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An Anti-Inflammatory Role for Plasmacytoid Dendritic Cells in Allergic Airway Inflammation

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It was previously shown that administration of recombinant human Fms-like tyrosine kinase receptor-3 ligand (Flt3L) before allergen challenge of sensitized mice suppresses the cardinal features of asthma through unclear mechanisms. Here, we show that Flt3L dramatically alters the balance of conventional to plasmacytoid dendritic cells (pDCs) in the lung favoring the accumulation of pDCs. Selective removal of pDCs abolished the antiinflammatory effect of Flt3L, suggesting a regulatory role for these cells in ongoing asthmatic inflammation. In support, we found that immature pDCs are recruited to the lungs of allergen-challenged mice irrespective of Flt3L treatment. Selective removal of pDCs during allergen challenge enhanced airway inflammation, whereas adoptive transfer of cultured pDCs before allergen challenge suppressed inflammation. Experiments in which TLR9 agonist CpG motifs were administered in vitro or in vivo demonstrated that pDCs were antiinflammatory irrespective of their maturation state. These effects were mediated through programmed death-1/programmed death ligand 1 interactions, but not through ICOS ligand, IDO, or IFN-α. These findings suggest a specialized immunoregulatory role for pDCs in airway inflammation. Enhancing the antiinflammatory properties of pDCs could be employed as a novel strategy in asthma treatment. The Journal of Immunology, 2009, 183: 1074–1082.

Allergic asthma is a chronic disease of the airways characterized by bronchial hyperreactivity to nonspecific stimuli, chronic eosinophilic airway inflammation, goblet cell hyperplasia, and airway structural changes in response to a chronic Th2 immune response to inhaled Ag, also reflected by Th2-dependent increased serum levels of allergen-specific IgE and IgG1 (1, 2).

Various forms of (anti-) cytokine therapy have been employed in an attempt to suppress allergic inflammation, with variable success. One such recently described strategy was to administer the hematopoietic growth factor Fms-like tyrosine kinase receptor-3 ligand (Flt3L)³ to allergic mice before allergen challenge. In these mice, all the cardinal features of asthma were abolished (3–6). The precise mechanism of this antiinflammatory effect has not been addressed. Flt3L has broad actions in the hematopoietic and immune system and was shown to selectively enhance the number of NK cells and dendritic cells (DCs) in vivo (7, 8). A specific subset of DCs induced by this cytokine in vivo and in vitro are the plasmacytoid DCs (pDCs), mainly known for their function in antiviral immunity and rapid production of type I IFNs (9). It was also shown that systemic administration of Flt3L to mice led to an expansion of semimature lung conventional DCs (cDCs) (10).

The effects of Flt3L on DCs could be important to understand the antiinflammatory effects of this cytokine in asthma. There is evidence for distinct roles of cDCs and pDCs in regulating T cell-mediated adaptive immunity in the lung (11, 12). Conventional DCs promote Th2 sensitization to inhaled Ag after reaching the mediastinal lymph nodes (13–15), whereas pDCs mediate tolerance to inhaled Ag through induction of regulatory T cells (16–19). Increasing evidence supports the notion that DCs also contribute to ongoing airway inflammation beyond the sensitization phase of asthma, as CD11c-diphteria toxin receptor transgenic mice no longer developed signs of asthma when airway DCs were depleted during secondary and tertiary challenge with Ag (15).

In this paper, we show that Flt3L treatment greatly enhanced the number of pDCs in the lungs of allergen-challenged mice, while reducing Th2-associated eosinophilic inflammation. Removing pDCs from Flt3L-treated mice before allergen challenge abolished the antiinflammatory actions of this cytokine. These studies also led us to investigate the contribution of pDCs in ongoing allergic responses to aerosolized Ag. Upon allergen challenge of sensitized mice, pDCs were recruited to the lungs. Selective removal of pDCs using a depleting Ab 120G8 enhanced inflammation whereas adoptive transfer of cultured pDCs suppressed it. These effects occurred irrespective of pDC maturation state, but through their

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expression of programmed death ligand 1 (PD-L1). Our data therefore demonstrate a previously unrecognized function of pDCs in controlling ongoing allergic inflammation that could be exploited for the better design of antiinflammatory compounds.

