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IL-12-Deficient Dendritic Cells, Generated in the Presence of Prostaglandin E₂, Promote Type 2 Cytokine Production in Maturing Human Naive T Helper Cells¹

Pawel Kaliński,² Catharien M. U. Hilkens, Alies Snijders, Frank G. M. Snijdewint, and Martien L. Kapsenberg

We studied to what extent the presence of an inflammatory mediator PGE₂, during the development of dendritic cells (DC) affects their subsequent ability to induce Th1- and Th2-type cytokines in maturing naive Th cells. PGE₂ (10⁻⁹-10⁻⁶ M) did not alter the morphology or the expression of class II MHC and costimulatory molecules on DC obtained from monocytes in the presence of granulocyte-macrophage CSF and IL-4, although at concentrations above 10⁻⁸ M, PGE₂ prevented the acquisition of CD1a marker. Both control DC and DC maturing in the presence of PGE₂ (PGE₂-DC) were potent stimulators of naive Th cells. In contrast to control DC, which produced high amounts of IL-12 and trace amounts of IL-10, PGE₂-DC produced no IL-12 and high amounts of IL-10 when stimulated in the absence of PGE₂. This distinct cytokine profile of PGE₂-DC was stable for at least 48 h of additional culture in the absence of PGE₂. Control DC induced the development of Th0-like cells from superantigen-activated naive Th cells, whereas PGE₂-DC promoted the development of Th cells that produced high amounts of IL-4 and IL-5. Experiments using IL-12-neutralizing Abs or rIL-12 indicated a crucial role of IL-12 deficiency in the induction of type 2 cytokine profiles. These findings suggest that elevated levels of PGE₂ promote type 2 Th responses by stably impairing the ability of maturing DC to produce IL-12. Since type 2 Th responses are protective in several Th1-related autoimmune disorders, PGE₂-DC may be considered for use in immunotherapy. *The Journal of Immunology*, 1997, 159: 28-35.

The release of inflammatory cytokines at the site of pathogen entry increases the turnover of local APC, inducing their emigration as well as the recruitment and maturation of precursor cells (1-3). These processes facilitate the delivery of antigenic signals to local lymph nodes and the initiation of Ag-specific immune responses. The major APC type involved in the primary stimulation of naive Th cells is the dendritic cell (DC)³ (4). A crucial DC factor, GM-CSF, is involved both in the recruitment of DC precursors (2, 3) to the tissues and in the induction of their maturation (5-7). Although the nature of additional factors contributing to the generation of DC in vivo is still unclear, it was shown that GM-CSF combined with TNF- α or IL-4 promotes the generation of CD1⁺ DC from human bone marrow (5, 6) or peripheral blood precursors (7, 8) in vitro. These in vitro models provide a tool to study the impact of microenvironmental factors on human DC maturation.

Although DC are effective inducers of both type 1 (IFN- γ) and type 2 (IL-4 and IL-5) cytokine production in naive Th cells (9), capable of inducing balanced Th0-like responses (10, 11), studies in humans and mice indicated their special role in the induction of type 1 Th responses. The capacity of DC to induce high levels of type 1 cytokines depends on their ability to produce high amounts of IL-12 (12-15), which selectively up-regulates IFN- γ production (16). IL-12 secretion is, therefore, critical for the induction of protective Th1 responses in a series of pathologic conditions, such as viral, mycobacterial, and other intracellular infections, and tumors (reviewed in Refs. 16-18). On the other hand, Th1 cells are involved in the development of human and mouse autoimmune disorders (reviewed in Refs. 18-20). In these cases DC may contribute to disease development, because IL-12 was shown to be essential in the pathogenesis of murine EAE, IDDM, collagen-induced arthritis, or allergen-induced colitis (16-18), models of human autoimmune diseases. Interestingly, neutralization of IL-12 or the induction of Ag-specific Th2 cells may prevent the onset of disease symptoms even when the destructive Th1 cells are already present (21-23).

To test the possibility of induction of such desirable Th2 responses by stably modified DC, we focused on the effects of PGE₂ on DC maturation. PGE₂ is an inflammatory mediator produced by stromal cells and infiltrating mononuclear cells (24). Various Th2 cell-related diseases are associated with elevated PGE₂ production (25-27). At concentrations >10⁻⁷ M, PGE₂ favors the production of type 2 cytokines in Th cells directly via the selective down-regulation of type 1 cytokines (28). The presence of much lower concentrations of PGE₂ during the stimulation down-regulates IL-12 production in whole blood cultures (29), which raises the possibility that PGE₂ may also promote Th2-type responses indirectly via the down-regulation of IL-12 production in APC. However, the skewing effect of PGE₂ at the stage of APC-Th cell interaction is counteracted by the concomitant impairment of IL-2

Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands

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² Address correspondence and reprint requests to Dr. Pawel Kaliński, Department of Cell Biology and Histology, University of Amsterdam, A.M.C., Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands. E-mail address: p.kalinski@amc.uva.nl

³ Abbreviations used in this paper: DC, dendritic cell; GM-CSF, granulocyte-macrophage colony-stimulating factor; IDDM, insulin-dependent diabetes mellitus; rhuGM-CSF, recombinant human granulocyte-macrophage colony-stimulating factor; PGE₂-DC, dendritic cells obtained in the presence of prostaglandin E₂; SEA, staphylococcal enterotoxin A; SAC, *Staphylococcus aureus* Cowan strain 1; ^{high}, high level of expression; EAE, experimental autoimmune encephalomyelitis.

production and, subsequently, the diminished clonal expansion of Th cells (30).

