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Dendritic Cells Are Required for the Development of Chronic Eosinophilic Airway Inflammation in Response to Inhaled Antigen in Sensitized Mice

Bart N. Lambrecht,²,³* Benoît Salomon,† David Klatzmann,† and Romain A. Pauwels*

Asthma is characterized by chronic eosinophilic inflammation of the airways, and allergen-specific Th2 lymphocytes are thought to play a major role in the development and maintenance of this type of inflammation in allergic asthma. It is generally accepted that airway dendritic cells (DC) are essential for stimulating naive T cells in a primary immune response to inhaled Ag and for the development of allergic sensitization. We have examined the role of airway DC in stimulating memory T cells in a secondary response to inhaled Ag and the subsequent development of chronic airway inflammation. In our mouse model of asthma, OVA aerosol challenge in OVA-sensitized mice leads to CD4-dependent peribronchial and perivascular eosinophilic inflammation, lung Th2 cytokine production, and systemic IgE production. We have used conditional depletion of airway DC by treatment of thymidine kinase-transgenic mice with the antiviral drug ganciclovir to deplete DC during the secondary exposure to OVA. In sensitized thymidine kinase-transgenic mice, a significant decrease in the number of bronchoalveolar CD4 and CD8 T lymphocytes and B lymphocytes was seen after ganciclovir treatment. In addition, Th2 cytokine-associated eosinophilic airway inflammation was almost completely suppressed. These studies demonstrate for the first time that the DC is essential for presenting inhaled Ag to previously primed Th2 cells in the lung, leading to chronic eosinophilic airway inflammation. Altering the function of airway DC may therefore be an important target for new anti-asthma therapy. The Journal of Immunology, 1998, 160: 4090–4097.

Th2 lymphocytes, secreting Th2 cytokines such as IL-4 and IL-5 in response to inhaled Ag, play a major role in the pathogenesis of allergic bronchial asthma (1). A critical step in the induction of a T cell immune response is the uptake, processing, and presentation of Ag by professional APCs. These cells present immunogenic epitopes of the Ag on the surface of MHC molecules and provide essential costimulatory signals for the induction and differentiation of a primary T cell response (2, 3). Recent studies have emphasized the importance of the dendritic cell (DC)³ as the most potent APC for the induction of a primary immune response to exogenous Ag (4–6). The ability of naive cells to respond vigorously to DC is largely attributable to the high expression of costimulatory ligands B7-1, B7-2, and ICAM-1 on the surface of DC (7). In contrast, in vitro experiments have shown that previously activated T cells are less dependent on costimulatory signals for optimal responses to rechallenge with Ag and can respond to a variety of APC, including resting B cells and macrophages (8, 9).

In the lung, the network of airway DC is particularly well developed to capture inhaled Ag (10–12). Its location above the basement membrane of the airway epithelium ensures accessibility to inhaled Ag. Upon encounter with inhaled Ag, airway DC migrate to the draining lymph nodes of the lung, up-regulate expression of costimulatory ligands, and interact with naive T lymphocytes, inducing a primary immune response (13–15). However, no data exist on whether DC are critical in the presentation of inhaled Ag to previously activated or memory T cells. We have addressed this question in a T cell-driven mouse model of asthma. We have previously reported that inhalational challenge with OVA aerosol in OVA-sensitized mice leads to Th2 cytokine-dependent eosinophilic airway inflammation, bronchial hyperreactivity, and IgE production, findings highly characteristic of human allergic asthma (16–18). In this study, we have used transgenic (TG) mice expressing the herpes simplex virus type 1 thymidine kinase (TK) suicide gene in the cells of the myeloid DC lineage, allowing the conditional ablation of DC by treatment with the nucleoside analogue ganciclovir (GCV) (19). This suicide gene technique allows a well-controlled time window of DC depletion during which experiments can be performed in a GCV-free environment. By depleting DC before challenge with inhaled Ag, we show that DC are essential for the presentation of Ag to previously activated T cells and are critical for the subsequent development of chronic allergic airway inflammation. These findings have important implications for understanding the APC requirements for reactivation of memory T cells. The requirement for functional DC for the development of eosinophilic airway inflammation in sensitized mice suggests that DC may provide a useful target for future anti-asthma therapy.