Materials and Methods

Animals

Female BALB/c mice were purchased from Harlan. PD-L1−/− mice were provided by Millennium Pharmaceuticals. All mice were housed under specific pathogen-free conditions and were between 6 and 8 wk of age at the start of an experiment. All experiments were conducted with the approval of the Animal Care Committee of Erasmus MC, Rotterdam, The Netherlands.

Induction of experimental asthma

Mice were sensitized by two i.p. injections of 10 μg of OVA (Worthington Biochemical) adsorbed onto 1 mg of aluminum hydroxide (alum, Inject; Pierce) in 500 μl of saline (OVA-alum) on days 0 and 7. Ten days after the last injection, the mice were challenged by inhalation of OVA aerosols. pDCs and cDCs were determined in the lung by flow cytometry. Results are expressed as means ± SEM for n = 5–6 mice/group; *, p < 0.05 and **, p < 0.01. Similar results were obtained in two different experiments.

Treatment of mice with CpG motifs

Unmethylated CpG motifs (10 μg; ISS-ODN 1680; CpG (5′-TGACTGTG AACGTTCCGAGATGA-3′); Sigma-Genosys) (21) were administered intratracheally (i.t.) in a volume of 80 μl of PBS in OVA-alum-sensitized mice. One day after the i.t. treatment mice were exposed to three OVA aerosols (see Fig. 5F).

Purification of pDCs

Bone marrow (from either BALB/c or PD-L1−/− mice) was cultured for 4 days with culture medium supplemented with 200 ng/ml Flt3L and 50 ng/ml recombinant murine stem cell factor (PeproTech) in a 6-well plate, at a concentration of 1 × 106 cells/ml. At day 4 of the culture, all nonadherent cells were collected and replaced into culture with 200 ng/ml Flt3L. On day 10 of the culture, the cells were pulsed with LPS-low OVA (Worthington Biochemical; LPS contamination of 2.9 ng/mg protein) and/or 10 μg/ml unmethylated CpG motifs. On day 11, the cells were sorted based on their expression of 120G8, B220, CD11c+, and CD11b using a FACSAria flow cytometer (BD Biosciences). A purity of ≥96% was obtained. Three days before the start of OVA aerosols, 1 × 106 pDCs were injected i.v. via the tail vein (see Figs. 4A and 6, A and D).

Isolation of bronchoalveolar lavage (BAL), lungs, and mediastinal lymph nodes (MLNs)

Mice were sacrificed by an overdose of 2.5% avertin, followed by bleeding. BAL was performed by inserting a canula in the trachea of the mouse. By lavage of 3 ml of PBS containing 0.01 mM EDTA, cells were extracted from the lung alveolar space. Lungs were either inflated with PBS/OCT for histology or perfused with PBS through the pulmonary artery before mincing them to obtain a single-cell suspension as described before (22). To determine the cytokine levels, MLN cells were subjected to three OVA aerosols.

