Prostaglandin D_2 Inhibits Airway Dendritic Cell Migration and Function in Steady State Conditions by Selective Activation of the D Prostanoid Receptor 1[1]

Hamida Hammad,[2]* Hendrik Jan de Heer,* Thomas Soullie,* Henk C. Hoogsteden,* Francois Trottein,† and Bart N. Lambrecht*

PGD_2 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 PGD_2 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 PGD_2 in the pulmonary immune response remains unclear. Here, we show that the intratracheal instillation of FITC-OVA together with PGD_2 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-PGD_2. 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 PGD_2 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.**

Prostaglandins are small lipid molecules that play an important role in the modulation of inflammatory diseases. They are produced from arachidonic acid, which is converted to PGH_2, a common precursor of several PGs, by the cyclooxygenase enzymes COX-1[3] and COX-2. PGH_2 can then be converted into a series of PGs, including PGE_2 and PGD_2 (1).

PGD_2 binds two receptors: the D prostanoid receptor 1 (DP1) (2) and DP2, also known as CRTH2 (3), which is preferentially expressed on Th2 cells, eosinophils, and basophils. PGD_2 has been associated with the development of pulmonary inflammatory diseases such as asthma (4). The major sources of PGD_2 include activated mast cells (5), Th2 cells (6), and dendritic cells (DCs) (7).

DCs are APCs that can initiate immune responses after they have captured Ags in peripheral tissues and have migrated to the T cell area in draining lymph nodes (LNs). The mechanisms that control DC migration from the periphery to the LNs are not completely understood. Chemokines and the pattern of chemokine receptors expressed by DCs seem to play a crucial role. However, more recently other factors, such as lipid mediators, also play a critical role in the migration of DCs from the periphery to the LNs. Leukotriene C_4 (LTC_4) and PGE_2 promote the migration of DCs from the skin to the LNs (8, 9), whereas PGD_2 has the opposite effect and prevents the departure of DCs from epidermis to draining LNs (10).

Here we show that the migration of airway DCs to the thoracic LNs in steady state conditions is mediated through DP1, not DP2, activation. Moreover, administration of the DP1 agonist BW245C reduces the proliferation of Ag-specific T cells and the cytokine production by LN cells.

Materials and Methods

Reagents and Abs

PGD_2, BW245C, DK-PGD_2 (13,14-dihydro-15-keto-PGD_2), and PGD_2 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 (RM4-5) 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-PGD_2, or PGD_2, 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.

*Department of Pulmonary Medicine, Erasmus Medical Center, Rotterdam, The Netherlands; and †Institut National de la Santé et de la Recherche Médicale, Unité 547, Institut Pasteur de Lille, Lille, France

Received for publication March 28, 2003. Accepted for publication August 4, 2003.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a European Community Marie Curie fellowship (to H.H.).

[2] Address correspondence and reprint requests to Dr. Hamida Hammad, Department of Pulmonary Medicine, Room Ee2253b, Erasmus Medical Center, Dr. Molewaterplein 50, 3015 GE Rotterdam, The Netherlands. E-mail address: h.hammad@erasmusmc.nl

[3] Abbreviations used in this paper: COX, cyclooxygenase; DC, dendritic cell; DP, D prostanoid receptor; LN, lymph node; LTC_4, leukotriene C_4.

Copyright © 2003 by The American Association of Immunologists, Inc.

