The Journal of Immunology

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J Immunol 2010; 184:677-684; Prepublished online 16 December 2009;
doi: 10.4049/jimmunol.0902028
http://www.jimmunol.org/content/184/2/677

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
1451 Rockville Pike, Suite 650, Rockville, MD 20852

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Prostaglandin E_2 Inhibits IFN-α Secretion and Th1 Costimulation by Human Plasmacytoid Dendritic Cells via E-Prostanoid 2 and E-Prostanoid 4 Receptor Engagement

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Plasmacytoid dendritic cell (PDC)-derived IFN-α plays a central role in antiviral defense and in Th1-driven autoimmune diseases, such as systemic lupus erythematosus (SLE). In the current study, we explored how PGE_2 effects the phenotype of PDCs from healthy and SLE subjects. Although PGE_2 is considered to mediate mainly proinflammatory effects, we show that PGE_2 and PG analogs potently inhibit secretion of IFN-α by TLR-activated PDCs. This effect is mainly mediated by PG receptors E-prostanoid 2 and E-prostanoid 4 and involves inhibition of IFN regulatory factor 7 expression. Of note, profound IFN-α inhibition by PGE_2 is also seen in PDCs from SLE subjects, independent of age, disease activity, and therapy. We show that TLR9-activated PDCs treated with PGE_2 exhibit DC2-like characteristics with enhanced expression of CD86 and CD62L, and decreased expression of CD80 and MHC class I. Consequently, PGE_2-treated PDCs suppress secretion of Th1 cytokines by T cells while increasing the secretion of Th2 cytokines. Prevention of CpG-induced CD62L downregulation by PGE_2 suggests that it may induce the retreat of PDCs from inflamed tissues. Our data on the effects of PGE_2 on PDCs may explain occasional reports about the induction of SLE-like symptoms by cyclooxygenase inhibitors as well as improvement of such symptoms by treatment with PG analogs. In conclusion, our data suggest that PGE_2 and certain PG analogs, some of which are already in clinical use, should be evaluated as a novel and inexpensive treatment approach for patients with SLE and other IFN-α–dependent, Th1-driven autoimmune diseases. ***The Journal of Immunology*****, 2010, 184: 677–684.**

Plasmacytoid dendritic cells (PDCs) represent a unique immune cell type, fundamentally different from other dendritic cell (DC) subsets. PDCs are specialized in the production and secretion of large amounts of IFNs, thereby initiating and orchestrating antiviral immune responses as well as activating further APC subsets (1, 2). IFN-α production can be triggered by viral nucleic acids as well as by synthetic CpG oligodeoxynucleotides (ODNs) in vitro. The decisive role of PDCs in antiviral immune defense is highlighted by the fact that low peripheral PDC numbers during certain virus infections, such as HIV, are associated with disease progression (3), whereas increased PDC numbers result in a protective effect in viral infections, such as respiratory syncytial virus (4).

Apart from their antiviral effects, PDCs play a crucial role in a variety of autoimmune diseases (1, 5–8). PDCs accumulate in affected tissues, such as in skin lesions from patients with lupus erythematosus (9) or in the synovial fluid from adult or juvenile subjects with rheumatoid arthritis (10, 11). IFN-α derived from autoantigen-activated PDCs appears to directly trigger the pathogenesis of certain autoimmune diseases, such as systemic lupus erythematosus (SLE), autoimmune thyroiditis, and diabetes mellitus type I (12, 13). Therefore, IFN-α inhibition may be a promising therapeutic strategy in this group of diseases (14).

PGE_2 is a proinflammatory mediator ubiquitously expressed by various cell types, including mononuclear and stromal cells (15, 16). PGE_2 functions primarily via four G protein-coupled receptors designated E-prostanoid (EP) 1–4 (17). PGE_2 has been described as an important factor for efficient migration and maturation of monocyte-derived DCs (18, 19). Furthermore, PGE_2 acts as an immunomodulator for B, T, and NK cells (15), mainly by inhibition of various cytokines including TNF-α (20), IFN-γ (21) and bioactive IL-12 (22). A series of PG analogs have been established as therapeutic agents for clinical indications, such as gastroduodenal ulcer (23) and severe chronic arterial obstructive disease (24), and could be readily translated into the clinic when proving effective as potential modulators of autoimmune processes.

Recently, we and others have shown that the immunomodulatory mediators vasoactive intestinal peptide (VIP) and IL-10 can efficiently inhibit IFN-α secretion by human PDCs and induce significant changes to their immune phenotype (25, 26). The effects of lipid mediators, such as PGE_2 and PG analogs, have been characterized mainly in murine models, partly with contradictory results on effector immune cell subsets, such as T cells (27, 28). In contrast, data relating to the effects of PG anologs on human

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Received for publication June 26, 2009. Accepted for publication November 14, 2009.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (German Research Foundation) and the Ministry of Science, Research, and the Arts Baden-Württemberg and the European Social Fund within the Schlieben-Lange-Programm (to D.F.).

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The online version of this article contains supplemental material.

Abbreviations used in this paper: CpG-A, type A CpG oligodeoxynucleotide 2336; CpG-B, type B CpG phosphorothioate oligodeoxynucleotide 2006; DC, dendritic cell; EP, E-prostanoid; IRF, IFN regulatory factor; MFI, mean fluorescence intensity; ODN, oligodeoxynucleotide; PDC, plasmacytoid dendritic cell; SLE, systemic lupus erythematosus; VIP, vasoactive intestinal peptide.

immune cells, particularly from subjects with autoimmune diseases, are limited (29, 30). In the current study, we investigated the biologic effects of PGE_2 on human PDCs from healthy and SLE subjects, as compared with VIP and IL-10, including its effects in combination with these agents.

We reveal PGE_2 as a potent IFN-α inhibitor in PDCs from healthy donors and from subjects with SLE. The overall IFN-α inhibition by PGE_2 is stronger than with VIP or IL-10, and PGE_2 is able to further enhance the inhibitory effects of VIP or IL-10. We demonstrate that these effects are mainly mediated by the PG receptors EP2 and EP4, because PG analogs with appropriate receptor preferences are at least as potent in IFN-α inhibition as PGE_2. Furthermore, we show that PGE_2-activated PDCs skew $CD4^+$ T cell responses from Th1 toward Th2, which by itself could have therapeutic implications in Th1-driven autoimmune diseases. Our data may provide the basis for novel and inexpensive treatment approaches for patients with SLE, particularly when conventional pharmacotherapy is insufficient or causes intolerable adverse reactions.

