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Suppression of Prostaglandin E_2–mediated *c-fos* mRNA Induction by Interleukin-4 in Murine Macrophages[1]

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When murine peritoneal macrophages were stimulated for 30 min with arachidonic acid, the growth-associated immediate early gene *c-fos* was induced in a concentration-dependent manner as assessed by Northern blot analysis. The arachidonic acid–induced *c-fos* mRNA expression was inhibited by a cyclooxygenase inhibitor, indomethacin, but not by a lipoxygenase inhibitor, nordihydroguaiaretic acid. Macrophages produced prostaglandin (PG) E_2 from arachidonic acid as determined by an enzyme immunoassay. Northern blot analysis revealed the expression of PGE receptor EP2 and EP4 subtypes, but not EP1 and EP3 in murine macrophages. PGE_2 brought about a marked elevation of cAMP, and *c-fos* mRNA expression was increased by PGE_2 and dibutyryl cAMP in these cells. These results suggest that arachidonic acid is transformed to PGE_2, which then binds to EP2 and EP4 receptors to increase intracellular cAMP and *c-fos* mRNA expression. Furthermore, the induction of *c-fos* by arachidonic acid, PGE_2, and cAMP was suppressed by pretreatment with interleukin (IL)-4. We also showed that the tyrosine phosphorylation of a Janus kinase, JAK3, is enhanced by IL-4 treatment, suggesting that the PGE_2-mediated *c-fos* mRNA induction is inhibited by IL-4 through the tyrosine phosphorylation of JAK3.

Key words: cAMP, *c-fos*, cyclooxygenase, IL-4, prostaglandin.

Macrophages exhibit diverse functions in host defense mechanisms against microbial infection and tumor cell destruction (*1*). They are activated by a variety of naturally occurring agents including bacterial lipopolysaccharides (*2–4*), granulocyte-macrophage colony-stimulating factor (*5*), tumor necrosis factor-α (*6*), 12-*O*-tetradecanoylphorbol 13-acetate (*7*), and so on. Upon activation by these compounds, growth-associated immediate early genes such as *c-fos* (*2–7*) and *c-myc* (*2*) are induced rapidly and transiently within the first few hours of stimulation without preceeding protein synthesis. The product of the nuclear oncogene *c-fos* has been suggested to play a pivotal role in the growth, differentiation, and development of a variety of cells (*8*). In addition, stimulation of macrophages by inflammatory stimuli leads to the release of arachidonic acid from membrane phospholipids by the activation of phospholipases (*9*, *10*). Arachidonic acid is then transformed either by cyclooxygenases to prostaglandins (PG), or by lipoxygenases to hydroperoxy acids (*11–13*). Although the induction of immediate early genes and the metabolism of arachidonic acid are well documented as the initial events in macrophage activation, the interaction of these apparently unrelated events has not yet been fully elucidated. The aim of this study is to investigate potential interaction mechanisms between immediate early gene induction and arachidonic acid metabolism in activated macrophages, which play a crucial role in modulating the initation and perpetuation of inflammatory responses. We found that the expression of *c-fos* mRNA is markedly increased by PGE_2 through the activation of EP2/EP4 receptors. Furthermore, interleukin (IL)-4 treatment was found to suppress PGE_2-mediated *c-fos* mRNA expression, and bring about tyrosine phosphorylation of Janus kinase (JAK) 3 in murine peritoneal macrophages.

MATERIALS AND METHODS

Materials—Arachidonic acid was purchased from Nu-Chek-Prep (Elysian, MN), γ-linolenic acid from Doosan Serdary Research Lab. (Englewood Cliffs, NJ), linoleic acid and Isogen from Wako (Osaka), oligo (dT)-cellulose and restriction enzymes from Toyobo (Osaka), eicosapentaenoic acid, PGE_2, $PGF_{2\alpha}$, PGD_2, beraprost, and U-46619 from Cayman (Ann Arbor, MI), dibutyryl cAMP from Sigma (St. Louis, MO), and Medium 199, penicillin G and streptomycin from Gibco BRL (Grand Island, NY). Brewer thioglycollate medium was supplied by Difco (Detroit, MI), fetal bovine serum was from JRH Biosciences (Lenexa, KS), AMV reverse transcriptase from Seikagaku Corporation (Tokyo), Quikhyb hybridization solution from Stratagene (La Jolla, CA), a radioimmunoassay kit for cAMP from Yamasa Shoyu (Chiba), recombinant murine IL-4 from Pepro Tech Inc. (London, UK), and Immobilon membranes from Millipore (Bedford, MA). Hot Tub DNA polymerase, Megaprime DNA labeling kit, [α-^{32}P]dCTP (110 TBq/mmol),

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Abbreviations: PG, prostaglandin; IL, interleukin; SSC, saline sodium citrate; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

Hybond-N$^+$ membrane, the PGE_2 enzyme immunoassay system, and protein A–Sepharose were obtained from Amersham (Bucks, UK), Ex Taq DNA polymerase was from Takara (Kyoto), antibodies against mouse JAK3 and phosphotyrosine were from Upstate Biotechnology (Lake Placid, NY), and peroxidase-labeled anti–rabbit IgG was from Funakoshi (Tokyo). Mouse PGE receptor cDNAs were generous gifts from Dr. S. Narumiya of Kyoto University.

