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Molecular mechanisms of lipopolysaccharide-induced cyclooxygenase-2 expression in human neutrophils: involvement of the mitogen-activated protein kinase pathway and regulation by anti-inflammatory cytokines

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Abstract

Neutrophils are an important cellular source of proinflammatory mediators, whose regulation may be of potential benefit for the treatment of a number of inflammatory diseases. However, the mechanisms of lipopolysaccharide (LPS)-induced neutrophil activation and its regulation by anti-inflammatory cytokines have not yet been fully elucidated. Recent studies have revealed that mitogen-activated protein kinases (MAPK) play a crucial role in the generation of proinflammatory mediators in some cell types. Therefore, we conducted this study to determine whether MAPK activation could be involved in prostaglandin E_2 (PGE_2) production and cyclooxygenase (COX)-2 expression in LPS-stimulated human neutrophils. PD98059 (MEK1 inhibitor) and SB203580 ($p38^{MAPK}$ inhibitor) reduced PGE_2 production as well as COX-2 expression in LPS-stimulated neutrophils. In addition, both extracellular signal-regulated protein kinase (ERK) and $p38^{MAPK}$ were phosphorylated and activated in time- and dose-dependent manners. Since we previously showed that IL-10 and IL-4 similarly inhibited COX-2 expression in LPS-stimulated neutrophils, we next tested the effects of IL-10 and IL-4 on the phosphorylation and activation of both kinases. IL-10 inhibited the phosphorylation and activation of $p38^{MAPK}$, but not ERK. In addition, IL-4 caused a marginal inhibition in the activation of $p38^{MAPK}$. Taken together, these results suggest that both ERK and $p38^{MAPK}$ pathways are involved in LPS-induced COX-2 expression and PGE_2 production in neutrophils, and IL-10 and IL-4 inhibit neutrophil prostanoid synthesis by down-regulating the activation of $p38^{MAPK}$.

Introduction

Human neutrophils are terminally differentiated cells, which constitute the first line of defense against microorganisms by means of their various functions, including phagocytosis, generation of superoxide anions and secretion of lytic enzymes. Although there is no question about the contribution of neutrophil activation to limiting microbial invasion, prolonged or excessive activation of neutrophils may be deteriorative for the body, and even cause self-damage such as adult respiratory distress syndrome and rheumatoid arthritis (1–3). Therefore, it is feasible to speculate that the regulation of synthesis of such mediators by neutrophils may be of potential benefit for the treatment of these pathological conditions.

Neutrophils stimulated with various agonists result in the activation of a large number of protein kinases (4,5). These include several kinases with a mol. wt of 42 kDa that undergo

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transient activation upon stimulation of these cells with lipopolysaccharide (LPS) (6). Mitogen-activated protein kinase (MAPK) is one of the candidate molecules, since it has been shown to be involved in the signal transduction pathway at the inflammatory state (7–9).

MAPK are proline-directed serine/threonine protein kinases which currently comprise three major subfamilies, i.e. classical MAPK [extracellular signal-regulated protein kinase (ERK)1 and ERK2], c-Jun N-terminal kinase (JNK) (p46 JNK1 and p54 JNK2) and $p38^{MAPK}$ (10,11). These distinct sets of MAPK can be activated by a variety of extracellular stimuli in many types of cells. Initial studies showed that in human neutrophils, LPS mainly activates $p38^{MAPK}$, while both 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and *N*-formyl-methionyl-leucyl-phenylalanine can activate ERK as well as $p38^{MAPK}$ (12–15). In addition, studies using the inhibitors of these kinases clearly demonstrated their relevance to the various cellular functions of neutrophils. For example, MAPK were involved in the signal transduction pathways of neutrophil phagocytosis (16). They also suggested that discrete MAPK could be utilized to display distinct functions. In other words, neutrophils would utilize each MAPK pathway to execute specific functions (15–21). Previous reports have indicated that neutrophils are induced to synthesize prostanoids in response to various types of stimulation (22–24). In cell types other than neutrophils, the expression of cyclooxygenase (COX)-2, which is an important rate-limiting enzyme in inducible prostanoid synthesis, required the activation of $p38^{MAPK}$ (25–28). However, the underlying mechanism involved in prostanoid synthesis in activated neutrophils has yet to be elucidated. It is thus intriguing to determine whether the activation of ERK and $p38^{MAPK}$ could be involved in COX-2 expression in LPS-stimulated human neutrophils.

