Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 2774–2778, April 1996
Immunology

Activation of c-Jun N-terminal kinase in bacterial lipopolysaccharide-stimulated macrophages

(c-Jun N-terminal kinase/endotoxin/macrophages)

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Communicated by J. Michael Bishop, University of California, San Francisco, CA, December 8, 1995 (received for review October 3, 1995)

ABSTRACT **Activation of macrophages by bacterial lipopolysaccharide (LPS) induces transcription of genes that encode for proinflammatory regulators of the immune response. Previous work has suggested that activation of the transcription factor activator protein 1 (AP-1) is one LPS-induced event that mediates this response. Consistent with this notion, we found that LPS stimulated AP-1-mediated transcription of a transfected reporter gene in the murine macrophage cell line RAW 264.7. As AP-1 activity is regulated in part by activation of the c-Jun N-terminal kinase (JNK), which phosphorylates and subsequently increases the transcriptional activity of c-Jun, we examined whether LPS treatment of macrophages resulted in activation of this kinase. LPS treatment of RAW 264.7 cells, murine bone marrow-derived macrophages, and the human monocyte cell line THP-1 resulted in rapid activation of the p46 and p54 isoforms of JNK. Treatment with wild-type and rough mutant forms of LPS and synthetic lipid A resulted in JNK activation, while pretreatment with the tyrosine kinase inhibitor herbimycin A inhibited this response. Binding of LPS–LPS binding protein (LBP) complexes to CD14, a surface receptor that mediates many LPS responses, was found to be crucial, as pretreatment of THP-1 cells with the monoclonal antibody 60b, which blocks this binding, inhibited JNK activation. These results suggest that LPS activation of JNK in monocyte/macrophage cells is a CD14- and protein tyrosine phosphorylation-dependent event that may mediate the early activation of AP-1 in regulating LPS-triggered gene induction.**

Upon exposure to bacterial lipopolysaccharide (LPS), a conserved component of the Gram-negative bacterium's outer membrane, macrophages release a large number of immunoregulatory molecules including tumor necrosis factor α, interleukin 1 (IL-1), IL-6, and arachidonic acid metabolites that recruit and activate other immune cells to help fight the bacterial infection (1, 2). The mechanism by which LPS induces these events is only partly understood. At physiological concentrations, LPS binds to the serum protein LPS-binding protein (LBP). This complex then interacts with CD14 on macrophages or neutrophils (3). Although CD14 is a glycosylphosphatidylinositol-linked protein that lacks an intracellular domain, LPS–LBP binding to CD14 results in rapid phosphorylation of various proteins on tyrosyl residues (3). Inhibitors of this protein tyrosine phosphorylation response abrogate cytokine secretion and generation of eicosinoids (3), emphasizing the importance of this event in mediating the macrophage response to LPS. Among the most prominently tyrosine-phosphorylated proteins in LPS-stimulated macrophages are three members of the mitogen-activated protein (MAP) kinase family, a group of related serine/threonine protein kinases that participate in transmitting extracellular signals to the cell interior, including the nucleus (4, 5). The LPS-activated MAP kinases include p42/extracellular signal-regulated kinase 2 (ERK2), p44/ERK1, and p38, the recently described mammalian homologue of HOG1, a MAP kinase-like protein that is activated in response to osmotic stress in yeast (3). These MAP kinases may be important mediators of LPS action in macrophages.

