vitro–primed $CD4^+$ Th2 cells from DO11.10 TCR Tg T cells in CD11c cell–depleted mice. Previous data have shown that such in vitro–differentiated Th2 cells mimic in vivo–generated memory T cells (31, 32) and can be used to transfer Th2 reactivity to OVA aerosol passively (24). 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 LNs of the lung, whereas they readily did in the presence of lung $CD11c^+$ DCs. Together, these data show that $CD11c^+$ cells are necessary for mounting the secondary response to OVA challenge in previously sensitized mice through the induction of effector function in primed Th2 cells. In view of the unique antigen-presenting characteristics of DCs in the airways, it is very likely that these effects were due to depletion of $CD11c^+$ DCs and not $CD11c^+$ autofluorescent alveolar macrophages because of several reasons. First, as direct proof, all the salient features of asthma were restored by i.t. adoptive transfer of wild-type bone marrow–derived $CD11c^+$ myeloid DCs, but not by transfer of $CD11c^+$ wild-type alveolar macrophages. Second, studies by Huh et al. and our own studies show a predominant interaction between $CD4^+$ T cells and DCs in the submucosa of the airways, at distant sites from alveolar macrophages, making a functional contribution of these cells less likely (10). Third, previous studies in mice in which alveolar macrophages were depleted using clodronate-filled liposomes have seen an actual enhancement of airway inflammation and T cell reactivity in the lung (33). This is explained by the normally suppressive influence of alveolar macrophages on activation of primed T cells and DCs in the lung (3). The fact that airway inflammation was severely suppressed in the combined absence of $CD11c^+$ DCs and $CD11c^+$ alveolar macrophages attests to the crucial role that $CD11c^+$ DCs play during the secondary response.

As the CD11c marker is also expressed weakly on activated $CD8^+$ T cells (34), we performed adoptive transfer reconstitution experiments of wild-type $CD8^+$ T cells to DT-treated mice, again failing to restore the eosinophilic airway inflammation and Th2 cytokine production. Moreover, CD8-deficient (35) and β_2-microglobulin–deficient mice (36) develop normal airway eosinophilia in mouse models of asthma. Eosinophils express very low levels of CD11c (37) and, thus, are likely to be less sensitive to DT compared with DCs. We cannot completely exclude that DT would target eosinophils directly. However, treatment with DT reduced not only eosinophilia but also effector Th2 cytokine secretion, goblet cell hyperplasia, and bronchial hyperreactivity, strongly suggesting that the main effect of DT treatment was on $CD11c^+$ DCs, leading to a failure of T cell activation. In support of this, i.t. reconstitution with wild-type eosinophils did not restore eosinophilia in the BAL compartment nor the secretion of effector Th2 cytokines.

These data of local depletion of $CD11c^+$ DCs immediately before antigen challenge to the lung support our previous work in which we systemically depleted all myeloid DCs from the lungs, LNs, bone marrow, and spleen of HIV-LTR promoter-driven thymidine kinase Tg mice before allergen challenge. In thymidine kinase Tg mice, conditional depletion of DCs took considerably more time as it depended on killing of dividing DC precursors and had to be performed before T cell activation as the HIV-LTR promoter used to drive transgene expression was also activated in effector T cells, a problem not encountered in the CD11c DTR Tg mice. Therefore, next we questioned what would be the role of lung DCs during ongoing inflammation at a time when fully activated effector $CD4^+$ Th2 cells are already causing all the features of asthma. When $CD11c^+$ DCs were depleted in the middle of the OVA aerosol period, all the salient features of asthma were strongly diminished, and effector cytokine secretion was strongly reduced. We observed that eosinophilia actually resolved despite ongoing allergen exposure when DCs were depleted from the airways, and the speed of resolution was similar to mice no longer exposed to allergen.

