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Cyclooxygenase-2 Expression in Macrophages: Modulation by Protein Kinase C-α

Mélanie Giroux and Albert Descoteaux

Cyclooxygenase-2 (COX-2) is an inducible enzyme responsible for high levels of PG production during inflammation and immune responses. Previous studies with pharmacological inhibitors suggested a role for protein kinase C (PKC) in PG production possibly by regulating COX-2 expression. In this study, we addressed the role of PKC-α in the modulation of COX-2 expression and PGE₂ synthesis by the overexpression of a dominant-negative (DN) mutant of this isoenzyme in the mouse macrophage cell line RAW 264.7. We investigated the effect of various stimuli on COX-2 expression, namely, LPS, IFN-γ, and the intracellular parasite Leishmania donovani. Whereas LPS-induced COX-2 mRNA and protein expression were down-regulated in DN PKC-α-overexpressing clones, IFN-γ-induced COX-2 expression was up-regulated in DN PKC-α-overexpressing clones with respect to normal RAW 264.7 cells. Measurements of PGE₂ levels revealed a strong correlation between PGE₂ secretion and IFN-γ-induced COX-2 mRNA and protein levels in DN PKC-α-overexpressing clones. Taken together, these results suggest a role for PKC-α in the modulation of LPS- and IFN-γ-induced COX-2 expression, as well as in IFN-γ-induced PGE₂ secretion. The Journal of Immunology, 2000, 165: 3985–3991.

Prostaglandins are important mediators of inflammatory and immune responses. Their secretion is induced by various stimuli including LPS, phorbol esters, cytokines, and phagocytosis (1). PGE₂ is one of the main PGs secreted in large quantities by macrophages and acts as an autocrine regulator of their activity (2, 3). Cyclooxygenases (COX), the key enzymes responsible for the conversion of arachidonic acid to PGs, exist in two isoforms with different physiological functions. Whereas COX-1 is constitutively expressed in most cell types and is responsible for regulating normal physiological functions (3, 4), COX-2 is inducible in cells playing a role in inflammation such as macrophages, fibroblasts, and endothelial cells (5, 6). In human and murine macrophages, COX-2 expression is induced by LPS, IL-1α and phorbol esters (6–9). Studies with the murine macrophage cell line RAW 264.7 indicated that accumulation of COX-2 mRNA can be induced by a combination of IFN-γ and LPS but not by IFN-γ alone (10). In addition to soluble mediators, pathogens such as the intracellular parasite Leishmania donovani can increase synthesis of PGE₂, possibly by inducing alterations in the COX pathway (11, 12).

Previous studies using protein kinase C (PKC) inhibitors and activators suggested that PGE₂ synthesis requires the activation of PKC in the mouse macrophage cell line RAW 264.7, as well as in peritoneal macrophages (1, 13). Twelve isoenzymes of PKC, a family of protein serine/threonine kinases, have been identified so far. Differences in their structure, requirement for activity, subcellular localization, and substrate specificity suggest that in a given cell, the various PKC isoenzymes may exert specific functions. Six of them are expressed in macrophages but their respective roles in the regulation of macrophage functions are poorly understood (14, 15). Using clones of the RAW 264.7 macrophage cell line overexpressing a dominant-negative (DN) mutant of PKC-α (DN PKC-α), we recently reported that PKC-α regulates selective LPS-induced responses, including inducible NO synthase (iNOS) and IL-1α expression (16). This study led us to propose a role for PKC-α in the regulation of inflammatory responses. Previous studies based on selective depletion of PKC isoenzymes and their differential sensitivities to pharmacological inhibitors led to the suggestion that PKC-α regulates zymosan-stimulated arachidonic acid metabolism and eicosanoid synthesis in peritoneal macrophages (17). To further investigate the role of PKC-α in the regulation of COX-2 expression and PGE₂ secretion, we used DN PKC-α-overexpressing clones of the RAW 264.7 macrophage line (16). We obtained evidence that PKC-α modulates COX-2 expression in macrophages exposed to both LPS and IFN-γ, thereby providing additional evidence that PKC-α is involved in the regulation of macrophage inflammatory responses.