LPS induces transcription of several genes encoding proinflammatory mediators. A key issue is how LPS-triggered signaling events lead to these gene inductions. One of the promoter elements that contributes to LPS induction of the tissue factor gene in THP-1 monocytes and of the IL-6 gene in PU-1.8 monocytes is a TPA-response element (TRE) (6, 7). The transcription factors that bind to TRE sites are referred to as the activator protein 1 (AP-1) complex, which consists of both homodimers and heterodimers of members of the Jun family (c-Jun, JunB, and JunD) and heterodimers of members of the Jun and Fos (c-Fos, FosB, Fra1, and Fra2) families. Regulation of AP-1 occurs by induced transcription of c-*jun* and c-*fos*, or by posttranslational modification of their products (5). c-Jun is constitutively present in cells in an inactive form that can be activated by receptors inducing phosphorylation of two serine residues (serines 63 and 73) within the transactivation domain of c-Jun (5). Phosphorylation of serines 63 and 73 appears to be mediated by either of two highly related c-Jun N-terminal protein kinases [the p46 and p54 isoforms of c-Jun N-terminal kinase (JNK)], which are members of the MAP kinase family (5). In this report, we show that physiological concentrations of LPS activate both isoforms of JNK in monocyte/macrophage cells in a CD14- and protein tyrosine phosphorylation-dependent manner.

MATERIALS AND METHODS

Materials and Antibodies. LPS preparations were purchased from List Biological Laboratories (Campbell, CA), with the exception that synthetic lipid A (diphosphoryl, *Escherichia coli*, and *Salmonella* types) and lipid IVa (LA-14-PP) were purchased from ICN Biomedicals (Irvine, CA). Herbimycin A, phorbol 12-myristate 13-acetate (PMA), β-estradiol, and myelin basic protein were purchased from Sigma. Anti-JNK and anti-ERK2 antibodies were purchased from Santa Cruz Biotechnology. 1α,25-dihydroxyvitamin D_3 was purchased from Biomol (Plymouth Meeting, PA). Ascites containing the 60b anti-CD14 monoclonal antibody (mAb) (8) was a gift from R. Todd (University of Michigan, Ann Arbor, MI), and the antibody was affinity-purified on a protein A-Sepharose column. The mAb anti-human IgM (IgG1) was purchased from Southern Biotech (Birmingham, AL).

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Abbreviations: LPS, lipopolysaccharide; IL, interleukin; AP-1, activator protein 1; MAP kinase, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; PMA, phorbol 12-myristate 13-acetate; GST, glutathione *S*-transferase; mAb, monoclonal antibody; LBP, LPS binding protein.

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Plasmids and Expression of Glutathione *S*-Transferase (GST) Fusion Proteins. A plasmid encoding the GST–c-Jun fusion protein, pGEX3X-cJun(1–79), was constructed by inserting a fragment encoding amino acids 1–79 from pBS-human c-Jun (generously provided by B. M. Peterlin, University of California, San Francisco) between the *Bam*HI and *Eco*RI sites of the GST-expressing vector pGEX3X (Pharmacia). The 1–79 amino acid fragment of c-Jun had been amplified by the polymerase chain reaction, using primers that incorporated *Bam*HI and *Eco*RI restriction sites at the 5′ and 3′ ends of the fragment, respectively. The GST fusion protein expression vector was transformed into the DH-5α strain of *E. coli*. Protein induction and purification were as described (9, 10).

Cell Culture, Stimulation, and Cell Lysis. The macrophage cell lines RAW 264.7 and THP-1 were obtained from American Type Culture Collection and maintained as described (11, 12). From 48 to 72 hr prior to an experiment, the THP-1 cells were seeded into a culture flask at $\approx 2.5 \times 10^5$ cells per ml in medium supplemented with 80 nM 1α,25-dihydroxyvitamin D_3 (vitamin D_3) to induce CD14 expression (12). Transfected RAW 264.7 cells expressing a truncated, oncogenic form of human $p74^{Raf\text{-}1}$ fused to the hormone-binding domain of the human estrogen receptor (ΔRaf-1:ER) were maintained in phenol red-free DMEM supplemented with G418 (1.5 mg/ml) (11). Murine bone marrow-derived macrophages were isolated and cultured in medium containing 20% L-cell-conditioned medium as described (13, 14). After at least 6 days *in vitro*, the murine bone marrow cells were seeded onto 100-mm plates and grown to 60–80% confluency for experiments. Examination of cellular responses and lysis conditions were as described (11, 12).

