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Elevated Levels of Cyclooxygenase-2 in Antigen-Stimulated Mast Cells Is Associated with Minimal Activation of p38 Mitogen-Activated Protein Kinase

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We have investigated possible factors that underlie changes in the production of eicosanoids after prolonged exposure of mast cells to Ag. Ag stimulation of cultured RBL-2H3 mast cells resulted in increased expression of cyclooxygenase (COX-2) protein and message. Other eicosanoid-related enzymes, namely COX-1, 5-lipoxygenase, and cytosolic phospholipase A\(_2\) were not induced. Activation of extracellular signal-regulated kinase, c-Jun N-terminal kinase, and p38 mitogen-activated protein (MAP) kinase preceded the induction of COX-2, whereas phosphatidylinositol 3’ kinase and its substrate, Akt, were constitutively activated in RBL-2H3 cells. Studies with pharmacologic inhibitors indicated that of these kinases, only p38 MAP kinase regulated expression of COX-2. The induction of COX-2 was blocked by the p38 MAP kinase inhibitor SB202190, even when added 12–16 h after stimulation with Ag when p38 MAP kinase activity had returned to near basal, but still minimally elevated, levels. Interestingly, expression of COX-2 as well as cytosolic phospholipase A\(_2\) and 5-lipoxygenase were markedly reduced by SB202190 in unstimulated cells. Collectively, the results imply that p38 MAP kinase regulates expression of eicosanoid-related enzymes, passively or actively, at very low levels of activity in RBL-2H3 cells. Also, comparison with published data suggest that different MAP kinases regulate induction of COX-2 in inflammatory cells of different and even similar phenotype and suggest caution in extrapolating results from one type of cell to another. The Journal of Immunology, 2001, 167: 1629–1636.

Mast cells are a major source of inflammatory mediators, some of which are preformed and stored in secretory granules and others such as the cytokines and lipid-derived eicosanoids, which are generated de novo. Release of these mediators can be stimulated through Ag-induced aggregation of receptors with high affinity for IgE (Fc\(_{\varepsilon}RI\)), which leads to activation of the tyrosine kinase Syk and, ultimately, to the activation of phosphatidylinositol 3’ kinase (PI-3) and the mitogen-activated protein (MAP) kinases, extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAP kinase (2–6), in addition to mobilization of calcium ions (7).

With respect to the generation of eicosanoids in mast cells, the initiating reaction is the release of free arachidonic acid by cytosolic phospholipase A\(_2\) (cPLA\(_2\)), which is activated through its phosphorylation by ERK-2 and increase in cytosolic calcium (2, 8). In cultured mast cell lines, which include the well-studied RBL-2H3 cell, free arachidonic acid then is rapidly metabolized to leukotriene C\(_4\)/B\(_4\) and PGD\(_2\) via the 5-lipoxygenase (5-LO) and cyclooxygenase (COX) pathways, respectively (9–11). Production of these metabolites is short lived (11–13), but with prolonged exposure to Ag, this initial phase is followed by sustained production of PGD\(_2\). This initial phase is dependent on constitutively expressed COX-1 and the second phase on the induction of synthesis of mRNA and protein for a second isoform of COX, COX-2 (12, 14, 15). A similar induction of COX-2 and the associated production of PGD\(_2\) occurs during c-kit-ligand-induced maturation of cultured bone marrow-derived mast cells (13). Currently, there is little information about the changes of other enzymes that are involved in eicosanoid synthesis, although induction of cPLA\(_2\) has been noted in other types of cells stimulated with cytokines (16).

COX-1 is expressed constitutively in many types of cells, where it is believed to perform “housekeeping” activities for normal cellular function, whereas expression of COX-2 is induced in certain types of cells by a variety of inflammatory stimuli (17, 18). In general, the inflammatory reactions are associated with an induction of COX-2 but not of COX-1. This induction is blocked by low concentrations of glucocorticoids, which disrupt transcriptional and posttranscriptional processes, although the molecular details are still unclear (for example, see Refs. 19 and 20). Recent studies in a variety of cultured cells indicate that induction of COX-2 is regulated by the MAP kinases (21), through stabilization of COX-2 mRNA (22), but the particular MAP kinase involved appears to vary from one cell type to another (see for example, Refs. 21, 23, and 24). In regard to mast cells, the Ras/MAP kinase-ERK kinase (MEKK) 1/JNK and Ras/Raf-1/ERK pathways have been implicated in studies with dominant negative proteins expressed in a murine mastocytoma cell line, MMC-34, transfected with COX-2 promoter/luciferase constructs (25).

