Immunomodulatory effects of aqueous birch pollen extracts and phytoprostanes on primary immune responses *in vivo*

Jan Gutermuth, MD,[a] Mayte Bewersdorff, MSc,[a] Claudia Traidl-Hoffmann, MD,[a] Johannes Ring, MD, PhD,[b] Martin J. Mueller, PhD,[c] Heidrun Behrendt, MD,[a] and Thilo Jakob, MD[a,d] *Munich, Würzburg, and Freiburg, Germany*

Background: We recently demonstrated that pollen not only function as allergen carriers but also as rich sources of bioactive lipids, such as phytoprostanes, that modulate human dendritic cell (DC) function in a way that results in an enhanced T_H2 polarization *in vitro*.
Objective: Here we analyzed the immunomodulatory capacities of *Betula alba* (white birch) aqueous pollen extracts (Bet-APEs) and pollen-associated phytoprostanes in the murine system *in vitro* and *in vivo*.
Methods: DC function was analyzed *in vitro* by using BALB/c bone marrow–derived DCs. T-cell responses were analyzed with DO11.10 peptide 323-339 from chicken ovalbumin (OVA)–specific CD4 T cells as responder cells. For *in vivo* studies, OVA-specific CD4 T cells were adoptively transferred into BALB/c mice. Twenty-four hours later, mice were challenged by means of intranasal application of OVA in the absence or presence of Bet-APEs or phytoprostanes. Polarization of T-cell responses *in vivo* was analyzed in draining lymph node cells.
Results: *In vitro* Bet-APEs and E_1–phytoprostanes dose-dependently inhibited LPS-induced IL-12p70 of DCs. In addition, Bet-APEs induced a T_H2 polarization *in vitro*. Similarly, intranasal instillation of Bet-APEs *in vivo*, together with the antigen, lead to increased IL-4, IL-5, and IL-13 secretion and decreased IFN-γ secretion from antigen-specific T cells in the draining lymph nodes. In contrast, intranasal E1- and F1–phytoprostanes downregulated both T_H1 and T_H2 cytokine production *in vivo*.
Conclusion: Pollen release water-soluble factors that display T_H2-polarizing capacities *in vivo* independently of E_1- and F_1–phytoprostanes.
Clinical implications: Identification of the underlying mechanisms might open new approaches for pharmacologic intervention.

Key words: *T_H1/T_H2 cells, T-cell polarization, pollen allergy, phytoprostanes, pollen-associated lipid mediators*

Atopic diseases are characterized by a predominance of T_H2-biased immune responses to environmental allergens.[1] Allergen-specific T_H2 cells are key players in allergic immune reactions, initiating and propagating inflammation through release of a number of T_H2 cytokines, such as IL-4, that regulate isotype switching to allergen-specific IgE or IL-5, which recruits and activates eosinophils.[2,3] Although the biology of T_H2 cells in allergy is well understood, little is known about the mechanisms that control the initial T_H2 polarization in response to exogenous allergens. Some studies suggest allergen-dependent mechanisms determined at the dendritic cell (DC) level because of particular attributes of the specific protein.[4,5] Others suggest T cell–dependent or individual factors leading to a predominance of the T_H2 response.[6-8] In the context of pollen allergy, we recently demonstrated that pollen, under physiologic exposure conditions, release not only allergens but also bioactive lipids that can activate human neutrophils and eosinophils *in vitro* and modulate maturation and cytokine release of human DCs that results in an increased capacity to induce T_H2 responses in naive T cells.[9-11]

Until recently, pollen grains have simply been regarded as allergen carriers, and little attention has been devoted to nonprotein compounds of pollen. However, individuals are rarely exposed to pure allergens but rather to particles releasing the allergen, such as pollen grains or pollen-derived granules.[12,13] Notably, lipids are major components of pollen exine and exsudate.[14] In addition, long-chain unsaturated fatty acids in pollen, such as linolenic acid, serve as precursors for the biosynthesis of several plant hormones, such as dinor isoprostanes, recently termed phytoprostanes.[15] Indeed, we recently demonstrated the presence of E_1-, F_1-, and B_1-phytoprostanes in *Betula alba* (white birch) aqueous pollen extracts (Bet-APEs) and showed that E_1-phytoprostanes (PPE_1), which are similar to Bet-APEs, dose-dependently inhibited IL-12 production and induced an increased T_H2-polarizing capacity of human DCs.[11]

