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Modulation of LPS-Induced Activation of Hepatic Map Kinases

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MODULATION OF LPS-INDUCED ACTIVATION OF HEPATIC MAP KINASES

By

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degree of Master of Science in Biology from the
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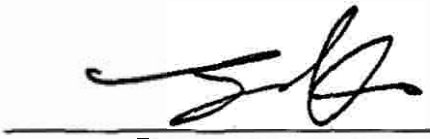
APPROVED BY

MENTOR



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ABSTRACT

Lipopolysaccharide (LPS) is a potent inflammagen that has been found to be primarily responsible for many symptoms caused by gram-negative bacterial infections. The LPS-initiated signal transduction pathways involve several terminal kinases, mainly p42/44 mitogen-activated protein kinase (MAPK), p38 MAPK and c-Jun N-terminal kinase (JNK), ultimately leading to increased expression of genes encoding such inflammatory cytokines as interleukin (IL)-1β, IL-6 and tumor necrosis factors (TNF)-α. In this study, the effects of age on LPS-induced activation of MAPKs in the liver of rats were examined. Results show that the basal level of phosphorylated p42/44 MAPK was increased in postnatal day (P) 21 and P70 compared to P1 animals treated with saline, and that LPS significantly increased the phosphorylation of p42/44 MAPK at P21 and P70. On the other hand, both basal and phosphorylated p38 MAPK did not show significant changes in P21 and P70 liver compared to P1. The effects of maternal exposure to LPS on the activation of MAPKs in P21 offspring liver were also examined. Results show that p42/44 MAPK activation was significantly decreased in the liver of pups born to LPS-treated dams as compared to those born to saline-treated dams following LPS stimulation. However, p38 MAPK and JNK activation was not affected by maternal exposure to LPS. These results indicate that MAPKs are differentially modulated by age and maternal exposure to LPS.

INTRODUCTION

Lipopolysaccharide (LPS) is found in the outer membrane of Gram-negative bacteria. It is an endotoxin that can trigger sepsis and septic shock in a person (Guha and Mackman, 2000). As an immune activator, LPS associates with LPS-binding protein (LBP) which brings it to CD-14, a glycosylphosphatidyl inositol (GPI)-anchored membrane receptor. LPS is then transferred by CD-14 to toll-like receptor (TLR)-4 which initiates the intracellular signaling cascades that include adaptor proteins such as myeloid differentiation factor 88 (MyD88) and MyD88-adaptor-like/TIR-associated protein (MAL/TIRAP), and the phosphorylation of mitogen-activated protein kinases (MAPK) (Wang et al., 2006).

The MAPKs involved in LPS signal transduction are p42/44 MAPK, also known as ERK1/2, p38 MAPK and c-Jun N-terminal kinase (JNK). The phosphorylation of these kinases lead to the production of cytokines responsible for proinflammatory immune response namely tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6, as well as the expression of IL-10, an anti-inflammatory cytokine (Ning et al., 2007). Inhibition of p38 MAPK activation decreases the expression of LPS-induced IL-1 and TNF-α without affecting mRNA accumulation in monocytes (Guha and Mackman, 2000; Lee et al., 1994). JNK and p38 MAPK signaling cascades have been implicated in the induction of TNF-α in LPS-stimulated microglia (Uesugi et al., 2006). It has been shown that p42/44 MAPK can work independently of p38 MAPK and JNK since inhibition of the mitogen-activated protein kinase ERK kinase (MEK) 1/2, which is

known to phosphorylate p42/44 MAPK (ERK1/2), decreases LPS-induced expression of IL-1, IL-8 and TNF-α without affecting p38 MAPK and JNK in monocytes (Guha and Mackman, 2000; Scherle et al., 1998). All three kinases have been implicated in the signal transduction of LPS but each has various effects on the expression of proinflammatory cytokines.

