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Regulation of ERK-Mediated Signal Transduction by p38 MAP Kinase in Human Monocytic THP-1 Cells

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SB 203580 has been widely used to specifically shut down the p38 MAP kinase-dependent pathway, although it is capable of inducing c-Raf kinase activity in cells. The present study demonstrates that SB 203580 activates members of the ERK cascade, c-Raf, MEK, and ERK, in human monocytic THP-1 cells. The activation of these kinases was sustained for at least 24 h after SB 203580 treatment and was also observed in U937 cells, suggesting that c-Raf efficiently transduces the signal even in the presence of the inhibitor in these cells. However, the expression of ERK cascade–dependent genes, such as c-*fos* and IL-1β, was extremely limited. Analysis of the cellular distribution of ERK in SB 203580-treated cells indicated that nuclear translocation of phosphorylated ERK was impaired. Also, nuclear translocation of ERK induced by 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) was inhibited by SB 239063, which does not associate with c-Raf and is highly selective for p38 MAP kinase. In addition, the forced expression of the dominant negative mutant of p38 MAP kinase suppressed serum responsive element-dependent transactivation induced by TPA. These results suggest that the steady-state level of p38 MAP kinase activity modulates ERK signaling.

Key words: cross talk, ERK, IL-1β, nuclear translocation, p38 MAP kinase.

Abbreviations: ERK, extracellular-signal-regulated kinase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IL-1β, interkeukin-1β; JNK, c-Jun N-terminal kinase; MAP kinase, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; TNFα, tumor necrosis factor α; TPA, 12-*O*-tetradecanoyl-phorbol-13-acetate.

The mitogen-activated protein (MAP) kinases constitute a family of proline-directed serine/threonine kinases included in least four distinct cascades; extracellular-signal-regulated kinase (ERK), c-Jun *N*-terminal kinase (JNK), p38 MAP kinase and ERK5. MAP kinases function as cellular signal transducers, and play a central role in the control of cellular proliferation, differentiation, and apoptosis in multicellular organisms. MAP kinase function has been analyzed in detail using commercially available low molecular weight compounds, which specifically inhibit kinase-dependent signal transduction. The compounds of choice for many researchers are PD 98059 (*1*) and U0126 (*2*), which demonstrate the participation of the ERK cascade in particular biological processes, and SB 202190 and SB 203580, which demonstrate the involvement of p38 MAP kinase (*3–5*). Since an inhibitor specific for JNK (*6*) has also been developed recently, the function of MAP kinases in cellular signal transduction pathways involved in complex biological processes may become clearer.

In the course of studying cellular signal transduction in monocytic THP-1 cells (*7*), we found that SB 203580 significantly reinforced interleukin (IL)-1β and tumor necrosis factor α (TNFα) gene expression induced by low concentrations of bufalin, a cardiotonic steroid that induces differentiation (*8*) and apoptosis (*9*) in leukemia cells. It would be interesting to determine the mechanism by which SB 203580 achieves these effects, since they have not been reported for other MAP kinase inhibitors. It has been shown, however, that in addition to p38 MAP kinase, SB 203580 binds to and inhibits c-Raf kinase *in vitro* (*10*). In contrast, the addition of SB 203580 to the culture medium resulted in activation of c-Raf kinase within the cells. Similar activation of c-Raf kinase has been observed when cells are treated with the c-Raf inhibitor ZM 336372 (*11*). Despite the increased c-Raf activity, these authors found no activation of downstream molecules, MAPK/ERK kinase (MEK) and ERK (*10*, *11*). In spite of the extensive use of SB 203580, there have been no previous reports describing ERK cascade activation by this p38 MAP kinase inhibitor.

In the present study, we demonstrate the activation of the ERK cascade by SB 203580 in human monocytic cells. We show that even though ERK was activated by SB 203580, the expression of the target genes of this kinase (such as IL-1β) was very limited. Our results suggest that the translocation of activated ERK from the cytosol to the nucleus is suppressed in SB 203580-treated cells, which leads us to conclude that p38 MAP kinase activity may modulate ERK signaling.

