Mitogen-activated Protein (MAP) Kinase Regulates Production of Tumor Necrosis Factor-α and Release of Arachidonic Acid in Mast Cells

INDICATIONS OF COMMUNICATION BETWEEN p38 AND p42 MAP KINASES*

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Aggregation of the high affinity IgE receptor (FceRI) in a mast cell line resulted in activation of the p42 and the stress-activated p38 mitogen-activated protein (MAP) kinases. Selective inhibition of these respective kinases with PD 098059 and SB 203580 indicated that p42 MAP kinase, but not p38 MAP kinase, contributed to the production of the cytokine, tumor necrosis factor-α, and the release of arachidonic acid in these cells. Neither kinase, however, was essential for FceRI-mediated degranulation or constitutive production of tumor growth factor-β. Studies with SB 203580 and the p38 MAP kinase activator anisomycin also revealed that p38 MAP kinase negatively regulated activation of p42 MAP kinase and the responses mediated by this kinase.

Stimulation of mast cells by aggregation of membrane IgE receptors (FceRI), leads to recruitment of the tyrosine kinase Syk and activation of Syk-dependent signaling cascades (1, 2). These cascades include activation of phospholipase C and sphingosine kinase for mobilization of calcium ions and PKC1 (3, 4) and the activation of p42 MAP kinase cascade through Ras (2, 5). These cascades lead ultimately to secretion of intracellular granules, a response primarily driven by the increase in [Ca2+]i, and activation of PKC (6), and a cPLA2-mediated release of arachidonic acid. The activation of cPLA2 is dependant on increase of [Ca2+]i, and phosphorylation by MAP kinase (2, 7, 8).

Stimulated mast cells also produce a variety of cytokines that include interleukins 1, 3, 4, 5, and 6 as well as TNFα and granulocyte-macrophage colony-stimulating factor (9, 10). Typically, increased expression of cytokine mRNA and protein is detectable 30 min to several hours after the addition of stimulant (11). These cytokines, particularly TNFα, are thought to mediate pathologic inflammatory reactions (10) and protective responses to bacterial infection (12). The production and release of TNFα are regulated through signals transduced by calcium and PKC, although there are indications that additional FceRI-mediated signals may operate for optimal production of TNFα in cultured RBL-2H3 mast cells. Compared with antigen, other stimulants are relatively weak inducers of TNFα production when doses of stimulants are matched for maximal stimulation of degranulation (13). Also, concentrations of Ro31–7549 that block PKC, secretion of granules, and release of TNFα only partially block production of TNFα (13).

The present objective was to determine whether stimulation of MAP kinases induces additional signals for production of TNFα. A linkage between these events has not been established in mast cells. Antigen-induced stimulation of p42 MAP kinase coincides with the activation of its upstream regulators, Ras, Raf, and MEK-1 (2, 5), and persists through the period when production of TNFα would be most apparent (14). As noted in this paper, however, RBL-2H3 cells also possess the mammalian homologue of the yeast HOG-1 protein kinase, p38 MAP kinase. We have utilized the MEK-1 inhibitor, PD 088059 (15, 16), and the p38 MAP kinase inhibitor, SB 203580 (17), to evaluate the role of these MAP kinases in the production of TNFα and, for comparison, the release of arachidonic acid, degranulation, and production of TGFβ. Release of arachidonic acid is thought to be dependent on phosphorylation of cPLA2 by MAP kinase, although the identity of the MAP kinase is uncertain (18). Degranulation and TGFβ production were assumed to be MAP-kinase-independent responses (7, 19). We show that, while p42 MAP kinase regulated production of both TNFα and arachidonic acid, p38 MAP kinase negatively regulated the activation of p42 MAP kinase and the responses mediated by this kinase.