This study addresses the question of whether the preexposure of DC to PGE₂ in the tissues may bear functional consequences for the subsequent priming of naive Th cells in lymph nodes. To this aim, we tested whether the exposure of DC to PGE₂ during their *in vitro* development from peripheral blood precursors modifies their subsequent ability to induce a particular cytokine profile in developing naive Th cells.

Materials and Methods

Culture media, Abs, and cytokines

All cultures were performed in Iscove's modified Dulbecco's medium (Life Technologies Ltd., Paisley, U.K.) supplemented with 10% FCS (HyClone, Logan, UT). The medium for T cell cultures was enriched with human transferrin (Behring-Werke, Marburg, Germany; 35 µg/ml) and insulin (Novo Nordisk A/S, Bagsvaerd, Denmark; 0.175 IU/ml). Growth factors used in the primary cultures of DC precursors were rhuGM-CSF (sp. act., 11.1×10^6 U/mg; Sandoz Pharma Ltd., Basel, Switzerland) and rhuIL-4 (sp. act., 10^7 U/mg; a gift from Dr. J. E. de Vries, DNAX Research Institute, Palo Alto, CA). T cells were stimulated using CD3 mAb (CLB-T3/3) and CD28 mAb (CLB-CD28/1), obtained from CLB (Amsterdam, The Netherlands). FACS analysis was performed using mAb against the following surface markers: CD1a (OKT6; Ortho Diagnostic Systems, Beersse, Belgium), CD4 (OKT4; Ortho), CD14 (Leu M3; Becton Dickinson, San Jose, CA), HLA-DR (L243; Becton Dickinson), HLA-DP (B7/21; Becton Dickinson), HLA-DQ (IOT-2d; Immunotech, Luminy, France), CD80 (B7-24) and CD86 (1G10; both provided by Innogenetics N.V., Ghent, Belgium), CD40 (EA-5; a gift from Dr. T. LeBien, University of Minnesota, Minneapolis, MN), CD45RA (2H4; Coulter, Hialeah, FL), and CD45RO (UCHL-1; a gift from Dr. P. Beverly, London, U.K.). Human rIL-12 (sp. act., 170×10^6 U/mg), IL-12 neutralizing goat Abs and the IL-12 p70-specific mAb 20C2 were gifts from Dr. M. K. Gately (Hoffmann-La Roche, Nutley, NJ). The IL-12 p40-specific mAb C8.6 was a gift from Dr. G. Trinchieri (The Wistar Institute, Philadelphia, PA). Human rIL-10 (sp. act., 5×10^{-5} U/mg) was obtained from PharMingen (San Diego, CA). IL-10 neutralizing mouse mAb (B-T10) was a gift from Dr. Wijdens (Diacione, Besancon, France). Human rIFN-γ (sp. act., 8×10^7 U/mg) was a gift from Dr. P. H. van der Meide (Biomedical Primate Research Center, Rijswijk, The Netherlands). Human rIL-2 was a gift from Cetus Corp. (Emeryville, CA; sp. act., 3×10^6 U/mg). For the purposes of FACS analysis, FITC-coupled goat F(ab')₂ anti-mouse IgG and IgM (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) was used as a secondary reagent. To prevent nonspecific Ab binding, DC were preincubated for 30 min with 5% heat-inactivated normal human pooled sera (BioWhittaker, Walkersville, MD) before the primary Ab application. Autofluorescence of DC was compensated for with use of the red fluorescence levels as a reference. Nonspecific isotype-matched control Abs MOPC-21 (IgG1; Sigma Chemical Co., St. Louis, MO), UPC-10 (IgG2a; Sigma Chemical Co.), and MOPC-141 (IgG2b; Sigma Chemical Co.) were used to set the nonspecific signal levels, which were similar in control DC and PGE₂-DC, at 10¹.

Isolation of monocytes and naive Th cells

PBMC from healthy donors were isolated from freshly drawn peripheral blood by density centrifugation on Lymphoprep (Nycomed, Torshov, Norway). Subsequently, the cells were separated on a Percoll (Pharmacia, Uppsala, Sweden) gradient (1.076, 1.059, and 1.045 g/ml). Monocytes were further purified by a 45-min adherence step. In the initial set of experiments the adherence step was replaced by immunomagnetic depletion of CD2-, CD16-, and CD20-positive cells, giving similar results. Naive CD45RA⁺ CD4⁺ T cells were isolated from the heavy fraction of PBMC in a two-step protocol. First, CD4⁺ cells were isolated by incubation with CD4-specific Dynabeads, followed by Detachabead treatment, as indicated by the manufacturer (Dynal, Oslo, Norway). In the second step, UCHL-1 and residual HLA-DR-positive cells were removed by panning, after labeling with appropriate Abs. This procedure yielded a population of >98% CD45RA⁺, CD4⁺ T cells, as determined by FACS analysis.

Generation of DC and PGE₂-DC

A modified protocol of Sallusto (7) was used. Adherent monocytes were cultured in 24-well flat-bottom culture plates (Costar, Cambridge, MA) at a density of 5×10^5 cells in 1 ml of medium. GM-CSF (500 U/ml), IL-4 (250 U/ml), and different concentrations of PGE₂ (Sigma Chemical Co.)

were added as indicated. Two-thirds of the medium, containing all factors, were replaced every second day. DC and PGE₂-DC were used for the functional studies on day 6. At this time point the cultures consisted of uniformly HLA-DR-positive cells without any detectable CD3-positive cells. More than 90% of the cells cultured in the absence of PGE₂ expressed CD1a marker. All functional studies were performed after removal of PGE₂, GM-CSF, and IL-4.