Mouse Peritoneal Macrophages—Specific pathogen-free C57BL/6 mice (8–12 weeks of age) were obtained from Nippon Charles River (Tokyo) and housed in our facility for experimental animals until use. Brewer thioglycollate medium (1 ml of 3% solution per mouse) was injected into the peritoneal cavity, and exudate cells were collected after 4 days by lavage with Medium 199 containing 10 units/ml heparin. The cells were centrifuged and suspended in Medium 199 supplemented with 10% fetal bovine serum, 100 units/ml penicillin G and 100 μg/ml streptomycin. The cells were plated in culture dishes, and kept at 37°C for 2 h in an atmosphere of 5% CO_2. Non-adherent cells were removed, and adherent cells were washed with phosphate-buffered saline. Approximately 10^7 adherent cells were obtained from one mouse, and more than 95% of these were identified as macrophages by nonspecific esterase staining (*14*).

Preparation of c-fos cDNA—Total RNA was prepared from NIH 3T3 fibroblasts stimulated for 30 min with 10% fetal calf serum. Total RNA (2 μg) was incubated at 42°C for 1 h with 20 units of reverse transcriptase in a first-strand reaction mixture containing 1 μM oligo $(dT)_{12-18}$, 20 mM dithiothreitol, 0.5 mM each deoxynucleotide triphosphates, and 20 units RNase inhibitor in a total volume of 20 μl. The reaction mixture (10 μl) was used to reconstitute a polymerase chain reaction mixture (40 μl) containing 0.25 μM 5′- and 3′-primers and 1.5 units Hot Tub DNA polymerase. Sequences of the 5′- and 3′-primers were 5′-CTTCGACCATGATGTTCTCGG-3′ and 5′-CCTTCTCTGACTGCTCACAGG-3′, respectively (*15*). Amplification was performed for 30 cycles at 94°C for 45 s, 55°C for 45 s, and 72°C for 90 s. The reaction mixture was subjected to agarose gel electrophoresis, and cDNA with the expected size (~1.2 kb) was subcloned into pBluescript pre-digested with *Eco*RV. The recombinant plasmid was digested with *Eco*RI and *Hin*dIII to yield a 1,177-bp DNA fragment, which was used as a *c-fos* probe as described below. The sequence of *c-fos* cDNA was confirmed by an automated DNA sequencer (ABI prism 310, PE Applied Biosystems, Foster City, CA) using a dRhodamine terminator cycle sequencing kit.

Isolation of RNA and Blot Analysis—Total RNA was isolated from murine macrophages and other tissues using Isogen according to the manufacturer's instruction. When necessary, poly(A)$^+$ RNA was selected using an oligo(dT)-cellulose column as described elsewhere (*16*).

For Northern blot analysis of *c-fos*, 20 μg of total RNA was denatured at 70°C for 5 min in 50 mM MOPS buffer, pH 7.0, containing 50% formamide and 2.2 M formaldehyde, and separated by electrophoresis in a 1% agarose gel containing 2.2 M formaldehyde. The RNA was transferred to a Hybond-N$^+$ membrane, and cross-linked using a gene linker UV chamber (Bio-Rad). For slot blot analysis of *c-fos*, 1 μg of total RNA was applied to a Hybond-N$^+$ membrane using a Milliblot system (Millipore). The cDNA for *c-fos* was labeled with [α-^{32}P]dCTP using a random primer labeling kit). The membranes were hybridized at 68°C for 1 h in Quikhyb solution containing the labeled *c-fos* probe. The membrane was finally washed with 0.1X saline sodium citrate (SSC)/0.1% SDS at 65°C for 10 min.

For Northern blot analysis of PGE receptors, 15 μg of poly(A)$^+$ RNA was resolved in an agarose gel, transferred to a membrane and hybridized as described above. cDNA probes for EP2 (1.7 kb) and EP4 (2.4 kb) receptors were excised with *Eco*RI, and the EP3 (1.4 kb) receptor with *Eco*RI and *Hin*dIII. An EP1 receptor probe was prepared by uni-directional polymerase chain reaction. The reaction mixture (40 μl) contained 2.5 units Ex Taq DNA polymerase, 400 ng of 1.5-kb DNA template, 200 μM each of dATP, dTTP, and dGTP, 10 μM dCTP, 1.5 μM [α-^{32}P]dCTP, and a primer (5′-AATTAACCCTCACTAAAGGGAACAAAAGC-3′). Amplification was performed for 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Membranes were hybridized as described above, and washed with 0.1X SSC/0.1% SDS at 68°C for 10 min (EP1 and EP2), at 65°C for 5 min (EP3), and at 80°C for 10 min (EP4). The membranes were analyzed by a Fujix BAS1000 bioimaging analyzer (Tokyo), and also exposed to a Fuji X-ray film for 60 h at –80°C with an intensifying screen.

Enzyme Immunoassay of PGE_2—Macrophages (10^6 cells/100 μl) were incubated for 30 min with various concentrations of arachidonic acid, and PGE_2 in the culture medium was measured using an enzyme immunoassay system according to the manufacturer's instructions.