The *in vitro* effects of Bet-APEs and PPE_1 on human DCs prompted us to investigate whether Bet-APEs and

From [a]the Division of Environmental Dermatology and Allergy GSF/TUM, GSF National Research Center for Environment and Health, ZAUM-Center for Allergy and Environment, Technische Universität München, Munich; [b]the Department of Dermatology and Allergy Biederstein, Technische Universität München, Munich; [c]the Julius-von-Sachs-Institute of Biosciences, Division of Pharmaceutical Biology, University of Würzburg; and [d]the Allergy Research Group, Department of Dermatology, University Medical Center Freiburg.

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Reprint requests: Thilo Jakob, MD, Allergy Research Group, Department of Dermatology, University Medical Center Freiburg, Haupstraße 7, 79104 Freiburg, Germany. E-mail: thilo.jakob@uniklinik-freiburg.de.

Abbreviations used
Bet-APE: *Betula alba* aqueous pollen extract
DC: Dendritic cell
OVA: Peptide 323-339 from chicken ovalbumin
PALM: Pollen-associated lipid mediator
PPE_1: E_1-phytoprostanes
PPF_1: F_1-phytoprostanes

PPE_1 also display T_H2-polarizing properties *in vivo*. For this, we first analyzed the effect of Bet-APEs and phytoprostanes on murine bone marrow–derived DCs and demonstrate that similar to their effect on human DCs, Bet-APEs and PPE_1 (but not F_1-phytoprostanes [PPF_1]) dose-dependently inhibit the IL-12 production of activated murine DCs. In addition, we demonstrate here that Bet-APEs exert adjuvant T_H2-polarizing capacities not only *in vitro* but also in the mucosa draining lymph nodes *in vivo*. In contrast, PPE_1 and PPF_1, when tested *in vivo*, exerted inhibitory effects on T_H1 and T_H2 cytokine production. Understanding the underlying mechanisms by which under natural exposure conditions allergen carriers, such as pollen, modulate the outcome of primary immune responses might open new approaches for therapeutic intervention.

METHODS

Animals

BALB/c wild-type mice were purchased from Charles River (Sulzfeld, Germany). Peptide 323-339 from chicken ovalbumin (OVA) T-cell receptor (TCR)–transgenic DO11.10 mice was a gift from Dr Dennis Loh (Howard Hughes Medical Institute, St Louis, Mo). Animals were housed under specific pathogen-free conditions. The study was conducted under federal guidelines for the use and care of laboratory animals and was approved by the government of the district of Upper Bavaria.

Reagents and antibodies

Recombinant murine IL-2, IL-4, IL-12, anti-IL-4, and anti-IL-12 were purchased from Peprotech (Frankfurt, Germany). OVA was purchased from Biosyntan (Berlin, Germany). Purified LPS was kindly provided by Stephanie Vogel (Department of Microbiology and Immunology, University of Maryland). Fluorescein isothiocyanate–, phycoerythrin-, or allophycocyanin-conjugated anti-CD4, anti-KJ1-26, anti-IL-4, anti-IFN-γ, anti-CD11c, anti-CD86, and anti-rat IgG1 were purchased from BD Biosciences (Heidelberg, Germany).

Preparation of Bet-APEs

Birch pollen grains *(Betula alba)* were obtained from Sigma (Munich, Germany), air-dried at about 30°C, and continuously stored at 2°C to 8°C in a desiccator. Microscopic examination revealed a purity of greater than 99%, with less than 0.1% foreign pollen, less than 0.1% mold spores, and less than 0.7% plant parts, as specified by the manufacturer. The aqueous birch pollen extracts were prepared from incubations of 10 mg/mL pollen for 30 minutes at 37°C in RPMI 1640 medium for *in vitro* studies and in Dulbecco's PBS (Gibco, Merelbeke, Belgium) for *in vivo* studies and subsequent centrifugation (500 g/20 minutes) and sterile filtration (0.2 nm).[11]

Preparation of PPE_1 and PPF_1

Racemic PPE_1 and PPF_1 were prepared by means of auto-oxidation of α-linolenic acid and purified as described previously.[16,17]

Generation and activation of DCs

Bone marrow–derived DCs were generated under the aegis of GM-CSF (50 ng/mL; Peprotech), as recently described.[18] For the last 20 hours, DCs were either stimulated with LPS (100 ng/mL) with or without concomitant Bet-APEs, PPE_1, or PPF_1 at indicated concentrations.