Materials and Methods

Cell lines

The murine macrophage cell line RAW 264.7 transfected with the expression vector pCIN-4, and the DN PKC-α-overexpressing clones B1 and C2 (16) were cultured in a 37°C incubator with 5% CO₂ in DMEM with glutamine (Life Technologies, Ontario, Canada), containing 10% heat-inactivated FBS (HyClone, Logan, UT), 10 mM HEPES pH 7.3, and antibiotics supplemented with 200 μg/ml G418 (Life Technologies).

Bone marrow-derived macrophages (BMM)

BMM were obtained as previously described (18). Briefly, bone marrow cells obtained from femurs of 6- to 8-wk-old female BALB/c mice (Charles River, St-Constant, Quebec, Canada), were freed of RBC by osmotic shock and resuspended in complete medium with 15% (v/v) L929 cell-conditioned medium. After 1 day in culture (37°C, 5% CO₂), nonadherent cells were transferred into new culture dishes and then allowed to differentiate
and adhere for 6 days. BMM were made quiescent by culturing them in CSF-1-free medium for 18 h before being used.

L. donovani

Promastigotes of L. donovani (Ethiopian strain LV9, obtained from G. Matlashewski, McGill University, Montréal, QC, Canada) were freshly derived from amastigotes isolated from the spleen of an infected hamster and were grown in 26°C in RPMI 1640 supplemented with 20% heat-inactivated FBS, 100 μM adenine, 5 μM hemin, 1 μM biotin, 20 mM [N-morpholino]ethanesulfonic acid, pH 5.5, and antibiotics. For infections with L. donovani, 2.5 × 10^5 adherent macrophages were incubated with 2.5 × 10^7 parasites for 8 h.

**Northern blot analyses**

Total RNA preparation and Northern blot analyses were performed essentially as described previously (16, 19). The probe for murine COX-2 consisted of the 1.2-kb EcoRI/Apal fragment from COX-2 cDNA amplified by PCR using oligodeoxynucleotides AD-24 (5'-CCCCTCTGGCAAGTTATC-3') and AD-25 (5'-GCATCAGGAGGTTTTC-3').

**Plasmids**

The luciferase reporter vector (pTIS10L) containing the promoter region of the mouse COX-2 gene (20) (~963-770 from the transcription initiation site) was provided by Harvey Herschman (University of California, Los Angeles, CA) and was used for transient transfections studies. The PKC-α expression vector (pcMV-PKC-α) was constructed by insertion of the human wild-type PKC-α cDNA (21) into the HindIII site of the expression vector pRcCMV (Invitrogen, San Diego, CA) and was used for overexpression analyses. The pRL-TK plasmid encoding the Renilla luciferase was obtained from Promega (Madison, WI).

**Transient transfections**

Adherent cells (2.5 × 10^5/well) were transfected using GenePorter (Gene Therapy Systems, San Diego, CA) with 0.25 μg of COX-2 luciferase reporter plasmid, and either 0.65 μg of pRcCMV (Invitrogen) or pcMV-PKC-α expression vector. All transfections included 0.1 μg of pRL-TK (Promega) as transfection efficiency control. Cells were transfected with 250 μg DNA/GenePorter mix for 5 h, and 1 ml of serum-free medium was added. Cells were treated 7 h later with 100 ng/ml LPS and harvested at 12 h in Reporter lysis buffer (Promega). Firefly and Renilla luciferase values were obtained by analyzing 20 μl of cell extracts according to standard instructions provided in the Dual Luciferase kit (Promega) using a Lumat LB 9507 luminometer (EG & G Berthold, Nashua, NH). Statistically significant differences were identified using the unpaired Student's t test. Values of p = 0.01 were considered statistically significant.

**Western blot analyses**

Western blot analyses were performed as described previously (16). Anti-COX-2 mAbs were obtained from Transduction Laboratories (Lexington, KY).