Immunoprecipitation and Kinase Assay. Triton X-100-soluble protein lysates were precleared with protein A-Sepharose (Pharmacia) and then incubated with anti-JNK antibody overnight at 4°C. The immunoprecipitates were washed twice with lysis buffer and once with kinase assay buffer (25 mM Hepes, pH 7.4/20 mM $MgCl_2$/20 mM β-glycerophosphate/0.1 mM sodium orthovanadate/2 mM dithiothreitol). Immunocomplex kinase assays were performed as described (15) with the exception that reactions were performed at 30°C for 15 min using 15–25 μg of GST or GST–c-Jun substrate.

In-Gel Kinase Assay. The kinase activity of JNK was also assessed by an in-gel kinase assay, wherein GST–c-Jun, the JNK substrate, was copolymerized into the polyacrylamide gel as described (16).

Transient Transfection. The AP-1 luciferase reporter plasmid, AP-1Lux, was generously provided by G. Crabtree (Stanford University, Palo Alto, CA). This plasmid, derived from X_3CAT, contains the luciferase gene under the transcriptional control of three synthetic AP-1 sites and the simian virus 40 early promoter (17). For transfection experiments, RAW 264.7 cells were dislodged from tissue culture dishes with a rubber policeman and transiently transfected in suspension using DEAE-dextran as described (18). Briefly, pools of 3×10^7 cells were each transfected with 22.5 μg of AP-1Lux in DEAE-dextran (0.2 mg/ml). Following a 90-min incubation and 3-min 10% dimethyl sulfoxide shock, all of the cells were pooled and then split onto 10-cm tissue culture dishes (10^7 cells per dish). Approximately 40 hr posttransfection, the dishes were treated with LPS or PMA as indicated. At the completion of the stimulation, the cells were lysed and the luciferase activity in each lysate was measured with an Optocomp I luminometer (MGM Instruments, Hamden, CT) according to the manufacturers' protocol (luciferase assay system catalog no. E1500; Promega).

RESULTS

LPS Stimulates AP-1 Transactivation in RAW 264.7 Macrophages. The promoters of the tissue factor and IL-6 genes contain binding sites for the AP-1 transcription factor complex, and these sites are necessary for full LPS responsiveness (6, 7). Moreover, LPS promotes AP-1 DNA binding activity (19–21). However, LPS stimulation of AP-1 transactivation has not yet been demonstrated. We examined this issue by using an AP-1-driven luciferase reporter plasmid transiently transfected into RAW 264.7 cells. LPS stimulated AP-1 transactivation, as indicated by an increase in luciferase activity, that was detected following 1 h of LPS treatment and was maximal by 2.5–3 h of stimulation (Fig. 1; data not shown). PMA also stimulated AP-1 transactivation, although the response lagged behind that with LPS. Thus, LPS induces rapid AP-1 transactivation, which is consistent with this transcription factor complex participating in the induction of LPS-inducible genes.

Activation of JNK Occurs in Response to LPS Stimulation of Macrophages. The ability of LPS to rapidly increase AP-1 transactivation suggested that it might be able to activate preexisting c-Jun in these cells via the JNK pathway. To test whether LPS stimulation of macrophages resulted in activation of JNK, the p46 and p54 isoforms of JNK were immunoprecipitated from protein lysates of unstimulated as well as LPS- and PMA-stimulated RAW 264.7 cells. The activity of the immunoprecipitated JNK was assessed using GST–c-Jun as a substrate. LPS stimulation resulted in increased phosphorylation of GST–c-Jun by JNK, but not of GST, demonstrating the specificity of the assay for c-Jun sequences (Fig. 2). Anti-JNK immunoprecipitates from LPS-stimulated RAW 264.7 cells catalyzed phosphorylation of myelin basic protein but to a much lesser extent than anti-ERK2 immunoprecipitates (data not shown). Anti-ERK2 immunoprecipitates did not phosphorylate the GST–c-Jun substrate (data not shown), and LPS-induced phosphorylation of GST–c-Jun was likely not due to activated p38 MAP kinase as purified p38 does not phosphorylate this substrate (22). Finally, we previously demonstrated that activation of a fusion protein between oncogenic Raf-1 and the ligand-binding domain of the estrogen receptor, ΔRaf-1:ER, expressed in RAW 264.7 cells was sufficient to activate ERK1 and ERK2 (11); however, it did not activate JNK (Fig. 2). The phorbol ester PMA, which also

