MHC Dextramer® – Detect with Confidence

Get the full picture of **CD8+** and **CD4+** T-cell responses

Even the low-affinity ones

Available also in GMP

IMMUDEX

PRECISION IMMUNE MONITORING

The Journal of Immunology

RESEARCH ARTICLE | MAY 15 2003

Prostaglandin D_2 Affects the Maturation of Human Monocyte-Derived Dendritic Cells: Consequence on the Polarization of Naive Th Cells[1]

Philippe Gosset; ... et. al

J Immunol (2003) 170 (10): 4943–4952.

https://doi.org/10.4049/jimmunol.170.10.4943

Related Content

Prostaglandin D_2 Causes Preferential Induction of Proinflammatory Th2 Cytokine Production through an Action on Chemoattractant Receptor-Like Molecule Expressed on Th2 Cells

J Immunol (November,2005)

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

J Immunol (October,2003)

Physiological Levels of 15-Deoxy-$\Delta^{12,14}$-Prostaglandin J_2 Prime Eotaxin-Induced Chemotaxis on Human Eosinophils through Peroxisome Proliferator-Activated Receptor-γ Ligation

J Immunol (November,2005)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

Prostaglandin D_2 Affects the Maturation of Human Monocyte-Derived Dendritic Cells: Consequence on the Polarization of Naive Th Cells[1]

Philippe Gosset,[2]*§ Fabrice Bureau,† Veronique Angeli,‡§ Muriel Pichavant,*§ Christelle Faveeuw,‡§ André-Bernard Tonnel,*§ and François Trottein‡§

Among the factors produced at inflammatory sites and those capable of modulating dendritic cell (DC) functions, PGD_2 may be important in the outcome of immune responses. The biological roles for PGD_2 are in part effected through two plasma membrane G protein-coupled receptors: the D prostanoid (DP) receptor and the chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2). In this report, we studied the effects of PGD_2 and of its major physiological metabolite, 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2), on the functions of human monocyte-derived DC. First, we show that PGD_2 exerts in vitro chemotactic effects on monocytes via CRTH2 activation while it inhibits the chemokine-driven migration of monocyte-derived DC through DP. We also report that PGD_2 and 15d-PGJ_2 alter the LPS- and allergen-induced DC maturation and enhance the CD80/CD86 ratio on mature DC in a DP- and CRTH2-independent manner. Moreover, PGD_2 and 15d-PGJ_2 strongly reduce the secretion of the Th1 promoting cytokine IL-12 and affect the synthesis of chemokines involved in Th1 cell chemotaxis, particularly CXCL10. Inhibition of cytokine/chemokine secretion implicates at least in part DP, but not CRTH2. The effects exerted by PGD_2 are associated with the phosphorylation of CREB, but do not parallel with the deactivation of the NF-κB and mitogen-activated protein kinase pathways. In contrast, 15d-PGJ_2 seems to target other cellular proteins. Finally, in a model of Th $CD45RA^+$ differentiation induced by allergen- and superantigen-pulsed DC, PGD_2 impacts on the orientation of the immune response by favoring a Th2 response. *The Journal of Immunology,* **2003, 170: 4943–4952.**

Prostaglandin D_2 is a major cyclooxygenase product that is generated at sites of inflammation and infection and exhibits important effector and regulatory functions during the inflammatory response. For example, PGD_2 production by allergen-activated mast cells (1) appears to be important in the development of allergic reactions (2, 3), which is an effect due, at least in part, to the ability of PGD_2 to increase leukocyte extravasation and to act as a chemoattractant for Th2 cells, eosinophils, and basophils (4–6). Along with its role in cell mobilization, PGD_2 appears to exert a dual function during the inflammatory response with the capacity to act as either a mediator or potent inhibitor of inflammation in response to certain physiological or pathological conditions (7–9). These biological roles for PGD_2 are effected through two plasma membrane G protein-coupled receptors: the D prostanoid (DP)[3] receptor (10) and the recently identified chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes (CRTH2) (4). In addition, because PGD_2 can be nonenzymatically converted by dehydration and isomerization to 15-deoxy-$\Delta^{12,14}$-PGJ_2 (15d-PGJ_2) (11, 12), its modes of action can be very diverse. For example, PGD_2 and 15d-PGJ_2 may interact directly with intracellular proteins, including transcription factor complexes and nuclear receptors such as the peroxisome proliferator-activated receptor (PPAR) (13, 14).

However, despite the role for PGD_2 as an important mediator during acute and chronic inflammation and our recent demonstration that PGD_2 greatly affects the migratory properties of dendritic cells (DC) (15), which are the most potent APC, its role during the early phases of the immune response is unknown. DC play key roles in orchestrating cellular and humoral immune responses to self- and foreign Ags (16, 17). Activation of naive T cells and their polarization into effector cells requires interactions with mature DC in the lymphoid organs. DC maturation results in a modification of its cell shape and in the regulation of the expression of chemokine receptors, which in turn allows DC migration from the site of Ag capture to the regional lymph nodes where they encounter naive T cells (18). Meanwhile, DC maturation results in an up-regulation of cell surface-expressed MHC and of costimulatory molecules in addition to enhanced secretion of immunomodulatory cytokines and chemokines. Complete DC maturation is not achieved in response to Ag capture alone but requires exposure to

*Institut National de la Santé et de la Recherche Médicale, Unité 416, Institut Pasteur de Lille, Lille, France; †Faculté de Médecine Vétérinaire, Université de Liège, Liège, Belgium; and ‡Institut National de la Santé et de la Recherche Médicale, Unité 547, and §Institut Fédératif de Recherche 17, Institut Pasteur de Lille, Lille, France

Received for publication August 28, 2002. Accepted for publication March 5, 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 the Institut National de la Santé et de la Recherche Médicale and the Pasteur Institute of Lille. V.A. and M.P. were the recipients of postdoctoral fellowships from the Ministère de l'Education Nationale and C.F. was the recipient of a postdoctoral fellowship from la Société de Secours des Amis des Sciences. F.B. is a research assistant at the National Fund for Scientific Research (Belgium). P.G. and F.T. are members of the Institut National de la Santé et de la Recherche Médicale and the Center National de la Recherche Scientifique, respectively.

[2] Address correspondence and reprint requests to Dr. Philippe Gosset, Institut National de la Santé et de la Recherche Médicale, Unité 416, Institut Pasteur de Lille, 1, rue du Professeur Calmette, BP 245, 59019 Lille Cédex, France. E-mail address: Philippe.Gosset@pasteur-lille.fr

[3] Abbreviations used in this paper: DP, D prostanoid; CRTH2, chemoattractant receptor-homologous molecule expressed on Th2 lymphocytes; 15d-PGJ_2, 15-deoxy-$\Delta^{12,14}$-PGJ_2; PPAR, peroxisome proliferator-activated receptor; DC, dendritic cells; MDDC, monocyte-derived DC; ERK, extracellular signal-regulated kinase; PKA, protein kinase A; CCL, C-C chemokine ligand; Dpt, *Dermatophagoides pteronyssinus*; MFI, mean of fluorescence intensity; SEA, staphylococcal enterotoxin Ag; MAPK, mitogen-activated protein kinase.

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

0022-1767/03/$02.00

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

microbial products and/or contact with T cells (16, 19). For example, production of IL-12, a key factor in the outcome of immune responses, is induced by some microbial constituents (such as LPS) or by CD40/CD40L interactions during DC contact with T cells (20). Conversely, it is reported that certain substances can block or modulate DC maturation. For example, maturation of DC in the presence of PGE_2 biases the development of naive T lymphocytes to cells producing type 2 cytokines (21). Because the nature of maturation signals received by DC at the peripheral sites and the type of locally released inflammatory mediators are critical to polarize immune responses, we investigated whether PGD_2 and its major metabolite 15d-PGJ_2 could affect the functions of DC, particularly their ability to polarize naive T cells. Our results showed that PGD_2 and 15d-PGJ_2 partially block the maturation of monocyte-derived DC (MDDC), and as a consequence, impact on the development of the immune response.

Materials and Methods

Reagents and Abs

PGD_2, 15d-PGJ_2, DK-PGD_2, and BW245C were purchased from Cayman Chemicals (Ann Arbor, MI), and LPS (type 055B5) was purchased from Sigma-Aldrich (St. Louis, MO). The chemokines used in this study were from R&D Systems (Abingdon, U.K.). The extracellular signal-regulated kinase (ERK) and p38 inhibitors, PD98059 and SB203580, as well as the protein kinase A (PKA) inhibitor Rp-8-Br-cAMP were purchased from Calbiochem (San Diego, CA). The allergen extract from *Dermatophagoides pteronyssinus* (Dpt) (Stallergènes, Paris, France) was detoxified by passage through a column of Detoxigel (Pierce, Rockford, IL). Anti-CD14- and anti-CD45RO-conjugated microbeads and CD4 T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) were used for magnetic cell separation of monocytes. Human recombinant GM-CSF and IL-4 were purchased from PeproTech (Rocky Hill, NJ) and C-C chemokine ligand (CCL)5 (also known as RANTES) and CCL19 (macrophage-inflammatory protein 3β) were from R&D Systems. KD36 (human DP-transfected K562 cells) and KB8 (human CRTH2-transfected K562 cells) were from Dr H. Hirai (R&D Center, BLM, Saitama, Japan) (5).