Materials and Methods

Human subjects and cell cultures

The present study was approved by the Ethics Committee at the University of Ulm. Peripheral blood from healthy volunteers was acquired after obtaining informed consent from each individual. PBMCs were isolated and RBCs were removed according to standard procedures. PDCs were magnetically purified using the BDCA-4^+ cell isolation kit II according to the manufacturer's instructions (Miltenyi Biotec, Bergisch Gladbach, Germany), resulting in >95% of cells with a lin-1^-, BDCA-2^+, CD123^{high}, or MHC class II^{high} phenotype. CD123 staining was performed as an additional marker because BDCA-2 can be downregulated upon PDC activation (31). For in vitro culture, cells were suspended in AIM-V medium (Life Technologies BRL, Grand Island, NY) supplemented with 10 ng/ml of the human PDC growth factor IL-3 and incubated for 48 h on 96-well flat-bottom plates (1×10^6 cells/ml, 200 μl/well, if not stated otherwise) at 37°C and 5% CO_2 in the presence of various agents as indicated. Purity after 2 d was regularly >99% BDCA-2^+ cells.

Reagents for functional assays

Human IL-3 (10 ng/ml) and IL-10 (25 ng/ml) were purchased from PeproTech (Hamburg, Germany). The type B CpG phosphorothioate ODN 2006 (1 μg/ml, called CpG-B) with the specific sequence 5′-TCG TCG TTT TGT CGT TTT GTC GTT-3′ and the type A CpG ODN 2336 (1 μg/ml, called CpG-A) with the specific sequence 5′-GGG GAC GAC GTC GTG GGG GGG-3′ were both purchased from Coley Pharmaceutical Group (Ottawa, Ontario, Canada), R848 (5 μM) from InvivoGen (San Diego, CA). PG E2 (PGE_2, 10^{-6} M) was purchased from Sigma-Aldrich (Schnelldorf, Germany). Brefeldin A (1 μg/ml) was purchased from Epicentre Technologies (Madison, WI). VIP (1×10^{-6} M) was purchased from Calbiochem (San Diego, CA). Recombinant human IFN-α (500 U/ml) was purchased from PBL Biomedical Laboratories (Piscataway, NY). The EP2 agonist butaprost, the EP2/3/4 agonist misoprostol, and the EP1/3 agonist sulprostone were purchased from Cayman Chemical (Ann Arbor, MI), the EP3/4 agonist alprostadil was purchased from Schwarz Pharma (Mannheim, Germany).

Flow cytometry

For FACS analysis, cells were harvested at the indicated time points and stained as described previously (25). FITC-, PE-, PE-Cy5-, PerCP-Cy5.5-, PE-Cy7-, or APC-labeled Abs to lin-1, CD3, CD4, CD8, CD123, and MHC class II were purchased from Becton Dickinson (Heidelberg, Germany). PE- or APC-labeled Abs to BDCA-2 (CD303) as well as PE- or APC-labeled anti-human IFN-α Abs and the corresponding isotype controls were purchased from Miltenyi Biotec (Bergisch Gladbach, Germany). For intracellular IFN-α detection, cells were incubated at 1×10^6/ml for 3 h. Next, brefeldin A was added to a final concentration of 1 μg/ml, and cells were cultured for another 3 h. Intracellular staining was performed after fixation and permeabilization with appropriate buffers (ADG, Bio Research, Vienna, Austria). FcR blocking reagent was used before anti–IFN-α or isotype control monoclonal Abs were added to decrease nonspecific binding. After another 15 min of incubation, cells were washed with PBS. Flow cytometric analyses were performed on a FACScan, a FACSCalibur, or an LSRII (BD Biosystems, San Jose, CA) and data analyzed using FlowJo software (version 8.7.1; Tree Star, Ashland, OR).

IFN-α ELISA

PDCs were isolated as described and cultured in IL-3–containing (10 ng/ml) AIM V medium (2×10^4 or 1×10^5 PDC/w) in the presence or absence of CpG-A (1 μg/ml), IL-10 (25 ng/ml), VIP (10^{-7} M), as well as PGE_2 or PG analogs at various concentrations as indicated. After 48 h, supernatants were harvested and IFN-α concentrations were determined using an ELISA kit for multisubtype human IFN-α (PBL Biomedical Laboratories, Piscataway, NY). All incubations were performed at 24°C. Supernatants (100 μl) or standards were placed in 96-well precoated ELISA plates and incubated for 1 h. After washing, plates were incubated with equal volumes of biotinylated anti–IFN-α for 1 h. Plates were washed and incubated for 1 h with HRP-conjugated avidin. After addition of the substrate tetramethyl-benzidine plates were read in an ELISA reader at 450 nm (Mitras, Berthold Technologies, Bad Wildbad, Germany). The lower detection limit for IFN-α was 76 pg/ml.

CFSE staining and proliferation assay

For coculture experiments, T cells from healthy individuals were isolated using the $CD4^+$ T cell Isolation kit II (Miltenyi Biotech). T cells were resuspended in PBS (1×10^7 cells/ml) containing 5-CFSE and 6-CFSE (Molecular Probes, Eugene, OR) at a final concentration of 1 μM, incubated at 37°C for 10 min, and washed three times. Next, 2×10^5 $CD4^+$ T cells were cocultured for 5 d with allogeneic purified PDCs that had been pretreated for 48 h with various reagents as indicated. Cells were plated in 200 μl AIM-V medium on 96-well round-bottom plates at a PDC:T cell ratio of 1:10. Flow cytometric analyses were performed as described above.

Cytometric bead array

For cytokine quantification, supernatants from T cell:PDC cocultures or from single PDC cultures were harvested as described above and analyzed using a sandwich Bio-Plex cytokine immunoassay following the manufacturer's specifications (Bio-Rad, Hercules, CA). This assay permits simultaneous detection of IL-2, IL-5, IL-10, IL-13, IFN-γ, and TNF-α. All incubations were performed at 24°C. Briefly, the anticytokine bead solution was diluted and 50 μl was placed on 96-well Durapore membrane plates (Millipore, Billerica, MA). After washing by vacuum filtration, 50 μl undiluted supernatants and standards were placed into wells and incubated for 30 min on a shaker. After washing, 25 μl detection Ab was added and incubated for another 30 min while shaking at 300 rpm. After washing, streptavidin–PE was added for 10 min. Next, plates were washed, resuspended in assay buffer, read on a flow cytometer (Luminex, Riverside, CA), and analyzed using Bio-Plex Manager software (Bio-Rad, Hercules, CA).