Radioimmunoassay of cAMP—Marophages (2×10^6 cells/2 ml) were preincubated at 37°C for 20 min in culture medium containing 0.1 mM 3-isobutyl-1-methylxanthine, and stimulated for 30 min with PGE_2. The medium was removed, and 0.5 ml of ice-cold 6% trichloroacetic acid was added to the cells. The cells were scraped off, and disrupted by sonication two times each for 30 s. The sonicate was extracted three times with 3 ml of water-saturated diethyl ether, and the aqueous phase was subjected to radioimmunoassay for cAMP according to the manufacturer's instructions.

Analysis of JAK3 Tyrosine Phosphorylation—Macrophages were incubated for 10 min at 37°C in the presence or absence of 10 ng/ml IL-4. Subsequent manipulations were carried out as described elsewhere (*17*). Briefly, the cells were washed with phosphate-buffered saline, and lysed in 50 mM Tris-HCl buffer, pH 7.5, containing 0.1% Triton X-100, 150 mM NaCl, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, and 10 μg/ml leupeptin. Insoluble materials were removed by centrifugation, and the supernatant was incubated for 1 h at room temperature with anti-JAK3 antibody bound to protein A–Sepharose. The complex was washed three times with 50 mM Tris-HCl buffer, pH 7.0, containing 0.1% Triton X-100. The immunoprecipitated proteins were eluted with 2X Laemmli's sample buffer, subjected to 8% SDS–polyacrylamide gel electrophoresis, and transferred to an Immobilon membrane. The membrane was probed at 25°C for 1 h with either anti-phosphotyrosine or anti-JAK3 antibody followed by washing with Tris-buffered saline containing 0.05% Tween 20. The membrane was incubated at 25°C for 1 h with peroxidase-labeled anti–rabbit IgG. Bands were visualized by the enhanced chemiluminescence (ECL) method (Amersham). Protein concentration was determined by the method of Lowry *et al.* (*18*).

RESULTS

Expression of c-fos by Arachidonic Acid—Murine peritoneal macrophages were stimulated for 30 min with various concentrations of arachidonic acid, and the expression of *c-fos* mRNA was analyzed by Northern blotting. As shown in Fig. 1A, arachidonic acid increased *c-fos* mRNA in a concentration-dependent manner up to 100 μM. Because of the limited solubility of arachidonic acid in aqueous solution, the effect at higher concentrations was not examined. Figure 1B shows the effects of various unsaturated fatty acids on *c-fos* mRNA expression. Only arachidonic acid increased *c-fos* mRNA expression, whereas γ-linolenic, linoleic and eicosapentaenoic acids were essentially without effects. These results indicate that the increased expression of the macrophage *c-fos* gene is specific for arachidonic acid as far as we examined.

PGE_2 Production in Macrophages—Since arachidonic acid has been shown to be actively metabolized in macrophages (*11–13*), we examined the effects of indomethacin and nordihydroguaiaretic acid, inhibitors of cyclooxygenase and lipoxygenase, respectively, on *c-fos* mRNA expression. Arachidonic acid–induced *c-fos* mRNA expression wa
ited by 10 μM indomethacin, but not by 10 μM
droguaiaretic acid (data not shown). These results
that cyclooxygenase is responsible for *c-fos* mRNA

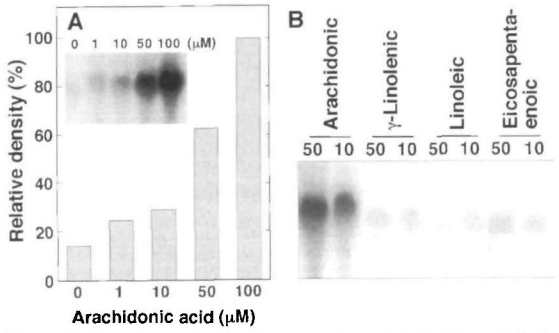

Fig. 1. **Induction of *c-fos* by unsaturated fatty acids in murine peritoneal macrophages.** (A) Macrophages were incubated for 30 min with arachidonic acid at the indicated concentrations, and total RNA (20 μg) was subjected to Northern blot analysis with ^{32}P-labeled *c-fos* cDNA probe as described under "MATERIALS AND METHODS." The density of the mRNA bands was measured by a Fujix BAS 1000 imaging analyzer. The inset shows an autoradiogram using X-ray film. (B) Macrophages were incubated for 30 min with arachidonic, γ-linolenic, linoleic, and eicosapentaenoic acids. Northern blot analysis was carried out as in (A).

TABLE I. **PGE_2 production from arachidonic acid in murine macrophages.**

Arachidonic acid (μM)	PGE_2 (pg/10^6cells)
—	297 ± 18
0.1	266 ± 44
1	592 ± 37
10	3,660 ± 170
50	8,790 ± 570

Murine peritoneal macrophages were incubated for 30 min with various concentrations of arachidonic acid, PGE_2 in the medium was measured by an enzyme immunoassay as described under "MATERIALS AND METHODS." Values are expressed as the mean ± SD (n = 10).

sion. We then measured the production of PGE_2, a major arachidonate metabolite of cyclooxygenase in murine macrophages (*12, 13*) by an enzyme immunoassay. As shown in Table I, the production of PGE_2 was increased depending on the concentration of arachidonic acid. Approximately 9 ng of PGE_2 was produced by 10^6 macrophages incubated with 50 μM arachidonic acid. This PGE_2 production represents an approximately 30-fold increase over the control.