The rat anti-human CRTH2 mAb (clone BM16) was a generous gift of Dr H. Hirai, and the isotype control mAb was from BD Biosciences (San Diego, CA). Polyclonal Abs against human DP were raised against a synthetic peptide corresponding to the C terminus of the protein. Briefly, Syrian hamsters were immunized by classical procedures with peptides coupled to maleimide-activated keyhole limpet hemocyanin (Pierce) in CFA. Abs were affinity-purified by passage through a column containing the corresponding DP peptide covalently coupled to *N*-hydroxysuccinimide -activated agarose (Bio-Rad, Hercules, CA). Affinity-purified hamster Abs directed against an irrelevant Ag were prepared in the laboratory and were used as a negative control. Ab binding performed after cell permeabilization was revealed by a FITC-conjugated anti-hamster IgG Ab (Jackson ImmunoResearch Laboratories, West Grove, PA). The specificity of the anti-human DP and CRTH2 Abs were checked on KD36 and KB8 cells, respectively (data not shown). The FITC-conjugated mouse mAbs (anti-CD1a, anti-CD14, anti-CD40, anti-CD80, anti-CD86, anti-HLA-DR) were obtained from BD Biosciences. The PE-conjugated mouse anti-CD11c, anti-CD54, anti-CD80, anti-CD86 Abs and the corresponding isotype controls were purchased by BD Biosciences, while the anti-CD83 mAb was from Beckman Coulter (Miami, FL).

Preparation of human MDDC and naive T cells

Blood monocytes were purified by positive selection over a MACS column using anti-CD14-conjugated microbeads and were then differentiated into DC by standard procedures (22, 23). Briefly, monocytes were cultivated at 10^6 cells/ml for 6 days in RPMI 1640 with 10% heat-inactivated FCS (Invitrogen, Paisley, U.K.) containing 15 ng/ml IL-4 and 25 ng/ml GM-CSF. At day 6, MDDC (99% pure) show phenotypic characteristics ($CD14^-$, $CD68^-$, $CD83^{low}$, $CD1a^{high}$, and $HLA\text{-}DR^{high}$) of immature DC. Autologous naive Th cells were isolated from the $CD14^-$ fraction by negative selection using the $CD4^+$ T cell isolation kit associated with CD45RO microbeads. After passage through the column, the negative fraction contained >95% of $CD4^+CD45RA^+$ cells as determined by flow cytometry.

MDDC stimulation

At day 6, immature MDDC were collected and resuspended at 0.5×10^6 cells/ml in RPMI plus 1% FCS and 10 ng/ml human recombinant GM-CSF. Cells were preincubated for 10 min with PGD_2, 15d-PGJ_2, BW245C, or DK-PGD_2 (1 μM) and then stimulated with LPS (1 μg/ml) or with the allergen extract from Dpt (1 reactivity index U/ml) for 24 h. To identify the signaling pathway(s) involved in the effect of PGD_2, PD98059 (25 μM), SB203580 (2 μM), or Rp-8-Br-cAMP (50 μM) were added 10 min before LPS-activation in the presence or absence of PGs. After 24 h, supernatants were collected for cytokine analysis, and MDDC were mechanically detached for FACS analysis. Cell death was assessed by trypan blue exclusion and by measurement of MTT oxidoreduction (Sigma-Aldrich). No significant cell death (<9%) was detected in any of the tested conditions. A dose-response curve was established for PGD_2 and 15d-PGJ_2 showing significant effects at 0.1 μM and maximal activity at 1 μM.

Chemotaxis assays

Chemotaxis assays were performed using 48-well Boyden microchambers (5 μm pore polycarbonate filter) (NeuroProbe, Cabin John, MD) (24). Briefly, cells were resuspended at 1×10^6 cells/ml in RPMI supplemented with 1% FCS and 45 μl was applied to the upper wells of the chamber. In the lower chamber, chemokines (200 ng/ml) or PGs (10 μM) were added. The modulation of chemotactic responses to CCL5 (monocytes and immature DC) or CCL19 (mature DC) by PGs was analyzed as previously described. In this case, however, cells were pretreated for 10 min with PGs (10 μM) before being applied to the upper chamber. After 90 min at 37°C, the number of cells that had migrated through the filter was determined microscopically in four random fields (magnification ×1000). Each assay was performed in quadriplicate, and the results are expressed as the mean ± SD of migrated cells per four fields.

Flow cytometric analysis

After stimulation, MDDC were collected in PBS containing 2 mM EDTA at 4°C, washed, and resuspended in PBS containing 1% normal human serum. Cells were incubated for 30 min at 4°C with the FITC-conjugated mouse mAbs anti-CD1a, anti-CD36, anti-CD40, anti-CD80, anti-CD83 or with the PE-conjugated anti-CD11c, anti-CD54, anti-CD86, and anti-HLA-DR mAbs or isotype controls. For analysis of micropinocytosis, DC were resuspended in RPMI plus 5% FCS and incubated for 1 h in the presence of 0.1 mg/ml FITC-conjugated dextran or human IgG. To evaluate CCR7 expression, binding of the mouse IgM Ab was detected by successive addition of biotinylated rat anti-mouse IgM and PE-conjugated streptavidin (BD PharMingen, San Diego, CA). Cells were washed, fixed with paraformaldehyde, and 5000 events were analyzed on a FACSCalibur flow cytometer with CellQuest software (BD Biosciences). Results are expressed as the mean of fluorescence intensity (MFI) obtained with specific Abs less the value obtained with the isotype control. In some cases, we calculated the percentage of variation of MFI as (PG-treated DC − untreated DC) × 100/untreated DC.

Cytokine assay

The concentrations of cytokines and chemokines in the culture supernatants were determined by ELISA as described by the manufacturers, R&D Systems, for the determination of IL-6, TNF-α, CCL5, CCL17 (thymus and activation-regulated chemokine), CCL22 (macrophage-derived chemokine) and CXCL10 (inducible protein-10) or Diaclone (Besançon, France) for IL-10 and IL-12p70.

Evaluation of mRNA expression by RT-PCR

Total RNA were obtained using the TRIzol reagent (Life Technologies, Grand Island, NY) and reverse transcripted using random hexamer primers and Superscript reverse transcriptase (Life Technologies). mRNA expression for DP, CRTH2, CD80, CD86, IL-12p40, and CCR7 was evaluated by a two-step semiquantitative RT-PCR using β-actin or 6-GAPDH mRNA as referenced. The primer sequences as well as the number of cycles are reported in Table I. After gel electrophoresis and staining with Sybrgreen (FMC, Rockland, ME), the intensity of each band was measured with Gel Analyst system (Claravision, Orsay, France). Results were expressed as the ratio gene of interest/GAPDH mRNA intensity.

EMSA

DC (5×10^6/well) were pretreated for 10 min with PGs and then incubated for 30 min with LPS. After washing the cells in cold PBS, nuclear proteins were extracted by standard procedures. The probes used for the gel retardation assay contained the consensus κB (5′-CAGCGGCAGGGGAAT TCCCCTCTCCTTAGGTT-3′) binding site. The 5′ end ^{32}P-labeling of the double-stranded oligonucleotide and EMSA were performed by standard procedures (25). Membranes were exposed to a PhosphorImager screen

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

Table I. *Sequences of primers used for PCR amplification of cDNA, product sizes, and PCR cycle numbers*

Gene	Primer	Sequence	Size (bp)	Cycle
DP	5′	5′-TCGGGAAGTTCGTGCAGTACTGCC	276	40
	3′	5′-GATCCAGCTCCTCCAGGGGCTGAG		
CRTH2	5′	5′-CTGCCCTTCTTCACCTACTTCTTGGC	265	40
	3′	5′-GTGTCCCGGAACACGAAATAGGGCAC		
IL-12p40	5′	5′-TCCCTGGTTTTTCTGGCATCT	560	24
	3′	5′-GCACTGTCCTCCTGGCACTC		
CCR7	5′	5′-CATGGACCTGGGGAAACCAA	1174	30
	3′	5′-CTGGGAGAGGTCCCTCTAGT		
CD80	5′	5′-AAACTCGCATCTACTGGCAAAAGG	840	30
	3′	5′-GGATCTTGGGAAACTGTTGTGTTGA		
CD86	5′	5′-GAGCTAGTAGTATTTTGGCAGGACC	932	25
	3′	5′-CCGCTTCTTCTTCTTCCATTT		
β-Actin	5′	5′-GCTGCTCACCGAGGCCCCCCTGAA	324	28
	3′	5′-CTTTAGCACGCACTGTAATTCCTC		
GAPDH	5	5′-GTCTTCACCACCATGGAGA	221	19
	3′	5′-CCAGTAGGTACTGTTGAAACC		

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

(Molecular Dynamics, Sunnyvale, CA), and the intensity of the bands was quantified by using a computer image-analysis system.

Western blot analysis

Equal amounts of whole-cell lysates or nuclear extracts (for the determination of phospho-CREB) were subjected to SDS-PAGE under reducing conditions, and proteins were electrotransferred to polyvinylidone difluoride membranes (Roche Diagnostics, Meylan, France). The membranes were blocked for 1 h at room temperature with 5% milk in TBS 1× plus 0.1% Tween 20 and incubated overnight at 4°C with Abs that recognize phosphorylated and nonphosphorylated forms of p38, ERK1/2, or CREB Abs (2 μg/ml) (New England Biolabs, Beverly, MA). Immunoreactive bands were revealed with HRP-conjugated secondary Abs using the ECL detection method (Amersham Pharmacia Biotech, Uppsala, Sweden).

Modulation of naive Th cell differentiation by activated DC in presence of PGs

Immature MDDC (5×10^4 cells/well) were incubated with or without allergen (Dpt 1 IRu/ml) in the presence or absence of PGD_2, 15d-PGJ_2, or BW245C as previously mentioned. After 6 h incubation, MDDC were washed and 2×10^6 autologous naive T cells (DC to T lymphocyte ratio = 1:200) were added in 1 ml of RPMI 1640 supplemented with 10% heated human AB^+ serum. After a 6-day incubation, T cells were collected, washed, and resuspended at 2×10^6 cells/ml in the same medium. Restimulation was performed by addition of anti-CD3 plus anti-CD28 mouse mAb (2 μg/ml) (BD PharMingen), and supernatants were collected after 24 h incubation for ELISA. In parallel, T cell proliferation was evaluated in 96-well plates in similar conditions and similar DC to T cell ratio. At day 5, [^{3}H]thymidine (0.5 mCi/well) was added for 12 h. Cells were harvested on glass fibers and analyzed in a beta-scintillation counter (Wallac OY, Turku, Finland).

