The p38 MAPK Pathway Mediates Transcriptional Activation of the Plasma Platelet-activating Factor Acetylhydrolase Gene in Macrophages Stimulated with Lipopolysaccharide*

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Administration of lipopolysaccharide (LPS) to experimental animals results in the up-regulation of expression of the plasma form of platelet-activating factor acetylhydrolase (PAF AH) in tissue macrophages. To investigate the mechanism underlying induction of PAF AH by LPS we used murine RAW264.7 and human THP-1 macrophages as model systems. We found that the p38 mitogen-activated protein kinase (p38 MAPK) pathway mediates transcriptional activation of the PAF AH gene through the participation of nucleotides ~68/316 relative to the transcriptional initiation site. This promoter region spans two Sp1/Sp3 binding sites (SP-A and SP-B) and is necessary and sufficient for the observed effect. Disruption of these Sp binding sites significantly reduces promoter activity in LPS-stimulated cells. The ability of LPS to induce transcriptional activation of PAF AH is not due to enhanced Sp1/Sp3 binding to the promoter but involves enhanced transactivation function of Sp1 via p38 MAPK activation. These studies characterize the mechanism by which LPS modulates expression of PAF AH at the transcriptional level, and they have important implications for our understanding of responses that occur during the development of LPS-mediated inflammatory diseases.

Platelet-activating factor (PAF, \(^1\)-O-alkyl-2-acetyl-sn-glycer-3-phosphocholine) is a potent pro-inflammation phospholipid synthesized upon exposure of monocytes/macrophages to lipopolysaccharide (LPS), one of the major components of the cell wall of Gram-negative bacteria (1). In addition to PAF, administration of LPS to animals and exposure of macrophages in culture to LPS also result in the accumulation of other mediators such as PGE\(_2\) and proinflammatory cytokines including IL-1, IL-6, IL-8, and tumor necrosis factor-\(\alpha\) and -\(\beta\) (2). Excess release of these molecules \textit{in vivo} is thought to play a key role in the pathogenesis of several human syndromes including sepsis, a severe inflammatory disease that causes ~20,000 annual deaths in the United States (1–3). The observation that PAF and LPS have common physiological and pathological activities led to the finding that this phospholipid is one of the key downstream intermediates produced after exposure to LPS (4, 5). The down-regulation of signals elicited by PAF (and PAF-like lipids) is mediated by PAF acetylhydrolases (PAF AHs), calcium-independent phospholipases A\(_2\) with specificity for hydrolysis of these lipid mediators (6). The secreted or plasma form of PAF AH circulates in blood as a complex with lipoproteins (7). Its precise role in pathophysiological processes is controversial (8–14) but our work and that of others has shown that the enzyme has anti-inflammatory properties \textit{in vivo} (15–20).

Results from studies in patients diagnosed with syndromes in which LPS is thought to participate as a mediator, such as the acute respiratory distress syndrome, sepsis, and septic shock have revealed alterations in the secreted form of PAF AH at the activity, protein, and mRNA levels. Endo et al. find that PAF AH activity levels correlate with markers of disease severity such as endotoxin, tumor necrosis factor-\(\alpha\), IL-8 (21) and thrombomodulin (22) during sepsis. Alveolar macrophages isolated from patients during the acute phase of acute respiratory distress syndrome have been reported to express higher PAF AH activity and mRNA levels compared with alveolar macrophages from patients at risk and healthy subjects (23). The combination of these observations suggests that LPS or the release of potent downstream mediators during sepsis, acute respiratory distress syndrome, and related syndromes results in macrophage activation followed by secretion of phospholipases and other key proteins (24). The increase in PAF AH levels may serve as a compensatory mechanism to down-regulate local and systemic activation mediated by PAF and PAF-like lipids through transcriptional activation of the PAF AH gene (25). A number of studies, however, reported decreased levels of PAF AH activity in plasma during severe sepsis (26, 27). Thus, seemingly opposing results have been obtained using relatively small patient populations, a number of different cells and body fluids, and various methodologies to detect PAF AH activity. Consequently, the role of PAF AH in sepsis and related human syndromes remains obscure.

In contrast to studies in humans, administration of LPS to
experimental animals has consistently resulted in robust increases in the levels of PAF AH at the activity, protein, and mRNA levels during the first 24–48 h (29–32). The origin of PAF AH synthesized in response to LPS seems to be limited to cells of the macrophage lineage including tissue macrophages from hepatic (29–32) and most likely pulmonary and peritoneal origins. This is consistent with the observation by Asano et al. (33) that most of the circulating PAF AH originates in cells of the hematopoietic lineage.