Effects of Various PGs on c-fos mRNA Expression—Since PGE_2 is produced by murine macrophages, we investigated whether this compound affects *c-fos* mRNA expression. As shown in Fig. 2A, PGE_2 induces *c-fos* mRNA expression in a dose-dependent manner. Near maximal induction was attained with 100 nM PGE_2. In the time course experiment shown in Fig. 2B, *c-fos* mRNA expression reached a maximal level at around 30 min, then gradually declined to the basal level at 16 h. This time course is essentially the same as previously reported for *c-fos* induction by lipopolysaccharides (*2*) and 12-*O*-tetradecanoylphorbol 13-acetate (*7*) in murine macrophages. We then examined the effects of various PGs and their analogues. As shown in Fig. 3A, PGE_2 gave by far the most dense *c-fos* band at 100 nM. Beraprost

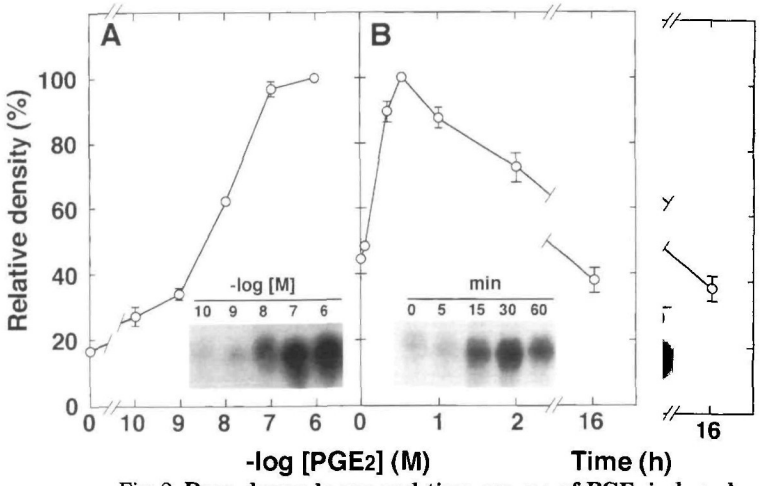

Fig. 2. **Dose dependency and time course of PGE_2-induced *c-fos* mRNA expression.** (A) Macrophages were stimulated for 30 min with various concentrations of PGE_2. (B) The cells were stimulated with 100 nM PGE_2 for various time periods. RNA blot analysis was carried out as described in Fig. 1. Data represent the mean ± SD of three independent experiments. Insets show representative blots.

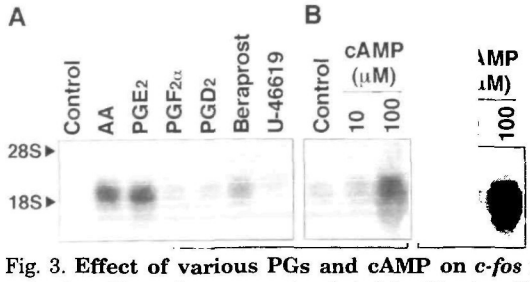

Fig. 3. **Effect of various PGs and cAMP on *c-fos* mRNA expression.** Macrophages were incubated for 30 min with 100 nM of various PGs and their analogues (A) or dibutyryl cAMP (B). Northern blot analysis was carried out as described in Fig. 1. Experiments with 50 μM arachidonic acid (AA) and an unstimulated control were also performed. Ribosomal RNAs (28S and 18S) are shown by arrows.

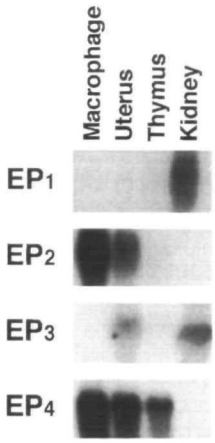

Fig. 4. **EP2 and EP4 receptors are expressed in murine macrophages.** Poly(A)$^+$ RNA (15 μg) from macrophages, uterus, thymus and kidney was resolved by agarose gel electrophoresis. RNA blotting onto a Hybond-N$^+$ membrane and hybridization were carried out as described under "MATERIALS AND METHODS."

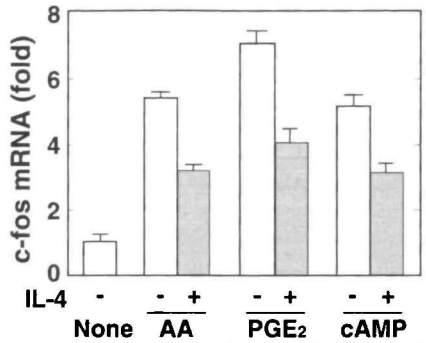

Fig. 5. **Suppression of *c-fos* induction by IL-4.** Macrophages were preincubated for 12 h in the presence or absence of 10 ng/ml IL-4, and treated for 30 min with 50 μM arachidonic acid (AA), 33 nM PGE_2, or 50 μM dibutyryl cAMP. Total RNA (1 μg) was subjected to slot blot analysis with ^{32}P-labeled *c-fos* cDNA probe as described under "MATERIALS AND METHODS." Data represent the mean ± SD of triplicate determinations.