During studies of the mechanism of induction of COX-2 synthesis in RBL-2H3 mast cells, it became apparent that p38 MAP kinase was essential not only for this induction but also for the constitutive expression of cPLA\(_2\) and 5-LO, as well as COX-2, in unstimulated cells. Furthermore, expression of these enzymes was regulated at very low levels of p38 MAP kinase activity and was suppressed by inhibitors of p38 MAP kinase and by the glucocorticoid, dexamethasone, which was found to be a potent inhibitor of the p38 MAP kinase pathway. This paper describes these results.
**Materials and Methods**

**Reagents**

Reagents were obtained from the following sources. All reagents for cell culture were obtained from Life Technologies (Rockville, MD). Polyclonal Abs against the phosphorylated MAP kinases, ERK, JNK, and p38 MAP kinase, and the phosphorylated substrates, c-Jun and Akt, as well as the p38 MAP kinase assay kit were obtained from New England Biolabs (Beverly, MA). mAbs against ERK-2, COX-2, and 5-LO were obtained from Transduction Laboratories (Lexington, KY). Polyclonal Ab against sheep COX-1 was obtained Caymen Chemical (Ann Arbor, MI). Monoclonal Ab against cPLA2 was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). PD098059 was obtained from Research Biomedicals (Natick, MA). Secondary Abs against mouse or rabbit IgG, SB202190 hydrochloride, and SB203580 were obtained from Calbiochem (San Diego, CA). Wortmannin and Ro31-7549 were obtained from Alexis (San Diego, CA). Minigels (10%) were obtained from Novex (San Diego, CA). QuickPrep micro mRNA purification kit was obtained from Amersham Pharmacia Biotech (Piscataway, NJ), and radiolabeled compounds were obtained from DuPont-New England Nuclear (Boston MA). All other chemical were molecular biology grade from several sources. The Ag, DNP-BSA, and DNP-specific monoclonal IgE were kindly supplied by Dr. H. Metzger (National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health, Bethesda, MD)

**Cell culture and experimental procedures**

RBL-2H3 cells were maintained in complete growth medium (SMEM) supplemented with 15% FCS, glutamine, antibiotic, and antymycotic agents. Trypsinized cells were plated into 24-well Costar cluster plates and were incubated overnight in complete growth medium with DNP-specific IgE (500 ng/ml; Refs. 26 and 27).

Cultures were washed the next day and repleted with the medium described above except that MEM was used instead of SMEM. For the assay of hexosaminidase, experiments were performed in a PIPES-buffered medium (25 mM PIPES, pH 7.2, 159 mM NaCl, 5 mM KCl, 0.4 mM MgCl2, 1.0 mM CaCl2, 5.6 mM glucose, and 0.1% fatty-acid-free fraction-V BSA). The inhibitors were added either 30 min before stimulation of release of the granule marker, hexosaminidase, which hydrolyses N-acetyl-β-D-glucosaminide to the chromophore, p-nitrophenol. Aliquots (10 μl) of medium and cell lysate were incubated with 10 μl of 1 mM p-nitrophenyl-N-acetyl-β-D-glucosaminide at 37°C in 0.1 M sodium citrate buffer (pH 4.5) for 1 h in 96-well microtiter plates. At the end of the incubation, 250 μl of a 0.1 M Na2CO3/0.1 M NaHCO3 buffer (pH 10) was added. Absorbance of the p-nitrophenate ion was read at 410 nm. Values were expressed as the percentage of intracellular hexosaminidase that was released into the medium after correction for spontaneous release (27). Release of radiolabeled arachidonic acid was determined in cultures labeled to equilibrium by incubation overnight with [14C]arachidonic acid (0.2 μCi/ml medium) as described previously (31).

**Presentation of data**

Data are presented either from representative experiments (immunoblots) or as numerical values (mean ± SEM) where at least three separate experiments were performed as described in the figure legends. Statistical differences were evaluated by ANOVA with the Statistica program from Statsoft (Tulsa, OK).