**PGE_2 production**

PGE_2 levels in the supernatants of macrophage were measured by competitive immunoassay (EIA; Cayman Chemicals, Ann Arbor, MI) after 8 h of incubation with different stimuli as recommended by the manufacturer. When indicated, the COX inhibitors NS398 (5 μM) and valeryl salicylate (1 mM) (Cayman Chemicals) or the iNOS inhibitor N^2-monomethyl-L-arginine monoacetate (L-NMMA; 500 μM) (Alexis, San Diego, CA) were used. Statistically significant differences were identified using the unpaired Student’s t test. Values of p = 0.01 were considered statistically significant.

**Results**

**Effect of DN PKC-α overexpression on LPS-induced COX-2 expression**

In macrophages, COX-2 expression is strongly induced by LPS, phorbol-ester, and several cytokines (22). To investigate the role of PKC-α in this process, we measured COX-2 mRNA accumulation and protein level expression in normal RAW 264.7 cells (containing the empty vector) and in DN PKC-α-overexpressing clones (B1 and C2; Ref. 16) after stimulation with LPS (10 and 100 ng/ml) for 8 h. In normal RAW 264.7 cells, LPS induced the expression of COX-2 mRNA accumulation and protein synthesis in a dose-dependent manner (Fig. 1, A and B, lanes 1–3). In DN PKC-α-overexpressing clones, LPS-induced COX-2 mRNA accumulation, and protein levels were significantly inhibited. Densitometric analyses revealed that in clone B1, COX-2 mRNA levels were reduced by 10- to 20-fold (Fig. 1A, lanes 4–6), and protein levels were reduced by ~4-fold (Fig. 1B, lanes 4–6) with respect to the levels observed in control cells. In clone C2, LPS-induced COX-2 mRNA levels were barely detectable (Fig. 1A, lanes 7–9), whereas COX-2 protein levels were reduced by ~3- to 5-fold with respect to control cells (Fig. 1B, lanes 7–9). Thus, similar to LPS-induced IL-1α and iNOS expression (16), DN PKC-α overexpression strongly inhibited LPS-induced COX-2 expression in RAW 264.7 macrophages.

**Overexpression of PKC-α increases LPS-induced COX-2 promoter activity**

The inhibition of LPS-induced COX-2 expression in DN PKC-α-overexpressing macrophages indicated that PKC-α plays a role in modulating COX-2 expression. To further demonstrate the involvement of PKC-α in the induction of COX-2 by LPS, we transiently transfected RAW 264.7 cells with a COX-2-luciferase reporter and a wild-type PKC-α expression vector. Overexpression of wild-type PKC-α had no effect on basal COX-2 promoter activity in untreated RAW 264.7 cells (Fig. 2). In contrast, PKC-α overexpression significantly increased LPS-stimulated COX-2 promoter activity by ~2-fold with respect to controls (Fig. 2, p = 0.0001, n = 3). These data are consistent with PKC-α playing a role in modulating COX-2 expression in LPS-stimulated macrophages.
FIGURE 2. Overexpression of PKC-α increases LPS-induced COX-2 promoter activity in RAW 264.7 macrophages. Adherent RAW 264.7 cells were transiently transfected with the COX-2/Luc reporter construct and pRL-TK for 5 h along with either the control vector (III) or the wild-type PKC-α expression vector (I). Cells were incubated for 7 h and then stimulated with 100 ng/ml LPS for 12 h. Firefly and Renilla luciferase activities were determined in cell extracts. Data are expressed as a ratio of firefly luciferase value/Renilla luciferase value. Experiments were performed in triplicate and are representative of results obtained in two separate experiments. **, p = 0.0001 as compared with LPS-stimulated cells transfected with control vector.