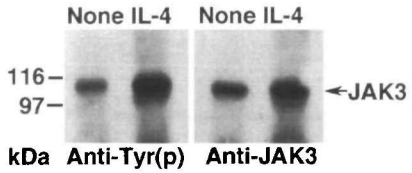

Fig. 6. **IL-4–dependent tyrosine phosphorylation of JAK3.** Macrophages were treated for 10 min with or without 10 ng/ml IL-4, and lysed in buffer containing 0.1% Triton X-100. The cell lysate was subjected to immunoprecipitation with anti-JAK3 antibody with the aid of protein A–Sepharose. Western blot analyses were carried out with antibodies against phosphotyrosine (left) and JAK3 (right).

(an analogue of PGI_2) caused a slight increase in *c-fos* mRNA. In contrast, $PGF_{2\alpha}$, PGD_2 and U-46619 (an analogue of thromboxane A_2) had essentially no effects. These results indicate that PGE_2 specifically increases *c-fos* mRNA expression in murine macrophages.

Expression of PGE Receptor Subtypes in Macrophages—PGE_2 has been shown to exert its effect through PGE receptors comprising 4 subtypes, EP1-EP4, in mice (*19–22*). We examined which subtype is expressed in murine macrophages by Northern blot analysis. As shown in Fig. 4, the expression of EP2 and EP4 mRNAs was observed, whereas EP1 and EP3 were not detected in murine macrophages. When RNAs from uterus, thymus and kidney were also subjected to Northern blot analysis, EP1 was present predominantly in kidney, EP2 in uterus, EP3 in uterus and kidney, and EP4 in uterus and thymus. The results are consistent with the reported occurrence of these receptors (*19–22*).

PGE_2 Enhances cAMP Production and c-fos mRNA Expression—Since the EP2 and EP4 receptors were expressed in murine macrophages and the activation of these receptors has been shown to increase intracellular cAMP (*20, 22*), we measured cAMP levels after stimulation by PGE_2. The cAMP level in murine macrophages was increased to 8.73 ± 0.33 pmol/10^6 cells by 100 nM PGE_2 over the control level of 1.33 ± 0.06 pmol/10^6 cells. We examined whether the increased cAMP could cause *c-fos* induction. To test this possibility, a membrane-permeable analogue of cAMP, dibutyryl cAMP, was employed. As shown in Fig. 3B, when murine macrophages were treated with 100 μM dibutyryl cAMP, the *c-fos* mRNA level was increased by approximately 3-fold, and a small increase was observed at 10 μM.

Suppression of c-fos mRNA Expression by IL-4—In order to investigate the regulatory mechanism of *c-fos* mRNA expression, the effect of IL-4 was examined, because cells of the monocyte/macrophage lineage have receptors for this cytokine, and IL-4 can suppress monocyte activation (*23*). Murine macrophages were preincubated for 12 h with 10 ng/ml IL-4, and then stimulated with arachidonic acid, PGE_2 and cAMP. As shown in Fig. 5, IL-4 suppressed the increase of *c-fos* mRNA expression caused by these compounds by 40–45%. We attempted to clarify the mechanism of the inhibition of *c-fos* mRNA expression by IL-4. Murine macrophages were incubated for 10 min with 10 ng/ml IL-4, and the tyrosine phosphorylation of JAK3 was examined. Extracts of IL-4–treated macrophages were subjected to immunoprecipitation with anti-JAK3 antibody followed by Western blot analysis. As shown in Fig. 6, IL-4 treatment caused an increase in the tyrosine phosphorylation of JAK3. When the density of the phosphorylated JAK3 was compared by NIH image software (version 1.60), it was found to be increased 1.5–2.5-fold by IL-4 treatment. In contrast, the JAK3 protein levels remained unchanged (right panel of Fig. 6).

DISCUSSION

We found that *c-fos* mRNA expression is markedly increased by 30-min incubation of murine peritoneal macrophages with arachidonic acid. It was previously reported that arachidonic acid is released by various stimuli including phorbol 12-myristate 13-acetate and IL-13 in mouse peritoneal macrophages (*24, 25*). Thus, we assumed that the released arachidonic acid might be metabolized to stimulate *c-fos* gene expression. The fact that the blockage of cyclooxygenase by indomethacin abolishes arachidonic acid–mediated *c-fos* mRNA expression suggests that this enzyme is involved in the activation of the *c-fos* gene. There are two isoforms of cyclooxygenase that differ in their

expression patterns and pathophysiological functions (*26*). In order to identify the cyclooxygenase isoform responsible for the synthesis of PGE_2, we carried out the reverse transcriptase–polymerase chain reaction with isoform-specific primers. Cyclooxygenase-2 mRNA was not detected in the thioglycollate-elicited macrophages even after stimulation for 30 min with 50 μM arachidonic acid, whereas cyclooxygenase-1 mRNA was present at the same levels in the stimulated and un-stimulated macrophages as well as in resident macrophages (data not shown). Moreover, our preliminary experiments showed no cyclooxygenase-2 protein detectable in arachidonic acid–stimulated macrophages as assessed by Western blotting. Therefore, we consider that cyclooxygenase-1 is responsible for PGE_2 production, since cyclooxygenase-2 would not be induced in such a short period (30 min) of incubation in murine peritoneal macrophages under our experimental conditions. In support of our observation, it has been reported that the expression of cyclooxygenase-2 protein is detectable after only 6 h in human macrophages promoted by IL-1β (*27*), and the maximum induction is observed between 12 and 18 h in lipopolysaccharide-stimulated $P388D_1$ macrophages (*28*).