Induction of proliferative responses in naive Th cells

In MLR experiments, allogeneic naive Th cells (6×10^4 /well) were cocultured with different numbers of 3000-rad irradiated DC, PGE₂-DC, or freshly isolated monocytes in 96-well flat-bottom culture plates in a total volume of 200 µl. Proliferation was measured after 6 days by [³H]TdR incorporation; [³H]TdR was present during the last 16 h of culture (13 kBq/well; Radiochemical Center, Amersham, U.K.). In the second approach, autologous naive Th cells (2×10^4 /well) were primed with SEA (1 ng/ml; Serva, Heidelberg, Germany) in the presence of different numbers of irradiated (3000 rad) DC, 10⁻⁷ M PGE₂-DC, or monocytes. [³H]TdR was added 16 h before termination of the assay (day 4).

Analysis of cytokine profiles of DC and PGE₂-DC

On day 6 the cells were harvested, washed four times in large volumes of medium, and seeded at a density of 4×10^4 cells in 200 µl of medium. The cells were either stimulated on the same day (in the absence of GM-CSF, IL-4, or PGE₂) or, alternatively, they were cultured for an additional 48-h period without the addition of PGE₂ in the presence of GM-CSF only, both GM-CSF and IL-4, or without any of these factors. After that additional culture period, the cells were washed within the culture plates by repetitive exchange of medium and stimulated. DC (4×10^4 /well) were stimulated in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in Iscove's modified Dulbecco's medium containing 10% FCS in a final volume of 200 µl. The following stimuli were used: fixed *Staphylococcus aureus* Cowan strain I (SAC; 75 µg/ml; Calbiochem, San Diego, CA), LPS (0.1 µg/ml; Difco, Detroit, MI), and soluble rCD40L containing a modified leucine zipper sequence (31) (1 µg/ml; provided by Immunex Research and Development Corp., Seattle, WA). This concentration of rCD40L was optimal for up-regulation of adhesion molecules and IL-6 production by freshly isolated monocytes (data not shown). Stimulation was performed in both the absence and the presence of IFN-γ (1000 U/ml). Supernatants were harvested after 24 h and analyzed for the contents of cytokines and/or were used to study the effects of DC-derived soluble factors on the development of naive Th cells.

Induction of memory-type lymphokines in maturing Th cells by different populations of APC

Autologous naive Th cells (2×10^4 /200 µl) were activated by irradiated (3000 rad) autologous DC (4×10^4 /200 µl) coated with SEA (1 ng/ml; Serva), resulting in a strong proliferative response, whereas in the absence of either DC or SEA no activation of T cells could be observed (data not shown). On day 7, IL-2 (10 U/ml) was added, and the lines were expanded for the next 7 days. On day 14 the quiescent Th cells, which had acquired the CD45RO^{high} phenotype (not shown) were harvested, washed, and restimulated with immobilized CD3 mAb and soluble CD28 mAb. Supernatants were harvested after 24 h, and the levels of IFN-γ, IL-4, and IL-5 were analyzed.

Accessory cell-independent maturation of naive Th cells in the presence of APC-soluble factors

To study the functional maturation of naive Th cells in the absence of DC we used a culture system described previously (32). Naive Th cells (2×10^4 /200 µl) were stimulated with immobilized CD3 mAb and soluble CD28 mAb in the presence of rIL-2 (5 U/ml). These conditions resulted in optimal proliferation of naive Th cells and resulted in the onset of production of both IL-4 and IFN-γ (32). Control experiments indicated that the addition of SAC alone did not affect the cytokine profile of maturing naive Th cells (data not shown). Supernatants obtained from 24-h SAC-stimulated DC or PGE₂-DC were added at the onset of cultures (final dilution, 1/2) in either the absence or the presence of IL-12 neutralizing Ab (5 µg/ml) or an excess of exogenous IL-12 (200 U/ml). After 12 days of culture, quiescent Th cells were harvested and restimulated in the absence of DC factors, as described above.

Cytokine measurements

Measurements of IL-12 p70 (detection limit, 2 pg/ml), IL-10 (detection limit, 25 pg/ml), IFN-γ (detection limit, 100 pg/ml), IL-4 (detection limit,

50 pg/ml), and IL-5 (detection limit, 10 pg/ml) were performed by specific solid phase sandwich ELISAs as previously described (33–36). The IL-1 β ELISA (detection limit, 3 pg/ml) was performed with the use of a pair of anti-IL-1 β mAb, M421B and biotinylated M420B, both obtained from Endogen (Cambridge, MA), while IL-1 β standard was obtained from Boehringer Mannheim (Mannheim, Germany).

Results

APC maturing in the presence of PGE₂ develop dendritic morphology and potent stimulatory function for naive Th cells

Six-day cultures of adherent monocytes performed in the presence of GM-CSF and IL-4 yielded a population of nonadherent and loosely adherent cells of dendritic morphology (Fig. 1A, left, light background). These control DC acquired high levels of expression of MHC class I, HLA-DR, -DP, and -DQ and the costimulatory molecules CD80, CD86, and CD40. Over 90% of these cells displayed the Langerhans cell-associated marker CD1a (Fig. 1B). Similar cultures performed in the presence of PGE₂ (10^{-9} – 10^{-6} M) yielded nonadherent cells of a similar dendritic morphology (PGE₂-DC; Fig. 1A, right, dark background) and a similarly high expression of MHC class I, HLA-DR, -DP, -DQ, CD80, CD86, and CD40, although the expression of CD80 and CD40 was lower in some experiments (Fig. 1B). Higher doses of PGE₂ ($\geq 10^{-8}$ M) prevented the disappearance of CD14 and the acquisition of CD1a by maturing cells (Fig. 1C) without any major influence on the expression of a panel of costimulatory molecules (Fig. 1B). PGE₂-DC and control DC obtained from the same donors showed comparable capacities to induce the proliferation of allogeneic CD4⁺CD45RA^{high} naive Th cells and to induce the responses of autologous naive Th cells to superantigen (Fig. 2). Monocytes from the same donors were much less effective in both assays.