Materials and Methods

Animals

TG mice carrying the TK transgene under the HIV-LTR promoter (19) were from the 12th backcross to C57BL/6 mice. As these mice show an

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3 Abbreviations used in this paper: DC, dendritic cell; BAL, bronchoalveolar lavage; GCV, ganciclovir; N-TG, nontransgenic; TK, thymidine kinase; TG, transgenic; WT, wild-type; LTR, long terminal repeat; SPF, specific pathogen free.
early wasting syndrome after treatment with GCV, most probably due to ectopic transgene expression in cells of the intestinal epithelium, we performed all experiments using irradiated (13 Gy) C57BL/6 hosts reconstituted with TG bone marrow, referred to as TK-TG (20). Wild-type (WT) C57BL/6 mice and C57BL/6 mice that had received bone marrow from nontransgenic (N-TG) littermates served as controls. All animals were housed in sterile microisolator units under specific pathogen free (SPF) conditions.

**Sensitization and challenge with OVA**

On day 0, mice (n = 8–10 per group) were actively immunized with OVA (grade III, Sigma Chemical, St. Louis, MO) by the i.p. injection of 10 μg OVA adsorbed to 100 μg of alum adjuvant. Sham-sensitized animals received i.p. PBS. From day 16 to day 20 after immunization, animals were exposed daily for 30 min to an aerosol of 1% (w/v) OVA in PBS. Groups of mice were sacrificed by sodium pentobarbital overdose (60 mg/kg body weight). BAL was performed with 2×10⁶ BAL cells were counted in a hemocytometer (Coulter Counter, Hertfordshire, UK). The cellular density of the airway DC network (cells/mm²) was calculated using the Impact image analysis system (Alcatel, TITN Answare, Oberkothen, Germany).

**Bronchoalveolar lavage (BAL)**

On day 21 of the experiment, 24 h after the last aerosol challenge, mice were sacrificed by sodium pentobarbital overdose (60 mg/kg body weight). BAL was performed with 2×10⁶ BAL cells were counted in a hemocytometer (Coulter Counter, Hertfordshire, UK.). Differential cell counts were performed on cytospin preparations (Cytospin 2, Shandon, Cheshire, UK.) stained with May-Grünwald-Giemsa by classification of 300 cells on standard morphologic criteria. The cytokine content in unconcentrated BAL fluid was determined using commercial available cytokine ELISA kits. The ELISA test for determination of murine IFN-γ and IL-2 had a sensitivity of 2 pg/ml and 3 pg/ml, respectively. The absolute numbers of BAL CD3⁺, CD4⁺, and CD8⁺ cells were significantly higher in actively sensitized compared with sham-sensitized animals (Fig. 1, A and B). The density of the network decreased from the upper to the lower trachea (Fig. 1D). Administration of GCV for 6 days to TK-TG mice led to a disappearance of more than 95% of airway DC (Fig. 1, C and D), a reduction maintained for at least 4 or 5 days after discontinuation of GCV treatment (not shown). After the same treatment regimen of 6 days, the cellular composition of BAL fluid of unimmunized TK-TG mice was identical in GCV- and PBS-treated animals (Table I).

Flow cytometric analysis of BAL fluid cells

Monoclonal antibodies conjugated to phycoerythrin or FITC were purchased from PharMingen (San Diego, CA). All reactions were performed on a Becton Dickinson FACScan flow cytometer (Becton Dickinson Immunocytometry Systems, San Jose, CA). Data were acquired in list mode on 1×10⁶ cells and analyzed using Cell Quest software (Becton Dickinson).

**Airway histology**

After BAL was performed, fixative (4% paraformaldehyde in PBS) was gently infused through the lavage catheter by a continuous release pump under pressure- and volume-controlled conditions. The lungs were resected and fixed for an additional 4 h. After routine paraffin embedding, 4-μm sections were stained with May-Grünwald-Giemsa and hematoxylin-eosin and examined by light microscopy for histologic changes.

**Measurement of serum IgE**

Blood was drawn by cardiac puncture for measurement of serum IgE by isotype-specific ELISA as described previously (18). Briefly, total serum IgE was determined by coating 96-well plates overnight with monoclonal rat anti-mouse IgE LO-ME-3 (H. Bazin, Experimental Immunology Unit, Brussels, Belgium). After blocking with 1% BSA, sample dilutions and known IgE standard were incubated, followed by biotin-conjugated monoclonal rat anti-mouse IgE LO-ME-2 (H. Bazin) and peroxidase-streptavidin conjugate. The detection limit of the assay was 0.05 μg/ml.

OVA-specific IgE was determined by coating plates overnight with OVA grade V (Sigma). Serial dilutions of serum were applied, followed by biotin-conjugated anti-mouse IgE. A serum pool of OVA-sensitized mice was used as internal laboratory standard. A 1:100 dilution of this pool was chosen as arbitrary unit. The lower detection limit of this assay is 0.00165 U/ml.