IL-10 and IL-4 have long been recognized as potent anti-inflammatory cytokines, because they commonly suppress the production of proinflammatory molecules by neutrophils and monocytes/macrophages (22,29–31). We previously showed that IL-10 as well as IL-4 inhibits the production of prostaglandin E_2 (PGE_2), an important inflammatory mediator, in LPS-stimulated neutrophils, through the inhibition of COX-2 expression (22). We also indicated that both cytokines inhibit the transcription of the COX-2 gene. However, upstream signaling events induced by these two cytokines in neutrophils remain largely unknown. Interestingly, one report previously indicated that IL-10 inhibited the phosphorylation of a 46–49-kDa protein in LPS-stimulated neutrophils, although this molecule was not further identified (6). In monocytes/macrophages, the mechanisms of inhibition by IL-10 and IL-4 of the induced expression of proinflammatory molecules could be partly explained by their regulatory effects on MAPK activation (27, 32). However, it should be noted here that IL-10 induced different signaling events between neutrophils and monocytes/macrophages (33,34). It is thus interesting to determine the effects of both cytokines on MAPK activation in neutrophils.

In the present study, using specific inhibitors of ERK and the $p38^{MAPK}$ pathway, we first questioned whether the activation of ERK and $p38^{MAPK}$ is involved in COX-2 expression in LPS-stimulated human neutrophils. In addition, we questioned whether IL-10 and IL-4 both regulate the phosphorylation and activation of ERK and $p38^{MAPK}$ in LPS-stimulated neutrophils.

Methods

Reagents

FBS and RPMI 1640 were purchased from Gibco (Grand Island, NY) and Nissui Chemical (Tokyo, Japan) respectively. RPMI 1640 supplemented with FBS (10%), glutamine (1 mmol/l), penicillin (100 U/ml) and kanamycin (80 μg/ml) was used for all experiments. LPS from *Escherichia coli* 0111:B4, purified by the Westphal method, was obtained from Difco (Detroit, MI). Human rIL-4 and human rIL-10 were generously provided by Schering-Plough (Bloomfield, NJ). The anti-COX-2 was a mouse mAb (Transduction Laboratories, Lexington, KY) that was found to be highly specific. The antibody did not cross-react with human COX-1 and it had no apparent cross-reactivity against other human cell proteins. PD98059, which selectively inhibits MAPK/ERK kinase (MEK)-1/2 resulting in the suppression of ERK activation, was obtained from New England Biolabs (Beverly, MA). 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridinyl) imidazole (SB203580), a specific inhibitor of $p38^{MAPK}$, was kindly provided by SmithKline Beecham (King of Prussia, PA). The phospho-specific ERK antibody (Tyr204), control anti-ERK antibody, phospho-specific $p38^{MAPK}$ antibody (Thr180/Tyr182) and control anti-$p38^{MAPK}$ antibody were all rabbit polyclonal antibodies purchased from New England Biolabs.

Isolation and culture of human neutrophils

Procedures of isolation and culture of human neutrophils were carried out as previously described (7), with some modification. Briefly, 30 ml of heparinized venous blood were mixed with 20 ml of 6% dextran/0.9% sodium chloride. After dextran sedimentation of erythrocytes, the plasma was layered onto Ficoll-Hypaque (Pharmacia LKB, Piscataway, NJ), centrifuged and the mononuclear cells at the interface were carefully removed with a Pasteur pipette. To further purify neutrophils, the remainder of the Ficoll-Hypaque phase (containing monocytes) was completely removed with a fresh Pasteur pipette. The tube wall was carefully washed with PBS and the cell pellet at the bottom of the tube was suspended in 5 ml of PBS. The suspension was transferred to a new tube and the residual contaminating erythrocytes were eliminated by hypotonic lysis. Subsequently, the cells were layered onto NycoPrep 1.063 (Nycomed Pharma, Oslo, Norway), centrifuged and the Nycoprep phase (containing platelets) was completely removed with a fresh Pasteur pipette. The recovered neutrophils were washed 3 times and resuspended at a density of 1×10^7 cells/ml in RPMI 1640 media. The cell preparation contained highly purified (>99.5%) neutrophils as judged morphologically. Cell viability was >98% as assessed by Trypan blue exclusion. These cells were further cultured in RPMI 1640 media with 10% FBS at 37°C in a humidified atmosphere with 5% CO_2 at a cell density of 1×10^6cells /ml.