PGE₂-DC have a stably impaired ability to produce IL-12

Since the presence of PGE₂ during the stimulation of whole blood cultures inhibits IL-12 production (29), we have compared the potential of DC and PGE₂-DC to produce IL-12. To this aim, DC obtained in the presence of different concentrations of PGE₂ were harvested on day 6, extensively washed, and stimulated with SAC, a potent IL-12 inducer in human PBMC and in murine and human DC (13, 37).⁴ Stimulation was performed in the absence of PGE₂, GM-CSF, and IL-4. As shown in Figure 3, preexposure of developing DC to PGE₂ dramatically reduced their subsequent ability to secrete IL-12. This effect was uniformly observed at a PGE₂ concentration of 10^{-9} M (Fig. 3A), while lower concentrations of this compound were not effective in most donors. Maximal inhibition of IL-12 production in response to SAC was already seen in the cells precultured with 10^{-8} M PGE₂ (Fig. 3A), while a PGE₂ concentration of 10^{-7} M induced a total IL-12 deficiency, which could not be even partially compensated by the addition of IFN- γ (Fig. 3B). This concentration was, therefore, used in the subsequent experiments. In parallel, IL-10 production by the cells obtained in the presence of increasing doses of PGE₂ increased dose dependently.

The inability of PGE₂-DC to produce IL-12 was observed after stimulation with bacterial products, such as SAC and LPS, as well as with soluble CD40L (Fig. 3B). Binding of CD40 by CD40 ligand was recently shown to be the critical event in the induction of IL-12 during APC-Th cell interaction (14, 15, 38, 39). Because in some models IL-12 production is dependent on priming by IFN- γ (40, 41), the tests were performed in both the absence and

the presence of IFN- γ (1000 U/ml). In the control DC, SAC and LPS alone induced considerable levels of IL-12, while the addition of IFN- γ increased IL-12 production 10 to 100 times. As reported (see Footnote 4), the induction of high levels of IL-12 in DC by soluble trimeric CD40L (31) required the presence of exogenous IFN- γ ; this issue requires further study. In sharp contrast, in all 11 donors tested, the stimulation of PGE₂-DC by LPS, SAC, or CD40L, with or without IFN- γ , resulted in low or undetectable IL-12 production.

DC were very poor producers of IL-10 in response to all the stimuli tested. PGE₂-DC produced high amounts of IL-10, after stimulation with SAC and LPS, but not with CD40L, despite the high expression of CD40 on the PGE₂-DC (Fig. 1C). Addition of IFN- γ reduced SAC- and LPS-induced IL-10 production. Both control DC and PGE₂-DC produced only minimal amounts of IL-1 β , e.g., 3.5 ± 2 and 2.5 ± 3 pg/ml after SAC stimulation (mean \pm SEM; $n = 6$ donors), while the monocyte-derived macrophages from the same donors obtained in parallel cultures performed in the presence of GM-CSF alone produced much higher amounts (186 ± 38 pg/ml; $n = 6$) of this cytokine.

To study the stability of PGE₂-induced IL-12 deficiency in DC, the cells were washed on day 6 and tested for their cytokine profile either on the same day or after an additional 2-day culture in the presence of GM-CSF only. This withdrawal of PGE₂ for 2 days did not reverse the deficient IL-12 production by PGE₂-DC (Fig. 3C). Also, the high IL-10 production by PGE₂-DC was maintained, although its levels were decreased. Similar data were also obtained in two experiments in which the additional 2-day cultures were performed in the presence of GM-CSF and IL-4 or in the absence of both these cytokines (not shown), confirming the stability of the PGE₂-induced cytokine profile.

PGE₂-DC promote the production of type 2 cytokines in maturing Th cells

To study whether the maturation of DC precursors in a PGE₂-rich environment has consequences for the type of immune responses initiated by these cells, we compared the ability of PGE₂-DC and control DC to induce the production of type 1 (IFN- γ) and type 2 (IL-4 and IL-5) cytokines in maturing naive Th cells. Since the precursor frequency of conventional Ag-specific Th cells is low in the naive Th population, autologous naive Th (CD4⁺CD45RA^{high}) cells were primed with the superantigen (SEA, 1 ng/ml) presented by the two types of DC. Within a 14-day period, Th cells acquired the CD45R0 memory marker (not shown) and the number of Th cells primed by either type of DC increased similarly about 200- to 500-fold. After restimulation with CD3 mAb in the absence of DC, Th cells primed with control DC displayed Th0-like cytokine profiles. Mean levels of cytokines induced by control DC in seven experiments from seven different donors were 3.83 ng/ml IFN- γ (range, 1.46–4.95), 0.75 ng/ml IL-4 (range, 0.21–1.8), and 0.96 ng/ml IL-5 (range, 0.01–2.7). Clearly, in these seven independent experiments, despite a donor-to-donor variation with respect to the absolute amounts of the cytokines produced, naive Th cells primed with the PGE₂-DC consistently produced higher amounts of IL-4 (3.28-fold; $p < 0.01$) and IL-5 (3.57-fold; $p < 0.01$), whereas the average IFN- γ production was only 70% ($p < 0.05$) of the levels induced by control DC (Fig. 4).