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MATERIALS AND METHODS

Materials—12-*O*-Tetradecanoyl-phorbol-13-acetate (TPA) was purchased from Sigma (St. Louis, MO). SB 203580 was purchased from Calbiochem (La Jolla, CA). SB 239063 was kindly supplied by Glaxo SmithKline. Antibodies for phosphorylated p44/42 MAPK (Thr202/Tyr204), phosphorylated MEK1/2 (Ser217/221), phosphorylated p38 (Thr180/Tyr182), phosphorylated IκBα (Ser32), p44/42 MAPK, MEK1/2 and p38 were purchased from Cell Signaling Technology (Beverly, MA). GST-Elk-1 (307–428) was also purchased from Cell Signaling Technology. Anti-ERK2 (C-14) and anti-c-Raf (C-12) antibodies for immunoprecipitation, and the full-length MEK-1 protein were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Other chemicals were of the highest grade commercially available.

Cell Culture—THP-1 cells were obtained from the Riken Cell Bank (Tsukuba). U937 and COS-7 cells were obtained from the Japanese Cancer Research Resources Bank (Osaka). THP-1 and U937 cells were cultured in RPMI 1640 medium comprising 10% fetal calf serum (FCS), 20 mM HEPES, 0.2% sodium bicarbonate and penicillin (100 units/ml)-streptomycin (100 μg/ml). These cells were seeded at a density of 1×10^5 cells/ml and logarithmic growth was maintained under a humidified 5% CO_2 atmosphere at 37°C. COS-7 cells were maintained in DMEM containing 10% FCS, 20 mM HEPES, 0.2% sodium bicarbonate and antibiotics. All experiments were performed in the presence of serum.

Northern Blot Analysis—Total cellular RNA was isolated by acid guanidinium thiocyanate-phenol-chloroform extraction as described by Chomczynski and Sacchi (*10*, *11*). Northern blot analysis was carried out as described previously (*7*). The probes used were a 1.1 kb human IL-1β cDNA *Pst*I insert purified from plasmid IL-1 X-14 (*12*) (American Type Culture Collection), and a 0.5 kb glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA insert purified from plasmid GD5 (*13*).

Immunoblot Analysis—Cells were washed with PBS and then lysed by boiling in SDS-sample buffer (62.5 mM Tris-HCl, pH 6.8, 5% 2-mercaptoethanol, 2% sodium dodecylsulfate, 10% glycerol, and 0.025% bromophenol blue). Denatured proteins were separated on a polyacrylamide gel (8%) and then transferred to a polyvinylidene difluoride membrane (Pall Biosupport Division, Port Washington, NY) using a semi-dry blotting apparatus at 120 mA for 1 h. The membrane was incubated for 1 h at room temperature with 0.2% casein-based I-Block (Tropix, Bedford, MA) dissolved in 25 mM Tris-HCl, pH 7.4, containing 137 mM NaCl, 2.68 mM KCl, and 0.1% Tween 20 (TTBS), washed with TTBS (3 × 15 min), and then incubated for 1 h with the primary antibodies which had been previously dissolved in the blocking solution overnight at 4°C. After washing with TTBS, the membrane was incubated for 1 h with secondary antibodies conjugated to horseradish peroxidase. Immunoreactive proteins were detected with an ECL system (Amersham-Pharmacia Biotech, Buckinghamshire, UK).

Immunocomplex Kinase Assay—Cells were washed with PBS and then lysed in lysis buffer [20 mM Tris, pH 7.4, containing 1% Triton X-100, 2 mM EDTA, 137 mM NaCl, 1 mM sodium vanadate, 2 mM sodium orthophosphate, 10% glycerol, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride, and 10 μg/ml leupeptin] for 10 min at 0°C. After centrifugation, 1 ml of the clear cell lysate was incubated with 4 μg of appropriate antibodies for 1 h at 4°C, followed by with lysis buffer-equilibrated protein G conjugated agarose for 1 h at 4°C. The immunocomplex was washed 3 times with lysis buffer containing 0.5 M NaCl and then once with kinase buffer (35 mM Tris, pH 7.5, containing 10 mM MgCl2, 0.5 mM EGTA, and 0.1 mM CaCl2). The kinase reaction was performed in kinase buffer containing 2 μg of an appropriate protein substrate, 50 μM ATP, and 8 μCi [γ-^{32}P]ATP for 10 min at 30°C. The phosphorylated proteins were resolved by 12% SDS–polyacrylamide gel electrophoresis.