FIGURE 1. Effect of Flt3L treatment is dependent on pDCs. A, Mice were sensitized by two i.p. injections of OVA-alum on days 0 and 7. Seven days after the last injection, the mice were treated with eight injections of Flt3L (10 μg i.p.) or with PBS as a control. Three days after the last Flt3L injection, mice were treated with mAb 120G8 or rat IgG (200 μg i.p.). Thereafter, mice were subjected to three OVA aerosols. B, Inflammation was analyzed in the BAL fluid with flow cytometry. C, A periodic acid-Schiff staining was done on lung slides (×50 magnification). D, Cytokine production in the supernatants of MLN cells 4 days after restimulation with OVA in vitro. E, Lung slides were stained for DCs (in blue 120G8 and red CD11c). F, Gating strategy for pDCs and cDCs on lung cells. pDCs are depicted as small cells, 120G8+ and CD11c−. cDCs are depicted as live, low autofluorescence MHC-II+ and CD11c+. An example is shown of a Flt3L-treated mouse. G, Numbers of pDCs and cDCs were determined in the lung by flow cytometry. Results are expressed as means ± SEM for n = 5–6 mice/group; *, p < 0.05 and **, p < 0.01. Similar results were obtained in two different experiments.
were plated in 96-well round-bottom plates at a density of 2 × 10^5 cells per well and restimulated with 10 or 100 μg/ml OVA for 4 days. After 4 days, supernatants were harvested and stored at -20°C until further examination. Using ELISA, the amount of IL-4, IL-5, IFN-γ (all eBioscience), IL-10 (BD Biosciences), and IL-13 (R&D Systems) was measured in the supernatants.

Flow cytometry of BAL, lung, and MLN

All staining reactions were performed at 4°C. In all staining reactions, 2.4G2 Fc receptor Ab (CD16/CD32) was added to reduce nonspecific binding. Dead cells were excluded using propidium iodide, and the cellular composition of the BAL fluid was determined as described before (23), discriminating macrophages, lymphocytes, eosinophils, neutrophils, and DCs. The BAL fluid, lung, and MLN were furthermore stained for DC subsets using 120G8-FITC, B220-PE (RA3-6B2) or MHC class II-PE (M5/114; BD Biosciences), and CD11c-allophycocyanin (HL3; eBioscience) or CD11b-allophycocyanin (MAC-1; BD Biosciences). In experiments in which the expression of the costimulatory molecules was investigated, 120G8-FITC and CD11c-allophycyanin, CD80-PE (16-10A1; BD Biosciences), ICOS ligand (ICOS-L)-PE (B7RP1; BD Biosciences), or isotype-PE were used.

Histology

Frozen sections (6 μm) of the lung were stained with periodic acid Schiff’s reagent (Sigma-Aldrich) to address the amount of inflammation and goblet cell hyperplasia. To identify pDCs, immunohistochemical staining was performed on formaldehyde-fixed sections. Endogenous peroxidase was blocked with 0.1% NaN₃/0.01% H₂O₂ in PBS for 30 min, nonspecific binding was blocked with 10% normal goat serum, and sections were subsequently incubated with 120G8, goat-anti-rat IgG (200 μg i.p.) as a control. Inflammation in the BAL fluid was determined by flow cytometry. Open bars represent the rat IgG-treated OVA-sensitized and OVA-challenged mice, and the filled bars represent the 120G8-treated OVA-sensitized and OVA-challenged mice. Mice were treated during challenge and/or during the resolution phase with 120G8 or rat IgG (200 μg i.p.). BAL inflammation was determined by flow cytometry. Open bars represent the rat IgG-treated OVA-challenged mice, the black bars represent the OVA-challenged mice treated with 120G8 during challenge, and the gray bars represent the OVA-challenged mice treated with 120G8 during challenge and resolution. E. Cellular composition of the BAL fluid was determined by flow cytometry. Open bars represent IgG-treated mice, and filled bars represent 120G8-treated mice during the resolution phase. Results are expressed as means ± SEM for n = 4–6 mice/group; *, p < 0.05 and **, p < 0.01. Similar results were obtained in two different experiments.

Statistical analysis

For all experiments, the difference between groups was calculated using the Mann-Whitney U test for unpaired data (GraphPad Prism version 4.0; GraphPad Software). Differences were considered significant when p < 0.05.
**Results**