MATERIALS AND METHODS

Reagents—Reagents were obtained from the following sources: all reagents for cell culture from Life Technologies, Inc.; ATP from Boehringer Mannheim; adenosine 5′-[(32)P]triphosphate tetra(triethyl-aminomonomium) salt and [14C]arachidonic acid from DuPont NEN; phenyl-Sepharose from Pharmacia (Uppsala, Sweden); MAP kinase substrates (myelin basic protein and a myelin basic peptide, residues 94–102), polyclonal antibodies against the COOH-terminal peptide of rat MAP kinase R2 (erk1-CT), anti-phosphotyrosine monoclonal antibody, 4G10, and p42 MAP kinase glutathione S-transferase fusion protein from Upstate Biotechnology Inc. (Lake Placid, NY); polyclonal antibodies against p38 MAP kinase, MEK, and cPLA2, from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA); TGFβ 1 kit and Factor-testTM Mouse TNF-α from Genzyme Corp. (Cambridge, MA). Ro31–7549 was obtained from LC Laboratories. The compounds PD 098059 and SB 203580 were synthesized in the Tsukuba Research Laboratories, Eisai Co., according to the procedures of Bridges et al. (20) and Adams et al. (21), respectively, and purified by column chromatography and recrystallization. These compounds were determined to be >95% pure on the basis of high

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The abbreviations used are: PKC, protein kinase C; PLA2, phospholipase A2; cPLA2, cytosolic PLA2; MAP, mitogen-activated protein; FceRI, high affinity receptor for IgE; DNP-BSA, antigen consisting of 24 molecules of O-dinitrophenol conjugated with one molecule of bovine serum albumin; TNFα and -β, tumor necrosis factor-α and -β, respectively; TGFβ, transforming growth factor-β; PIPES, 1,4-piperazineethanesulfonic acid; MOPS, 4-morpholinepropanesulfonic acid; MEK, MAP kinase.
terphoresis and detected by autoradiography.

MBP (nase R2 and assayed for MAP kinase activity using myelin basic protein MAP kinase was immunoprecipitated with antibody against MAP kinase (2, 14). The additional retarded band represents the activated tyrosine-phosphorylated form of p42 MAP kinase (p42\textsuperscript{p42MAPkinase}).

Assay of MAP Kinase Activity in Cell Extracts—

Measurement of Degranulation, Release of \[^{14}C\]Arachidonic Acid, and Production of TNFα and TGFβ—Release of the granule marker, hexosaminidase, was determined by colorimetric assay of medium and cell lysates by previously described procedures (6). For measurement of release of arachidonic acid, cells were labeled with \[^{14}C\]arachidonic acid (0.1 μCi/ml). Cultures were washed the next day and replenished with the required medium. For the assay of hexosaminidase or \[^{14}C\]arachidonic acid, experiments were performed in a PIPES-buffered medium (25 mM PIPES, pH 7.2, 150 mM NaCl, 5 mM KCl, 0.4 mM MgCl\(_2\), 1.0 mM CaCl\(_2\), 5.6 mM glucose, and 0.1% fatty acid-free fraction V bovine serum albumin). For \[^{32}P\]phosphorylation of proteins, cultures were incubated for 90 min with \[^{32}P\]pyrophosphate (200 μCi/ml). Cells were harvested by scraping, and cell lysates (in 0.1% Triton X-100) were assayed for hexosaminidase and -14C]arachidonic acid release, \[^{32}P\]pyrophosphate fusion protein (extracellular signal-regulated kinase) as substrate with \[^{32}P\]pyrophosphate and then phosphorylated peptide was isolated by centrifugation of the incubation substrate peptide (peptide 94–102 of bovine myelin basic protein). The mixture was kept on ice for 5 min for binding of MAP Kinase to the beads. After centrifugation, the phenyl-Sepharose was washed beforehand with 300 ml of a Tris buffer (25 mM Tris, pH 7.5, 25 mM NaCl, 1 mM Na\(_3\)VO\(_4\), 2 mM EGTA, 1.5 mM dithiothreitol, 2.5 mM p-nitrophenyl phosphate, and 20 μg/ml leupeptin and antiproteinase). Cells were disrupted by freezing and thawing three times. The lysate was centrifuged (15,800 \(\times g\) for 10 min), and 450 μl of the supernatant fraction was mixed with 50 μl of ethylene glycol and 80 μl of washed phenyl-Sepharose. The phenyl-Sepharose was washed beforehand with 300 μl of the Tris buffer. The mixture was kept on ice for 5 min for binding of MAP Kinase to the beads. After centrifugation, the phenyl-Sepharose beads were washed with 1 ml of 10% (v/v) ethylene glycol and then with 30% (v/v) ethylene glycol. Finally, MAP kinase was eluted by incubating the beads with 75 μl of 60% ethylene glycol for 5 min on ice. After centrifugation of the suspension, 15 μl of supernatant was incubated (15 min, 30°C) in a solution that contained 50 mM Tris (pH 7.5), 10 mM MgCl\(_2\), \[^{32}P\]pyrophosphate (10 Ci/mmol, 37 kBq/μl), and 25 μg of MAP kinase substrate peptide (peptide 94–102 of bovine myelin basic protein). The phosphorylated peptide was isolated by centrifugation of the incubation mixture through phosphocellulose membrane (Spinzyme; Pierce), which was then washed twice with 500 μl of 75 mM H\(_3\)PO\(_4\) for the assay of radioactivity.