0022-1767/03/$02.00

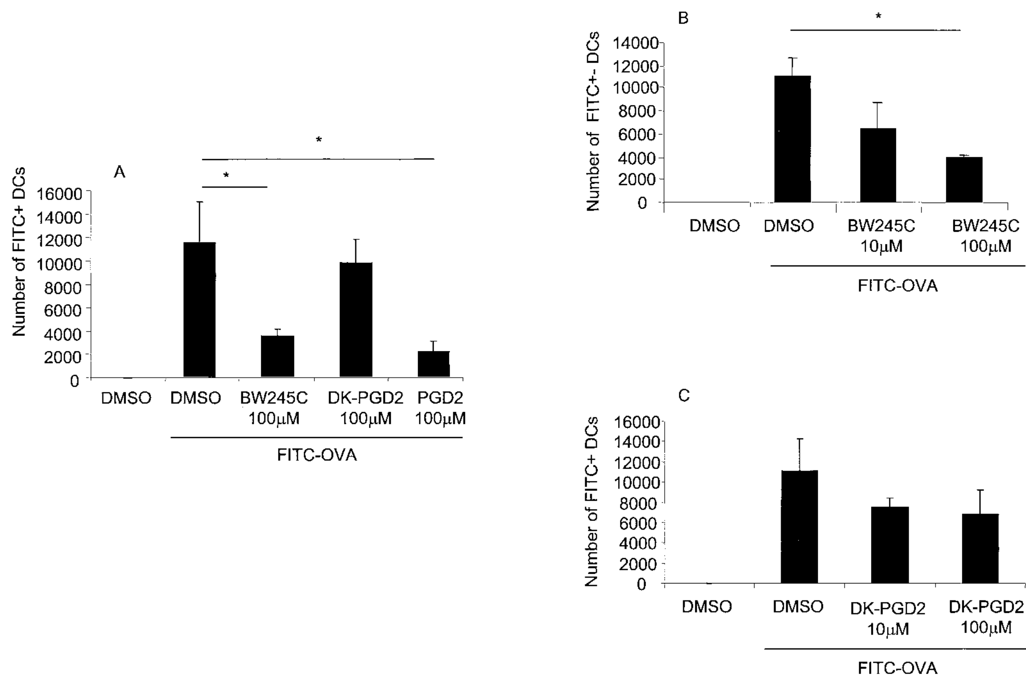

FIGURE 1. Effects of PGD_2, BW245C, and DK-PGD_2 on lung DC migration to the thoracic draining LNs. On day 0, mice were instilled intratracheally with 10 mg/ml FITC-OVA with or without different doses of PGD_2, BW245C (*A* and *B*), and DK-PGD_2 (*A* and *C*). On day 2, the presence of $FITC^+$ migrating DCs in thoracic draining LNs was analyzed by flow cytometry. Results are shown as the mean ± SD and are representative of 8–12 mice/group.

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, 10×10^6 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^+ OVA-specific T cells. 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

PGD_2 impairs lung DC migration to draining LNs through DP1

As shown in Fig. 1*A*, 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 PGD_2. To identify which of the PGD_2 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-PGD_2. 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-PGD_2 (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.

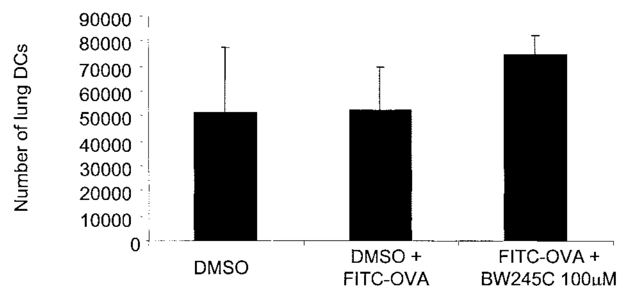

FIGURE 2. Effect of BW245C on lung DCs. On day 0, mice were instilled intratracheally with 10 mg/ml FITC-OVA with or without BW245C. On day 2, lungs were enzymatically digested and stained for the presence of $FITC^+$ MHC II^+ $CD11c^+$ DCs. Results are shown as the mean ± SD and are representative of 10–12 mice/group.