This study demonstrates that PGE_2 induces *c-fos* in murine macrophages (Figs. 2 and 3). PGE_2 also induces *c-fos* mRNA expression in other cell types such as Swiss 3T3 fibroblasts (*29*) and rat glomerular mesangial cells (*30*). It is of interest that lipoxygenase metabolites, hydroperoxyeicosatetraenoic acids, are involved in the increased *c-fos* mRNA expression in rat aortic smooth muscle cells (*31*) and adipogenic TA1 cells (*32*). The difference in arachidonate metabolites involved in *c-fos* mRNA expression may be attributed to the cell types used in the experiments.

Northern blot analysis of PGE receptor subtypes revealed the specific expression of EP2 and EP4 in murine peritoneal macrophages (Fig. 4). A recent report showed that EP2 and EP4 are expressed in a mouse macrophage-like cell line, J774.1 (*33*). With regard to signal transduction, intracellular cAMP is elevated by the activation of these receptors (*20*, *22*). In fact, PGE_2 causes an increase in cAMP in murine macrophages (data not shown). Therefore, it is apparent that cAMP acts as a second messenger in PGE_2-stimulated *c-fos* mRNA expression. This was clearly shown by Northern blot analysis of the dibutyryl cAMP-stimulated macrophages (Fig. 3B). It has also been shown that cAMP enhances *c-fos* mRNA expression in murine bone marrow-derived macrophages (*34*). On the other hand, macrophage colony-stimulating factor-1 induces *c-fos* mRNA expression in these cells by a mechanism involving the breakdown of membrane phospholipids by phospholipase C with the ensuing activation of protein kinase C and intracellular release of Ca^{2+} (*35*). Thus, macrophages seem to act by two distinct mechanisms to regulate *c-fos* transcription, the phospholipase C and the cAMP pathways; the former may be a mechanism common to many cell types, while the latters is cell type–specific (*35*).

Th2-derived cytokines such as IL-4 have the potential to antagonize the inflammatory process by suppressing Th1-dependent activities (*36*, *37*). The anti-inflammatory function of IL-4 is attributed to its ability to inhibit the gene expression of proinflammatory cytokines by monocytes and macrophages including tumor necrosis factor-α (*38*). We demonstrated in this study that IL-4 suppresses the increased *c-fos* mRNA expression by PGE_2 and cAMP in murine peritoneal macrophages (Fig. 5). A nuclear oncoprotein, fos, has been suggested to play a pivotal role during cell growth and differentiation (*8*). Therefore, IL-4 may exert its anti-inflammatory effects partly through the inhibition of *c-fos* mRNA expression in acute and chronic inflammatory responses where arachidonic acid is released in macrophages (*24*, *25*). In accordance with our observation, IL-4 inhibits *c-fos* induction in lipopolysaccharide-stimulated human monocytes (*39*). We also demonstrated that IL-4 treatment brings about an increase in the tyrosine phosphorylation of JAK3 in murine peritoneal macrophages (Fig. 6). Upon IL-4 binding to its membrane receptor, JAK3 is tyrosine-phosphorylated and activated in human monocytes (*17*) and T cells (*40*). Thus, it is possible that the suppression of *c-fos* mRNA expression by IL-4 is mediated, at least in part, by the tyrosine phosphorylation of JAK3 in murine macrophages. It should be mentioned that IL-4 stimulates the tyrosine phosphorylation of the signal transducer and activator of transcription (STAT) 6 (*41*) , and the IL-4–dependent suppression of interferon γ-induced monokine gene expression is abolished in mouse peritoneal macrophages from which the STAT6 gene has been deleted (*42*). Alternatively, since PGE_2 production is inhibited by IL-4 in activated human monocytes (*23*), the inhibition of arachidonic acid–mediated *c-fos* mRNA expression by IL-4 may be explained by the decreased PGE_2 production in murine macrophages. However, the IL-4–mediated inhibition of *c-fos* mRNA expression by PGE_2 and cAMP can not be explained by this mechanism. It is of interest that the lipoxygenase pathway, but not cyclooxygenase, is associated with the IL-4–mediated inhibition of *c-fos* mRNA expression in human monocytes (*43*). Further investigations are necessary to clarify the precise regulatory mechanisms of the IL-4–mediated inhibition of *c-fos* mRNA expression in relation to macrophage proliferation and differentiation.

