Journal of Toxicology and Environmental Health Part A

ISSN: 1528-7394 (Print) 1087-2620 (Online) Journal homepage: https://www.tandfonline.com/loi/uteh20

ROLE OF MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IN THE PRODUCTION OF INFLAMMATORY MEDIATORS: DIFFERENCES BETWEEN PRIMARY RAT ALVEOLAR MACROPHAGES AND MACROPHAGE CELL LINES

K. Murali Krishna Rao , Terence Meighan & Linda Bowman

To cite this article: K. Murali Krishna Rao , Terence Meighan & Linda Bowman (2002) ROLE OF MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IN THE PRODUCTION OF INFLAMMATORY MEDIATORS: DIFFERENCES BETWEEN PRIMARY RAT ALVEOLAR MACROPHAGES AND MACROPHAGE CELL LINES, Journal of Toxicology and Environmental Health Part A, 65:10, 757-768, DOI: 10.1080/00984100290071027

To link to this article: https://doi.org/10.1080/00984100290071027

Published online: 07 Jan 2011.

Journal of Toxicology and Environmental Health, Part A, 65:757–768, 2002

1528-7394/02 $12.00 + .00
DOI: 10.1080/0098410029007102 7

Taylor&Francis
healthsciences

ROLE OF MITOGEN-ACTIVATED PROTEIN KINASE ACTIVATION IN THE PRODUCTION OF INFLAMMATORY MEDIATORS: DIFFERENCES BETWEEN PRIMARY RAT ALVEOLAR MACROPHAGES AND MACROPHAGE CELL LINES

K. Murali Krishna Rao, Terence Meighan, Linda Bowman

Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia, USA

Stimulation of macrophages has been shown to activate all three families of mitogen activated protein kinases (MAPKs). However, variable results are reported in the literature with respect to the particular kinases activated with any given stimulus. In this study, the role of activation of MAPKs was examined in the production of inflammatory mediators by measuring the phosphorylation of the kinases and their ability to phosphorylate specific substrates in rat primary alveolar macrophages, a rat alveolar macrophage cell line (NR8383), and two mouse monocytic cell lines (RAW 264.7 and J774A.1). In the three cell lines examined, all three families of MAPKs were activated upon stimulation with either lipopolysaccharide (LPS) or LPS plus interferon-γ; in contrast, only ERK1/2 was activated in primary rat alveolar macrophages upon stimulation with LPS. Inhibition of ERK1/2 activation by the MEK inhibitor PD98059 abrogated nitric oxide and tumor necrosis factor-α (TNF-α) production in primary rat alveolar macrophages, but the p38 inhibitor SB203580 had no effect on the production of these two inflammatory mediators. These observations indicate that MAPK activation is cell specific and explain some of the conflicting results reported in the literature. These studies emphasize the need to exercise caution in extrapolating data from cell lines to primary cells.

Treatment of macrophages with bacterial lipopolysaccharide activates macrophages, leading to production of several inflammatory mediators. The signal transduction events involved in LPS stimulation are not yet defined completely. However, several studies have implicated mitogen-activated protein kinases (MAPKs) in stimulus-induced activation of macrophages. MAPKs are a family of serine/threonine protein kinases that participate in signal transduction events associated with a number of stimuli (Cobb, 1999; Force & Bonventre, 1998; Davis, 1994). The three members of this family are extracellular signal regulated kinases 1 and 2 (ERK1/2), p38 MAP kinase, and c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK). All three MAPKs undergo phosphorylation as a result of the action of upstream phosphokinases. Phosphorylation, therefore, is one measure of activation of MAPKs. MAPKs in turn phosphorylate specific substrates that are involved in further signal propagation. Transcription factors are the

Received 4 June 2001; sent for revision 4 July 2001; accepted 8 August 2001.
Address correspondence to K. Murali Krishna Rao, MD, PPRB/HELD/NIOSH, M/S 2015, 1095 Willowdale Road, Morgantown, WV 26505, USA. E-mail: mir8@cdc.gov

major targets for MAPKs. The transcription factors phosphorylated include ELK1 (Marais et al., 1993; Janknecht et al., 1993), ATF-2 (Livingstone et al., 1995), and c-Jun (Derijard et al., 1994). These, in turn, induce transcription of several early response genes involved in cell activation, proliferation, and transformation. The p38 MAPK has been implicated in interleukin-1β (IL-1β) transcription in lipopolysaccharide (LPS)-stimulated RAW264.7 and J774 cell lines (Baldassare et al., 1999), and in cytokine production in the human monocytic cell line THP-1 (Lee & Young, 1996). Similarly, p38 MAPK seems to be required for inducible nitric oxide synthase (iNOS) induction in LPS-stimulated RAW 264.7 macrophages (Chen & Wang, 1999). Other studies indicate that LPS stimulation of RAW 264.7 cells activates all three families of MAPKs (Sanghera et al., 1996).