Measurement of cytokines in cell-culture supernatants by means of ELISA

Aliquots of cell-culture supernatants were assayed for IL12p70, IFN-γ, IL-4, IL-5, and IL-13 by means of 2-site ELISA with antibodies from BD Biosciences, as described previously.[18]

Enrichment of DO11.10 CD4 cells

Spleens from DO11.10 mice were collected, and single-cell suspensions were obtained by mincing through 70-μm cell strainers. Red cell lysis was carried out for 10 minutes in lysis buffer (150 nmol/L NH_4-Cl, pH 7.5) on ice. DO11.10 CD4 cells were MACS sorted by using a mouse CD4 T-cell isolation kit from Miltenyi Biotech (Bergisch Gladbach, Germany), according to the manufacturer's protocol.

Chimeric DO11.10/BALB/c splenocyte assay

MACS-sorted DO11.10 CD4 T cells were titrated into total BALB/c wild-type splenocytes, ensuring that 5% of all $CD4^+$ T cells were specific for OVA. Chimeric DO11.10/BALB/c splenocytes were stimulated for 6 days with OVA (0.2 μmol/L) in the presence or absence of Bet-APEs at indicated concentrations. Cells were cultured in 96-well plates (2×10^5/200 μL) in T-cell medium (RPMI 1640 supplemented with IL-2 [25 U/mL], FCS [10%], HEPES [0.025 mol/L], nonessential amino acids [0.1 mmol/L], L-glutamine [2 mmol/L], penicillin [100 U/mL], streptomycin [100 μg/mL; GIBCO], and β-mercaptoethanol [0.05 mmol/L; Merck, Darmstadt, Germany]).

Adoptive transfer and *in vivo* priming of DO11.10 OVA-specific CD4 cells

MACS-sorted DO11.10 CD4 T cells (5×10^6/200 μL DPBS) were injected into the tail veins of naive BALB/c wild-type mice. Twenty-four hours later, mice were anesthetized and intranasally exposed to OVA (2 mmol/L) in DPBS (10 μL) per nare in the presence or absence of Bet-APEs or phytoprostanes. For *in vivo* exposure studies, mice received a single dose of 3% aqueous birch pollen extracts generated at 10 mg/mL, which corresponds to as little as 6 μg of pollen per mouse. PPE_1 have been detected in aqueous extracts of birch pollen in concentrations of 0.5×10^{-6} mol/L, as determined by means of GCMS analysis. The concentrations used in the present study were chosen to be in the same range as 1×10^{-6} mol/L and 10 times higher at 1×10^{-5} mol/L. Forty-eight hours later, draining cervical lymph nodes from DO11.10/BALB/c chimeric mice were excised, and single-cell suspensions were obtained. Two hundred thousand cells were stimulated with 0.2 μmol/L OVA in T-cell medium (see above). After 72 hours, 100-μL aliquots were replaced by fresh medium (50 U/mL IL-2) and analyzed for cytokine content by means of ELISA (see above). On day 6, OVA-specific $KJ1\text{-}26^+$ T cells were analyzed for intracellular accumulation of IL-4 and IFN-γ by means of flow cytometry (see below).

Intracellular cytokine staining

For the final 6 hours, T cells were stimulated with PMA (20 ng/mL; Sigma, St Louis, Mo) and ionomycin (1 μg/mL, Sigma), and for

the final 3 hours, Brefeldin A (Golgiplug, BD Biosciences; 0.25 μL/mL) was added to prevent cytokine secretion. Cells were fixed and permeabilized (Cytofix/Cytoperm Kit, BD Biosciences), according to the manufacturer's protocol, and analyzed by means of flow cytometry for accumulation of IL-4 and IFN-γ production in OVA-specific T cells identified by gating on KJ1-26$^+$ cells.

Statistics

The paired Student *t* test was used to compare levels of cytokine secretion in cell-culture supernatants, as well as intracellular cytokine accumulation in OVA-specific T cells between experimental groups. A *P* value of less than .05 was considered significant.