Radioimmunoassay (RIA) for PGE_2

Neutrophils (1×10^6cells) were incubated in RPMI 1640 containing 10% FBS in the presence or absence of LPS (1 μg/g/ml) for 18 h and then the supernatant was collected. Analysis of PGE_2 levels was performed with a commercially available [^{125}I]PGE_2 RIA kit (NEN, Boston, MA), as described elsewhere.

Analysis of COX-2 protein expression

Neutrophils (1×10^7 cells) were incubated at 37°C in RPMI 1640 containing 10% FBS in the presence or absence of PD98059, SB203580, IL-4 or IL-10 for 1 h before stimulation with LPS (1 μg/g/ml). After appropriate time intervals, cell pellets were lysed in SDS sample buffer [62.5 mM Tris–HCl, pH 6.8, 10% glycerol, 2% (w/v) SDS, 50 mM DTT and 0.1% (w/v) BPB] at 25°C. The proteins were then separated on 10% SDS–PAGE and transferred onto a PVDF membrane. The membrane was incubated with 0.25 μg/ml of mouse anti-COX-2 mAb at 25°C for 2 h. The membrane was then subsequently incubated with anti-mouse Ig, a peroxidase-linked species-specific $F(ab')_2$ fragment (from sheep) (1:2000 dilution) and analyzed using an ECL system (Amersham, Arlington Heights, IL). Densitometric analysis of the intensities of signals was carried out with the National Institutes of Health Image 1.55 program.

Analysis of MAPK phosphorylation

Neutrophils (1×10^7 cells) were incubated at 37°C in RPMI 1640 containing 10% FBS in the presence or absence of PD98059, SB203580, IL-4 or IL-10 for 1 h before stimulation with LPS (1 μg/ml). After appropriate time intervals, cell pellets were then lysed on ice with lysis buffer (1% Triton X-100, 50 mM HEPES, pH 7.4, 150 mM NaCl, 30 mM sodium pyrophosphate, 50 mM NaF, 1 mM Na_3VO_4 and 1 mM PMSF) for 30 min. After centrifugation, supernatants were subsequently mixed at a ratio of 1:1 with SDS sample buffer. Aliquots of the samples were subjected to Western blot analysis using a rabbit polyclonal control or phospho-specific MAPK (ERK, p38^{MAPK}) antibodies (1:1000 dilution). The membrane was subsequently incubated with anti-rabbit Ig, a peroxidase-linked species-specific $F(ab')_2$ fragment (from donkey) (1:2000 dilution) and analyzed using an Amersham ECL system. Densitometric analysis of the intensities of signals of phospho-MAPK in reference to those of non-phospho-MAPK was carried out with the National Institutes of Health Image 1.55 program.

MAPK assay

ERK and p38^{MAPK} assays were carried out with a commercially available kinase assay kit (New England Biolabs). Briefly, neutrophils (1×10^7 cells) were incubated at 37°C in RPMI 1640 containing 10% FBS in the presence or absence of PD98059, SB203580, IL-4 or IL-10 for 1 h before stimulation with LPS (1 μg/ml). After appropriate time intervals, cell pellets were then lysed on ice with 500 μl of 1 × cell lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerolphosphate, 1 mM Na_3VO_4 and 1 μg/ml leupeptin) containing 1 mM PMSF for 30 min. Then 200 μl of the supernatants was incubated with phospho-specific ERK mAb (1:100 dilution) or anti- p38^{MAPK} antibody (1:50 dilution) in the presence of 20 μl of Protein G Plus/Protein A–agarose suspension (Calbiochem, La Jolla, CA) at 4°C for 6 h. The cell pellet was then rinsed twice with 500 μl of 1 × cell lysis buffer (25 mM Tris, pH 7.5, 5 mM β-glycerolphosphate, 2 mM DTT, 0.1 mM Na_3VO_4 and 10 mM $MgCl_2$). The pellet was incubated in 50 μl 1 × kinase buffer with 2 μg of Elk-1 or ATF-2 fusion protein in the presence of 200 μM ATP at 30°C for 30 min. The reaction was terminated with 3 × SDS sample buffer. The samples were subjected to Western blot analysis using rabbit polyclonal phospho-specific Elk-1 or ATF-2 antibodies (1:1,000 dilution). Densitometric analysis of the intensities of signals of phospho-Elk-1 or -ATF-2 was carried out with the National Institutes of Health Image 1.55 program.