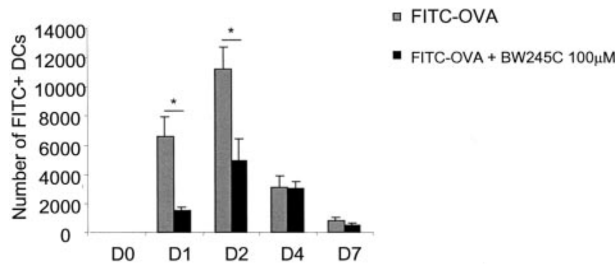

FIGURE 3. Kinetics of action of BW245C on lung DC migration to draining LNs. On day 0, mice were instilled intratracheally with 10 mg/ml FITC-OVA with or without 100 μM BW245C. On days 1, 2, 4, and 7 following instillation, thoracic LNs were digested and stained for the presence of FITC$^+$ MHC II$^+$ CD11c$^+$ DCs. Results are shown as the mean $\pm$ SD and are representative of 10 mice/group.

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 of 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.

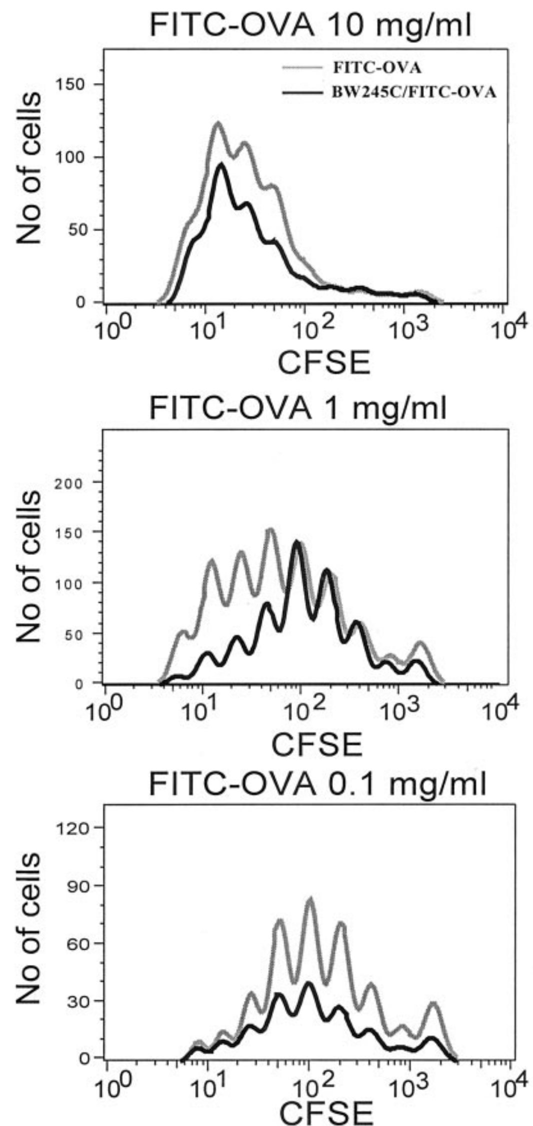

FIGURE 4. Effect of BW245C on DC-induced T cell proliferation in draining LNs. On day -2, mice were injected i.v. with OVA-specific naive T cells from DO11.10 mice. On day 0, mice were instilled intratracheally with increasing doses of FITC-OVA (0.1, 1, and 10 mg/ml; ▨) in the absence or the presence of 100 μM BW245C (■). On day 4, the proliferation of KJ1–26$^+$ CD4$^+$ CFSE$^+$ T cells was analyzed by flow cytometry. Results show one representative experiment with 10–12 mice/group.

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 (LTC_4 and PGE_2) 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-PGD$_2$. DP1 activation has been reported to inhibit Langerhans