**Effect of systemic Flt3L administration on allergic airway inflammation in OVA-sensitized mice**

It has been shown that administration of Flt3L for 10 days before a secondary OVA allergen challenge in OVA-sensitized mice suppresses the cardinal features of asthma (4). Using a modified protocol, we administered Flt3L i.p. for 8 days before giving a series of three OVA aerosols to OVA-sensitized mice (Fig. 1A) and focused on DCs. To allow later comparisons with pDC-depleting Abs, mice received isotype control rat IgG Abs during secondary challenge (see below). As expected in OVA-sensitized and OVA-challenged mice, PBS-treated mice developed a strong eosinophilic and lymphocytic airway inflammation seen in eosinophilic and lymphocytic recruitments to the lung interstitium (Fig. 1C). Also, cytokine levels produced by MLN cells were restored to the levels produced by PBS-treated OVA-challenged mice (Fig. 1D, gray bars). These data clearly demonstrate that the anti-inflammatory effects of Flt3L are in large part mediated by pDCs.

**Effect of OVA aerosol challenge on the number of pDCs in OVA-sensitized mice**

We next addressed if the number of pDCs in the lungs would also be affected during airway inflammation in the absence of Flt3L treatment. In these experiments, OVA-sensitized mice were subjected to different numbers of OVA aerosols (Fig. 2A). As expected, the degree of eosinophilic inflammation increased with increasing numbers of OVA aerosols (Fig. 2B). Previous work by van Rijt et al. (24) reported that different number of PBS aerosols did not change the composition of the BAL fluid. Therefore, as a control, mice were only exposed to the maximum number of PBS aerosols. The number of pDCs in the BAL fluid of allergic mice was increased up to 6-fold with increasing numbers of aerosols (Fig. 2C). The increase in CDC numbers in the BAL fluid followed the same trend as for pDCs. We next looked at whether the level of maturation of pDCs and cDCs would be affected by increasing numbers of OVA aerosols. In the BAL fluid, lungs, and MLNs of OVA-challenged mice, pDCs showed no increase in the expression CD40, CD80, CD86, MHC class II, PD-L1, PD-L2, or ICOS-L. However, we could observe an up-regulation of costimulatory molecules (CD40, PD-L2) on cDCs in the BAL fluid as previously reported (Ref. 15 and data not shown).

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**FIGURE 4.** Effect of exogenous pDCs in the secondary response of lung inflammation. A, Mice were sensitized by two i.p. injections of OVA-alum on days 0 and 7. Three days before the first aerosol, mice received $1 \times 10^6$ pDCs i.v. B, Inflammation of the BAL was analyzed. Open bars represent mice receiving PBS, black bars represent mice receiving unpulsed pDCs, and gray bars represent OVA-pulsed pDCs before challenge. C, Cytokine levels in the supernatants of MLN cell cultures restimulated in vitro with OVA for 4 days. Results are expressed as means $\pm$ SEM for $n$ = 4–6 mice/group; *, $p < 0.05$. Similar results were obtained in two different experiments.
Effect of selective removal of pDCs on OVA-induced airway inflammation in OVA-sensitized mice

As OVA-induced airway inflammation caused an increase in the number of pDCs, we wondered what effect depletion of pDCs would have on the severity of eosinophilic inflammation. To address this point, we depleted pDCs using 120G8 Abs, administered at the time of challenge (Fig. 3A). Injection of 120G8 Abs decreased the number of pDCs in most organs by 80–90%, and the effect of 120G8 administrations was observed for at least 4 days after the last injection (data not shown). As expected in OVA-sensitized and OVA-challenged groups, mice injected with rat IgG control Ab developed eosinophilic airway inflammation (Fig. 3, B, D, and E, open bars) and showed a Th2 cytokine profile in the draining MLN cell cultures restimulated in vitro with OVA for 4 days. Results are expressed as means ± SEM for n = 4–6 mice/group; *, p < 0.05 and **, p < 0.01. Similar results were obtained in two different experiments.