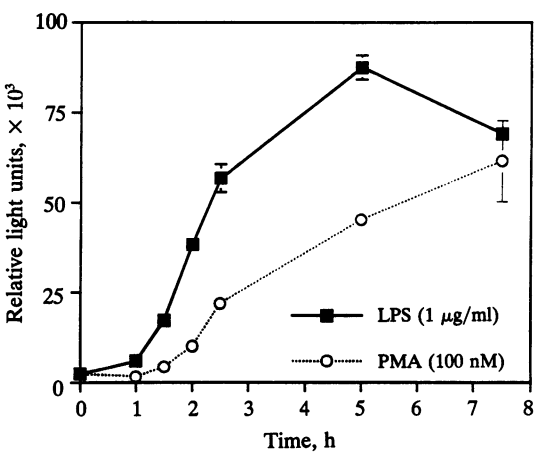

FIG. 1. LPS stimulates AP-1 transactivation in RAW 264.7 macrophages. Cells were transiently transfected with an AP-1-regulated luciferase plasmid, pooled, and then evenly split onto tissue culture dishes. Approximately 40 h after transfection, the cells were treated with *S. minnesota* R595 LPS (1 μg/ml) or 100 nM PMA for the indicated times. Following stimulation, the cells were lysed and the luciferase activity was measured with a luminometer. Each data point is the average of two independent samples; error bars indicate range. Two additional experiments gave similar results.

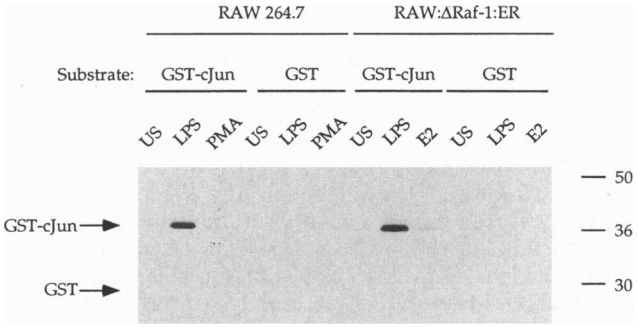

FIG. 2. Enzymatic activation of JNK in response to LPS. Anti-JNK immunoprecipitates from unstimulated (US), R595 LPS (1 μg/ml; 15 min)- or PMA (100 nM; 15 min)-treated RAW 264.7 cells or from unstimulated (US), R595 LPS (1 μg/ml; 15 min)- or estradiol (E2; 1 μM; 20 min)-treated RAW:ΔRaf-1:ER transfectants were incubated with either GST–c-Jun or GST in the presence of [γ-^{33}P]ATP and resolved on SDS/PAGE. Molecular size standards (kDa) are indicated to the right of the gel.

activates ERK1 and ERK2 strongly in RAW 264.7 cells (11, 23), did not activate JNK, despite prolonged exposure up to 6 h (Fig. 2; data not shown). These data demonstrate that activation of Raf-1 or the ERK pathway is not sufficient to activate JNK in macrophages.

Activation of JNK in response to LPS was rapid, occurring by 5 min of stimulation, reached a maximum by 20 min, and declined thereafter (Fig. 3*A*). This induced activity was detectable at 100 pg/ml and was maximal at 1 μg/ml (Fig. 3*B*). LPS-induced JNK activation also occurred in murine bone marrow-derived macrophages and in the human monocyte cell line THP-1 (Fig. 4), suggesting that this is a generalized response in macrophage cells. The kinetics and dose–response that characterized JNK activation in LPS-stimulated THP-1 cells were similar to those found in the RAW 264.7 cells (Fig. 5; data not shown).

