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Immunomodulatory Mediators from Pollen Enhance the Migratory Capacity of Dendritic Cells and License Them for Th2 Attraction

Valentina Mariani,* Stefanie Gilles,* Thilo Jakob,** Martina Thiel,* Martin J. Mueller,§ Johannes Ring,† Heidrun Behrendt,* and Claudia Traidl-Hoffmann2*

The immune response of atopic individuals against allergens is characterized by increased levels of Th2 cytokines and chemokines. However, the way in which the cytokine/chemokine profile is matched to the type of invading allergen, and why these profiles sometimes derail and lead to disease, is not well understood. We recently demonstrated that pollen modulates dendritic cell (DC) function in a way that results in anenhanced capacity to initiate Th2 responses in vitro. Here, we examined the effects of aqueous birch pollen extracts (Bet.-APE) on chemokine receptor expression and chemokine production by human monocyte-derived DCs. Bet.-APE strongly induced expression and function of CXCR4 and reduced CCR1 and CCR5 expression on immature DCs. In addition, DC treatment with Bet.-APE significantly reduced LPS-induced production of CXCL10/IP-10, CCL5/RANTES; induced CCL2/macrophage-derived chemokine; and did not significantly change release of CCL17/thymus and activation-regulated chemokine. At a functional level, Bet.-APE increased the capacity of LPS-stimulated DCs to attract Th2 cells, whereas the capacity to recruit Th1 cells was reduced. Bet.-APE significantly and dose-dependently enhanced intracellular cAMP, suggesting that water-soluble factors from pollen grains bind a Gαi-protein-coupled receptor. E1-Phytoprostanes were identified to be one player in the Th2-polarizing potential of aqueous pollen extracts. In summary, our results demonstrate that pollen itself releases regulatory mediators which generate a Th2-promoting microenvironment with preferential recruitment of Th2 cells to the site of pollen exposure. The Journal of Immunology, 2007, 178: 7623–7631.

Dendritic cells (DCs)3 are professional APCs capable of Ag transport and presentation in secondary lymphoid organs, which is crucial for the initiation and maintenance of T cell-mediated immune responses (1, 2). They reside in the periphery in an immature state, taking up pathogens or allergens through pinocytosis or receptor-mediated endocytosis, leading to the induction of DC maturation. DCs undergo a maturation process, induced by inflammatory cytokines, bacterial or viral products, which leads to their migration to lymph nodes, where they efficiently attract and activate T cells (3).

The trafficking of immature DCs to sites of inflammation and of mature DCs to the T cell area of secondary lymphoid organs is regulated by the expression of different chemokines and chemokine receptors (4, 5). Immature DCs express inflammatory chemokines (CCL2/MCP-1, CCL3/MIP-1α, CCL4/MIP-1β, CCL5/RANTES, and CCL20/MIP-3α) and chemokine receptors that bind to inflammatory chemokines (CCR1, CCR2, CCR5, CCR6, and CXCR1). On maturation, DCs down-regulate the inflammatory chemokines and their receptors and up-regulate chemokines such as CXCL10/IP-10, CCL17/thymus and activation-regulated chemokine (TARC), CCL18/pulmonary and activation-regulated chemokine, CCL19/MIP-3β, CCL22/macrophage-derived chemokine (MDC), and the chemokine receptor CCR7, ligand of the lymph node-derived chemokines CCL19/MIP-3β and CCL21/secondary lymphoid tissue chemokine (6). PGE2 has been shown to up-regulate CCR7 and CXCR4 on mature monocyte-derived DCs (MoDC) and license their migration to CCL19/MIP-3β and CCL21/secondary lymphoid tissue chemokine and CXCL12/SDF-1α (7).

In allergic individuals, the uptake of allergens by dendritic cells ends in an allergen-specific Th2-biased immune response that ultimately leads to clinical manifestations of IgE-mediated hypersensitivity (8). Allergen-specific Th2 cells are the key orchestrators of allergic reactions, initiating and propagating inflammation through the release of a number of Th2 cytokines such as IL-4 and IL-13. Although the biology of Th2 cells in allergy is well understood, little is known about the mechanisms that control the initial Th2 polarization in response to exogenous allergens.