The fact that at day 7 after the last aerosol, airway inflammation was no longer different between 120G8 Ab- and rat IgG-treated mice could suggest that pDC depletion was no longer complete during the resolution phase of airway inflammation. Therefore, mice were additionally given 120G8 Ab during the resolution phase (Fig. 3C). Mice treated with rat IgG throughout challenge and resolution still demonstrated a strong BAL lymphocytosis and eosinophilia 7 days after the last aerosol exposure (Fig. 3D, open bars), accompanied by Th2 cytokine production by MLN cells (data not shown). In mice depleted of pDCs only during challenge (black bars), the number of inflammatory cells in the BAL fluid was not different from this response. However, in mice depleted of pDCs during the challenge and resolution phase, the degree of airway inflammation was again dramatically increased (Fig. 3D, gray bars). Moreover, when mice were only depleted of pDCs during the resolution phase, eosinophilia and lymphocytosis in the BAL fluid also significantly increased (Fig. 3E, black bars).

Effect of exogenous pDC administration on OVA-induced airway inflammation in OVA-sensitized mice

Since depletion of pDCs during the secondary response altered the inflammatory response (Fig. 3), and the antiinflammatory effect of
Flt3L was pDC-dependent (Fig. 1), we examined if ex vivo-cultured pDCs would also have an effect on airway inflammation. Mice were injected with bone marrow (BM)-derived pDCs 3 days before submitting them to OVA aerosols (Fig. 4A). As expected in OVA-sensitized and OVA-challenged mice not injected with pDCs, lymphocytosis and eosinophilia were observed in the BAL fluid (Fig. 4B, open bars) and Th2 cytokine production by MLN cells (Fig. 4C, open bars) was induced. A strong decrease in the number of BAL fluid eosinophils and lymphocytes was observed in pDC-injected mice (Fig. 4B, black and gray bars). This decrease occurred irrespective of whether pDCs were pulsed in vitro with OVA. The administration of pDC had no effect on the levels of Th2 cytokines produced by MLN cells (Fig. 4C, black and gray bars).

Effect of pDC maturation state on the capacity to control airway inflammation

Since endogenous and exogenous pDCs can suppress allergen-induced eosinophilic airway inflammation (Figs. 1, 3, and 4), we next studied if this effect was dependent on their maturation status. BM-derived pDCs were exposed to unmethylated CpG motifs (ISS ODN 1680) in vitro before injecting them into mice. pDCs exposed to CpG showed an up-regulation of CD80 (Fig. 5A), ICOS-L, PD-L1, CD40, CD86, and MHC-II (data not shown) (27, 28). When injected, fully mature pDCs pulsed with OVA and CpG were as efficient as immature OVA-pulsed pDCs or unpulsed pDCs in reducing the number of inflammatory cells in BAL fluids (Fig. 5B). These data show that cultured pDCs are able to dampen airway inflammation irrespective of their degree of maturation. We next addressed whether endogenous maturing pDCs exposed to CpG motifs in vivo would have the same capacity. Direct instillation of unmethylated CpG motifs in naive mice induced a rapid and massive increase in the number of pDCs in the lungs compared with PBS instillation (Fig. 5C). In mice injected with CpG motifs, lung pDCs showed a mature phenotype as assessed by the up-regulation of CD80, ICOS-L, and PD-L1 compared with pDCs of mice injected with PBS (Fig. 5E). It has been shown by others that instillation of CpG motifs in sensitized animals before...
subjecting them to OVA aerosols led to a marked decrease in eosinophilic inflammation (29–32). In support, we also observed a decrease in airway inflammation and Th2 cytokine profile following local CpG motif treatment (Fig. 5H, black bars). Similar to the effects of CpG motifs in naive mice, the lungs of CpG motif-treated OVA-challenged mice (CpG/IgG) showed an increase in the number of pDCs compared with PBS-treated OVA-challenged mice (Fig. 5G, black bars compared with open bars). To address a role for pDCs in the CpG motif-induced reduction in airway inflammation, mice were concomitantly treated with CpG motifs and 120G8 Abs (Fig. 5F). The increase in pDC number by CpG motif instillation was neutralized by 120G8 treatment (Fig. 5G, gray bars). A partial restoration of the airway eosinophilia could be observed when pDCs were depleted in CpG motif-treated mice (Fig. 5H, gray bars). This could also be seen in the cytokines, such as IL-5 produced by MLN cells (Fig. 5I, gray bars).