The absence of IL-12 is crucial for the induction of Th2-like cytokine profiles in maturing naive Th cells

To gain insight into the mechanism of induction of high levels of IL-4 and IL-5 by PGE₂-DC, we tested to what extent the soluble factors of DC were involved in the differential steering of Th cell

⁴ C. M. U. Hilkens, P. Kalinski, M. de Boer, and M. L. Kapsenberg. 1997. Human dendritic cells direct the development of naive Th cells toward Th0 phenotype: induction of Th1 development requires activation of dendritic cells by exogenous factors. *Blood*. *In press*.

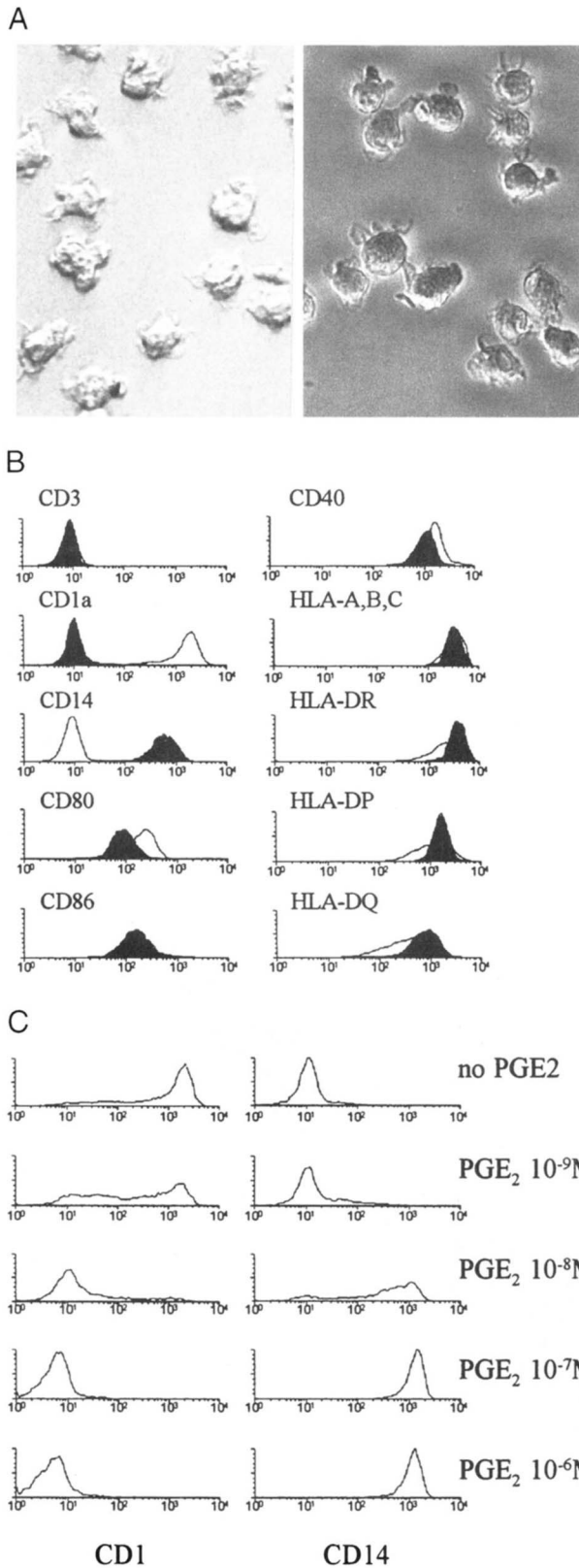


FIGURE 1. Morphology and cell surface phenotype of DC maturing in the absence or the presence of PGE₂. *A*, Six-day cultures of DC in the absence (*left*, light background) and the presence of 10⁻⁷ M PGE₂ (*right*, dark background). *B*, Cell surface phenotype of control DC, obtained in the absence of PGE₂ (open profiles) and in the presence of 10⁻⁷ M PGE₂-DC (solid profiles). The data shown are from one of >30 experiments performed. *C*, Effect of the increasing concentrations of PGE₂ on CD1a and CD14 expression in 6-day cultures. The data shown are from one experiment of three performed.