The actions of LPS are mediated by TLR4, a member of the toll-like receptor (TLR) superfamily (34). LPS associates with LPS-binding protein (LBP) and is delivered to the cell surface receptor the CD14. The LPS-LBP-CD14 complex interacts with TLR4 and activates the intracellular mitogen-activated protein kinase kinase kinases (MAPKKK(MKK)) (34), leading to stimulation of three distinct MAPKs including p38 MAPK, extracellular signal-regulated kinases (ERK1/2), and c-Jun N-terminal kinase (JNK) (35, 36). LPS is thought to activate ERK1/2 via the Ras/Raf-1/MAPK kinase-1 (MKK1 or MEK1) cascade (35). On the other hand, p38 MAPK and JNK are phosphorylated and activated by MKK3/6 and MKK4/7, respectively (37, 38). Active JNK and p38 MAPK participate in the LPS-mediated regulation of diverse pro-inflammatory genes by stimulating multiple transcription factors including the Ap1 complex (c-Jun and c-Fos), ATF-2, cAMP-response element-binding protein (CREB), and C/EBP (39).

To investigate the mechanisms that underlie PAF AH up-regulation by LPS, we developed a model system that recapitulates PAF AH responses to LPS similar to those observed in vivo. Second, we established that the ability of LPS to enhance PAF AH expression involves participation of the p38 MAPK signaling pathway combined with the presence of essential Sp1/Sp3 binding sites in the PAF AH promoter. Finally, we demonstrated that transactivation by Sp1 is the mechanism by which this transcription factor enhances PAF AH expression.

EXPERIMENTAL PROCEDURES

Cell Culture and Reagents—Murine RAW264.7 macrophages were purchased from American Type Culture Collection and were maintained in a 5% CO₂ atmosphere at 37 °C in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 10% fetal bovine serum (HyClone). Human THP-1 monocytes overexpressing surface CD14 (generously provided by Dr. Richard Ulevitch, Scripps Research Institute, San Diego, CA) were maintained at 37 °C in RPMI 1640 (Invitrogen) supplemented with 500 μg/ml Geneticin (Invitrogen). Lipoprotein-free LPS from Salmonella enteriditis (Sigma-Aldrich) was sterilized by γ-irradiation and used at 1 μg/ml unless otherwise specified. The inhibitors PD98059 (Biomol), U0126, SB202190, and SB203580 (Alexis Biochemicals) were dissolved in Me₂SO before addition to the culture medium. Cells were treated with LPS and/or inhibitors in the presence of essential Sp1/Sp3 binding sites in the PAF AH promoter. In cell lineages subsequently transfected with the p38 MAPK and JNK-overexpressing cell lines, we used 1 ng/ml recombinant rat IL-1α (PeproTech).

Electrophoretic Mobility Shift Assay (EMSA)—Nuclear protein extracts were prepared from cells using a passive lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1 mM Na₂EDTA, 1 mM EDTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na₂VO₄, and 1 μg/ml leupeptin). After sonication, the cell lysates were clarified by centrifugation at 16,000 × g for 15 min at 4 °C. The nuclear proteins were then transferred to a polyvinylidene fluoride membrane (PerkinElmer Life Sciences). The membrane was blocked in 1× TBST buffer (50 mM Tris-HCl, pH 7.5, 250 mM NaCl, 1% Tween 20) containing 5% nonfat dry milk and then probed with the primary antibody indicated in each case at 4 °C overnight. After three washes in 1× TBST, the membranes were exposed to a horseradish peroxidase-conjugated secondary antibody (BIOSOURCE) for 1 h at room temperature. Immunoreactive bands were visualized using chemiluminescence detection reagents (PerkinElmer Life Sciences).

Kinase Activity Assays—The cellular activities of p38 MAPK and JNK were measured using p38 MAPK and JNK assay kits from Cell Signaling following the instructions suggested by the manufacturer.

Results

1. PAF AH expression: PAF AH expression in murine peritoneal monocyte/macrophage cell line RAW264.7 and a human monocytic cell line overexpressing CD14 (THP-1/CD14). Resting RAW264.7 and THP-1/CD-14 cells expressed extremely low levels of PAF AH mRNA (Fig. 1, A and B) and protein (Fig. 1C), thus resembling the characteristic phenotype of primary human monocytes (43). Stimulation with LPS at low concentrations (1–10 ng/ml) consistently increased PAF AH mRNA levels; maximal expression was observed at 1 μg/ml LPS (Fig. 1, A and B). In a time-course analysis, PAF AH mRNA levels were increased 3 h after the addition of 1 μg/ml.
LPS and were sustained up to 12 h after stimulation (Fig. 1, A and B). These changes were followed by robust increases in PAF AH protein that remained elevated for at least 24 h (Fig. 1C).