Most of the studies implicating MAPKs in macrophage activation have been conducted in cell lines. However, considerable differences in responses between different cell lines have been described. For example, LPS treatment induces activation of cytosolic phospholipase-2 (PLA2) in RAW 264.7 cells (a mouse monocytic cell line), but not in P338D1 cells (a mouse macrophage cell line) (Barbour et al., 1998). In particular, with respect to MAPKs, LPS stimulation causes activation of ERK in RAW 264.7 cells but not in P388D1 cells (Barbour et al., 1998). Similar differences are found between different cell types in other kinase cascade systems, such as phosphatidylinositol-3 kinase (P13K) and p^{70S6} kinase. Inhibition of DNA synthesis by rapamycin in bone-marrow-derived macrophages (BMM) is much less than that observed in a CSF-1-dependent cell line, BAC1.2F5 (a mouse macrophage cell line) (Hamilton et al., 1998). The authors of these studies have cautioned, rightly, that data from cell lines should not be extrapolated to primary cells, much less to in vivo situations. In view of the reports of activation of MAPKs in LPS-stimulated macrophage cell lines, it was decided to investigate if similar activation of MAPKs occurs in primary rat alveolar macrophages. The results of our studies comparing MAPK activation in rat alveolar macrophages and three macrophage cell lines—two of mouse origin, RAW 264.7 and J774, and one of rat origin, NR8383—are reported here.

MATERIALS AND METHODS

Animals and Reagents

Male Sprague-Dawley rats, Hla:(SD)CVF, from Hilltop Lab Animals (Scottdale, PA), monitored free of endogenous viral pathogens, parasites, *Helicobacter*, and CAR *Bacillus*, weighing 250–300 g and approximately 7–8 wk old, were used for all experiments. The rats were quarantined for at least 7 d after arrival and were kept in filtered ventilated cages in specific pathogen-free and environmentally controlled conditions in AAALAC-accredited facilities, on Alpha-Dri virgin cellulose chips and hardwood Beta

chips as bedding; they were provided HEPA-filtered air, autoclaved Prolab 3500 diet, and tap water ad libitum.

Lipopolysaccharide B (from *Escherichia coli* 026:136) was obtained from Difco Laboratories (Detroit, MI). The culture medium consisted of Dulbecco's modified Eagle's medium (DMEM: Bio-Whittaker, Walkersville, MD), 1 m*M* glutamine (GIBCO, Life Technologies, Grand Island, NY), supplemented with 2 m*M* L-glutamine (Sigma, St. Louis, MO), 100 U/ml penicillin-streptomycin (GIBCO), 100 μg/ml streptomycin (GIBCO), Fungizone (GIBCO), and 10% (v/v) fetal bovine serum (Bio-Whittaker). Kits for detection of activated phoshpo-MAPKs, assay kits for measuring the activities of the MAPKs, and the MEK1 inhibitor (PD98059) were obtained from New England Biolabs, Inc. (Beverly, MA). The p38 MAPK inhibitor, SB 203580, was from Upstate Biotechnology (Lake Placid, NY). The inhibitors were dissolved in dimethyl sulfoxide (DMSO) to 20 m*M* concentration, and final dilutions were made up in the culture medium.

Cell Lines

NR8383 (CRL-2192), a cell line derived from rat alveolar macrophages RAW 264.7, and J774A.1 (ATCC, TIB-67, and TIB-71, respectively), and cell lines derived from mouse macrophages were obtained from American Type Culture Collection and were grown according to their instructions. RAW 264.7 and J774A.1 were cultured in the DMEM medium already described. NR8383 cells were cultured in F-12 medium (Ham's) supplemented with 2 m*M* L-glutamine and 15% fetal bovine serum with the antibacterial and antifungal agents mentioned earlier.

Isolation of Alveolar Macrophages

The animals were anesthetized with pentobarbital sodium (150 mg/kg body weight) and exsanguinated by cutting the abdominal aorta. Alveolar macrophages were obtained by bronchoalveolar lavage according to the method of Myrvik et al. (1961). The lungs from each animal were lavaged 8 times with 5 ml phosphate-buffered medium (145 m*M* NaCl, 5 m*M* KCl, 9.4 m*M* Na_2HPO_4, and 1.9 m*M* NaH_2PO_4, pH 7.4). The cells were separated from the lavage fluid by centrifugation at 300 × g for 5 min and then washed 3 times by alternate centrifugation and resuspension in phosphate-buffered medium. The cells were then resuspended in the culture medium for use in all experiments. Cell number was determined by an electronic cell counter (model ZB, Coulter Electronics, Hialeah, FL).

Cell Stimulation for Measuring MAPK Phosphorylation and Activity

Cell lines were grown in 25-cm^2 flasks to confluence. On the day of the experiment, the medium was replaced with fresh medium and stimulated with LPS (1 μg/ml) for the time periods indicated at 37°C in a CO_2 incubator. The flasks were taken out and placed on ice and the cells were scraped with a cell scraper. The cells were spun in the cold and washed

twice with cold Hanks balanced salt solution (HBSS). Finally, the cells were lysed in the lysis buffer (pH 7.4) provided with the MAPK kits, containing a cocktail of protease inhibitors. The lysate was then subjected to sonication to ensure complete cell disruption. Following sonication, the lysate was spun at high speed (21,000 × g) to precipitate insoluble material and the supernatant was used for MAPK assays. The protein concentration of the lysates was determined using a Bio-Rad (Richmond, CA) protein assay. In the case of freshly isolated rat alveolar macrophages, the cells (2 million cells in 1 ml) were plated in 6-well culture plates and incubated for 2 h at 37°C in a CO_2 incubator. Nonadherent cells were washed off with the culture medium and 1 ml fresh culture medium was added. Next, the cells were stimulated with LPS (1 μg/ml) for 30 min or the times indicated, and the protein was isolated as described for the cell lines.