In the context of allergy, pollen grains have simply been regarded as allergen carriers, and little attention has been devoted to
further compounds of pollen. However, individuals are rarely exposed to purified allergens, but rather particles releasing the allergen such as pollen grains or pollen-derived granules (9–11).

We recently demonstrated that pollen, under physiological exposure conditions, release not only allergens but also bioactive lipids that activate human neutrophils and eosinophils in vitro (12–14). Furthermore, pollen-associated phytoprostane (PPE) reduces in human MoDCs the LPS/CD40L-induced production of IL-12 p70 leading to a Th2 induction in naïve heterogeneous T-lymphocytes (15). Here we describe the ability of *Betula alba* L. aqueous birch pollen extracts (Bet.-APE) to affect chemokine receptor expression and chemokine release of human DCs that can affect the DC capacity to home to lymphoid organs and to attract Th2 cells. In functional migration assays, we found that stimulation of DCs with Bet.-APE enhanced migration of type 2 T cells, suggesting an increased capacity to amplify type 2 immune responses. Furthermore, we provide data pointing to a cAMP-related mechanism leading to the observed effects. Understanding the mechanisms that regulate DC function after contact with allergen carriers will ultimately benefit the development of new therapeutic strategies in preventing and treating allergy.

**Materials and Methods**

**Reagents and Abs**

Human rIL-4 was obtained from Promocell, human rGM-CSF was from Essex, and soluble CD40L was from Alexix. Purified LPS (Escherichia coli K253-derived LPS, <0.008% protein) was kindly provided by Dr. Stephanie Vogel (Department of Microbiology and Immunology, University of Maryland, Baltimore, MD). Anti-CD4 and anti-CD45RA microbeads were from Miltenyi Biotec.

**Preparation of Bet.-APE**

Birch pollen grains (*B. alba* L.) were obtained from Allergon. Bet.-APEs were generated by incubation of pollen grains in RPMI 1640 (30 mg/ml) for 30 min at 37°C followed by centrifugation (20 min at 3345 × g) and sterile filtration (0.2 μm). In a previous study, this procedure was shown to be the most effective to release pollen-associated lipid mediators from pollen (12). LPS was measured by Luminol amebocyte lysate assay (Cambrex Bio Science). To deplete LPS, Bet.-APE were eluted over polymyxin B columns (Pierce), leading to LPS concentrations below the detection limit of the assay (<0.05 endotoxin units (EU)/ml). LPS-depleted Bet.-APE was used for all subsequent experiments.

**MoDCs**

Healthy, nonatopic blood donors were characterized by screening for total and specific IgE for common allergens as recently described (15). All volunteers were without medication for at least 15 days before blood sampling. The ethical committee of the Technical University of Munich approved the study and volunteers were enrolled in the study after written informed consent. MoDCs were prepared from peripheral blood of healthy individuals, as described recently (16). In brief, adherent PBMC (>90% pure CD14+ cells) were cultured at 1 × 10^6 cells/ml in RPMI 1640 supplemented with 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 0.05 mM 2-ME, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Invitrogen Life Technologies) supplemented with 10% FBS, 500 U/ml human rGM-CSF (Tebu Bio), and 500 U/ml human rIL-4 (Promocell; complete DC medium) at 37°C under 5% CO2. After 6M, cells were harvested and recultured in complete DC medium for 24 h at 37°C with or without indicated stimuli in the presence or absence of LPS (100 ng/ml) or soluble CD40L (1 μg/ml; Alexix) followed by addition of a cross-linker (1 μg/ml; Alexix). PGE2 was used as control (1 × 10^-6 M).