RESULTS

Bet-APEs and PPE_1 inhibit IL-12 production of murine DCs

Before analyzing the *in vivo* effects of soluble factors released from pollen, we first analyzed the *in vitro* effect of Bet-APEs or phytoprostanes on the function of murine DCs. For this, immature bone marrow–derived DCs were exposed to Bet-APEs or phytoprostanes, and phenotypic and functional DC maturation was analyzed. As reported previously, analysis of Bet-APEs by using the Limulus amebocyte lysate test revealed substantial quantities of LPS (range, 10-200 EU/mL). Elution over polymyxin B columns allowed efficient removal of LPS (<0.05 EU/mL). Bet-APEs with LPS levels of less than the detection level of the Limulus amebocyte lysate test were used for all subsequent experiments. Exposure of immature DCs to Bet-APEs, PPE_1, or PPF_1 alone did not induce DC maturation, as determined by means of analysis of MHC class II, CD40, CD80, and CD86 surface expression (data not shown). When DCs were stimulated simultaneously with LPS, neither Bet-APEs nor PPE_1 or PPF_1 affected the LPS-induced DC maturation (data not shown). This was also reflected at a functional level, where addition of Bet-APEs had no significant effect on the DC-induced proliferation of allogeneic CD4 T cells (data not shown). In contrast, the presence of Bet-APEs or PPE_1 resulted in a dose-dependent inhibition of LPS-induced IL-12p70 production by DCs (Fig 1), whereas PPF_1 did not affect LPS-induced IL-12 production.

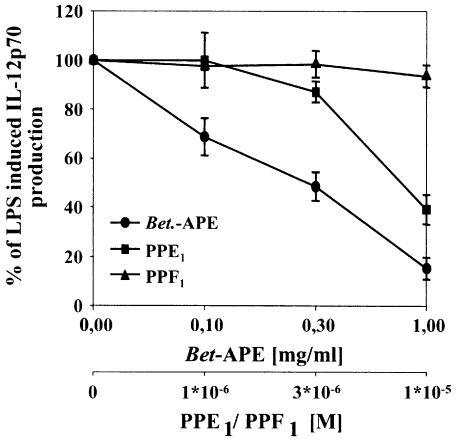

FIG 1. Bet-APEs and PPE_1 inhibit murine DC IL-12 production. DCs were stimulated with 100 ng/mL LPS in the presence of increasing concentrations of Bet-APEs, PPE_1, or PPF_1. After 20 hours, IL-12p70 concentrations were determined in culture supernatants by means of ELISA. Data are presented as percentages of LPS-induced IL-12p70 production (means ± SEMs) to equalize donor-specific variability (IL12p70: 100% = 1599 ± 437 pg/mL; Bet-APE: n = 7; PPE_1: n = 5; PPF_1: n = 3).

Bet-APE exposure shifts T_H differentiation from T_H1 to T_H2 *in vitro*

The inhibitory effects of Bet-APEs on DC IL-12 production prompted us to investigate the phenotype of primary immune responses toward an innocuous antigen (OVA) in the presence of Bet-APEs. In preparation for the *in vivo* studies described below, we used OVA-specific TCR-transgenic CD4 T cells obtained from DO11.10 mice as responder cells to analyze the T_H cell differentiation. MACS-sorted DO11.10 CD4 cells were added to BALB/c wild-type spleen suspensions, creating chimeric culture conditions with defined numbers of OVA-specific CD4 cells. Antigen-induced cytokine production of OVA-specific T cells was analyzed at the single-cell level by means of intracellular cytokine staining. In cultures stimulated with OVA alone, a T_H1-dominated response was observed, as demonstrated by IFN-γ$^+$ KJ1-26$^+$ cells (Fig 2). Simultaneous stimulation with Bet-APEs (0.3 mg/mL) changed the OVA-induced T_H differentiation, with an increase of cells producing the T_H2 marker cytokine IL-4 and a reduction of IFN-γ–producing cells (Fig 2).