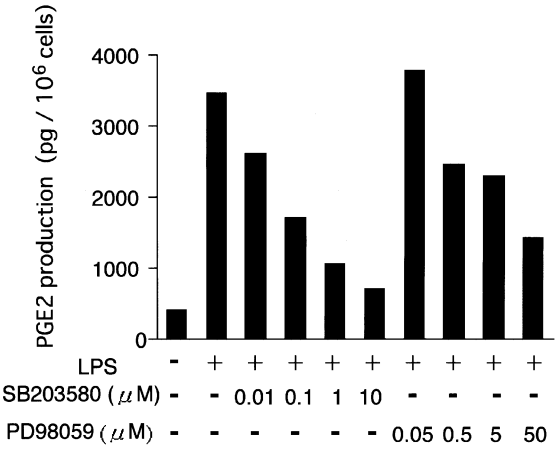

Fig. 1. Effects of PD98059 and SB203580 on PGE_2 induction in LPS-stimulated neutrophils. Human neutrophils (1×10^6 cells) were treated with the indicated concentrations of either PD98059 or SB203580 for 1 h before stimulation with LPS (1 μg/ml). After 18 h, culture supernatants were assayed for PGE_2 by RIA. Similar results were obtained in three separate experiments.

Results

Involvement of ERK and p38^{MAPK} pathways in prostanoid synthesis and COX-2 expression in LPS-stimulated neutrophils

As previously reported by our group and others (22–24), neutrophils produce a large amount of prostanoids, including PGE_2, in response to LPS. This induction was caused by the expression of COX-2 protein. First, we determined the effects of the specific inhibitors of ERK and p38^{MAPK} (PD98059 and SB203580 respectively) on LPS-stimulated PGE_2 production in neutrophils. The specificity of both inhibitors was previously confirmed in our laboratory (27). After 18 h culture with or without LPS, cell-free supernatants were used for measurements of PGE2. As a result, LPS-induced PGE_2 production in neutrophils was inhibited in a dose-dependent manner by PD98059 (with 61% inhibition at 50 μM) and SB203580 (with 80% inhibition at 10 μM) (Fig. 1). Regulation of PGE_2 synthesis includes several important steps. Since the most important rate-limiting enzyme is COX-2, we next focused on the effects

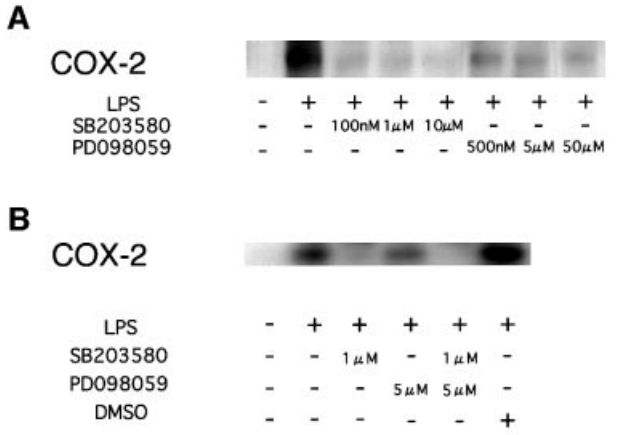

Fig. 2. Effects of PD98059 and SB203580 on COX-2 protein expression in LPS-stimulated neutrophils. Human neutrophils (1×10^7 cells) were treated with the indicated concentrations of either PD98059 or SB203580 (A) and with the mixture of both inhibitors (PD98059 5 μM + SB203580 1 μM) (B) for 1 h before stimulation with LPS (1 μg/ml). After 18 h, cell lysates were used to determine COX-2 expression. Western blot analysis was carried out using anti-COX-2 mAb. Similar results were obtained in three separate experiments.

