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Prostaglandins Inhibit 5-Lipoxygenase-Activating Protein Expression and Leukotriene B₄ Production from Dendritic Cells Via an IL-10-Dependent Mechanism¹

Hedi Harizi, Monique Juzan, Jean-François Moreau, and Norbert Gualde²

PGs produced from arachidonic acid by the action of cyclooxygenase enzymes play a pivotal role in the regulation of both inflammatory and immune responses. Because leukotriene B₄ (LTB₄), a product of 5-lipoxygenase (5-LO) pathway, can exert numerous immunoregulatory and proinflammatory activities, we examined the effects of PGs on LTB₄ release from dendritic cells (DC) and from peritoneal macrophages. In concentration-dependent manner, PGE₁ and PGE₂ inhibited the production of LTB₄ from DC, but not from peritoneal macrophage, with an IC₅₀ of 0.04 μM. The same effect was observed with MK-886, a 5-LO-activating protein (FLAP)-specific inhibitor. The decreased release of LTB₄ was associated with an enhanced level of IL-10. Furthermore, the inhibition of LTB₄ synthesis by PGs was significantly reversed by anti-IL-10, suggesting the involvement of an IL-10-dependent mechanism. Hence, we examined the effects of exogenous IL-10 on the 5-LO pathway. We demonstrate that IL-10 suppresses the production of LTB₄ from DC by inhibiting FLAP protein expression without any effect on 5-LO and cytosolic phospholipase A₂. Taken together, our results suggest links between DC cyclooxygenase and 5-LO pathways during the inflammatory response, and FLAP is a key target for the PG-induced IL-10-suppressive effects. The Journal of Immunology, 2002, 169: 139–146.

Prostaglandins and leukotrienes (LTs)³ are potent eicosanoid lipid mediators derived from phospholipase-released arachidonic acid (AA) that are involved in numerous homeostatic biological functions and inflammation (1). They are produced predominately by cells of myeloid origin (2), by the action of cyclooxygenase (COX) isozymes and 5-lipoxygenase (5-LO), respectively (3). For many years, macrophages have been considered as a major source of eicosanoids. More recently, we have reported that the in vitro generated dendritic cells (DC) produce a full range of eicosanoids, in particular PGE₂ and LTB₄ (4).

The 5-LO pathway is the major route of AA metabolism leading to the formation of LTs (5). In the first steps of LT biosynthesis, AA liberated by the action of phospholipase A₂ (PLA₂), in particular cytosolic PLA₂ (cPLA₂), is transferred to 5-LO by 5-LO-activating protein (FLAP) (6, 7). Upon activation, 5-LO translocates to the nuclear envelope, in which FLAP is localized (8, 9). FLAP binds free AA, presents it to 5-LO, and thus makes easier 5-LO substrate interaction (10). The 5-LO catalyzes the insertion of molecular oxygen into AA to form 5-hydroxyperoxyeicosatetraenoic acid as well as its subsequent dehydration to LTA₄ (11, 12). LTA₄ is then modified by LTA₄ hydrolase to generate LTB₄ (13).

LTB₄ is a powerful leukotrophic, proinflammatory, and immunoregulatory mediator (14, 15). The overproduction of LTB₄ plays an important role in the pathogenesis of a variety of inflammatory diseases, including asthma, glomerulonephritis, psoriasis, inflammatory bowel diseases, and acute lung injury (16, 17). Considering their role in host defense against microbial infections (18) and their important pathophysiologic roles in inflammatory states, synthesis of LTs must be highly regulated. Two steps seem to be involved in the control of the formation of these lipid mediators, that is, liberation of AA and regulation of 5-LO activity (19). Moreover, the expression of 5-LO and FLAP, which is subject to regulation in monocytic cells (20), has been considered as the major determinant of cellular LT synthesis (21).