Preparation of Subcellular Fractions—Cells were washed with PBS and then homogenized in buffer A [20 mM Tris, pH 7.4, containing 2 mM EDTA, 137 mM NaCl, 1 mM sodium orthovanadate, 2 mM sodium pyrophosphate, 10% glycerol, 1 mM 4-(2-aminorthyl)-benzenesulfonyl fluoride and 10 μg/ml leupeptin] using a Dounce homogenizer. The homogenized suspension was clarified by centrifugation at 750 ×*g* for 20 min at 4°C. The supernatant thus obtained was subjected to further centrifugation at 10,000 ×*g* for 20 min at 4°C to obtain a cellular lysate, which was used as the cytosol fraction. The pellet obtained on the first centrifugation at 750 ×*g* was washed with buffer A and then lysed with buffer A containing 2% SDS. After sonication, this solution was used as the nuclear fraction.

Expression Vectors and Reporter Constructs—Firefly luciferase reporter plasmid pSRE-Luc (BD Biosciences Clontech, Palo Alto, CA) contains 3 tandem copies of the serum responsive element (SRE) upstream of the Herpes simplex virus thymidine kinase promoter. The PCR-amplified coding region of human p38α was inserted into the pcDNA3.1 vector (Invitrogen, Carlsbad, CA) (p38/pcDNA3.1). A vector encoding a dominant negative mutant of p38 (p38DN/pcDNA3.1) was generated by replacing Thr^{180} and Tyr^{182} by Ala and Phe, respectively (*14*), using a Gene Editor Site Directed Mutagenesis System (Promega, Madison, WI).

Reporter Assay—THP-1 cells seeded at a density of 1×10^5 cells in a 12-well plate were transfected in triplicate with 0.1 μg of DNA consisting of pSRE-Luc, pRL-TK (Promega), as an internal control, p38/pcDNA3.1, p38DN/pcDNA3.1, and/or the corresponding empty vector using Effecten Transfection Reagent (Qiagen, Hilden, Germany). COS-7 cells seeded at a density of 0.8×10^5 cells of 24-well plate 1 day in advance were transfected in triplicate with 0.8 μg of DNA using Lipofectamine 2000 (Invitrogen). Cells were either untreated or treated with 10 nM TPA 12 h after transfection and then incubated for another 12 h. Firefly and *Renilla* luciferase activities were measured with a Dual-Luciferase Reporter Assay System (Promega). Firefly luciferase activity was normalized with respect to *Renilla* luciferase activity.

RESULTS

Previous studies revealed that c-Raf activity in a lysate of cells incubated with SB 203580 or ZM 336372 increases significantly, when measured *in vitro*, but paradoxically,