Effect of DN PKC-α overexpression on COX-2 expression following a stimulation with IFN-γ

IFN-γ is a potent regulator of macrophage function (23). In addition to inducing the expression of several genes, incubation of macrophages with IFN-γ enhances their responsiveness to LPS (10, 24). To determine whether PKC-α plays a role in the regulation of IFN-γ-induced responses, we have measured the induction of COX-2 mRNA accumulation and protein synthesis in control RAW 264.7 cells and in the DN PKC-α-overexpressing clones B1 and C2 in response to 100 U/ml IFN-γ alone or in combination with 100 ng/ml LPS. Macrophages were primed with 100 U/ml IFN-γ for 18 h before the addition of either 100 U/ml IFN-γ or the combination of 100 U/ml IFN-γ and 100 ng/ml LPS. IFN-γ induced an important increase of COX-2 mRNA accumulation in DN PKC-α-overexpressing cells (20-fold for clone B1 and 60-fold for clone C2) (Fig. 3A, lanes 6 and 10) compared with control cells (Fig. 3A, lane 2). Similar results were obtained with the levels of COX-2 protein expression, as in clone B1 (Fig. 3B, lane 6) and in clone C2 (Fig. 3B, lane 10) COX-2 levels were increased by 2- and 4-fold, respectively, compared with the levels observed in control cells (Fig. 3B, lane 2). This significant increase in IFN-γ-induced COX-2 expression in DN PKC-α-overexpressing RAW 264.7 cells suggested that PKC-α negatively modulates IFN-γ-induced COX-2 expression. When macrophages were exposed to a combination of both IFN-γ and LPS, high levels of COX-2 mRNA and protein were induced independently of DN PKC-α overexpression (Fig. 3, A and B, lanes 4, 8, and 12). Thus, DN PKC-α overexpression had little effect on the synergistic effect of LPS and IFN-γ on the induction of COX-2 mRNA accumulation and protein synthesis.

COX-2 expression following a phagocytic stimulation with L. donovani promastigotes

Infection with the intracellular protozoan L. donovani stimulates macrophages to secrete PGE2, possibly by inducing COX-2 expression (11, 12). Thus we determined whether PKC-α was involved in this process by comparing the induction of COX-2 mRNA accumulation and protein synthesis in normal RAW 264.7 cells and in DN PKC-α-overexpressing clones following phagocytosis of L. donovani promastigotes. For priming experiments, cells were incubated for 18 h with 100 U/ml IFN-γ before the addition of either 100 U/ml IFN-γ alone or in combination with L. donovani promastigotes for an additional 8 h. Phagocytic stimulation with L. donovani promastigotes failed to induce COX-2 mRNA accumulation as well as protein synthesis in control RAW 264.7 macrophages (Fig. 4, A and B, lane 3) and in the two DN PKC-α-overexpressing clones (Fig. 4, A and B, lane 7 for clone B1 and lane 11 for clone C2). Priming with IFN-γ had no effect on the induction of COX-2 expression following phagocytosis of L. donovani, as COX-2 mRNA and protein levels induced by IFN-γ alone (Fig. 4, A and B, lane 2 for control cells, lane 6 for clone B1, and lane 10 for clone C2) were similar to those induced by the combination of IFN-γ and L. donovani (Fig. 4, A and B, lane 4 for control cells, lane 8 for clone B1, and lane 12 for clone C2). In naïve BMM, L. donovani evaded the induction of COX-2 expression (Fig. 5, A and B, lane 5), whereas priming with IFN-γ led to the induction of COX-2 mRNA and protein synthesis by L. donovani promastigotes in BMM (Fig. 5, A and B, lane 6).

Effect of DN PKC-α overexpression on PGE2 secretion

We compared the ability of control RAW 264.7 cells and clones B1 and C2 to secrete PGE2 in response to either LPS (10 or 100 ng/ml), 100 U/ml IFN-γ, or L. donovani. As shown in Fig. 6A, in the presence of 10 ng/ml (III) and 100 ng/ml (I) LPS, control RAW 264.7 cells as well as DN PKC-α-overexpressing clones B1 and C2 secreted PGE2 in a dose-dependent manner. In contrast to
COX-2 mRNA and protein levels, overexpression of DN PKC-α did not affect LPS-induced PGE₂ secretion by RAW 264.7 cells. (For 10 ng/ml LPS, ρ = 0.15 for B1 vs control cells, and p = 0.02 for C2 vs control cells, n = 3. For 100 ng/ml LPS, ρ = 0.103 for B1 vs control cells, and p = 0.07 for C2 vs control cells, n = 3.)