**Generation of polarized Th1 and Th2 cell clones**

Human CD4^+ CD45RA^- T cells were purified from nonadherent PBMC from healthy nonatopic donors using MACS column separators with anti-CD4 and anti-CD45RA microbeads (Miltenyi Biotec). Differentiated MoDC (24 h) were washed and cocultured with MACS-purified allogeneic naïve CD4^+ CD45RA^- T cells (1 × 10^5 cells/well) in complete RPMI with autologous 5% human serum. LPS-activated DC (100 ng/ml, 24 h) were used to generate Th1 polarized T cell lines. Th2-polarized T cells were generated by using DCs that were activated (24 h) with LPS in the presence of PGE2 (10^-6 M; Alexix). In addition, neutralizing anti-IL-12 mAb (10 μg/ml; BD Biosciences) was added at the beginning of the T cell/DC coculture to generate a maximal Th2 polarization. The T cell lines were cloned after 10 days by limited dilution. As analyzed by flow cytometry, the Th1 clones used in the migration assay were strongly positive for CXCR3 and CCR5 and negative of CCR4 and CRTh2, whereas Th2 clones were negative for CCR5 and CXCR3 and positive for CCR4 and CRTh2 (Fig. 5; all Abs from BD Biosciences).

**Flow cytometry of DCs and T cells**

Surface expression of DC maturation marker CD83 and chemokine receptors of DCs were analyzed using multicolor flow cytometry. In brief, DCs, either untreated or stimulated for 24 h with LPS in the presence or absence of pollen extracts, were harvested, washed, and suspended in cold PBS containing 5% FCS and 0.02% NaN₃, and then incubated with saturating concentrations of FITC-conjugated mAb (CD83), and PE-conjugated mAb (CCR1, CCR5, and CXCR4, all from BD Biosciences). Matched isotype control mAb were used in control samples. Stained cells were analyzed using a FACSCalibur flow cytometer equipped with CellQuest software (BD Biosciences). Propidium iodide-permeable (nonviable) cells were excluded from analysis.

**Quantitative mRNA analysis**

Total RNA was extracted from purified DC after 6 h of incubation with the indicated stimuli using peqGOLD RNAPure buffer (Peqlab). RNA was
reverse transcribed using random hexamer primers (Roche). PCRs for the chemokine receptors CCR1, CCR5, CXCR4 and the chemokines CXCL10, CCL5, CCL22, and CCL17 (Assay on demand; Applied Biosystems) were run on an ABI PRISM 7700 Sequence Detection System device (Applied Biosystems Division of PerkinElmer) using the following program: 10 min at 94°C followed by 40 cycles of 15 s at 95°C, and 60 s at 55°C; 18s RNA served as housekeeping gene.

Migration assay
Chemotaxis of DCs and T cells was evaluated by measuring their migration through 5-µm-pore polycarbonate filters in 24-well Transwell chambers (Corning Costar), as described previously (13). Briefly, the chemotactic property of DC supernatants was evaluated by adding 10⁵ T cells suspended in complete RPMI with 0.5% BSA to the top chamber and various dilutions of the supernatants (0.6 ml) to the bottom chamber of a Transwell insert (Costar). To analyze the role of cAMP-induction in the chemokine receptor regulation by Bet-APE, day 5 MoDC were preincubated for 1 h in complete medium (+GM-CSF/IL-4) in the absence or presence of 6 mM adenylyl cyclase inhibitor SQ-22536 (Calbiochem). The cells were then stimulated with medium, 100 ng/ml E. coli LPS, Bet-APE (10 mg/ml, 25% v/v), or 10 µM forskolin (Sigma-Aldrich). The stimuli Bet-APE and forskolin were applied either alone or in combination with LPS. After 24 h, cells were harvested, washed twice, and resuspended in complete medium at a density of 1 x 10⁶ cells/ml for the

**FIGURE 2.** Bet-APE augment the response of LPS-stimulated DCs to the CXCR4 ligand CXCL12 by a cAMP-dependent mechanism. MoDCs were incubated for 24 h with medium, Bet-APE (3 mg/ml), PGE₂ (1 x 10⁻⁹ M), LPS, LPS plus PGE₂, LPS plus Bet-APE (B), or with LPS, LPS plus Bet-APE and LPS plus forskolin (FSK) in the absence or presence of adenylyl cyclase inhibitor SQ-22536 (C). Stimulated DCs were processed for migration assays in 24-well Transwell chambers. As migratory stimuli the following chemokines were chosen: 1 µg/ml CCL16 (ligand for CCR1); 100 ng/ml CCL4 (ligand for CCR5); 30 ng/ml CCL21; 100 ng/ml CCL19 (both ligands for CCR7); and 10 ng/ml CXCL12 (ligand for CXCR4). Data are expressed as total migrated cells ± SD. Values are for one representative experiment of five performed in triplicate. *, Significant changes of DC migration (p ≤ 0.05).
migration assay, 100 μl of the cell suspension were pipetted into the upper and the diluted chemokines (CXCL12, CCL19, medium as control) into the lower chamber of the Transwell. After 1 h of incubation at 37°C with 5% carbon dioxide, cells that transmigrated into the lower chamber were recovered and acquired with a FACSCalibur device for 60 s at a flow rate of 60 μl/min. Data acquisition and analysis were restricted to events with the forward and side scatter properties of cells and not cell debris.