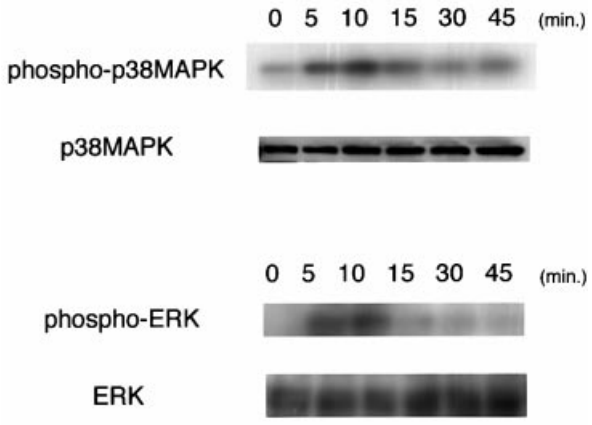

Fig. 3. LPS induces the phosphorylation and activation of ERK2 and $p38^{MAPK}$ in dose-dependent manner. Human neutrophils (1×10^7 cells) were treated with LPS (1 μg/ml) for the indicated time periods and cell lysates were collected. Western blot analysis was carried out using phospho-specific ERK antibody, control anti-ERK antibody, phospho-specific $p38^{MAPK}$ antibody and control anti-$p38^{MAPK}$ antibody. Similar results were obtained in three separate experiments.

of both inhibitors on LPS-stimulated COX-2 expression in neutrophils. As shown in Fig. 2(A), LPS-induced COX-2 protein expression in neutrophils was inhibited in a dose-dependent manner by both PD98059 and SB203580. Complete inhibition of COX-2 expression was consistently observed at 50 μM of PD98059 as well as at 10 μM of SB203580. Moreover, the addition of both inhibitors at the suboptimal dose (PD98059 5 μM + SB203580 1 μM) resulted in the complete inhibition of COX-2 expression (Fig. 2B). Collectively, these results suggest that the activation of ERK and $p38^{MAPK}$ is independently involved in the induction of COX-2 protein and in PGE_2 synthesis in LPS-stimulated neutrophils.

LPS induces the phosphorylation and activation of ERK2 and $p38^{MAPK}$ in neutrophils

An initial report described how LPS phophorylates and activates $p38^{MAPK}$, but not ERK (15). However, a more recent report has shown that LPS is able to phosphorylate ERK (35). So, we next determined whether LPS phosphorylates and activates ERK and $p38^{MAPK}$ in human neutrophils. The phosphorylation of ERK by LPS peaked at 10 min and rapidly decreased thereafter (Fig. 3). Since LPS increased ERK phosphorylation in a dose-dependent manner (Fig. 4), the dose of 1 μg/ml of LPS was used in the following experiments. Although both ERK1 and ERK2 proteins were detectable, ERK2 seemed to be a predominant isoform in LPS-stimulated neutrophils, as has been previously reported (Fig. 5A). Consistent with LPS-induced phosphorylation of ERK2 on the Tyr residue, the activation of ERK, as assessed by the phosphorylation of Elk-1, was clearly induced (Fig. 5B).

As for $p38^{MAPK}$, unstimulated neutrophils showed a weak basal phosphorylation on the Thr and Tyr residues (Fig. 6A), but the activation of this kinase was not observed under this condition (Fig. 6B). LPS stimulation significantly induced the phosphorylation (Fig. 6A) and activation of $p38^{MAPK}$ (Fig. 6B).

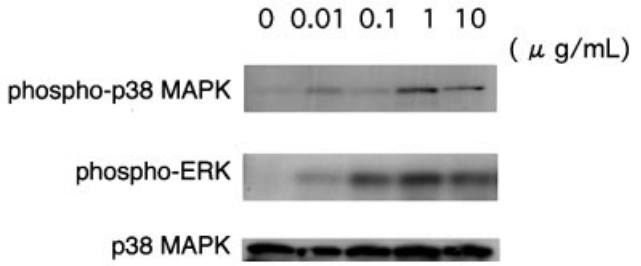

Fig. 4. LPS induces the phosphorylation and activation of ERK2 and $p38^{MAPK}$ in time-dependent manner. Human neutrophils (1×10^7 cells) were treated with the indicated concentrations of LPS. After 10 min culture, cell lysates were collected. Western blot analysis was carried out using phospho-specific ERK antibody, control anti-ERK antibody, phospho-specific $p38^{MAPK}$ antibody and control anti-$p38^{MAPK}$ antibody. Similar results were obtained in three separate experiments.