Determination of the Phosphorylation of MAPKs

Phosphorylation of the MAPKs was determined by Western blot analysis. Equal amounts (60 μg for p38 and ERK; 80 μg for JNK/SAPK) of proteins from cell lysates from cells treated with various agents were separated on 12% polyacrylamide gels and transferred to nitrocellulose membranes with an electrophoretic transfer unit (Hoefer Scientific Instruments, San Francisco, CA). The composition of the transfer buffer was 25 m*M* Tris base, 0.2 *M* glycine, 20% methanol (pH 8.5). After transfer, the nitrocellulose membrane was washed with 25 ml TBS (50 m*M* Tris-HCl, 150 m*M* NaCl, pH 7.4) for 5 min at room temperature. The membrane was then incubated in 25 ml blocking buffer (1 × TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk) for 1 h at room temperature. The blocking buffer was poured off and the membrane was incubated with the primary antibody in 10 ml primary antibody dilution buffer (antibody dilution 1:1000, dilution buffer 1 × TBS, 0.1% Tween-20 with 5% bovine serum albumin, BSA) overnight at 4°C with gentle agitation. The membrane was washed 3 times for 5 min each at room temperature with 25 ml TBST (TBS with 0.1% Tween-20). Next the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (dilution 1:2000) in 10 ml blocking buffer with gentle agitation for 1 h at room temperature. The membrane was washed 3 times for 5 min each at room temperature with 25 ml TBST. The protein bands were detected by incubating the membrane in 10 ml LumiGLO (0.5 ml of 20× LumiGLO, 0.5 ml of 20× peroxide, and 9 ml Milli-Q water) with gentle agitation for 1 min at room temperature. The membrane was then drained of excess developing solution and wrapped in Saran Wrap to prevent drying and exposed to x-ray film.

After determining the phosphorylation of MAPKs, the same membranes were stripped by incubating for 20 min at 50°C with a buffer containing 62.5 m*M* Tris-HCl (pH 6.7), 2% sodium dodecyl sulfate (SDS), and 200 μ*M* mercaptoethanol. The membranes were washed twice with Tris-HCl buffer and then incubated in blocking buffer for 1 h. Then it was probed

using antibodies for specific MAPK proteins. Usually, a separate membrane was used for each MAPK.

Assays for Activities of MAPKs

Cell treatment was similar to that described earlier for determination of phosphorylation of MAPKs. The kits used for measuring MAPK activities use phosphorylation of specific substrates, namely, ATF-2, ELK-1, and c-Jun fusion protein, for detecting the activities of p38, ERK1/2, and SAPK/JNK, respectively. The assays were performed according to the manufacturer's instructions. The procedure involves immunoprecipitating the specific MAPKs and performing the phosphorylation studies on the specific substrates.

Measurement of Nitric Oxide (NO) Production

The amount of NO in the supernatants was measured as the stable oxidation products of NO, nitrate and nitrite. All samples were first incubated with *E. coli* reductase to convert the nitrate to nitrite. NO production was then measured by using the Greiss reaction (Green et al., 1982). The amount was calculated from a standard curve using sodium nitrite.

Measurement of Tumor Necrosis Factor-α

TNF-α was measured in culture supernatants by an enzyme-linked immunosorbent assay (ELISA) kit obtained from Biosource International (Camarillo, CA), according to the manufacturer's instructions.

Statistical Analysis

The data were analyzed by paired *t*-test, and significance was set at $p < .05$.

RESULTS

LPS Induces Only ERK1/2 Phosphorylation in Rat Alveolar Macrophages

Figure 1 shows the phosphorylation of the three families of MAPKs in primary rat alveolar macrophages and a rat alveolar macrophage cell line, NR8383. The results indicate that in primary alveolar macrophages significant phosphorylation of only ERK1/2 occurs ($n = 5$), whereas in the NR8383 cell line all three families of MAPKs undergo phosphorylation upon LPS stimulation. Since a combination of LPS and interferon (IFN)-γ is used to stimulate cell lines in several studies, this combination was used to stimulate NR8383 cells. The results were similar to those seen with LPS alone. MAPK phosphorylation studies in J774.A1 and RAW264.7 cells were similar to those seen with NR8383 cells with LPS-induced phosphorylation of all three MAPKs (data not shown). A time-course study for p38 phosphorylation revealed that there was no marked increase in p38 phosphorylation over a

period of 90 min (Figure 2). Similarly, there was no increase in phosphorylation of JNK/SAPK over a period of 2 h (data not shown).