Suppression of airway inflammation by CpG-matured pDCs occurs via PD-L1/PD-1 interaction

It has been shown in various models that pDCs induce the formation of regulatory T cells through expression of IDO, ICOS-L, and/or PD-L1. Moreover, OVA-pulsed CpG-stimulated pDCs are a copious source of IFN-α. To address any of these pathways, we cultured pDCs from IDO−/−, ICOS-L−/−, and PD-L1−/− mice (treatment shown in Fig. 6A) or treated with blocking type I IFN receptor Abs (treatment shown in Fig. 6D). A strong mechanistic role for the expression of PD-L1 on pDCs was shown when using PD-L1−/− pDCs (Fig. 6F), which could not decrease the eosinophilic airway inflammation, whereas the control wild-type (WT) pDCs could (Fig. 6G). IFN-α, IDO, or ICOS-L were not involved in the suppression of eosinophilic airway inflammation, as in these blocking or knockout conditions suppression could still be observed (Fig. 6, B, C, and E).

Discussion

Several key findings in this paper point to an intrinsic antiinflammatory role for pDCs in eosinophilic airway inflammation in allergically sensitized mice. First, pDCs are recruited to the airways of allergen-challenged mice with kinetics similar to other inflammatory cells. Second, their removal using a specific pDC-depleting Ab enhances inflammation and prevents the resolution of inflammation once allergen challenge is stopped. Finally, increasing the numbers of pDCs through adoptive transfer of cultured pDCs, or expansion of endogenous pDCs by administration of Flt3L, dampens inflammation.

In previous studies, Flt3L was shown to suppress all the cardinal features of asthma in models of experimental airway inflammation (3, 4, 6). In this study, we specifically set out to study the mechanism of this antiinflammatory effect. Systemic administration of Flt3L is known to expand the number of NK cells, cDCs, and pDCs in vivo (7, 8, 33). All of these cell types have been implicated in the pathogenesis of asthma. Although we did not directly test the effect of Flt3L on lung NK cell distribution or function in asthma, it is unlikely that the antiinflammatory effects of Flt3L were caused by NK cell alterations. Korsgren et al. have previously shown that depletion of NK cells in a very similar model of asthma decreased disease severity, suggesting that it is a proinflammatory player in this disease (34). We therefore favor the hypothesis that the effects of Flt3L were due to alterations in DC homeostasis. DCs are thought to be the most important APCs that determine the outcome of allergen inhalation in the lung (12). cDCs and inflammatory DCs are held responsible for inducing Th2 sensitization to inhaled Ag and are similarly crucial for the establishment of secondary immune responses to inhaled allergen in allergen-sensitized mice (13, 15, 24). As administration of cDCs and/or inflammatory DCs to mice exacerbates the severity of the disease, and their depletion ameliorates disease (15), it is unlikely that Flt3L would have its antiinflammatory effects via increasing the number and/or function of these subsets. We hypothesized that it is predominantly through the recruitment of pDCs that Flt3L suppresses inflammation. In support of this hypothesis, we have previously reported that pDCs play an essential role in maintaining tolerance in the lung when naive mice were exposed to harmless inhaled Ags (16). The critical involvement of various DC subsets in promoting or dampening inflammation has been reported in several other models, in which also viral Ags were used (35–40). However, these experiments were done in the context of a primary immune response, as pDCs were given or depleted at the time of sensitization. In the present paper, we show that pDCs, like cDCs, play a role beyond the sensitization phase. Flt3L treatment given before the secondary challenge induced a massive recruitment of pDCs, accompanied by a suppression of eosinophilic airway inflammation. Importantly, this pDC recruitment was necessary for the antiinflammatory effect, as removal of pDCs using the specific pDC-depleting Ab 120G8 restored airway eosinophilia and Th2 cytokine levels in draining nodes.