Although these depletion studies show that DCs are required for eosinophilic airway inflammation in vivo, we looked for additional proof to show that they are also sufficient to induce all asthma features. Repeated i.t. injection of OVA-pulsed DCs in the absence of any adjuvant or aerosol, commonly used in all other asthma models, induced all the cardinal features of asthma, including Th2 cytokine activation and bronchial hyperreactivity. As this response did not occur in SCID mice devoid of lymphocytes, it seems that DCs mediate their effect by first sensitizing naive T cells to become Th2 cells, and subsequently restimulate these cells to cause disease, although it is not completely excluded that DCs also have direct effector activities.

Based on studies on the functional interaction between mucosal T cells and DCs (10) and based on our current findings, it is clear that effector Th2 responses in vivo in the lung continuously depend on antigen-presenting DCs. One possible explanation would be that effector T cells in vivo remain dependent on costimulation in contrast with their in vitro counterparts (20, 38). Numerous models of asthma have demonstrated that blocking the interaction of costimulatory molecules of the B7 superfamily (CD80, CD86, ICOS-L) or TNF-R family (OX40L) can reduce the features of asthma, even when given during challenge in sensitized mice and even when given together with in vitro–primed Th2 cells (39–43). As lung DCs are the predominant cell

type expressing these molecules (Fig. 1 A), an absence of DCs might have the same effect as blocking these costimulatory molecules. An alternative explanation would be that lung DCs are essential for the recruitment of Th2 cells by producing Th2 selective chemokines. Indeed, it was 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, acting on $CCR4^+$ Th2 cells (18, 44). Therefore, depletion of lung DCs might lead to an "unfavorable" chemokine gradient, failing to attract primed Th2 cells to the lung.

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 shown recently 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 (45). Similarly, in T cell–mediated peri-insulitis of prediabetic NOD mice, DCs are prominently present and cluster with pathogenic T cells (46). Although formally not yet proven, it is very likely that in light of our data, DCs are essential for activation of pathogenic T cells in these diseases as well.

In conclusion, our findings identify lung DCs as key proinflammatory cells that are necessary and sufficient for Th2 cell stimulation during ongoing airway inflammation and validate the airway DC as a novel target for directed therapy of asthma.

MATERIALS AND METHODS

Animals

Wild-type BALB/c and CD11c-DTR Tg BALB/c mice were housed under specific pathogen-free conditions at the animal facility of Erasmus MC and experiments were approved by the animal ethical committee. The generation and screening of CD11c-DTR Tg mice has been reported previously (23).

Culture and antigen pulsing of bone marrow DCs

Bone marrow cells were collected from naive mice, depleted of red blood cells using ammonium chloride, and grown in RPMI 1640 culture medium containing 5% FCS (Sigma-Aldrich) and rmGM-CSF (provided by K. Thielemans, Free University, Brussels, Belgium) for 9 d as described previously (47). At day 9, they were pulsed in vitro overnight with 100 μg/ml OVA (Worthington Biochemical Corp.; LPS contamination <29 EU/mg LPS by LAL assay) (OVA-DC) or sham-pulsed with PBS (PBS-DC).

Asthma model using OVA-pulsed DC immunization followed by OVA aerosol challenge

To induce sensitization to inhaled OVA, 10^6 BM-derived OVA-DCs were injected intratracheally into the airways of naive anesthetized mice, as described previously (8). 10 d after i.t. immunization, mice were challenged with OVA aerosol (1% wt/vol in PBS; Sigma-Aldrich; using a jet nebulizer) during a daily 30-min challenge on three to six consecutive days, generating vigorous eosinophilic airway inflammation (8). Experiments using $Ly5.1^+$ DC adoptive transfer into Ly5.2 recipients demonstrated that none of the transferred DCs remained in the lungs at the time of allergen challenge (unpublished data). To address their functional role in generating airway inflammation, endogenous $CD11c^+$ DCs were depleted before or during OVA challenge.

In a first series of experiments, CD11c-DTR Tg mice and nTg littermates were sensitized to OVA at day 0. At day 10, all mice received an i.t injection of 50 ng DT to deplete $CD11c^+$ cells and were subsequently challenged with three daily OVA or control PBS aerosols from days 11–13. Mice were killed at day 14.