Data obtained with the specific COX-2 inhibitor NS-398 (5 μM) (25) confirmed that COX-2 activation is the major pathway responsible for LPS-stimulated PGE₂ secretion (Table I). The observation that valeryl salicylate, a COX-1 inhibitor (26), reduced LPS-induced PGE₂ production by 50% suggested a role for COX-1, although it is possible that COX-2 activity was also inhibited at the concentration used (1 mM) (Table I). As shown in Fig. 6B, IFN-γ induced the secretion of minimal PGE₂ levels in control RAW 264.7 cells, whereas DN PKC-α-overexpression increased IFN-γ-induced PGE₂ secretion by 35-fold by clone B1 (ρ = 0.005, n = 3) and 70-fold by clone C2 (ρ = 0.01, n = 3). Collectively, these results indicated that DN PKC-α overexpression had no effect on LPS-induced PGE₂ secretion but strongly up-regulated IFN-γ-induced PGE₂ secretion. When macrophages were exposed to a combination of IFN-γ and LPS, control RAW 264.7 cells and the DN PKC-α-overexpressing clones B1 and C2 secreted similar PGE₂ levels (p = 0.04 for B1 vs control cells, and p = 0.795 for C2 vs control cells, n = 3) (Fig. 6C). As observed for COX-2 mRNA and protein synthesis, L. donovani promastigotes failed to induce PGE₂ secretion in control RAW 264.7 cells as well as in DN PKC-α-overexpressing clones B1 and C2 (data not shown).

Discussion
PGs are important regulatory mediators for the maintenance of numerous physiological functions and are synthesized by most mammalian tissues (3, 27). In inflammatory reactions, macrophages are the main producers of large quantities of PGE₂ (10, 28). COX-2, the inducible COX isoform, has been identified in activated macrophages and constitutes the key enzyme responsible for the high production of inflammatory PGs such as PGE₂ (3, 5, 29).

A role for PKC in the regulation of PG production (possibly by regulating COX-2 expression) has been suggested after treatment of macrophages with PKC inhibitors or activators (1, 13). In this study, we investigated the role of PKC-α in the regulation of COX-2 expression in macrophages. To this end, we have stably overexpressed a DN mutant of this isoenzyme in the murine macrophage cell line RAW 264.7 (16). We obtained evidence suggesting that PKC-α activity is important for the modulation of COX-2 expression in macrophages exposed to either LPS or IFN-γ.

Previous studies suggested that PKC is involved in the regulation of COX-2 expression. A role for PKC-α in the regulation of
zymosan-induced PGE$_2$ secretion in mouse peritoneal macrophages has been previously proposed based on the selective down-regulation of PKC isoenzymes and on their differential sensitivities to pharmacological inhibitors (17). Recently, it has been reported that overexpression of wild-type PKC-$\alpha$ in mouse epidermis increases phorbol esters-induced expression of specific proinflammatory mediators, including COX-2, suggesting that PKC-$\alpha$ plays a role in cutaneous inflammation (30). Our data obtained with DN PKC-$\alpha$-overexpressing clones suggest that this isoenzyme is required for COX-2 expression in the RAW 264.7 macrophage cell line. However, the possibility exists that stable overexpression of the DN PKC-$\alpha$ might have affected basal PKC-$\alpha$ activity levels, thereby altering the maintenance of normal cellular functions. A possible consequence of such alterations could be the loss of regulator(s), which could potentially affect signal transduction pathways leading to COX-2 expression. Accordingly, PKC-$\alpha$ would play a secondary role in LPS-induced COX-2 expression. In contrast, our observation that overexpression of wild-type PKC-$\alpha$ increases LPS-induced COX-2 promoter activity is not consistent with this possibility and argues in favor of a direct role for PKC-$\alpha$ (Fig. 2). Thus, our observations further support a role for PKC-$\alpha$ in the modulation of COX-2 expression in macrophages, and hence in the regulation of inflammatory responses.

