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**References**

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Prostaglandin D₂ Inhibits Airway Dendritic Cell Migration and Function in Steady State Conditions by Selective Activation of the D Prostanoid Receptor 1

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PGD₂ is the major mediator released by mast cells during allergic responses, and it acts through two different receptors, the D prostanoid receptor 1 (DP1) and DP2, also known as CRTH2. Recently, it has been shown that PGD₂ inhibits the migration of epidermal Langerhans cells to the skin draining lymph nodes (LNs) and affects the subsequent cutaneous inflammatory reaction. However, the role of PGD₂ in the pulmonary immune response remains unclear. Here, we show that the intratracheal instillation of FITC-OVA together with PGD₂ inhibits the migration of FITC⁺ lung DC to draining LNs. This process is mimicked by the DP1 agonist BW245C, but not by the DP2 agonist DK-PGD₂. The ligation of DP1 inhibits the migration of FITC-OVA⁺ DCs only temporarily, but still inhibits the proliferation of adoptively transferred, OVA-specific, CFSE-labeled, naive T cells in draining LNs. These T cells produced lower amounts of the T cell cytokines IL-4, IL-10, and IFN-γ compared with T cells from mice that received FITC-OVA alone. Taken together, our data suggest that the activation of DP receptor by PGD₂ may represent a pathway to control airway DC migration and to limit the activation of T cells in the LNs under steady state conditions, possibly contributing to homeostasis in the lung. The Journal of Immunology, 2003, 171: 3936–3940.

Materials and Methods

Reagents and Abs
PGD₂, BW245C, DK-PGD₂ (13,14-dihydro-15-keto-PGD₂), and PGD₂ were purchased from Cayman Chemicals (Ann Arbor, MI). FITC-labeled OVA and CFSE were obtained from Molecular Probes (Eugene, OR). Collagenase type II was purchased from Worthington Biochemical Corp. (Lakewood, NJ). DNase I was obtained from Sigma-Aldrich ( Zwijndrecht, The Netherlands). The PE-conjugated anti-I-Ad/I-Ed (M5/114.5.2) was obtained from BD PharMingen (Heidelberg, Germany). The PE-conjugated KJ1-26 (clonotypic OVA-TCR) was purchased from Caltag Laboratories (Burlingame, CA). The allophycocyanin-labeled anti-CD4 (RM-5) and anti-CD11c (HL3) were obtained from BD PharMingen. The endotoxin level of FITC-OVA determined by a Limulus amebocyte assay (BioWhittaker, Walkersville, MD) was <0.001 μg, which was previously reported not to affect DCs (11).

Mice
BALB/c mice (6–8 wk old) were purchased from Harlan (Zeist, The Netherlands). OVA-TCR transgenic mice (DO11.10) on a BALB/c background were bred at Erasmus University (Rotterdam, The Netherlands).

Intratracheal administration of reagents
Mice were anesthetized by i.p. injection of avertin. Eighty microliters of FITC-OVA (10 mg/ml), with or without BW245C, DK-PGD₂, or PGD₂, was administered intratracheally under direct vision through the opening vocal cords using a 18-gauge polyurethane catheter connected to the outlet of a micropipette as previously described (12). Control mice received 80 μl of PBS/DMSO.
Preparation of single-cell suspension from LNs

At several time points following FITC-OVA instillation, animals were killed by an overdose of avertin. Thoracic draining LN cells and lung cells were obtained as previously described (13). Briefly, thoracic LNs or lungs were digested for 1 h at 37°C in RPMI 1640 containing 5% FCS, 1 mg/ml of collagenase II, and 2 IU/ml of DNase I. The reaction was stopped by addition of PBS containing 10 mM EDTA. LN cells (95% viability) were washed, stained with anti-MHC class II and anti-CD11c Abs, and analyzed by flow cytometry for FITC positivity. Dead cells and debris were excluded using propidium iodide.