The interactions between eicosanoids may represent means to regulate the release of inflammatory mediators, and may be important for the regulation of cell functions and inflammatory disorders, such as allergic asthma. Previous studies have reported that eicosanoid products of AA metabolism are important modulators of macrophage COX and 5-LO pathways (22). For example, macrophage 5-LO activity may be inhibited by some metabolites of the lipooxygenase pathways (23, 24). PGE₂ can modulate the synthesis and metabolism of other lipid mediators (25). It also has been reported that in rat neutrophils, PGE₁ or PGE₂ (26) could inhibit FMLP-induced synthesis of LTB₄. Although many studies involving eicosanoids have been performed on macrophages, little is known about the interactions between these lipid mediators in DC and the mechanism by which PGs may inhibit 5-LO metabolism. Because a report related the inhibited production of macrophage LTB₄ production by limiting AA availability (27), expression of 5-LO and FLAP by human DC has been noted (28, 29).

High concentrations of exogenous AA have been shown to activate 5-LO enzyme activity as well as providing substrate for LT synthesis (30, 31). However, it has been reported that exogenous AA did not modify macrophage eicosanoid release (32). Early theories have suggested that the blockade of COX pathways by non-steroidal anti-inflammatory drugs causes shunting of AA into the

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³ Abbreviations used in this paper: LT, leukotriene; AA, arachidonic acid; CM, complete medium; COX, cyclooxygenase; PLA₂, phospholipase A₂; cPLA₂, cytosolic PLA₂; DC, dendritic cell; 5-LO, 5-lipoxygenase; FLAP, 5-LO-activating protein; NDGA, nor-dihydroguaiaretic acid; PM, peritoneal macrophage; PMN, polymorphonuclear leukocyte; TXB₂, thromboxane B₂.
5-LO pathway, resulting in the production of high levels of LTB₄ and symptoms of asthma. However, the demonstration of the bronchoprotective effect of inhaled PGE₂ against aspirin-induced bronchospasm has suggested the inhibitory effect of PGE₂ on the 5-LO pathway (33).

Because the interactions between COX and 5-LO pathways may regulate tissue homeostasis and contribute to the modulated production of AA metabolites, we have examined the effects of PGs on the 5-LO pathway, and the mechanism by which PGs modulate 5-LO metabolism. We found that LTB₄ does not affect COX expression and PGE₂ production from either DC or peritoneal macrophage (PM). By contrast, PGE₂ decreases the production of LTB₄ from DC. In concentration-dependent manner, PGE₂ enhances the production of endogenous IL-10, which inhibits FLAP protein expression and LTB₄ production without any effect on cPLA₂ and 5-LO protein levels. We obtained the same results with exogenous IL-10, but not with IL-6. Compared with DC, PM exhibited no suppression by PGs of 5-LO metabolism and no induction of endogenous IL-10. Together with the effects of anti-IL-10, our study demonstrates that PGE₂ down-regulates DC 5-LO metabolism by inhibiting FLAP protein expression via an IL-10-dependent mechanism.

Materials and Methods

**Media, reagents, and cell cultures**

Complete medium (CM) was RPMI 1640 (Life Technologies, Paisley, U.K.) supplemented with 10% heat-inactivated FCS (Dominique Dutcher, Brumath, France), 1% streptomycin (Life Technologies; 1000 μg/ml), 2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO), 50 μM 2-ME (Sigma-Aldrich), and 2 mM sodium pyruvate (Life Technologies). DC were generated from mouse bone marrow cells, as we have reported previously (34). Briefly, bone marrow cells isolated from BALB/c mice (Iffa Credo, Lyon, France) were cultured in CM in the presence of GM-CSF (20 ng/ml) and flt3, bone marrow cells isolated from BALB/c mice (Iffa Credo, Lyon, France) were cultured in CM in the presence of GM-CSF (20 ng/ml) and 2 mM sodium pyruvate (Life Technologies). DC were generated from mouse bone marrow cells, as we have reported previously (34). By contrast, PGE₂ enhances the translocation of 5-LO and cPLA₂ to the nuclear envelope and increases the release of LTB₄.