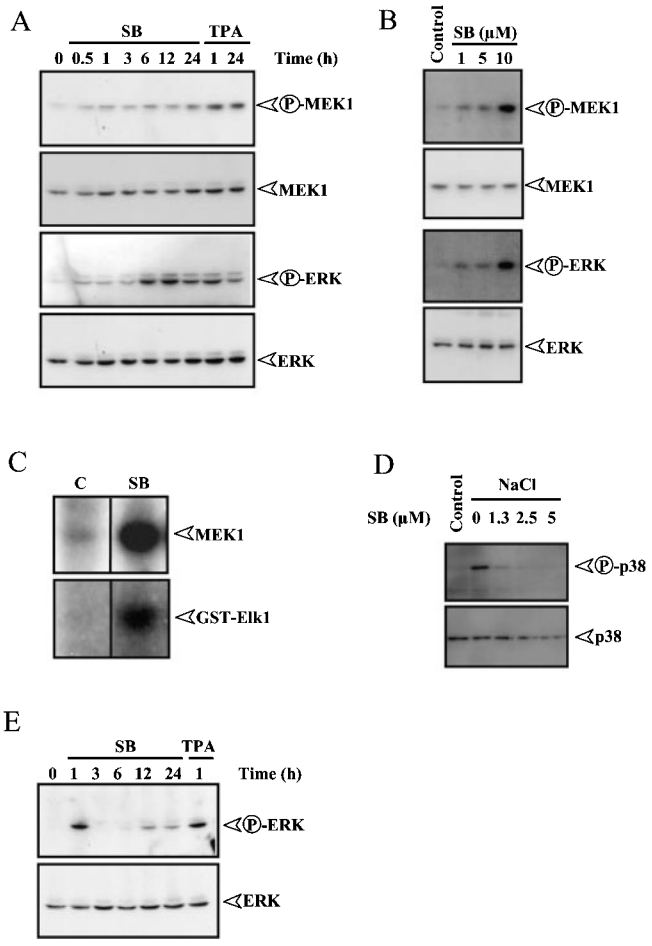

Fig. 1. **SB 203580 induces c-Raf, MEK and ERK activation in monocytic cells.** A: THP-1 cells were either untreated (0 h) or treated with 5 μM SB 203580 (SB) or 10 nM TPA for the times indicated. Cell lysates were subjected to immunoblot analysis using anti-Ser217/221 phosphorylated MEK and anti-Thr202/Tyr204 phosphorylated ERK antibodies. Blots were probed sequentially with anti-MEK and ERK antibodies to determine the total kinase loaded. B: THP-1 cells were either untreated (Control) or treated with SB 203580 (SB) at the indicated concentrations for 1 h. Phospho-MEK and ERK were analyzed as described above. C: THP-1 cells were either untreated (C) or treated with 5 μM SB 203580 (SB) for 2 h. Cell lysates were subjected to immunoprecipitation with anti-c-Raf or -ERK antibodies, and *in vitro* kinase assaying to determine c-Raf and ERK activities using MEK1 and GST-Elk1 as the substrates, respectively. Incorporation of ^{32}P into the substrate protein is illustrated. D: THP-1 cells were either untreated or pretreated for 30 min with SB203580 at the indicated concentrations followed by 45 min stimulation with 0.5 M NaCl. Cell lysates were subjected to immunoblot analysis using anti-Thr180/Tyr182 phosphorylated p38 antibodies. Blots were probed sequentially with anti-p38 antibodies to determine the total kinase loaded. E: U937 cells were either untreated (0 h) or treated with 5 μM SB 203580 (SB) or 10 nM TPA for the times indicated. Cell lysates were subjected to immunoblot analysis using anti-Thr202/Tyr204 phosphorylated ERK antibodies. Blots were probed sequentially with anti-ERK antibodies to determine the total kinase loaded.

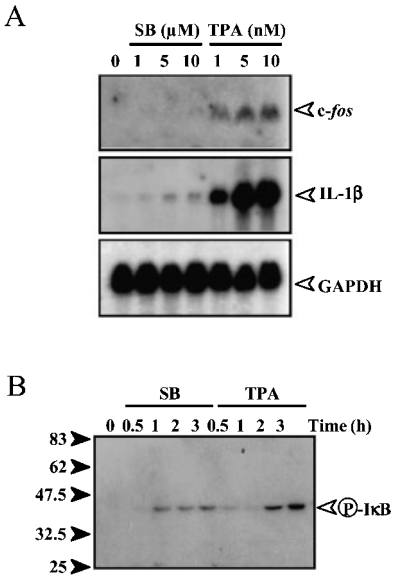

Fig. 2. **Lack of ERK cascade-dependent gene expression in SB 203580-treated THP-1 cells.** A: cells were either untreated (0 h) or treated with SB 203580 (SB) or TPA at the indicated concentrations for 12 h. Northern blot analysis was carried out using ^{32}P-labeled c-*fos* and IL-1β specific probes. The membranes were subsequently hybridized with a GAPDH probe to assess equal loading. B: cells were either untreated (0 h) or treated with 5 μM SB 203580 (SB) or 10 nM TPA for the times indicated. Cell lysates were subjected to immunoblot analysis to detect Ser32 phosphorylated IκBα.