REFERENCES

1. Adams, D.O. and Hamilton, T.A. (1984) The cell biology of macrophage activation. *Annu. Rev. Immunol.* **2**, 283–318
2. Introna, M., Hamilton, T.A., Kaufman, R.E., Adams, D.O., and Bast, R.C., Jr. (1986) Treatment of murine peritoneal macrophages with bacterial lipopolysaccharide alters expression of *c-fos* and *c-myc* oncogenes. *J. Immunol.* **137**, 2711–2715
3. Introna, M., Bast, R.C., Jr., Tannenbaum, C.S., Hamilton, T.A., and Adams, D.O. (1987) The effect of LPS on expression of the early "competence" genes JE and KC in murine peritoneal macrophages. *J. Immunol.* **138**, 3891–3896
4. Barbour, S.E., Wong, C., Rabah, D., Kapur, A., and Carter, A.D. (1998) Mature macrophage cell lines exhibit varible responses to LPS. *Mol. Immunnol.* **35**, 977–987
5. Schaberg, T. and Filderman, A.E. (1990) Colony-stimulating factor induction of protooncogene expression in rat alveolar macrophages. *Am. J. Physiol.* **259**, L144–L150
6. Descoteaux, A. and Matlashewski, G. (1990) Regulation of tumor necrosis factor gene expression and protein synthesis in murine macrophages treated with recombinant tumor necrosis factor. *J. Immunol.* **145**, 846–853
7. Radzioch, D. and Varesio, L. (1991) mRNA expression in macrophages is downregulated by interferon-γ at the posttranscriptional level. *Mol. Cell. Biol.* **11**, 2718–2722
8. Sassone-Corsi, P., Sisson, J.C., and Verma, I.M. (1988) Transcriptional autoregulation of the proto-oncogene *fos*. *Nature* **334**, 314–319
9. Balsinde, J., Barbour, S.E., Bianco, I.D., and Dennis, E.A. (1994) Arachidonic acid mobilization in $P388D_1$ macrophages is

