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c-Jun N-Terminal Kinase Negatively Regulates Lipopolysaccharide-Induced IL-12 Production in Human Macrophages: Role of Mitogen-Activated Protein Kinase in Glutathione Redox Regulation of IL-12 Production

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Although c-Jun N-terminal kinase (JNK) plays an important role in cytokine expression, its function in IL-12 production is obscure. The present study uses human macrophages to examine whether the JNK pathway is required for LPS-induced IL-12 production and defines how JNK is involved in the regulation of IL-12 production by glutathione redox, which is the balance between intracellular reduced (GSH) and oxidized glutathione (GSSG). We found that LPS induced IL-12 p40 protein and mRNA in a time- and concentration-dependent manner in PMA-treated THP-1 macrophages, and that LPS activated JNK and p38 mitogen-activated protein (MAP) kinase, but not extracellular signal-regulated kinase, in PMA-treated THP-1 cells. Inhibition of p38 MAP kinase activation using SB203580 dose dependently repressed LPS-induced IL-12 p40 production, as described. Conversely, inhibition of JNK activation using SP600125 dose dependently enhanced both LPS-induced IL-12 p40 protein production from THP-1 cells and p70 production from human monocytes. Furthermore, JNK antisense oligonucleotides attenuated cellular levels of JNK protein and LPS-induced JNK activation, but augmented IL-12 p40 protein production and mRNA expression. Finally, the increase in the ratio of GSH/GSSG induced by glutathione redox regulated LPS-induced IL-12 p40 production in PMA-treated THP-1 cells. GSH-OEt augmented p38 MAP kinase activation, but suppressed the JNK activation induced by LPS. Our findings indicate that JNK negatively affects LPS-induced IL-12 production from human macrophages, and that glutathione redox regulates LPS-induced IL-12 production through the opposite control of JNK and p38 MAP kinase activation. The Journal of Immunology, 2003, 171: 628–635.

M any extracellular stimuli elicit specific biological responses through the activation of mitogen-activated protein (MAP)1 kinase cascades (1). The MAP kinases constitute an important group of serine/threonine signaling kinases that modulate the phosphorylation, and therefore the activation status of transcription factors, and link transmembrane signaling with gene induction events in the nucleus. Extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAP kinase constitute three major subfamilies of MAP kinases that seem to mediate cellular responses, including proliferation, differentiation, and apoptosis (2). Generally, ERK play a major role in cell proliferation and differentiation, as well as in survival via various growth factors (3). In contrast, JNK and p38 MAP kinase are activated by environmental stress such as irradiation, heat shock, hyperosmotic shock, and inflammatory cytokines (4), and play important roles in apoptosis and cytokine expression (5–7).

IL-12 is a heterodimeric 70-kDa (p70) cytokine composed of two disulfide-linked glycosylated chains of 40 (p40) and 35 kDa (p35), encoded by two distinct genes (8, 9). This cytokine is mainly produced by monocytes, macrophages, and dendritic cells in response to bacterial products such as LPS and intracellular pathogens (8, 10), or upon interaction with activated T cells (11, 12). IL-12 plays a pivotal role in the regulation of cell-mediated immunity, exerting pleiotropic effects on T and NK cells. It also induces the development of Th1 responses, leading to IFN-γ and IL-2 production, and has an important role in maintaining the balance between Th1 and Th2 responses in vivo (8). The expression of IL-12 appears to be regulated primarily at the level of transcription, and the p40 gene promoter region has been analyzed in detail. Multiple regulatory elements, such as NF-kB (13) and F1 complex, including Ets-2, IFN-regulatory factor-1, c-Rel, and Ets-related factors (14, 15), are implicated in p40 regulation. However, little is known about the early signaling events underlying the up-regulation of IL-12 expression by LPS in macrophages. Recent reports suggest that LPS activates p38 MAP kinase, which subsequently promotes the induction of IL-12 production in murine APC (7, 16). We also reported that p38 MAP kinase activation positively regulates LPS-induced IL-12 production in human monocytes (17).