the kinase activity within intact cells is suppressed by these inhibitors, preventing further signal transduction to down-stream kinases (*10*, *11*). In monocytic THP-1 cells, however, we found that treatment with 5 μM SB 203580 actually induced MEK phosphorylation, which was detected with the phospho-MEK1/2 (Ser217/221) antibody (Fig. 1A), and is a hallmark of c-Raf activation *in vivo*. The increased MEK phosphorylation was observed as early as 30 min after SB 203580 treatment and sustained for at least 24 h (Fig. 1A). Vigorous c-Raf activation by SB 203580 was also detected in an immunocomplex kinase assay involving anti–c-Raf antibodies for immunoprecipitation and MEK1 protein as the substrate (Fig. 1C). Enhanced phosphorylation of ERK detected with the phospho-p44/42 MAPK (Thr202/Tyr204) antibody was also observed 30 min after SB 203580 treatment, with a further increase 6 h or more after treatment (Fig. 1A). An immunocomplex kinase assay involving anti-ERK antibodies and Elk-1 protein also demonstrated the induction of ERK activation by SB 203580 (Fig. 1C). Phosphorylation of MEK and ERK induced by SB203580 was detected at concentrations as low as 1 μM and increased in a dose-dependent manner (Fig. 1B). The kinetics of the activation of MEK and ERK by SB203580 appeared to be similar to those observed for inhibition of the p38 activation induced by osmotic shock. ERK phosphorylation was also detected in SB 203580-treated U937 cells, another monocytic cell line; however, the time course for the change in phosphorylation differed from that observed for THP-1 cells. In this case, ERK phosphorylation was induced only transiently at 1 h but reappeared at 12 h or more after treatment (Fig. 1D).

It is known that activation of the ERK cascade in monocytic cells stimulates the expression of early response genes and inflammatory cytokines (*7*, *15*). Although SB 203580 induced MEK and ERK phosphorylation to similar extents to TPA (Fig. 1A), which signifi-

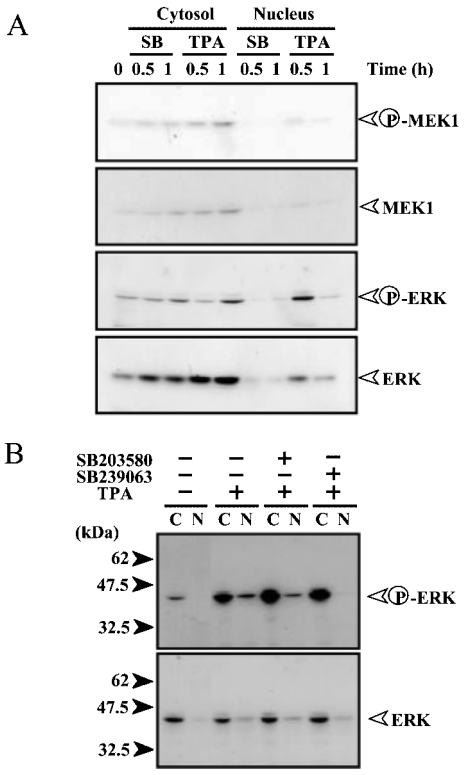

Fig. 3. **Lack of nuclear translocation of activated ERK in SB 203580-treated THP-1 cells.** A: Cells were either untreated (0 h) or treated with 5 μM SB 203580 (SB) or 10 nM TPA for the times indicated. Cytosolic and nuclear preparations were subjected to immunoblot analysis using antibodies, as illustrated. B: Cells were treated 5 μM SB 203580, 5 μM SB 239063 and/or 10 nM TPA for 1 h. Cytosolic (C) and nuclear (N) preparations were subjected to immunoblot analysis using anti-phospho ERK and -ERK antibodies, as illustrated.