**Statistical analysis**

Total and OVA-specific IgE levels were logarithmically transformed before calculation of the mean ± SEM. Comparison of means between different groups was performed with the Mann-Whitney U test for unpaired data using the SPSS software (SPSS, Chicago, IL) statistical package (22). Differences were considered significant if p < 0.05.

**Results**

**Conditional depletion of DC from the airways**

To determine whether DC could be depleted from the airways of TK-TG mice, animals were treated with a continuous release infusion of GCV delivered via miniosmotic pumps, as previously described (19). The cellular density of the network lining the airways was visualized in tracheal whole mounts. The pattern of MHC class II staining in the airways of WT mice revealed a network of strongly positive cells with a dendritic morphology in the upper layers of the respiratory epithelium (Fig. 1, A and B). The density of the network decreased from the upper to the lower trachea (Fig. 1D). Administration of GCV for 6 days to TK-TG mice led to a disappearance of more than 95% of airway DC (Fig. 1, C and D), a reduction maintained for at least 4 or 5 days after discontinuation of GCV treatment (not shown). After the same treatment regimen of 6 days, the cellular composition of BAL fluid of unimmunized TK-TG mice was identical in GCV- and PBS-treated animals (Table I).

**Effect on cellular composition of BAL fluid of DC depletion during secondary challenge with OVA**

In initial experiments, WT C57BL/6 mice were sensitized to OVA by i.p. injection of OVA in alum and challenged 2 wk later with OVA-aerosol. The cellular composition of BAL fluid of unimmunized TK-TG mice was identical in GCV- and PBS-treated animals (Table I).
identical in PBS-treated TK-TG and WT mice, illustrating that there was no intrinsic difference in the response of TG mice. However, when TK-TG mice were treated with GCV to deplete DC, there was a significant reduction in the number of CD3⁺, CD4⁺, CD8⁺, and B cells in BAL fluid compared with PBS-treated TK-TG and GCV-treated WT mice (p < 0.01) (Fig. 2, B and C). This effect of GCV was not observed in control N-TG chimeric mice, which developed similar changes in BAL fluid as WT animals. The number of eosinophils induced by OVA aerosol was reduced to background levels by GCV treatment (Fig. 2A). However, considerably more T lymphocytes were recovered from lavage in DC-depleted OVA-sensitized animals compared with sham-sensitized animals, illustrating the presence of a primary immunization effect in OVA-sensitized TG animals (p < 0.01). Alveolar macrophages constituted 88% of cells in the BAL fluid of GCV-treated TK-TG mice.

Effect on airway histology of DC depletion during secondary challenge with OVA

Histologic analysis of the lungs of sensitized and challenged mice revealed that OVA-aerosol challenge in OVA-sensitized mice led to the development of peribronchial and perivascular inflammatory lesions characterized by a predominance of eosinophils and mononuclear cells, as previously described (Fig. 3, B and C) (16). Occasionally, giant cells were seen in the infiltrates. These changes were absent from the lungs of sham-sensitized mice (Fig. 3A). Similar tissue lesions were observed in WT mice treated with GCV and TK-TG mice treated with PBS (Fig. 3, D and E). However, when DC were depleted by GCV treatment in TK-TG mice, the tissue response to secondary challenge with OVA was suppressed such that no peribronchial and perivascular infiltrates were seen (Fig. 3F).

Table I. Cellular distribution of BAL fluid in unimmunized TK-TG mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Cells (×10⁶)</th>
<th>Macrophages (% of Cells)</th>
<th>CD3⁺ (×10⁶)¹</th>
<th>CD4⁺ (×10⁶)²</th>
<th>CD8⁺ (×10⁶)²</th>
<th>B Cells (×10⁶)²</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>2.7 ± 0.2</td>
<td>98.2 ± 0.6</td>
<td>25.4 ± 2.8</td>
<td>10.2 ± 1.8</td>
<td>7.8 ± 0.8</td>
<td>6.3 ± 1.6</td>
</tr>
<tr>
<td>GCV</td>
<td>2.4 ± 0.3</td>
<td>98.5 ± 0.5</td>
<td>30.3 ± 6.5</td>
<td>11.4 ± 2.8</td>
<td>7.3 ± 0.7</td>
<td>7.8 ± 0.5</td>
</tr>
</tbody>
</table>

¹Animals (n = 4 per group) were treated for 6 days by i.p. implantation of a GCV- or PBS-filled pump. BAL was performed 24 h after removal of the pumps.