The liver is an important organ involved in LPS detoxification (Jirillo et al., 2002). Kupffer cells are macrophages found in the liver and are the first to respond to the presence of LPS. These cells express TLR-4 and produce proinflammatory cytokines when stimulated by LPS (Schwabe et al., 2006), and both p38 MAPK and JNK have been shown to be involved in LPS-stimulated TNF-α production in Kupffer cells (Shen et al., 2005). LPS-stimulated IL-6 release has also been found to be regulated by p38 MAPK in rat Kupffer cells (Bode et al., 1998). Cytokines released by Kupffer cells, TNF-α for instance, may in turn propagate the signals to other parenchymal cells such as hepatocytes (Milosevic and Maier, 2000). Additionally, hepatocytes express TLR-4, suggesting that hepatocytes may also use the TLR-4 signaling cascade to produce inflammatory cytokines in response to LPS (Migita et al., 2004). Indeed LPS has been shown to increase the expression of IL-6 in hepatocytes, and LPS-induced IL-6 expression is regulated by p42/44 MAPK (Jawan et al., 2008). This suggests that different liver cells use various MAPK pathways to produce inflammatory mediators.

There is increasing evidence that the activity of MAPKs may vary at different periods of the development. It has been characterized that 25-month old mice liver has increased JNK activity compared to 4-month old mice liver in response to oxidative

stress (Hsieh et al., 2003). The basal level of p38 MAPK is higher in 25-month old mice liver compared to 4-month old liver. In addition, the activation of p38 MAPK by oxidative stress present in the young liver was not observed in the aged liver (Hsieh and Papaconstantinou, 2002). In skeletal muscles of 36-month old rats, stretching stimulates p42/44 MAPK phosphorylation but not JNK and p38 MAPK when compared to 6- and 30-month old rat skeletal muscle (Mylabathula et al., 2005). A study done on the developing rat visual system showed that p38 MAPK activation in the retina increases at postnatal day (P) 4, and then goes back to its basal level at P9. While from P15 to P45, p42/44 MAPK phosphorylation increases along with p38 MAPK. These studies suggest that p38 MAPK and p42/44 MAPK are differentially regulated during postnatal development (Oliveira et al., 2007). However, it is not known how LPS-induced activation of MAPKs in the liver is affected at different stages of life.

Maternal exposure to LPS has a number of deleterious effect on the offspring, including intrauterine fetal death (IUFD), intrauterine growth retardation (IUGR), preterm labor, spontaneous abortion and embryonic resorption (Xu et al., 2007; Ning et al., 2007; Xu et al., 2005). Maternal LPS has been shown to increase the level of TNF-α in the fetal liver and fetal brain (Ning et al., 2008), slow skeletal development (Xu et al. 2006), and delay the development of the innate immune response of the offspring to LPS (Hodyl et al., 2007) in mice. Postnatal exposure to LPS of pups born to LPS-treated dams has also shown decreased levels of various cytokines including TNF-α, IL-1β and IL-6 in the blood and inhibited mRNA levels of the same cytokines as well as the chemokines, macrophage inflammatory protein (MIP)-1β, MIP-2 and keratinocyte-

derived chemokine (KC) in the brain, as compared to pups born to saline-treated dams (LaSala and Zhou, 2007). It remains to be seen how maternal exposure to LPS affects the activation of MAPKs in the liver of the offspring.

This study focused on the effects of age and maternal exposure to LPS on LPS-induced hepatic MAPK activation. Phosphorylation of the three terminal kinases, namely p42/44 MAPK, p38 MAPK and JNK, was examined in saline or LPS treated rats at postnatal (P) 1, P21 and P70. Activation of these kinases was also examined in pups born to dams treated with saline or LPS at day 18 of pregnancy and subsequently stimulated with saline or LPS for 2 hours at P21.

METHODS AND MATERIALS

Animals

Adult male and female Sprague-Dawley rats were purchased from Harlan Inc. (Indianapolis, IN). The rats were acclimated for at least one week at a temperature- and humidity-controlled animal facility with a 12-hour light/dark cycle and fed a standard rat diet and water *ad libitum*. Animal studies were conducted with the approval of the Institutional Animal Care and Use Committee (IACUC) at Seton Hall University. Each male rat (250-300g) was placed in the same cage with 1~3 female rats (200-230g) at night for mating. To confirm copulation, each female rat was visually inspected for the presence of the vaginal plug. This day was noted as day 0 of pregnancy and the female rat was moved to a separate cage in the above-mentioned conditions.