**Preparation of cytoplasmic and nuclear extracts and Western blot analyses**

Cytoplasmic lysates were prepared, as described previously (35). Briefly, following the designated treatments, PM and the in vitro generated DC obtained at 98% purity were washed twice with PBS and lysed in ice-cold lysis buffer containing 10 mM HEPES (pH 7.6), 3 mM MgCl₂, 40 mM KC₁, 2 mM DTT, 0.5% Nonidet P-40, 8 μg/ml leupeptin, and 10 μg/ml PMSF. Nuclei were removed by centrifugation (1250 × g at 4°C for 5 min). Nuclei were then resuspended and sedimented twice in the lysis/extraction buffer to avoid contamination by cytoplasmic proteins. Nuclear extracts were prepared from the pelleted nuclei in the nuclear extraction buffer containing 10 mM HEPES (pH 7.9), 0.1 mM EGTA, 1.5 mM MgCl₂, 420 mM NaCl, 0.5 mM DTT, 0.5 mM PMSF, and 25% glycerol. After incubation in ice for 20 min, cellular debris were removed by centrifugation (1250 × g at 4°C for 5 min). The bicinchoninic acid protein assay (Pierce, Rockford, IL) was used to analyze protein concentration. Nuclear and cytoplasmic extracts (15 μg protein/lane) were resolved on SDS-polyacrylamide gels, and Western blotting analyses were performed using an ECL kit (Amersham, Little Chalfont, U.K.). The blots were probed with specific Abs directed against cPLA₂ (1/1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), 5-LO (1/500 dilution; BD Transduction Laboratories, Lexington, KY), and α-tubulin (2/10,000; Sigma-Aldrich). The rabbit polyclonal antiserum to FLAP, which have been extensively characterized (8), was generously provided by J. F. Evans (Merck Frosst Center for Therapeutic Research, Pointe Claire-Dorval, Quebec, Canada). Used at 1/300 dilution, the antiserum to FLAP (designated H1-4) was raised against the thyroglobulin conjugate of α residues 41–52 of FLAP. This region is completely conserved between human and murine FLAP. Protein bands were detected with the ECL Western blotting analysis system from Amersham.

**Assessment of IL-10 production and effects of anti-IL-10 and exogenous cytokines on LTB₄ release**

IL-10 level was measured using the commercially available ELISA kit, according to the manufacturer’s instructions (Quantikine mouse IL-10 immunoassay; R&D Systems, Abingdon, U.K.), in the supernatants of cells treated for 18 h with increasing concentrations of TXB₂ or PGE₂. The detection limit of the endogenously produced IL-10 is 4 pg/ml. To investigate whether PGE₂-induced IL-10 mediated suppression of LTB₄ production, PGE₂-treated cells were incubated for 18 h with 100 ng/ml of anti-IL-10-neutralizing Ab (purified rat anti-mouse IL-10 mAb, clone JES5-16E3; BD Pharmingen, San Diego, CA) or isotype control (purified rat IgG1 mAb clone 5E10 IP-2D19; BD Pharmingen). The effects of exogenous cytokines on LTB₄ production from A-23187-stimulated cells treated for 18 h with graded concentrations of IL-10 or IL-6 (mouse rIL-10, rIL-6; PeproTech, London, U.K.). The detection limits for PGE₂ and LTB₄ determined in the supernatants of cells treated for 18 h with graded concentrations of IL-10 or IL-6 (mouse rIL-10, rIL-6; PeproTech) are 4 pg/ml.

**Assessment of IL-10 production and effects of anti-IL-10 and exogenous cytokines on LTB₄ release**

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**Effect of exogenous LTB₄ on COX pathways**

To investigate the interaction between COX and 5-LO pathways, DC and LPS-stimulated DC were treated with increasing concentrations of exogenous LTB₄ (Sigma-Aldrich) for 18 h. The various concentrations used did not affect the number of viable cells quantified by MTT assay. At the end of culture period, cells were collected for the analysis of COX protein expressions by Western blotting, as we have recently reported (4), and supernatants were used for the measurement of PGE₂ production by ELISA.

