Human monocyte-derived dendritic cells are deficient in prostaglandin E2 production

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Abstract Monocyte-derived dendritic cells (moDCs) are increasingly used in clinical settings to stimulate tumor immunity. Prostaglandin E2 (PGE2), which is a member of the eicosanoid family of oxygenated arachidonic acid derivatives generated through the action of cyclooxygenases (COXs), is frequently used to enhance the tumor necrosis factor-α-induced terminal maturation of moDCs. We show here that one effect of interleukin (IL)-4, which is used together with GM-CSF to generate moDCs, is the suppression of endogenous PGE2 production in moDCs. IL-4 inhibits the cytoplasmic form of phospholipase A2, the enzyme that specifically liberates arachidonic acid from membrane phospholipids. Although moDCs failed to mobilize endogenous arachidonic acid, they converted exogenous arachidonic acid into PGE2 in a COX-1- and COX-2-dependent fashion. IL-4-mediated suppression of PGE2 biosynthesis in human moDCs explains the previously reported maturation-enhancing effect of exogenous PGE2. The general suppression of eicosanoid biosynthesis may, however, limit the immunological efficacy of moDCs generated with IL-4. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Dendritic cell; Monocyte-derived; Arachidonic acid; Phospholipase A2; Eicosanoid

1. Introduction

Dendritic cells (DCs) represent a small leukocyte subset that appears to play a central role in the immune system (reviewed by Banchereau and Steinman [1]). DCs can induce and modulate antigen-specific immune responses. In vitro, DCs can be differentiated from $CD14^+$ monocytes in a two-step culture system [2,3]. In the first step, GM-CSF and interleukin (IL)-4 promote the differentiation of immature monocyte-derived DCs (moDCs). In the second step, pro-inflammatory factors induce the terminal maturation of $CD83^+$ immunostimulatory moDCs. We and others have shown that exogenous prostaglandin E2 (PGE2) can enhance the tumor necrosis factor (TNF)-α-induced maturation of moDCs [4,5]. MoDCs have been used in numerous clinical studies to induce or enhance tumor immunity in cancer patients and in some studies PGE2 was used along with TNF-α to generate mature moDCs [6–8].

PGE2 is a member of the eicosanoid family of oxygenated arachidonic acid derivatives [9]. The first step of PGE2 biosynthesis is the release of arachidonic acid from membrane phospholipids by phospholipases such as phospholipase A2 (PLA2) [10]. Macrophages contain at least three different PLA2s, which have no sequence homology: a cytosolic and a secretory PLA2 (cPLA2 and sPLA2), which both require Ca^{2+} as well as a Ca^{2+}-independent PLA2 (iPLA2). cPLA2 is considered a highly specific enzyme for arachidonic acid and generates a major pool of arachidonic acid in response to pro-inflammatory factors [9,10]. Free arachidonic acid is further metabolized towards PGH2 via PGG2 by two cyclooxygenase (COX) isoforms [11]. COX-1 is expressed constitutively in most cell types and generates PGs, which are required for the maintenance of normal physiological functions. In contrast, COX-2 is inducible and is thought to contribute to prostaglandin formation at sites of inflammation. PGE2 synthase finally converts PGH2 into PGE2 [12].

In the present report, we have investigated the ability of moDCs to generate PGE2 from endogenous or exogenous arachidonic acid.

2. Materials and methods

2.1. Material

Lipopolysaccharide (LPS) from *Salmonella abortus equi* and arachidonic acid were obtained from Sigma (Vienna, Austria). Indomethacin (*Liometacen*) was from Chiesi Farmaceutici S.p.a. (Parma, Italy). NS-398 was obtained from Cayman Chemical Company (Ann Arbor, MI, USA). Calcium ionophore A23187 was purchased from Sigma.