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**FIGURE 1.** Time-course of induction of COX-2 protein in Ag-stimulated RBL-2H3 cells. RBL-2H3 cells were stimulated with 0.2 ng/ml or 20 ng/ml DNP-BSA for the indicated times. The COX-2 content of cell lysates were analyzed by immunoblotting (inset) and quantified by densitometric scanning. Data points were the mean ± SEM of values from three or more experiments and are expressed in this and subsequent figures as a percentage of COX-2 protein in unstimulated cells collected at the same time point.

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**FIGURE 2.** Effect of kinase inhibitors on expression of COX-2 protein in RBL-2H3 cells. Vehicle (Control) or the indicated inhibitor was added to cell cultures 1 h before addition of 20 ng/ml DNP-BSA or the equivalent volume of vehicle. Expression of COX-2 protein was determined 24 h later in unstimulated (Ag) and stimulated (+Ag) cells by immunoblotting and densitometric scanning. The inhibitors tested included SB202190 (25 μM), PD098059 (50 μM), and wortmannin (100 nM). Data points are expressed as a percentage of COX-2 protein in unstimulated cells and are the mean ± SEM of three experiments. The symbols indicate significant decrease (p < 0.05) compared with unstimulated control (++) or stimulated control (*). The inset shows an immunoblot from a representative experiment.
Results

Prolonged exposure of RBL-2H3 cells to Ag leads to induction of COX-2

Initial experiments showed that COX-1, COX-2, cPLA2, and 5-LO were present in unstimulated RBL-2H3 cells, but only COX-2 showed a significant increase in levels after prolonged exposure of cells with Ag (DNP-BSA; data not shown but see later experiments). The increase in COX-2 protein was biphasic (Fig. 1) whether cells were stimulated with concentrations of DNP-BSA that were suboptimal (0.2 ng/ml) or optimal (20 ng/ml) for secretion of hexosaminidase (data not shown). In both cases, a small increase between 3 and 6 h was followed by a larger increase over the course of 10 and 20 h. As shown in Fig. 1 (inset), there was an increase in basal levels of COX-2 with time as cells reached confluence over the course of 20 h. This and subsequent data were corrected for this increase by determining basal levels for each time point or treatment group.

Effects of kinase inhibitors on Ag-induced expression of COX-2 and other eicosanoid-related enzymes in RBL-2H3 cells

To identify potential signals for induction of COX-2, cells were stimulated with Ag for 24 h in the absence or presence of various

![FIGURE 3](#). Comparison of the effects of Ag and SB202190 on the expression of COX-2 and other eicosanoid-related enzymes. Vehicle (−), 25 μM SB202190 (+), and 20 ng/ml DNP-BSA (Ag) were added to cultures as indicated. A, The amounts of COX-2, COX-1, cPLA2, and 5-LO were determined 24 h later in whole cell lysates by immunoblotting and densitometric scanning. The data were the mean ± SEM of values from at least five experiments and are expressed as a percentage of the level of protein in untreated cells (i.e., no drug or Ag). **, Significant difference (p < 0.01) from untreated cells. †, Significant difference (p < 0.05) from Ag-stimulated cells without drug. B, Levels of mRNA for COX-2 were determined at the indicated times by RT-PCR with mRNA from cell lysates. The experiment was representative of two similar experiments. C, In additional experiments levels of mRNA for COX-2, COX-1, cPLA2, and 5-LO were determined at 2 h and 24 h by RT-PCR as in B. The experiment was representative of two similar experiments.

![FIGURE 4](#). Effect of SB202190 on cPLA2 activity as indicated release of arachidonic acid and cPLA2 gel shift. [14C]Arachidonic acid-labeled cells were stimulated with 20 ng/ml DNP-BSA (Ag) or left unstimulated (control) for 30 min, in the absence or presence of the indicated concentrations of SB202190, for determination of release of labeled arachidonic acid into the medium and electrophoretic migration of cPLA2. Data points show the mean ± SEM arachidonic acid release from three cultures and the inset illustrates the Ag-induced gel-shift of cPLA2 from one of four similar experiments.