Chemokine production of DC
MoDCs were harvested, seeded into 96-well plates (Nunc) at a density of 100,000 cells/well, and treated with medium or 100 ng/ml LPS, alone or in combination with different concentrations of Bet.-APE. After 24 h, supernatants were analyzed for the presence of CXCL10/IP-10, CCL5/ RANTES, CCL22/MDC and CCL17/TARC by ELISA. ELISA kits used were from BD Biosciences (CXCL10) and R&D Pharmaceuticals (CCL5, CCL22, CCL17).

cAMP measurements
DC, generated as described above, were stimulated at 10^5 cells/100 μl/well in 96-well flat-bottom culture plates (Nunc) with various concentrations of PGE2 (Cayman) or Bet.-APE. After 40 min, cells were lysed by addition of equal volumes of sample/lysis buffer (cAMP-Screen System; Applied Biosystems), resuspension, and incubation for 30 min at 37°C. The lysates were then analyzed for cAMP by ELISA (cAMP-Screen System) following the supplier’s instructions.

FIGURE 3. Bet.-APE block the LPS-induced CCL5 and CXCL10 mRNA expression, whereas LPS-induced CCL22 and CCL17 expressions are enhanced. Human MoDCs were stimulated for 6 h with LPS (100 ng/ml) and/or Bet.-APE (3 mg/ml), processed for RNA extraction, and analyzed by real-time PCR for the expression of CXCL10/IP-10, CCL5 (RANTES), CCL22 (MDC), and CCL17 (TARC). Data are expressed as relative expression (2^-ΔΔCT) and one representative experiment of five performed is shown.

FIGURE 4. Bet.-APE inhibit the LPS-induced CXCL10 and CCL5 release and augment LPS-induced CCL22 secretion in a dose-dependent manner, whereas CCL17 production is not affected. Human MoDCs were stimulated with LPS (100 ng/ml) and graded concentrations of Bet.-APE (0.1 to 3 mg/ml). After 24 h, cell-free supernatants were harvested and analyzed by ELISA for CXCL10/IP-10, CCL5 (RANTES), CCL22 (MDC) and CCL17 (TARC). Differences in LPS-stimulated DCs in the presence of Bet.-APE were significant (p < 0.05) for CXCL10 at a concentration of 0.1 mg/ml, for CCL5 at 0.3 mg/ml, and for CCL22 at 1 mg/ml, whereas LPS-induced CCL17 release did not change significantly in the presence of Bet.-APE. Results are given as the percentage (mean ± SD) of LPS-induced chemokine production (CXCL10 x = 17,766.7 ± 2,786.3 pg/ml; CCL5 x = 12,766.7 ± 2,254.6; CCL22 x = 4,666.7 ± 723.6; CCL17 x = 15,136 ± 4,265.3).
+ Significant changes (p ≤ 0.05). Values are for one representative experiment of three performed in triplicate.
Preparation of E1-phytoprostanes

Racemic E1-phytoprostanes were prepared by autooxidation of α-linolenic acid and purified as described previously (15).

Statistics

Student’s paired t test was used to compare differences in chemokine release, cAMP levels and cell migration. p values of 0.05 or less were considered to indicate significance.