**Cell viability**

The viability of DC and PM was measured using the MTT assay, which is routinely used in our laboratory. In brief, cells (4 × 10⁶/ml) were incubated in flat-bottom 96-well plates in the presence of 5 mg/ml MTT (Sigma-Aldrich) for 4 h at 37°C. The precipitates were dissolved by adding 0.115 ml of 0.015 M isopropyl alcohol containing 1% formic acid, and the absorbance was measured at 570 nm (Tiettek Lasksystems Multiskan reader, Turku, Finland).

**Statistical analysis**

The results are expressed as the mean ± SEM. Analysis of data was performed using Student’s t test; p < 0.01 was considered significant.

**Results**

A-23187 enhances the translocation of 5-LO and cPLA₂ to the nuclear envelope and increases the release of LTB₄

Compared with control cells, the incubation of DC with calcium ionophore A-23187 for 18 h had no significant effect on the percentage of viable cells (96%) quantified by MTT assay. Because we knew little about the intracellular location of the LT enzymatic pathway, resulting in the production of high levels of LTB₄ and symptoms of asthma. However, the demonstration of the bronchoprotective effect of inhaled PGE₂ against aspirin-induced bronchospasm has suggested the inhibitory effect of PGE₂ on the 5-LO pathway (33).
FIGURE 1. Calcium ionophore A-23187 stimulates the translocation of 5-LO and cPLA$_2$ to the nuclear envelope and enhances the production of LTB$_4$ from endogenous AA. Cells (1 $\times$ 10$^6$/ml) were cultured for 18 h in CM with (Activated) and without (Resting) 5 $\mu$M of calcium ionophore A-23187. Subsequently, A, cellular expression of 5-LO, FLAP, and cPLA$_2$ was analyzed by Western blot in the nuclear (N) and cytoplasmic (C) extracts from resting and activated cells. B, PGE$_2$ and LTB$_4$ production was measured in cell-free supernatants by ELISA. One of six representative Western blots is shown. Each value of eicosanoid level represents the mean ($\pm$SEM) of six independent experiments.

Role of FLAP in 5-LO metabolism

Several lines of evidence have advanced a model for the initiation of LT biosynthesis in which FLAP plays a critical role in LT production. This evidence includes studies of LT biosynthesis in cells transfected with transgenes encoding 5-LO and FLAP (38), as well as studies of LT inhibitors, such as MK-886. MK-886 is known to bind to FLAP and prevent the synthesis of LTs from endogenous AA in intact cells, by blocking membrane association of 5-LO (39). However, other reports state that MK-886 may inhibit LT biosynthesis without any effect on membrane association (9, 40), suggesting that LT biosynthesis can be a two-step process consisting of FLAP-independent binding of 5-LO to the membrane of the nuclear envelope, followed by FLAP-dependent activation of the enzyme. In our study, treatment of A-23187-stimulated DC with graded concentrations of MK-886, a FLAP-specific inhibitor (Fig. 3A), or NDGA, a 5-LO inhibitor (Fig. 3B), concentration dependently decreases the production of LTB$_4$ with an IC$_{50}$ of 2.8 nM and 5 $\mu$M, respectively. There is no significant difference between DC and PM in terms of drug-inhibitory effect on 5-LO metabolism (data not shown). A concentration of 4 nM of MK-886, which did not affect cell viability, totally suppresses LTB$_4$ release from endogenous AA. However, we observed no effect of MK-886 on the production of PGE$_2$, suggesting that the blockade of FLAP did not affect COX metabolism. The same results were observed with NDGA.