EP2, EP4, and IFN regulatory factor 7 RT-PCR

PDCs from healthy individuals were magnetically isolated to a purity of >98%, and mRNA from 1×10^6 PDC per sample was prepared using TRIZOL reagent (Invitrogen, Carlsbad, CA). Total RNA (0.5 μg) was reverse-transcribed with RT Superscript III (Invitrogen, Carlsbad, CA); 2.5 μl cDNA was used per 12.5 μl PCR reaction with Taq DNA polymerase (Qiagen, Hilden, Germany). Primers used were for EP2, forward: 5′-GCTGCTGCT TCTCATTGTCTCG-3′, reverse: 5′-TCCGACAACAGAGGACTGAACG-3′ (392 bp), and for EP4, forward: 5′-ATCTTACTCATTGCCACC-3′, reverse: 5′-TCTATTGCTTTACTGAGCAC-3′ (212 bp; Biomers.net, Ulm, Germany) (32). PCR products were run on a 1.5% agarose gel and stained with ethidium bromide for UV detection. For quantitative mRNA expression analysis of IFN regulatory factor (IRF) 7, EP2 and EP4, PDCs from three healthy donors were purified and incubated with IL-3 in the presence or absence of PGE_2, and CpG-A for 15 h. Total RNA was isolated and reverse-transcribed as described above, followed by real-time RT-PCR using SYBR Green to detect accumulation of PCR products. Primers for EP2 and EP4 were used as above; primers for IRF7 were: forward primer 5′-ACGCTATACCA TCTACCTGGGCTT-3′, reverse 5′-TATCCAGGGAAGACACACCCTCA-3′, RPL-32 (forward primer 5′-AGTTCCTGGTCCACAACGTC-3′, reverse primer 5′-GATGCCAGATGGCAGTTTTT-3′) and TATA box binding protein (forward primer 5′-GCCGAAACGCCGAATA-3′, reverse primer 5′-CGTGGCTCTCCATGAT-3′), served as housekeeping genes. Cycle threshold (ΔC_T) was determined by subtracting the average C_T value of both housekeeping genes from the average C_T values of EP2, EP4, and IRF7, respectively. $\Delta\Delta C_T$ values were calculated by subtraction of ΔC_T values of treated PDC from those of IL-3-treated PDCs, and the final value was determined as $2^{-\Delta\Delta CT}$ (Applied Biosystems, Foster City, CA).

Fluorescence microscopy

Isolated PDCs from healthy individuals were pooled and transferred into a FACS tube (BD Biosciences, Heidelberg, Germany). Cells were then surface-stained with FITC-labeled mouse Ab for human BDCA-2 for 15

min (Miltenyi Biotec). Subsequently, the cells were washed, incubated with rabbit anti-human EP4 Ab (Santa Cruz Biotechnologies, Santa Cruz, CA) for 1 h, followed by incubation with a Texas Red-labeled goat anti-rabbit secondary Ab (Vector Laboratories, Burlingame, CA). After two washing steps, cells were transferred onto 8-well Lab-TekII chamber slides (USA Scientific, Orlando, FL), settled for 20 min, covered with slips attached with Vectashield mounting medium (Vector Laboratories), and examined using an Olympus AX70 fluorescence microscope (Hamburg, Germany).

Statistics

Data are expressed as means ± SEM. To determine statistical differences between the means of two data columns, the paired two-tailed Student *t* test was used. *p* Values were corrected using the Bonferroni method when applicable. Results were considered significant at $p < 0.05$.

Results

PGE_2 and IL-10 are potent inhibitors of IFN-α secretion by PDCs and enhance VIP-mediated IFN-α suppression

In an earlier study, we reported an inhibitory effect of VIP on IFN-α production by PDCs (25). The primary goal of the current study was to identify additional IFN-α inhibitors that support the inhibitory effect of VIP, and that are efficient not only in healthy individuals but also in subjects with SLE. We cultured purified PDCs in the presence of CpG-A and various combinations of VIP with IL-10 and PGE_2 for 48 h and then harvested cell culture supernatants. An IFN-α ELISA of cell culture supernatants revealed that PGE_2 and IL-10 were not only able to inhibit IFN-α as single agents, but also enhance VIP-mediated suppression of IFN-α. IFN-α secretion was found only in the presence of CpG-A, with CpG-A alone inducing average IFN-α levels of 132 ± 69 ng/ml with 1×10^5 PDCs per well. PGE_2 and IL-10 reduced IFN-α levels to 42 ± 15% and 60 ± 29% of CpG-A–induced levels, respectively. In combination with VIP, PGE_2 suppressed IFN-α levels to 31 ± 7% and IL-10 levels to 29 ± 16%. (Fig. 1*A*, 1*B*). Similar results were obtained for TNF-α after PDC treatment with PGE_2 and IL-10 for 48 h, measured by a cytometric bead array with supernatants from 1×10^5 PDCs (data not shown). Moreover, PGE_2 inhibited IFN-α induced by the TLR9 ligand CpG-A as well as IFN-α induced by the TLR7/8 ligand R848 (Supplemental Fig. 1*A*, 1*B*).

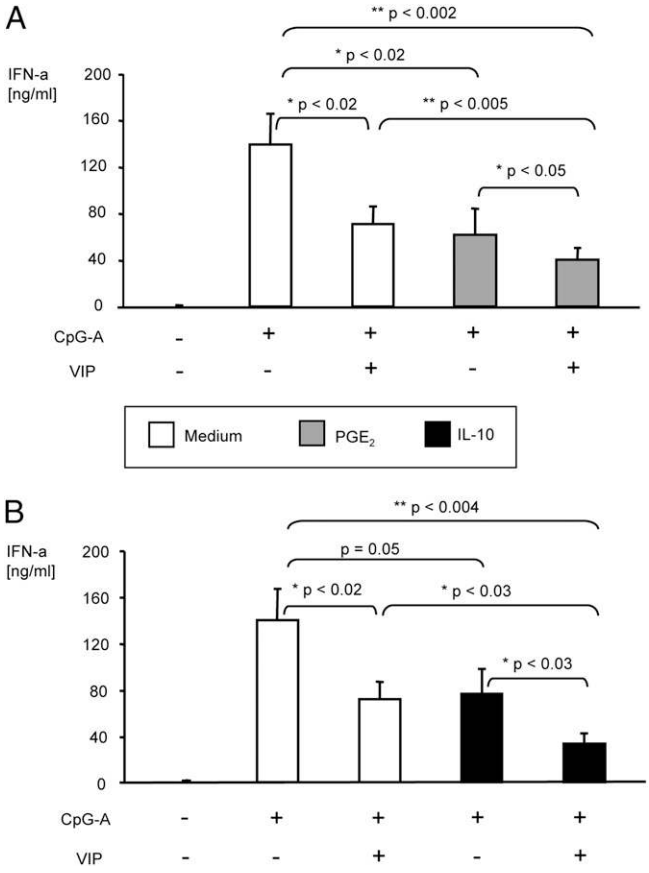

FIGURE 1. PGE_2 and IL-10 inhibit secretion of IFN-α by isolated PDCs and enhance VIP-mediated suppression of IFN-α. PDCs from healthy individuals were purified (>90%) and cultured for 48 h in IL-3–containing (10 ng/ml) AIM-V medium (1×10^5 PDCs per well) in the presence or absence of CpG-A (1 μg/ml) and the combination of VIP (10^{-7} M) with PGE_2 (10^{-6} M) or IL-10 (25 ng/ml). Supernatants were then harvested and IFN-α concentrations determined by ELISA. Bar graphs represent average IFN-α concentrations secreted by PDCs from five different donors stimulated with VIP in combination with PGE_2 (*A*) or IL-10 (*B*). Error bars indicate SEM.