The mechanism by which PKC-$\alpha$ modulates COX-2 expression remains obscure. One possibility is that PKC-$\alpha$ is required for the activation of specific transcription factors. In this regard, few studies have addressed the identity of the transcription factor(s) regulating COX-2 expression (31-33). The ubiquitous transcription factor NF-$\kappa$B, one of the main mediators of LPS responses (34), binds to regulatory sequences within the promoter region (−403 to −395 bp) of both the human and mouse COX-2 genes to regulate COX-2 expression (35). Because LPS-induced NF-$\kappa$B activation takes place normally in DN PKC-$\alpha$-overexpressing macrophages (16), it is likely that transcription factor(s) other than NF-$\kappa$B and required for LPS-induced COX-2 expression may be defective in our DN PKC-$\alpha$ overexpressing clones. Consensus binding sites for NF-IL6 have been identified within the COX-2 promoter region, and recent evidence indicated that this regulatory sequence is responsible for the induction of human COX-2 by LPS, through NF-IL6$\beta$ (C/EBP$\beta$) (33). More recently, it has been established that although that NF-$\kappa$B is not required, NF-IL6 is essential for LPS-induced COX-2 gene expression in RAW 264.7 cells (36). Further studies will be required to examine whether a defective activation of NF-IL6 could account for the inhibition of LPS-induced COX-2 expression in the DN PKC-$\alpha$-overexpressing RAW 264.7 macrophages. In this regard, preliminary evidence indicated that DN PKC-$\alpha$ overexpression inhibited LPS-induced NF-IL6 activation in RAW 264.7 cells (F. Chano and A. Descoteaux, unpublished data).

IFN-$\gamma$ is a pleiotropic cytokine that plays a key role in modulating immune and inflammatory responses (37) and regulates several macrophage functions (38). Previous studies in human macrophages demonstrated that IFN-$\gamma$ priming is required for the induction of COX-2 expression following stimulation with either IFN-$\gamma$ or TNF-$\alpha$. Moreover, IFN-$\gamma$, in combination with either LPS or TNF-$\alpha$, induced a synergistic increase in the accumulation of COX-2 mRNA (24). However, this synergistic effect is not universal, as IFN-$\gamma$ priming down-regulated COX-2 gene transcription in response to IL-1$\beta$ but not to LPS in human macrophages (6). Despite these observations, no data exist on the regulation of COX-2 expression by PKC following stimulation with IFN-$\gamma$. In contrast to LPS-induced COX-2 expression, we found that levels of COX-2 mRNA were significantly enhanced in DN PKC-$\alpha$-overexpressing macrophages following a stimulation with IFN-$\gamma$. These data suggest that PKC-$\alpha$ negatively modulates COX-2 expression in response to IFN-$\gamma$. Two possible mechanisms may account for these results. First, overexpression of DN PKC-$\alpha$ influences the transcriptional activity of the COX-2 promoter, possibly by regulating the activation of IFN-$\gamma$-induced transcription factor(s). IFN consensus sequence binding protein (ICSBP), which is primarily expressed in cells of the macrophage and lymphocytic lineages, is a member of the IFN regulatory factor family that binds to a DNA sequence, known as the IFN-stimulated response element (ISRE), which mediates IFN-$\gamma$ responsiveness for several genes (39, 40). ICSBP mRNA levels become elevated in response of COX-2 mRNA (42). However, this synergistic effect is not universal, as IFN-$\gamma$ priming down-regulated COX-2 gene transcription in response to IL-1$\beta$ but not to LPS in human macrophages (6). Despite these observations, no data exist on the regulation of COX-2 expression by PKC following stimulation with IFN-$\gamma$. In contrast to LPS-induced COX-2 expression, we found that levels of COX-2 mRNA were significantly enhanced in DN PKC-$\alpha$-overexpressing macrophages following a stimulation with IFN-$\gamma$. These data suggest that PKC-$\alpha$ negatively modulates COX-2 expression in response to IFN-$\gamma$. Two possible mechanisms may account for these results. First, overexpression of DN PKC-$\alpha$ influences the transcriptional activity of the COX-2 promoter, possibly by regulating the activation of IFN-$\gamma$-induced transcription factor(s). IFN consensus sequence binding protein (ICSBP), which is primarily expressed in cells of the macrophage and lymphocytic lineages, is a member of the IFN regulatory factor family that binds to a DNA sequence, known as the IFN-stimulated response element (ISRE), which mediates IFN-$\gamma$ responsiveness for several genes (39, 40). ICSBP mRNA levels become elevated in response to IFN-$\gamma$, but not IFN-$\alpha$/$\beta$, in macrophage cell lines and in thiglycollate-elicited peritoneal macrophages (41). Thus, the selectivity of ICSBP for macrophages and other cells of the immune system, coupled with its strong inducibility and long half-life in macrophages, suggests that it could play a critical role in the down-regulation of macrophage activity after activation by IFN-$\gamma$ (42).
Recent studies provided evidence that ICSBP can selectively suppress the expression of IFN-responsive genes (40). Furthermore, induction of ICSBP mRNA by IFN-γ was found previously to be inhibited by PKC inhibitors (41). Considering these observations, it will be of interest to verify the role of ICSBP in IFN-γ-induced COX-2 expression in DN PKC-α-overexpressing RAW 264.7 cells in response to IFN-γ. Second, the steady-state levels of COX-2 transcripts are the result of a balance between the rate of gene transcription and the rate of degradation of the mRNA produced. The 3’ untranslated region of COX-2 mRNA contains conserved AUUA repeats also found in other short-lived mRNA species, such as GM-CSF mRNA (20, 43), that are important in determining mRNA stability and translation (44, 45). Whether PKC-α activity negatively regulates the binding of putative cytosolic factors to the 3’ untranslated region of the COX-2 transcripts, and hence influences COX-2 mRNA stability in IFN-γ-stimulated macrophages, is an hypothesis that will deserve further attention.