The exposure of OVA-sensitized mice to OVA aerosols resulted in a Th2-dependent allergic eosinophilic inflammation and in the recruitment of cDCs to the lung and the BAL fluid, as already reported (15). Interestingly, the number of pDCs was also increased following OVA challenges and correlated with the degree of airway inflammation. In the absence of any Flt3L treatment, the function of these cells was again to dampen inflammation as 120G8 treatment exacerbated the degree of immunopathology. How pDCs are recruited to inflamed airways is still unclear but could involve several inflammatory mediators (41). Indeed, inflammatory chemokines such as CCL2 (MCP-1), CCL3 (MIP-1), CCL5 (RANTES), CCL12 (MCP-5), CxCL10 (IFN-γ-inducible protein 10), and CxCL12 (stromal cell-derived factor 1) are up-regulated in allergic lungs (42) and pDCs express the receptors for most of these chemokines (CCR1, CCR2, CCR5, CCR7, CXCR3, and CXCR4 (43–45)). A second possibility would be the involvement of ChemR23, a receptor involved in DC migration and expressed on pDCs (46). Its ligand, chemerin, is found in inflammatory fluids (47), but more importantly directs DC migration to inflamed tissues (46, 48, 49). Whether chemerin is expressed in the lungs after repeated exposures to OVA aerosols remains to be established. Alternatively, DC subsets might be recruited to the airways by osteopontin, a cytokine involved in autoimmune and allergic diseases. Osteopontin is increased in BAL fluids of OVA-airways by osteopontin, a cytokine involved in autoimmune and allergic diseases. Osteopontin is increased in BAL fluids of OVA-sensitized mice (13, 15, 24). As administration of cDCs and/or inflammatory DCs to mice exacerbates the severity of the disease, and their depletion ameliorates disease (15), it is unlikely that Flt3L would have its antiinflammatory effects via increasing the number and/or function of these subsets. We hypothesized that it is predominantly through the recruitment of pDCs that Flt3L suppresses inflammation. In support of this hypothesis, we have previously reported that pDCs play an essential role in maintaining tolerance in the lung when naive mice were exposed to harmless inhaled Ags (16). The critical involvement of various DC subsets in promoting or dampening inflammation has been reported in several other models, in which also viral Ags were used (35–40). However, these experiments were done in the context of a primary immune response, as pDCs were given or depleted at the time of sensitization. In the present paper, we show that pDCs, like cDCs, play a role beyond the sensitization phase. Flt3L treatment given before the secondary challenge induced a massive recruitment of pDCs, accompanied by a suppression of eosinophilic airway inflammation. Importantly, this pDC recruitment was necessary for the antiinflammatory effect, as removal of pDCs using the specific pDC-depleting Ab 120G8 restored airway eosinophilia and Th2 cytokine levels in draining nodes.

Increasing pDC numbers at the site of allergic airway inflammation may be part of an inherent protective mechanism, as suggested by the fact that the disease was exacerbated following removal of these cells at the time of challenge, and by the fact that administration of pDCs before secondary challenge diminished the severity of inflammation. One striking observation was the fact that before adoptive transfer, pDCs did not have to be pulsed with OVA Ag for the suppressive effect to occur. This is in contrast to the situation in naive mice, where pDCs had to be pulsed with OVA Ag for prevention of alumn-induced sensitization, as previously reported by our group (16). The most likely explanation is that in sensitized mice, unpulsed pDCs acquired and processed inhaled Ag delivered via aerosol exposure. In already sensitized...
mice, the uptake of OVA could be facilitated by the presence of OVA-specific IgG (our unpublished data) (50–52).