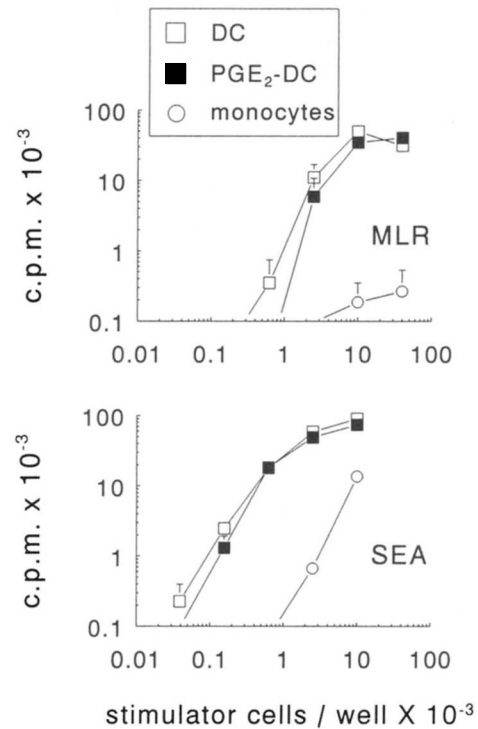


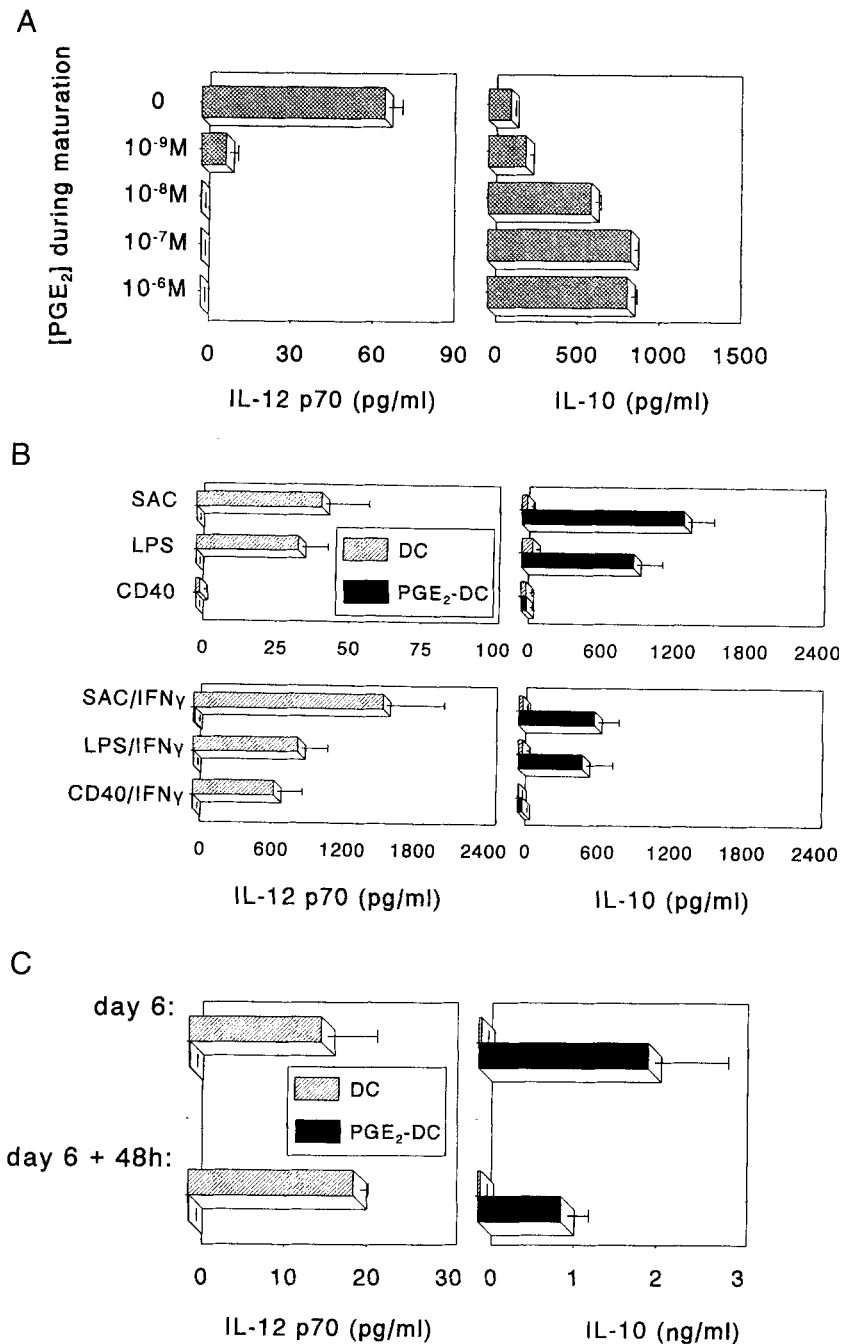
FIGURE 2. Capacity of control DC, PGE₂-DC, and monocytes to stimulate the proliferation of naive (CD45RA^{high}) Th cells. Naive Th cells were stimulated by allogeneic APC (*top*) or by the superantigen SEA (1 ng/ml) presented by autologous APC (*bottom*). Data represent the mean (±SD) of triplicate cultures from one representative experiment of four performed.

development, and what were the roles of the different IL-12 productions in this respect. To this aim, we studied the impact of SAC-activated DC supernatants on the maturation of naive (CD4⁺CD45RA^{high}) Th cells. After stimulation with immobilized CD3 mAb and costimulation with CD28 mAb, naive Th cells mature into CD4⁺CD45RO^{high} memory cells, able to produce IFN- γ and IL-4 at comparable levels to memory Th cells directly isolated from peripheral blood (32). When restimulated, the Th cells primed in the presence of PGE₂-DC supernatants produced significantly less IFN- γ , but more IL-4 and IL-5, compared with the cells primed in the presence of supernatants from control DC (Fig. 5), indicating the involvement of soluble factors in the differential steering effects of the two DC types. Comparison to the Th cells matured in the absence of either supernatant indicated that the Th2-promoting capacity of PGE₂-DC is not related to any particular Th2-promoting soluble factor but, rather, to the absence of Th1-steering activity. This activity, selectively present in the supernatants of control DC, was identified as IL-12 by showing that the differential modulation of Th cell development by the supernatants of the two DC types was abolished by the addition of IL-12 neutralizing Ab. This was further supported by the fact that in the presence of exogenous rIL-12, which by itself enhanced IFN- γ levels and decreased IL-4 and IL-5 levels in maturing Th cells, no modulatory effect of PGE₂ supernatants was seen.