LPS Treatments

For time-course studies, the pups were allowed to mature in above-mentioned conditions until postnatal day (P) 21 after birth. At P21, rat pups were treated with 250 μg/kg LPS (*Salmonella enteric* serovar Typhimuriuml Sigma, St, Louis, MO), dissolved in sterile pyrogen-free saline, for 0, 0.5 h, 1 h, 2h, and 6 h (four pups per group) via i.p. injection. At the end of each treatment, the pups were then sacrificed and their liver tissues harvested and stored at -80°C for total protein extraction.

For age-dependency studies, the pups were maintained in the above-mentioned conditions and randomly assigned to receive saline or 250 μg/kg LPS at P1, P21 and P70

via i.p. injection. Two hours after stimulation, the pups were sacrificed and their liver tissues were collected and stored at -80°C for total protein extraction.

To study the effects of maternal exposure to LPS on the phosphorylation of MAPKs in the liver of the offspring, the pregnant dams were randomly assigned to a saline control group and an LPS group. Dams exposed to LPS have a high rate of fetal resorption so the LPS group had twice as many animals as the saline group. One intraperitoneal (i.p.) injection of 500 μg/kg LPS was given to the dams in the LPS group while the dams in the saline group received one i.p. injection of saline on day 18 of pregnancy. The dams were then housed in above-mentioned condition and allowed to mature until P21. An i.p. injection of250 μg/kg LPS or saline was randomly given to the pups. Five pups from each group were then sacrificed 2 hours after saline or LPS treatment and their liver tissues harvested for total protein extraction. Another five pups from each group were anesthetized with euthasol and perfused transcardially with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. After perfusion, the liver tissues were harvested and postfixed overnight at 4°C. They were then rinsed with PBS and dehydrated through a series of washes starting with 50% ethanol (30 min, twice at room temperature), 70% ethanol (30 min, at room temperature), 85% ethanol (30 min, at room temperature), 95% ethanol (30 min, twice at room temperature), 100% ethanol (30 min, twice at room temperature), xylenes (30 min, twice at room temperature), xylenes/wax (1:1 v/v, 30 minutes at 60°C), and wax (30 min, at 60°C). Finally, the liver tissues were embedded in wax.

Total Protein Extraction

The liver tissues were finely ground and 150 µl of lysis buffer containing 50 mM HEPES (pH 7.5), 5 mM EDTA, 50 mM NaCl, 10 mM NaPPi, 50 mM NaF, 1% IGEPAL, 2 mM Na_3VO_4, and 1X protease inhibitor cocktail (Roche, Indianapolis, IN) was added to every 100 µg of tissue. The liver tissues were then homogenized with an Eppendorf (EP) tube pestle and sonicated 3 times with a Branson Sonifier 250 for 15 pulses and 10 seconds on ice between each time. A 30-minute incubation on ice and a 15-minute centrifugation at 14,000g in 4°C followed. The supernatants were collected for protein concentration determination and western blotting.

Protein Concentration Determination

Protein concentration was determined using the bicinchoninic acid (BCA) Protein Assay Reagent from Pierce biotechnology Inc. (Rockford, IL) following the manufacturer's instructions.

Western Blot

Liver samples from the rats were prepared using a volume having 100 µg of proteins mixed with an equal volume of 2X sample loading buffer, boiled for 5 minutes, centrifuged at 14,000g for 5 minutes at 4°C. The samples were resolved using a 12% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto nitrocellulose membranes. The membranes were blocked with 5% (w/v) nonfat milk for 2 hours and incubated overnight at 4°C with primary antibodies that recognize phosphorylated forms of p38 MAPK, JNK (Santa Cruz Biotechnology, Santa Cruz, CA) or p42/44 MAPK (Cell Signaling Technology, Boston, MA). Following four

10-minute rinses with tris-buffered saline containing 0.1% Tween-20 (TBST), the membranes were incubated in horseradish peroxidase (HRP)-conjugated rabbit IgG antibody (Vector Laboratories, Burlingame, CA) for 1 hour at room temperature. The membranes were washed 4 times with TBST before development with ECL^{plus} chemiluminescence detection kit (GE Healthcare bio-Sciences Corp., Piscataway, NJ) applied directly. Scanning was done using Storm 860 molecular Imager (Molecular Dynamics), after which, membranes were stripped and reprobed with antibodies for total p38 and p42/44 (Cell Signaling, Boston, MA) as well as an antibody immunoreactive to β-actin (Sigma, St. Louis, MO). The scanned images were digitized using Molecular Dynamics ImageQuant software.