Effect of BW245C on T cell proliferation in thoracic LNs

Because the frequency of OVA-specific T cells is very low in immunized animal, naive T cells purified from DO11.10 mice were adoptively transferred into BALB/c mice. Briefly, LNs and spleen were harvested from DO11.10 mice and homogenized, and after RBC lysis, cell suspensions were labeled with CFSE as previously described (14). On day 2, 10^7 live cells were injected i.v. in the lateral tail vein of each mouse (day 2). On day 0, mice received an intratracheal injection of FITC-OVA (0.1, 1, or 10 mg/ml) with or without 100 μM BW245C (final concentration, 8 nmol). On day 4, thoracic LNs were collected and stained for the presence of KJ1-26^+CD4^+ MHC II^+CD11c^+ DCs. Some of the LN cells (2 × 10^5 cells/well in triplicate) were resuspended in RPMI 1640 containing 5% FCS and antibiotics and placed in round-bottom, 96-well plates. Four days later, supernatants were harvested and analyzed for the presence of IL-4, IL-10, and IFN-γ (BD PharMingen).

Statistical analysis

For all experiments the difference between the various groups was calculated using the Mann-Whitney U test for unpaired data. Differences were considered significant at p < 0.05.

Results

PGD2 impairs lung DC migration to draining LNs through DP1

As shown in Fig. 1A, migrating MHCII^+CD11c^+FITC^+ DCs are detected in the thoracic LNs 2 days after the instillation of FITC-OVA. The OVA-induced migration of DCs was strongly inhibited by PGD2. To identify which of the PGD2 receptors (DP1 or DP2) was involved in reducing DC migration to the LNs, FITC-OVA was injected intratracheally together with the DP1 agonist BW245C or the DP2 agonist DK-PGD2. BW245C dose-dependently inhibited the migration of lung DCs to thoracic LNs (BW245C/FITC; Fig. 1, A and B). Interestingly, the migration of lung DCs was not affected by DK-PGD2 (Fig. 1, A and C), suggesting that the migration of lung DCs to the nodes was mediated mainly through DP1, not DP2. As the maximal effect was obtained with a dose of 100 μM BW245C, additional experiments were performed with this dose. Moreover, to exclude a possible toxic effect of BW245C on lung DCs, total lungs were digested and stained for MHCII^+CD11c^+ DCs. The total number of DCs detected in the lungs of animals that were instilled with FITC-OVA alone and that in animals given BW245C/FITC-OVA was not significantly different (52,520 ± 16,678 and 74,080 ± 8,120, respectively; Fig. 2), indicating that the effect induced by BW245C was not due to cell death, but, rather, to the immobilization of DCs in the airways.
BW245C temporarily blocks DC migration in vivo

As the selective activation of DP1 impaired lung DC migration, we studied how long a single injection of BW245C would affect the migration of endogenous DCs to the thoracic LNs. For this purpose, BALB/c mice were injected intratracheally with 10 mg/ml FITC-OVA with or without 100 μM BW245C. Mice were killed 1, 2, 4, or 7 days later. Compared with mice that received FITC-OVA alone, the number of migrating FITC+ DCs was reduced up to day 2 in mice that received an instillation of BW245C. However, no difference was observed in the number of FITC+ DCs reaching draining LNs at 4 or 7 days in mice that received either FITC-OVA alone or FITC-OVA containing BW245C (Fig. 3).

BW245C impairs OVA-specific T cell proliferation in thoracic LNs

As BW245C inhibited the migration of endogenous lung DCs, we next hypothesized that it could also impact T cell activation in thoracic LNs. To test this we first set up an experiment in which BALB/c mice were adoptively transferred with cells from DO11.10 mice on day 2 and were injected with increasing doses of FITC-OVA (0.1, 1, and 10 mg/ml) with or without 100 μM BW245C on day 0. Mice were killed on day 4, and cell divisions were evaluated by flow cytometry. As expected, in mice that received the higher dose of FITC-OVA (10 mg/ml), T cells had undergone seven divisions (Fig. 4). Interestingly, the less FITC-OVA the mice received, the fewer T cells entered into divisions. BW245C reduced the number of T cells entering into division without affecting the number of cell divisions independently from the dose of FITC-OVA. However, the effect of BW245C on T cell proliferation appeared to be dependent on the dose of FITC-OVA. The stronger effect was obtained with the lower concentration of FITC-OVA (0.1 mg/ml). We chose this dose of OVA to perform the following experiment.