LPS Increases Only ERK1/2 Activity in Alveolar Macrophages

Figure 3 shows the effect of stimulation of alveolar macrophages and NR8383 cells on MAPK activities as measured by their ability to phosphorylate specific kinase substrates. The results were consistent with MAPK phosphorylation studies. In alveolar macrophages, only an increase in ERK1/2 activity was seen. In contrast, in the-NR8383 cell line, there was very little basal activity in unstimulated cells; LPS or LPS plus IFN-γ treatments markedly activated all three MAPKS.

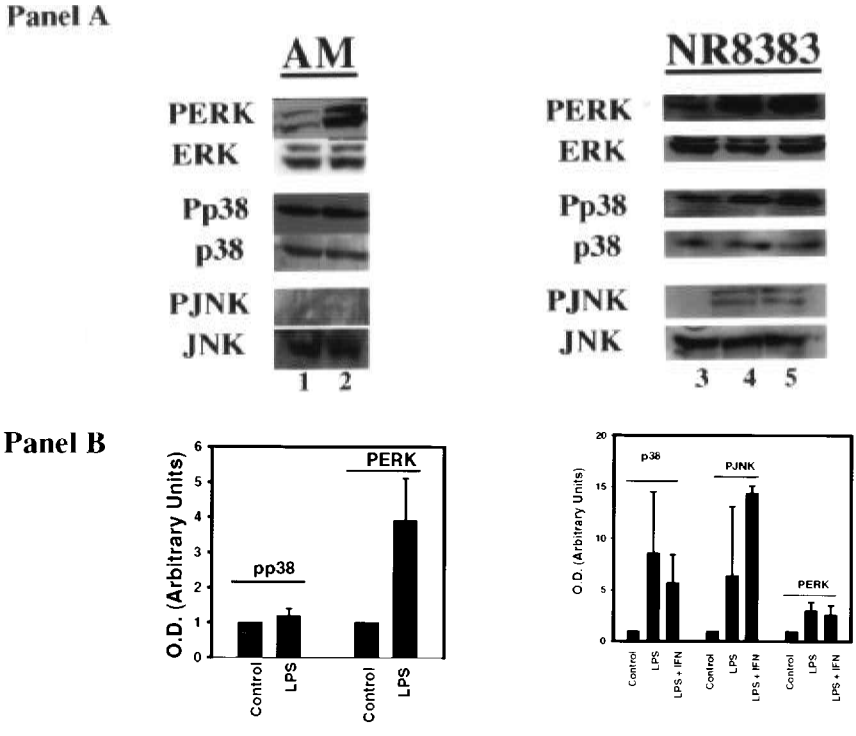

FIGURE 1. Phosphorylation of MAP kinases in rat primary alveolar macrophages and NR8383 cell line. Two million rat primary alveolar macrophages in 1 ml culture medium were incubated in 6-well culture plates for 2 h at 37°C. Nonadherent cells were washed off, and the adherent cells were stimulated with LPS (1 μg/ml) for 15 min in the case of p38 and JNK/SAPK and 30 min for ERK. In the case of NR8383 cells, cells were grown in 25-cm^2 flasks to near confluence. On the day of the experiment, the medium was replaced with fresh medium and stimulated with LPS (1 μg/ml) or LPS + IFN-γ (25 U/ml) for 30 min. Cell lysates were prepared and Western blot analysis was performed as described in the methods. PERK, Pp38, and PJNK are the phosphorylated forms of the respective MAPKs. Lane 1, control cells; lane 2, LPS-stimulated cells; lane 3, control cells; lane 4, LPS-stimulated cells; lane 5, cells stimulated with LPS + IFN-γ. Results shown are representative of a minimum of three experiments. (A) Representative Western blots. (B) Densitometric measurements of phosphoproteins normalized to the respective total MAPK proteins from at least three separate experiments. The bars represent the ratios; the ratio of the control cells was set to 1.

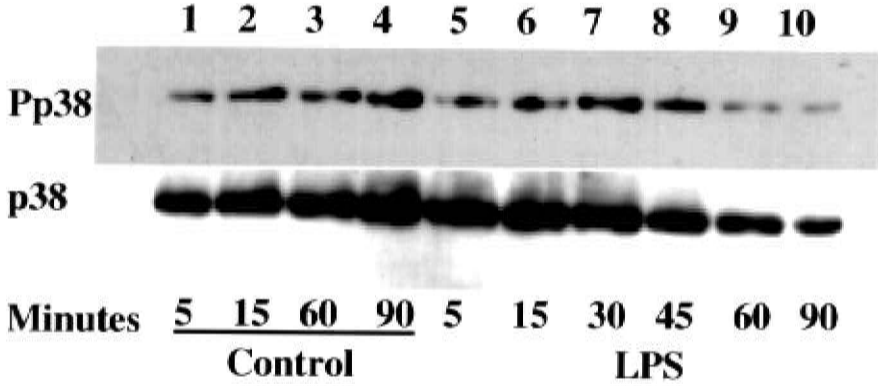

FIGURE 2. Time course of p38 MAP kinase phosphorylation in primary rat alveolar macrophages. Two million cells were cultured and assayed as described in Figure 1. Pp38 designates the phosphorylated form of p38. Data from one of two experiments are shown.