Intranasal exposure to Bet-APEs promotes a T_H2 shift in draining cervical lymph nodes, whereas phytoprostanes inhibit cytokine production *in vivo*

Having confirmed the T_H2-promoting capacities of Bet-APEs in the murine system *in vitro*, we addressed the effect of Bet-APEs and phytoprostanes on T-cell priming *in vivo*. Analysis of early events during T_H polarization *in vivo* is often hampered by the low frequency of antigen-specific T cells in naive wild-type animals. To overcome this, OVA-specific T cells from TCR-transgenic DO11.10 mice were adoptively transferred to wild-type BALB/c mice, as described previously, so that the resulting DO11.10/BALB/c chimera carried approximately 2% OVA-specific CD4 cells in peripheral lymphoid tissue.[19]

Forty-eight hours after *in vivo* antigen exposure, draining cervical lymph node cell suspensions were prepared and restimulated with OVA *ex vivo*. Subsequent analysis of culture supernatants revealed a pronounced T_H2 shift with increased levels of IL-4, IL-5, and IL-13, accompanied by decreased IFN-γ release in cultures derived from mice that had been exposed intranasally to OVA in the presence of Bet-APEs compared with cultures from mice exposed to OVA alone. PPE_1 and PPF_1 also inhibited IFN-γ secretion but did not affect the secretion of IL-4 and IL-5 and even reduced IL-13 secretion (Fig 3, *A*). Similar results were obtained when cytokine production was analyzed at the single-cell level. *In vivo* exposure to Bet-APEs

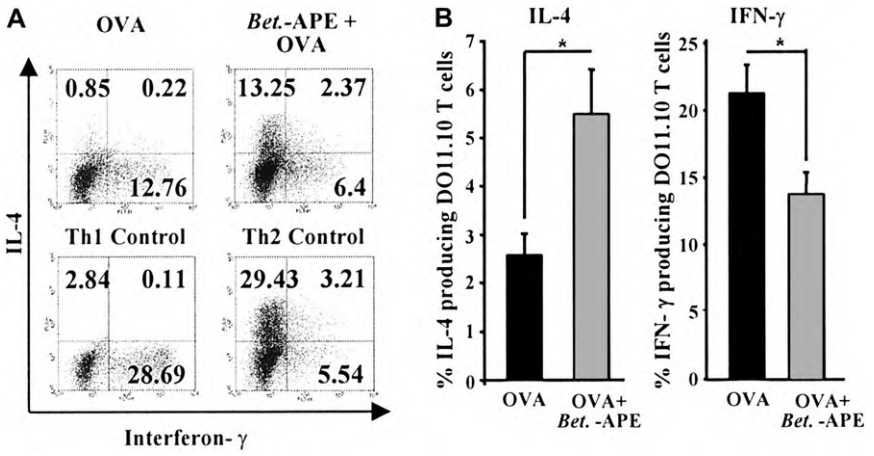

FIG 2. T_H2-polarizing effects of Bet-APEs on primary immune responses *in vitro*. The intracellular cytokine accumulation in OVA-specific DO11.10 T cells 6 days after antigen stimulation in a chimeric DO11.10/BALB/c splenocyte culture is shown. The percentage of IL-4/IFN-γ–producing OVA-specific T cells (gated on KJ1-26^+ cells) after antigen stimulation in the absence (OVA) or presence of Bet-APEs (OVA, Bet-APEs) is also shown. T_H1 and T_H2 controls were stimulated with OVA, IL-12, and anti-IL-4 or OVA, IL-4, and anti-IL-12, respectively. **A**, One representative experiment of 5. **B**, Means ± SEMs of 5 experiments.

simultaneously to the antigen lead to an increased percentage of IL-4–producing and a reduced percentage of IFN-γ–producing OVA-specific T cells, whereas PPE_1 and PPF_1 downregulated the number of IFN-γ– and IL-4– producing DO11.10 T cells (Fig 3, *B*). Thus water-soluble factors from birch pollen did exert T_H2-polarizing capacity on the antigen-specific immune response not only *in vitro*[11] but also when applied *in vivo* under natural exposure conditions through a physiologic route. At the same time, phytoprostanes displayed inhibitory effects on cytokine production of antigen-specific T cells in draining lymphoid tissue.