In some cases, MDDC were pretreated with the staphylococcal enterotoxin Ag (SEA, Sigma-Aldrich) at 2 μg/ml in the presence or absence of vehicle, PGD_2, 15d-PGJ_2, or BW245C as previously described. Naive T cells were added after 6 h incubation and half of the medium was changed every 2 days. At day 7, frozen autologous MDDC were thawed, washed, and stimulated by SEA in the presence or absence of these PGs. After 6 h activation, T lymphocytes were transferred and restimulated with these MDDC. At day 14, T cells were collected, washed, and activated by addition of anti-CD3 plus anti-CD28 mouse mAb for 6 h. The percentage of $CD4^+$ cells expressing IFN-γ and IL-4 was determined by secretion assays (Miltenyi Biotec). Another batch of cells was activated for 24 h, and IL-4 and IFN-γ secretion was quantified in the corresponding supernatants.

Statistical analysis

Data from flow cytometry analysis were numbered as the difference of the percentage of positive cells or of the MFI obtained either with the specific Ab or the isotype control. Results were expressed as mean ± SEM. Statistical analysis was performed using of the Wilcoxon test for paired data or by the Student *t* test (chemotaxis assays). A value of $p < 0.05$ was considered as significant.

Results

Expression of plasma membrane PGD_2 receptors in MDDC

We first examined the expression of the plasma membrane PGD_2 receptors on immature DC, which were generated in vitro from monocytes in the presence of GM-CSF and IL-4, and on DC matured in the presence of LPS. As Fig. 1*A* shows, RT-PCR analysis

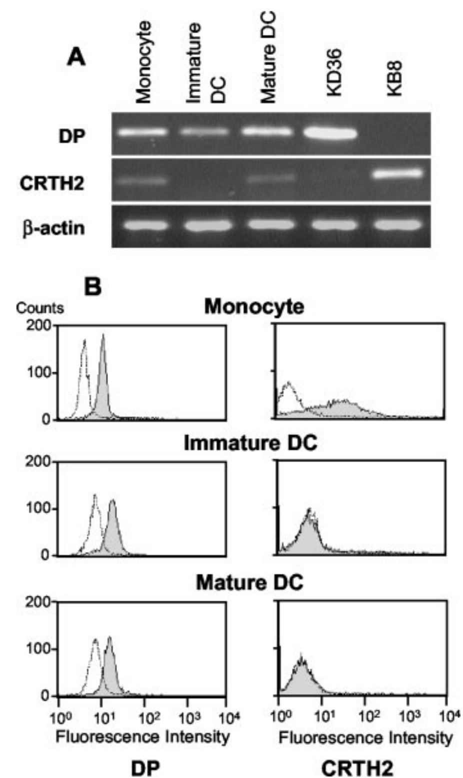

FIGURE 1. DP and CRTH2 are expressed in monocytes and/or in immature and mature MDDC as assessed by RT-PCR (*A*) and flow cytometry (*B*). *A*, Monocytes were cultured in the presence of GM-CSF and IL-4 for 6 days to generate DC, and DC maturation was induced by LPS. As positive controls, cDNA from human DP-transfected (KD36) or CRTH2-transfected (KB8) cells were used (30 cycles). *B*, Freshly isolated monocytes or immature and mature DC were analyzed by FACS analysis for DP and CRTH2 expression. Histograms were reported for specific mAbs (shaded histogram) in comparison with the isotype control (hatched histogram). One representative experiment of three is shown.

on freshly isolated cells revealed transcripts for DP, and to a lesser extent, for CRTH2 in monocytes. In immature and mature MDDC, DP mRNA expression was also observed, and this result was confirmed by FACS analysis using the specific anti-human DP Ab as a probe (Fig. 1*B*). In contrast, whereas the mAb BM16 confirmed the surface expression of CRTH2 on monocytes (Fig. 1*B*), CRTH2 mRNA was expressed at a very low level (Fig. 1*A*) and the protein was almost undetectable in immature and mature MDDC (Fig. 1*B*). Taken together, this suggests that monocytes express both DP and CRTH2 and that in our differentiation condition CRTH2 synthesis is down-regulated while that of DP is sustained in DC. Moreover, our data suggest that DC maturation by LPS does not modify the extent of DP and CRTH2 synthesis in DC.

Chemotactic response of monocytes and MDDC to PGD$_2$ receptor agonists and to chemokines

Because DP and CRTH2 are involved in cell motility (4, 15), we next examined the effects of DP and CRTH2 agonists on monocyte and DC migration in vitro. To this end, PGD$_2$, BW245C (a specific DP agonist), or DK-PGD$_2$ (a specific CRTH2 agonist) were first tested for their ability to attract monocytes. As shown in Fig. 2*A*, monocytes were attracted by the chemokine CCL5 (used as a positive control) and by, but in a less potent manner, PGD$_2$ and DK-PGD$_2$. In contrast BW245C did not induce monocyte migration. We also evaluated whether pretreatment of monocytes or MDDC with the different PGD$_2$ receptor agonists could affect their chemotactic response to chemokines. As shown in Fig. 2*B*, PGD$_2$ and BW245C, but not DK-PGD$_2$, strongly inhibited the CCL5- and

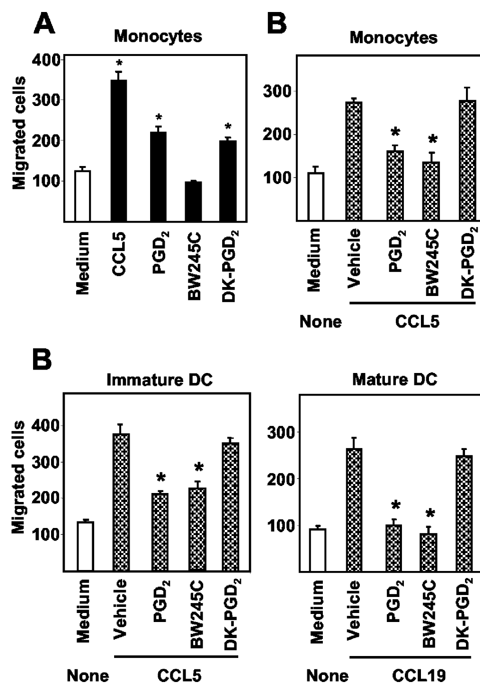

FIGURE 2. CRTH2 activation induces monocyte migration, whereas DP ligands inhibit the chemotactic response of immature and mature DC toward chemokines. *A*, Chemotactic response of monocytes to CCL5 (200 ng/ml) and PGD$_2$ receptor agonists (10 μM). *B*, Inhibition of the chemotactic responses of monocytes and immature and mature DC to chemokines. Cells were treated with PGD$_2$, BW245C, or DK-PGD$_2$ (10 μM) or the vehicle before being subjected to the chemotactic effect of CCL5 (monocyte and immature DC) or CCL19 (mature DC). The number of migrated cells is indicated and results are mean $\pm$ SD. Asterisks indicate statistical significance with a $p < 0.01$ in Student's t test compared with medium (*A*) and compared with vehicle (*B*).

CCL19-driven cell migration of monocytes/immature DC and mature DC, respectively. Taken together, this suggests that PGD$_2$ induces the migration of monocytes via CRTH2 activation, whereas it inhibits the chemokine-driven migration of DC in a DP-dependent manner.

Modulation of DC maturation by PGD$_2$

We next studied the effects of PGD$_2$ on the maturation of MDDC triggered by LPS and by Dpt, which is the major allergen of house dust mite. Moreover, to evaluate its mode of action, we also studied the effects of DP and CRTH2 agonists and of the PGD$_2$ metabolite 15d-PGJ$_2$ on DC maturation. As depicted in Fig. 3, DC maturation is characterized by the induction of CD83 and CCR7 synthesis and by a decreased incorporation of FITC-dextran (a marker for micropinocytosis). Treatment of immature DC with PGD$_2$ significantly reduced the LPS-triggered expression of CD83 and CCR7, whereas 15d-PGJ$_2$ nearly blocked their expression (Fig. 3). In parallel, the down-regulation of micropinocytosis was partially prevented by 15d-PGJ$_2$ but was not markedly affected by PGD$_2$. Moreover, these PGs had no effect on CD1a and CD14 expression (data not shown). Surprisingly, treatment of immature DC with the DP or CRTH2 agonist did not mimic the effect of PGD$_2$. As seen in Fig. 3, BW245C moderately amplified the expression of CD83 and CCR7 in DC, whereas DK-PGD$_2$ had no action on DC maturation (Fig. 3*B*).

