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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 Cells

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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 (10⁻³-10⁻⁶ 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.


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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, dendritic cells obtained in the presence of prostaglandin E₂; SEA, staphylococcal enterotoxin A; SAC, Staphylococcus aureus Cowan strain I; hae, high level of expression; EAE, experimental autoimmune encephalomyelitis.

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

This study addresses the question of whether the preexposure of DC to PGE$_2$ 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 PGE$_2$, 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 Nordish 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 X 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, Becton Dickinson), CD14 (OKT4; Ortho Diagnostic Systems, San Jose, CA), HLA-DR (L243; Becton Dickinson), HLA-DP (L241; Becton Dickinson), HLA-DQ (IOT-2; Immunotech, Luminex, 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 rHL-12 (sp. act., 170 X 10$^{6}$ U/mg), IL-12 neutralizing mouse mAb (B-T10) was a gift from Dr. Wijdenes (Diaclone, Besancon, France); Human rIL-2 (sp. act., 8 X 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 X 10$^{7}$ U/mg). For the purposes of FACS analysis, FITC-coupled goat Fab’ (1 μg/ml), 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 PGE$_2$-DC, at 10$^{4}$.

**Isolation of monocytes and naive Th cells**

PBMC from healthy donors were isolated from freshly drawn peripheral blood by density centrifugation on Lymphoprep (Nycomed, Trondheim, 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 step of the experiment, 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 Detatchabead 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 PGE$_2$-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 X 10$^{5}$ cells in 1 ml of medium, GM-CSF (500 U/ml), IL-4 (250 U/ml), and different concentrations of PGE$_2$ (Sigma Chemical Co.) were added as indicated. Two-thirds of the medium, containing all factors, were replaced every second day. DC and PGE$_2$-DC were used for the functional studies on day 6. At this point the cultures consisted uniformly HLA-DR-positive cells without any detectable CD3-positive cells. More than 90% of the cells cultured in the absence of PGE$_2$ expressed CD1a marker. All functional studies were performed after removal of PGE$_2$, GM-CSF, and IL-4.

**Induction of proliferative responses in naive Th cells**

In MLR experiments, allogeneic naive Th cells (6 X 10$^{5}$/well) were cocultured with different numbers of 3000-rad irradiated DC, PGE$_2$-DC, or freshly isolated monocytes in 96-well flat-bottom culture plates. Freshly isolated monocytes were cultured 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 stimulants were used: fixed Streptococcus faecalis Cowan strain (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 X 10$^{5}$/200 μl) were irradiated (3000 rad) autologous DC (4 X 10$^{5}$/200 μl) coated with SEA (1 ng/ml). SEA, 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$^{+}$ phenotype (not shown) were harvested, washed, and re-stimulated 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 X 10$^{5}$/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 PGE$_2$-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 recombinant 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, 25 pg/ml), and IL-6 (detection limit, 10 pg/ml) were performed.
the presence of IL-12 during APC-Th cell interaction (14, 38, 39). Because the precursor frequency of conventional Ag-specific Th cells is low in the naive Th population, autologous naive Th (CD4+CD45RAhigh) cells primed by either type of DC increased similarly about 200- to 500-fold. After restimulation with CD3 mAb in the absence of DC, the average IFN-γ production was only 70% 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...
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 (CD45RA\textsuperscript{neg} CD4\textsuperscript{pos}) Th cells. After stimulation with immobilized CD3 mAb and costimulation with CD28 mAb, naive Th cells mature into CD4\textsuperscript{pos} CD45R0\textsuperscript{neg} memory cells, able to produce IFN-\gamma 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 PGE\textsubscript{2}-DC supernatants produced significantly less IFN-\gamma, 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 PGE\textsubscript{2}-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-\gamma levels and decreased IL-4 and IL-5 levels in maturing Th cells, no modulatory effect of PGE\textsubscript{2} supernatants was seen.

Discussion

In this study, we addressed the question of whether elevated levels of PGE\textsubscript{2} affect the maturation of precursor DC and, subsequently, the type of immune response initiated by the PGE\textsubscript{2}-preexposed cells. The key observation is that the naive Th cells primed by
FIGURE 3. DC obtained in the presence of PGE$_2$ are IL-12 deficient, but produce increased levels of IL-10. A, Effect of increasing concentrations of PGE$_2$ on the subsequent ability of maturing DC to produce IL-12 and IL-10 in the absence of PGE$_2$. The data (bars represent the mean ± 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 PGE$_2$-DC (10$^{-7}$ M) 24 h after stimulation with SAC (75 μg/ml), LPS (100 ng/ml), or CD40L (1 μg/ml) in the absence or the presence of IFN-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 (±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 PGE$_2$-DC, stimulated directly after removal of PGE$_2$, or 48 h after removal of PGE$_2$ (see Materials and Methods). Data represent the mean (±SD) of triplicate cultures from one donor. Similar data were obtained in three additional donors.

Control DC or PGE$_2$-DC developed different cytokine profiles. Similarly to several other studies (10, 11), control DC induced the development of memory Th0-like cells, whereas PGE$_2$-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 cell-free 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 PGE$_2$-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 PGE$_2$-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 PGE$_2$-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
milliliters) 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 plasmacytoma 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 (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). Similar 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-1β. 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 DC. In addition to PGE2, tissue promote type 2 Th responses via a stable impairment of IL-12-producing capacity in DC by exogenous PGE2, suggesting that tissue-related signals contribute to the initiation of Th2-related autoimmune disorders. Interestingly, two recent studies suggested a correlation between elevated PGE2 production and prevention of diabetes in NOD mice (54, 55).

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References

human myeloid leukemia cell lines and peripheral blood mononuclear cells.