Immunohistochemistry

The embedded liver tissues were cut into 10-μm sections on a microtome (Leitz) and transferred onto slides. The liver sections were then dewaxed and rehydrated through xylenes (5 min, three times), 100% ethanol (5 min, twice), 95% ethanol (5 min), 70% ethanol (5 min), 25% ethanol (5 min), and TBS (5 min). After rehydration, the liver sections were heated for 3 min in 10 mM sodium citrate (pH 6.0) in a microwave and cooled down for 20 min at room temperature. The liver sections were subsequently rinsed with TBS and incubated with 0.3% H_2O_2 in methanol for 30 min at room temperature. The sections were then blocked with 5% normal host serum in TBS containing 0.2% Triton X-100, incubated with antibodies for phosphorylated p38 MAPK, phosphorylated JNK (Santa Cruz Biotechnologies, Santa Cruz, CA) or phosphorylated p42/44 MAPK (Cell Signaling Technologies) overnight at 4°C, and detected with the

VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, CA) following manufacturer's instructions.

Statistical Analysis

All data were presented as means ± SE and analyzed by two-way analysis of variance (ANOVA) followed by Bonferroni post-tests. Results with $p<0.05$ were considered statistically significant.

RESULTS

Time-course of MAPK phosphorylation in P21 rat liver

Rat pups were treated at postnatal day (P) 21 with one i.p. injection of 250 μg/kg of LPS for 0, 0.5 h, 1 h, 2 h, or 6 h. They were then sacrificed, their liver tissues harvested, and total proteins extracted for western blot analysis of MAPK activation. Antibodies reactive to phosphorylated p42/44 MAPK, phosphorylated p38 MAPK and β-actin were used to evaluate the levels of phosphorylated p42/44 and p38 MAPKs.

Western blot analysis showed a significant increase in p-p42/actin and p-p44/actin ratios at 1 h. The levels remained elevated at 2 h and finally returned to basal levels at 6 h after LPS stimulation (Figs. 1A and 1B). In contrast, the p-p38/actin ratio was slightly decreased at 1 h and 2 h. This decrease, however, was not significant (Fig. 2A and 2B). These data indicate that LPS stimulation in the offspring increased phosphorylated p42/44 MAPK level but not phosphorylated p38 MAPK in rat liver. In addition, p42/44 MAPK activation reaches peak levels 2 h after exposure to LPS (Surriga et al., 2009).

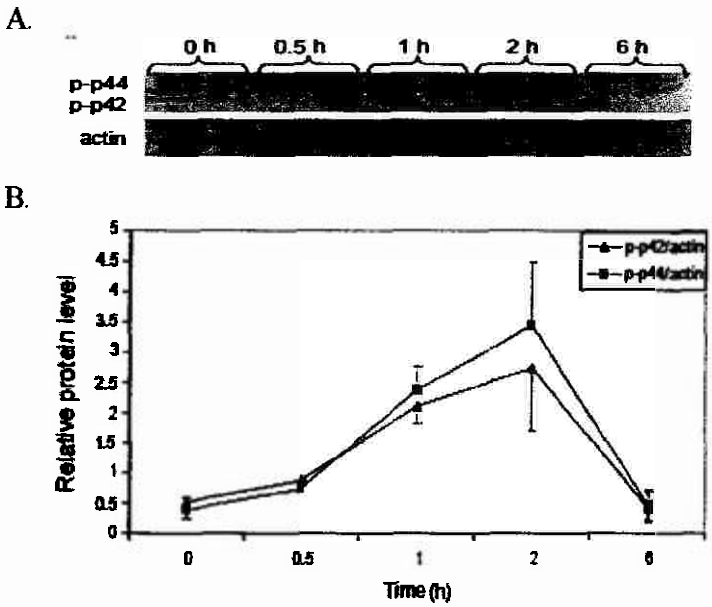


Figure 1. Time-course of p42/44 MAPK in the liver of P21 pups following LPS stimulation. Pups were treated with one dose of 250 μg/kg LPS for 0 h, 0.5 h, 1 h, 2 h and 6 h and levels of phosphorylated p42/44 MAPK were determined using Western Blot. (A) Representative Western Blot image of p-p42/44 MAPK with its respective β-actin two samples for each time period. (B) Quantitation of p-p42 and p-p44 MAPK normalized to β-actin. Values are means ± SE, n=4.