Discussion

In this study, we addressed the question of whether elevated levels of PGE₂ affect the maturation of precursor DC and, subsequently, the type of immune response initiated by the PGE₂-preexposed cells. The key observation is that the naive Th cells primed by

FIGURE 3. DC obtained in the presence of PGE₂ are IL-12 deficient, but produce increased levels of IL-10. *A*, Effect of increasing concentrations of PGE₂ on the subsequent ability of maturing DC to produce IL-12 and IL-10 in the absence of PGE₂. The data (bars represent the mean \pm SD of triplicate cultures) were obtained in the same experiment as the data shown in Figure 1C. Similar results were obtained with two additional donors. *B*, Production of IL-12 and IL-10 by control DC and PGE₂-DC (10⁻⁷ M) 24 h after stimulation with SAC (75 μ g/ml), LPS (100 ng/ml), or CD40L (1 μ g/ml) in the absence or the presence of rIFN- γ (1000 U/ml). No IL-12 or IL-10 production could be detected in nonstimulated cells or in the presence of IFN- γ only. Data are shown as the mean (\pm SEM) of 11 independent experiments, using DC obtained from different donors. *C*, Comparison of SAC-induced cytokine profiles of control DC and 10⁻⁷ M PGE₂-DC, stimulated directly after removal of PGE₂ or 48 h after removal of PGE₂ (see *Materials and Methods*). Data represent the mean (\pm SD) of triplicate cultures from one donor. Similar data were obtained in three additional donors.



control DC or PGE₂-DC developed different cytokine profiles. Similarly to several other studies (10, 11), control DC induced the development of memory Th0-like cells, whereas PGE₂-DC strongly promoted type 2 cytokine production. This effect was strongly pronounced after a single stimulation cycle. The observed bias most likely represents the acquisition of a different cytokine profile by naive Th cells, rather than a selective outgrowth of contaminating Th2-like memory cells, since the purity of the starting population was >98%, and both DC types induced similarly strong proliferative responses, yielding similar numbers of Th cells after 14 days of culture. Additional evidence against a selective outgrowth of memory Th cells comes from the observation that the supernatants of the two DC types showed the adequate steering effects in the accessory cellfree model, in which we previously demonstrated that naive and memory Th cells have similar proliferation rates (32).

In the latter series of experiments, the deficient production of IL-12 by PGE₂-DC was shown to be responsible for their ability to induce high levels of the type 2 cytokines IL-4 and IL-5. These experiments indicated that IL-12 contributed to the down-regulation of IL-4 and IL-5 levels in memory Th cells maturing in the presence of DC. Although these data indicate the dominant role of IL-12 differences in the differential steering of Th development by PGE₂-DC vs control DC, they do not exclude the possible participation of other soluble or cell surface-related APC factors. In this respect, the possible involvement of the different CD80 expressions (42) by DC and PGE₂-DC has yet to be studied.

Previous studies showed that DC produce high amounts of IL-12 in response to bacterial products (13) and after direct contact with T cells, in which case IL-12 is induced by the ligation of CD40 or MHC class II molecules (14, 15). Accordingly, control DC produced significant levels (in the range of several units per

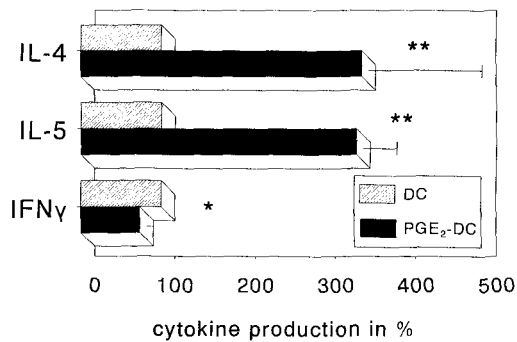


FIGURE 4. PGE₂-DC promote type 2 cytokine production in maturing Th cells. Induction of type 1 (IFN- γ) and type 2 (IL-4 and IL-5) cytokine production in naive (CD45RA^{high}) Th cells primed with SEA (1 ng/ml) presented by PGE₂-DC or by control DC. Th cells were restimulated on day 14 with a combination of CD3 and CD28 mAb. The data represent the mean (\pm SEM) of seven independent experiments from seven different donors. To bypass the variation in different donors in the absolute amounts of cytokines induced (see *Results* for the absolute values and the ranges), the level of each cytokine induced by PGE₂-DC was calculated as a percentage of the production induced by the control DC from the same donor. Paired Wilcoxon test was used for statistical analysis. *, Indicates $p < 0.05$; **, $p < 0.01$.

milliliter) of IL-12 in response to SAC and LPS alone, while the addition of exogenous IFN- γ resulted in further elevation of IL-12 production. Interestingly, induction of IL-12 production by a soluble trimeric CD40L (31) required the presence of IFN- γ , which is in contrast to a recent study (14) in which CD40L-transfected plasmocytoma cells were shown to effectively induce IL-12 in human DC in the absence of exogenous IFN- γ . The difference observed between the two models will be a subject of further study.

In contrast to control DC, PGE₂-DC were deficient in IL-12 production, which was observed in all donors and after all modes of stimulation. Importantly, this deficit was not compensated in the presence of high amounts of IFN- γ , which highly up-regulates IL-12 production in APC in most of the experimental conditions described to date (40, 41). In the same experiments in which IFN- γ only marginally up-regulated IL-12 production in PGE₂-DC, IFN- γ elevated IL-12 production in monocyte-derived macrophages to the levels produced by control DC stimulated in the presence of IFN- γ (see Footnote 4). Although IL-10 is a potent inhibitor of IL-12 production (37), the deficient IL-12 production in PGE₂-DC does not result from elevated IL-10 production by this subset, since the neutralization of IL-10 activity by IL-10-specific mAb did not restore IL-12 production in PGE₂-DC. In addition, PGE₂-DC did not produce IL-12 after the stimulation with CD40L plus IFN- γ , a mode of stimulation that induced only marginal IL-10 production. Further support for the lack of IL-10 involvement in the down-regulation of IL-12 production by PGE₂ comes from the experiments in which PGE₂ was added to the cultures of developing DC not on day 0 but only 48 h before harvesting of the cells. In such a case, a strong IL-12 down-regulation was observed, which was not accompanied by elevated IL-10 production (data not shown).