In another series of experiments to address the role of DCs in already established inflammation, CD11c-DTR Tg mice and nTg littermates were sensitized to OVA at day 0 and subsequently challenged with three OVA aerosols on days 10–12. On day 13, all mice received an i.t. injection of 50 ng DT. On days 14–16, mice were further aerosolized with OVA or as a control with PBS. Mice were killed at day 17.

Adoptive transfer reconstitution experiments were performed in DT-treated CD11c DTR Tg mice with the following wild-type cells: unpulsed bone marrow–derived DCs; alveolar macrophages (98% pure from pooled BALF of naive mice); eosinophils (89% pure sorted based on CCR3 expression from pooled BALF of allergic mice as described previously [37]); and $CD8^+$ cells (purified to 85% using negative depletion beads [Miltenyi Biotec] from the spleen and LNs of OVA-sensitized and -challenged mice).

Asthma model using only adoptive transfer of DCs

In separate experiments, OVA aerosol challenge was compared with challenge with OVA-pulsed DCs in OVA-DC– or PBS-DC–sensitized mice. Mice received two i.t. injections of 10^6 OVA-DCs at day 10 and day 12. Alternatively, mice were aerosolized with OVA at days 10–13. Animals were killed 24 h after the last aerosol or 48 h after the last DC injection.

Adoptive transfer of OVA-specific Th2 cells

In some experiments, naive mice received adoptive transfer of in vitro–differentiated Th2 cells obtained from DO11.10 OVA-TCR Tg mice. DO11.10 LN cells were stimulated with OVA for 6 d in the presence of 10 ng/ml IL-4, 10 μg/ml anti-IFNγ, 1 μg/ml anti–IL-12, and 5 μg/ml OVA peptide. Th2 differentiation was confirmed by quantitative PCR trough a 360-fold increase in mRNA for IL-4, 892-fold increase in IL-5, and 608-fold increase in IL-13, whereas mRNA for IFNγ was unaffected compared with naive DO11.10 T cells. After 6 d, cells were washed, and 3×10^6 cells were injected i.v. into naive CD11c-DTR Tg mice treated simultaneously or not with DT to deplete DCs. Mice were challenged with daily OVA aerosols for the subsequent 4 d. Th2 cytokine production by lung draining LNs was determined ex vivo 24 h after the last aerosol challenge.

Collection and analysis of cells and tissues

BALF. 24 h after the last aerosol, BAL was performed with 3× 1 ml of Ca^{2+}- and Mg^{2+}-free PBS. After red blood cell lysis using ammonium chloride lysis buffer, cells were stained with MHCII-FITC (2G9), CD11c-APC (HL3), B220-CyChr (RA3-6B2), CD3-CyChr (145-2C11; BD Biosciences), and CCR3-PE (R&D Systems). 2.4.G2 was used to prevent nonspecific binding. The cellular composition of BALF cells was determined using flow cytometry as described previously (37) on a FACScalibur flow cytometer using CELLQuest (Becton Dickinson) and FlowJo software (Treestar).

LNs. LN cell suspensions were plated in 96-well round bottom plates at a density of 2×10^5 cells per well and were restimulated for 4 d with 10 μg/ml OVA. After 4 d, levels of IL-4, IL-5, IFN-γ (OptEIA; BD Biosciences and Becton Dickinson) and IL-13 (R&D Systems) were measured by ELISA. Numbers of $CD11c^+$ $MHCII^+$ DCs were measured using flow cytometry in homogenized lung draining LNs at 48 h after DT injection. LNs were homogenized after collagenase and DNase I treatment as described previously (7).

Airway histology. Frozen sections were stained with hematoxylin eosin or with Periodic Acid Schiff's reagent (Sigma-Aldrich). For immunohistochemical identification of $CD11c^+$ DCs and $CD4^+$ T cells, lung sections were fixed in acetone, and endogenic peroxidase was blocked with 0.1%

SoAz/0.01% H_2O_2 in PBS for 30 min, blocked with NGS and NRS, and incubated with hamster anti-CD11c followed by goat anti–Armenian hamster PO (Jackson ImmunoResearch Laboratories); to stain for CD4, sections were incubated with rat anti–mouse CD4-FITC (BD Biosciences), followed by rabbit anti-FITC–AP (DakoCytomation and ITK). Signal was developed with AEC and Fast blue, respectively.