The p38 MAPK Pathway Mediates LPS Induction of PAF AH mRNA in Macrophages—Previous studies have shown that LPS activates multiple pathways including those involving MAPK participation (36). Our next goal was to elucidate the signaling pathway(s) that participates in LPS-mediated up-regulation of PAF AH expression. We initially examined the effect of various MAPK inhibitors on the ability of LPS to up-regulate PAF AH expression in RAW264.7 cells. We found (Fig. 2A) that the p38 MAPK inhibitors SB203580 (10 μM) and SB202190 (15 μM) completely prevented LPS-mediated up-regulation of PAF AH mRNA in RAW264.7 cells. The inhibitor SB203580 attenuated the phosphorylation of ATF-2, a reaction catalyzed by p38 MAPK (Fig. 2B) and had no effect on ERK and JNK activation, as expected (Fig. 2B). In contrast, the MEK1/2 inhibitor U0126 and the JNK inhibitor SP600125 had no effect on the up-regulation of PAF AH mediated by LPS but were effective inhibitors of ERK and JNK activation, respectively (Fig. 2B). We also observed (Fig. 2C) a robust increase in PAF AH mRNA levels after LPS stimulation of human THP-1/CD14 cells. The stimulation of human PAF AH mRNA production was almost completely inhibited by p38 MAPK inhibitors (Fig. 2D), which were also effective inhibitors of LPS-mediated up-regulation of IL-10 mRNA levels, as expected (44). In contrast, the ability of LPS to mediate up-regulation of PAF AH mRNA levels was completely independent of MEK1/2 activation because supplementation of U0126 was without effect (Fig. 2, A and C). The effectiveness of U0126 as a MEK1/2 inhibitor was demonstrated by its ability to inhibit the increase in IL-6 mRNA levels in response to LPS, a process that requires active MEK1/2 (Fig. 2C). These results demonstrate the participation of p38 MAPK, but not ERK or JNK, as one of the key intermediates in the ability of LPS to induce expression of PAF AH mRNA in cells of the monocytic lineage.

The Proximal Region of the PAF AH Promoter Participates in LPS-mediated Increases in PAF AH mRNA Levels; Role of p38 MAPK—The effect of LPS on PAF AH mRNA levels could be due to increased mRNA synthesis, increased stability, or both. We first investigated what regions of the PAF AH promoter are necessary for the observed effects. We transfected RAW264.7 cells with various mouse and human PAF AH promoter constructs fused to a luciferase reporter and then stimulated the cells with LPS for 24 h. We found (Fig. 3A) that LPS treatment increased the activity of the transfected human and mouse promoter constructs by 4–5-fold. In contrast, the promoter activity of the backbone vector (pGL3-Basic) was only moderately increased by LPS (1.8-fold, data not shown). These data suggest that one of the mechanisms by which LPS up-regulates expression of PAF AH includes transcriptional activation.

We next investigated whether the participation of p38 MAPK in LPS-mediated effects occurred at the promoter level using dominant-negative constructs of various MAPKs. We co-transfected RAW264.7 macrophages with the mouse PAF AH promoter construct that spans nt −1806/+86 and an expression plasmid encoding either dominant-negative ERK1 (dnERK1), ERK2 (dnERK2), JNK1 (dnJNK1), or p38 MAPKα (dnp38α). We found (Fig. 3B) that promoter activation mediated by LPS was completely abolished by overexpression of dnp38α; in contrast, expression of dnERK1, dnERK2, or dnJNK1 had no effect. These results demonstrate that LPS-induced PAF AH expression requires participation of the p38 MAPK pathway at the transcriptional level.

To precisely map the promoter regions necessary for LPS-mediated effects, we transfected RAW264.7 cells with a series of previously characterized mouse promoter constructs fused to a luciferase reporter (40). All the promoter constructs exhibited 4–5-fold LPS-dependent increases in promoter activity except a very short construct, which spanned nt −68/+86 (2.2-fold activation, Fig. 3C). The minimal sequence necessary for maximal LPS-dependent promoter activation included 316 nt upstream of the transcriptional initiation site of the mouse gene. This 316-nt sequence contained elements targeted by the p38 MAPK pathway as co-expression of dnp38α, but not dnERK1, dnERK2, or dnJNK1, inhibited LPS-mediated promoter activation (Fig. 3D). Thus, LPS elicits signals from the p38 MAPK pathway that target the proximal mouse PAF AH promoter region between nt −68 and −316.