2.2. Generation of moDCs

DCs were generated from monocytes in the presence of GM-CSF and IL-4 as described previously [2–4]. $CD14^+$ monocytes were affinity-purified from peripheral blood mononuclear cells using the MACS CD14 isolation-kit (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Monocytes (1×10^6 cells) were subsequently cultured in 1 ml of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (FCS), 50 U/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM pyruvate, and 5×10^{-5} M 2-mercaptoethanol as well as 1000 U/ml of each GM-CSF and IL-4. Monocytes were also cultured with GM-CSF alone (GM-MOs). Monocytes could not be cultured with IL-4 alone. Cells were fed on day 2 with 1 ml of fresh medium and the cytokines indicated. Day 5 moDCs or GM-MOs were stimulated with LPS at the indicated concentrations for 24–48 h.

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2.3. PGE2 measurement

PGE2 was measured by a radioimmunoassay (Biotrak™ PGE2 (^{125}I) assay system, Amersham Pharmacia Biotech, UK) according to the manufacturer's instructions. Culture supernatants (0.5 ml) were mixed with one volume of water:ethanol (1:4) and 10 μl of glacial acetic acid and applied to a Amprep C18 minicolumn, which had been primed with ethanol (10% in water). PGE2 was eluted using ethylacetate and dried under a stream of nitrogen. PGE2 was reconstituted in phosphate-buffered saline (PBS), pH 7.0, containing 0.1% gelatin. Methyl oximation reagent was added and derivation was performed overnight at room temperature. In this assay, PGE2 present in the test sample is converted by methyloxiamine hydrochloride to the stable methyl oximate derivative and competes with a fixed quantity of ^{125}I-labeled PGE2 tracer (methyl oximate derivative) for the limited number of binding sites of an antiserum specific for the methyl oximate derivative of PGE2. An aliquot of the processed test sample was mixed with tracer and antiserum in assay buffer and incubated for 2 h at 25°C in a water bath. The antibody-bound fraction was separated using magnetic separation. After 15 min separation on an Amerlex-M separator base, the supernatants were discarded. Radioactivity present in each tube was determined by counting for at least 60 s in a gamma scintillation counter. The amount of PGE2 was calculated by comparing the percentage binding of the test sample with a standard curve.

2.4. Western blotting analysis

On day 5, the cells were treated with 100 ng/ml of LPS for 20 h. Cells were harvested, washed two times with ice-cold PBS, lysed in ice-cold buffer containing 50 mM Tris–HCl (pH 7.3), 5 mM EDTA, 50 mM NaCl, 5 mM $Na_4P_2O_7{\cdot}10\ H_2O$, 5 mM NaF, 5 mM Na_3VO_4, 2% Triton X-100, 1 mM PMSF, 5 μg/ml aprotinin and 5 μg/ml leupeptin. Lysates were left on ice for 10 min and were subsequently clarified by centrifugation at 10 000 rpm for 15 min at 4°C. Supernatants were collected and 20 μg of proteins was separated on an 8% SDS–PAGE and transferred to a PVDF membrane (Immobilon-P, Millipore). After blocking in 1% non-fat dry milk, TBST membranes were incubated with anti-cPLA2 antibody (4-4B-3C, Santa Cruz Biotechnology) followed by horseradish peroxidase-conjugated goat anti-mouse IgG, washed and developed with enhanced chemoluminescence (ECL Western blotting kit, Amersham Life Sciences).

2.5. Immunoprecipitation

cPLA2 was immunoprecipitated using rabbit polyclonal anti-cPLA2 antibody (N-216, Santa Cruz Biotechnology) and Protein A Micro-Beads according to the manufacturer's instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). Immunoprecipitated proteins were separated on an 8% SDS–PAGE and transferred to a PVDF membrane. Immunostaining was performed using an anti-phosphotyrosine (PY20; Santa Cruz Biotechnology, CA, USA) or an anti-phosphoserine antibody (PSR-45; Sigma, Vienna, Austria) and the immunoreactive band was visualized using ECL.