Stimulus Specificity of JNK Activation. Wild-type LPS consists of two structural domains: a carbohydrate O-region, which is variable among different bacteria, and a conserved lipid region, lipid A. The predominant biological activity of the LPS molecule is contained in the lipid A domain, although the variable O-antigen structures may be the major target for the host's anti-LPS immune response (3). Therefore, we tested whether different forms of LPS could stimulate JNK activation

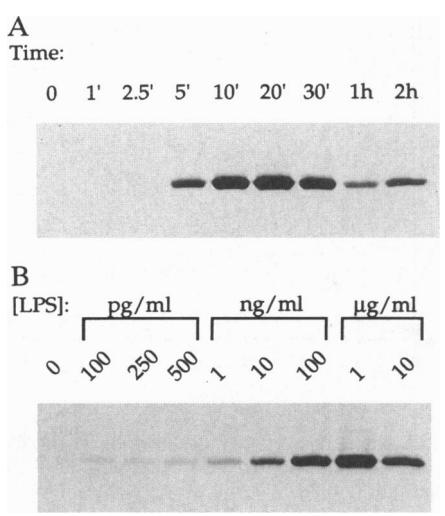

FIG. 3. Kinetics and dose–response of LPS-induced JNK activation in RAW 264.7 cells. Cells were incubated with R595 LPS (1 μg/ml) for the indicated times (*A*) or with the indicated concentrations of R595 LPS for 15 min (*B*). JNK activity was assessed as in Fig. 2.

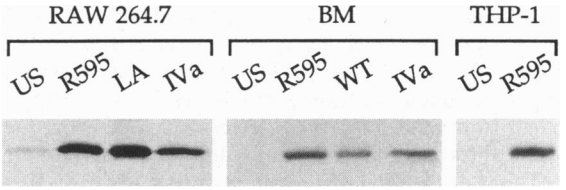

FIG. 4. Activation of JNK in different macrophage populations by different forms of LPS. RAW 264.7 cells, murine bone-marrow-derived macrophages (BM), and vitamin D_3-treated THP-1 cells were incubated (in separate experiments) in the presence of *S. minnesota* R595 LPS, synthetic lipid A (LA), lipid IVa, or *S. minnesota* smooth LPS (WT) for 15 min (all [LPS] 1 μg/ml). JNK activity was determined as in Fig. 2.

in the RAW 264.7 and murine bone marrow-derived macrophages. Stimulation with rough LPS from *Salmonella minnesota* R595, which lacks most of the O-region carbohydrate domain, wild-type smooth LPS from both *S. minnesota* and *E. coli* (K235, 055:B5, and 0111:B4), synthetic lipid A, or lipid IVa, which is a biosynthetic precursor of LPS, induced activation of JNK in the RAW 264.7 cells (Fig. 4; data not shown). Similarly, R595 LPS, wild-type LPS, and lipid IVa induced JNK activation in the murine bone marrow-derived macrophages (Fig. 4). These results indicated that JNK activation by LPS is generalizable to various forms of LPS and that the lipid A moiety is sufficient to induce this signaling event.

LPS Activates Both Isoforms of JNK. Two isoforms of JNK, p46 and p54, have been identified (24). To determine whether the LPS-induced phosphorylation of GST–c-Jun by anti-JNK immunoprecipitates was due to $p46^{JNK}$ or $p54^{JNK}$ or both, the anti-JNK immunoprecipitates were subjected to an in-gel kinase assay, wherein proteins were resolved on an SDS/polyacrylamide gel containing GST–c-Jun and then renatured and allowed to phosphorylate the substrate *in situ* (16). As shown in Fig. 6, LPS induced strong activation of both JNK isoforms with a similar time course to that noted in the immune complex kinase assays. Anti-JNK immunoprecipitates from LPS-stimulated RAW 264.7 cells resolved on an SDS/polyacrylamide gel containing GST alone or anti-ERK2 immunoprecipitates from LPS-stimulated RAW 264.7 cells resolved on a GST–c-Jun-containing SDS/polyacrylamide gel did not phosphorylate the gel substrate (data not shown), indicating that phosphorylation of the substrate required c-Jun sequences and was specific for activated JNK.