The impact of exogenous PGE₂ on the cytokine profile of maturing DC was observed over a wide concentration range, with a strong effect even at 10^{-9} M. This indicates the physiologic relevance of the presently discussed PGE₂ effect, since these and higher PGE₂ levels are found in inflamed tissues (25, 43) or are secreted by tumor cells or tumor stroma (44–46). Although the distinct effect on cytokine profiles was seen at concentrations as low as 10^{-9} M, even the highest concentrations of PGE₂ tested

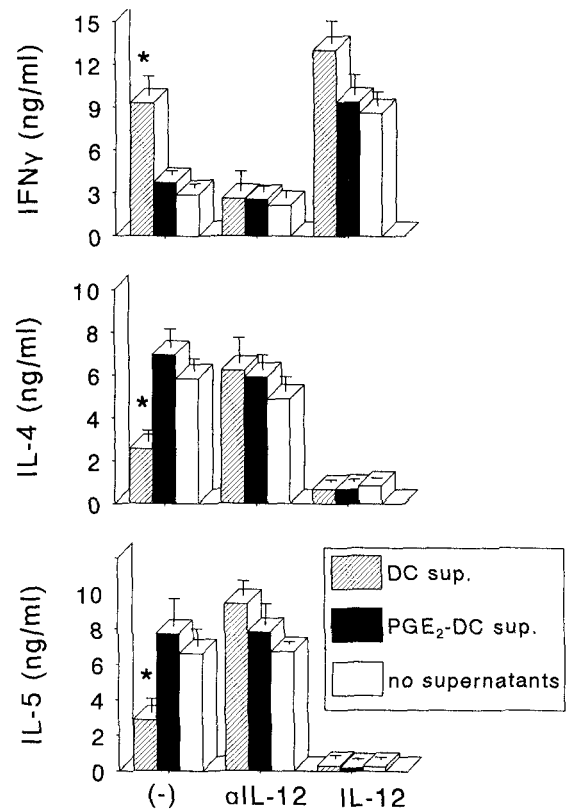


FIGURE 5. Role of DC-derived soluble factors in the differential priming of naive Th cells. Cytokine production in the restimulated Th cells obtained from naive precursors that were primed in the presence of supernatants of SAC-stimulated DC and PGE₂-DC. CD45RA^{high} Th cells were primed with immobilized CD3 mAb in the presence of CD28 mAb (32) and the DC supernatants. As a control, Th cells primed in the absence of supernatants were used. Primary cultures were performed without further additions or were supplemented with IL-12-neutralizing Ab (5 μ g/ml) or rhuIL-12 (200 U/ml), as indicated. Data are shown as the mean (\pm SD) of triplicate priming cultures and represent results obtained in one donor of three that gave similar results. Statistical analysis was performed using Student's test. *, Indicates $p < 0.05$ between the cultures performed in the presence of control DC supernatants vs the cultures performed in the presence of PGE₂-DC supernatants or in the absence of either.

(10^{-6} M) did not prevent the acquisition of dendritic morphology, high expression of class I and II MHC and co-stimulatory molecules by DC, or their high activity in stimulating naive Th cells. Concentrations $>10^{-8}$ M selectively prevented the appearance of CD1a marker and the disappearance of CD14 (Fig. 1B). Such CD1⁻ cells obtained in high concentrations of PGE₂ resembled CD1⁺ dendritic cells in respect to their morphology, potent stimulatory capacity for naive Th cells, and low production of an inflammatory cytokine IL-1 β . Their relation to CD14⁺ DC observed in the epithelia of upper airways (47), the lung (48), the dermis (49), or the inflamed thyroid (50) has yet to be established.

Importantly, the PGE₂-induced IL-12 deficiency in maturing DC is stable for at least 48 h. This stability implies that modulation of the cytokine pattern of DC in tissue compartments will be preserved after emigration of DC to the draining lymph nodes, where they thus contribute to the development of Th2-biased responses.

The stability of PGE₂-induced IL-12 deficiency in maturing DC may also be important for their possible therapeutic application. Since the induction of Ag-specific Th2 responses may prevent the

onset or even ameliorate the ongoing autoimmune processes in EAE and IDDM, murine models of human multiple sclerosis and diabetes (21–23), the immunostimulatory PGE₂-DC of a stably impaired IL-12 production may be used in Ag-specific therapies of Th1-related autoimmune disorders. Interestingly, two recent studies suggested a correlation between elevated PGE₂ production and protection against disease in both EAE and IDDM (51, 52).

The present data suggest that elevated levels of PGE₂ in the tissue promote type 2 Th responses via a stable impairment of IL-12 production in locally maturing DC. In addition to PGE₂, IL-12 levels in DC are probably modified by the factors that modulate IL-12 production in monocytes or macrophages, such as IFN- γ (40, 41), TNF- α (41), IL-10 (37), and TGF- β (37), factors that may be induced in tissues by a variety of signals, including pathogen-derived molecules and tissue damage. It was proposed (53) that tissue-related signals contribute to the initiation of Ag-specific immune responses by enhancement of the stimulatory capacity of local APC. Demonstration of a stable modulation of IL-12-producing capacity in DC by exogenous PGE₂ suggests that such tissue-related signals may also participate in the initial bias of Ag-specific immune responses toward a particular cytokine profile.

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