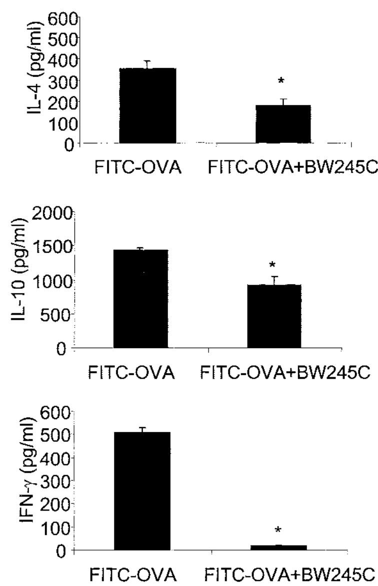

FIGURE 5. Effect of BW245C on cytokine production by T cells of the LNs. On day −2, mice were injected i.v. with OVA-specific naive T cells from DO11.10 mice. On day 0, mice were instilled intratracheally with FITC-OVA (0.1 mg/ml) in the absence or the presence of 100 μM BW245C. On day 4, LN cells were collected and cultured in 96-well plates for 4 days. The presence of IL-4, IL-10, and IFN-γ in the supernatants was analyzed by ELISA. Results are shown as the mean $\pm$ SD and are representative of 10–12 mice/group.

cell migration in a model of parasite infection (10). However, to our knowledge, this is the first report showing the inhibition of DC migration with a high turnover (17) and under steady state conditions. The direct effect of BW245C on lung DCs remains to be determined. Lung DCs are difficult to purify in sufficient amounts to perform functional studies. However, we have evidence that in vitro treatment of bone marrow-derived DCs with BW245C reduces their potential to migrate from the bronchoalveolar lavage fluid compartment to the lung draining LNs in vivo (our unpublished observations and Ref. 14). However, an indirect effect of the agonist on airway DCs cannot be ruled out in this study, as BW245C was administered into the trachea of mice.

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/CCL21) 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 PGD_2, 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 PGD_2 or its metabolites might have a physiological meaning. PGD_2 is produced by mast cells and APCs, such as alveolar macrophages. Under steady state conditions, macrophages might secrete PGD_2 to suppress DC and T cell activation (25). During inflammation, PGE_2 and LTC_4 are produced and induce differentiation and maturation of tissue-resident DCs (8, 19). Under these conditions, the anti-inflammatory effects of PGD_2 are overridden by the proinflammatory effects of PGE_2, LTC_4, and proinflammatory cytokines. Also, at the resolution of inflammation, COX-2 enzyme mainly generates PGD_2, rather than PGE_2 (26). The release of PGD_2 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).

References

1. Harris, S. G., J. Padilla, L. Koumas, D. Ray, and R. P. Phipps. 2002. Prostaglandins as modulators of immunity. *Trends Immunol. 23:144*.
2. Boie, Y., N. Sawyer, D. M. Slipetz, K. M. Metters, and M. Abramovitz. 1995. Molecular cloning and characterization of the human prostanoid DP receptor. *J. Biol. Chem. 270:18910*.
3. Nagata, K., H. Hirai, K. Tanaka, K. Ogawa, T. Aso, K. Sugamura, M. Nakamura, and S. Takano. 1999. CRTH2, an orphan receptor of T-helper-2-cells, is expressed on basophils and eosinophils and responds to mast cell-derived factor(s). *FEBS Lett. 459:195*.
4. Kay, A. B., L. Barata, Q. Meng, S. R. Durham, and S. Ying. 1997. Eosinophils and eosinophil-associated cytokines in allergic inflammation. *Int. Arch. Allergy Immunol. 113:196*.
5. O'Sullivan, S., S. E. Dahlen, K. Larsson, B. M. Larsson, P. Malmberg, M. Kumlin, and L. Palmberg. 1998. Exposure of healthy volunteers to swine house dust increases formation of leukotrienes, prostaglandin D_2, and bronchial responsiveness to methacholine. *Thorax 53:1041*.
6. Tanaka, K., K. Ogawa, K. Sugamura, M. Nakamura, S. Takano, and K. Nagata. 2000. Cutting edge: differential production of prostaglandin D_2 by human helper T cell subsets. *J. Immunol. 164:2277*.
7. Urade, Y., M. Ujihara, Y. Horiguchi, K. Ikai, and O. Hayaishi. 1989. The major source of endogenous prostaglandin D_2 production is likely antigen-presenting cells: localization of glutathione-requiring prostaglandin D synthetase in histiocytes, dendritic, and Kupffer cells in various rat tissues. *J. Immunol. 143:2982*.
8. Robbiani, D. F., R. A. Finch, D. Jager, W. A. Muller, A. C. Sartorelli, and G. J. Randolph. 2000. The leukotriene C_4 transporter MRP1 regulates CCL19 (MIP-3β, ELC)-dependent mobilization of dendritic cells to lymph nodes. *Cell 103:757*.
9. Scandella, E., Y. Men, S. Gillessen, R. Forster, and M. Groettrup. 2002. Prostaglandin E_2 is a key factor for CCR7 surface expression and migration of monocyte-derived dendritic cells. *Blood 100:1354*.
10. Angeli, V., C. Faveeuw, O. Roye, J. Fontaine, E. Teissier, A. Capron, I. Wolowczuk, M. Capron, and F. Trottein. 2001. Role of the parasite-derived prostaglandin D_2 in the inhibition of epidermal Langerhans cell migration during schistosomiasis infection. *J. Exp. Med. 193:1135*.
11. 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*.
12. 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*.
13. Vremec, D., and K. Shortman. 1997. Dendritic cell subtypes in mouse lymphoid organs: cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. *J. Immunol. 159:565*.
14. 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*.
15. Steinman, R. M., S. Turley, I. Mellman, and K. Inaba. 2000. The induction of tolerance by dendritic cells that have captured apoptotic cells. *J. Exp. Med. 191:411*.