PGE_2, but not IL-10, decreases the percentage of IFN-α–producing PDCs

In regard to recent data underlining the reduction of PDC survival by both PGE_2 and IL-10 (26, 30, 33), we evaluated PDC viability and percentage of IFN-α^+ PDCs after 48 h incubation. In our evaluation, neither IL-10 nor VIP significantly reduced PDC viability. Although PGE_2 decreased survival by 18 ± 15% (Fig. 2*C*), this reduction was too low to explain the extent of IFN-α inhibition by PGE_2. We therefore determined the percentages of IFN-α–producing viable PDCs after stimulation. The percentage of IFN-α^+ PDCs was decreased by PGE_2, particularly in the presence of VIP, whereas IL-10 and VIP had no such effects (Fig. 2*A*, 2*B*). Of note, PGE_2 suppressed not only the percentage of PDCs positive for IFN-α, but also the amount of IFN-α on a single PDC level as determined by median fluorescence intensity for IFN-α (data not shown). Overall, these results suggested that PGE_2 had a stronger inhibitory effect on PDC IFN-α production and secretion than did IL-10 or VIP.

PGE_2 and IL-10 differentially regulate the PDC-immune phenotype

Another important function of PDCs, apart from IFN-α secretion, is their ability to interact with various cell types, such as T, B, and endothelial cells. We therefore studied which effects CpG ODN, PGE_2, IL-10, and VIP had on a panel of molecules enabling PDCs to communicate with other cells. These molecules included costimulatory molecules, Ag-presenting molecules and a PDC-typical homing and adhesion molecule. Of note, the CpG ODN we used for these studies was CpG-B rather than CpG-A, because of its stronger effects on the immune phenotype of PDC. We found that PGE_2 preferentially inhibited MHC class I expression, whereas IL-10 had a strong suppressive effect on MHC class II expression. PGE_2 skewed the expression of costimulatory molecules of the B7 family from CD80 to CD86, whereas IL-10 did the opposite (Fig. 3). Finally, CpG-induced downregulation of the adhesion molecule CD62L was prevented by PGE_2, whereas IL-10 strongly decreased CD62L expression (Fig. 3). As with IFN-α expression, PGE_2 had similar effects on the phenotype of PDCs stimulated with the TLR7/8 agonist R848 instead of the TLR9 agonist CpG-B, with the only exception being that the combination of R848 and PGE_2 enhanced expression of both CD86 and CD80 (Supplemental Fig. 2).

PGE_2-treated PDCs induce $CD4^+$ T cell proliferation and skew the cytokine profile toward Th2

PDCs regulate the immune response by a series of interactions with other immune cells including T cells. We therefore investigated how modulation of PDC phenotype effects T cell proliferation and cytokine profile by gating on viable $CD3^+CD4^+$ T cells (Supplemental Fig. 3*A*). Cocultures of differentially activated PDCs with CFSE-stained T cells revealed that PGE_2 strongly increased the capacity of PDCs to induce $CD4^+$ T cell proliferation, in both the

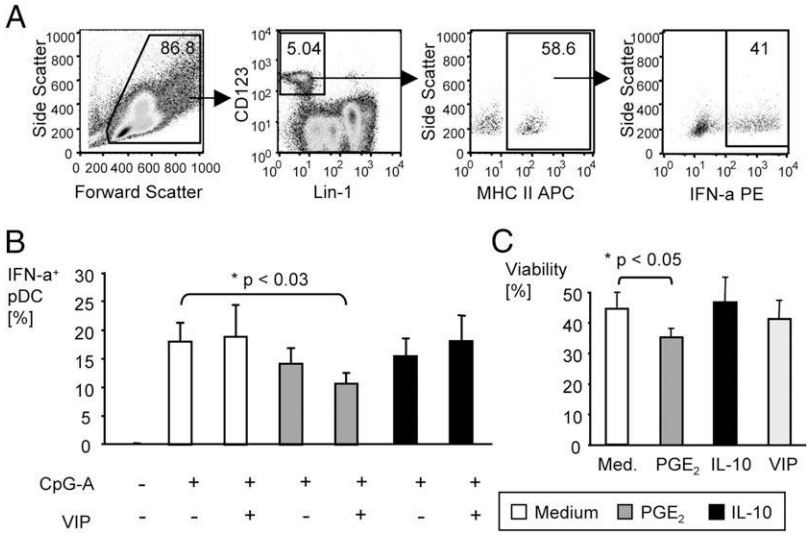

FIGURE 2. The combination of PGE_2 and VIP decreases the percentage of IFN-α–producing PDCs. PBMC from healthy subjects were depleted of $CD3^+$ cells by positive magnetic bead selection to enrich for PDCs. Cells were then cultured in IL-3–containing (100 ng/ml) AIM-V medium in the presence of CpG-A (1 μg/ml) and combinations of VIP (10^{-6} M) with PGE_2 (10^{-6}M) or IL-10 (25 ng/ml). After 6 h, cells were stained for IFN-α and analyzed by FACS. *A*, Dot plots show the gating strategy applied for the identification of viable $lin1^-$, $CD123^+$, MHC II^+, and IFN-α^+ PDCs. *B*, Bar graph shows average percentages of IFN-α^+ PDCs from five independent experiments. *C*, Viability of PDCs was determined based on scatter characteristics after 48 h culture in IL-3–containing medium as described above. Average values from at least seven independent donors are shown. Error bars indicate SEM.

presence or absence of CpG-B. In contrast, IL-10 enhanced the capacity of PDCs to stimulate $CD4^+$ T cell proliferation in only the absence, not in the presence, of CpG-B (Fig. 4*A*). To characterize the cytokine profile of $CD4^+$ T cells in the presence of differentially activated PDCs, we performed a multiplex cytokine assay of coculture supernatants. We found that PGE_2, IL-10, and VIP decreased IFN-γ and IL-2 concentrations, whereas increasing IL-5 (Fig. 4*B*) and IL-13 (Supplemental Fig. 3*B*). Therefore, all three modulators tested appeared to skew the $CD4^+$ T cell cytokine profile toward Th2. T cell secretion of the immunosuppressive cytokine IL-10 was decreased by PDCs activated with PGE_2 or VIP, but not with IL-10 itself (Fig. 4*B*). The addition of exogeneous IFN-α to PDC–T cell cocultures did not significantly