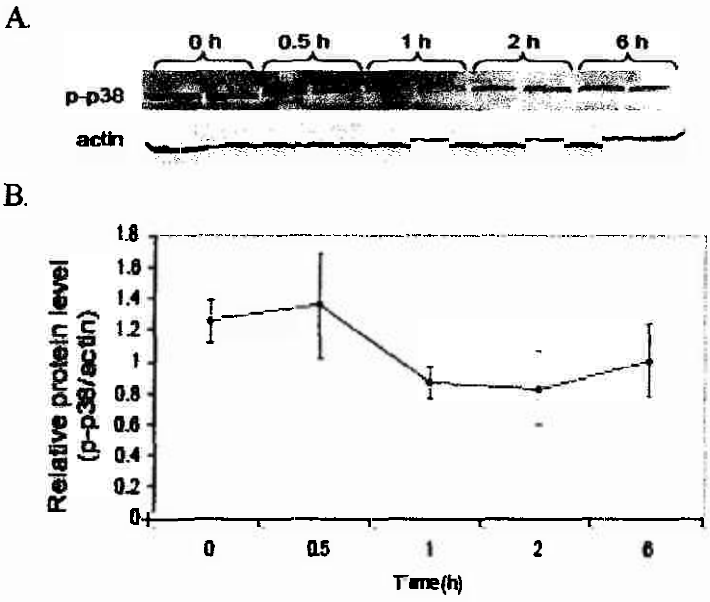


Figure 2. Time-course of p38 MAPK in the liver of P21 pups following LPS stimulation. Pups were treated with one dose of 250 μg/kg LPS for 0 h, 0.5 h, 1 h, 2 h and 6 h and levels of phosphorylated p38 MAPK were determined using Western Blot. (A) Representative Western Blot image of p-p38 MAPK with its respective β-actin, two samples for each time period. (B) Quantitation of p-p38 MAPK normalized to β-actin. Values are means± SE, n=4.

LPS-induced activation of MAPKs in P1, P21 and P70 rat liver

To examine how age affects the LPS-induced activation of MAPKs, pups were treated with 250 μg/kg of LPS or saline at postnatal day (P) 1, P21 and P70. Two hours after stimulation, the pups were sacrificed and their liver harvested for total protein extraction. Western blot was used to analyze the level of phosphorylated MAPKs probed with antibodies reactive specifically to either phosphorylated p42/44 MAPK or phosphorylated p38 MAPK.

The ratios of p-p42 MAPK (p42/actin) and p-p44 MAPK (p44/actin) to their respective β-actin were significantly increased in LPS-treated rats compared to saline-treated rats at P21 and P70 while in P1 rats, the LPS-induced increase in p-p42 MAPK and p-p44 MAPK was only marginal (Figs. 3A and 3B). The basal levels of the ratios of p-p42 MAPK and p-p44 MAPK were also noticeably elevated in saline-treated P21 and P70 rats compared to saline-treated P1 rats (Figs 3A and 3B). However, p-p38 MAPK to β-actin ratio did not show significant increases in basal levels of saline-treated rats nor in LPS-treated rats compared to saline-treated rats at P1, P21 and P70 (Figs. 4A and 4B).