Results

Bet.-APE induces CXCR4 and down-regulates CCR1 and CCR5 expression

Maturation of DCs results in substantial changes in the expression of chemokine receptors and the ability to migrate toward chemokine gradients. Therefore, expression of selected chemokine receptors and its modulation by Bet.-APE on human MoDCs was analyzed at mRNA (real time PCR) and protein level (flow cytometry). LPS-induced DC maturation, as determined by CD83 expression, was accompanied by a down-regulation of CCR1 and CCR5 and an up-regulation of CXCR4 (Fig. 1A). Even though stimulation with Bet.-APE did not induce a significant DC maturation (CD83), it still led to a down-regulation of CCR1 and CCR5 and a pronounced up-regulation of CXCR4 surface expression on immature DCs. Chemokine receptor mRNA expression was analyzed by real-time PCR (Fig. 1B). LPS treatment resulted in a moderate down-regulation of CCR1 and CCR5 mRNA expression whereas CXCR4 mRNA was 3-fold up-regulated. Consistent with flow cytometric analysis of receptor surface expression, stimulation with Bet.-APE alone led to reduced levels of CCR1 and CCR5 mRNA and a substantial induction of CXCR4 mRNA. In addition, Bet.-APE had an additive effect on the CXCR4 mRNA up-regulation induced by LPS.

Bet.-APEs enhance migration of DCs toward the CXCR4 ligand, CXCL12

The functional activity of chemokine receptors was examined by measuring the migration to ligands of CCR1, CCR5, CCR7, and CXCR4. DCs were stimulated with LPS and/or Bet.-APE (3 mg/ml). Because the presence of PGE₂ has been described to be important for the response to the CCR7 and CXCR4 ligands (17, 18), DCs were stimulated with PGE₂ (1 × 10⁻⁵) plus LPS or PGE₂ alone as positive control. Immature DCs showed a baseline, non-directional migration to all stimuli used that was comparable with that of medium control. After LPS stimulation, baseline migration to medium and migration toward the CCR7 ligands (CCL16 and CCL4, respectively) was absent, whereas high migratory activity was observed to the CCR7 ligands CCL21 and CCL19. The CXCR4 ligand CXCL12 induced only a weak migratory response in LPS-stimulated DCs. When PGE₂ was added to the LPS-stimulated culture, the migration toward CCL19 and CCL21 was enhanced 2.2 and 1.2-fold, respectively. Notably, PGE₂ enhanced the migration of LPS-stimulated DCs toward CXCL12 55-fold. Similarly, when DCs were costimulated with LPS and Bet.-APE, the migration toward CXCR4 ligand CXCL12 was enhanced 37-fold. In contrast, the migration to the CCR7 ligands CCL21 and CCL19, although enhanced, did not change significantly when Bet.-APE was added. The stimulation with PGE₂ or Bet.-APE in the absence of LPS led only to a marginal variation...
in the random migration but not in the directed migration toward
on of the tested ligands.

The enhanced migration of Bet.-APE-stimulated DCs toward
CXCL12 is dependent on the induction of adenylyl cyclase

We observed an enhanced chemotaxis of DC toward the Th2 chemo-
kine CXCL12 after simultaneous stimulation with LPS and either
PGE_2 or Bet.-APE (Fig. 2B). Because PGE_2 is a known inducer of
adenylyl cyclase, we investigated whether the enhanced tendency of
DC to migrate toward CXCL12 when stimulated with LPS and Bet.-
APE might be due to elevated levels of the second messenger cyclic
AMP (cAMP). DC migration to chemokines was assessed in the ab-

dence or presence of an inhibitor of adenylyl cyclase, SQ22536. As a
positive control for cAMP induction, forskolin, an adenylyl cyclase
inhibitor, was included as stimulus. LPS treatment alone led to migra-
tion toward CCL19, whereas no chemotaxis occurred toward
CXCL12 (Fig. 2B and C). Interestingly, the cAMP agonist forskolin

significantly induced the migration of LPS-stimulated DC toward
CXCL12 and enhanced the migrations toward the CCR7 ligand
CCL19, as did Bet.-APE (Fig. 2B and C). This effect of forskolin or
Bet.-APE on the migratory behavior of LPS-stimulated DCs was sig-
ificantly reversed in the presence of SQ-22536. The CCL19-respon-
siveness was even more sensitive to the cAMP antagonist than
CXCL12. Comparable results were obtained with the combined stim-
ulation of DCs with LPS and PGE_2 (data not shown). Yet unexplained
is the induction of the CXCL19 responsiveness of LPS-stimulated
DCs in the presence of the cAMP-antagonist SQ22536.