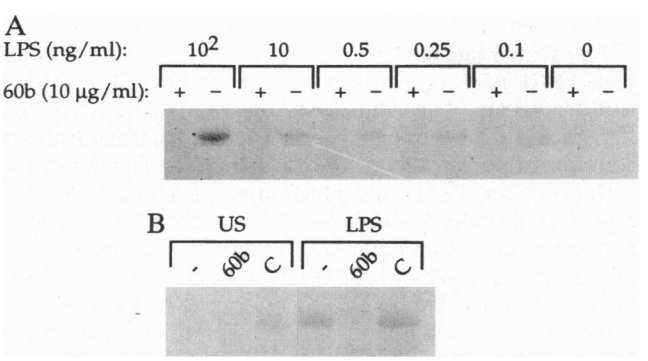

FIG. 5. LPS-induced activation of JNK is inhibited by anti-CD14 antibodies in THP-1 cells. Vitamin D_3-treated THP-1 cells were incubated at 37°C for 15 min in the presence (lanes +) or absence (lanes −) of 60b anti-CD14 mAb (IgG1; 10 μg/ml) and then stimulated with R595 LPS at the indicated concentrations for 15 min (*A*). In a separate experiment, cells were pretreated with 60b (5 μg/ml), an isotype-matched control antibody (C; anti-IgM; 5 μg/ml), or no antibody (lanes −), and then stimulated with R595 LPS (1 μg/ml) for 15 min (*B*). Activation of JNK was determined as in Fig. 2.

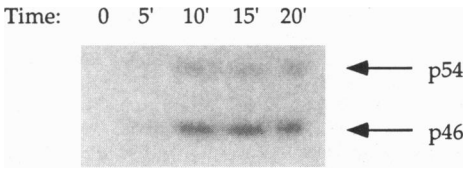

FIG. 6. LPS-induced enzymatic activation of the p46 and p54 isoforms of JNK as determined by the in-gel kinase assay. Anti-JNK immunoprecipitates from unstimulated (time 0) RAW 264.7 cells or from cells treated with R595 LPS (1 μg/ml) for the indicated times were electrophoresed into SDS/polyacrylamide gels containing GST–c-Jun. After denaturation and renaturation of proteins, the kinase activity was determined *in situ* by phosphorylation of the incorporated GST–c-Jun. p46 and p54 indicate the activated isoforms of JNK.

Effects of Herbimycin A on LPS-Induced JNK Activation. Inhibitors of LPS-induced protein tyrosine phosphorylation, such as herbimycin A, block the secretion of cytokines and the generation of eicosanoids (3). To test whether JNK activation is upstream or downstream of tyrosine kinase action following LPS stimulation, RAW 264.7 cells were incubated for 4 h with herbimycin A before being stimulated with LPS or PMA. JNK activation by LPS was completely inhibited by herbimycin A (10 μg/ml) (Fig. 7). As previously reported (23, 25), this dose of herbimycin A did not inhibit the activation of ERK2 by PMA (data not shown), indicating that the inhibition of herbimycin A was specific for protein tyrosine phosphorylation events and not due to general cellular toxicity.