![FIGURE 5](#). Action of SB202190 on phosphorylation of proteins associated with the activation of p38 MAP kinase and JNK. SB202190 was added to RBL-2H3 cells at the indicated concentrations 1 h before addition of vehicle (−) or 20 ng/ml DNP-BSA (+). Cells were lysed 15 min later for detection of phosphorylated (Thr71)-ATF-2 (p-ATF-2), doubly phosphorylated (Thr180/Tyr182)-p38 MAP kinase (p-p38), (Thr/Tyr)JNK (p-JNK), phosphorylated c-Jun (p-cJun), and c-Jun protein (cJUN) by immunoblotting. Quantitative data from this and other experiments are shown in the next figure.
inhibitors. The induction of COX-2 in stimulated cells was blocked by the p38 MAP kinase inhibitor SB202190 (32), but not by PD098059 and wortmannin, which inhibit MEK (33) and PI-3’ kinase, respectively (Fig. 2). In addition, SB202190 partially inhibited the constitutive expression of COX-2 in unstimulated cells. Similar results were obtained with another p38 MAP kinase inhibitor, SB203580 (data not shown). In addition to inhibiting constitutive and induced expression of COX-2, SB202190 partially reduced levels of cPLA₂ and 5-LO protein even though these enzymes were not induced by Ag (Fig. 3A).

SB202190 substantially reduced expression of COX-2 mRNA, in addition to COX-2 protein in both unstimulated and stimulated cells, although some recovery in levels of message was apparent after 24 h of exposure to the drug (Fig. 3B). A transient increase in levels of COX-2 mRNA was observed 2 h and 4 h after Ag stimulation in SB20219-treated cells, but the levels of message remained well below those observed in untreated cells. In other experiments, this transient increase was apparent at 1 or 2 h but not at later time points. Consistent with the increase in COX-2 protein as noted in Fig. 1, levels of COX-2 mRNA also increased over

FIGURE 6. Comparison of the effects of SB202190 on the activation of phosphorylation of p38 MAP kinase, induction of COX-2, and the phosphorylation of JNK and its substrate c-Jun. RBL-2H3 cells were treated with the indicated concentrations of SB202190 and stimulated with DNP-BSA exactly as described for Fig. 4 except that cells were stimulated for 15 min for the assay p38 MAP kinase activity (A) and phosphorylated proteins (B, D, and E) and for 24 h for the assay of COX-2 (C). A, immunoprecipitated p-38 MAP kinase was assayed for kinase activity by phosphorylation of ATF-2 peptide in vitro and measurement of the phosphorylated peptide by immunoblotting and densitometric scanning. B–E, whole cell lysates were used for immunoblotting and densitometric analysis of phosphorylated proteins and COX-2 as described for previous figures. The data were mean ± SEM of values from at least five experiments and indicate relative amounts in arbitrary units.
time in unstimulated cells (Fig. 3B). However, in contrast to the effects on COX-2 mRNA, SB202190 had no effect on levels of mRNA for COX-1, cPLA₂, or 5-LO (Fig. 3C). These results suggested that other mechanisms, such as decreased translation or increased degradation of protein, were responsible for the decreased levels of cPLA₂ and 5-LO in SB202190-treated cells. However, the activation of cPLA₂ by Ag was not impaired by treatment with SB202190. This drug was found not to inhibit release of arachidonic acid nor the shift in gel migration of cPLA₂ in Ag-stimulated cells at concentrations up to 25 μM. Fig. 4 shows data from a typical experiment. The change in migration of cPLA₂ has been attributed to phosphorylation of this protein by ERK2 and possibly other kinases (Ref. 34 and citations therein). As in previous studies (5), the release of arachidonic acid was blocked by PD098059, verifying that ERK was involved in the activation of cPLA₂. The data in total imply that the constitutive expression of COX-1 and the activation of cPLA₂ in RBL-2H3 cells were not dependent on this enzyme.

**Activation of p38 MAP kinase and JNK and their relationship to induction of COX-2**

The above results did not exclude a role for JNK in regulating expression of COX-2 because SB202190 can inhibit JNKβ at high concentrations (35, 36) in addition to the α and β isoforms of p38 MAP kinase (37). Moreover, both p38 MAP kinase and JNK were activated in Ag-stimulated RBL-2H3 cells. This was indicated by the appearance of phosphorylated (Thr²¹⁷)-activating transcription factor (ATF)-2, a phosphorylation catalyzed by either p38 MAP kinase or JNK (Ref. 38 and citations therein); the doubly phosphorylated (Thr¹⁸⁰/Tyr¹⁸²)-p38 MAP kinase (39) and (Thr¹⁸³/Tyr¹⁸⁵)JNK (40); phosphorylated (Ser⁷⁵)J-Jun, a product of JNK activation (41, 42); and additional retarded bands of c-Jun (Fig. 5)