Assay of MAP Kinase and MEK Activities by Immunoprecipitation—

performance liquid chromatography and NMR analysis. The antigen, DNP-BSA, and O-dinitrophenol-specific monoclonal IgE were kindly supplied by Dr. Henry Metzger (NIAMS, National Institutes of Health, Bethesda, MD).

Cell Culture and Measurement of Stimulatory Responses—

The RBL-2H3 cell line was maintained in complete growth medium (minimum essential medium) supplemented with 15% fetal calf serum, glutamine, antibiotic, and antimycotic agents. Trypsinized cells were plated into 150-mm culture dishes or six-well Costar cluster plates and were incubated overnight in complete growth medium with O-dinitrophenol-specific IgE (0.5 μg/ml) and, for measurement of arachidonic acid release, \[^{32}P\]pyrophosphate and then autoradiography as described under "Materials and Methods." C, cell lysates were analyzed by Western blotting for p42 MAP kinase. The lower band, previously identified as p42 MAP kinase (p42\textsuperscript{p42MAPkinase}), is indicated. The additional retarded band represents the activated tyrosine-phosphorylated form of p42 MAP kinase (p42\textsuperscript{p42MAPkinase}).

Assay of MAP Kinase and MEK Activities by Immunoprecipitation—

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p42 and p38 MAP kinases were immunoprecipitated with the appropriate polyclonal antibodies by procedures described elsewhere (2). Equal amounts of immunoprecipitated proteins from 5 × 10⁶ cells were incubated in a MOPS buffer (25 mM β-glycerol phosphate, 1 mM EGTA, 1 mM sodium orthovanadate, 1 mM dithiothreitol, and 25 mM MOPS, pH 7.2) with Mg²⁺-[γ³²P]ATP (10 μCi in 150 μM cold ATP, 25 μM MgCl₂) and 5 μM myelin basic protein (18 kDa) as substrate in a final volume of 30 μl. The mixture was incubated at 30 °C for 12 min. The reaction was terminated by the addition of 30 μl of 4× SDS sample buffer. MEK was immunoprecipitated with anti-MEK antibody and assayed similarly except that p42 MAP kinase glutathione S-transferase fusion protein (1 μg/assay) was used as substrate for phosphorylation. Proteins were separated by 12% SDS-PAGE. Radioactive proteins were detected by autoradiography.