²As determined by flow cytometry on lavage cells.
We have previously studied the selectivity of the GCV-induced depletion of various populations of APC in TK-TG mice (19, 20). We found no effect on the number of monocytes and B cells in the peripheral blood of animals given an identical treatment regimen consistent with the previous observation that all MHC class II staining in the airways of SPF-reared mice is attributable to DC (10, 12), indicates that these cells constitute the DC population. After discontinuation of GCV treatment, DC gradually replenish the airways, allowing for a time window of DC depletion in which experiments can be performed in a GCV-free environment. Since GCV is toxic only to dividing cells, the depletion of DC in TK-TG mice by 6-day treatment with GCV indicates a rapid turnover rate of airway DC in mice, consistent with the previous report of a turnover rate of about 2 or 3 days in rats (23).

We have previously studied the selectivity of the GCV-induced depletion of various populations of APC in TK-TG mice (19, 20). We found no effect on the number of monocytes and B cells in the peripheral blood of animals given an identical treatment regimen of GCV. FACS analysis of spleen and lymph nodes of these animals revealed an identical percentage of Mac-1-positive macrophages and B-220-positive B cells. Immunohistochemical analysis of spleen and lymph node sections of TK-TG mice treated with GCV indicates a rapid turnover rate of airway DC in mice, consistent with the previous report of a turnover rate of about 2 or 3 days in rats (23).
GCV has demonstrated a complete depletion of CD11c-positive marginal zone DC, without effects on red pulp macrophages, marginal metallophilic macrophages (MOMA-1⁺), and marginal zone macrophages (24). Moreover, the CD8α⁻ NLDC-145⁻ population of interdigitating DC were not affected by treatment with GCV, suggesting that these cells have a separate lymphoid lineage or turnover rate, as recently shown by others (24, 25). In our experiments, GCV treatment in TK-TG mice had no effect on the numbers of alveolar macrophages and B cells recovered from lavage. However, the depletion of DC in the airways of GCV-treated TK-TG mice was almost complete.

As the HIV LTR-promoter sequence used to drive the expression of TK in DC can also be transcribed in activated T cells, we have also addressed the question of whether T cell function is normal in GCV-treated animals at the time of antigenic stimulation (i.e., 24 h after the discontinuation of GCV). Under these experimental conditions, T cell responses are not affected as assessed by the generation of a normal CTL response after challenge with lymphocytic choriomeningitis virus (R. Zinkernagel, B. Salomon, and D. Klatzmann, unpublished observations). Moreover, TK-TG mice demonstrated normal proliferation in Vβ8.1-2 T cells 48 h after the i.v. injection of staphylococcal enterotoxin B superantigen. CD4⁺ Vβ8.1-2 cells increased from

**FIGURE 3.** Effect on the development of eosinophilic airway inflammation of depleting DC during the secondary challenge to inhaled Ag in sensitized mice. Animals were sensitized to OVA (day 0) and challenged daily (days 16–20) to OVA aerosol. Twenty-four hours after the last challenge, lungs were resected and processed for histologic analysis. H & E staining; ×200. A, Sham-sensitized mice challenged with OVA do not develop any histologic lesions. B, Control WT animals in which a PBS-filled pump was implanted from days 9 to 15 developed perivascular and peribronchial infiltrates. Occasional giant cells were noticed. C, Higher magnification (×1000) showing the predominance of mononuclear cells and eosinophils. D, Control WT animals treated with GCV develop similar lesions as PBS-treated animals upon inhalation of OVA. E, TK-TG mice treated with PBS develop similar degrees of airway inflammation as WT animals. F, TK-TG mice treated with a GCV-filled pump to deplete DC. Absence of the eosinophil-rich mononuclear infiltrates that were readily noticed in all other groups.
15 to 25% and CD8+ Vβ8.1-2 cells from 24 to 35% (Salomon, unpublished observations). These experiments also demonstrate that other non-DC APCs must be functional during the period of GCV-induced DC depletion.