In contrast, JNK is also activated by inflammatory stimuli (LPS, IL-1, TNF-α) and upon infection of cultured cells by various pathogens, including Gram-negative bacteria. LPS binds to LPS-binding protein in plasma and is delivered to the cell surface receptor CD14. Next, LPS is transferred to the transmembrane signaling receptor Toll-like receptor 4 and its accessory protein MD2

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3 Abbreviations used in this paper: MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; GSH, reduced glutathione; GSH-OEt, glutathione reduced form ethyl ester; GSSG, oxidized glutathione; JNK, c-Jun N-terminal kinase.
(18). This signaling pathway leads to the activation of NF-κB and JNK, which initiate the transcription of proinflammatory cytokine genes (19). The transcription factors c-Jun (part of AP-1 complex), Jun D, ATF-2, ATFa, Elk-1, Sap-1, NF-ATc1, and NF-ATF4 are all phosphorylated by JNK (20–23). Three JNK genes have been identified: JNK1, JNK2, and JNK3, and their alternative splicing results in at least 10 isoforms of JNK (24). JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 is expressed mainly in the brain, testis, and heart. Mice deficient in JNK1 (25) and JNK2 (26) have distinct defects in T cell differentiation. CD4 T cells from JNK1-deficient mice preferentially differentiate into the Th2 phenotype, and the enhanced production of Th2 cytokines by JNK1-deficient cells is associated with an increase in the accumulation of the transcription factor NF-ATc in the nucleus (25). However, the role of JNK in IL-12 production from human macrophages has not been determined.

The present study examines whether the JNK pathway is required for LPS-induced IL-12 production in human macrophages and investigates the role of JNK in gluatathione redox-regulated LPS-induced IL-12 production (17).

Materials and Methods

Reagents
Glutathione reduced form ethyl ester (GSH-OEt), PMA, and LPS (from Escherichia coli, serotype 011:B4) were purchased from Sigma-Aldrich (St. Louis, MO). SB203580 to specifically inhibit p38 MAP kinase activity (27) was purchased from Calbiochem-Novabiochem (La Jolla, CA) and dissolved in DMSO (Sigma-Aldrich). SP600125 to specifically inhibit JNK activity (28) was obtained from Biomol Research Labs (Plymouth Meeting, PA) and dissolved in DMSO.

Cell culture
Human monocytic THP-1 cells (American Type Culture Collection, Manassas, VA) in RPMI 1640 medium (Life Technologies, Rockville, MD), with 4.5 g/L glucose, 10 mM HEPES, 1 mM sodium pyruvate, and 50 μM 2-ME supplemented with 10% FBS (Equitech-Bio, Ingram, TX), were cultured under a humidified 5% CO2 atmosphere at 37°C. Phorbol esters can induce this cell line to differentiate into macrophages (29). This was accomplished by washing and resuspending the cells in fresh RPMI-10% FBS containing 100 ng/ml PMA (30). After 48 h, the cells were washed three times and cultured overnight in fresh RPMI 1640 medium containing 1% FBS; then processed as follows for cytokine production. Cells (1 × 10⁶/500 μl) were stimulated with 1 μg/ml LPS for 24 h, and sedimented by centrifugation. The supernatants were stored at −20°C.

Human monocytes were separated, as described (17). Briefly, PBMC were separated by density-gradient centrifugation from leukocyte concentrates obtained from healthy volunteers. Monocytes were separated by incubating PBMC in RPMI-10% FBS for 1 h at 37°C. Adherent cells comprised over 90% monocytes, according to differential counting on May-Giemsa-stained smears. Cytokine production was measured after monocytes (2 × 10⁵/500 μl) were cultured in RPMI-10% FBS and stimulated, as described above.

Quantitation of IL-12 p40 and p70
Cytokine concentrations in the supernatants were determined using specific ELISA kits. Levels of IL-12 p40 and p70 (heterodimeric form) were measured using ELISA kits purchased from R&D Systems (Quantikine; Minneapolis, MN), according to the manufacturer’s instructions. The assay detected >15 pg/ml of IL-12 p40 and >5 pg/ml of IL-12 p70.

Preparation of cRNA probes and Northern blot analysis
Human IL-12 p40 and G3PDH cDNA were cloned, as described (17). cRNA probes for human IL-12 p40 and G3PDH were synthesized using (α-32P)-5’[γ-ATP (ICN Biomedicals, Aurora, OH) and T7 RNA polymerase (Promega, Madison, WI)]. Northern blots were performed, as described (17). Total RNA was extracted from THP-1 cells using a modified acid guanidinium thiocyanate-phenol-chloroform method. Aliquots of total RNA (15 μg) were resolved by electrophoresis in 1.4% agarose/0.66 M formaldehyde gels and transferred onto nylon membranes (Hybond-N; Amersham Pharmacia Biotech, Tokyo, Japan). The membranes were hybridized to a human IL-12 p40 cRNA probe at 60°C overnight, and autoradiographic signals were visualized by exposure to x-ray film (Hyperfilm: Amersham Pharmacia Biotech) at −70°C. After detecting IL-12 mRNA, the probes were stripped and the blots were rehybridized with a control human G3PDH cRNA probe.