MAPKs Are Activated in All Cell Lines Studied

Initially, the RAW 264.7 cells were used to compare the activation of MAPKs with respect to alveolar macrophages. Because RAW 264.7 cells are derived from the mouse, and alveolar macrophages were from rats, it was important to exclude any possible species differences. Figure 4 shows MAPK activities in two mouse cell lines, RAW 264.7 and J774.1A. All three MAPKs show increased activity upon stimulation with either LPS or LPS + IFN-γ. Thus, activation of MAPKs appears to be a feature of all cell lines investigated in this study.

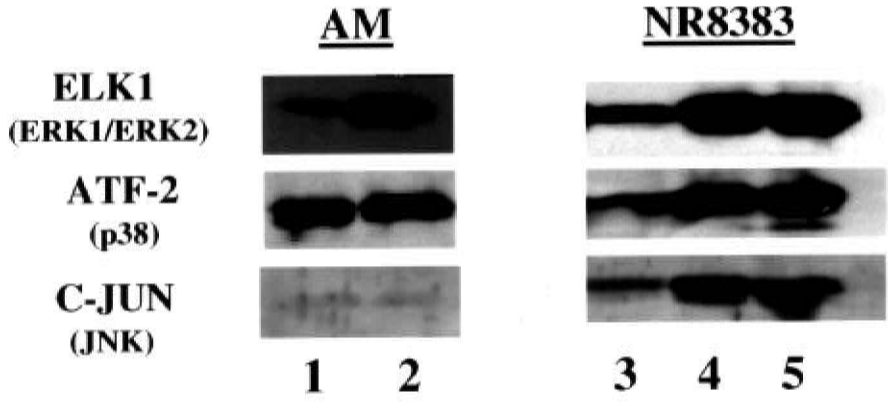

FIGURE 3. Activities of MAP kinases in alveolar macrophages and NR8383 cells stimulated with LPS. The cells were stimulated with LPS (1 μg/ml) or LPS plus IFN-γ (25 U/ml) for 15 min, except in the case of alveolar macrophages for ERK (30 min). Cell lysates were prepared and activities assayed according to the protocol of the manufacturer of the assay kits. The figure shows the phosphorylation of specific transcription factors associated with MAP kinases. Lane 1, control cells; lane 2, LPS-stimulated cells; lane 3, control cells; lane 4, LPS-stimulated cells; lane 5, cells stimulated with LPS + IFN-γ. Results shown are representative of a minimum of three experiments.

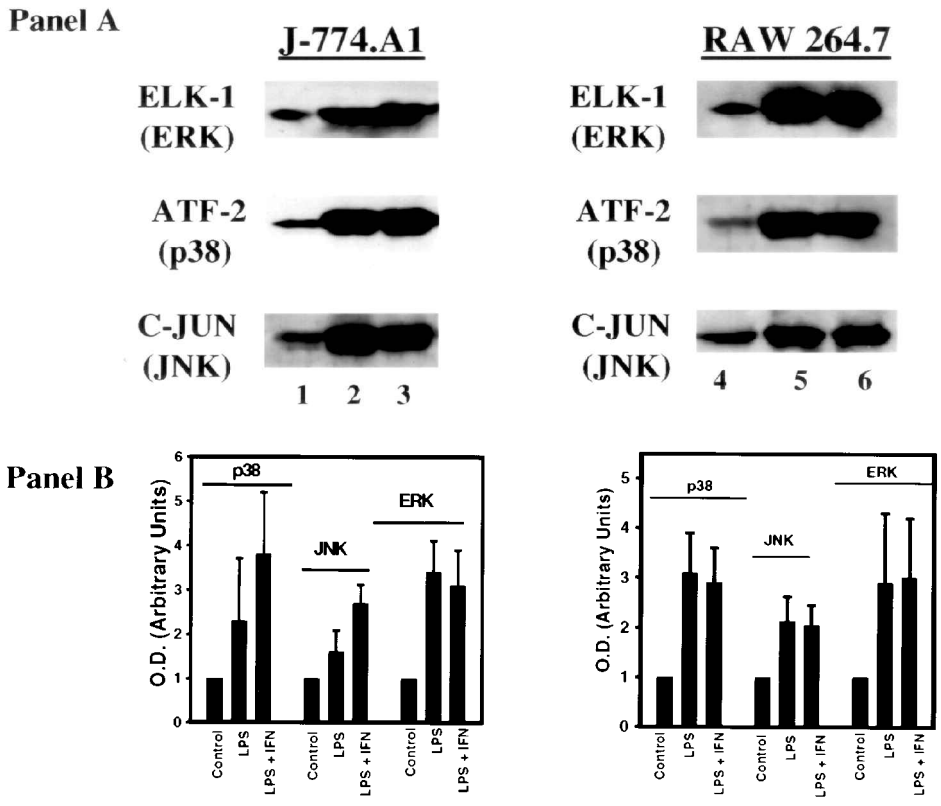

FIGURE 4. Activities of MAP kinases in RAW 264.7 and J774.A1 cell lines following stimulation with LPS (1 μg/ml) or LPS + IFN-γ (25 U/ml) for 30 min. The experimental conditions were as described in Figure 3. Lanes 1 and 4, control cells; lanes 2 and 5, LPS-stimulated cells; lanes 3 and 6, cells stimulated with LPS + IFN-γ. (A) Representative Western blots. (B) Densitometric measurements from at least three separate experiments. The optical density (OD) for the control cells was set to 1.