Measurement of AHR using whole-body plethysmography

Airway responsiveness to inhaled metacholine was measured by barometric plethysmography in conscious mice (EMKA Technologies) as described previously (48). 24 h after the last aerosol challenge, nonspecific responsiveness was measured by exposing mice to aerosolized PBS to set a baseline value, followed by increasing concentrations of aerosolized metacholine (1.5625, 3.125, 6.25, 12.5, and 25 mg/ml in PBS for 3 min; Sigma-Aldrich) using ultrasonic nebulizers. PenH (enhanced pause) values were measured during 3 min after each metacholine aerosol. The average PenH values were expressed for each MCH concentration as the percentage increase over baseline PenH values measured after PBS exposure.

Statistical analysis

All experiments were performed using 5–10 mice per group. Comparison of means between different groups was performed with a Kruskal-Wallis test for equality and, in case of a significant difference the Mann-Whitney U test for unpaired data, was used for comparing two groups (SPSS 11.0 for Windows) separately. Differences were considered significant if $P < 0.05$. To compare histology sections, we ordered sections based on presence of goblet cell hyperplasia, perivascular and peribronchial eosinophilic infiltrates, and parenchymal inflammatory cells. By assigning each section a number, starting with the least inflamed section assigning 1, the scores per group were compared with a Kruskal-Wallis test and, in case of significance, with a Mann-Whitney U test. Degree of inflammatory infiltrates around airways was quantified by measuring the area of inflammatory infiltrate using an image analyzing system. The inflammatory surfaces were corrected for the size of the airway by the following formula: (area inflammatory infiltrate − area airways)/area airways. These values were also compared with a Kruskal-Wallis test and, in case of significance, with a Mann-Whitney U test.

L. van Rijt and N. Vos are supported by a grant from the Netherlands Asthma Foundation and by a grant from ICES/KIS (grant VIRGO), and Bart N. Lambrecht is supported by a VIDI grant of the NWO Netherlands. S. Jung is an incumbent of the Pauline Recanati Research Development Chair and a Scholar of the Benoziyo Center for Molecular Medicine. C. Duez is supported by a grant from Institut National de la Santé et de la Recherche Médicale.

The authors have no conflicting financial interests.