The phosphorylation of $p38^{MAPK}$ by LPS peaked at 10 min, rapidly decreasing thereafter (Fig. 3). Reprobing of the immunoblot with either anti-ERK or -$p38^{MAPK}$ antibody showed the equal loading of proteins in each lane.

Effect of IL-4 and IL-10 on COX-2 expression as well as MAPK phosphorylation and activation in LPS-stimulated neutrophils

As previously reported by our group (22), both cytokines completely inhibited LPS-induced COX-2 expression and PGE_2 synthesis in neutrophils. We next determined the effects of IL-4 and IL-10 on the phosphorylation and activation of ERK and $p38^{MAPK}$ in LPS-stimulated neutrophils (Fig. 6). Neither of these cytokines alone induced the phosphorylation or activation of these kinases (data not shown). Interestingly, IL-10 partly inhibited LPS-induced phosphorylation of $p38^{MAPK}$ by 39%. Inhibition of LPS-induced $p38^{MAPK}$ activation by IL-10

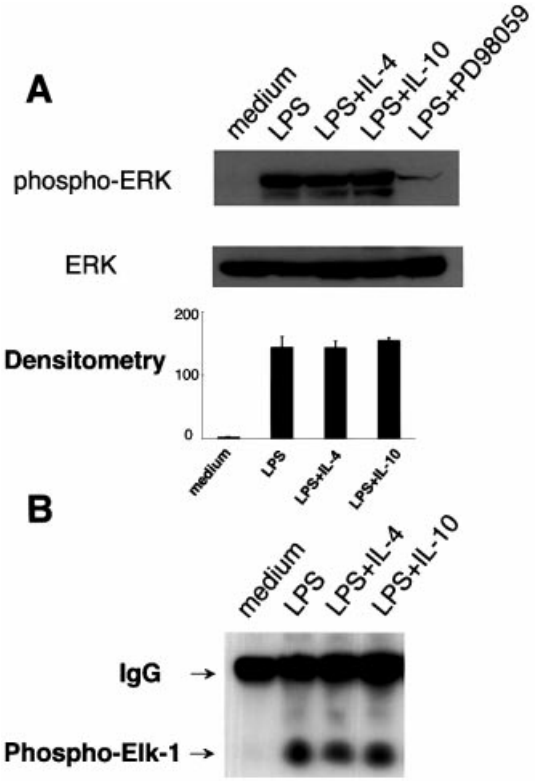

Fig. 5. Effects of IL-4 and IL-10 on the phosphorylation (A) and activation (B) of ERK in LPS-stimulated neutrophils. Human neutrophils (1×10^7 cells) were treated with or without IL-4 (10 ng/ml) or IL-10 (10 ng/ml) for 1 h before stimulation with LPS (1 μg/ml) for 10 min. After stimulation, cell lysates were used to determine the phosphorylation (A) and activation (B) of ERK2. Western blot analysis was carried out using phospho-specific Elk-1 antibody, phospho-specific ERK antibody and control anti-ERK antibody. Similar results were obtained in five separate experiments. Densitometry results are expressed as a percentage of phospho-protein signal intensity to total protein signal intensity (A). The relative activity of ERK was calculated relative to the amounts of immunoprecipitated ERK protein used in this assay (B).