We also studied the effects of PGD$_2$ and 15d-PGJ$_2$ on the expression of HLA-DR and costimulatory molecules in mature DC. The expression of CD54 and HLA-DR (data not shown) was not significantly modified by any of the tested molecules, whereas 15d-PGJ$_2$ slightly decreased CD40 expression (Fig. 3*B*) on mature DC. Although the allergen Dpt moderately regulated the expression of DC markers, the effects of the tested molecules were similar to those observed after LPS stimulation (data not shown). By contrast, addition of PGD$_2$ and 15d-PGJ$_2$ significantly up-regulated the LPS-induced expression of CD80, whereas that of CD86 was markedly reduced. As observed before, BW245C had an opposite effect because it increased the CD86 and decreased the CD80 expression, whereas DK-PGD$_2$ had no activity. Interestingly, inhibition of CD80 expression was due to the lack of CD80 synthesis in a subset of MDDC (Fig. 3*A*), as evidenced by a decrease in the percentage of positive cells (data not shown). Similar results were obtained after allergen stimulation. In this case, compared with unstimulated cells, the allergen dramatically increased the percentage of CD86$^+$CD80$^-$ and CD86$^+$CD80$^+$ cells (Fig. 4). In contrast, in the presence of PGD$_2$ or 15d-PGJ$_2$, the percentage of CD86$^+$CD80$^-$ was markedly decreased and that of CD86$^+$CD80$^+$ was enhanced. Yet, BW245C had an opposite effect compared with PGD$_2$ and 15d-PGJ$_2$, whereas DK-PGD$_2$ had no activity. Measurement of mRNA levels by RT-PCR (Fig. 5) indicated that the effects of PGD$_2$ and 15d-PGJ$_2$ on the synthesis of CD80 and CD86 probably occur at the transcriptional level. Collectively, these data suggest that PGD$_2$, probably via its rapid conversion into 15d-PGJ$_2$, exerts marked inhibitory effects on DC maturation and profoundly increases the CD80/CD86 ratio on mature DC (in a DP- and CRTH2-independent manner). On the other hand, DP activation appears to exert an opposite effect on DC maturation and on the CD80/CD86 ratio.

Inhibition of IL-12 secretion by PGD$_2$

We next tested the effect of PGD$_2$ and 15d-PGJ$_2$ on the LPS- and allergen-induced secretion of the bioactive form of IL-12 (IL-12p70), IL-10, IL-6, and TNF-α by MDDC. As shown in Fig. 6, PGD$_2$ and particularly 15d-PGJ$_2$ down-regulated the LPS-induced secretion of IL-12 (47% vs 69% inhibition, respectively). Similar

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

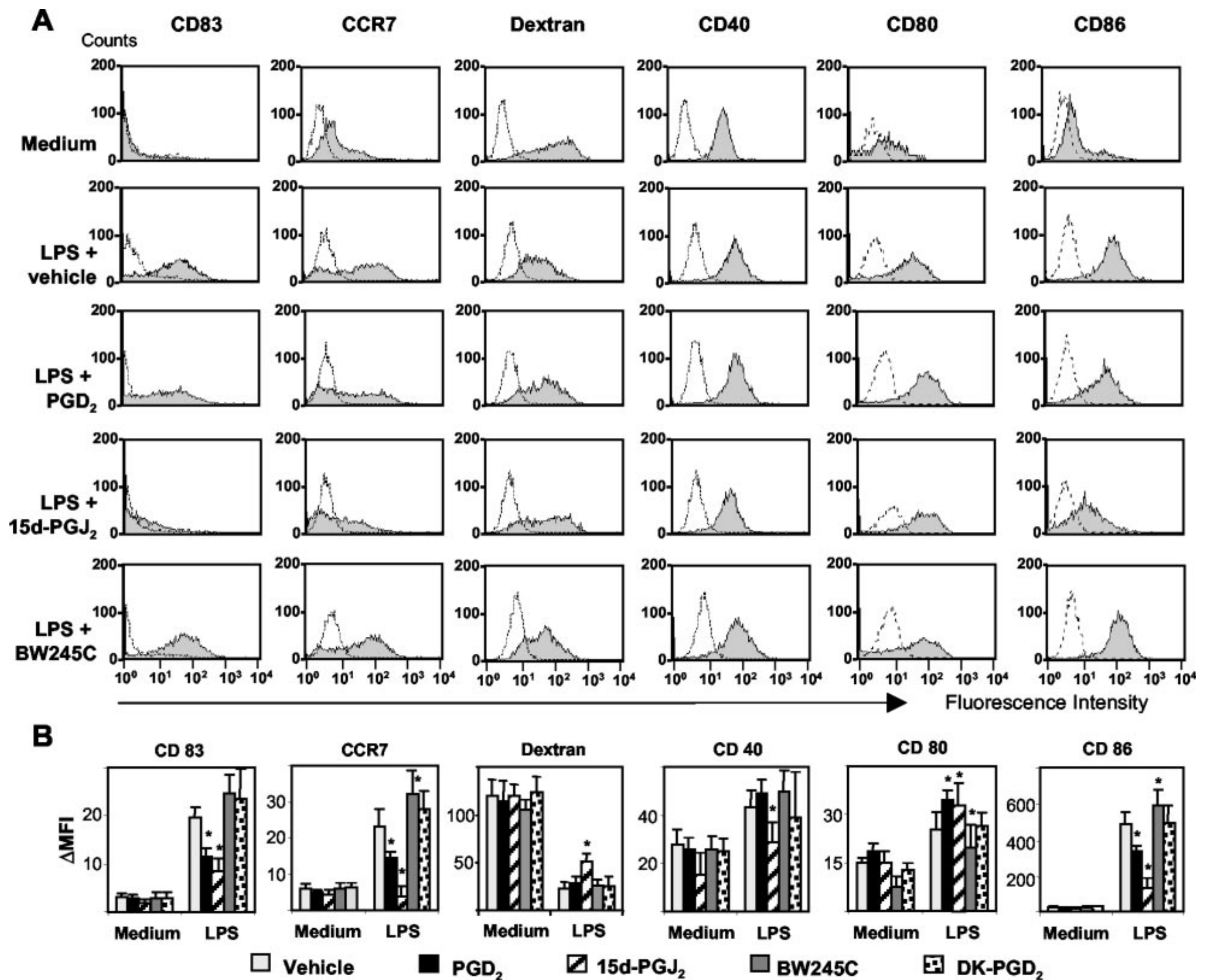

FIGURE 3. PGD_2, 15d-PGJ_2, and BW245C modulate the expression of maturation markers and of costimulatory molecules in LPS-stimulated DC. *A*, Immature MDDC were incubated with medium alone or with LPS in the absence or presence of PGD_2, 15d-PGJ_2, or BW245C for 24 h. The expression of CD83, CCR7, CD40, CD80, and CD86 was evaluated by flow cytometry by incubation with specific mAb (shaded histogram) in comparison with the isotype control (hatched histogram). Micropinocytosis activity was measured by incubation of DC with FITC-conjugated dextran (shaded histogram) in comparison with FITC-conjugated human IgG (hatched histogram). One representative experiment is shown. *B*, Data are expressed as the mean ± SEM of the differences calculated for each experiment between the MFI obtained with the specific Ab and the isotype control Ab (ΔMFI). Histograms summarize the results of 12 independent experiments. *, $p < 0.05$ as compared with controls (MDDC cultured without PGs).

levels of inhibition were obtained after stimulation with allergen (52% and 71% inhibition). Assessment of mRNA levels in MDDC by RT-PCR indicated that the effects exerted by 15d-PGJ_2 and by PGD_2 on the synthesis of IL-12 probably occur at the transcriptional level (Fig. 5). Interestingly, BW245C, but not DK-PGD_2, also significantly down-regulated the secretion of LPS- and allergen-triggered IL-12 secretion (Fig. 6). Analysis of the LPS-induced IL-10 secretion by DC revealed that PGD_2 and BW245C were ineffective, whereas 15d-PGJ_2 attenuated the production of IL-10 by 58%. Finally, PGD_2 and 15d-PGJ_2 did not affect the TNF-α (Fig. 6) and IL-6 (data not shown) production by LPS- and allergen-stimulated DC, whereas BW245C significantly decreased (by 62%) the LPS-induced production of TNF-α. Therefore, PGD_2 appears to selectively inhibit the production of IL-12 at least in part by activating DP, but not CRTH2, as well as through its conversion into 15d-PGJ_2.

Modulation of chemokine production by PGD_2

The influence of PGD_2 and 15d-PGJ_2 on the expression of chemokines by activated DC was next investigated. To this end, CXCL10 and CCL5, which are two ligands for CXCR3 and CCR5 (two receptors preferentially present on Th1 cells), respectively, and CCL17 and CCL22, which are two ligands for CCR4 (a chemokine receptor preferentially expressed on Th2 cells), were quantified (26, 27). As shown in Fig. 7, the LPS- and allergen-induced secretion of CXCL10 were reduced by PGD_2 (~45% inhibition) and by 15d-PGJ_2 (55% inhibition), whereas that of CCL5 was more moderately affected (28% and 50% inhibition, respectively). Similarly, treatment with BW245C also decreased the synthesis of CXCL10 (by 60%) and to a lesser extent, CCL5 (by 25%). In contrast, although the induction was low, none of the tested products modulated significantly the LPS- or allergen-induced production of CCL17. Finally, we found that 15d-PGJ_2 decreased the allergen- and LPS-induced CCL22 secretion (by 30%), whereas BW245C, but not PGD_2, increased its synthesis in both conditions (by 50%). In these assays, DK-PGD_2 had no modulatory activity on the secretion of the chemokines tested. Taken together, PGD_2 and its metabolite 15d-PGJ_2 strongly reduce the production of chemokines involved in Th1 cell chemotaxis. DP activation has a similar effect on the secretion of chemokines active on Th1 cells in

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

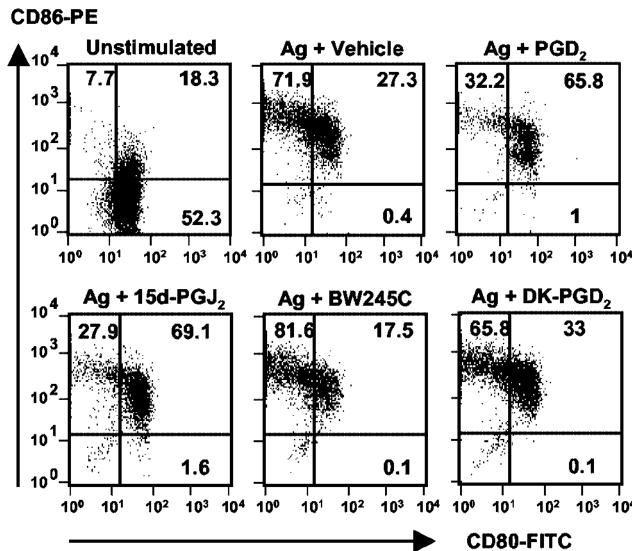

FIGURE 4. PGD$_2$, 15d-PGJ$_2$, and BW245C affect the surface expression of CD80 and CD86 in allergen-stimulated MDDC. Immature MDDC were stimulated or not with allergen for 24 h in the presence of PGs. After incubation, cells were stained with the indicated Abs and were analyzed by flow cytometry. The results are reported as dot blot, and the percentage of CD80$^-$CD86$^+$, CD80$^+$CD86$^-$, and CD80$^+$CD86$^+$ cells are mentioned in the corresponding area. DC stained with the isotype control have <1% of positive cells. One representative experiment of six is shown.