![Figure 7](http://www.jimmunol.org/)

**Figure 7.** Effects of the PI-3 kinase inhibitor wortmannin on phosphorylation of JNK, phosphorylation of Akt, and secretion in Ag-stimulated RBL-2H3 cells. The indicated amounts of wortmannin were added to RBL-2H3 cultures 1 h before addition of 20 ng/ml DNP-BSA. Cells were stimulated for 15 min for assay of phosphorylated Akt (p-Akt) and JNK (p-JNK) by immunoblotting and densitometric scanning and for secretory response by measurement of release of hexosaminidase. Immunoblots are shown in the inset for a typical experiment. The data points show mean ± SEM of results from three or more separate experiments and are expressed as percentage of values for Ag-stimulated cells without drug (stimulated control).

![Figure 8](http://www.jimmunol.org/)

**Figure 8.** Suppression of expression of COX-2 by SB202190 and cycloheximide in unstimulated and Ag-stimulated RBL-2H3 cells. Cells were stimulated with 20 ng/ml DNP-BSA or left unstimulated before addition of vehicle, SB202190 (25 μM; A), or cycloheximide (1 μM; B) and incubated for additional periods of time. The times shown indicate the duration of exposure to Ag and drug. The cell lysates were assayed for COX-2 by immunoblotting and densitometric scanning. The data shown indicate levels of COX-2 expressed as a percentage of COX-2 levels in unstimulated cells without drug (control). Values were the mean ± SEM from three to five identical experiments. Asterisks indicate significant differences from control (no Ag, no drug) at the $p < 0.05$ (*) or $p < 0.01$ (**) level, and the symbol (†) indicates a significant difference ($p < 0.05$) from the Ag-stimulated group at the corresponding time point.

SB202190 suppressed the phosphorylation of p38 MAP kinase in a dose-dependent manner, and to some extent ATF-2, but it had no marked effect on the phosphorylation of JNK. With respect to c-Jun, the amounts of both phosphorylated c-Jun and c-Jun itself were decreased by SB202190, but the proportion of immunoreactive c-Jun present in retarded bands remained unchanged. These and additional experiments indicated that although expression of c-Jun was reduced by SB202190, the extent of c-Jun phosphorylation, and presumably JNK activation, were undiminished. Additional experiments were conducted to compare the effects of SB202190 on the protein phosphorylations as described in Fig. 5, with the effects on the activity of immunoprecipitated p38 MAP kinase and the expression of COX-2. These experiments revealed that suppression of the activation (Fig. 6A) and phosphorylation (Fig. 6B) of p38 MAP kinase by SB202190 was dose-dependent and best correlated with suppression of COX-2 levels (Fig. 6C) in unstimulated and stimulated cells, whereas no correlation was observed with the phosphorylation of JNK (Fig. 6D) and c-Jun (Fig. 6E).

Another indication that JNK was not involved in the regulation of COX-2 expression came from studies with the PI-3 kinase inhibitor wortmannin. This inhibitor suppresses JNK activation in...
two other mast cell lines, namely mouse bone marrow-derived mast cells (6) and MC/9 cells (43) and weakly so in RBL-2H3 cells (4). In our hands, wortmannin did not inhibit but may have enhanced the phosphorylation of JNK (Fig. 7) and c-Jun (data not shown) in RBL-2H3 cells while potently inhibiting secretion and the phosphorylation of Akt (protein kinase B), a reaction dependent on PI-3 kinase (44) (Fig. 7). Thus, wortmannin inhibited PI-3 kinase and enhanced JNK activities, but it neither enhanced nor inhibited induction of COX-2 (as noted in Fig. 2).