Western blotting
Western blotting proceeded as described (31). Briefly, cell lysates were electrophoresed and immunoblotted against primary Abs, then specific reactive proteins were detected using ECL. The primary Abs that selectively recognize phosphorylated forms of JNK (Thr183/Tyr185), p38 MAP kinase (Thr180/Tyr182), ERK (Thr202/Tyr204), and c-Jun (Ser63) were purchased from the same source (Cell Signaling Technology, Beverly, MA). To determine the amounts of precipitated JNK, p38 MAP kinase, ERK, and c-Jun, blots were stripped and reprobed using phosphorylation state-independent anti-JNK (SC-571; Santa Cruz Biotechnology, Santa Cruz, CA), anti-p38 MAP kinase (Cell Signaling Technology), anti-ERK (Cell Signaling Technology), and c-Jun (Cell Signaling Technology) Abs.

Antisense oligonucleotides
We synthesized and purified JNK phosphorothioate antisense oligonucleotides by HPLC (Kurabo, Osaka, Japan), as described (32). The specific antisense oligonucleotides were as follows: antisense JNK1 (AS-JNK1), 5’-CTTCTCTGTAGCCGCCTTGG-3’ and antisense JNK2 (AS-JNK2), 5’-GTCGGGGGCAGGGCCTCTC-3’. Control oligonucleotides consisted of the scrambled (Scr) version of each antisense sequence, as follows: Scr-JNK1, 5’-CTTCTCTGTAGCCGCCTTGG-3’ and Scr-JNK2, 5’-GTCGGGGGAGGGCCTCTC-3’. Oligonucleotides (3 μg) were added to 94 μl of RPMI 1640 medium containing 6 μl of FuGene 6 transfection reagent (Roche, Indianapolis, IN). After a 40-min incubation at room temperature, the mixture was added to cells in flasks containing RPMI medium supplemented with 1% FBS and incubated in humidified 5% CO2 at 37°C for 36 h. We then determined the JNK1 or JNK2 content by Western blotting whole cell lysates using a commercial Ab raised against JNK1 (SC-571) that also recognizes JNK2.

Measurement of JNK kinase activity
We measured the activity of JNK using commercially available kits (SAPK/JNK Assay Kit; Cell Signaling Technology), according to the manufacturer’s instructions.

Measurements of intracellular glutathione concentrations
The concentrations of GSH and oxidized glutathione (GSSG) were assayed by the GSSG-reductase 5’,5’-dithio-bis(2-nitrobenzoic acid) recycling procedure, as described (17).

Statistical analysis
All values are expressed as means ± SEM of the indicated numbers of experiments. Data were compared by Student’s t test with the Bonferroni correction for multiple comparisons. A P value below 0.05/m (in which m is the number of comparisons) was considered statistically significant in the Bonferroni method.

Results
Induction of IL-12 p40 protein production and mRNA expression by LPS stimulation in a human macrophage cell line
Human monocytic THP-1 cells can be induced to differentiate into macrophages by phorbol esters (29). Therefore, we examined the induction of IL-12 by LPS in PMA-treated THP-1 cells. In THP-1 cells without PMA treatment, IL-12 p40 protein induced by LPS was undetectable (data not shown). However, Fig. 1A shows that at the mRNA level, 1 g/ml LPS time dependently increased IL-12 p40 mRNA expression, reaching a maximal response after 24 or 48 h. Fig. 1C shows that at the mRNA level, 1 μg/ml of LPS time dependently increased IL-12 p40 mRNA expression, reaching a maximal response 12 or 24 h after LPS stimulation. Thus, we stimulated cells with 1 μg/ml LPS for 24 h to induce IL-12 p40 protein production and for 12 h to induce IL-12 p40 mRNA expression in subsequent experiments.
LPS-induced JNK, p38 MAP kinase, and ERK activation in human macrophages

We determined the roles of the three MAP kinases in LPS signal transduction by detecting their dually phosphorylated (Thr/Tyr) forms by Western blotting against specific anti-phosphokinase Abs. The amounts of phosphorylated threonine and tyrosine of JNK significantly increased and reached a maximum at 30 min, then returned to near basal levels at 120 min after adding LPS (Fig. 2A). Levels of phosphorylated p38 threonine and tyrosine similarly increased, reaching a maximum at 30 min and returning to near basal levels at 120 min after adding LPS (Fig. 2B). The amounts of phosphorylated ERK threonine and tyrosine in PMA-treated THP-1 cells were not affected by LPS (Fig. 2C).