MEK1 Inhibitor PD98059 Decreases ERK1/2 Activation and the Production of Inflammatory Mediators in Alveolar Macrophages

Alveolar macrophages were treated with 50 μ*M* PD98059, 1 h prior to the addition of LPS, and were incubated for an additional 60 min for the determination of MAP kinase activation. As expected, there was marked decrease in ERK activity (Figure 5). For determination of NO and TNF production, the incubation was carried out for 18 h. PD98059 significantly decreased both NO and TNF production in alveolar macrophages stimulated with LPS (Figure 6). In contrast, the p38 MAPK inhibitor SB 20358.0 (20 μ*M*) had no effect on either NO or TNF production under identical experimental conditions.

DISCUSSION

MAPKs are serine/threonine kinases that are activated in response to a wide array of stimulants (Cobb, 1999; Force & Bonventre, 1998; Davis,

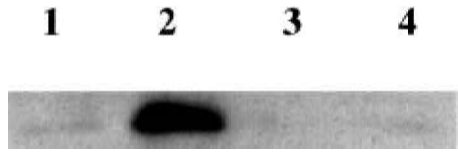

FIGURE 5. Effect of MEK1 inhibitor PD98059 on ERK1/2 activity in rat primary alveolar macrophages. Two million cells in 1 ml culture medium were incubated in 6-well culture plates for 2 h at 37°C. Nonadherent cells were washed off and 1 ml fresh medium was added to the adherent cells along with 50 μ*M* or 100 μ*M* PD98059 for 1 h. Then LPS (1 μg/ml) was added for an additional hour. After treatment the cell lysates were prepared and phosphorylation detection and assays were performed as described in the methods. Lane 1, control cells; lane 2, LPS-stimulated cells; lane 3, PD98059 (50 μ*M*) + LPS; lane 4, PD98059 (100 μ*M*) + LPS.

1994). MAPKs phosphorylate a variety of substrates in vitro and have been implicated in the regulation of several fundamental cellular processes (Kyriakis & Avruch, 1996). Stimulation of macrophage cell lines with LPS has been shown to activate all three families of MAPKs, namely, p38, ERK1/2, and SAPK/JNK, within minutes (Sanghera et al., 1996; Swantek et al., 1997). Activation of p38, in particular, has been implicated in the in-

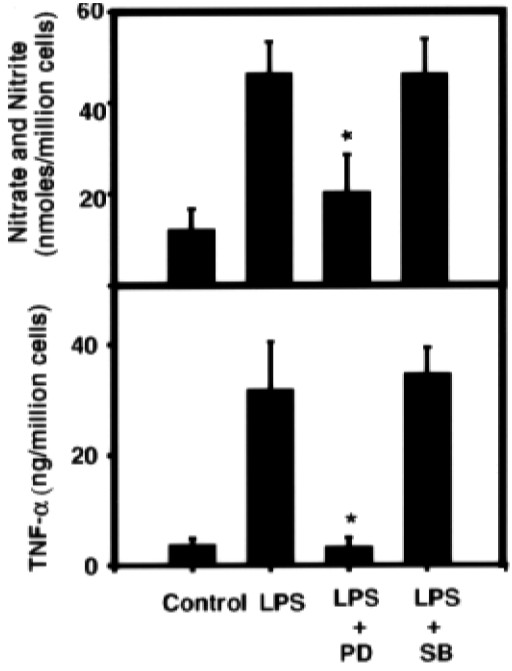

FIGURE 6. Effect of the MEK1 inhibitor PD98059 and the p38 MAPK inhibitor SB 203580 on the production of nitric oxide and tumor necrosis factor-α (TNF-α) by rat primary alveolar macrophages. Two million cells in 1 ml culture medium were incubated in 6-well culture plates for 2 h at 37°C. Nonadherent cells were washed off and 1 ml fresh medium was added to the adherent cells along with 50 μ*M* PD98059 or 20 μ*M* SB 203580 for 1 h. Then LPS (1 μg/ml) was added and incubation continued for an additional 18 h. Cell-free supernatants were assayed for nitric oxide and TNF-α. Asterisk indicates significantly decreased from LPS treatment alone ($p < .05$, $n = 3$).

duction of nitric oxide synthase (Chen & Wang, 1999) and IL-1β (Baldassare et al., 1999) in RAW 264.7 cells, following LPS stimulation. In contrast, using human monocytes, it was demonstrated that all three MAPKs are activated following LPS stimulation, but activation of MEK/ERK was found to be critical for cytokine and prostaglandin E2 production (Scherle et al., 1998).