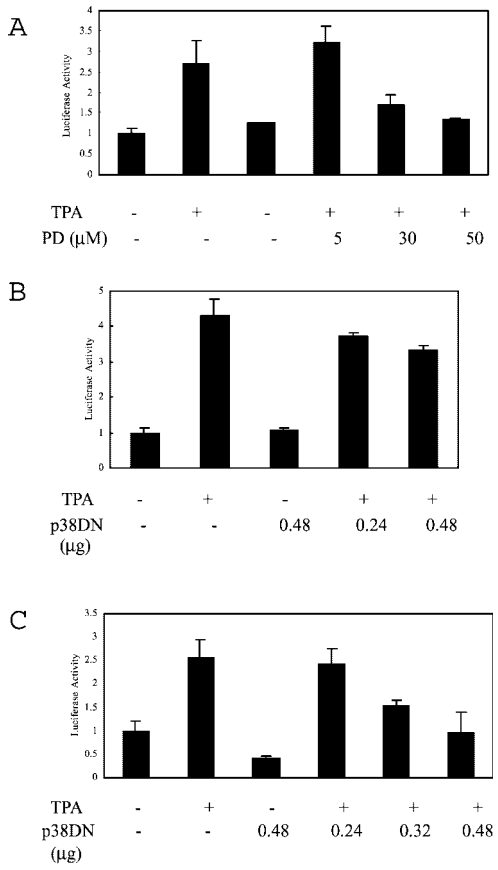

Fig. 4. **p38 dominant negative mutant suppresses SRE-dependent transactivation.** A: THP-1 cells seeded on a 12-well multidish (1×10^6 cells/well) were transfected with pSRE-Luc (0.48 μg), pRL-TK (0.24 μg), and an empty vector (pcDNA3.1, 0.48 μg). Twelve hours after transfection, the cells were pretreated with the indicated concentrations of PD98059 (PD) for 1 h and then with 10 nM TPA for 6 h. Cells were lysed and subjected to the dual luciferase assay. Firefly luciferase activity was normalized with respect to Renilla luciferase activity, and is expressed as the ratio to the unstimulated control. The data illustrated are means ± SD (n = 3). B: THP-1 cells were transfected with pSRE-Luc (0.48 μg), pRL-TK (0.24 μg), and the indicated amount of p38DN/pcDNA3.1 or the empty vector. Twelve hours after transfection, the cells were treated with 10 nM TPA for 6 h. The luciferase activity was determined and is expressed as described above. C: COS-7 cells (0.8×10^5 cells/well) were seeded on a 24-well multidish a day before transfection. Cells were transfected with pSRE-Luc (0.24 μg), pRL-TK (0.08 μg), and the indicated amount of p38DN/pcDNA3.1. Twelve hours after transfection, the cells were treated with 10 nM TPA for 6 h. The luciferase activity was determined and is expressed as described above.

cantly induced c-*fos* and IL-1β gene expression, the inhibitor only slightly increased these mRNA levels at higher concentrations (Fig. 2A). Within the enhancer element of the human IL-1β gene, the TPA responsive element (TRE) is located next to the binding site of nuclear transcription factor NFκB (*16*). These *cis*-acting elements have been shown to act cooperatively under conditions that stimulate expression of this cytokine gene. Under non-stimulated conditions, IκB binds to NFκB to suppress its nuclear translocation. Following stimulation, IκB phosphorylation and the subsequent release of NFκB occur. In order to determine the effect of SB 203580 on NFκB signaling, phosphorylation of IκB was analyzed using phospho-IκB-α (Ser32) antibodies. SB 203580 induced phosphorylation of IκB at 1 h or later after exposure (Fig. 2B), suggesting that NFκB activation should occur.