Effect of SB203580 on LPS-induced IL-12 p40 protein production and mRNA expression in human macrophages

Others and we have shown that p38 MAP kinase regulates LPS-induced IL-12 production in human macrophages and dendritic cells (33). Therefore, to understand the p38 MAP kinase-dependent production of IL-12 induced by LPS in PMA-treated THP-1 cells, we used SB203580, a specific inhibitor of p38 MAP kinase activity. SB203580 dose dependently inhibited LPS-induced IL-12 p40 protein production (p < 0.05, Fig. 3A). Consistent with the protein data, SB203580 dose dependently suppressed IL-12 p40 mRNA expression induced by LPS (Fig. 3B).

FIGURE 1. IL-12 p40 protein production and mRNA expression induced by LPS in human macrophages. To induce macrophage differentiation, THP-1 cells were incubated with 100 ng/ml PMA for 48 h. A, Dose-dependent protein production of IL-12 p40 after 24-h stimulation with LPS. B and C, Time-dependent protein production (B) and mRNA expression (C) of IL-12 p40 after stimulation with 1 μg/ml LPS. IL-12 p40 protein in culture supernatants was evaluated by ELISA. Values represent mean ± SEM of four experiments. IL-12 p40 mRNA was detected by Northern blotting. Total RNA was extracted and hybridized with human p40 cRNA probe. Blots were stripped, then human G3PDH probe was the loading control.

FIGURE 2. Effect of LPS on JNK, p38 MAP kinase, and ERK activation in human macrophages. PMA-treated THP-1 cells were stimulated with LPS (1 μg/ml) for indicated periods. Activities of JNK, p38 MAP kinase, and ERK were determined by Western blotting whole cell lysates using Abs specific for phosphorylated, activated forms of JNK (A, top), p38 MAP kinase (B, top), and ERK (C, top). Corresponding bottom panels are Western blots using Abs to total JNK (A, bottom), p38 MAP kinase (B, bottom), and ERK (C, bottom), indicating amounts of precipitated enzymes.

FIGURE 3. Effect of SB203580 on LPS-induced IL-12 p40 protein production and mRNA expression in human macrophages. PMA-treated THP-1 cells were cultured with 0.1% DMSO (control vehicle) and SB203580 (specific inhibitor of p38 MAP kinase) for 30 min before stimulation with LPS (1 μg/ml). A, Dose-dependent protein production of IL-12 p40 after 24-h stimulation with LPS. B, After 24 h, IL-12 p40 protein in culture supernatants was evaluated by ELISA. Values represent mean ± SEM of four experiments. IL-12 p40 mRNA was detected by Northern blotting. Total RNA was extracted and hybridized with human p40 cRNA probe. Blots were stripped, then human G3PDH probe was the loading control.
Effect of SP600125 on LPS-induced IL-12 p40 and p70 protein production and p40 mRNA expression in human macrophages

To further understand the mechanism of LPS-induced IL-12 production, we investigated the role of JNK using a selective inhibitor of this kinase, SP600125. Fig. 4A shows that SP600125 dose dependently suppressed the phosphorylation of c-Jun, which is phosphorylated and activated by JNK, induced by LPS. Fig. 4B demonstrates that SP600125 dose dependently enhanced IL-12 p40 protein production from human macrophages stimulated with LPS. Fig. 4C shows that this compound dose dependently augmented LPS-induced IL-12 p40 mRNA expression. Furthermore, SP600125 enhanced LPS-induced IL-12 p70 production in human monocytes (Fig. 4D). These results suggest that the JNK pathway negatively regulates LPS-induced IL-12 production.