Bet.-APEs up-regulate DC production of Th2-chemokines,
whereas Th1 chemokines are blocked

In the next series of experiments, we investigated the effect of
Bet.-APE on chemokine production by DCs. Consistent with pre-
vious observations (19, 20), immature DCs constitutively pro-
duced CCL22 and CCL17 (532.6 ± 96.3 pg/ml and 682.6 ± 78.4

FIGURE 7. PPE_1 blocks LPS-in-
duced CCL5, CXCL10, and CCL22 expression and leads to reduced Th1
and enhanced Th2 attraction via DCs.

A, Human MoDCs were stimulated
for 6 h with E_1-phyoprostanes (1 × 10^{-3} M) in the presence of LPS (100
ng/ml). Analysis of chemokine ex-
pression was performed by real-time
PCR. Data are expressed as relative
expression (2^{-ΔΔCT}) of three inde-
pendent experiments performed in
triplicate. B, DCs were stimulated for
24 h with LPS alone or together with
PPE_1, 1 × 10^{-3} M. Cell-free super-
natants were used for cell migration
studies of polarized Th1 and Th2 T

cell clones. In each experiment, posi-
tive controls for a Th1 (CCL3, 100
ng/ml) and a Th2 (CCL22, 100 ng/
ml) chemokine were added. Data are
given as migration index ± SD. Mi-
gration index: ratio of cells migrated
to chemoattractant or DC-supernatant
and medium. Values are for one rep-
resentative experiment of three per-
formed in triplicate.
BET-APES enhance the capacity of DCs to attract type 2 T lymphocytes

Depending on their polarization, T lymphocytes differentially express receptors for CXCL10, CCL5, CCL22, and CCL17, with type 1 cells preferentially expressing CXCR3 and CCR5 and type 2 cells expressing CCR3, CCR4, and CRTh2 (6, 21–23). We thus investigated whether BET-APES could affect the capacity of DCs to attract type 1 and type 2 T lymphocytes. Polarized T cell clones were generated and characterized for IL-4 and IFN-γ production and for expression of CXCR3, CCR5, CCR3, and CRTh2. As expected, type 1 lymphocytes showed high levels of IFN-γ and low levels of IL-4, whereas type 2 cells had high IL-4 and low IFN-γ production (data not shown). CCR5 and CCRX3 were expressed by Th1 clones, whereas CCR4 and CRTh2 were present on type 2 T cell clones (Fig. 5A). Furthermore, Th2 clones showed a significant migration (migration index, >2) to CCL22, whereas migration to CCL3 was negative. Equally, Th1 clones migrated significantly to CCL3 but not to CCL22 (Fig. 5B). As expected, both Th1 and Th2 T cell clones showed a higher migratory response to supernatants from LPS-stimulated DCs than did those from non-stimulated immature DCs. Here, Th1 cells migrated more efficiently than Th2 cells to supernatants from LPS-stimulated DCs (Fig. 5B). In contrast, supernatants from BET-APES-treated DCs induced a preferential induction of Th2 cells. This was consistent with the finding that BET-APES alone was sufficient to induce DC production of CCL22. In line with the inhibitory effect of BET-APES on the LPS-induced production of CXCL10 and CCL5, BET-APES stimulation reduced the capacity of LPS-stimulated DCs to attract Th1 cells. The immunomodulatory capacity of BET-APES on DC chemokine production and migratory response of Th1 and Th2 cells was comparable with that of PGE2, well known to induce in DCs the release of Th2 chemokines (24).

BET-APES enhance cAMP level in human MoDCs

PGE2 exerts its immunomodulatory effects on DC chemokine expression via its receptors, EP2 and EP4 (25), both of which signal primarily via the cAMP/protein kinase A pathway. To investigate whether an induction of the intracellular cAMP level might also be involved in the observed modulation of chemokine expression by aqueous pollen extracts, CAMP was measured in lysates of DCs stimulated with various concentrations of PGE2 or BET-APES. Although PGE2 treatment led to only a 2-fold induction of intracellular cAMP, BET-APES dose-dependently increased cAMP levels up to 7-fold (Fig. 6).