A persistent p38 MAP kinase-generated signal of low intensity appears necessary for induction and continued synthesis of COX-2

SB202190 blocked induction of COX-2 whether added before (as in the above experiments) or after addition of Ag. As shown in Fig. 8A, this inhibitor effectively blocked further increases in COX-2 when added 12 and 16 h after the addition of Ag. As in the earlier experiments, the constitutive expression of COX-2 in unstimulated cells was suppressed. Similar studies with cyclohexamide, an inhibitor of protein synthesis, indicated declines in levels of COX-2 in both unstimulated and stimulated cells (Fig. 8B). The extent of these declines suggested similar turnover-times for COX-2 under both conditions. Comparison of data in Fig. 8, A and B, suggested that SB202190 substantially, if not totally, inhibited synthesis of COX-2 and that levels of COX-2 reflected the dynamic balance between synthesis and degradation in both unstimulated and stimulated cells.

Our initial determination of the time-course of p38 MAP kinase phosphorylation showed that the activating phosphorylations (i.e., Thr180/Tyr182) occurred rapidly after the addition of Ag to reach a maximum by 10 min or 60 min with the optimal and suboptimal doses of Ag, respectively (Fig. 9). Thereafter, the extent of these phosphorylations declined to near unstimulated levels by 6 h even though SB202190 was shown in the previous experiment (Fig. 8A) to block induction of COX-2 when added 12 or 16 h after the addition of Ag. This paradox was resolved by increasing the sensitivity of the immunoblotting procedure where it became apparent that p38 MAP kinase was phosphorylated in unstimulated cells and that this phosphorylation was still minimally enhanced 24 h after Ag stimulation (Fig. 9B, inset). The amount of immunoreactive p38 MAP kinase remained unchanged throughout this period (data not shown).

Phosphorylation of p38 MAP kinase and induction of COX-2 are equally sensitive to the inhibitory actions of dexamethasone

The phosphorylation and activation of ERK and JNK are suppressed after treatment of RBL-2H3 cells with nanomolar concentrations of dexamethasone (4, 45) via glucocorticoid receptors (D. Cissel and M. A. Beaven, unpublished data). We examined whether or not dexamethasone also suppressed phosphorylation of p38 MAP kinase as a potential mechanism for the inhibitory action of this steroid on COX-2 induction. As shown in Fig. 10A, treatment of RBL-2H3 cells with low concentrations of dexamethasone before stimulation with Ag suppressed phosphorylation of the p38 MAP kinase. This suppression was time-dependent, as suppression was not observed after 1 h (data not shown) but was apparent after 5 h. As shown in Fig. 10B, dexamethasone (or vehicle) was added to cultures at the indicated concentrations 5 or 24 h before addition of 20 ng/ml DNP-BSA and the amount of doubly phosphorylated (Thr180/Tyr182)-p38 MAP kinase was determined 15 min later. Values are expressed as a percentage of phosphorylated p38 MAP kinase (A) or COX-2 protein (B) in stimulated cells in the absence of dexamethasone. Data are mean values ± SEM from three separate experiments. Asterisks indicate significant decreases (*, p < 0.05; **, p < 0.01) in phosphorylated enzyme or protein levels.
5 h and more so after 24 h of exposure to dexamethasone (Fig. 10A). Induction of COX-2 was suppressed by the same low concentrations of dexamethasone (Fig. 10B). These effects were apparent with as little as 1 nM dexamethasone.

Discussion

We show that the p38 MAP kinase inhibitor SB202190 partially suppresses the expression of COX-2 message and protein in unstimulated RBL-2H3 cells and their induction in stimulated cells. All three MAP kinases, namely ERK, JNK, and p38 MAP kinase, exhibit similar time courses of activation in response to Ag stimulation (Ref. 46 and T. R. Hundley, unpublished data). However, it would appear from our studies that ERK and JNK (as well as PI-3 kinase) do not play a significant role in the regulation of the COX-2 expression and induction in RBL-2H3 cells. Other eicosanoid-related enzymes, namely COX-1, cPLA2, and 5-LO, are not induced by Ag, although basal levels of cPLA2 and 5-LO protein but not message are reduced by SB202190.

These findings could indicate that p38 MAP kinase plays both a permissive role in maintaining steady-state levels of COX-2 in unstimulated cells and an active role in inducing COX-2 in stimulated cells. Alternatively, another unidentified Ag-mediated signal may provide a necessary stimulatory signal for induction of COX-2 in stimulated cells. In contrast to COX-2, expression of COX-1 is unaffected by SB202190. This observation allayed concern that SB202190 can cause apoptotic changes in RBL-2H3 cells after 24 h at doses higher than those used here (i.e., < 50 μM; T. R. Hundley, unpublished data), as has been noted for inhibitors of p38 MAP kinase in other types of cells (47, 48). The stability of COX-1 suggested instead that the SB202190-induced effects on other eicosanoid-related enzymes were specific and not attributable to preapoptotic changes in RBL-2H3 cells.