Effect of COX inhibitors on the production of eicosanoids from DC and PM

The in vitro generated DC have all the enzymatic tools to synthesize PGE$_2$ and LTB$_4$, the major COX and 5-LO metabolites, respectively. The production of PGE$_2$ and LTB$_4$ did not require the addition of exogenous substrate, because DC possess an active cPLA$_2$, which catalyzes the liberation of endogenous AA from membrane phospholipids. As shown in Fig. 2B (Control), DC produce low levels of PGE$_2$ and LTB$_4$, when compared with PM (Fig. 2A, Control), from which production of LTB$_4$ is 2-fold higher. In addition, results obtained in Fig. 2A show that PM COX and 5-LO pathways prevail in terms of eicosanoid production from endogenous AA. However, in DC, COX metabolism of endogenous AA is greater than 5-LO pathway, in particular when cells were stimulated with LPS (Fig. 2B). In fact, we have recently demonstrated that following LPS stimulation, DC strongly express COX-2 protein, the predominant isoform of COX, which induces a production of high levels of PGE$_2$ (4). The production of PGE$_2$ from LPS-stimulated DC is 3-fold higher when compared with control cells (Fig. 2B). The blockade of COX pathway by COX inhibitors significantly increased the production of LTB$_4$ from DC (+100%), but not from PM (Fig. 2). These results suggest that COX metabolites, in particular PGE$_2$, may inhibit the production of LTB$_4$ from DC. These observations argue in favor of links between COX and 5-LO pathways in DC. However, these two oxidative pathways of AA metabolism seem to be independent in PM.

FIGURE 2. COX inhibitors enhance the production of LTB$_4$ from DC, but not from PM. A-23187-stimulated cells (1 $\times$ 10$^6$/ml) were incubated for 18 h with 1 $\mu$g/ml LPS in the absence and the presence of COX inhibitors. Control included activated cells cultured in CM alone. The production of PGE$_2$ and LTB$_4$ was measured by ELISA in cell-free supernatants from PM (A) and DC (B). Each value represents the mean ($\pm$SEM) of six independent experiments.
the 5-LO enzyme. Interestingly, we found that the exogenously added AA failed to significantly enhance the production of LTB₄ from DC, and did not significantly reverse the inhibitory effect of PGE₂ on 5-LO metabolism (Fig. 4C). Although the inhibitory effect of PGE₂ on the biosynthesis of LTs from endogenous AA is clearly established, the modulation of 5-LO metabolism from exogenous substrate appears to be complex. Our results, however, suggest that PGs did not inhibit 5-LO metabolism by limiting substrate availability. Together with the results obtained with COX inhibitors, we demonstrate that the shunting of AA into 5-LO pathway did not explain the increased level of LTB₄ following the treatment of cells with COX inhibitors, and PGs down-regulate LTB₄ release via an AA-independent mechanism.

**PGE₂ enhances the production of endogenous IL-10 from DC, but not from PM**

Supernatants from various culture conditions (see Materials and Methods) were analyzed by ELISA to determine the production of IL-10 and IL-6 in response to prostanoid treatment. As shown in Fig. 5A, TXB₂ has no effect on IL-10 release from DC and from PM. We also observed no effect on IL-6 release (data not shown). However, when DC were cultured for 18 h in the presence of graded concentrations of PGE₂, significant stimulation of endogenous IL-10 was observed in DC, but not in PM (Fig. 5B). The same results were obtained with PGE₁ (data not shown). A concentration of 1 μM of PGE₂, which causes a 94% decrease in LTB₄ release (see Fig. 4B), resulted in the production of 2 ng/ml...
of endogenous IL-10. In contrast, PGE₂ lacked any significant effect on the production of IL-6 (data not shown).

Inhibition of LTB₄ release by PGE₂ was totally reversed by anti-IL-10

To determine whether the increase in IL-10 production by PGE₂ was responsible for its inhibitory effect on 5-LO metabolism of endogenous AA, PGE₂-treated DC were incubated in the presence of 100 ng/ml anti-IL-10 or isotype control Abs for 18 h. As we reported before (4), the concentration of 100 ng/ml anti-IL-10 induced the neutralization of >95% of the endogenously induced IL-10. Results obtained in Fig. 6 showed that the inhibitory effect of PGE₂ on LTB₄ release was totally reversed by anti-IL-10, but not by isotype control, suggesting the involvement of an IL-10-dependent mechanism in the inhibition of DC LTB₄ release by PGs.