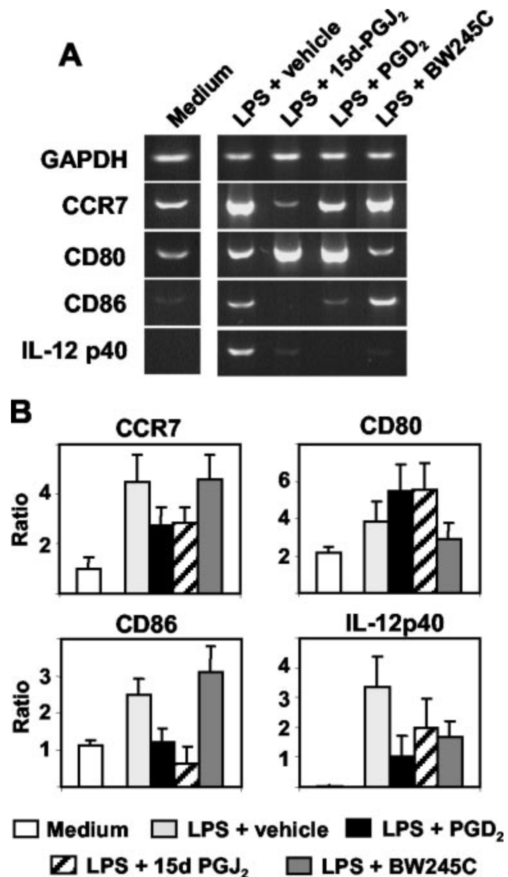

FIGURE 5. PGD$_2$, 15d-PGJ$_2$, and BW245C affect the expression of mRNAs for CCR7, CD80, CD86, and IL-12p40 in DC matured in the presence of LPS. *A*, After 6 h incubation, total RNA was extracted and RT-PCR was conducted using the primers shown in Table I. mRNA expression was evaluated by semiquantitative RT-PCR, and GAPDH was used as a reference. This is one representative experiment of three conducted. *B*, Quantification of each band was performed by image analysis. Results are expressed as the ratio of the intensity obtained for the specified mRNA/GAPDH mRNA, and the mean ± SEM of three experiments is shown.

addition to up-regulating the synthesis of CCL22, a chemokine active on Th2 cells.

Evaluation of the signaling pathway involved in the effect induced by PGD$_2$

DC maturation induced by LPS has been associated with NF-κB and to mitogen-activated protein kinase (MAPK) activation. To determine whether PGD$_2$ interferes with the NF-κB pathway, we monitored its ability to modify NF-κB translocation by EMSA. Surprisingly, PGD$_2$, 15d-PGJ$_2$, and BW245C did not interfere with the LPS-induced NF-κB binding activity (Fig. 8*A*). Moreover, these PGs did not modify the LPS-induced degradation of IκB-α in DC (data not shown). We further studied the effect of PGD$_2$ on the MAPK activation pathway by focusing on p38 and ERK1/2, two key kinases involved in DC maturation and IL-12 regulation (28, 29). After a preliminary kinetic experiment, we found that the LPS-induced phosphorylation of ERK1/2 and p38 was not modified by PGD$_2$ nor by 15d-PGJ$_2$ and BW245C (Fig. 8*B*). To confirm this, we next studied the effects of MAPK inhibitors on the PGD$_2$-, 15d-PGJ$_2$-, and BW245C-induced inhibition of IL-12 and CXCL10. As represented in Table II and consistent with later result, PD98059 and SB203580 had no effect on the PGD$_2$-, 15d-PGJ$_2$-, and BW245C-induced down-regulation of IL-12 and CXCL10 in LPS-treated MDDC. Finally, because some of the effects induced by PGD$_2$ on cytokine/chemokine secretion appear to be mediated by DP, a receptor known to activate PKA, we studied the effect of PGD$_2$ on the phosphorylation state of the CREB, one of the main targets of PKA (30). As shown in Fig. 8*B*, PGD$_2$ and BW245C, but not 15d-PGJ$_2$, increased the extent of phospho-CREB in LPS-activated DCs, whereas the level of unphosphorylated CREB was unchanged. We next investigated whether PKA activation may be responsible for some of the effects exerted by PGD$_2$ in DC. As represented in Table II, Rp-8-Br-cAMP (a highly potent PKA inhibitor) partially reversed (by 50%) the inhibitory effect of PGD$_2$ and BW245C on LPS-induced IL-12 and CXCL10 secretion. Taken together, these findings indicate that inhibition of NK-κB binding activity and deactivation of the MAPK pathway do not appear to be involved in the effects exerted by PGD$_2$ and 15d-PGJ$_2$. However, PGD$_2$ might exert some of its biological effects on DC by recruiting DP and in turn by activating the PKA pathway.

Promotion of type 2 response by DC matured in the presence of PGD$_2$

We then studied whether DC pulsed in vitro with Ag in the presence of PGD$_2$, 15d-PGJ$_2$, or BW245C could modulate the sensitization of autologous naive CD45RA CD4$^+$ T cells. To this end, we compared the ability of DC pretreated or not with PGD$_2$, 15d-PGJ$_2$, or BW245C, plus allergen to induce the proliferation and the production of type 1 (IFN-γ) and type 2 (IL-4) cytokines by in vitro differentiated T cells. As Fig. 9*A* shows, DC sensitized with allergen resulted in T cell priming, as assessed by the increased allergen-dependent proliferation. Although 15d-PGJ$_2$ had a relatively low inhibitory effect on T cell proliferation (37%, $p < 0.05$), treatment of DC with PGD$_2$ or BW245C did not affect it. After restimulation with anti-CD3 and anti-CD28 Abs, allergen-pulsed DC induced the CD4$^+$ T cells to secrete IL-4 and IFN-γ (IFN-γ/IL-4 ratio = 0.032). Interestingly, DC pulsed

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

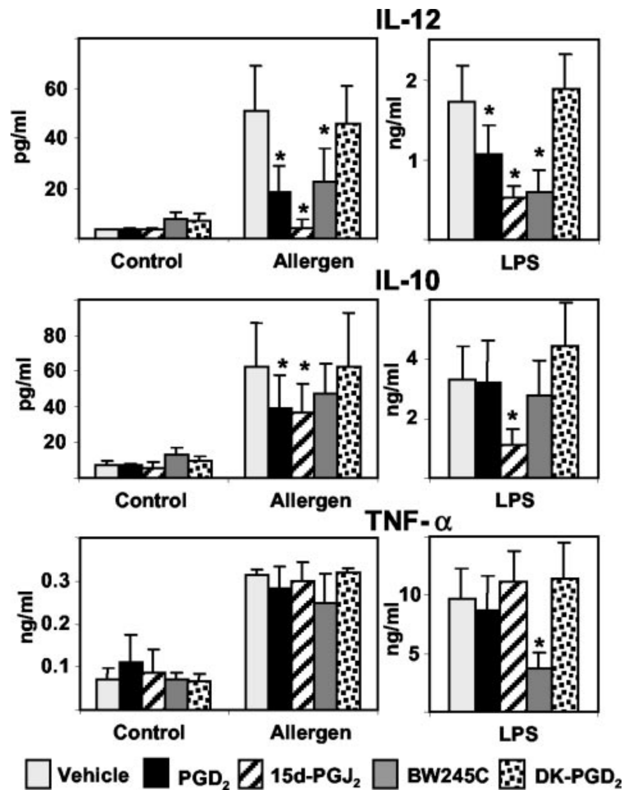

FIGURE 6. PGD_2, 15d-PGJ_2, and BW245C but not DK-PGD_2 affect the secretion of cytokines by allergen- and LPS-stimulated MDDC. Immature MDDC were incubated or not (control) with allergen or LPS in the absence (vehicle) or presence of PGs. After 24 h, IL-12 p70, IL-10, and TNF-α production were quantified by ELISA. Results represent the mean ± SEM of six independent experiments. *, $p < 0.05$ as compared with vehicle.

in the presence of PGD_2 or BW245C significantly increased the production of IL-4, whereas they moderately diminished the level of IFN-γ (ratio = 0.014) (p <0.05). In contrast, 15d-PGJ_2 only down-regulated, in a significant manner, the secretion of IFN-γ (ratio = 0.019, $p < 0.05$).