E1-Phytoprostanes block LPS-induced Th1 chemokines in DCs and consequently lead to reduced Th1 attraction and enhanced Th2 attraction compared with LPS-stimulated DCs

We recently demonstrated the presence of phytoprostanes with a predominance of PPE1 in aqueous birch pollen extracts (BET-APES). E1-phytoprostanes, similar to BET-APES, dose-dependently inhibited IL-12 production and induced an increased Th2-polarizing capacity of human MoDCs (15). To evaluate whether PPE1 was also responsible for the reduction of Th1 and induction of Th2 chemokines, DCs were stimulated with PPE1 (3 × 10⁻⁸M–1 × 10⁻⁵ M) together with LPS. Here, PPE1 lead to a significant reduction of LPS-induced CXCL10, CCL5, and CCL22 at the concentration of 1 × 10⁻⁵ M (Fig. 7A). LPS-induced CCL17 did not change significantly in the presence of PPE1, being neither enhanced nor blocked. On a functional level, DCs stimulated with PPE1 alone (1 × 10⁻⁵ M) showed a similar attraction pattern of Th1 and Th2 as nonstimulated DCs. However, LPS plus PPE1 (1 × 10⁻⁵ M) stimulated DCs emerged as low Th1 and strong Th2 attracting cells compared with LPS-stimulated DCs (Fig. 7B). This points to the fact that PPE1 is one player in the Th2-polarizing effect of BET-APES albeit not being the only one. The CCL22 inducing factor remains elusive to date.

Discussion

In the current study, water-soluble factors from pollen were found to be a critical switch factor for the acquisition of migratory capacity and T cell attraction profile of DCs. This underlines a novel role of pollen in the decision-making process of allergic responses that goes beyond the allergen carrier model. BET-APES led, together with LPS, to an up-regulation of the lymphoid chemokine receptor CXCR4 and the coordinated down-regulation of CCR1 and CCR5. Functionally, DCs exposed to BET-APES plus LPS migrated vigorously to CCL19 and CCL21, whereas chemotaxis to CCL16 and CCL4 was reduced compared with immature DCs. BET-APES alone induced CXCR4 expression at mRNA and protein level which, however, appeared to be nonfunctional because no significant migration toward CXCL12 was observed. However, BET-APES stimulation in the presence of a maturation signal such as LPS induced in DCs a strong migratory response to the CXCR4 ligand CXCL12. Because CXCL12 is constitutively expressed in lymphoid tissue (26), this could be critical for maintenance of DC levels in such tissues during allergic inflammation. In line with previous studies (6, 27), DC stimulation with LPS alone also induced a moderate up-regulation of CXCR4, which did, however, not lead to a significant migration to CXCL12. PGE2 has recently been described to be required for human DC migration turning CXCR4 into a functionally expressed receptor (7). Also in our hands, LPS-stimulated DCs migrated only after costimulation with PGE2 toward the CXCR4 ligand CXCL12. Also, pollen extracts contain a factor turning the CXCR4 expression of DCs in to a functional receptor, which in turn can favor DC migration to lymphoid organs (28–30). It has previously been reported that cholera toxin induces DC maturation that is associated with membrane CXCR4 expression (27). Cholera toxin may lead to CXCR4 expression on DC membranes by elevating the intracellular levels of cAMP. Consistent with this hypothesis, Cole et al. (31) reported that cAMP up-regulates membrane CXCR4 expression on lymphocytes by decreasing receptor internalization without affecting the level of gene expression. The observed chemotaxis toward CXCL12 of DCs stimulated with LPS and PGE2, forskolin or BET-APES was reduced significantly in the presence of an adenylyl cyclase inhibitor. Consistent with this finding, BET-APES led to a rapid and significant up-regulation of cAMP in DCs. This suggests
that water-soluble factors from pollen bind to a cAMP-coupled receptor that may account for the observed chemokine receptor regulation. Whether the recently described prostaglandin-like, pollen-associated phytoprostanes (15, 32, 33) are responsible for this effects is currently under investigation.