Effects of JNK1 and JNK2 antisense oligonucleotides on LPS-induced JNK activation and IL-12 p40 production in human macrophages

To further confirm the role of JNK in LPS-induced IL-12 production in human macrophages, we performed experiments using JNK1 and JNK2 antisense oligonucleotides. The apparent molecular mass values of 46 and 54 kDa represent JNK1 and JNK2 isoforms (34). JNK1 antisense (AS-JNK1) and JNK2 antisense (AS-JNK2) oligonucleotides attenuated the 46- and 54-kDa components (Fig. 5, A and B). Furthermore, AS-JNK1 and AS-JNK2 together suppressed LPS-induced JNK phosphorylation (Fig. 5, A and B) and kinase activity (Fig. 5C) compared with control oligonucleotides (Scr-JNK1 and Scr-JNK2). We examined susceptibility to LPS-induced IL-12 p40 production under these conditions. Fig. 5D shows that AS-JNK1 and AS-JNK2 significantly ($p < 0.05$) suppressed LPS-induced IL-12 p40 production.
0.05) enhanced LPS-induced IL-12 p40 protein production compared with control oligonucleotides. Consistent with the protein data, JNK antisense oligonucleotides augmented LPS-induced IL-12 p40 mRNA expression (Fig. 5E).

**Effect of GSH-OEt on the ratio of GSH/GSSG and LPS-induced IL-12 p40 production in human macrophages**

We reported that IL-12 production is positively or negatively polarized by augmenting or depleting the intracellular contents of GSH in murine peritoneal macrophages (35) and in human monocytes (17). In PMA-treated THP-1 cells, GSH-OEt, which is a membrane-permeable compound that quickly increases the intracellular soluble pool of GSH (36), promoted a significant increase in the intracellular GSH concentration and the ratio of GSH/GSSG ($p < 0.05$, Fig. 6A). Fig. 6B and C, demonstrates that GSH-OEt-treated human macrophages dose dependently enhanced LPS-induced IL-12 p40 protein production ($p < 0.05$) and mRNA expression, consistent with the increase in the GSH/GSSG ratio. These results indicate that the glutathione redox balance, namely the intracellular GSH/GSSG ratio, regulates LPS-induced IL-12 p40 production in human macrophages.

**Effect of GSH-OEt on LPS-induced JNK and p38 MAP kinase activation in human macrophages**

We investigated whether or not the glutathione redox balance affects the LPS-induced JNK and p38 MAP kinase activation induced by LPS in human macrophages. We found that GSH-OEt dose dependently inhibited LPS-induced JNK phosphorylation, and that inhibition was maximal at a GSH-OEt concentration of 5 mM (Fig. 7A). Densitometry showed that 5 mM GSH-OEt significantly ($p < 0.05$) inhibited LPS-induced JNK phosphorylation (Fig. 7D). In contrast, GSH-OEt enhanced LPS-induced p38 MAP kinase phosphorylation, and increases were maximal at a GSH-OEt concentration of 0.5 mM (Fig. 7B). Densitometry showed that 0.5 mM GSH-OEt significantly ($p < 0.05$) enhanced LPS-induced p38 MAP kinase phosphorylation (Fig. 7E). Neither LPS nor GSH-OEt affected ERK activity in PMA-treated THP-1 cells (Fig. 7C).
compared with LPS-stimulated THP-1 cells. GSH-OEt-treated THP-1 cells stimulated with LPS.

Additionally, cells were incubated with or without GSH-OEt (5 mM) for 4 h before stimulation. A, After 30-min stimulation, the phosphorylation of c-Jun was determined by Western blotting whole cell lysates using an Ab specific for a phosphorylated, activated form of c-Jun. Corresponding bands for total c-Jun were determined. B recruit-ment of the present study was that JNK plays a negative role in the production of IL-12 from human macrophages stimulated by LPS. Another was that glutathione redox regulates LPS-induced IL-12 production through the suppression of JNK activation as well as through the enhancement of p38 MAP kinase activation. We are the first to report that JNK negatively regulates IL-12 production and that glutathione redox paradoxically regulates JNK and p38 MAP kinase in human macrophages.

Although many investigators have reported that several regulatory elements in the IL-12 p40 promoter region are implicated in the regulation of p40 expression (13–15), little is known about the early signaling events underlying the up-regulation of p40 expression in human macrophages. Mice deficient in MAP kinase kinase 3, the specific upstream MAP kinase kinase for p38 MAP kinase, have defective p38 MAP kinase activation and IL-12 p40 promoter activity (7), suggesting that LPS-induced IL-12 production depends on activation of the p38 MAP kinase pathway in murine APC. Saccani et al. (33) have recently demonstrated that p38 MAP kinase-dependent histone H3 phosphorylation initiates LPS-induced p40 mRNA expression through increased NF-κB recruitment. We also considered that p38 MAP kinase activation positively regulates LPS-induced p40 production in human macrophages, because LPS was a powerful stimulator of p38 MAP kinase activation (Fig. 2B) and a selective blocker of this kinase activation inhibited LPS-induced p40 production (Fig. 3).