Activation of JNK by LPS Is CD14 Dependent. The glycosylphosphatidylinositol-linked surface protein CD14 is expressed primarily on monocyte/macrophage cells and neutrophils and has been shown to mediate many of the responses of these cells to LPS (3). Moreover, antibodies directed against CD14 inhibit LPS induction of protein tyrosine phosphorylation and secretion of various cytokines (3). We therefore tested whether the ability of LPS to activate JNK in monocyte/macrophage cells was also mediated by CD14. The human monocytic THP-1 cells were pretreated with various concentrations of the anti-CD14 mAb 60b (IgG1), which prevents LPS–LBP complexes from binding to CD14 (26) and inhibits LPS-induced tyrosine phosphorylation in these cells (12). Cells were then stimulated with LPS at various doses, after which JNK activation was assessed by the immune complex kinase assay. Inhibition of JNK activation was complete when the cells were incubated with at least 5 μg of anti-CD14 antibody per ml prior to LPS stimulation (Fig. 5; data not shown). Moreover, pretreatment of the THP-1 cells with 60b mAb at 5 and 10 μg/ml inhibited JNK activation at all doses of LPS tested (Fig. 5; data not shown). Pretreatment of these cells with other anti-CD14 mAbs blocked ERK2 phosphorylation only at low concentrations of LPS treatment (<10 ng/ml), whereas the 60b antibody blocked at low as well as high concentrations of LPS treatment (ref. 12; data not shown). These observations likely reflect different properties of these anti-CD14 blocking antibodies.

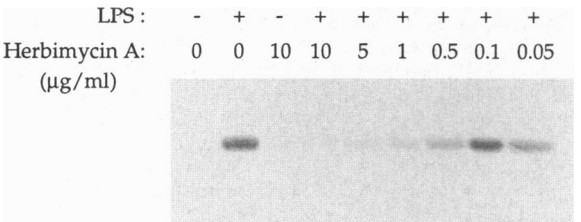

FIG. 7. Herbimycin A inhibition of LPS-induced JNK activation. RAW 264.7 cells were pretreated for 4 h with the indicated concentrations of herbimycin A and then stimulated with R595 LPS (1 μg/ml) for 15 min. Activation of JNK was determined as in Fig. 2. Lanes − and +, absence and presence of LPS.

Pretreatment of the THP-1 cells with 10 μg of 60b per ml did not block the activation of ERK2 by PMA (data not shown), and pretreatment with an isotype-matched control antibody did not inhibit the LPS-induced activation of JNK (Fig. 5*B*), arguing that this event is mediated by CD14.

DISCUSSION

LPS stimulation of macrophages results in a rapid increase in tyrosine phosphorylation and activation of p42/p44 ERK and p38/HOG1 MAP kinase pathways (3). Recently, a third mammalian MAP kinase-like pathway has been identified involving JNK, also known as the stress-activated protein kinase (SAPK). Activation of this pathway results in phosphorylation of the N-terminus of c-Jun and subsequent increase in its ability to activate transcription (5). LPS stimulation results in activation of the tissue factor and IL-6 genes in an AP-1-dependent manner (6, 7) and, as shown in this report, LPS rapidly stimulates AP-1 transactivation. Therefore, we wished to examine whether JNK was also activated in response to LPS in macrophages. We have found that LPS stimulates activation of JNK in RAW 264.7 macrophage cells in a rapid and dramatic fashion. The activation of JNK was apparent after 5 min of LPS stimulation, and was maximal by 20 min. Moreover, JNK activation appeared to be a general feature of the LPS response of macrophages as LPS also stimulated JNK activation in murine bone marrow-derived macrophages and in human THP-1 monocytes. Recently, LPS has also been reported to activate JNK in Chinese hamster ovary cells transfected with human CD14 (22), indicating that this signaling event may occur in other LPS-responsive cell types as well.

Treatment with wild-type LPS, a rough mutant form of LPS, and the biologically active moiety of LPS, lipid A activated this kinase in the macrophage cells tested. This response was CD14 dependent, as pretreatment of THP-1 cells with a mAb against CD14 abrogated the response. Additionally, activation of JNK was inhibited by the tyrosine kinase inhibitor herbimycin A, indicating that this event, as with many early LPS-induced events, is dependent on intact tyrosine phosphorylation. We cannot rule out that herbimycin A directly inhibits JNK, but this seems unlikely as JNK is a serine/threonine kinase and herbimycin A does not directly inhibit p42/p44 ERK activation. We presume that the tyrosine phosphorylation requirement reflects an important role for one or more tyrosine kinases in LPS-induced activation of the related MAP kinase pathways.