BW245C reduces cytokine production by draining LN T cells

We next investigated whether the administration of BW245C could affect FITC-OVA-induced cytokine production by thoracic LN T cells. In these experiments, 4 days following the injection of FITC-OVA with or without BW245C, thoracic LN cells were collected and cultured for 4 days in the absence of exogenous OVA. Supernatants were then tested for the presence of IL-4, IL-10, and IFN-γ. As shown in Fig. 5, compared with levels in mice that received FITC-OVA alone, treatment with BW245C reduced the amounts of all cytokines tested.

Discussion

The migration of DCs from the periphery to draining LNs is a key step leading to the initiation of immune responses or to tolerance, depending on their maturation stage (15, 16). The molecular events that induce or control DC migration have been the purpose of extensive research in the past few years. The factors involved in the migration of Langerhans cells, which are known to have low turnover (17), have been widely studied. The presence of inflammatory cytokines, such as TNF-α or IL-1β, in their microenvironment induces the departure of Langerhans cells (18). More recently, it has been shown that some products of arachidonic acid (LTC4 and PGE2) could up-regulate the chemokine-driven migration of DCs (8, 9, 19). However little is known about the migration of DCs from other organs, such as the lung. In this study we show that airway DCs migrate very efficiently to thoracic LNs following intratracheal injection of FITC-OVA, as previously reported (20). Interestingly, the OVA-induced migration was inhibited by BW245C, a selective agonist for DP1, and not by the DP2 agonist, DK-PGD2. DP1 activation has been reported to inhibit Langerhans...
As the migration of airway OVA-loaded DCs reaching the LNs was less strong and delayed over time by BW245C treatment, we looked at the effect of BW245C administration on T cell activation. In mice that received only FITC-OVA, T cells from the LNs had already undergone eight divisions by day 4 following instillation. These data are in agreement with those from other groups showing that under steady state conditions, DCs reaching the LNs show high levels of Ag presentation to specific T cells (21). However, when mice received BW245C together with FITC-OVA, the number of T cells recruited into divisions was lower, but the number of divisions undergone by some T cells was not affected. Moreover, the levels of all cytokines produced by T cells of BW245C-treated mice were reduced compared with those in mice instilled with only FITC-OVA. This suggests that BW245C can impair the primary activation of T cells by DCs within draining LNs. The lower T cell response observed in BW245C-treated mice may be explained 1) by the lower number of DCs reaching the LNs or 2) by the fact that BW245C could limit the maturation of airway DCs by affecting the levels of costimulatory molecules or/and the expression of the chemokine receptor CCR7 whose ligands (CCL19/CLL21) are known to direct mature DCs to draining LNs (22). The unresponsiveness of DCs to the ligands of CCR7 may subsequently affect the primary immune response, as previously reported in plt mice (23) or CCR7-deficient mice (24).

Our data show for the first time that PGD₂, through selective activation of DP₁, can reduce the migration of lung DCs. Moreover, T cell activation within the thoracic draining LNs was also significantly reduced. This is another example of interference of DC migration by lipid mediators. Regulation of DC migration by PGD₂ or its metabolites might have a physiological meaning. PGD₂ is produced by mast cells and APCs, such as alveolar macrophages. Under steady state conditions, macrophages might secrete PGD₂ to suppress DC and T cell activation (25). During inflammation, PGF₂α and LTα₁ are produced and induce differentiation and maturation of tissue-resident DCs (8, 19). Under these conditions, the anti-inflammatory effects of PGD₂ are overridden by the proinflammatory effects of PGE₂, LTC₄, and proinflammatory cytokines. Also, at the resolution of inflammation, COX-2 enzyme mainly generates PGD₂, rather than PGE₂ (26). The release of PGD₂ might suppress DC migration to prevent further immune stimulation, thus contributing to the resolution of inflammation. Additional experiments will be necessary to understand the differential effects of PGs on DC functions. These data may have important consequences to improve the treatments of lung diseases such as asthma, where the migration of DCs to the thoracic LNs is known to induce or enhance the Ag-specific Th₂ response (12, 14, 27, 28).

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**References**