Effects of exogenous IL-10 and PGE₂ on the expression of cPLA₂, FLAP, and 5-LO proteins

We next determined whether PGE₂-mediated inhibition of LTB₄ release is associated with changes in 5-LO, FLAP, or cPLA₂ protein expressions. Western blot analyses showed that PGE₂ had no effect on the expression of 5-LO either in DC or in PM (Fig. 7A).

It also did not affect the cPLA₂ protein expression and AA release from DC (data not shown). In contrast, incubation of cells with increasing concentrations of PGE₂ inhibits, in concentration-dependent manner, the expression of FLAP protein in DC, but not in PM. A reproducible and representative Western blot is shown in Fig. 7B. The same results were obtained when cells were incubated for 18 h with graded concentrations of exogenous IL-10 (Fig. 8A), which caused a concentration-dependent inhibition of LTB₄ release from DC (Fig. 8B). A concentration of 1 ng/ml IL-10 significantly down-regulates FLAP protein expression and decreases (minus 80%) LTB₄ production from A-23187-stimulated DC. However, no effect on the expression of either FLAP or 5-LO proteins was observed in response to exogenous IL-6 (data not shown).

Effects of exogenous IL-10 and PGE₂ on the expression of cPLA₂, FLAP, and 5-LO proteins

We next determined whether PGE₂-mediated inhibition of LTB₄ release is associated with changes in 5-LO, FLAP, or cPLA₂ protein expressions. Western blot analyses showed that PGE₂ had no effect on the expression of 5-LO either in DC or in PM (Fig. 7A).

Exogenous PGE₂ concentration dependently inhibits the expression of FLAP in DC, but not in PM, without any effect of 5-LO. PM and DC were treated with graded concentrations of PGE₂ as described in Materials and Methods. After 18 h of incubation, the expression of FLAP and 5-LO protein was analyzed by Western blot in the nuclear extracts. A. No change in the 5-LO-immunoblotting signal was observed either in PM or in DC. B, PGE₂ decreases in concentration-dependent fashion the expression of FLAP in DC, but not in PM. One of six representative Western blots is shown.

Exogenous PGE₂ concentration dependently inhibits the expression of FLAP in DC, but not in PM, without any effect of 5-LO. PM and DC were treated with graded concentrations of PGE₂ as described in Materials and Methods. After 18 h of incubation, the expression of FLAP and 5-LO protein was analyzed by Western blot in the nuclear extracts. A. No change in the 5-LO-immunoblotting signal was observed either in PM or in DC. B, PGE₂ decreases in concentration-dependent fashion the expression of FLAP in DC, but not in PM. One of six representative Western blots is shown.

LTB₄ does not affect COX expression and PGE₂ production from DC. DC and DC stimulated with 1 µg/ml LPS were incubated in the presence of increasing concentrations of exogenous LTB₄, which had no effect on the cell viability. A, The expression of both isoforms of COX was analyzed in the cytoplasmic extracts, as we have recently reported (4), and B, the production of PGE₂ was assessed by ELISA in the cell-free supernatants. One of six representative Western blots is shown. Each value represents the mean (±SEM) of six independent experiments.
shown). Taken together, our results demonstrate that FLAP is a target for the PG-induced IL-10-suppressive effects.

**LTB₄ does not affect COX pathways**

The findings that PGE₂ release is associated with inflammatory states suggest that other lipid mediators, such as LTB₄, might modulate its production. Moreover, the 5-LO metabolite of AA, LTB₄, has been shown to exert numerous proinflammatory activities, such as the induction of IL-6 gene expression and cytokine release from human monocytes (41). Recently, we have shown that LTB₄ had no effect on the production of IL-10 and IL-12 from DC (4). When we examined the effect of LTB₄ on the COX pathways, we found that this 5-LO metabolite did not affect COX expression (Fig. 9A) and PGE₂ production (Fig. 9B) from in vitro generated DC. These results confirm the effects of MK-886 and NDGA used to block the endogenous biosynthesis of LTB₄ (Fig. 3), and demonstrate that the endogenously produced or the exogenously added LTB₄ had no effect on COX metabolism.