As noted earlier, the mechanism of induction of COX-2 varies from one cell type to another. In regard to tumor mast cells, a recent report suggests that COX-2 levels are regulated by ERK and JNK in the murine MMC-34 mast cell line (25), whereas neither kinase appears to have a role in regulating COX-2 in RBL-2H3 cells. Regulatory mechanisms for COX-2 thus may vary among mast cell lines. These variations suggest caution in extrapolating results from one cell line to another or even to their cognates in vivo.

The present findings also raise additional issues. The inhibitory actions of SB202190 described here suggest that COX-2 is regulated by the relatively low p38 MAP kinase activity in unstimulated RBL-2H3 cells and by the minimally elevated activity in chronically stimulated RBL-2H3 cells. Thus, a signal of low intensity may be sufficient for maintenance of COX-2 levels. Indeed, we initially failed to detect the small stimulation of p38 MAP kinase in chronically stimulated cells and the low but measurable p38 MAP kinase activity in unstimulated cells. A possible implication is that low intensity signals such as these may be masked by overstimulation of alternate pathways by pharmacologic or genetic intervention and thus distort the physiological situation.

Another issue concerns the actions of drugs used in this study. Pyridinylimidazole inhibitors such as SB202190 and SB203580 were initially thought to compete with ATP within the ATP-binding pocket of phosphorylated p38 MAP kinase rather than to impede the initial phosphorylation and activation of this enzyme (37). However, in agreement with another report (49), we find that SB202190 inhibits the phosphorylation of p38 MAP kinase in vivo. The affinity (Kd ~40 nM) of SB202190 for p38 MAP kinase (49) makes it likely that much of the drug is lost during extensive washing of immunoprecipitates. Therefore, the measured p38 MAP kinase activities may not represent actual enzyme activities in vivo but rather reflect the reduction in phosphorylation of p38 MAP kinase. Our finding that dexamethasone also inhibits phosphorylation of p38 MAP kinase extends the range of known inhibitory actions of this drug to include all three MAP kinases in RBL-2H3 cells (Refs. 4 and 45 and this paper). Ongoing studies indicate that dexamethasone and other glucocorticoids disrupt signaling at the level of the MAP kinase kinases, namely Raf-1, MEKK-1, and transforming growth factor β-activated kinase (Ref. 45 and D. S. Cissel and M. A. Beaven, unpublished data). We suggest that the inhibition of the MAP kinase pathways may contribute, at least in part, to the suppression of COX-2 induction, as both actions are observed with nanomolar concentrations of dexamethasone.

Stimulation of mast cells via FceRI leads to activation of several Syk-dependent signaling cascades, which include activation of phospholipase C, phospholipase D, protein kinase C (1), the mobilization of calcium ions from internal and external sources (7), and the activation of the MAP kinases (2, 3). Studies in cultured mast cell lines, primarily the RBL-2H3 cell line, indicate that these cascades lead ultimately to secretion of intracellular granules, a response primarily driven by the increase in cytosolic calcium and activation of certain isoforms of protein kinase C (27), and a cPLA2-mediated release of arachidonic acid. The activation of cPLA2 is dependent on increase of cytosolic calcium (8) and the phosphorylation of the enzyme by ERK-2. Thus, PD98059, but not SB203580, inhibits activation of ERK-2, release of arachidonic acid, and gel mobility shift of cPLA2 in stimulated RBL-2H3 cells (5). Similar correlations have been reported for human basophils (50) and neutrophils (34). Therefore, these and the present findings suggest that production of eicosanoids is modulated by at least two MAP kinases. ERK-2 appears to regulate cPLA2 activity and release of arachidonic acid, both of which are inhibited by PD98059 (5). p38 MAP kinase appears to regulate expression of several eicosanoid-related enzymes, including COX-2, and this expression is inhibited by SB202190 (this paper). Inhibitors of ERK and p38 MAP kinase may thus suppress production of the eicosanoids at distinct points of the eicosanoid cascade.

References

INDUCTION OF COX-2 IN MAST CELLS