To confirm these results and because the percentage of IL-4 positive cells defined by flow cytometry was very low in this model (~1–2%), another stimulus was used to induce T cell differentiation. As shown in Fig. 9*B* compared with unpulsed cells, MDDC pulsed with the superantigen SEA induced the differentiation of naive $CD4^+$ T cells to IL-4 and IFN-γ producing cells. Pretreatment of MDDC with PGD_2 or BW245C slightly increased the percentage of IL-4 positive cells, whereas it markedly inhibited the percentage of IFN-γ producing cells. Incubation of MDDC with 15d-PGJ_2 only affected the percentage of IFN-γ positive cells. As found previously, analysis of cytokine secretion by ELISA confirmed that in this model of stimulation, PGD_2 (IFN-γ/IL-4 ratio = 0.21) and BW245C (ratio = 0.23) impacted on the differentiation of naive T cells, compared with vehicle (ratio = 0.45). Altogether, compared with DMSO-treated DC, these data suggest that DC matured in the presence of PGD_2 skew the immune response toward an increased Th2 response, at least in part through DP activation.

Discussion

Among DC-polarizing mediators produced at sites of inflammation or during DC/T cell contact, PG metabolites may be important in the outcome of immune responses. Among them, the most intensively studied PG member is PGE_2. Recent studies show that depending on the mode of stimulation, the effects of PGE_2 on DC functions are different (21, 31, 32). In this report, we investigated whether PGD_2, one of the main PGH_2 metabolite produced in vivo during inflammation and/or infection, could affect the functionality of DC. We show that PGD_2, and its major metabolite 15d-PGJ_2, affect the maturation of DC by modulating the expression of cell surface molecules, by down-regulating the production of cytokines known to be important in the type 1/type 2 cytokine responses, and by affecting the synthesis of chemokines involved in the mobilization of Th lymphocytes.

We first investigate the expression of plasma membrane PGD_2 receptors on monocytes and MDDC. RT-PCR analysis indicates

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

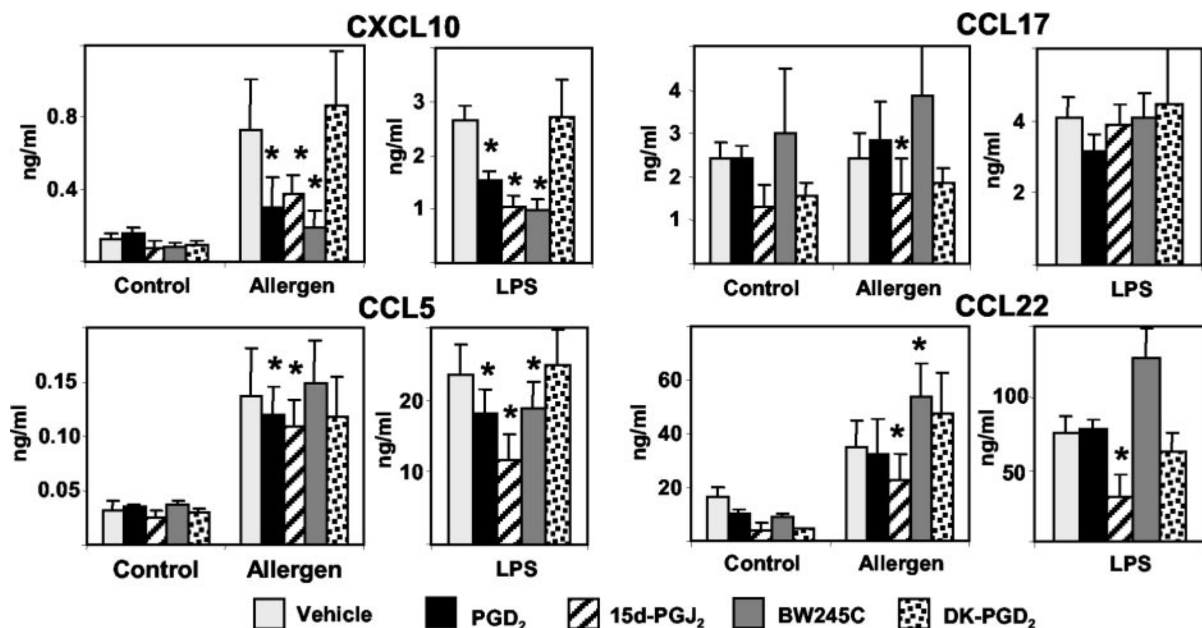

FIGURE 7. PGD_2, 15d-PGJ_2, and BW245C modulate the secretion of chemokines by allergen- and LPS-stimulated MDDC. Results represented the mean ± SEM of six independent experiments. *, $p < 0.05$ as compared with vehicle.

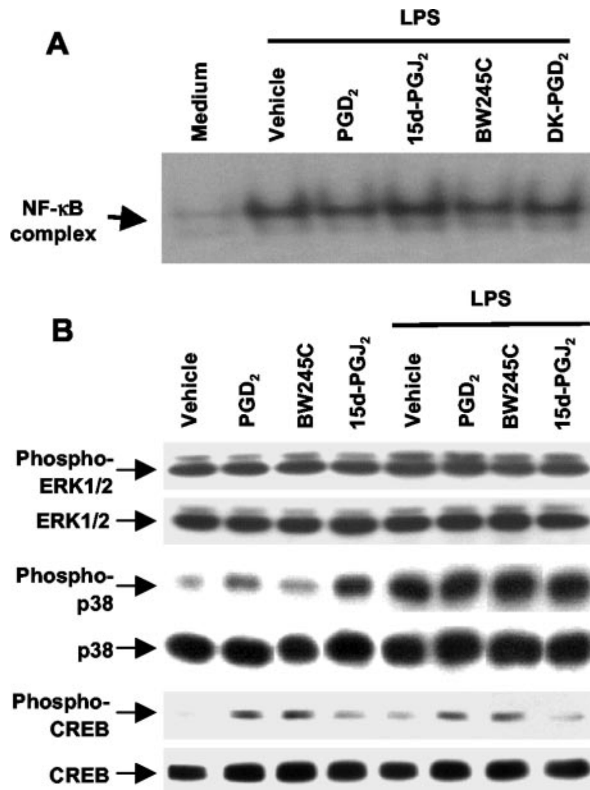

FIGURE 8. PGD$_2$, 15d-PGJ$_2$, and BW245C do not affect the NF-κB and MAPK pathways, whereas PGD$_2$ and BW245C up-regulate CREB phosphorylation in mature DC. *A*, Effect of PGD$_2$, 15d-PGJ$_2$, BW245C, and DK-PGD2 on the LPS-induced NF-κB DNA binding. DC were pretreated with PGs for 10 min and afterward they were cultured with LPS (1 μg/ml) for 2 h. Nuclear proteins from DC were incubated with the ^{32}P-labeled κB probe and electrophoresed on a 5% polyacrylamide gel. Specific binding of NF-κB complexes was controlled by competition with a 50-fold excess of cold κB probe (data not shown). *B*, Effect of PGD$_2$, 15d-PGJ$_2$, and BW245C on the phosphorylation of ERK1/2, p38, or CREB in unstimulated or LPS-stimulated DC. After treatment, whole-cell lysates or nuclear proteins were analyzed by Western blotting with specific anti-phospho-ERK1/2, p38, or CREB Abs. Equal loading of protein was controlled by reprobing the blots with specific ERK1/2, p38 and CREB Abs. Note that PGD$_2$ and 15d-PGJ$_2$ slightly increased the phosphorylation of p38 in unstimulated cells. One representative experiment of three is shown.

that DP is expressed in freshly isolated monocytes and in immature and mature DC. Attempts to prepare anti-human DP Abs have led for the first time to the obtainment of a probe that allowed us to confirm this result by FACS. In contrast, although a faint signal was detected by RT-PCR in DC, CRTH2 expression appears to be restricted to monocytes. We next attempted to demonstrate that these two receptors, when activated by specific agonists, were functional as assessed by chemotactic assays. Importantly, we found that the CRTH2-dependent cell migration is not restricted to Th2 cells, eosinophils, and basophils as initially reported (4, 6, 33), but also to monocytes. This suggests that the local production of PGD$_2$ may contribute to monocyte recruitment, and through this mechanism may influence the inflammatory response. Moreover, because newly recruited monocytes can differentiate into DC in peripheral tissues (34), it would be interesting to study the impact of PGD$_2$ on DC differentiation. Conversely, our results confirm in humans our recent data (15) showing that PGD$_2$ inhibits the migration of DC in a DP-dependent manner (Fig. 2*B*). We also found that PGD$_2$ interferes, in a DP-independent fashion, with the protein (Fig. 3) and mRNA (Fig. 4) expression of CCR7, a receptor known to drive DC migration to the T cell areas of lymph nodes (35) in mature DC. Therefore, PGD$_2$ may alter DC migration to the lymphoid organs through a direct inhibition of cell motility (mediated by DP activation) and also by down-regulating the expression of CCR7 (probably via its metabolite 15d-PGJ$_2$). Although future in vivo studies are clearly warranted, our data suggest that the dual role of PGD$_2$ in monocyte/DC migration may be important in DC homeostasis and in the induction of the primary immune response.