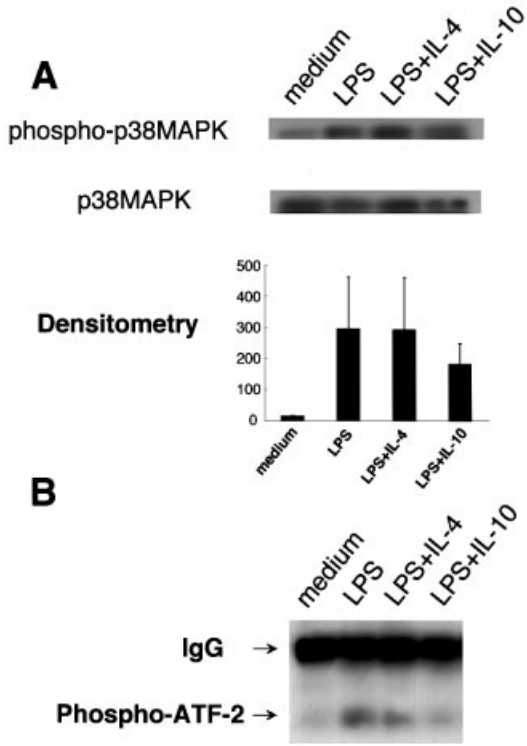

Fig. 6. Effects of IL-4 and IL-10 on the phosphorylation (A) and activation (B) of $p38^{MAPK}$ in LPS-stimulated neutrophils. Human neutrophils (1×10^7 cells) were treated with or without IL-4 (10 ng/ml) or IL-10 (10 ng/ml) for 1 h before stimulation with LPS (1 μg/ml) for 10 min. After stimulation, cell lysates were used to determine the phosphorylation (A) and activation (B) of $p38^{MAPK}$. Western blot analysis was carried out using phospho-specific ATF-2 antibody, phospho-specific $p38^{MAPK}$ antibody and control anti-$p38^{MAPK}$ antibody. Similar results were obtained in five separate experiments. Densitometry results are expressed as a percentage of phospho-protein signal intensity to total protein signal intensity (A). The relative activity of $p38^{MAPK}$ was calculated relative to the amounts of immunoprecipitated $p38^{MAPK}$ used in this assay (B).

was somewhat more pronounced (52% inhibition). Moreover, IL-4 exerted a marginal inhibitory effect on LPS-induced activation of $p38^{MAPK}$ (19% inhibition) (Fig. 6). On the other hand, LPS-induced phosphorylation and activation of ERK2 was not affected at all by these cytokines (Fig. 5). Since 1 h pretreatment with these cytokines might not long enough to exert a maximal effect, we conducted experiments with longer pretreatment periods (up to12 h). However, the results were virtually the same (data not shown). Taken together, these results suggest that LPS-induced phosphorylation and activation of $p38^{MAPK}$ was partly regulated by IL-10 and IL-4.

Discussion

Previously, we reported that human neutrophils were induced to produce prostanoids in the presence of LPS. This induction was caused by an increase in COX-2 expression. Against such an induction of COX-2, both IL-4 and IL-10 inhibited its expression at the level of gene expression (22). To date, the precise molecular mechanism of the signal transduction pathways of COX-2 expression and its regulation by these anti-inflammatory cytokines has not been investigated.

In the present study, we were able to show for the first time that the activation of both the ERK and $p38^{MAPK}$ pathways is independently involved in COX-2 expression in LPS-stimulated neutrophils. $p38^{MAPK}$ activation has been recently shown be involved in COX-2 expression in LPS-stimulated human monocytes (25–28). In contrast to this study, Pouliot *et al.* previously showed that SB203580 did not inhibit LPS-induced COX-2 expression in human neutrophils (23). However, the kinetic profile of LPS-induced COX-2 expression of Pouliot *et al.* seems to differ from our findings. This apparent discrepancy might be partly due to the concentration of serum used in the culture (1 versus 10%). Indeed, they treated the cells with SB203580 for 1 h, while we did so for 18 h. Whether or not this inhibitor exerts a similar inhibitory effect on COX-2 expression at earlier time points in our system remains to be clarified. ERK was originally shown not to become phosphorylated or activated in LPS-stimulated neutrophils (13–15). However, consistent with recent report (35), we were

able to clearly show the phosphorylation and activation of ERK in LPS-stimulated neutrophils. This may have been due to recent advances in the method of detecting a weak signal, because we observed that LPS-induced ERK activation in neutrophils was much weaker than TPA-induced activation (data not shown). ERK activation was also evident in LPS-stimulated monocytes (36,37). Based on these findings, a similar signaling pathway appears to function in a similar way between neutrophils and monocytes; however, a difference in the MAPK pathways also seems to exist for the following reasons. First, as mentioned below, the regulation of ERK activation by IL-10 and IL-4 was quite different between the two cell types. This suggests that the upstream pathways involved in LPS-induced ERK activation may be different. Secondly, consistent with other reports (38), LPS-induced JNK activation was observed in monocytes, but not in neutrophils in our study (data not shown). Therefore, the utilization of MAPK subfamilies may vary depending on the cell types used, even in the presence of the same stimuli.