In vivo proliferation of OVA-specific T cells is not affected by Bet-APEs but is reduced by phytoprostanes

To provide a functional basis for the observed immunomodulatory capacity of pollen-derived factors, we analyzed the effects on *in vivo* proliferation of antigen-specific T cells in draining lymph nodes. Intranasal exposure to OVA induced, as expected, a marked proliferation of adoptively transferred carboxyfluorescein diacetate succinimidyl ester–labeled OVA-specific KJ1-26^+ CD4 cells in draining lymph nodes, whereas no proliferation was observed in nondraining lymph nodes (eg, inguinal; data not shown) or in PBS-challenged DO11.10/BALB/c chimeras (Fig 4). Antigen-induced T-cell proliferation was not affected when OVA was applied together with Bet-APEs but was strongly reduced when PPE_1 or PPF_1 were coadministered (Fig 4).

DISCUSSION

In the context of the allergic immune response, pollen not only function as allergen carriers but also as a rich source of bioactive lipids. We recently demonstrated that on contact with the aqueous phase, pollen release substantial amounts of oxylipins and phytoprostanes that can modulate cells of the innate immune response.[9-11] Aqueous pollen extracts, as well as PPE_1, were demonstrated to change human DC functions in a way that results in an enhanced T_H2 polarization *in vitro*.[11] Here we extend these findings to the murine system and demonstrate relevant immunomodulatory effects also *in vivo*. Similar to effects on human DCs, Bet-APEs and PPE_1 (but not PPF_1) inhibited the LPS-induced IL-12 production of murine bone marrow–derived DCs. When applied intranasally with innocuous antigen (OVA), Bet-APEs promoted a T_H2 differentiation of antigen-specific T cells in the draining lymph nodes *in vivo*. In contrast, PPE_1 and PPF_1 inhibited OVA-induced T-cell proliferation and cytokine production *in vivo*.

IL-12 secretion by DCs is a key stimulus for induction of T_H1 responses in naive CD4 T cells, and T_H2 responses, among others, are thought to be established as a default pathway in the absence of T_H1 stimuli.[20] The reduced capacity of murine DCs to secrete IL-12 on LPS stimulation in the presence of Bet-APEs and PPE_1 (but not PPF_1) might be an underlying mechanism for the T_H2 shift exerted by Bet-APEs and verifies previous human findings. The precise mechanisms of how Bet-APEs and PPE_1 reduce the capacity of DCs to produce IL-12 are not clear yet. Toxicity as a possible factor for suppression of cytokine release is unlikely because DC viability, as analyzed on the basis of annexin V/propidium iodide staining and the production of other cytokines, such as IL-6, was not affected (data not shown). PPE_1, the most abundant phytoprostane in aqueous birch pollen extracts,[11] shows high structural homology to prostaglandin E_2,[17,21] which has been demonstrated to inhibit the production of T_H1-polarizing cytokines by human DCs.[22] Whether PPE_1