Sp1/Sp3 Binding Sites Participate in the Transcriptional Activation of PAF AH by LPS—We recently demonstrated that nt −68/−316 in the mouse PAF AH promoter contain three functional Sp1/Sp3 binding sites (SP-A, -B, and -C) and that SP-A and SP-B are essential for the constitutive expression of murine PAF AH in macrophages (40). We next tested the hypothesis that these Sp1/Sp3 sites also participate in LPS-mediated transcriptional effects. We systematically mutated SP-A, SP-B, SP-C, and combinations therein and then asked if the promoter activity of the mutant constructs was altered by LPS. We found (Fig. 4) that disruption of SP-A or SP-B, but not
SP-C, significantly reduced promoter activation mediated by LPS. Mutation of both SP-A and SP-B completely abolished LPS-induced promoter activation in RAW264.7 macrophages, thus demonstrating that these sites are essential for the ability of LPS to activate the murine PAF AH gene. We next conducted similar experiments within the context of the human PAF AH promoter. We previously showed that constitutive expression of the human PAF AH gene is largely dependent on the presence of one Sp1/Sp3 binding site homologous to murine SP-B (40). We found (Fig. 4) that mutation of this Sp1/Sp3 site completely blocked LPS-induced stimulation of the minimal human PAF AH promoter.

LPS Stimulation Enhances the Transactivation Function of Sp1, but Not Its DNA Binding Activity, in a p38 MAPK-dependent Manner—To further investigate the mechanism of LPS-mediated increases in PAF AH mRNA levels, we tested the hypothesis that enhanced binding of Sp1/Sp3 to SP-A and SP-B takes place after LPS stimulation. We performed EMSA using oligonucleotides spanning SP-A and SP-B combined with nuclear extracts from RAW264.7 or THP-1/CD14 cells treated with LPS for various time periods. We found no changes in the extent of DNA-protein complex formation (Fig. 5, A and B) or the amount of nuclear Sp1 levels (not shown) after LPS treatment. In contrast, LPS stimulation resulted in significant increases in NF-κB and C/EBP binding activities (Fig. 5, A and B, compare lane 1 with lanes 6 and 7), suggesting adequate activation efficiency. Our results demonstrate that the ability of LPS to enhance PAF AH mRNA levels does not occur because of increased DNA binding activity of Sp1 and Sp3.

We next considered additional mechanisms to account for the participation of Sp1/Sp3 in LPS-mediated up-regulation of PAF AH mRNA levels. We co-transfected RAW264.7 cells with a Gal4-luciferase reporter plasmid and plasmids expressing either the Gal4 DNA binding domain (Gal4DBD) or Gal4DBD fused to the transactivation domains of TAT, Elk1, Sp1 (Sp1B), or the entire Sp1 protein. We found that the transactivation domain of Elk1, a known target of Ras/MEK signaling, enhanced transcriptional activity in response to LPS, as expected (Fig. 5C). In contrast, transfection with Gal4DBD or Gal4DBD-TAT fusion protein showed undetectable transcriptional activ-
The transactivation domain of Sp1 (Sp1B) conferred responsiveness to LPS to the same extent as full-length Sp1, in agreement with previous findings (45). These data demonstrate that Sp1 can mediate transcriptional induction by LPS without an increase in its DNA binding activity.

To investigate what intracellular signaling pathways were involved in this process, we treated RAW264.7 cells transfected with Gal4DBD-Sp1B with inhibitors targeting the MEK, JNK, and p38 MAPK pathways. After transfection, we supplemented LPS along with inhibitors or vehicle (Me2SO) for 18 additional hours and then determined luciferase activity to assess the activation status of the signaling events. LPS-mediated induction of luciferase expression was considerably reduced by the p38 MAPK inhibitor SB202190 (Fig. 5D) and by dnp38/H9251 (not shown). In contrast, inhibition of the ERK- and JNK-signaling pathways using specific inhibitors (Fig. 5D) or dominant negative approaches (not shown) failed to inhibit luciferase expression. Interestingly, SP600125 further increased promoter activation induced by LPS, suggesting an inhibitory role for the JNK signaling in p38 MAPK-mediated Sp1 activation. These results indicate that p38 MAPK plays a key role in LPS responses mediated by the transactivating domain of Sp1. In contrast, the remaining MAPK pathways activated by LPS (ERK and JNK) do not participate in the regulation of PAF AH expression by LPS.