Having established that DC could be efficiently depleted from the airways of TK-TG mice, we used these mice in an existing model of allergic airway inflammation in which WT C57BL/6 mice are actively sensitized to OVA and subsequently develop eosinophilic peribronchial and perivascular inflammation, demonstrable by analysis of BAL fluid eosinophil content and lung histology, in response to inhalational challenge with OVA (16–18). This is accompanied by increased numbers of BAL fluid T lymphocytes secreting Th2 cytokines. Furthermore, challenge of sensitized animals increases the number of B cells recovered in BAL fluid and systemic production of total and OVA-specific IgE. In view of the dependence of IgE synthesis on IL-4 and of the development of tissue eosinophilia on IL-5, these results in WT mice indicate that systemic sensitization and aerosol reexposure to OVA induce a Th2-associated eosinophilic airway inflammation (26). Brusselle et al. have previously shown that this response is critically dependent on the presence of T lymphocytes and IL-4 (18) and can be inhibited by the systemic administration of IL-12 during sensitization (17), further strengthening the hypothesis that the development of airway inflammation in the mouse is controlled by Th2 lymphocytes, and B lymphocytes recovered from BAL fluid. IL-4 was decreased the number of eosinophils, CD4+ and CD8+ T lymphocytes, and B lymphocytes recovered from BAL fluid. GCV treatment in TK-TG mice significantly decreased the number of eosinophils, CD4+ and CD8+ T lymphocytes, and B lymphocytes recovered from BAL fluid. IL-4 was detected in only 1 out of 9 BAL fluid samples tested compared with 4 out of 10 in the controls. On histologic analysis, GCV treatment led to the disappearance of aerosol-induced peribronchial and perivascular eosinophilic infiltrates. The levels of both total and OVA-specific IgE were significantly lower in GCV-treated compared with PBS-treated TK-TG mice, suggesting that T cell help for IgE synthesis was deficient. These effects of GCV

**FIGURE 4.** Effect on levels of serum IgE of depleting DC during the secondary challenge to inhaled Ag in sensitized mice. Experimental groups are coded as follows: immunization/pump filling/challenge. A, Total IgE levels were determined 24 h after the last aerosol challenge as described in Materials and Methods. B, OVA-specific IgE levels. Results are expressed as the mean ± SEM from 8 to 10 mice per group.
treatment were not apparent in transgene-negative littermate controls, indicating that the depletion of myeloid DC was specifically implicated in the suppression of eosinophilic airway inflammation.

These data strongly suggest that depletion of DC during secondary exposure to inhaled Ag affects presentation of Ag to memory Th2 cells, leading to the suppression of Th2 effector functions. Although in vitro experiments suggest that memory T cells are less dependent than naive T cells on costimulatory signals derived from the APC, and can respond to a variety of professional APCs such as resting B cells and macrophages (8, 9), our results suggest that the requirements for costimulation of resting memory T cells may be more stringent in vivo. Thus DC appear to be required for generation of effector function by cells exposed to Ag 16 days previously. This interpretation is consistent with recent studies showing that lung DC constitutively express costimulatory ligands and up-regulate their expression upon maturation (15) and that aerosol-induced effector function in memory T cells can be inhibited by blocking the function of these costimulatory ligands B7-1 (28) or B7-2 (29, 30). It will be interesting to study whether generation of effector function in memory cells in models of infectious or autoimmune diseases are similarly dependent on costimulation and DC function (reviewed in Ref. 31).

An alternative hypothesis is that DC are critical for the transport of inhaled Ag to the draining lymph nodes of the lung, by analogy with the primary response (13, 14). In contrast to previous data suggesting that activated/memory T cells can recirculate via non-lymphoid tissues, possibly reacting to Ag presented by APCs resident in these tissues (32), recent studies have shown that resting memory cells may follow a similar pathway of recirculation as naive T cells, leaving the bloodstream at the high endothelial venules of secondary lymphoid organs (33, 34). Thus it is possible that one of the functions of airway DC in the generation of effector function is to carry Ag to the draining nodes of the lung where recirculating memory cells can be stimulated.

Whatever the mechanism of suppression of airway inflammation by DC depletion, our findings suggest that other APCs are unable to substitute for DC function in the airways. This is further supported by previous studies that have addressed the role of macrophages and B cells in presenting inhaled Ag to sensitized animals. Aerosol-induced inflammatory lung lesions developed to a greater extent and IgE production was higher in animals depleted of alveolar macrophages by administration of toxic liposomes into the trachea before the secondary Ag challenge. Thus alveolar macrophages not only have poor Ag-presenting capacity, but even suppress the activity of other APC (35, 36). Similarly, recent studies using the same experimental model as ours in B cell-deficient mice have indicated that these cells do not appear to play an important role in the induction of airway inflammation (37).

In view of the dependence on DC for the development of eosinophilic airway inflammation in this animal model, targeting DC could provide a novel therapeutic strategy for human asthma. The airways of atopic asthmatics are known to contain increased numbers of DC (38), some of which carry the high affinity receptor for IgE (39); however, their role in the pathogenesis of the disease remains largely unknown (40). It has been shown that treatment with inhaled glucocorticosteroid drugs leads to a reduction in the number of DC in the airways in both man and rodents (38, 41). The current study suggests that this reduction in DC numbers could be an important means by which these drugs reduce eosinophilic airway inflammation. Finding new therapeutic strategies that target the function of the airway DC selectively, without inducing pulmonary or systemic immunodeficiency, will be a major future challenge.

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References