16. Jonuleit, H., E. Schmitt, M. Stassen, A. Tuettenberg, J. Knop, and A. H. Enk. 2001. Identification and functional characterization of human $CD4^{+}CD25^{+}$ T cells with regulatory properties isolated from peripheral blood. *J. Exp. Med. 193:1285*.
17. 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*.
18. Schwarzenberger, K., and M. C. Udey. 1996. Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression in situ. *J. Invest. Dermatol. 106:553*.
19. Kabashima, K., D. Sakata, M. Nagamachi, Y. Miyachi, K. Inaba, and S. Narumiya. 2003. Prostaglandin E_2-EP4 signaling initiates skin immune responses by promoting migration and maturation of Langerhans cells. *Nat. Med. 9:744*.
20. 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*.
21. Crowley, M., K. Inaba, and R. M. Steinman. 1990. Dendritic cells are the principal cells in mouse spleen bearing immunogenic fragments of foreign proteins. *J. Exp. Med. 172:383*.
22. Cyster, J. G. 1999. Chemokines and cell migration in secondary lymphoid organs. *Science 286:2098*.
23. Mori, S., H. Nakano, K. Aritomi, C. R. Wang, M. D. Gunn, and T. Kakiuchi. 2001. Mice lacking expression of the chemokines CCL21-ser and CCL19 (plt mice) demonstrate delayed but enhanced T cell immune responses. *J. Exp. Med. 193:207*.
24. Forster, R., A. Schubel, D. Breitfeld, E. Kremmer, I. Renner-Muller, E. Wolf, and M. Lipp. 1999. CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell 99:23*.
25. Bilyk, N., and P. G. Holt. 1995. Cytokine modulation of the immunosuppressive phenotype of pulmonary alveolar macrophage populations. *Immunology 86:231*.
26. Gilroy, D. W., P. R. Colville-Nash, D. Willis, J. Chivers, M. J. Paul-Clark, and D. A. Willoughby. 1999. Inducible cyclooxygenase may have anti-inflammatory properties. *Nat. Med. 5:698*.
27. Hammad, H., B. N. Lambrecht, P. Pochard, P. Gosset, P. Marquillies, A. B. Tonnel, and J. Pestel. 2002. Monocyte-derived dendritic cells induce a house dust mite-specific Th2 allergic inflammation in the lung of humanized SCID mice: involvement of CCR7. *J. Immunol. 169:1524*.
28. 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 $CD31^{hi}Ly\text{-}6C^{neg}$ bone marrow precursors in a mouse model of asthma. *Blood 100:3663*.