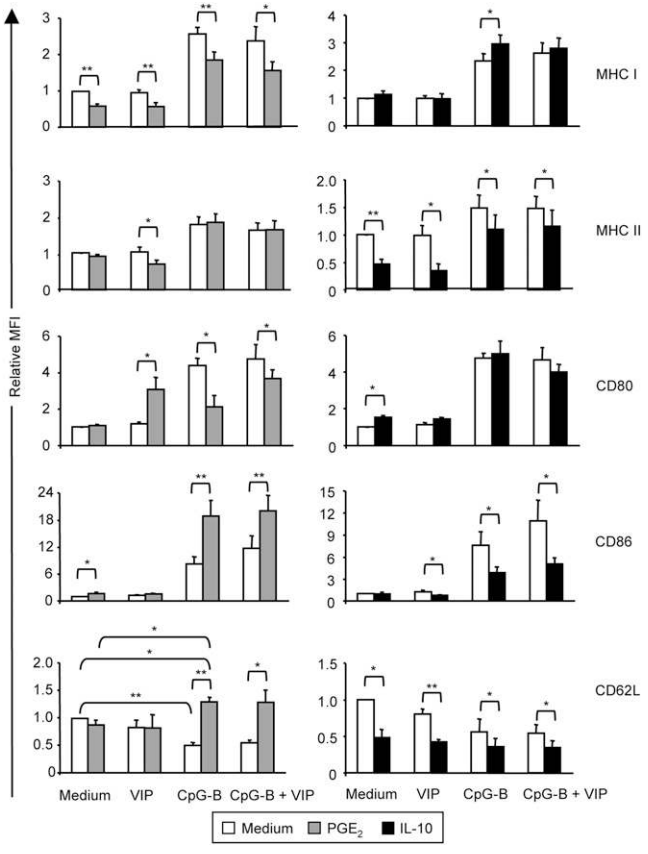

FIGURE 3. PGE_2 and IL-10 differentially regulate the immune phenotype of PDCs. PDCs from healthy subjects were isolated and incubated (1×10^6 cells/ml, 200 μl/well) for 48 h in IL-3–containing AIM-V medium in the presence of CpG-B (1 μg/ml), PGE_2 (10^{-6} M), IL-10 (25 ng/ml) and VIP (10^{-7} M) as indicated. Cells were then harvested, and expression of various cell surface molecules as stated was determined by FACS analysis. Bar graphs show relative average median fluorescence intensities (MFIs) of treated versus untreated PDCs (medium). Results are from at least four independent experiments for each individual marker. $p < 0.05$ was considered statistically significant ($*p < 0.05$; $**p < 0.01$). Error bars indicate SEM.

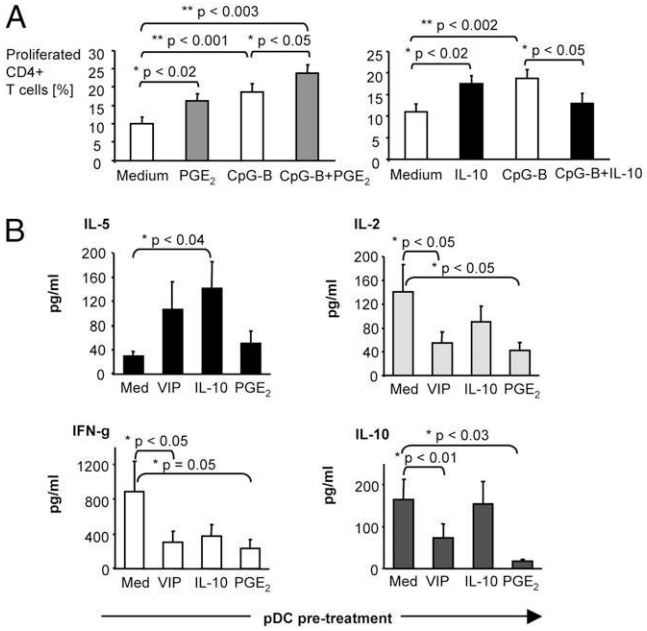

FIGURE 4. PGE_2-treated PDCs induce $CD4^+$ T cell proliferation and skew their cytokine profile toward Th2. PDCs from healthy volunteers were isolated and cultured in IL-3–containing (10 ng/ml) AIM-V medium in the presence of CpG-B (1 μg/ml), PGE_2 (10^{-6} M), or IL-10 (25 ng/ml) as indicated. After 48 h, cells were harvested, washed, counted, and cocultured at a PDC:T ratio of 1:10 with 2×10^5 allogeneic CFSE-labeled $CD4^+$ T cells for 5 d in 200 μl AIM-V medium. At day 5, proliferation was assessed using flow cytometry by gating on viable $CD3^+CFSE^{low}$ T cells. *A*, Bar graphs represent average T cell proliferation from six independent experiments. Error bars indicate SEM. *B*, Supernatants from cocultures of T cells with PDCs pretreated as described above were harvested and analyzed for cytokine production using a cytometric bead array that permits simultaneous detection of indicated cytokines. Bar graphs show T cell cytokine production from six independent cocultures. Error bars indicate SEM.

affect Th1 cytokines, and it mildly suppressed the Th2 cytokines IL-4, IL-5, and IL-13 in the presence of PDCs, irrespective of pretreatment (data not shown).

IFN-α production in response to CpG-A is higher in PDCs from individuals with SLE, but its overall secretion can be suppressed more effectively than in normal PDCs

To compare the IFN-α–producing capacity of PDCs from individuals with SLE and healthy age- and sex-matched controls, PBMCs from either group were isolated, incubated with PGE_2, VIP, or both in the presence of CpG-A for 48 h, and subsequently analyzed by flow cytometry. Only CpG-A–treated PDCs produced detectable IFN-α levels. In normal PDCs, the percentage of IFN-α^+ PDCs could be reduced by treatment with PGE_2, whereas the percentage of IFN-α^+ PDCs from SLE individuals could not be suppressed and remained significantly higher than in PDCs from healthy individuals (Fig. 5*A*, 5*B*). In contrast, secretion of IFN-α by both PDCs from healthy and SLE individuals was effectively reduced by PGE_2 and VIP (Fig. 5*C*). Importantly, inhibition was more prominent in PDCs from SLE subjects than in those from healthy controls, with VIP reducing IFN-α concentrations by up to 55%, and PGE_2 by up to 85% (Fig. 5*C*). Combination of PGE_2 with VIP did not enhance the inhibitory effect achieved with PGE_2 alone (Fig 5C). IFN-α inhibition by PGE_2 occurred independently of age, therapy, and disease activity as determined by the SLE disease activity index (Table I).