Since SB 203580 had only a limited effect on ERK cascade-dependent gene expression, the possibility was considered that the ERK signal was not transmitted to the nucleus. MEK and ERK activities were analyzed in nuclear and cytoplasmic fractions of THP-1 cells treated with TPA, and compared to the MEK and ERK activities in SB 203580–treated cells. Irrespective of the stimulus, MEK existed in the cytoplasm and was hardly detected in the nucleus (Fig. 3A). In cells treated with TPA, nuclear translocation of phosphorylated ERK was detected after 30 min, and the phosphorylated ERK level in the nucleus had decreased 1 h later, suggesting the action of MAP-kinase specific nuclear phosphatases, such as MAPK phosphatase-1 (*17*). However, in cells treated with SB 203580, ERK remained only in the cytoplasm. There was no sign of nuclear translocation of ERK under these experimental conditions (Fig. 3A).

To determine the nature of the relationship between the impaired nuclear translocation of ERK caused by SB 203580 and the inhibitory effect it has on p38 MAP kinase, we utilized recently developed SB 239063, a p38

MAP kinase inhibitor with greater selectivity (*18*). SB 239063 showed no detectable effect on MEK and ERK phosphorylation or on the nuclear translocation of ERK (data not shown). However, SB 239063 did inhibit ERK nuclear translocation induced by TPA almost completely, indicating that inhibition of p38 MAP kinase activity causes impaired ERK translocation (Fig. 3B). On the other hand, SB203580, which induces the activities of the ERK cascade in addition to its inhibitory effect on p38 MAP kinase, had little effect on TPA-mediated ERK nuclear translocation (Fig. 3B).

It is widely known that SRE is activated in an ERK-dependent manner through the phosphorylation of ternary complex factors such as Elk-1 (*19*, *20*). Therefore, we utilized a SRE-dependent reporter assay system to demonstrate the regulation of the ERK function by p38 MAP kinase activity. The luciferase activity in THP-1 cells transfected with the reporter gene was induced by TPA and this response was cancelled by pretreatment of the cells with PD98059 (Fig. 4A), an inhibitor of MEK, indicating that the TPA-mediated increase in luciferase activity is mainly mediated through ERK-dependent signal transduction. Co-transfection with the p38 T180A and Y182F double mutant, which is known to disturb the p38 activity in a dominant negative manner (*14*), suppressed TPA-induced luciferase activity in a transfected DNA amount-dependent manner (Fig. 4B), although the observed net inhibitory effect was not high. Because the transfection efficiency and the expression level of the exogenously introduced gene were very low in THP-1 cells, we next utilized COS-7 cells, which are widely used for experiments on ectopic expression. In COS-7 cells, expression of the dominant negative mutant of p38 MAP kinase efficiently inhibited TPA-induced transactivation. This effect was observed in a transfected DNA amount-dependent manner and almost complete inhibition was observed with the highest DNA amount (Fig. 4C). On the other hand, co-transfection of wild type p38 MAP kinase had no effect on the SRE-dependent transactivation (data not shown).

DISCUSSION

It has been reported that treatment of cells with c-Raf inhibitors such as SB 203580 and ZM 336372 paradoxically induces kinase activation (*10*, *11*). This phenomenon is explained by a putative negative feedback mechanism in which c-Raf activity is suppressed by its own kinase activity. Cutting the negative feed back loop by means of inhibitors allows accumulation of the active form, resulting in a striking increase in the kinase activity, when measured in the absence of the inhibitor *in vitro* (*11*). In cells, however, the kinase activity remains repressed by the inhibitor, resulting in impaired signal transduction to downstream molecules, such as MEK and ERK (*11*). The present study provided experimental evidence that SB 203580 effectively activates c-Raf, MEK and ERK in THP-1 monocytic cells. Sustained ERK phosphorylation by SB 203580 was also observed in U937 cells. These data suggest that in monocytic cells, c-Raf interacts efficiently with MEK after release from feedback inhibition, even in the presence of SB 203580, resulting in increased MEK and ERK activation. It is obscure why SB203580 activates the ERK cascade in THP-1 cells; however, a recent report described that a high concentration of SB203580 induces the differentiation of certain leukemia cells, such as HL60 and ML-1, but not of THP-1, U937 and K562 cells (*21*). Therefore, activation of ERK by SB203580 could occur in several cell types, although the signal is not transmitted in THP-1 cells.