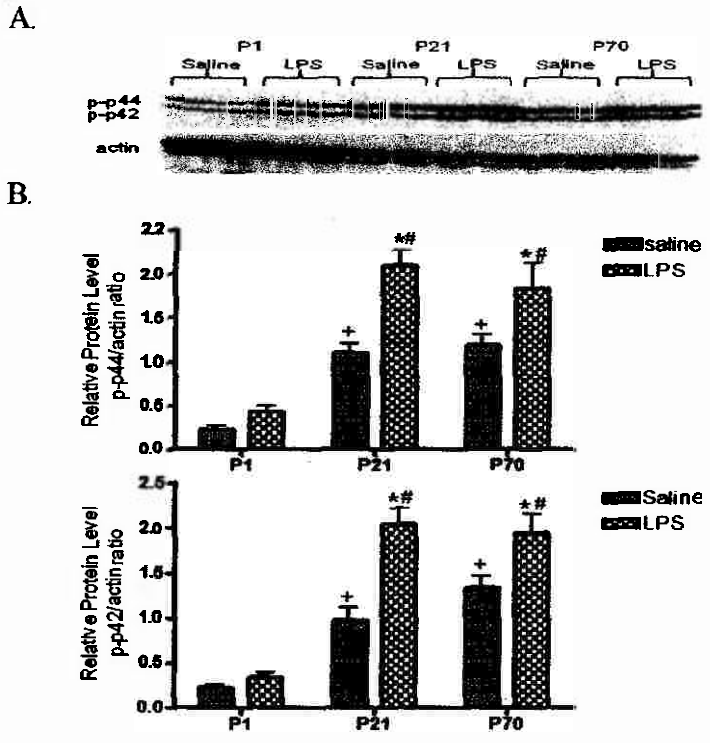


Figure 3. Western blot analysis of the age-dependent activation of p42/44 MAPK in LPS-treated pups. Pups were treated for 2 h with either 250 μg/kg of LPS or saline at P1, P21 and P70. (A) Representative Western blot of p-p42/44 MAPK with its respective total p42/44 MAPK and β-actin, two samples for each treatment of all postnatal days. (B) Quantitation of Western blots of p-p42 and p-p44 MAPK normalize to β-actin. Values are means± SE, n=4. + represents significant difference between P1 saline and P21 and P70 saline at $p<0.05$. * represents significant difference between P21 saline and P21 LPS as well as P70 saline and P70 LPS at $p<0.05$. # represents significant different between P1 LPS and P21 and P70 LPS, $p<0.05$.

A.

B.

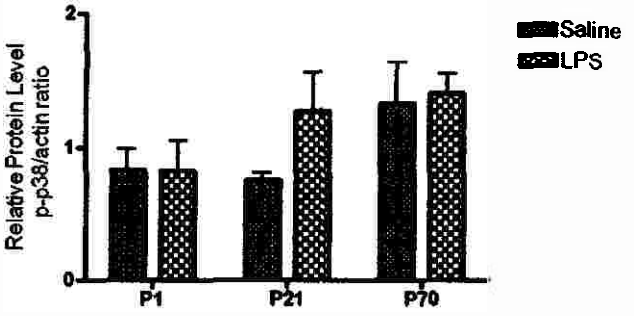


Figure 4. Western blot analysis of the age-dependent activation of p38 MAPK in LPS-treated pups. Pups were treated for 2 h with either 250 μg/kg of LPS or saline at P1, P21 and P70. (A) Representative Western blot of p-p38 MAPK with its respective total p38 MAPK and β-actin, two samples for each treatment of all postnatal days. (B) Quantitation of Western blots of p-p38 MAPK normalized to β-actin. Values are means ± SE, n=4.

Effects of maternal exposure to LPS on MAPK phosphorylation in offspring liver

Pregnant dams were injected with 500 μg/kg of LPS or saline on day 18 of pregnancy. The offspring were then stimulated with 250 μg/kg of LPS or saline at P21. To evaluate effects of maternal exposure to LPS on the activation of MAPKs in the offspring liver, phosphorylated MAPK, total MAPK levels, as well as, β-actin levels were measured using Western blot assay with antibodies immunoreactive to phosphorylated MAPKs, total MAPKs and β-actin respectively. (Surriga et al., 2009)

Western blot analysis showed significant increase in the ratios of p-p42/actin and p-p44/actin born to saline-treated dams and subsequently stimulated with LPS (S+L) compared with animals born to saline-treated dams and subsequently stimulated with saline (S+S) at P21 (Figs. 5A and 5B). Animals born to LPS-treated dams and subsequently stimulated with LPS (L+L) showed some increase in p-p42/actin and p-p44/actin ratios compared to animals born to LPS-treated dams and subsequently stimulated with saline (L+S) (Figs. 5A and 5B). Furthermore, p-p42/actin and p-p44/actin ratios in L+L showed a substantial decrease compared to those in S+L (Figs. 5A and 5B). In contrast, p-p38/actin (Figs. 6A and 6B) and p-JNK/actin (Figs. 7A and 7B) ratios were not significantly affected by LPS exposure (Surriga et al., 2009).