We then studied the effects of PGD$_2$ on the activation of DC triggered by LPS and by Dpt, the major allergen of house dust mite. Although these agents initiate different signaling pathways in DC, we found that PGD$_2$ and 15d-PGJ$_2$, but not BW245C, alters the LPS- and allergen-induced DC maturation. Moreover, cytokine production and phenotype in DC stimulated by CD40 ligation were similarly affected by these agents (data not shown). Interestingly, PGD$_2$ and 15d-PGJ$_2$, but not BW245C, also greatly affected the CD80/CD86 ratio on mature DC, and this may have an important role in the orientation of T cell-mediated immune responses. For instance, up-regulation of CD86 is associated with an increased Th2 response (36–38). In addition, PGD$_2$ selectively inhibited the secretion of IL-12, a key factor in the development of Th1 cells. Considering the activities of PGD$_2$ (and 15d-PGJ$_2$) on the LPS- or allergen-induced DC maturation, we next assessed their impact in the DC-mediated differentiation of naive T cells. Pretreatment of allergen- and superantigen-pulsed DC with PGD$_2$ had no inhibitory effect on T cell proliferation and favored the development of Th2 cells as evidenced by the increased synthesis of IL-4 and the coincident decrease in IFN-γ production by T cells, an effect reproduced by BW245C. Moreover, using another stimulus (SEA),

Table II. *Effect of MAPK and PKA inhibitors on the PGD$_2$-, 15d-PGJ$_2$-, and BW245C-mediated inhibition of IL-12 and CXCL10 secretion in LPS-stimulated MDDC*[a]

Cytokine	Inhibitor	Medium	LPS + Agonist: Vehicle	PGD$_2$	15d-PGJ$_2$	BW245C
IL-12	None	6.4 ± 0.2	667.7 ± 214	136.7 ± 16	75 ± 7.2	17.7 ± 23
	PD98059		773.3 ± 295	189.3 ± 68	92 ± 42	176.7 ± 50
	SB203580		85.3 ± 10	54 ± 26.6	54 ± 22	103.3 ± 74.2
CXCL10	None	175 ± 112	2109 ± 361	847 ± 256	752 ± 274	605 ± 250
	PD98059		2635 ± 317	1283 ± 450	995 ± 292	903 ± 260
	SB203580		1028 ± 210	723 ± 178	451 ± 378	620 ± 207
IL-12	None	3.5 ± 1.5	331.5 ± 103	261 ± 101	227 ± 71	230.5 ± 85
	RP-8-Br-cAMP		353 ± 118	296.5 ± 116	311 ± 101	324.5 ± 91
CXCL10	None	24 ± 15	4098 ± 190	2406 ± 376	3210 ± 61	1215 ± 565
	RP-8-Br-cAMP		3850 ± 110	3085 ± 566	4567 ± 48	2429 ± 838

[a] MDDC were pretreated with MAPK (PD98059 and SB203580) and PKA (Rp-8-Br-cAMP) inhibitors 10 min before being stimulated with LPS in the presence of PGs (1 μM). Data are expressed as the mean ± SEM (picograms per milliliter) of three independent experiments.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

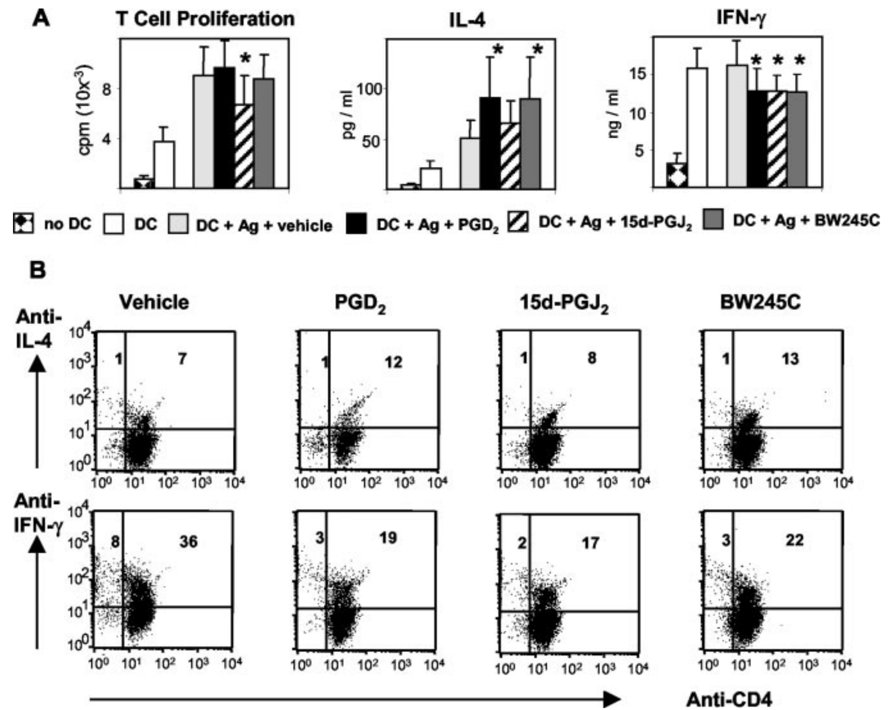

FIGURE 9. PGD_2, 15d-PGJ_2, and BW245C favor Th2 development in allergen-stimulated naive autologous Th cells. *A*, Naive Th cells were cocultured without or with immature DC or allergen-pulsed DC in the absence (vehicle) or presence of PGD_2, 15d-PGJ_2, or BW245C. Cell proliferation was measured by incorporation of [^{3}H]thymidine at day 5. At day 6, Th cells were restimulated with anti-CD3 and anti-CD28 mAbs for 24 h. Supernatants were collected and analyzed by ELISA for the production of IL-4 and IFN-γ. Results are expressed as mean ± SEM of ten independent experiments. *, $p < 0.05$ as compared with controls. *B*, Naive T cells were stimulated two times (at day 0 and day 7) with SEA-pulsed MDDC in the presence or not of the mediators previously cited. At day 14, Th cells were restimulated with anti-CD3 and anti-CD28 mAbs for 6 h and the percentage of IL-4 and IFN-γ-secreting cells was measured by flow cytometry. The percentage of positive cells were reported in the corresponding region. This is one representative experiment of three.

the percentage of IL-4-secreting T cells increased and that of IFN-γ-secreting T cells decreased in the presence of PGD_2- and BW245C-treated DC. This suggests as already reported (20, 21, 39) that the decreased secretion of IL-12 induced by PGD_2 and BW245C, rather than their effects on the CD80/CD86 ratio, is responsible for the Th2-biased response observed in these two models. Of note, along with its relatively moderated effect in the control of Th1/Th2 response, 15d-PGJ_2-treated DC appear to reduce T cell proliferation, a phenomenon associated with their reduced endocytosis ability and to the altered expression of CD40. Collectively, our data suggest that PGD_2 affects, at least in part through DP activation, the functions of DC to polarize the primary immune response toward an increased Th2 response.

Our data also raise the possibility that by targeting DC, PGD_2 may play key roles in the physiopathology of Th2-mediated inflammatory responses. During asthma attack, PGD_2 is released in large amounts (2, 3) and appears to contribute to the infiltration of CRTH2-expressing cells, including Th2 lymphocytes and eosinophils (4, 6, 33). Moreover, using an experimental murine model of allergic asthma, Matsuoka et al. (40) showed that DP is important in the development of the pulmonary Th2 response, through unknown mechanisms. Our data suggest that PGD_2, by affecting DC functions, may be important during allergic reactions. First, PGD_2 may increase, through CRTH2, the recruitment of DC precursors into inflamed tissues. Secondly, PGD_2 could also, through DP, inhibit the emigration of maturing DC and favor their retention in the site of allergen exposure. Thirdly, via its action on the production of DC-derived chemokines, PGD_2 may strongly contribute to the preferential recruitment of Th2 lymphocytes into allergic sites. Whether or not these CRTH2- and/or DP-mediated events contribute to the enhanced local Th2 response in peripheral tissues and/or impact the inflammatory response in allergic sites is an open question that deserves further in vivo investigations.

We next attempted to explore the molecular mechanisms involved in the observed effects. As previously mentioned, DP is expressed in immature and mature DC while CRTH2 appears to be absent. In addition, because we did not observe any effects of DK-PGD_2 on DC functions, it is likely that CRTH2 is not implicated in the effects of PGD_2. This does not rule out the possibility that the lack of action of DK-PGD_2 may also be due to a defect in CRTH2-mediated calcium signaling in MDDC. On the other hand, the involvement of DP is likely to occur in some effects (IL-12 and CXCL10 inhibition and increased Th2 response) induced by PGD_2. We show that the DP-mediated PKA activation in DC is in part responsible for the observed effects of PGD_2 and is associated with the phosphorylation of the transcription factor CREB. Conversely, some of the effects mediated by PGD_2 (i.e., CD80/CD86 ratio) are not mimicked by BW245C, suggesting the implication of (an)other DP-independent mechanism(s). It is known that PGD_2, and particularly 15d-PGJ_2, can also transactivate nonprostanoid receptors and signal cells through activation of MAPK or conversely directly inhibit some kinases implicated in NF-κB activation pathway (14, 41, 42). However, we found that PGD_2 and 15d-PGJ_2 do not activate MAPK (at least ERK and p38) in MDDC nor do they affect the DNA binding activity of NF-κB in these cells. Another more likely possibility is that some of the effects exerted by PGD_2, and particularly by 15d-PGJ_2, are mediated through PPARγ, a nuclear transcription factor described recently to impact inflammatory and immune responses (23, 43, 44). Our report and others have indeed shown that PPARγ is functional in MDDC and that its activation reproduced some (IL-12 and CXCL-10 inhibition) but not all (CD80/CD86 ratio) the effects mediated by PGD_2 and/or 15d-PGJ_2 (23, 44).

Taken together, our data show that PGD_2, one of the major arachidonic acid metabolite produced at sites of inflammation, impacts on the functions of DC through DP-dependent and DP-independent manners to favor the development of Th2 cells. We also suggest that PGD_2, by targeting DC, may be an important component in the development and/or the maintenance of Th2-mediated inflammatory diseases.

Acknowledgments

We are grateful to Drs. H. Hirai and K. Nagata (R & D Center, BML, Saitama, Japan) for kindly providing the BM16 mAb and the DP- and CRTH2-transfected cells. We greatly thank C. Fourneau and J. Fontaine for

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024

their helpful technical assistance and Margaret Bracher for the critical reading of the manuscript.