2.6. Measurement of [^{3}H]arachidonic acid release

Radiolabeling of moDCs was performed by incubating day 5 moDCs with 0.5 μCi/ml [5,6,8,9,11,12,14,15-^{3}H]arachidonic acid (specific activity 100 Ci/mmol; NEN Life Science Products) overnight in culture medium containing 1% FCS. [^{3}H]Arachidonic acid that had not been incorporated was removed by washing the cells four times with culture medium containing 1% FCS. An aliquot of the labeled cells was removed to determine the incorporated radioactivity by scintillation counting. Incorporation of [^{3}H]arachidonic acid was similar in GM-MOs and moDCs. The labeled cells were then replated in 96-well plates (2×10^5 cells in 200 μl) in culture medium containing 10% FCS before the addition of increasing concentrations of LPS (0–1000 ng/ml). Supernatants were removed after 30 min and 20 h, respectively, cleared by centrifugation and assayed for radioactivity by liquid scintillation counting. Results are mean values ± S.D. of duplicate measurements.

3. Results and discussion

To examine the ability of moDCs to produce PGE2 from endogenous arachidonic acid, day 5 moDCs were stimulated with increasing concentrations of LPS (0–1000 ng/ml) and PGE2 was measured in culture supernatants after 48 h using a radioimmunoassay. To study the role of IL-4, monocytes were also cultured with GM-CSF alone (GM-MOs) [13]. While GM-MOs produced large amounts of PGE2 in response to LPS stimulation (Fig. 1A), the PGE2-producing capacity was strongly diminished in moDCs (Fig. 1B) indicating that IL-4 suppresses PGE2 biosynthesis in human moDCs.

To determine whether liberation of arachidonic acid from membrane phospholipids was limiting PGE2 production, moDCs were matured with LPS for 48 h and then incubated with exogenous arachidonic acid for 30 min. MoDCs converted exogenous arachidonic acid into PGE2 in a dose-dependent fashion (Fig. 1C). The non-selective COX inhibitor indomethacin strongly blocked PGE2 formation by moDCs from exogenous arachidonic acid (Fig. 1D), whereas the COX-2-selective inhibitor NS-398 reduced PGE2 synthesis by approximately 50% indicating that both isoforms contributed substantially.

Since cPLA2 is regulated by an increase in intracellular calcium, which binds to an amino-terminal C2 domain and induces its translocation to the nuclear envelope and endoplasmic reticulum [9], we also tested the influence of the highly selective calcium ionophore A23187. However, A23187 failed