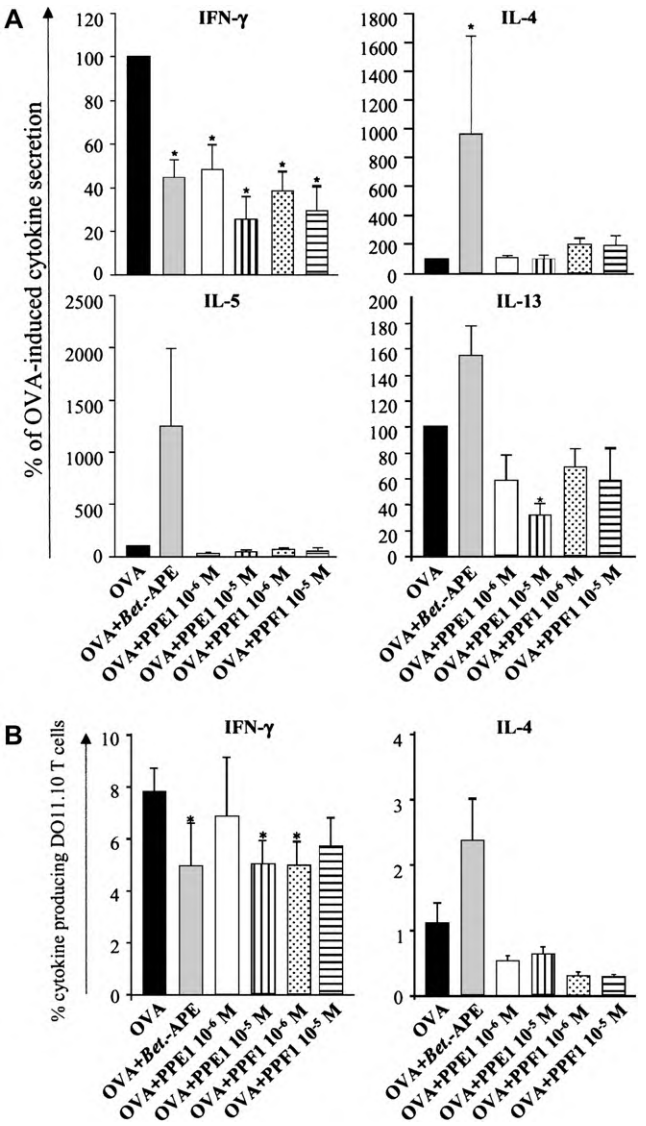

FIG 3. Intranasal Bet-APEs induce a T_H2 shift in draining cervical lymph nodes, whereas exposure to phytoprostanes inhibit T_H1 and T_H2 induction. DO11.10/BALB/c chimeric mice were exposed intranasally to 2 mM OVA (20 μL) with or without Bet-APEs (6 μg/20 μL) or PPE_1 or PPF_1 at indicated concentrations. After 48 hours, nose-draining cervical lymph nodes were excised, single-cell suspensions were restimulated *ex vivo*, and supernatants were analyzed after 3 days for IL-4, IL-5, IL-13, and IFN-γ by means of ELISA **(A)** or antigen-specific T cells were analyzed after 6 days for intracellular cytokine production at the single-cell level by means of flow cytometry **(B)**. ELISA data are presented as percentages of OVA-induced cytokine production (mean ± SEM: IFN-γ 100% = 626 ± 111 pg/mL; IL-4: 100% = 14 ± 8 pg/mL; IL-5: 100% = 12 ± 4 pg/mL; IL-13: 100% = 149 ± 39 pg/mL). Fig 3, A and *B*: 5 independent experiments with 3 animals per group.

act through the same mechanisms and use the same E prostanoid receptors is currently under investigation.

When analyzing the effects of pollen-derived factors *in vivo*, we confirmed the T_H2-promoting activity of Bet-APEs, as demonstrated by a T_H2 shift of antigen-specific T cells in the draining lymph nodes after intranasal application. These data provide good evidence that water-soluble factors released from pollen affect the local microenvironment of the nasal mucosa in a fashion that results in a T_H2 polarization of the ensuing immune response in draining lymphoid tissue. Based on *in vitro* findings in human and murine DCs, we speculated that PPE_1 would exert similar effects *in vivo*. However, when coadministered with innocuous antigen, PPE_1 did not induce a T_H2 polarization but rather inhibited antigen-induced IFN-γ production, as well as IL-13 production, whereas no significant effect was observed on the production of IL-4 and IL-5. Similar effects were observed when PPF_1 was applied, suggesting a mechanism others than DC IL-12 regulation. Administration of Bet-APEs with antigen did not influence the proliferative response of antigen-specific T cells in the draining lymph node. In contrast, both PPE_1 and