**Electrophoretic Separation and Immunoblotting of [³²P]MEK, p42 MAP Kinase, and cPLA₂**—The preparation of cell lysates and immunoprecipitates, analysis of proteins by SDS-PAGE, and transfer to nitrocellulose paper were performed as described elsewhere (2, 7) with the following exception: cPLA₂ was separated on NOVEX 10% Tris/glycine gels for 3 h at 35 mA and 4 °C as described by Kramer and co-workers (25). Previously described procedures were used for isolation and detection of [³²P]MEK (7). Otherwise, proteins were detected by the immunoblotting technique with antibodies against MEK, p42 MAP kinase, cPLA₂, or anti-phosphotyrosine. Secondary antibodies included horse-radish peroxidase-conjugated antibody against rabbit IgG or mouse IgG. Finally, proteins were visualized by the ECL System (Amersham Corp.) or by autoradiography.

**RESULTS**

**MEK Inhibitor PD 098059 Inhibits the Activity of p42 MAP Kinase, Release of Arachidonic Acid, and Production of TNFα**—As shown in Fig. 1, the MEK inhibitor PD 098059 attenuated antigen-induced [³²P]phosphorylation of MEK (panel A) and the activation of MEK as determined by in vitro assay of immunoprecipitated MEK (panel B). Activation of p42 MAP kinase was also attenuated, as indicated by the change in electrophoretic migration of p42 MAP kinase (panel C) or by the assay of MAP kinase activity of immunoprecipitated p42 MAP kinase (panel D). The extent of these inhibitions was dependent on the concentration of PD 098059. As shown in Fig. 2, the suppression of MAP kinase activation by PD 098059 (panel A) was associated with similar dose-dependent suppression of arachidonic acid release (panel B) and TNFα production (panel C). The suppression of the latter two responses was highly correlated (r > 0.95). All three responses were inhibited by 50% with 10 μM PD 098059. As will be described later, activation of cPLA₂ was also inhibited by PD 098059. These results suggested that release of arachidonic acid and production of TNFα were both regulated by p42 MAP kinase.

**The Effect of PD 098059 on Degranulation and TGFβ Production**—To test the selectivity of PD 098059, we next examined the effects of this compound on antigen-stimulated degranulation and the constitutive production of TGFβ, which are thought not to be regulated by MAP kinase (7, 19). PD 098059 had only minimal effects on stimulated release of the hexosaminidase (Fig. 3A) and the production of TGFβ in unstimulated cells (Fig. 3B). The only significant effect was partial inhibition (<30%) of degranulation at 50 μM PD 098059 (3A).

The p38 MAP Kinase Inhibitor, SB 203580, Enhances Activation of p42 MAP Kinase, Release of Arachidonic Acid, and Production of TNFα in Antigen-stimulated Cells—Antigen stimulation also resulted in increased activity of p38 MAP kinase (Fig. 4A, compare lanes 1 and 2). The p38 kinase inhibitor, SB 203580, inhibited this activation (Fig. 4A, lanes 3 and 4). Interestingly, antigen activation of p42 MAP kinase was enhanced significantly by SB 203580. This enhancement was apparent when cells were stimulated with 20 or 200 ng/ml antigen (Fig. 4B). The latter concentration of antigen was known to elicit maximal activation of p42 MAP kinase.² These results suggested that p38 MAP kinase negatively regulates p42 MAP kinase and that this regulation is alleviated by SB 203580.

The enhanced activation of p42 MAP kinase in the presence of SB 203580 was associated with increased release of arachidonic acid (Fig. 5A) and production of TNFα (Fig. 5B). In the experiment shown in Fig. 5B, cells were stimulated with a low concentration of antigen (6 ng/ml) to maximize enhancement of the TNFα response (250% increase in Fig. 5B). At optimal doses of antigen enhancement of TNFα production was less (40–80% increase) but still significant (data not shown).

Because pyridinyl imidazoles that are closely related to SB 203580 have cyclooxygenase-inhibitory activity (25, 26), experiments were conducted to determine whether blockade of cyclooxygenase activity with indomethacin (27) altered accumulation of radiolabel in the medium by suppressing metabolism of [¹⁴C]arachidonate via this enzyme. Unlike SB 203580, 10 μM indomethacin did not significantly alter release of radiolabel from antigen-stimulated cells (7.8 ± 0.4% release over 15 min versus 7.2 ± 0.2% release in the absence of indomethacin; mean ± S.E. in eight cultures from two experiments). It seemed probable, therefore, that SB 203580 enhanced release rather than the accumulation of [¹⁴C]arachidonic acid in the medium.