In spite of the striking and sustained activation of the ERK cascade by SB 203580, c-*fos* and IL-1β gene expressions were still extremely limited. In the present study we also demonstrated that SB 203580 induces IκBα phosphorylation, which may in turn activate an NFκB transcriptional factor. It was recently reported that SB 203580 enhances NFκB-dependent transcriptional activation in human erythroleukemia cells (*22*). Therefore, it is suggested that the leaky IL-1β gene expression in SB 203580-treated THP-1 cells was, at least in part, due to NFκB-dependent signal transduction, which is known to regulate IL-1β gene expression (*16*).

Since activation of the ERK cascade, and expression of c-*fos* and inflammatory cytokines are often observed in parallel in THP-1 cells (*7*), it was expected that the signal transduction is disturbed in SB 203580-treated cells. Phosphorylated ERK dissociates from MEK, which functions as its cytosolic anchor, and is translocated to the nucleus (*23*), where it phosphorylates ternary complex factors (*19*, *20*). Analysis of the cellular distribution of ERK in SB 203580-treated cells indicated that nuclear translocation of phosphorylated ERK was impaired. Conversely, nuclear translocation of ERK induced by TPA was inhibited by SB 239063, which does not associate with c-Raf and is highly selective to p38 MAP kinase (*18*), suggesting that a steady-state level of the p38 MAP kinase activity is required for the nuclear translocation of ERK. On the other hand, SB 203580 showed only a limited effect on TPA-mediated ERK translocation, suggesting that nuclear translocation is dependent on the balance between the activities of the p38 MAP kinase and the ERK. The levels of phosphorylated p38 MAP kinase and its activity are high in unstimulated THP-1 cells cultured in the presence of serum (*7*). In addition, the data we obtained in the reporter gene assay indicate that inhibition of the endogenous p38 MAP kinase activity by ectopic expression of the dominant negative mutant of the enzyme suppressed the ERK-dependent SRE activation. All these data suggest that the p38 MAP kinase activity participates in ERK nuclear translocation and thereby positively regulates its function.

Recently, direct interaction between ERK and activated p38 MAP kinase was reported (*24*). It is thus possible that p38 MAP kinase may act as a cytoplasmic anchor of activated ERK in cells that confers relatively high basal activity on the former MAP kinase. However, Zhang *et al.* demonstrated that p38 MAP kinase sequesters ERK and blocks its activation by MEK (*24*). They used anisomycin and an activated form of MKK6 to achieve p38 MAP kinase activation. It is therefore possible that the results for excessively enhanced kinase activity could be somewhat different from those for basal activity. Although the possibility still remains that p38 MAP kinase acts on the ERK function through downstream molecules, such as MAPKAPK2 (*25*), MNK1 (*26*),

MSK1 (*27*), and PRAK (*28*), the potential for cross-talk between the MAP kinases that regulate their intracellular distribution is a novel idea and should be examined carefully in further studies. In addition, results in a recent report that p38 MAP kinase activity is required for AP-1-dependent transactivation elicited by TPA in THP-1 cells (*29*) are entirely consistent with the present study. However, this report demonstrates that p38 MAP kinase regulates AP-1–driven transcription, in part, by modulating the activation of the basal transcriptional module. In conjunction with our present results, it is, therefore, suggested that p38 MAP kinase modulates ERK-mediated signal transduction at multiple steps.

In conclusion, the present study demonstrates that SB 203580 activates the ERK cascade without transducing the signal into the nucleus. However, the impaired ERK signal transduction caused by the inhibitor may turn over and lead to synergistic enhancement of ERK cascade-dependent gene expression, when cells are treated in conjunction with other stimulants, such as bufalin (*7*). Consequently, care should be taken when applying p38 MAP kinase inhibitors (particularly compounds with affinity to c-Raf) to the treatment of inflammatory diseases.

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