Chemokines regulate leukocyte trafficking by inducing firm integrin-dependent adhesion of blood leukocytes to endothelial cells and by inducing directional migration. Because pollen modifies the capacity of mature DCs to produce IL-12 and consequently the outcome of the ensuing Th cell polarization (15), we wanted to assess whether they also might induce effects on the ability of DCs to attract different polarized Th cells. Indeed, Bet.-APE affected the pattern of chemokine release from DCs by up-regulating the constitutive production of CCL22 and CCL17 whereas Th1 chemokines such as CXCL10 and CCL5 were not induced by Bet.-APE alone. Furthermore, Bet.-APE substantially inhibited the LPS-induced secretion of CXCL10 and CCL5, whereas CCL22 release was up to 6-fold increased. The pattern of chemokine release from DCs matured in the presence of Bet.-APE suggested an altered capacity of DCs to attract T cell subsets, because Bet.-APE up-regulated the Th2-related chemokine CCL22 and inhibited the release of the LPS-induced Th1 chemokines CXCL10 and CCL5. To assess whether Bet.-APE might enhance Th2 recruitment, we tested the capacity of supernatants from DC cultures to induce migration of type 1 or type 2 polarized T cells.

We found that DCs matured in the presence of Bet.-APE attracted type 1 polarized Th1 clones less efficiently then Th2 clones. Furthermore, Bet.-APE blocked the LPS-induced chemotrafficking of Th1 cells, and increased the LPS-induced attraction of Th2 cells. These results confirm the hypothesis that DCs exposed to Bet.-APE have a diminished capacity to amplify type 1 immune responses (15) and at the same time favor the attraction of type 2 Th cells to the site of allergic inflammation.

Several data indicate that PGE2 up-regulates CCL22 production by LPS or CD40L-stimulated DCs (24, 34). Furthermore, reports indicate that PGE2 also suppresses chemokine mRNA expression and chemokine production in various types of cells. For example, PGE2 or intracellular accumulation of cAMP suppresses CCL5 production by murine mesangial cells (35) and CXCL10 mRNA expression by cultured keratinocytes (36). In our hands, the stimulation of DCs with LPS and PGE2 lead to a preferential attraction of Th2 cells, confirming previous data showing that PGE2 enhances LPS-induced Th2 chemokines (31). As already mentioned, PGE2 exerts its immunomodulatory effects on DC chemokine expression via its receptors, EP2 and EP4 (25), both of which are coupled to the induction of adenyl cyclase and a consecutive rise in intracellular cAMP. Interestingly, the increase in intracellular cAMP levels by Bet.-APE in DCs was even more pronounced than that induced by PGE2. The observed up-regulation of CCL22 by stimulation with LPS and Bet.-APE together with the regulation of CXCR4 indicates that Bet.-APE contains bioactive compounds with immunomodulatory characteristics similar to that of PGE2, most probably acting via a cAMP-dependent mechanism.

To find the responsible substance for the observed effects, E1-phytoprostanes were tested in our DC system. Here we demonstrate that PGE2, accounts in part for the observed effects such as the reduction of CXCL10 and CCL5. However, it is not responsible for the CCL22 induction, pointing to the fact that the in vitro effects of Bet.-APE very likely reflect summative effects of various substances, which may act synergistically in the Th2-polarizing capacity of Bet.-APE. Aqueous pollen extracts contain a large number of different substances with potential immunomodulatory capacities. Besides pollen-associated lipid mediators such as phytoprostanes and oxilipins, carbohydrates or proteins might also exert Th-polarizing effects.

In conclusion, we have demonstrated that water-soluble mediators from pollen change the chemokine receptor profile of DCs, which may result in an enhanced capacity to localize to lymph nodes. In addition, we have described a mechanism by which pollen can promote immune deviation toward a type 2 response, i.e., by preventing DC recruitment of type 1 and enhancing the attraction of type 2 Th lymphocytes. These findings support the hypothesis that pollen grains themselves appear to harbor important tools to pave the way toward a Th2-dominated immune response against pollen-associated allergens.

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Disclosures

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