**DISCUSSION**

LPS has been demonstrated to act as a potent inducer of PAF AH expression in animals (29–32), but some studies show that this agent down-regulates expression of either PAF AH activity or mRNA levels in cultured decidual macrophages (41), RAW264.7 cells (25), liver Kupffer cells (29), and TPA-differentiated HL-60 cells (41). These seemingly contradictory results can be explained by careful examination of the experimental conditions used in each case, the state of cellular differentiation or activation, and the type and timing of the measurements. First, in our previous studies using RAW264.7 cells we found that LPS decreased the promoter activity of a human PAF AH promoter construct that spanned nt −3416/ +206 (25). This large construct included additional sequences...
that may have silenced LPS-mediated events involving proximal sites. Second, studies using terminally differentiated macrophages and/or activated macrophages may have limited value in the assessment of mechanisms aimed at characterizing the effect of cytokines and other effectors of cellular function on PAF AH expression. The significant heterogeneity observed in macrophage populations both in the resting state and when activated by inflammatory stimuli is likely to pay important roles in this process (46–48). For example, the kinetics and magnitude of PAF AH expression are different in primary macrophage populations that differ in CD14 and CD16 expression levels. Moreover, differentiation of monocytes into dendritic cells results in significantly lower levels of intracellular and secreted PAF AH activity compared with macrophages (49). A key contribution of the present study is the utilization of a murine model system that mimics in vivo responses in cells that are not terminally differentiated and, thus, potentially recapitulates events that occur when monocytic cells are recruited and induced to differentiate in inflamed tissues in vivo.

The RAW264.7 and THP-1/CD14 macrophages used here express extremely low levels of PAF AH in the basal state. LPS robustly up-regulated expression of PAF AH mRNA, and this effect was maximal 12 h after stimulation. However, PAF AH expression subsequently decreased at later time points in RAW264.7 cells, as in previous studies (>24 h, Fig. 1A), suggesting that the ability of LPS to regulate PAF AH expression is dependent on the state of cellular maturation and activation.

The work presented here also shows that the p38 MAPK pathway participates in LPS-mediated modulation of PAF AH expression. First, inhibitors of the p38 MAPK pathway prevented up-regulation of PAF AH by LPS (Fig. 2). Second, overexpression of dominant-negative p38 MAPK attenuated the transcriptional activation of PAF AH (Fig. 3B). The p38 MAPK pathway plays key roles in the expression of genes involved in stress-induced responses, such as tumor necrosis factor-α, IL-1β, inducible nitric-oxide synthase, and cyclooxygenase-2 (50, 51). In addition, this pathway mediates increases in the expression of IL-10, a cytokine that inhibits inflammatory and cell-mediated immune responses (44). Thus, the role of the p38 MAPK kinase signaling pathway is complex because it controls expression of factors that both amplify and modulate responses to stress, injury, and inflammatory stimuli.

We recently demonstrated that non-canonical Sp1/Sp3 binding sites in the human and mouse PAF AH promoters are essential for the constitutive expression of PAF AH in macrophages (40). The present study established that these Sp1/Sp3 binding motifs (SP-A and SP-B) are also critical for increased PAF AH expression in response to LPS (Fig. 4). Both studies showed that SP-A and SP-B are essential for the constitutive activity of the mouse PAF AH promoter because combined disruption of these sites reduced promoter activity by 70–80%. However, mutation of sites SP-A, SP-B, and SP-C in combination resulted in promoter activity levels significantly lower compared with the wild-type construct but appreciably higher than those of the double mutation (SP-A and SP-B, Fig. 4 and Ref. 40). We also consistently observed that mutation of SP-C alone weakly increases promoter activity, suggesting that SP-C may contain negative regulatory elements.