In contrast to the increased release of arachidonic acid and production of TNFα, SB 203580 had no significant effect on antigen-induced degranulation (Fig. 5C) or constitutive production of TGFβ (Fig. 5D). Collectively, these results provided further evidence for the notion that release of arachidonic acid and TNFα production are both regulated by p42 MAP kinase. In addition, the results suggested that p38 MAP kinase negatively modulates these responses through p42 MAP kinase.

The above results suggested that release of arachidonic acid,

² C. Zhang, R. A. Baumgartner, K. Yamada, and M. A. Beaven, unpublished data.
as well as production of TNFα, was regulated by p42 MAP kinase. As in other systems (25, 28), the phosphorylation of cPLA2 in stimulated RBL-2H3 cells leads to decreased electrophoretic mobility of the enzyme (7). The connection between p42 MAP kinase and the release of arachidonic acid via cPLA2 was further demonstrated by the finding that the antigen-induced retardation of electrophoretic migration of cPLA2 (25) was suppressed by PD 098059 but not by SB 203580 (Fig. 6).

Activation of p38 MAP Kinase by Anisomycin Partially Suppresses Activation of p42 MAP Kinase and Release of Arachidonic Acid—The p38 MAP kinase activator, anisomycin, markedly activated this kinase (Fig. 7A) but much less so p42 MAP kinase (Fig. 7B). The combination of anisomycin and antigen revealed inhibitory communication between these two kinase. For example, the combination of stimulants resulted in less activation of p38 MAP kinase (Fig. 7C, lane 3) than that induced by antigen (Fig. 7C, lane 2) or anisomycin (Fig. 7A, lane 2) alone. The combination also caused less activation of p42 MAP kinase (Fig. 7D, lane 3) than that by antigen alone (Fig. 7D, lane 2). Thus, stimulation of p42 MAP kinase by antigen appeared to block activation of p38 MAP kinase by anisomycin, and conversely stimulation of p38 MAP kinase by anisomycin appeared to partially block activation of p42 MAP kinase by antigen. Consistent with the latter situation, anisomycin partially suppressed antigen-induced release of arachidonic acid (25 ± 4% reduction, mean of three experiments). This reduction corresponded to an approximately 25% reduction in p42 MAP kinase activation as determined by densitometric analysis of the blots shown in Fig. 7D and two other experiments. Anisomycin almost totally blocked (by 83 ± 4%) antigen-induced production of TNFα, probably as a consequence, however, of its known inhibitory actions on protein synthesis at the translation step (29). Presumably, de novo synthesis of TNFα would be especially sensitive to inhibitors of protein synthesis.

DISCUSSION

Past studies have shown that the responses evoked by antigen in RBL-2H3 cells were dependent on calcium and signals generated through PKC or MAP kinase. These studies indicated, for example, that PKC regulated degranulation (6) as well as the production and secretion of TNFα, although it appeared likely that additional FcεRI-mediated signals facilitated TNFα production (13). Activation of p42 MAP kinase, in contrast, was associated with phosphorylation of cPLA2 and release of arachidonic acid (2, 7). These studies, however, did not address the issue of whether other MAP kinases, such as p38 MAP kinase, regulated cPLA2.