In contrast, stimulating THP-1 cells with LPS rapidly activates the JNK pathway (37) (Fig. 2A). However, because a JNK-specific inhibitor is unknown, few studies have examined the role of JNK in cellular responses including the expression of IL-12 compared with other MAP kinases, such as p38 MAP kinase and ERK. SP600125, which is a specific and ATP-competitive inhibitor of JNK1, JNK2, and JNK3, almost completely blocks TNF-α production from human monocytes induced by LPS (28). The present study showed that SP600125 simultaneously dose dependently suppressed LPS-induced c-Jun phosphorylation (Fig. 4A), and dose dependently enhanced IL-12 p40 production from PMA-treated THP-1 macrophages (Fig. 4, B and C) as well as IL-12 p70 production from human monocytes (Fig. 4D). Furthermore, our studies demonstrated that a decrease in cellular amounts of JNK in human macrophages caused by JNK1 and JNK2 antisense oligonucleotides (Fig. 5, A and B) potently inhibited JNK activity (Fig. 5C), but significantly enhanced IL-12 production (Fig. 5, D and E) induced by LPS. These results indicate that negative signaling pathways for IL-12 production in human macrophages are mediated by JNK. IL-10 is involved in a major negative regulatory mechanism of IL-12 production because mice deficient in IL-10 spontaneously develop autoimmune disease-like phenotypes that are associated with increased IL-12 production (38). In contrast, IL-10 is probably not involved in the JNK-mediated suppression of IL-12 p40 expression because the kinetics of

Effects of SP600125 and SB203580 on LPS-induced IL-12 p40 production in GSH-OEt-treated human macrophages

Finally, we investigated whether or not JNK and p38 MAP kinase are involved in IL-12 production regulated by glutathione redox. Exposure to the JNK-specific inhibitor, SP600125, suppressed the subsequent phosphorylation of LPS-induced c-Jun (Fig. 8A) and enhanced LPS-induced IL-12 production (Fig. 8B). Like SP600125, prior exposure to GSH-OEt enhanced LPS-induced IL-12 production (Fig. 8B, lanes 2 and 3), but suppressed the phosphorylation of LPS-induced c-Jun to a lesser extent (Fig. 8A, lanes 4 and 5). The suppression of LPS-induced c-Jun phosphorylation attributable to prior SP600125 exposure in cells also pretreated with GSH-OEt was comparable to that in the cells without GSH-OEt (Fig. 8A, lanes 5 and 6). However, the amount of LPS-induced IL-12 production attributable to prior SP600125 exposure was more enhanced in cells also pretreated with, than without GSH-OEt (Fig. 8B, lanes 3 and 4). However, the degree of enhancement was weaker than that of LPS-induced IL-12 production enhanced by prior GSH-OEt exposure. These findings suggest that although JNK is involved in glutathione redox-regulated IL-12 production, other pathways are also involved. Furthermore, the p38 MAP kinase-specific inhibitor, SB203580, suppressed LPS-induced IL-12 production (Fig. 8C). The IL-12 production in the presence of GSH-OEt was suppressed by SB203580 at the same rate as that in the absence of GSH-OEt (Fig. 8C). These findings suggest that although p38 MAP kinase is involved in glutathione redox-regulated IL-12 production, other pathways are also involved. Hence, both JNK and p38 MAP kinase are involved in glutathione redox-regulated IL-12 production.

FIGURE 8 Effects of SP600125 and SB203580 on LPS-induced IL-12 p40 production in GSH-OEt-treated human macrophages. A–C, PMA-treated THP-1 cells were cultured with 0.1% DMSO (control vehicle) or 30 μM SP600125 (A, B), and 0.1% DMSO (control vehicle) or 10 μM SB203580 (C) for 30 min before stimulation with LPS (1 μg/ml). In addition, cells were incubated with or without GSH-OEt (5 mM) for 4 h before stimulation. A, After 30-min stimulation, the phosphorylation of c-Jun was determined by Western blotting whole cell lysates using an Ab specific for a phosphorylated, activated form of c-Jun. Corresponding lanes consist of Western blots using an Ab to total c-Jun, indicating amounts of precipitated enzymes. B and C, After 24-h stimulation, IL-12 p40 protein in culture supernatants of THP-1 cells was evaluated by ELISA. Values represent mean ± SEM of four experiments. * p < 0.05 compared with LPS-stimulated THP-1 cells. § p < 0.05 compared with GSH-OEt-treated THP-1 cells stimulated with LPS.
IL-10 production induced by LPS from PMA-treated THP-1 macrophages were similar to those of IL-12 p40 production (data not shown).