controlled by two distinct Ca^{2+}-dependent phospholipase A_2 enzymes. *Proc. Natl. Acad. Sci. USA* **91**, 11060–11064
10. Lin, W.W. and Chen, B.C. (1998) Pharmacological comparison of UTP- and thapsigargin-induced arachidonic acid release in mouse RAW 264.7 macrophages. *Br. J. Pharmacol.* **123**, 1173–1181
11. Bonney, R.J., Opas, E.E., and Humes, J.L. (1985) Lipoxygenase pathways of macrophages. *Federation Proc.* **44**, 2933–2936
12. Humes, J.L., Opas, E.E., Galavage, M., Soderman, D., and Bonney, R.J. (1986) Regulation of macrophage eicosanoid production by hydroperoxy-and hydroxy-eicosatetraenoic acids. *Biochem. J.* **233**, 199–206
13. Schade, U.F., Moll, H., and Rietschel, E.T. (1987) Metabolism of exogenous arachidonic acid by mouse peritoneal macrophages. *Prostaglandins* **34**, 401–412
14. Hamilton, T.A., Weiel, J.E., and Adams, D.O. (1984) Expression of the transferrin receptor in murine peritoneal macrophages is modulated in the different stages of activation. *J. Immunol.* **132**, 2285–2290
15. Van Beveren, C., van Straaten, F., Curran, T., Muller, R., and Verma, I.M. (1983) Analysis of FBJ-MuSV provirus and *c-fos* (mouse) gene reveals that viral and cellular *fos* gene products have different carboxy termini. *Cell* **32**, 1241–1255
16. Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) *Molecular Cloning. A Laboratory Manual 2nd Ed.* pp. 7.26–7.29, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
17. Musso, T., Johnston, J.A., Linnekin, D., Varesio, L., Rowe, T.K., O'Shea, J.J., and McVicar, D.W. (1995) Regulation of JAK3 expression in human monocytes: phosphorylation in response to interleukins 2, 4, and 7. *J. Exp. Med.* **181**, 1425–1431
18. Lowry, O.H., Rosenberg, N.J., Farr, A.L., and Randall, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**, 265–275
19. Watabe, A., Sugimoto, Y., Honda, A., Irie, A., Namba, T., Negishi, M., Ito, S., Narumiya, S., and Ichikawa, A. (1993) Cloning and expression of cDNA for a mouse EP1 subtype of prostaglandin E receptor. *J. Biol. Chem.* **268**, 20175–20178
20. Katsuyama, M., Nishigaki, N., Sugimoto, Y., Morimoto, K., Negishi, M., Narumiya, S., and Ichikawa, A. (1995) The mouse prostaglandin E receptor EP2 subtype: cloning, expression, and Northern blot analysis. *FEBS Lett.* **372**, 151–156
21. Sugimoto, Y., Namba, T., Honda, A., Hayashi, Y., Negishi, M., Ichikawa, A., and Narumiya, S. (1992) Cloning and expression of a cDNA for mouse prostaglandin E receptor EP3 subtype. *J. Biol. Chem.* **267**, 6463–6466
22. Honda, A., Sugimoto, Y., Namba, T., Watabe, A., Irie, A., Negishi, M., Narumiya, S., and Ichikawa, A. (1993) Cloning and expression of a cDNA for mouse prostaglandin E receptor EP2 subtype. *J. Biol. Chem.* **268**, 7759–7762
23. Hart, P.H., Vitti, G.F., Burgess, D.R., Whitty, G.A., Piccoli, D.S., and Hamilton, J.A. (1989) Potential antiinflammatory effects of interleukin 4: suppression of human monocyte tumor necrosis factor α, interleukin 1, and prostaglandin E_2. *Proc. Natl. Acad. Sci. USA* **86**, 3803–3807
24. Qiu, Z.H., de Carvalho, M.S., and Leslie, C.C. (1993) Regulation of phospholipase A_2 activation by phosphorylation in mouse peritoneal macrophages. *J. Biol. Chem.* **268**, 24506–24513
25. Rey, A., M'Rini, C., Sozzani, P., Lamboeuf, Y., Beraud, M., Caput, D., Ferrara, P., and Pipy, B. (1998) IL-13 increases the cPLA2 gene and protein expression and the mobilization of arachidonic acid during an inflammatory process in mouse peritoneal macrophages. *Biochim. Biophys. Acta* **1393**, 244–252
26. Dubois, R.N., Abramson, S.B., Crofford, L., Gupta, R.A., Simon, L.S., Van De Putte, L.B., and Lipsky, P.E. (1998) Cyclooxygenase in biology and disease. *FASEB J.* **12**, 1063–1073
27. Barrios-Rodiles, M. and Chadee, K. (1998) Novel regulation of cyclooxygenase-2 expression and prostaglandin E_2 production by IFN-γ in human macrophages. *J. Immunol.* **161**, 2441–2448
28. Shinohara, H., Balboa, M.A., Johnson, C.A., Balsinde, J., and Dennis, E.A. (1999) Regulation of delayed prostaglandin production in activated $P388D_1$ macrophages by group IV cytosolic and group V secretory phospholipase A_2s. *J. Biol. Chem.* **274**, 12263–12268
29. Danesch, U., Weber, P.C., and Sellmayer, A. (1994) Arachidonic acid increases *c-fos* and *Egr-1* mRNA in 3T3 fibroblasts by formation of prostaglandin E_2 and activation of protein kinase C. *J. Biol. Chem.* **269**, 27258–27263
30. Simonson, M.S., Herman, W.H., and Dunn, M.J. (1994) PGE_2 induces *c-fos* expression by a cAMP-independent mechanism in glomerular mesangial cells. *Exp. Cell. Res.* **215**, 137–144
31. Rao, G.N., Glasgow, W.C., Eling, T.E., and Runge, M.S. (1996) Role of hydroperoxyeicosatetraenoic acids in oxidative stress-induced activating protein 1 (AP-1) activity. *J. Biol. Chem.* **271**, 27760–27764
32. Haliday, E.M., Ramesha, C.S., and Ringold, G. (1991) TNF induces *c-fos via* a novel pathway requiring conversion of arachidonic acid to a lipoxygenase metabolite. *EMBO J.* **10**, 109–115
33. Katsuyama, M., Ikegami, R., Karahashi, H., Amano, F., Sugimoto, Y., and Ichikawa, A. (1998) Characterization of the LPS-stimulated expression of EP2 and EP4 prostaglandin E receptors in mouse macrophage-like cell line, J774.1. *Biochem. Biophys. Res. Commun.* **251**, 727–731
34. Wilson, N.J., Jaworowski, A., Ward, A.C., and Hamilton, J.A. (1998) cAMP enhances CSF-1-induced ERK activity and *c-fos* mRNA expression *via* a MEK-dependent and Ras-independent mechanism in macrophages. *Biochem. Biophys. Res. Commun.* **244**, 475–480
35. Bravo, R., Neuberg, M., Burckhardt, J., Almendral, J., Wallich, R., and Muller, R. (1987) Involvement of common and cell type-specific pathways in *c-fos* gene control: stable induction of cAMP in macrophages. *Cell* **48**, 251–260
36. Spergel, J.M., Mizoguchi, E., Oettgen, H., Bhan, A.K., and Geha, R.S. (1999) Roles of TH1 and TH2 cytokines in a murine model of allergic dermatitis. *J. Clin. Invest.* **103**, 1103–1111
37. Lubberts, E., Joosten, L.A.B., van Den Bersselaar, L., Helsen, M.M., Bakker, A.C., van Meurs, J.B.J., Graham, F.L., Richards, C.D., and van Den Berg, W.B. (1999) Adenoviral vector-mediated overexpression of IL-4 in the knee joint of mice with collagen-induced arthritis prevents cartilage destruction. *J. Immunol.* **163**, 4546–4556
38. Levings, M.K. and Schrader, J.W. (1999) IL-4 inhibits the production of TNF-α and IL-12 by STAT6-dependent and -independent mechanisms. *J. Immunol.* **162**, 5224–5229
39. Dokter, W.H.A., Esselink, M.T., Halie, M.R., and Vellenga, E. (1993) Interleukin-4 inhibits the lipopolysaccharide-induced expression of *c-jun* and *c-fos* messenger RNA and activator protein-1 binding activity in human monocytes. *Blood.* **81**, 337–343
40. Witthuhn, B.A., Silvennoinen, O., Miura, O., Lai, K.S., Cwik, C., Liu, E.T., and Ihle, J.N. (1994) Involvement of the Jak-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature* **370**, 153–157
41. Kotanides, H. and Reich, N.C. (1993) Requirement of tyrosine phosphorylation for rapid activation of a DNA binding factor by IL-4. *Science* **262**, 1265–1267
42. Ohmori, Y. and Hamilton, T.A. (1998) STAT6 is required for the anti-inflammatory activity of interleukin-4 in mouse peritoneal macrophages. *J. Biol. Chem.* **273**, 29202–29209
43. Dokter, W.H.A., Sierdsema, S.J., Esselink, M.T., Halie, M.R., and Vellenga, E. (1994) Interleukin-4-mediated inhibition of C-*c-fos* mRNA expression: role of the lipoxygenase directed pathway. *Leukemia* **8**, 1181–1184