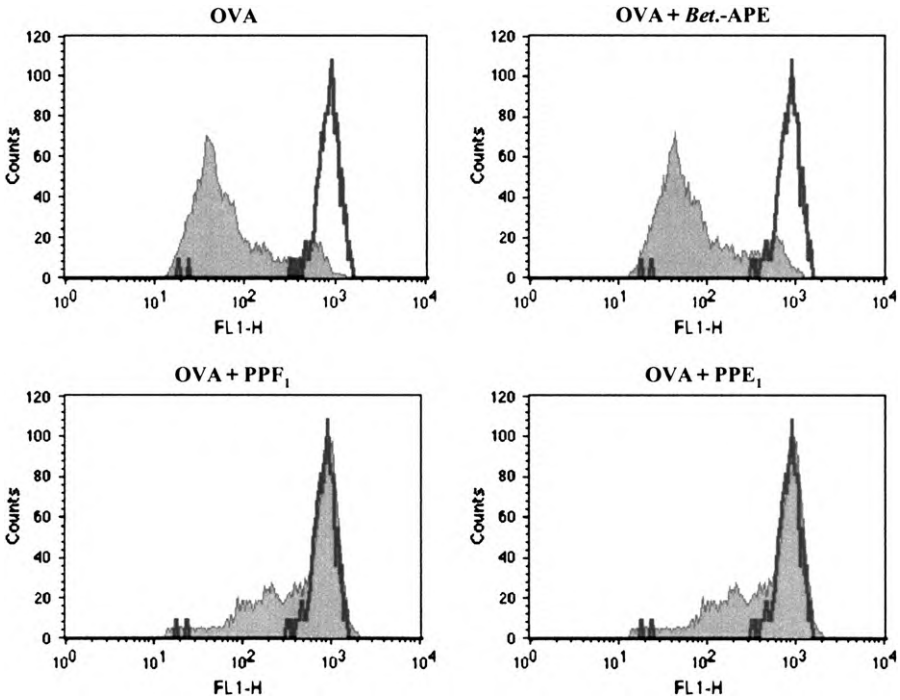

FIG 4. Intranasal exposure to phytoprostanes but not to Bet-APEs inhibits antigen-induced T-cell proliferation in draining lymph nodes *in vivo*. *In vivo* division kinetics of carboxyfluorescein diacetate succinimidyl ester–labeled OVA-specific T cells in nose-draining cervical lymph nodes of DO11.10/BALB/c chimeras 48 hours after intranasal exposure to PBS *(solid line)* or OVA (2 mM, 20 μL) in the presence or absence of Bet-APEs (6 μg/20 μL) or phytoprostanes (10^{-6} M; *filled curve*) are shown.

PPF_1 markedly inhibited the antigen-induced T-cell proliferation. This might in part account for the observed reduction of cytokine release. Currently, we do not know whether the reduced T-cell proliferation is simply the result of an impaired antigen presentation or delivery to the draining lymph node or if it reflects the induction of regulatory mechanisms, such as regulatory T cells. Investigations that address these questions are currently underway.

The *in vivo* effect of Bet-APEs very likely reflects summative effects of various substances, which might act synergistically in the induction phase of the sensitization process. Aqueous pollen extracts contain a large number of different substances with potential immunomodulatory capacities. In addition to pollen-associated lipid mediators (PALMs), such as phytoprostanes and oxylipins, carbohydrates or proteins might also exert T_H-polarizing effects. In addition, pollen grains release oxidases with profound effects on reactive oxygen species production of the local microenvironment that might affect the outcome of primary immune responses initiated within the airways.[23-25]

Although mouse models mirror human biology remarkably well, a number of differences in the immune system of both species have been well documented.[26] Thus translation of findings from mice to men and *vice versa* is not always possible and might explain the different effects of PPE_1 and PPF_1 in the mouse system compared with human *in vitro* findings.[11] Human exposure studies of naive individuals to xenogenic antigen, such as keyhole limpet hemocyanin, have been used to address the adjuvant effects of diesel exhaust particles.[27] Similar approaches might provide an answer on the *in vivo* T_H2-promoting effect of PALMs in human subjects.

For clinical implications, the *in vivo* effects of Bet-APEs would depend on concentrations of bioactive mediators in the nasal or bronchial microenvironment. As reported earlier, the concentrations of PALM precursors (linolic and linolenic acid) in pollen are high,[9,10] and other bioactive substances (eg, oxidases and lipoxygenases) are also present in pollen.[24] Hence during the pollen season, especially during peak days of pollination, we assume biologically relevant exposure to various immunomodulatory substances released by pollen. Provided that we characterize the mode of action by which PALMs, such as phytoprostanes, regulate immune responses (ie, identify the receptor and signaling pathway involved), we can identify novel target molecules that can be modulated pharmacologically by using selective agonists or antagonists. Previous observations[9,10] that the proinflammatory effects of PALMs on neutrophils and eosinophils can be blocked by a selective leukotriene B_4 receptor antagonist seem to support this perspective. Collectively, our data provide evidence that water-soluble factors released from pollen act as biogenic adjuvants, which exert critical influences on the decision-making process whether an antigen induces primary T_H1 or T_H2 responses *in vivo*.

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