The present results demonstrate that both p38 and p42 MAP kinases are activated in antigen-stimulated cells. Activation of the latter kinase appears to be most closely related to release of arachidonic acid and production of TNFα. All three events are
inhibited by the MEK inhibitor, PD 098059 (Fig. 2), and enhanced by the p38 MAP kinase inhibitor, SB 203580 (Figs. 4 and 5). Both compounds are reported to be selective inhibitors of MEK (i.e. PD 098059) and p38 MAP kinase (i.e. SB 203580) when tested against a wide range of kinases (15–17). In addition, the enhancement of responses in the presence of SB 203580, in contrast to the attenuation of p42 MAP kinase activation by the p38 MAP kinase activator, anisomycin (Fig. 7), suggest that p38 MAP kinase negatively regulates activation of p42 MAP kinase and its associated responses. Antigen-stimulated degranulation and the constitutive production of TGFβ in RBL-2H3 cells are minimally affected by the inhibitors (Figs. 3 and 5). Collectively, the results support the notion that p42 MAP kinase regulates release of arachidonic acid and promotes an additional signal for stimulating TNFα production but does not regulate degranulation. Interestingly, the p38 MAP kinase inhibitor, SB 203580, was first identified as an inhibitor of cytokine biosynthesis in lipopolysaccharide-stimulated human monocytes (17) and was subsequently shown to suppress TNFα production in lipopolysaccharide-injected mice (30). The compound also possessed anti-inflammatory activity in mouse models of arthritis (collagen- and adjuvant-induced), whereas cellular immune responses measured ex vivo were unaffected (30). It is possible, therefore, that different MAP kinase pathways are utilized for activating gene transcription for cytokine synthesis when synthesis is induced by inflammatory agents or through multimeric immunologic receptors such as FccRI.

The question has been raised whether p38 rather than p42 MAP kinase is responsible for the activation of cPLA2 (18, 25). cPLA2 is phosphorylated by both kinases in thrombin-stimulated platelets, although the phosphorylation by p38 MAP kinase does not appear to activate cPLA2 (25). Our results indicate that p42 MAP kinase regulates phosphorylation of cPLA2 and release of arachidonic acid and suggest, therefore, that this kinase is the activator of cPLA2 at least in RBL-2H3 cells.

Most mast cells, including RBL-2H3 cells, also contain a low molecular weight secreted form (type II) of PLA2 (31) in secretory granules, and this form is presumably released along with other granule constituents in activated cells (32). A role for secreted PLA2 is unlikely, however, because total suppression of degranulation by selective inhibitors of PKC, such as Ro31–7549 and calphostin C, minimally affects release of arachidonic acid in RBL-2H3 cells (Refs. 7 and 33 and see below). In addition to the correlations between activation of the p42 MAP kinase/cPLA2 pathway and release of arachidonic acid noted here with PD 098059 and SB 203580, similar correlations have been noted in previous studies with less specific MAP kinase inhibitors. Activation of the entire Raf/MEK/p42 MAP kinase pathway and release of arachidonic acid were suppressed equally by the glucocorticoid, dexamethasone, and the kinase inhibitor, quercetin, while effects on degranulation were apparent only at relatively high concentrations of these agents (7, 34). Correlations were noted with the PKC inhibitor, Ro31–7549. This inhibitor transiently delayed activation of p42 MAP kinase in antigen-stimulated RBL-2H3 cells. There was a corresponding transient delay in the release of arachidonic acid, although the cumulative release eventually equaled that observed in the absence of Ro31–7549 (14). On the basis of these and other results, we have suggested that the p42 MAP kinase/cPLA2 pathway, although transiently activated by PKC, was predominantly activated by Ras through recruitment of Shc/Grb2/Sos or Vav by FccRI (14). Others have reported that fatty acids, particularly arachidonic acid, activate p42 and p44 MAP kinases through PKC (35). This scenario is unlikely in antigen-stimulated RBL-2H3 cells, however, because of the predominance of the PKC-independent (i.e. Ro31–7549-resistant) pathway in RBL-2H3 cells.