References

1. Lewis, R. A., and K. F. Austen. 1981. Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. *Nature 293:103.*
2. Murray, J. J., A. B. Tonnel, A. R. Brash, L. J. Roberts II, P. Gosset, R. Workman, A. Capron, and J. A. Oates. 1986. Release of prostaglandin D_2 into human airways during acute antigen challenge. *N. Engl. J. Med. 315:800.*
3. Wenzel, S. E., J. Y. Westcott, and G. L. Larsen. 1991. Bronchoalveolar lavage fluid mediator levels 5 minutes after allergen challenge in atopic subjects with asthma: relationship to the development of late asthmatic responses. *J. Allergy Clin. Immunol. 87:540.*
4. 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.*
5. Hirai, H., K. Tanaka, O. Yoshie, K. Ogawa, K. Kenmotsu, Y. Takamori, M. Ichimasa, K. Sugamura, M. Nakamura, S. Takano, and K. Nagata. 2001. Prostaglandin D_2 selectively induces chemotaxis in T helper type 2 cells, eosinophils, and basophils via seven-transmembrane receptor CRTH2. *J. Exp. Med. 193:255.*
6. Monneret, G., S. Gravel, M. Diamond, J. Rokach, and W. S. Powell. 2001. Prostaglandin D_2 is a potent chemoattractant for human eosinophils that acts via a novel DP receptor. *Blood 98:1942.*
7. 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.*
8. Ajuebor, M. N., A. Singh, and J. L. Wallace. 2000. Cyclooxygenase-2-derived prostaglandin D_2 is an early anti-inflammatory signal in experimental colitis. *Am. J. Physiol. Gastrointest. Liver Physiol. 279:G238.*
9. Ianaro, A., A. Ialenti, P. Maffia, B. Pisano, and M. Di Rosa. 2001. Role of cyclopentenone prostaglandins in rat carrageenin pleurisy. *FEBS Lett. 508:61.*
10. Narumiya, S., Y. Sugimoto, and F. Ushikubi. 1999. Prostanoid receptors: structures, properties, and functions. *Physiol. Rev. 79:1193.*
11. Fitzpatrick, F. A., and M. A. Wynalda. 1983. Albumin-catalyzed metabolism of prostaglandin D_2: identification of products formed in vitro. *J. Biol. Chem. 258: 11713.*
12. Shibata, T., M. Kondo, T. Osawa, N. Shibata, M. Kobayashi, and K. Uchida. 2002. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2: a prostaglandin D_2 metabolite generated during inflammatory processes. *J. Biol. Chem. 10:10.*
13. Forman, B. M., P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans. 1995. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPAR γ. *Cell 83:803.*
14. Straus, D. S., G. Pascual, M. Li, J. S. Welch, M. Ricote, C. H. Hsiang, L. L. Sengchanthalangsy, G. Ghosh, and C. K. Glass. 2000. 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits multiple steps in the NF-κB signaling pathway. *Proc. Natl. Acad. Sci. USA 97:4844.*
15. 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.*
16. Banchereau, J., and R. M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature 392:245.*
17. Steinman, R. M., M. Pack, and K. Inaba. 1997. Dendritic cells in the T-cell areas of lymphoid organs. *Immunol. Rev. 156:25.*
18. Cella, M., F. Sallusto, and A. Lanzavecchia. 1997. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol. 9:10.*
19. Gallucci, S., M. Lolkema, and P. Matzinger. 1999. Natural adjuvants: endogenous activators of dendritic cells. *Nat. Med. 5:1249.*
20. Kalinski, P., C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today 20:561.*
21. Kalinski, P., C. M. Hilkens, A. Snijders, F. G. Snijdewint, and M. L. Kapsenberg. 1997. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E_2, promote type 2 cytokine production in maturing human naive T helper cells. *J. Immunol. 159:28.*
22. Sallusto, F., and A. Lanzavecchia. 1994. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J. Exp. Med. 179:1109.*
23. Gosset, P., A. S. Charbonnier, P. Delerive, J. Fontaine, B. Staels, J. Pestel, A. B. Tonnel, and F. Trottein. 2001. Peroxisome proliferator-activated receptor-γ activators affect the maturation of human monocyte-derived dendritic cells. *Eur. J. Immunol. 31:2857.*
24. Tillie-Leblond, I., H. Hammad, S. Desurmont, J. Pugin, B. Wallaert, A. B. Tonnel, and P. Gosset. 2000. CC chemokines and interleukin-5 in bronchial lavage fluid from patients with status asthmaticus: potential implication in eosinophil recruitment. *Am. J. Respir. Crit. Care Med. 162:586.*
25. Bureau, F., S. Delhalle, G. Bonizzi, L. Fievez, S. Dogne, N. Kirschvink, A. Vanderplasschen, M. P. Merville, V. Bours, and P. Lekeux. 2000. Mechanisms of persistent NF-κB activity in the bronchi of an animal model of asthma. *J. Immunol. 165:5822.*
26. Sallusto, F., D. Lenig, C. R. Mackay, and A. Lanzavecchia. 1998. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes. *J. Exp. Med. 187:875.*
27. Panina-Bordignon, P., D. Mazzeo, P. D. Lucia, D. D'Ambrosio, R. Lang, L. Fabbri, C. Self, and F. Sinigaglia. 1997. β2-agonists prevent Th1 development by selective inhibition of interleukin 12. *J. Clin. Invest. 100:1513.*
28. Aicher, A., G. L. Shu, D. Magaletti, T. Mulvania, A. Pezzutto, A. Craxton, and E. A. Clark. 1999. Differential role for p38 mitogen-activated protein kinase in regulating CD40-induced gene expression in dendritic cells and B cells. *J. Immunol. 163:5786.*
29. Puig-Kroger, A., M. Relloso, O. Fernandez-Capetillo, A. Zubiaga, A. Silva, C. Bernabeu, and A. L. Corbi. 2001. Extracellular signal-regulated protein kinase signaling pathway negatively regulates the phenotypic and functional maturation of monocyte-derived human dendritic cells. *Blood 98:2175.*
30. Parry, G. C., and N. Mackman. 1997. Role of cyclic AMP response element-binding protein in cyclic AMP inhibition of NF-κB-mediated transcription. *J. Immunol. 159:5450.*
31. Rieser, C., G. Bock, H. Klocker, G. Bartsch, and M. Thurnher. 1997. Prostaglandin E_2 and tumor necrosis factor α cooperate to activate human dendritic cells: synergistic activation of interleukin 12 production. *J. Exp. Med. 186:1603.*
32. Steinbrink, K., L. Paragnik, H. Jonuleit, T. Tuting, J. Knop, and A. H. Enk. 2000. Induction of dendritic cell maturation and modulation of dendritic cell-induced immune responses by prostaglandins. *Arch. Dermatol. Res. 292:437.*
33. Gervais, F. G., R. P. Cruz, A. Chateauneuf, S. Gale, N. Sawyer, F. Nantel, K. M. Metters, and G. P. O'Neill. 2001. Selective modulation of chemokinesis, degranulation, and apoptosis in eosinophils through the PGD_2 receptors CRTH2 and DP. *J. Allergy Clin. Immunol. 108:982.*
34. Randolph, G. J., S. Beaulieu, S. Lebecque, R. M. Steinman, and W. A. Muller. 1998. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science 282:480.*
35. 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.*
36. Haczku, A., K. Takeda, I. Redai, E. Hamelmann, G. Cieslewicz, A. Joetham, J. Loader, J. J. Lee, C. Irvin, and E. W. Gelfand. 1999. Anti-CD86 (B7.2) treatment abolishes allergic airway hyperresponsiveness in mice. *Am. J. Respir. Crit. Care Med. 159:1638.*
37. Jirapongsananuruk, O., M. F. Hofer, A. E. Trumble, D. A. Norris, and D. Y. Leung. 1998. Enhanced expression of B7.2 (CD86) in patients with atopic dermatitis: a potential role in the modulation of IgE synthesis. *J. Immunol. 160: 4622.*
38. Larche, M., S. J. Till, B. M. Haselden, J. North, J. Barkans, C. J. Corrigan, A. B. Kay, and D. S. Robinson. 1998. Costimulation through CD86 is involved in airway antigen-presenting cell and T cell responses to allergen in atopic asthmatics. *J. Immunol. 161:6375.*
39. Faries, M. B., I. Bedrosian, S. Xu, G. Koski, J. G. Roros, M. A. Moise, H. Q. Nguyen, F. H. Engels, P. A. Cohen, and B. J. Czerniecki. 2001. Calcium signaling inhibits interleukin-12 production and activates $CD83^+$ dendritic cells that induce Th2 cell development. *Blood 98:2489.*
40. Matsuoka, T., M. Hirata, H. Tanaka, Y. Takahashi, T. Murata, K. Kabashima, Y. Sugimoto, T. Kobayashi, F. Ushikubi, Y. Aze, et al. 2000. Prostaglandin D_2 as a mediator of allergic asthma. *Science 287:2013.*
41. Rossi, A., P. Kapahi, G. Natoli, T. Takahashi, Y. Chen, M. Karin, and M. G. Santoro. 2000. Anti-inflammatory cyclopentenone prostaglandins are direct inhibitors of I-κB kinase. *Nature 403:103.*
42. Tilley, S. L., T. M. Coffman, and B. H. Koller. 2001. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J. Clin. Invest. 108:15.*
43. Faveeuw, C., S. Fougeray, V. Angeli, J. Fontaine, G. Chinetti, P. Gosset, P. Delerive, C. Maliszewski, M. Capron, B. Staels, M. Moser, and F. Trottein. 2000. Peroxisome proliferator-activated receptor γ activators inhibit interleukin-12 production in murine dendritic cells. *FEBS Lett. 486:261.*
44. Nencioni, A., F. Grunebach, A. Zobywlaski, C. Denzlinger, W. Brugger, and P. Brossart. 2002. Dendritic cell immunogenicity is regulated by peroxisome proliferator-activated receptor γ. *J. Immunol. 169:1228.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/170/10/4943/1160192/4943.pdf by guest on 22 May 2024