PG analogs that preferentially target EP2 and EP4 are as efficient as PGE_2 in suppressing IFN-α secretion by PDCs

To narrow down the receptor subtypes, which may mediate IFN-α inhibition in PGE_2, we compared the inhibitory potential of PGE_2 and that of various PG analogs with different receptor preferences. As in the previous experiments, IFN-α levels were determined in PDC supernatants by ELISA. We found that apart from PGE_2, the EP3/4 agonist alprostadil, the EP2/3/4 agonist misoprostol, and the EP2 agonist butaprost had a potent dose-dependent suppressive effect on IFN-α secretion (Fig. 6*A*). In contrast, the EP1/3 agonist sulprostone showed inhibition of IFN-α secretion only at a higher concentration (Supplemental Fig. 4*A*). Because these data suggested that both EP2 and EP4 were involved in the mediation of IFN-α inhibition in PDCs, we performed RT-PCR for both receptors in highly purified PDCs,

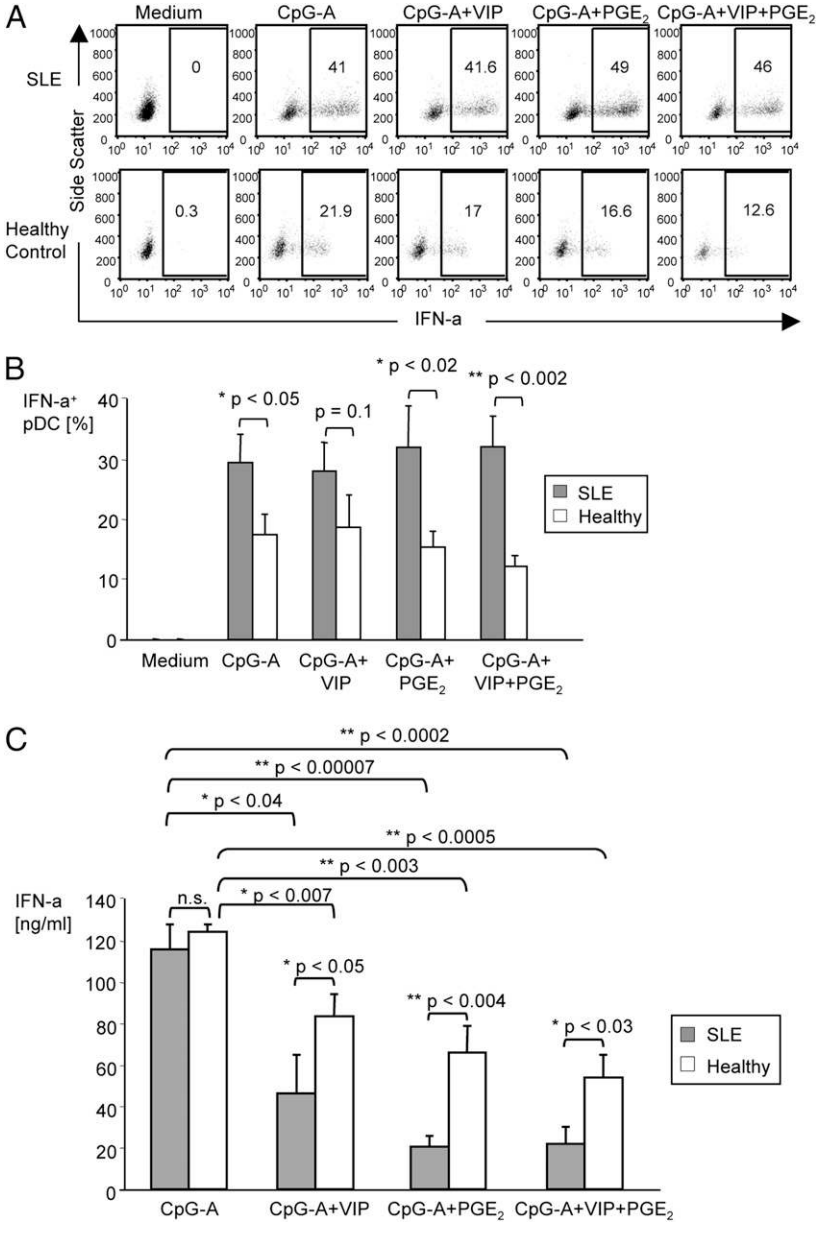

FIGURE 5. IFN-α production in response to CpG-A is higher in PDCs from individuals with SLE, but its overall secretion can be suppressed more effectively than in PDCs from normal donors. PBMCs from SLE and from age- and sex-matched healthy individuals were magnetically depleted of $CD3^+$ cells to enrich for PDCs, and 2×10^4 PDCs per well were cultured in IL-3–containing AIM-V medium in the presence of CpG-A, PGE_2, and VIP. After 6 h, cells were harvested, stained for IFN-α, and analyzed by FACS. IFN-α analysis was performed on viable lin-1^- and BDCA-2^+ PDCs. *A*, Dot plots from one representative healthy individual and one representative subject with SLE are shown. Gates indicate percentages of IFN-α–producing PDCs. *B*, Average percentages of IFN-α–producing PDCs from five (healthy) and four (SLE) independent experiments are shown as bar graphs. Error bars indicate SEM. *C*, PDCs from healthy and from SLE individuals were magnetically purified and cultured in IL-3–containing AIM-V medium in the presence of CpG-A, PGE_2, and VIP. After 24 h, culture supernatants were harvested and IFN-α concentrations were determined by ELISA. Bar graphs represent average IFN-α concentrations from seven different SLE patients and nine healthy donors. Error bars indicate SEM.

Table I. *Clinical profile of SLE subjects*

Patient Number	Age	Disease Duration (y)	Current Therapy	SLEDAI Score	IFN-α (ng/ml)	IFN-α^+ PDC (%)	Inhibition (%)
1	31	6	Co	18	ND	24	ND
2	51	14	None	11	ND	41	ND
3	30	1	None	2	ND	11	ND
4	63	12	None	8	ND	32	ND
5	50	1	Pred	14	94	ND	50
6	62	13	Co, Aza	16	0.27	ND	60
7	37	10	Co, MTX	20	480	ND	80
8	45	7	Co, Chl	10	488	ND	96
9	38	18	Co, Myco	8	26	ND	74
10	42	10	Co, MTX, CyA	16	6141	ND	92
11	68	18	Co, Chl	10	3	ND	92
12	40	16	Flu, Chl, Myco	13	7	ND	89

All subjects were of female gender. IFN-α (ng/ml) is CpG-A-induced IFN-α secretion per 1×10^5 cells; inhibition (%) indicates PGE_2-induced suppression of IFN-α secretion, both determined by an IFN-α ELISA.