**Discussion**

Considering the many actions of the PGs and LTs, some in mimicry and some in opposition to each other, it is reasonable to expect important interactions to occur between these two groups of compounds. This is truer in view of the fact that products of both pathways derive from the same precursor, AA, and possibly even from the same substrate pool. The inflammatory response induced by AA could be virtually eliminated by the COX inhibitor, indomethacin, in 5-LO-deficient mice, but not in normal animals, suggesting links between COX systems and 5-LO pathway during inflammatory reaction (42).

There are a number of reports that nonsteroidal anti-inflammatory drugs, acting by inhibiting PG synthesis, enhance LT biosynthesis (43, 44), and the existence of a group of people who respond to aspirin and other such drugs with symptoms of asthma emphasizes the importance of these phenomena (45). Because AA is a substrate common to both pathways, this enhancement of LT synthesis has been attributed to the shunting of substrate from the PG to the LT pathway (43, 44). In considering the question of the shunting of AA from one pathway to the other, it is necessary to consider whether added AA alone can result in the synthesis of LTs. The cytosolic nature of 5-LO does not foster the concept that exogenously added AA would necessarily stimulate LT synthesis. Such an action would require circumvention of triglycerides and phospholipid-forming enzymes present throughout the cell for the accumulation of free AA within the cytosol in quantities sufficient to result in the formation of LTs. In agreement with other investigators (32), we show that the addition of exogenous substrate to the DC cultures had no significant effect on the generation of any of the eicosanoids measured. These results suggest that the enhanced level of LT synthesis is not due to the shunting of AA from COX to 5-LO pathway. The inability of exogenous PGs to affect cPLA₂ expression and AA release from DC would argue in favor of an AA-independent mechanism, and PGs did not suppress 5-LO metabolism by limiting substrate availability.

The enzyme 5-LO catalyzes the initial steps in the synthesis of LT from AA. For this reason, LT synthesis is critically dependent on processes that modulate 5-LO activity. The 5-LO activity and product generation might be modulated in multiple ways (46). For example, it has recently become apparent that the intracellular compartmentalization of the 5-LO metabolism affects the integrated output of this biosynthetic pathway (47). Another possibility is modulating the expression and activity of the enzymatic components through transcriptional or posttranscriptional mechanisms. It has also been demonstrated that the nuclear import of 5-LO can modulate LT biosynthesis, because nuclear import of 5-LO can strongly enhance (48) or suppress (49) 5-LO activity. Recently, Flammand et al. (50) have reported that PGE₂ and other CAMP-elevating agents down-regulated LT biosynthesis by inhibiting 5-LO translocation to the nuclear envelope in human polymorphonuclear leukocytes (PMN). In our study, we observed that 5-LO protein was found in the nuclear envelope when activated DC were treated with exogenous PGE₂ (data not shown), suggesting that this lipid mediator did not suppress LT biosynthesis by inhibiting 5-LO translocation to the nuclear envelope of DC. Our results may only apply to our experimental conditions, and they may differ with alternate matrices, cell types, or culture conditions. In considering the question of 5-LO translocation and its localization, it is necessary to consider that 5-LO can move in or out of the nucleus in response to in vivo or in vitro experimental conditions (47).