To verify these results, immunohistochemistry was used to detect LPS-induced phosphorylated MAPKs in the liver. Consistent with the western blot findings, the number of cells immunoreactive to p-p42/44 in the S+L group was significantly increased compared to those in the S+S group (Figs. 8A and 8C). The number of p-p42/44-positive cells in L+L was also notably increased compared to L+S (Figs. 8B and 8D). Results

also show that number of p-p42/44-immunoreactive cells in the L+L group was considerably lower than that in the S+L group (Figs. 8C and 8D). Furthermore, the decrease in p-p42/44 immunoreactive cells in the L+L group the S+L group was more region-dependent than cell-type dependent (Figs. 8C and 8D). For p-p38 MAPK and p-JNK, prenatal or postnatal LPS treatment did not affect the immunoreactivity in the offspring liver (Figs. 9A-9D and Figs. 10A-10D).

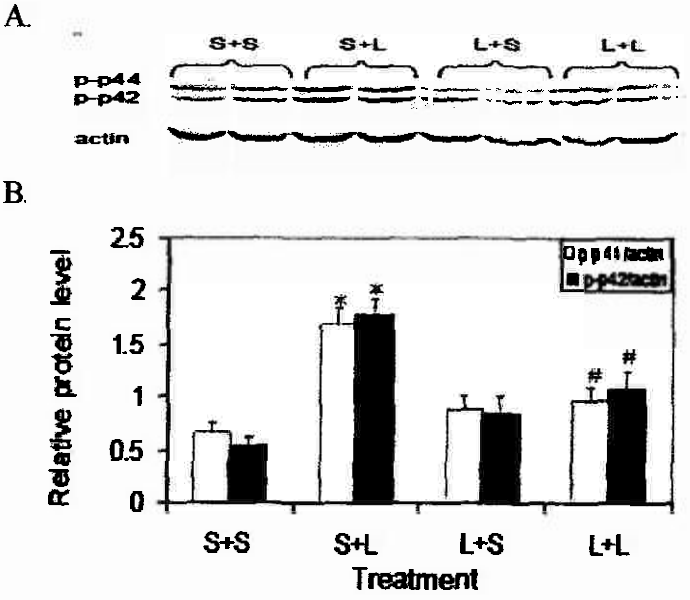
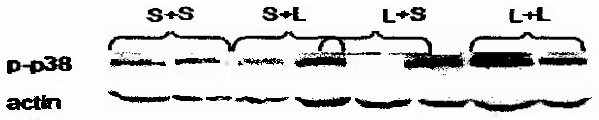


Figure 5. p-p42/44 MAPK Western blot analysis of P21 rat liver tissue. Pregnant dams were treated with either 500 μg/kg of LPS or saline on day 18 of pregnancy, pups were then treated with either 250 μg/kg of LPS or saline at P21 for 2 hours. (A) Representative Western blot of p-p42/44 MAPK with its respective β-actin, two samples for each treatment. (B) Quantitation of Western blots of p-p42 and p-p44 MAPK normalize to β-actin. Values are means± SE: n=5. * represents significant difference from the S+S group at $p < 0.05$; # represents significant difference from the S+L group at $p < 0.05$.

A.



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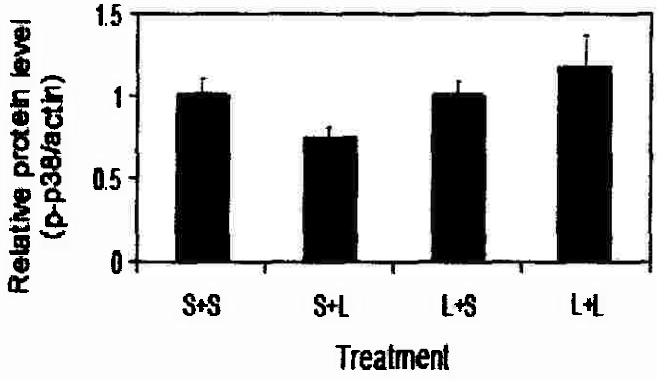

Figure 6. p-p38 MAPK Western blot analysis of P21 rat liver tissue. Pregnant dams were treated with either 500 μg/kg of LPS or saline on day 18 of pregnancy, pups were then treated with either 250 μg/kg of LPS or saline at P21 for 2 hours. (A) Representative Western blot of p-p38 MAPK with its respective β-actin, two samples for each treatment. (B) Quantitation of Western blots of p-p38 MAPK normalize to β-actin. Values are means ± SE. n=5.