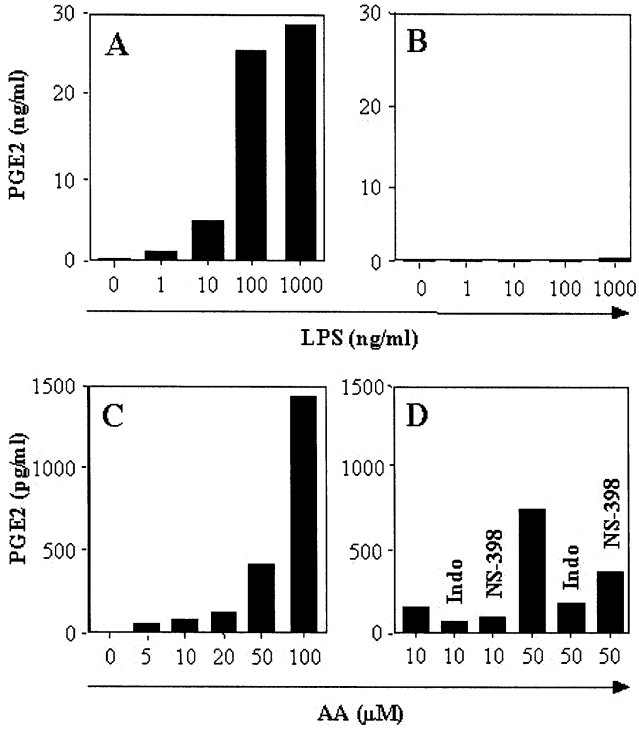

Fig. 1. IL-4 suppresses PGE2 production from endogenous arachidonic acid in human moDCs: conversion of exogenous arachidonic acid into PGE2. A: Monocytes were cultured with GM-CSF alone (GM-MOs) or (B) with GM-CSF plus IL-4 (moDCs). On day 5, cells were stimulated with different concentrations of LPS. After 48 h, supernatants were harvested and analyzed for PGE2 contents. C: After LPS-induced maturation for 48 h moDCs were harvested, washed and resuspended in fresh medium (3×10^5 moDCs/ml) containing arachidonic acid (AA) at the indicated concentrations. After a 30 min incubation, the cells were centrifuged and PGE2 was measured in the supernatant. D: MoDCs were incubated for 30 min with the non-selective COX inhibitor indomethacin (10 μM) or with the COX-2-selective inhibitor NS-398 (10 μM) prior to the addition of arachidonic acid. Results are from one experiment representative of four independent experiments.

to stimulate PGE2 production in GM-MOs and in moDCs, respectively (not shown).

The failure to generate PGE2 from endogenous arachidonic acid and the ability to convert exogenous arachidonic acid into PGE2 suggested that moDCs were deficient in cPLA2 activity. Previous work has reported inhibitory effects of IL-4 on cPLA2 activity [14]. We therefore compared cPLA2 expression and activity in human moDCs (GM-CSF plus IL-4) and in GM-MOs (GM-CSF only). Western blotting experiments revealed that although cPLA2 protein could also be detected in moDCs, the level of cPLA2 protein expression was significantly reduced in moDCs as compared to GM-MOs (Fig. 2A). Since cPLA2 activation has been shown to require cPLA2 phosphorylation [15], we also used antibodies directed against phosphotyrosine and phosphoserine to examine the phosphorylation state of cPLA2. For this purpose, cPLA2 was immunoprecipitated from GM-MOs and moDCs, respectively, and subjected to gel electrophoresis followed by membrane blotting and staining for either phosphotyrosine or phosphoserine. The comparatively weak phosphotyrosine and phosphoserine staining of cPLA2 immunoprecipitated from moDCs as compared to cPLA2 from GM-MOs (Fig. 2A) only reflected the reduced level of cPLA2 in moDCs but did not indicate a suppression of cPLA2 phosphorylation by IL-4.

Finally, we compared the ability of radiolabeled moDCs to liberate [^{3}H]arachidonic acid in response to LPS stimulation. Arachidonic acid mobilization by macrophages and mast cells usually occurs in two phases [16]. The immediate phase, which is characterized by a burst of arachidonic acid liberation, takes minutes. The delayed phase, which spans several hours, depends on the continuous supply of arachidonic acid and involves cPLA2 activation. GM-MOs and moDCs incorporated similar amounts of [^{3}H]arachidonic acid (approximately 20 000 cpm/2×10^5 cells). While GM-MOs induced the expected delayed arachidonic acid mobilization response, detectable at 20 h but not at 30 min, moDCs failed to mount a similar response (Fig. 2B). Taken together, these data suggested that only GM-MOs but not moDCs were able to induce cPLA2 activity upon LPS stimulation and that IL-4 prevented the induction of cPLA2 protein and enzyme activity.

In conclusion, we have shown that IL-4 causes suppression of PGE2 production in human moDCs. The failure of moDCs to generate PGE2 also explains the previously reported effects of exogenous PGE2, which cooperates with TNF-α to promote the terminal maturation of CD83$^+$ immunostimulatory moDCs [4,5]. PGE2 is both product and mediator of inflammation in vivo. PGE2 plays a major role in tissue edema, hyperalgesia and IL-6 production at sites of inflammation [17] and inhibitors of COX, the key enzyme in PGE2 production, are used clinically to treat inflammatory diseases [11]. Thus, it is tempting to argue that PGE2 also contributes to DC activation in vivo.