Aza, azathioprine; Chl, hydroxychloroquinsulfate; Co, cortisone; CyA, Cyclosporin A; Flu, flucortolone; MTX, methotrexate; Myco, mycophenolatemofetil; Pred, prednisolone; SLEDAI, SLE disease activity index.

which showed strong mRNA expression for EP4 and weak expression for EP2 (Fig. 6*B*). Of note, expression of EP4 mRNA was negatively regulated by PGE_2 in TLR9-stimulated PDCs (Fig. 6*C*). Finally, surface expression of EP4 protein by PDCs could be confirmed using fluorescence microscopy (Supplemental Fig. 4*B*).

Suppression of IFN-α by PGE_2 is associated with downregulation of IRF7 expression

Because the suppressive effects of PGE_2 on R848-mediated induction of PDC IFN-α appeared to be comparable to its effects on CpG ODN-mediated IFN-α induction, we hypothesized that the effects of PGE_2 occurred downstream of TLR9. We therefore performed RT-PCR for IRF7 mRNA expression, because PDCs constitutively express high levels of this key transcription factor for IFN-α expression (6, 34). We found that PGE_2 markedly suppressed IRF7 mRNA expression in TLR9-stimulated, but not in unstimulated, PDCs (Fig. 7).

Discussion

IFN-α is mainly produced by PDCs and exhibits a broad spectrum of effects on tumor cells (35), virus-infected cells (36), and various immune cell subsets, such as T cells, NK cells, monocytes, and DCs (37). Importantly, IFN-α plays a key pathophysiologic role in certain autoimmune diseases, such as SLE, where it induces differentiation of monocytes into DCs that present self-Ag, and where it directly stimulates autoreactive T and B cells (12, 14). Because of its potentially deleterious effects on the immune system, IFN-α secretion requires tight control by an extensive network of modulators. These modulators may include immunosuppressive cytokines, such as IL-10 or VIP (25, 26), but also lipid mediators of the PG family, such as PGE_2, as demonstrated in the current study. Although PG were so far thought to mediate mainly proinflammatory effects, their involvement in IFN-α regulation may explain not only occasional reports about the induction of SLE-like symptoms by cyclooxygenase inhibitors (38), but also improvement of such symptoms by treatment with PGE_1 (39) and PGI analogs (40). Such beneficial effects have been mainly attributed to vasodilation and inhibition of thrombosis, whereas the effect of PG analogs on PDC-dependent IFN-α secretion and the costimulatory phenotype of PDC has so far not been considered.

In the current study, we characterize the biologic responses by PDCs from healthy individuals and subjects with SLE to PGE_2 and related analogs in comparison with earlier established PDC modulators, including VIP (25) and IL-10 (26). One of the striking effects of PGE_2 was a potent inhibition of IFN-α secretion by human PDCs from healthy donors and from subjects with SLE. This inhibition was the result of both a decrease in the number of viable PDCs producing IFN-α and a lower amount of IFN-α produced and secreted on the single PDC level. Although individuals with SLE displayed higher percentages of IFN-α–producing PDCs than did healthy donors after stimulation with CpG-A, the suppressive effect of PGE_2 on IFN-α secretion appeared to be stronger in PDCs from SLE than from healthy subjects. By using various PG analogs with different PG receptor preferences, we were able to delineate EP4 and EP2 as the main receptors responsible for PG-mediated IFN-α inhibition in PDCs. This finding is in line with a previous report on conventional DCs, in which PG-induced effects were also shown to be mainly mediated via EP2 and EP4 (19). Importantly, we show that mRNA expression of EP4 is negatively regulated by PGE_2 and that PGE_2-induced inhibition of IFN-α is not TLR9-specific, because PGE_2 effectively inhibits TLR7/8-mediated IFN-α induction as well. These results, and the fact that PGE_2 markedly suppresses IRF7 mRNA expression in TLR9-stimulated PDCs, suggest that the effect of PGE_2 occurs on a transcriptional level downstream of TLR7 or TLR9.

In addition to its effects on IFN-α, PGE_2 exhibited a high capacity to alter the surface immune phenotype of activated PDCs on several levels, to what is consistent with a DC2 phenotype (26, 30, 33). First, PGE_2, but not IL-10 or VIP, strongly skewed the expression of costimulatory molecules of the B7 family toward CD86, which has been described to preferentially costimulate a Th2-type T cell response characterized by initial production of IL-4 (41). This finding is supported by our data showing a decreased secretion of Th1 and an increased secretion of Th2 cytokines by $CD4^+$ T cells after stimulation with PGE_2-activated PDCs. Furthermore, expression of Ag-presenting molecules, particularly of MHC class I, was suppressed by PGE_2, whereas IL-10 suppressed MHC class II expression, as described earlier (42). The finding that PGE_2 was able to enhance $CD4^+$ T cell proliferation clearly requires further study in terms of T cell phenotype, particularly in light of recent reports concerning the induction of regulatory T cells by various immune modulators, including VIP (43, 44) and CpG ODN (45). Finally, PGE_2, but not IL-10, effectively prevented downregulation of CD62L by CpG ODN on activated PDCs. CD62L, also referred to as L-selectin, is highly expressed on naive T cells and PDC precursors. A decrease of CD62L expression on PDCs is a sign of maturation, and high expression of CD62L enables PDCs to recirculate from the peripheral blood to secondary lymphoid organs through high endothelial venules (46). Our results suggest that PGE_2 inhibits PDC maturation and triggers the migration and homing to the lymph nodes after pick-up of microbial Ags at the site of active inflammation.