*L. donovani* is an obligate intracellular protozoan that resides within mononuclear phagocytes of infected mammals (46). A previous study demonstrated that infection of murine peritoneal macrophages with *L. donovani* induced specific alterations in COX and lipoxygenase pathways. This response involved selective increase of some metabolites, such as PGE₂ (11). Another study in spleen cells indicated an ex vivo evidence for increased COX activity (12). Because LPS- and IFN-γ-induced COX-2 expression are modulated by PKC-α it was of interest to determine whether DN PKC-α overexpression would influence COX-2 expression during phagocytosis of *L. donovani* promastigotes. However, we failed to detect COX-2 expression in RAW 264.7 cells exposed to *L. donovani* promastigotes. In contrast to RAW 264.7 cells, IFN-γ treatment of BMM before infection with *L. donovani* promastigotes allowed the induction of COX-2 expression.

Whereas COX-2 mRNA and protein synthesis were inhibited, LPS-induced PGE₂ secretion was normal in DN PKC-α-overexpressing macrophages. A recent study reported that secretion of NO attenuates PGE₂ production in response to LPS in RAW 264.7 macrophages (47). Moreover, it was shown that NO suppresses the activity and expression of COX-2 mRNA in LPS-stimulated rat peritoneal macrophages (48). However, data obtained with the iNOS inhibitor L-NMMA (Table I) ruled out the possibility that our data are related to the low levels of NO secreted by LPS-stimulated DN PKC-α-overexpressing clones (16). In contrast, PGE₂ secretion was increased in DN PKC-α-overexpressing clones compared with control RAW 264.7 cells in response to IFN-γ.

In summary, we have provided evidence suggesting a role for PKC-α in the modulation of COX-2 expression in macrophages. Further knowledge of the mechanism that regulates COX-2 expression may potentially lead to the development of novel anti-inflammatory therapies.

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