The present data extend previous findings on the regulation of TNFα synthesis and release. This cytokine is synthesized de novo and subsequently secreted via Golgi in a PKC- and calcium-dependent manner (13). The PKC inhibitor, Ro31–7549, blocks secretion of TNFα but only partially suppresses synthesis of TNFα (6, 13, 19). Thus, additional signals may be necessary for optimal stimulation of TNFα synthesis. Antigen is a particularly potent stimulant of TNFα production when compared with the combination of calcium ionophore and PKC agonist, phorbol 12-myristate 13-acetate (13, 19). These observations and the present studies with the MAP kinase inhibitors suggest that optimal production of TNFα is achieved through activation of both PKC and p42 MAP kinase.

The present findings may explain antigen-induced activation of p42 MAP kinase (34), release of arachidonic acid (22, 34) and production of TNFα (36) exhibit similar sensitivity to dexamethasone. All three responses are totally suppressed in RBL-2H3 cells that have been treated with 10 μM dexamethasone, whereas antigen-stimulated hydrolysis of phosphoinositides, increase in [Ca2+]i, and degranulation (22, 34) are only partially suppressed by treatment of cells with 100 μM dexamethasone. Dexamethasone, as noted above, inhibits the entire

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In addition to the cytosolic (cPLA2) and secreted (sPLA2) PLA2, a calcium-independent form of PLA2, (iPLA2) has been described in mouse and rat platelets and smooth muscle cells (40). iPLA2 is inhibited by bromoelaidin lactone (41) and activated by depletion of intracellular calcium stores or by the calmodulin inhibitor, W7 (40). By use of the same experimental strategies, we find no evidence for the presence of iPLA2 in RBL-2H3 cells (T. Hundley and M. A. Beaven, unpublished data).

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**MAP Kinase Regulation of TNFα Production**

**FIG. 6.** Effect of PD 098059 and SB 203580 on antigen-induced retardation of electrophoretic migration of cPLA2. RBL-2H3 cells were stimulated with 20 ng/ml DNP-BSA (Ag. +) or left unstimulated (Ag. −) for 7 min in the presence or absence of the indicated concentrations of PD 098059 (PD) or SB 203580 (SB). Proteins in cell lysates were separated by SDS-PAGE, and cPLA2 was detected by probing with anti-cPLA2 antibody as described under "Materials and Methods."

**FIG. 7.** Activation of p38 and p42 MAP kinases by anisomycin and antigen. Intact RBL-2H3 cells were incubated with vehicle (Ag. −) or 10 μM of anisomycin for 30 min (Ag. +) and then left unstimulated (Ag. −) or stimulated with 20 ng/ml DNP-BSA (Ag. +) for 5 min before the assay of immunoprecipitated p38 or p42 MAP kinases with myelin basic protein as substrate as described in previous figure legends.
Raf/MEK/p42 MAP kinase/cPLA$_2$ pathway at nanomolar concentrations (34). Therefore, if p42 MAP kinase is the common regulator of TNF$\alpha$ production and arachidonic acid release, the similar sensitivities to dexamethasone would be expected.

The connections between the p42 MAP kinase pathway and cytokine production are unknown for mast cells, but recent reports indicate the following connections in other types of cells. The overexpression of RafI (37, 38) or p42 MAP kinase (39) results in enhanced expression of a variety of cytokine genes in T cells and macrophages (37, 39), the inactivation of IcB (38), and the enhanced binding activity of cytokine transcription factors such as NF-xB and AP-1 (39).

In conclusion, the above results provide the first indication that p42 MAP kinase regulates antigen-mediated production of TNF$\alpha$ in a mast cell line and that p38 MAP kinase may negatively regulate the p42 MAP kinase/cytokine pathway. We can, for the first time, broadly define the regulatory pathways for all three functional responses of mast cells to antigen as follows. Along with elevated [Ca$^{2+}$], the additional primary signals are as follows: for degranulation (6) and secretion of newly formed cytokines (13), activation of PKC (6); for cPLA$_2$-mediated release of arachidonic acid, activation of p42 MAP kinase (Refs. 2, 7, and 34, and this paper); and for production of TNF$\alpha$, the coactivation of PKC and p42 MAP kinase (Ref. 13 and this paper).

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