Since cPLA2 appears to be a major target of the suppressive effect of IL-4, moDCs must probably also fail to generate other arachidonic acid derivatives such as leukotrienes [9]. Spanbroek and colleagues recently demonstrated that IL-4 also suppresses 5-lipoxygenase in human moDCs [18]. Thus, a double blockade induced by IL-4 appears to prevent leukotriene formation in moDCs. IL-4 prevents the liberation of arachidonic acid by suppressing cPLA2 activity (Fig. 2). In addition, IL-4 prevents the conversion of arachidonic acid towards leukotrienes by suppressing 5-lipoxygenase [18]. However, leukotrienes may play an important role in DC biology. Leukotriene C4 has been shown to be important for the CCR7-directed migration of DCs to lymph nodes [19] and leukotriene B4 plays a role in B [20] and natural killer (NK) cell activation [21]. Thus, failure to produce leukotrienes such as leukotriene B4 and C4 may hamper moDC migration as well as the ability of moDCs to activate B and NK cells [22].

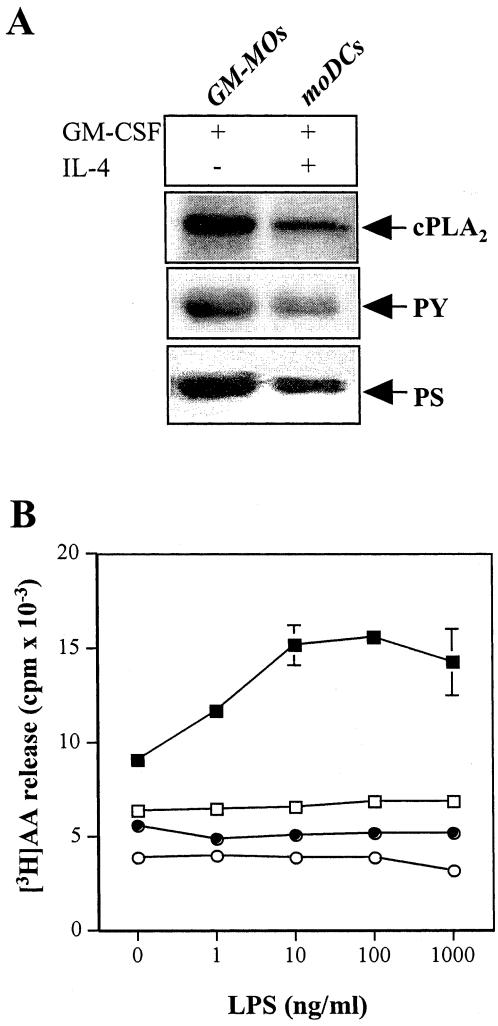

Fig. 2. IL-4 suppresses cPLA2 protein expression and enzyme activity in human moDCs. Monocytes were cultured with GM-CSF alone (GM-MOs) or with GM-CSF plus IL-4 (moDCs). A: Cells were treated with 100 ng/ml of LPS for 20 h. Lysates (20 μg) were separated by SDS–PAGE and immunoblots were stained with an anti-cPLA2 antibody. cPLA2 phosphorylation was examined after immunoprecipitation. cPLA2 was immunoprecipitated from cell extracts using anti-cPLA2 antibodies, subjected to SDS–PAGE and immunoblots were stained with an anti-phosphotyrosine antibody (PY) or an anti-phosphoserine antibody (PS). B: GM-MOs or moDCs were labeled with [^{3}H]arachidonic acid. Similar amounts of [^{3}H]arachidonic acid were incorporated by GM-MOs and moDCs (approximately 20 000 cpm per 2×10^5 cells). The LPS-induced release of arachidonic acid from GM-MOs (squares) and moDCs (circles) was measured after 30 min (open symbols) and 20 h (closed symbols). Results are mean values ± S.D. of duplicate measurements. One out of three experiments with similar results is shown.

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