Reports from various other groups support our findings that PGE_2 may have anti-inflammatory effects in certain autoimmune diseases (20, 47). Furthermore, drugs that induce the release of PGE_2, such as

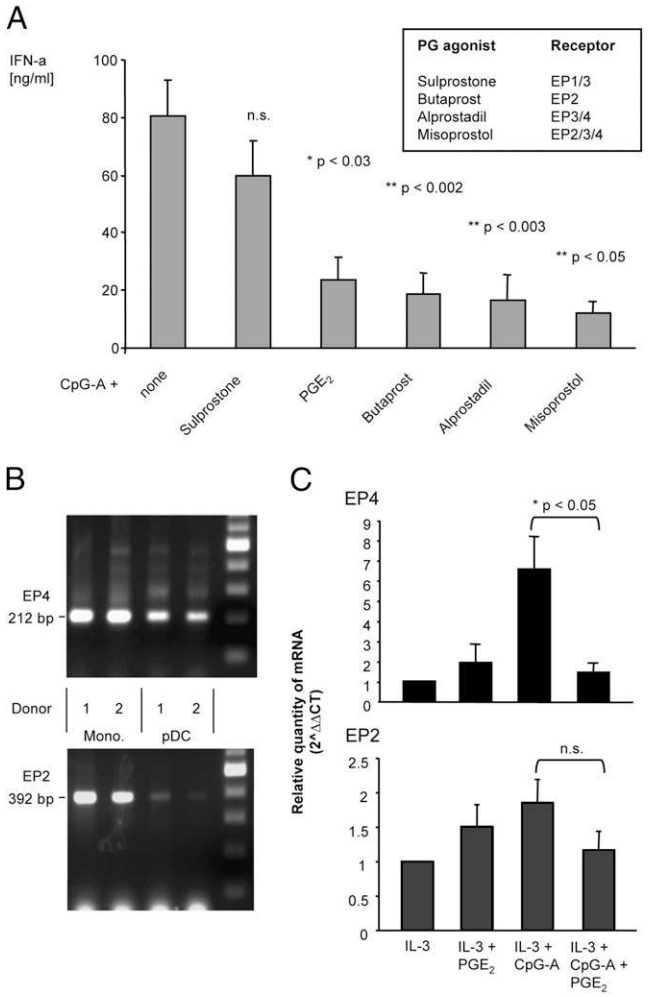

FIGURE 6. PG analogs that preferentially target EP2 and EP4 are as efficient as PGE_2 in suppressing IFN-α secretion by PDCs. PDCs from healthy individuals were magnetically purified and cultured in IL-3–containing AIM-V medium in the presence of CpG-A. After 24 h, supernatants were harvested and IFN-α concentrations determined by ELISA. *A*, Bar graph represents average IFN-α concentrations from seven independent experiments with PGE_2, EP3/4 agonist alprostadil, EP2/3/4 agonist misoprostol, EP1/3 agonist sulprostone, and EP2 agonist butaprost, all at 10^{-5} M. Bar graphs were normalized. Error bars indicate SEM. *B*, Conventional RT-PCR for EP2 and EP4 was performed on purified PDCs (>98%) from healthy individuals. One representative experiment of three with similar results is shown. *C*, Real-time RT-PCR for EP2 and EP4 was performed with PDCs from four healthy donors after incubation with IL-3 in the presence or absence of PGE_2 (10^{-6} M) and CpG-A (1 μg/ml) for 15 h. To determine the relative quantity of EP2 and EP4 mRNA, the comparative C_T method was used as described in *Materials and Methods*. Bar graphs represent the mean relative EP4 (upper graph) and EP2 (lower graph) expression (fold increase) compared with IL-3 alone ($n = 4$). Error bars indicate SEM.

cetirizine, were also described to exert beneficial effects in a series of autoimmune disorders (48). Finally, the observation that PGE_2 production is decreased in monocytes from SLE patients (49) and that such monocytes are poor inhibitors of IFN-α production when added to PDC–NK cell cocultures (50) further supports the view that PGE_2 is involved in limiting autoimmune reactions and that this mechanism may be deficient in SLE. Although other groups showed that PGE_2 could promote inflammation in certain models of murine autoimmune diseases, including experimental autoimmune encephalomyelitis (28) or pristane-induced murine lupus (51), this discrepancy with the findings mentioned above may be due to interspecies variations and pathogenetic differences of the models used. For example, in murine

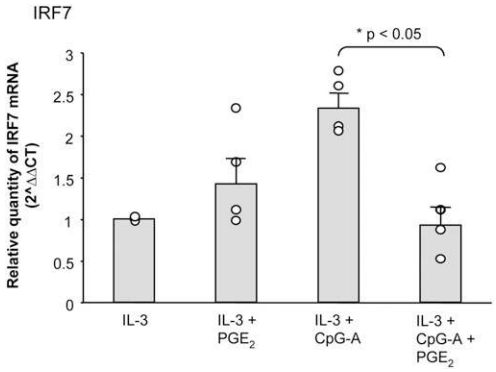

FIGURE 7. PGE_2 reduces IRF7 mRNA expression. PDCs from four healthy donors were purified and incubated with IL-3 in the presence or absence of PGE_2 (10^{-6} M) and CpG-A (1 μg/ml) for 15 h. After incubation, total RNA was isolated and real-time RT-PCR was performed for quantification of IRF7 mRNA. Relative quantity of mRNA was determined with the comparative C_T method as described in *Materials and Methods*. Bar graphs represent the mean relative IRF7 expression (fold increase) compared with IL-3 alone. Dots represent individual values ($n = 4$). Error bars indicate SEM.

lupus models, excessive IFN-α induces disease only in certain genetic backgrounds (14, 52, 53), resulting in highly variable responses to IFN-α inhibition in general. Therefore, a translation of results concerning the involvement of PGE_2 in murine autoimmune models into the human system appears problematic.

In conclusion, we demonstrate that PDCs express the PG receptors EP2 and EP4, enabling PGE_2 and PG analogs to potently inhibit secretion of IFN-α and, on a lower level, TNF-α by CpG ODN-stimulated PDCs. Furthermore, we show that the secretion of Th1 cytokines, such as IL-2 and IFN-γ, by T cells is suppressed by PGE_2-activated PDCs. Thus, the direct effects of PGE_2 and PG analogs on PDCs, as well as the effect of PGE_2-activated PDCs on T cells, suggest a high potential for PGE_2 modifying the activity of autoimmune diseases, such as SLE. Because many autoimmune diseases, such as SLE, are triggered by Th1 cytokines (54, 55), skewing of the immune response from Th1 to Th2 by PGE_2 may result in a beneficial effect on disease activity. Furthermore, PGE_2-mediated inhibition of PDC maturation and promotion of L-selectin expression, such as CD62L, may induce a retreat of PDCs from inflamed tissues. This again may intervene with disease perpetuation by preventing further uptake of autoantigens and activation of autoreactive T and B cells at the site of active inflammation. The use of PGE_2 and PG analogs may therefore represent a novel and inexpensive approach for the treatment of SLE and other IFN-α–dependent, Th1-driven autoimmune diseases when conventional treatment options are not sufficient or bear unacceptable adverse effects.

Acknowledgments

We thank Yves Laumonnier and Kai Sontheimer for assistance with PCR and Gisela Sailer for support with cytometric bead array measurements (University of Ulm, Ulm, Germany).

Disclosures

The authors have no financial conflicts of interest.

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