We next performed experiments aimed at characterizing the mechanism by which Sp1 participates in LPS-induced transcriptional activation of the PAF AH gene. The level of Sp1 activity is modulated by post-translational modifications, alterations of Sp1 protein abundance, or both (52). The possibility that changes in the total amount of Sp1 accounted for increased PAF AH expression in response to LPS was ruled out when we found no significant change in the levels of Sp1 and Sp3 expressed by both RAW and THP-1 cells throughout the entire period of LPS treatment (data not shown). Likewise, we found no changes in the ability of either the SP-A or SP-B sites to bind nuclear proteins present in LPS-treated extracts (Fig. 5, A and B). Brightbill et al. (45) also report no changes in the binding of oligonucleotides containing Sp1 sites present in the murine IL-10 promoter after treatment with LPS. Interestingly, the TC-rich sequence found in site SP-B of the murine and human PAF AH promoters is highly homologous to a TC-rich motif in the murine IL-10 promoter that is required for constitutive and LPS-induced IL-10 expression in RAW264.7 cells (45). Bethea et al. (53) report similar findings in studies that utilized probes spanning Sp1 binding sites present in the tumor necrosis factor receptor II (TNFR-II) promoter. In contrast, Jarvis and Qureshi (54) and Ma et al. (44) observe robust increases in Sp1 binding activity after treatment of RAW264.7 and human THP-1/CD14 cells with LPS, respectively. These investigators utilized consensus Sp1 probes whose sequences differ from those present in the CD14 and IL-10 promoters they were studying, respectively. Marco et al. (55) presented the notion that a
regulation code" exists that correlates specific residues in the recognition helix of Sp1 with particular bases in the DNA site. Although the code is known to tolerate some level of degeneracy, small alterations in the sequences within both the core motif and the flanking regions result in altered affinities. These observations may account at least in part for the observed variability in Sp1 binding to various promoter sequences in response to LPS.

We investigated another potential mechanism by which Sp1 could mediate LPS-induced effects on PAF AH expression. Sp1 has been reported to induce gene expression in response to TGF-β (56) or LPS (45) through increases in the function of a domain known as the B transactivation domain. Our studies confirmed that LPS activates the transactivating domain of Sp1. In addition, we demonstrated that p38 MAPK is necessary for this activation to occur. The mechanism that mediates this effect is not known, but it may involve phosphorylation of serine and/or threonine residues in the Sp1 “B” domain resulting in enhanced transactivation (28). Thus, our results are consistent with the hypothesis that the ability of LPS to enhance PAF AH expression involves phosphorylation (and activation) of the transactivating domain of Sp1 through the p38 MAPK pathway.

In summary, our data demonstrate that LPS is a key effector of PAF AH expression in surrogate monocytic cell models and that LPS induction of PAF AH is mediated through a p38

Fig. 5. LPS increases the transcriptional, but not the binding, activity of Sp1. A, EMSA using 32P-labeled probes containing either site SP-A (5′-CCATCATCACCTCCCCGCCCCAGGACACGT(AG), SP-B (AGGAGGGGGGCCACACGCTCTCCCCCGGTCCCTT), consensus NF-κB (5′-AGTTAGGGGAACCTCCAGGC), or consensus C/EBP (5′-CGGTCCGTCCGAACCTACT) oligonucleotides and nuclear extracts from RAW264.7 cells treated with LPS for 15 min to 6 h. B, EMSA using 32P-labeled probes containing the human SP-B site (hSP-B, 5′-CACACGCTCTCCCTCCCCGGTGATCC) or the consensus NF-κB probe and nuclear extracts from THP-1/CD14 cells treated with LPS for 15 min to 4 h. C, RAW264.7 cells were co-transfected with 1 μg of a reporter plasmid (5XGal4-TATA-Luc), 1 μg of an empty vector (pcDNA3), and 50 ng of an expression plasmid encoding the Gal4 Gal4DBD or Gal4DBD fused to the transactivation domains of TAT (Gal4DBD-TAT), Elk1 (Gal4DBD-Elk1), or Sp1B (Gal4DBD-Sp1B) or to full-length Sp1 (Gal4DBD-Sp1). We then treated the cells with LPS for 18 h and determined luciferase activity. D, RAW264.7 cells transfected with Gal4DBD-Sp1B were pretreated with Me_2SO, PD98059 (10 μM), SP600125 (20 μM), or SB202190 (15 μM) for 1 h before LPS stimulation (18 h). The luciferase activity of each sample was determined as described previously. The results represent the mean ± S.D. of three separate experiments.
MAPK-dependent signaling pathway. PAF AH regulation by LPS occurs at the transcriptional level and requires specific Sp1/Sp3 binding sites within the proximal promoter region. The most likely mechanism involves enhanced transactivation mediated by Sp1. This study has furthered our understanding of the molecular mechanisms that participate in the induction of this enzyme during endotoxic challenge.

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