Submitted: 10 November 2004
Accepted: 26 January 2005

REFERENCES

1. Wills-Karp, M. 1999. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu. Rev. Immunol.* 17:255–281.
2. Herrick, C.A., and K. Bottomly. 2003. To respond or not to respond: T cells in allergic asthma. *Nat. Rev. Immunol.* 3:405–412.
3. Holt, P.G., M.A. Schon-Hegrad, and J. Oliver. 1988. MHC class II antigen-bearing dendritic cells in pulmonary tissues of the rat. Regulation of antigen presentation activity by endogenous macrophage populations. *J. Exp. Med.* 167:262–274.
4. Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature.* 392:245–252.
5. Brimnes, M.K., L. Bonifaz, R.M. Steinman, and T.M. Moran. 2003. Influenza virus-induced dendritic cell maturation is associated with the induction of strong T cell immunity to a coadministered, normally nonimmunogenic protein. *J. Exp. Med.* 198:133–144.
6. Lambrecht, B.N., R.A. Peleman, G.R. Bullock, and R.A. Pauwels. 2000. Sensitization to inhaled antigen by intratracheal instillation of dendritic cells. *Clin. Exp. Allergy.* 30:214–224.
7. Vermaelen, K.Y., I. Carro-Muino, B.N. Lambrecht, and R.A. Pauwels. 2001. Specific migratory dendritic cells rapidly transport antigen from the airways to the thoracic lymph nodes. *J. Exp. Med.* 193:51–60.
8. Lambrecht, B.N., M. De Veerman, A.J. Coyle, J.C. Gutierrez-Ramos, K. Thielemans, and R.A. Pauwels. 2000. Myeloid dendritic cells induce Th2 responses to inhaled antigen, leading to eosinophilic airway inflammation. *J. Clin. Invest.* 106:551–559.
9. Lambrecht, B.N., R.A. Pauwels, and B. Fazekas De St Groth. 2000. Induction of rapid T cell activation, division, and recirculation by intratracheal injection of dendritic cells in a TCR transgenic model. *J. Immunol.* 164:2937–2946.
10. Huh, J.C., D.H. Strickland, F.L. Jahnsen, D.J. Turner, J.A. Thomas, S. Napoli, I. Tobagus, P.A. Stumbles, P.D. Sly, and P.G. Holt. 2003. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *J. Exp. Med.* 198:19–30.
11. Vermaelen, K., and R. Pauwels. 2003. Accelerated airway dendritic cell maturation, trafficking, and elimination in a mouse model of asthma. *Am. J. Respir. Cell Mol. Biol.* 29:405–409.
12. Lambrecht, B.N., and H. Hammad. 2003. Taking our breath away: dendritic cells in the pathogenesis of asthma. *Nat. Rev. Immunol.* 3:994–1003.
13. Stampfli, M.R., R.E. Wiley, G.S. Neigh, B.U. Gajewska, X.F. Lei, D.P. Snider, Z. Xing, and M. Jordana. 1998. GM-CSF transgene expression in the airway allows aerosolized ovalbumin to induce allergic sensitization in mice. *J. Clin. Invest.* 102:1704–1714.
14. Eisenbarth, S.C., D.A. Piggott, J.W. Huleatt, I. Visintin, C.A. Herrick, and K. Bottomly. 2002. Lipopolysaccharide-enhanced, toll-like receptor 4–dependent T helper cell type 2 responses to inhaled antigen. *J. Exp. Med.* 196:1645–1651.
15. Jahnsen, F.L., E.D. Moloney, T. Hogan, J.W. Upham, C.M. Burke, and P.G. Holt. 2001. Rapid dendritic cell recruitment to the bronchial mucosa of patients with atopic asthma in response to local allergen challenge. *Thorax.* 56:823–826.
16. Moller, G.M., S.E. Overbeek, C.G. Van Helden-Meeuwsen, J.M. Van Haarst, E.P. Prens, P.G. Mulder, D.S. Postma, and H.C. Hoogsteden. 1996. Increased numbers of dendritic cells in the bronchial mucosa of atopic asthmatic patients: downregulation by inhaled corticosteroids. *Clin. Exp. Allergy.* 26:517–524.
17. van Rijt, L.S., J.B. Prins, P.J. Leenen, K. Thielemans, V.C. de Vries, H.C. Hoogsteden, and B.N. Lambrecht. 2002. Allergen-induced accumulation of airway dendritic cells is supported by an increase in CD31(hi)Ly-6C(neg) bone marrow precursors in a mouse model of asthma. *Blood.* 100:3663–3671.
18. Vermaelen, K.Y., D. Cataldo, K. Tournoy, T. Maes, A. Dhulst, R. Louis, J.M. Foidart, A. Noel, and R. Pauwels. 2003. Matrix metalloproteinase-9-mediated dendritic cell recruitment into the airways is a critical step in a mouse model of asthma. *J. Immunol.* 171:1016–1022.
19. Lambrecht, B.N., B. Salomon, D. Klatzmann, and R.A. Pauwels. 1998. Dendritic cells are required for the development of chronic eosinophilic airway inflammation in response to inhaled antigen in sensitized mice. *J. Immunol.* 160:4090–4097.
20. London, C.A., M.P. Lodge, and A.K. Abbas. 2000. Functional responses and costimulator dependence of memory CD4+ T cells. *J. Immunol.* 164:265–272.
21. Dubey, C., M. Croft, and S.L. Swain. 1996. Naive and effector CD4 T cells differ in their requirements for T cell receptor versus costimulatory signals. *J. Immunol.* 157:3280–3289.
22. Gause, W.C., V. Mitro, C. Via, P. Linsley, J.F. Urban Jr., and R.J. Greenwald. 1997. Do effector and memory T helper cells also need B7 ligand costimulatory signals? *J. Immunol.* 159:1055–1058.
23. Jung, S., D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, et al. 2002. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. *Immunity.* 17:211–220.
24. Cohn, L., R.J. Homer, A. Marinov, J. Rankin, and K. Bottomly. 1997. Induction of airway mucus production By T helper 2 (Th2) cells: a critical role for interleukin 4 in cell recruitment but not mucus