One interesting feature of these studies was that JNK was not activated in response to stimulation of macrophages by the phorbol ester PMA, in contrast to what has been seen in a number of other cell types (15, 16). JNK activity was also not increased upon activation of ΔRaf-1:ER in RAW 264.7 transfectants expressing this chimeric protein. PMA treatment and ΔRaf-1:ER activation do, however, result in rapid and near-complete activation of ERK1 and ERK2 (11, 23). Therefore, activation of Raf-1 or the ERK pathway are not sufficient to activate JNK in macrophages. In contrast, PMA treatment did result in increased AP-1-mediated transactivation, although it was slower than that seen upon LPS treatment. Thus, it may be that AP-1 activity could be increased in two ways, one of which was independent of JNK activation. As PMA stimulation resulted in accumulation of c-*fos* mRNA in the RAW 264.7 cells (data not shown), perhaps activation of the ERK pathway increased AP-1 activity by inducing expression of c-*fos*, whose protein product could then bind to preexisting c-Jun (5). This hypothesis is consistent with the slower activation of AP-1 seen by PMA treatment compared to that induced by LPS. LPS induces expression of c-*fos* (ref. 27; data not shown) but in addition activates JNK, and this could lead to the more rapid AP-1 activity.

Activation of the three distinct MAP kinase subfamilies (ERK, JNK, and p38) involves dual phosphorylation of tyrosine and threonine residues separated by a single residue in the catalytic domain of these enzymes (4). In each of these three known subfamilies, there is a distinct amino acid between the phosphorylated threonine and tyrosine residues, and this likely provides target specificity for distinct activators of each subfamily (4). Reports to date indicate that Raf-1 and MEK1 activate the ERK cascade, MEKK-1 and MKK4 activates the JNK/SAPK cascade, and MKK3 activates p38 (5, 28). Thus, in principle, different stimuli can activate one or more of the known MAP kinase family pathways. Numerous mitogens and differentiative stimuli activate the ERK cascade (29), whereas it is primarily proinflammatory cytokines and environmental stress that result in p38 activation (22). The JNK pathway is also activated by cytokines and cellular stress (5), as well as by costimulation of T lymphocytes (30), and activation of small GTPases (31). Interestingly, LPS strongly stimulates all three of these MAP kinase-like pathways in macrophages and is the only cellular stimulant characterized to date that does this. The mechanisms by which LPS activates these pathways is not yet established.

Upon activation, members of the ERK and JNK subfamilies have been shown to translocate to the nucleus (5), whereby phosphorylation of transcription factors may occur. Activated ERK phosphorylates the ternary complex factor (TCF), Elk-1/p62^{TCF}, which mediates c-*fos* induction via modulation of the serum response element (5). Activated ERK also phosphorylates NF-IL6, which mediates induction of various genes, such as IL-6 and IL-8 (32–35). Activation of JNK, on the other hand, results in phosphorylation of the transcription factors c-Jun and activating transcription factor 2 (ATF2) (36), which, in turn, mediate regulation of the AP-1 complex and c-*jun* promoter (5). While it appears that these pathways are distinct signaling cascades, they may also converge on particular targets, such as the transcription factors Elk-1 (37) and c-Jun (38). Thus, simultaneous activation of the three MAP kinase pathways in LPS-stimulated macrophages is an important mechanism by which LPS-induced signaling events culminate in transcription of a number of immunoregulatory genes that then coordinate the host's immune response.

Note Added in Proof. A number of recent reports indicate that TNF-α and IL-1 probably activate all three of the MAP kinase pathways, as does LPS stimulation of monocyte/macrophage cells.

We thank Robert Finney and Byron Gallis for their technical assistance and S. Harmer, P. Mittlestadt, and J. Richards for their critical review of the manuscript. This work was supported by grants from the National Institutes of Health (K11 AI-01164 and RO1 AI-33443). S.W. was supported by the Freddy Cunha IV Fellowship, Cancer Research Institute.

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