It should be noted that in some studies activation of MAPKs was conducted under low serum conditions (Swantek et al., 1997). Although the background MAPK activation is low under those conditions, it was felt essential to study MAPK activation under conditions similar to those used for the production of inflammatory mediators, namely, under normal serum concentrations. Under these conditions, whatever the basal activation of MAPKs might be, it does not seem to have much influence on the inducibility of inflammatory mediator production, since LPS stimulation produced a severalfold increase in inflammatory mediators over the basal activity.

Our studies indicate, unlike what is reported in several macrophage cell lines (Chen & Wang, 1999; Baldassare et al., 1999), that LPS stimulation of rat primary alveolar macrophages causes activation of only ERK1/ERK2. Furthermore, inhibition of ERK1/2 activity with PD98059 markedly reduced both LPS-induced nitric oxide and TNF-α production. In contrast, SB 203580 treatment had no effect on LPS-induced production of these two inflammatory mediators. This is different from what is shown in human alveolar macrophages, where LPS stimulation has been shown to cause both ERK and p38 activation (Carter et al., 1999), JNK/SAPK activation was not studied in human alveolar macrophages specifically, but simultaneous inhibition of both ERK and p38 kinase pathways reduced cytokine expression to near control levels, suggesting that the JNK/SAPK pathway by itself is not sufficient for cytokine expression (Carter et al., 1999). In our studies, no activation of JNK/SAPK was found in rat alveolar macrophages stimulated with LPS. So with respect to JNK/SAPK activation, the studies in rat and human alveolar macrophages seem to agree. There is agreement on ERK activation also, but no activation of p38 with LPS was found in rat alveolar macrophages. This observation is consistent with the finding that in mouse alveolar macrophages, inhibition of ERK with PD98059 abrogates TNF-α transcription (Means et al., 2000).

It is known that signaling pathways differ in macrophages based on their maturation state (Lucas et al., 1998). The expression of different isoforms of p38 varies according to cell type and their differentiation stage (Hale et al., 1999). Therefore, it is not surprising that there are differences in signal transduction events in macrophage cell lines and primary alveolar macrophages. The original study implicating MAPKs in the regulation of cytokine biosynthesis was conducted in a human monocytic cell line, THP.1 (Lee et al., 1994). Other studies used various murine cell lines to demonstrate a role for MAPKs in cytokine and iNOS regulation (Baldassare et al., 1999; Chen & Wang, 1999). In this study, we show that LPS does activate all three MAP kinases in two mouse cell lines and one rat cell

line. So it appears that cell lines are particularly sensitive with regard to MAPK activation following LPS stimulation.

The differences detected in this study in MAPK activation in LPS-stimulated cell lines and primary cells do raise some concerns regarding the extrapolation of data obtained from cell lines to primary cells. Further, differences in signal transduction mechanisms in different cell types may account for varying observations with regard to regulation of certain inflammatory modulators. In RAW 264.7 cells stimulated with LPS, activation of p38 was found to be important in regulating iNOS expression (Chen & Wang, 1999). In another study using RAW 264.7 cells stimulated with a combination of LPS and IFN-γ, both ERK1/2 and p38 were implicated in iNOS expression (Ajizian et al., 1999). Similarly, in rat microglial cells and astrocytes treated with LPS or the combination of LPS/IFN-γ, ERK1/2 and p38 were shown to cooperate in iNOS induction (Bhat et al., 1998). Yet, in another study of BMM stimulated with TNF-α, it was reported that JNK/SAPK was involved in regulating NO production (Chan et al., 1999). Perhaps differences in signal transduction events leading to selective MAPK activation or activation of other mediators not yet discovered may account for these diverse observations. The findings reported in this study emphasize the need to exercise caution in extrapolating data from one cell type to the other with regard to signal transduction mechanisms involved in cytokine regulation.

REFERENCES

Ajizian, S. J., English, B. K., and Meals, E. A. 1999. Specific inhibitors of p38 and extracellular signal–regulated kinase mitogen activated protein kinase pathways block inducible nitric oxide synthase and tumor necrosis factor accumulation in murine macrophages stimulated with lipopolysaccharide and interferon-γ. *J. Infect. Dis.* 179:939–944.

Baldassare, J. J., Bi, Y., and Bellone, C. J. 1999. The role of p38 mitogen-activated protein kinase in IL-1β transcription. *J. Immunol.* 162:5367–5373.

Barbour, S. E., Wong, C., Rabah, D., Kapur, A., and Carter, A. D. 1998. Mature macrophage cell lines exhibit variable responses to LPS. *Mol. Immunol.* 35:977–987.

Bhat, N. R., Zhang, P., Lee, J. C., and Hogan, E. L. 1998. Extracellular signal-regulated kinase and p38 subgroups of mitogen-activated protein kinases regulate inducible nitric oxide synthase and tumor necrosis factor-α gene expression in endotoxin-stimulated primary glial cultures. *J. Neurosci.* 18:1633–1641.

Carter, A. B., Monick, M. M., and Hunninghake, G. W. 1999. Both Erk and p38 kinases are necessary for cytokine gene transcription. *Am. J. Respir. Cell Mol. Biol.* 20:751–758.