production. *J. Exp. Med.* 186:1737–1747.
25. Demangel, C., A.G. Bean, E. Martin, C.G. Feng, A.T. Kamath, and W.J. Britton. 1999. Protection against aerosol *Mycobacterium tuberculosis* infection using *Mycobacterium bovis* Bacillus Calmette Guerin-infected dendritic cells. *Eur. J. Immunol.* 29:1972–1979.
26. De Heer, H.J., H. Hammad, T. Soullie, D. Hijdra, N. Vos, M.A. Willart, H.C. Hoogsteden, and B.N. Lambrecht. 2004. Essential role of lung plasmacytoid dendritic cells in preventing asthmatic reactions to harmless inhaled antigen. *J. Exp. Med.* 200:89–98.
27. Lambrecht, B.N., I. Carro-Muino, K. Vermaelen, and R.A. Pauwels. 1999. Allergen-induced changes in bone-marrow progenitor and airway dendritic cells in sensitized rats. *Am. J. Respir. Cell Mol. Biol.* 20:1165–1174.
28. Shin, T., G. Kennedy, K. Gorski, H. Tsuchiya, H. Koseki, M. Azuma, H. Yagita, L. Chen, J. Powell, D. Pardoll, and F. Housseau. 2003. Cooperative B7-1/2 (CD80/CD86) and B7-DC costimulation of $CD4^+$ T cells independent of the PD-1 receptor. *J. Exp. Med.* 198:31–38.
29. Hammad, H., H. Jan de Heer, T. Soullie, H.C. Hoogsteden, F. Trottein, and B.N. Lambrecht. 2003. Prostaglandin D2 inhibits airway dendritic cell migration and function in steady state conditions by selective activation of the D prostanoid receptor 1. *J. Immunol.* 171:3936–3940.
30. Holt, P.G., S. Haining, D.J. Nelson, and J.D. Sedgwick. 1994. Origin and steady-state turnover of class II MHC-bearing dendritic cells in the epithelium of the conducting airways. *J. Immunol.* 153:256–261.
31. Hu, H., G. Huston, D. Duso, N. Lepak, E. Roman, and S.L. Swain. 2001. CD4(+) T cell effectors can become memory cells with high efficiency and without further division. *Nat. Immunol.* 2:705–710.
32. Harbertson, J., E. Biederman, K.E. Bennett, R.M. Kondrack, and L.M. Bradley. 2002. Withdrawal of stimulation may initiate the transition of effector to memory CD4 cells. *J. Immunol.* 168:1095–1102.
33. Tang, C., M.D. Inman, N. van Rooijen, P. Yang, H. Shen, K. Matsumoto, and P.M. O'Byrne. 2001. Th type 1-stimulating activity of lung macrophages inhibits Th2-mediated allergic airway inflammation by an IFN-gamma-dependent mechanism. *J. Immunol.* 166:1471–1481.
34. Huleatt, J.W., and L. Lefrancois. 1995. Antigen-driven induction of CD11c on intestinal intraepithelial lymphocytes and CD8+ T cells in vivo. *J. Immunol.* 154:5684–5693.
35. Gonzalo, J.A., C.M. Lloyd, L. Kremer, E. Finger, A.C. Martinez, M.H. Siegelman, M. Cybulsky, and J.C. Gutierrez-Ramos. 1996. Eosinophil recruitment to the lung in a murine model of allergic inflammation. The role of T cells, chemokines, and adhesion receptors. *J. Clin. Invest.* 98:2332–2345.
36. Zhang, Y., K.H. Rogers, and D.B. Lewis. 1996. β2-microglobulin–dependent T cells are dispensable for allergen-induced T helper 2 responses. *J. Exp. Med.* 184:1507–1512.