There is evidence showing that pDCs can down-modulate immune responses by directly interfering with effector T cell function, by inhibiting cDC function, or by inducing regulatory T cells (18, 53). Increasing regulatory T cell numbers and functions during the secondary phase is a strategy to inhibit airway inflammation and even reverse ongoing inflammation (54–57). We have previously reported that immature lung OVA-pulsed pDCs could induce regulatory T cells when cocultured with naive CD4+ T cells (16). One way by which pDCs might promote the formation of regulatory T cells is their poor expression of costimulatory molecules (53). In our experiments, the instillation of CpG motifs strongly reduced airway inflammation, similar to previous reports (29–31). The explanation for this effect has been the inhibition of both Th2 cell activation and IgE-mediated cytokine induction (32) and the induction of IDO in the epithelial layer of the lungs (58).

Here, we show not only that the effect induced by CpG motifs was partially mediated by pDCs, but also that the degree of maturation of these pDCs did not seem to influence their suppressive effect. Indeed, CpG motif-exposed pDCs, although very mature, were still very potent at suppressing eosinophilic airway inflammation in vivo. Various costimulatory molecules, such as CD80, ICOS-L, and PD-L1, were induced by CpG motifs. In various models it has been shown that pDCs induce the formation of regulatory T cells through expression of IDO, ICOS-L, and/or PD-L1. Moreover, CpG motif-stimulated pDCs are an abundant source of IFN-α. We show that pDCs exert their suppressive function in vivo via their expression of costimulatory molecule PD-L1, as PD-L1−/− pDCs did not down-regulate eosinophilic inflammation. During peripheral tolerance, high PD-L1 expression on pDCs was directly associated with higher numbers of regulatory T cells (59, 60). PD-L1 was shown to be directly responsible for the generation of regulatory T cells when expressed by CD8α− cDCs and when TGFβ was present (61). However, we could not find clear differences in the number of regulatory T cells in our pDC-treated allergic mice (our unpublished data), probably due to the high degree of inflammation present. Our experiments also revealed that other pathways, such as ICOS-L on pDCs inducing IL-10-producing regulatory T cells (17, 62), did not play a role. When we injected mice with ICOS-L−/− pDCs, inflammation could still be reduced (Fig. 6C). Second, pDCs have been shown express the tryptophan metabolizing enzyme IDO especially after activation with CpG motifs (63, 64). Although, it has been shown that IDO expression can block experimental asthma (58), IDO−/− pDCs were still capable of reducing eosinophilic airway inflammation (Fig. 6B). Third, pDCs stimulated with unmethylated CpG motifs are a major source of IFN-α. There is evidence that IFN-α can block the recruitment of eosinophils and CD4+ T cells to the site of inflammation (65). However, when the type I IFN receptor was blocked using specific Abs at the time of pDC administration (i.e., before the secondary challenge), no differences in degree of airway inflammation were found between anti-IFN-α receptor mAb and isotype-treated allergic mice (Fig. 6E), ruling out a role for this cytokine in the pDC-induced suppression.

pDCs could furthermore suppress eosinophilic airway inflammation by down-regulating the function of cDCs (18). cDCs are known to be critical in the maintenance of airway inflammation, as their removal at the time of challenge reduced the features of asthma (15). Moreover, cDCs of allergen-sensitized-and–challenged animals selectively produce CCR4 ligands CCL17 (thymus and activation-regulated chemokine, TARC) and CCL22 (monocyte-derived chemokine) as a means to recruit Th2 cells to sites of inflammation (18, 66). Kohl et al. elegantly showed that in conditions where the cDC:pDC ratio is shifted to pDCs, the levels of cDCs and TARC were decreased (18). This might offer a likely explanation as to why Th2-dependent airway inflammation is decreased when the number of pDCs is increased in the Airways. Our own unpublished data suggests that pDCs keep cDCs immature, as depleting pDCs results in more mature cDCs measured by their costimulatory molecules (data not shown).

In conclusion, our paper provides definite proof that pDCs serve an antinflammatory role during eosinophilic airway inflammation irrespective of their maturation state through their expression of the inhibitory costimulatory molecule PD-L1. The therapeutic effectiveness of Flt3L on airway inflammation validates the concept that enhancing pDC functions during established airway inflammation could be a potential new strategy for the treatment of asthma.

Disclosures

The authors have no financial conflicts of interest.

References