Chan, E. D., Winston, B. W., Uh, S.-T., Wynes, M. W., Rose, D. M., and Riches, D. W. H. 1999. Evaluation of the role of mitogen-activated protein kinases in the expression of inducible nitric oxide synthase by IFN-γ and TNF-α in mouse macrophages. *J. Immunol.* 162:415–422.

Chen, C.-C., and Wang, J.-K. 1999. p38 but not p44/42 mitogen-activated protein kinase is required for nitric oxide synthase induction mediated by lipopolysaccharide in RAW 264.7 macrophages. *Mol. Pharmacol.* 55:481–488.

Cobb, M. H. 1999. MAP kinase pathways. *Prog. Biophys. Mol. Biol.* 71:479–500.

Davis, R. J. 1994. MAPKs: new JNK expands the group. *Trends Biochem. Sci.* 19:470–473.

Derijard, B., Hibi, M., Wu, I. H., Barrett, T., Su, B., Deng, T., Karin, M., and Davis, R. J. 1994. JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell.* 76:1025–1037.

Force, T., and Bonventre, J. V. 1998. Growth factors and mitogen-activated protein kinases. *Hypertension* 31:152–161.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., and Tannenbaum, S. R. 1982. Analysis of nitrate, nitrite, and [^{14}N]nitrite in biological fluids. *Anal. Biochem.* 126:131–138.

Hale, K. K., Trollinger, D., Rihanek, M., and Manthey, C. L. 1999. Differential expression and activation of p38 mitogen-activated protein kinase α, β, γ, and δ in inflammatory cell lineage. *J. Immunol.* 162:4246–4252.

Hamilton, J. A., Byrne, R., Whitty, G., Vadiveloo, P. K., Marmy, N., Pearson, R. B., Christy, E., and Jaworowski, A. 1998. Effects of wortmannin and rapamycin on CSF-1-mediated responses in macrophages. *Int. J. Biochem. Cell Biol.* 30:271–283.

Janknecht, R., Ernst, W. H., Pingoud, V., and Nordheim, A. 1993. Activation of ternary complex factor Elk-1 by MAP kinases. *EMBO J.* 12:5097–5104.

Kyriakis, J. M., and Avruch, J. 1996. Sounding the alarm: Protein kinase cascades activated by stress and inflammation. *J. Biol. Chem.* 271:24313–24316.

Lee, J. C., and Young, P. R. 1996. Role of CSBP/p38/RK stress response kinase in LPS and cytokine signaling mechanisms. *J. Leukocyte Biol.* 59:152–157.

Lee, J. C., Laydon, J. T., McDonnell, P. C., Gallagher, T. F., Kumar, S., Green, D., McNulty, D., Blumenthal, M. J., Heys, J. R., Landvatter, S. W., Strickler, J. E., McLaughlin, M. M., Siemens, I. R., Fisher, S. M., Livi, G. P., White, J. R., Adams, J. L., and Young, P. R. 1994. A protein kinase involved in the regulation of inflammatory cytokine biosynthesis. *Nature* 372:739–746.

Livingstone, C., Patel, G., and Jones, N. 1995. ATF-2 contains a phosphorylation-dependent transcriptional activation domain. *EMBO J.* 14:1785–1797.

Lucas, D. M., Lokuta, M. A., McDowell, M. A., Doan, J. E. S., and Paulnock, D. M. 1998. Analysis of the IFN-γ-signalling pathway in macrophages at different stages of maturation. *J. Immunol.* 160:4337–4342.

Marais, R., Wynne, J., and Treisman, R. 1993. The SRF accessory protein Elk-1 contains a growth factor-regulated transcriptional activation domain. *Cell.* 73:381–393.

Means, T. K., Pavlovich, R. P., Roca, D., Vermeulen, M. W., and Fenton, M. J. 2000. Activation of TNF-α transcription utilizes distinct MAP kinase pathways in different macrophage populations. *J. Leukocyte Biol.* 67:885–893.

Myrvik, Q. N., Leake, E. S., and Fariss, B. 1961. Lysozyme content of alveolar and peritoneal macrophages from the rabbit. *J. Immunol.* 86:133–136.

Sanghera, J. S., Weinstein, S. L., Aluwalia, M., Girn, J., and Pelech, S. L. 1996. Activation of multiple proline-directed kinases by bacterial lipopolysaccharide in murine macrophages. *J. Immunol.* 156:4457–4465.

Scherle, P. A., Jones, E. A., Favata, M. F., Daulerio, A. J., Covington, M. B., Nurnberg, S. A., Magolda, R. L., and Trzakos, J. M. 1998. Inhibition of MAP kinase kinase prevents cytokine and prostaglandin E_2 production in lipopolysaccharide-stimulated monocytes. *J. Immunol.* 161: 5681–5686.

Swantek, J. L., Cobb, M. H., and Geppert, T. D. 1997. Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK) is required for lipopolysaccharide stimulation of tumor necrosis factor alpha (TNF-α) translation: Glucocorticoids inhibit TNF-α translation by blocking JNK/SAPK. *Mol. Cell. Biol.* 17:6274–6282.