The mechanism by which both ERK and $p38^{MAPK}$ activation eventually induces the transcriptional activation of the COX-2 gene has yet to be elucidated in neutrophils. However, the activation of several transcriptional factors may be involved. The 5′ promoter region of the COX-2 gene was shown to contain NF-κB, NF-IL-6 and CRE sites (39–42). Although little is known about the transcriptional regulation of the COX-2 gene in neutrophils, there are some conflicting findings about the importance of these transcription factors in monocytes/macrophages stimulated with LPS. The NF-κB site was involved in LPS-induced COX-2 expression in human monocytic lines (41), while CRE and two NF-IL-6 sites were crucial in COX-2 induction in LPS-stimulated murine macrophage lines (43). This discrepancy may simply be due to a difference in species. In addition, the issue about whether or not NF-κB and $p38^{MAPK}$ pathways might work independently is not completely clear (44–47). However, in the case of neutrophils, one recent paper has shown that NF-κB activation was completely blocked in the presence of $p38^{MAPK}$ inhibitor (48), suggesting the scenario that $p38^{MAPK}$ is a upstream kinase regulating NF-κB activation in neutrophils. Furthermore, another interesting aspect of the $p38^{MAPK}$ pathway is its effect on COX-2 mRNA stability. Dean *et al.* showed that treatment of LPS-stimulated monocytes with $p38^{MAPK}$ inhibitors caused a rapid degradation of COX-2 mRNA (28,49). This suggests that $p38^{MAPK}$ might play a role in both transcriptional and post-transcriptional regulation of the COX-2 gene.

As we have previously reported (27), in LPS-stimulated monocytes, both IL-10 and IL-4 significantly inhibited the phosphorylation and activation of ERK, while IL-10, but not IL-4, also inhibited the phosphorylation and activation of $p38^{MAPK}$. Surprisingly, in neutrophils, IL-10 only down-regulated the activation of $p38^{MAPK}$, but not that of ERK. In the light of our previous findings that IL-10 regulates COX-2 expression at both the transcriptional and post-transcriptional levels (22), the same may be true in neutrophils and accordingly this is now under investigation. However, the clear difference in the regulation of ERK activation by IL-10 also makes us speculate that regulatory mechanisms by IL-10 of further upstream signaling events leading to MAPK activation may differ between neutrophils and monocytes/macrophages. This is reminiscent of previous findings showing that IL-10 induced different signaling events between neutrophils and monocytes/macrophages (33,34). In the case of IL-4, the activation of $p38^{MAPK}$ was only marginally inhibited. In light of its significant inhibitory effects on COX-2 expression (22), IL-4 seems likely to utilize a MAPK-independent novel pathway, thereby regulating COX-2 expression. In this regard, an intriguing paper recently showed that IL-4-activated STAT6 and NF-κB may compete for a limited supply of a transcriptional co-activator such as CREB-binding protein, resulting in suppression of NF-κB-dependent transcription (50). Whether or not such a mechanism is involved in regulation of neutrophil COX-2 expression by IL-4 awaits further investigation.

The molecular mechanisms underlying relatively unique anti-inflammatory actions induced by IL-10 and IL-4 in neutrophils are still not completely clear. Further understanding of such mechanisms would be potentially of great interest in terms of designing more ideal therapeutic approaches for inflammatory diseases, such as rheumatoid arthritis.

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Abbreviations

COX	cyclooxygenase
ERK	extracellular signal-regulated protein kinase
JNK	c-Jun N-terminal kinase
LPS	lipopolysaccharide
MAPK	mitogen-activated protein kinase
MEK	MAPK/ERK kinase
PGE_2	prostaglandin E_2
RIA	radioimmunoassay
TPA	12-*O*-tetradecanoylphorbol-13-acetate

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