37. Van Rijt, L.S., N. Vos, D. Hijdra, V.C. De Vries, H.C. Hoogsteden, and B.N. Lambrecht. 2003. Airway eosinophils accumulate in the mediastinal lymph nodes but lack antigen-presenting potential for naive T cells. *J. Immunol.* 171:3372–3378.
38. Schweitzer, A.N., and A.H. Sharpe. 1998. Studies using antigen-presenting cells lacking expression of both B7-1 (CD80) and B7-2 (CD86) show distinct requirements for B7 molecules during priming versus restimulation of Th2 but not Th1 cytokine production. *J. Immunol.* 161:2762–2771.
39. Coyle, A.J., S. Lehar, C. Lloyd, J. Tian, T. Delaney, S. Manning, T. Nguyen, T. Burwell, H. Schneider, J.A. Gonzalo, et al. 2000. The CD28-related molecule ICOS is required for effective T cell-dependent immune responses. *Immunity.* 13:95–105.
40. Mathur, M., K. Herrmann, Y. Qin, F. Gulmen, X. Li, R. Krimins, J. Weinstock, D. Elliott, J.A. Bluestone, and P. Padrid. 1999. CD28 interactions with either CD80 or CD86 are sufficient to induce allergic airway inflammation in mice. *Am. J. Respir. Cell Mol. Biol.* 21:498–509.
41. Deurloo, D.T., M.A. van Berkel, B.C. van Esch, F. Hofhuis, F.P. Nijkamp, M.A. Oosterwegel, and A.J. van Oosterhout. 2003. CD28/CTLA4 double deficient mice demonstrate crucial role for B7 co-stimulation in the induction of allergic lower airways disease. *Clin. Exp. Allergy.* 33:1297–1304.
42. Harris, N., R. Peach, J. Naemura, P.S. Linsley, G. Le Gros, and F. Ronchese. 1997. CD80 costimulation is essential for the induction of airway eosinophilia. *J. Exp. Med.* 185:177–182.
43. Keane-Myers, A., W.C. Gause, P.S. Linsley, S.J. Chen, and M. Wills-Karp. 1997. B7-CD28/CTLA-4 costimulatory pathways are required for the development of T helper cell 2-mediated allergic airway responses to inhaled antigens. *J. Immunol.* 158:2042–2049.
44. Hammad, H., H.H. Smits, C. Ratajczak, A. Nithiananthan, E.A. Wierenga, G.A. Stewart, A. Jacquet, A.B. Tonnel, and J. Pestel. 2003. Monocyte-derived dendritic cells exposed to Der p 1 allergen enhance the recruitment of Th2 cells: major involvement of the chemokines TARC/CCL17 and MDC/CCL22. *Eur. Cytokine Netw.* 14:219–228.
45. Malmstrom, V., D. Shipton, B. Singh, A. Al-Shamkhani, M.J. Puklavec, A.N. Barclay, and F. Powrie. 2001. CD134L expression on dendritic cells in the mesenteric lymph nodes drives colitis in T cell-restored SCID mice. *J. Immunol.* 166:6972–6981.
46. Rosmalen, J.G., F. Homo-Delarche, S. Durant, M. Kap, P.J. Leenen, and H.A. Drexhage. 2000. Islet abnormalities associated with an early influx of dendritic cells and macrophages in NOD and NODscid mice. *Lab. Invest.* 80:769–777.
47. Lutz, M.B., N. Kukutsch, A.L. Ogilvie, S. Rossner, F. Koch, N. Romani, and G. Schuler. 1999. An advanced culture method for generating large quantities of highly pure dendritic cells from mouse bone marrow. *J. Immunol. Methods.* 223:77–92.
48. Hamelmann, E., J. Schwarze, K. Takeda, A. Oshiba, G.L. Larsen, C.G. Irvin, and E.W. Gelfand. 1997. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* 156:766–775.