Glutathione is the most abundant nonprotein tripeptide containing a sulfhydryl group in virtually all cells, and it plays a significant role in many biological processes. It also constitutes the intracellular redox buffer in many cell types (36). Evidence suggests that the intracellular redox status regulates various aspects of cellular function (39). We recently demonstrated that glutathione levels in murine APC play a central role in determining which of the Th1 and Th2 cytokine responses predominate during immune states through IL-12 production (35). In addition, we reported that glutathione redox regulates LPS-induced IL-12 production at the transcriptional level of p40 and p35 subunit mRNA through p38 MAP kinase activation in human monocytes (17). The present investigation confirmed that the increase in the ratio of GSH/GSSG induced by GSH-OEt dose dependently enhanced LPS-induced IL-12 p40 protein production and mRNA expression in human macrophages (Fig. 6). Contrary to our previous observation (17), we could not confirm that IL-12 production up-regulated via the increased GSH/GSSG ratio was merely due to enhanced p38 MAP kinase activity, because the maximal concentration (0.05–5 mM) augmentation of IL-12 production by GSH-OEt. We also found that GSH-OEt dose dependently suppressed LPS-induced JNK activation (Fig. 7, A and D), which might negatively regulate IL-12 p40 expression, as noted above. We considered that the inhibitory effect of GSH-OEt on LPS-induced JNK activation was not due to toxicity, because GSH-OEt did not affect ERK activity induced by LPS (Fig. 7C). Furthermore, prior exposure to SP600125, which is a JNK-specific inhibitor, further enhanced the LPS-induced IL-12 production enhanced by GSH-OEt, but to a lesser degree than that enhanced by GSH-OEt alone (Fig. 8B). Prior exposure to SB203580, which is a p38 MAP kinase-specific inhibitor, suppressed the LPS-induced IL-12 production at the same rate in the presence or absence of GSH-OEt (Fig. 8C). These findings suggest that both JNK and p38 MAP kinase are involved in glutathione redox-regulated IL-12 production.

We discovered that LPS-induced IL-12 synthesis is negatively controlled by JNK-mediated signaling, and that JNK and p38 MAP kinase function negatively and positively, respectively, in the mechanisms involved in the regulation of IL-12 p40 mRNA expression mediated by glutathione redox in human macrophages. Some enzyme activities depend on protein S-thiolation/dethiolation, namely, the oxidation of protein sulfhydryls to mixed disulfides and their reduction back to sulfhydryls (40). Therefore, the balance of the reaction from cellular thiol to disulfide, including that of glutathione redox, must be able to regulate the activity of these enzymes (41). We postulate that glutathione redox regulates LPS-induced JNK and p38 MAP kinase activation through protein S-thiolation/dethiolation in the JNK- and p38 MAP kinase-signaling pathway. Moreover, the enhancement of LPS-induced p38 MAP kinase activity was not dose dependent on GSH-OEt. To explain this finding, we postulate that the primary target protein(s) of glutathione redox is situated upstream of p38 MAP kinase. However, further study is required to clarify these notions.

We provide evidence of a JNK-mediated negative feedback mechanism of LPS-induced IL-12 production from human macrophages. We demonstrated that LPS stimulates both JNK and p38 MAP kinase, and that these MAP kinases play antagonistic roles in regulating the signals leading to the induction of IL-12 p40 mRNA. In addition, we showed that glutathione redox, specifically the intracellular GSH/GSSG ratio, regulates LPS-induced IL-12 production during the mediation of both suppressed JNK activation and enhanced p38 MAP kinase activation in human macrophages. IL-12 is a key cytokine that differentiates Th0 to Th1. Thus, JNK and p38 MAP kinase could be new targets for controlling IL-12 production. In addition, the regulation of these MAP kinases by modulators of glutathione redox may aid the development of new therapeutic strategies for altering the Th1-Th2 balance through IL-12 production in allergic and autoimmune diseases, as well as in other pathological conditions.

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References