Ham et al. (26) were the first to demonstrate that PGE₂ inhibited LT biosynthesis. Although much effort has been focused on understanding the mechanism by which PG inhibit 5-LO metabolism in cells, such as PMN, little is known about the regulation of LT metabolism in DC. The results presented in this work represent the first description of a new mechanism by which PGs suppress LT biosynthesis in DC. Compared with LTB₄, which had no effect on COX pathways and cytokine release (4), PGE₂ concentration dependently enhances the production of endogenous IL-10, which inhibits FLAP expression and LTB₄ production from DC. These results demonstrate that IL-10 plays an essential role in modulating LT biosynthesis by inhibiting the expression of FLAP in DC. Although we and others have reported that some inhibitory effects of PGs on DC functions are mediated by the endogenously induced IL-10 (4, 51), to our knowledge, there have been no reports that the expression of FLAP is down-regulated by IL-10. Recently, other investigators have reported that Th2-derived cytokines, such as IL-4 and IL-13, enhanced the A-23187-stimulated production of LTB₄ and increased the expression of LTA₄ hydrolase, but not those of cPLA₂ and 5-LO in human PMN leukocytes (52). They also have demonstrated that IL-10 had no effect on the 5-LO metabolism. One can explain the discrepancy between the two reports because authors adopted a quite different model from the one we used. The present study demonstrates that the down-regulation of 5-LO metabolism by the PG-induced IL-10 is specifically attributable to FLAP, but not to cPLA₂ or 5-LO. However, it does not exclude a possible action of PGs on LTA₄ hydrolase, which catalyzes the final step in LTB₄ synthesis. Because FLAP is the target of the PG-inhibitory effect, other 5-LO products, in particular LTC₄ and LTD₄, which are increasingly incriminated in the pathogenesis of several inflammatory diseases, such as asthma, would expect to decrease after PG treatment.

Our study shows that FLAP plays pivotal role in the interaction between COX and 5-LO pathways. However, a number of questions remain concerning the mechanism by which FLAP stimulates 5-LO activity. The translocation of 5-LO from cytosol to nuclear envelope in which FLAP is located, and the inability of the exogenously added AA to increase 5-LO metabolism suggest that FLAP is not only required for binding and presenting AA to 5-LO, but also involved in modulating 5-LO activity. Accordingly, a stable complex would be required to form at the membrane between 5-LO, FLAP, cPLA₂, and possibly other proteins, and could regulate the interaction of 5-LO with its substrate for an efficient LT production.

The general consensus is that PGs, in particular PGE₂, act to shift the immune response toward a type 2 cytokine profile. For example, DC are not refractory to the effects of PGE₂ because the exogenously added or the endogenously released PGE₂ acts on the DC themselves by inhibiting IL-12 production (4) and MHC class
II expression (34), limiting their ability to act as potent APC. Moreover, this lipid mediator can also up-regulate IgE production (53), and may consequently support the development of type 2 cytokine-associated inflammatory disorders. However, there is evidence for a bronchoprotective role for PGE₂ in asthma (33, 54, 55). The effects of this lipid mediator on Th1/Th2 responses are sometimes opposite. For example, it has been shown recently that in BALB/c mouse, COX inhibition enhances the production of IL-13 (56). Additional cofactors or cellular interactions may be lacking in the in vitro models.

Our study shows some differences between DC and PM in terms of FLAP expression and eicosanoid production. The blockade of COX pathways enhanced the 5-LO metabolism of endogenous AA and resulted in a significant increase in LTβ₂ production from DC, but not from PM, suggesting no links between PM COX and 5-LO pathways. It is quite possible that COX and 5-LO metabolites derive from the same pool of AA in DC and from two distinct pools of substrates in PM, as was reported by others (57). We also observed that DC and PM differ in terms of cellular response to exogenous PGE₂. This may be due to a different pattern of E prostanoid receptor expression and signaling pathways.

Cells that produce lipid mediators, such as PGE₂, or become targets of their actions can also produce other inflammatory mediators, such as AA metabolites and cytokines. The limited capacity of DC to synthesize LTs in the presence of PGE₂ issued from the induced COX-2 enzyme or produced by other cells may understate their potential contribution to the generation of these lipid mediators at the site of inflammation. The inhibition of LT release by PGE₂ via endogenous IL-10 could be of particular significance as a potential anti-inflammatory and possibly also an antiinflammatory, obstructive effect. However, although this reduction of 5-LO metabolism in the setting of PG or IL-10 exposure may represent an endogenous mean to limit the inflammatory response, it may at the same time increase susceptibility to infections, and COX inhibitors may have utility in restoring LT release.

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