A.

B.

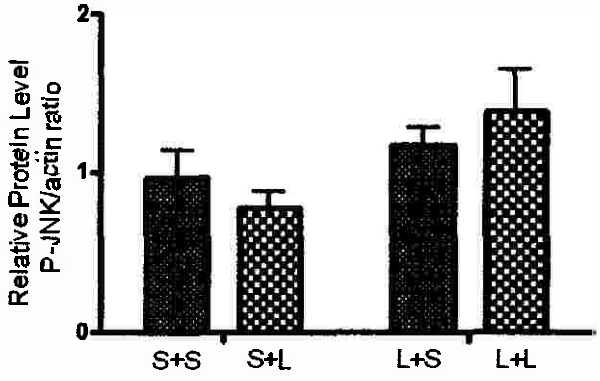

Figure 7. p-JNK Western blot analysis of P21 rat liver tissue. Pregnant dams were treated with either 500 μg/kg of LPS or saline on day 18 of pregnancy, pups were then treated with either 250 μg/kg of LPS or saline at P21 for 2 hours. (A) Representative Western blot of p-JNK with its respective β-actin, two samples for each treatment. (B) Quantitation of Western blots of p-JNK normalize to β-actin. Values are means± SE: n=5.

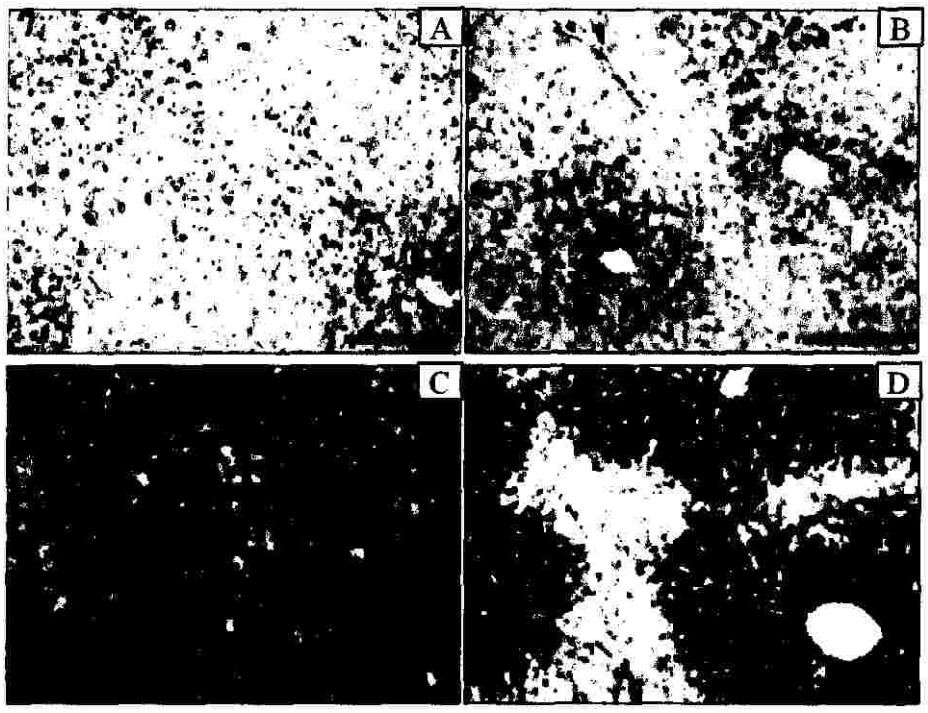

Figure 8. Representative images of p-p42/44 MAPK immunohistochemistry. Pregnant dams were treated with either 500 μg/kg of LPS or saline on day 18 of pregnancy, pups were then treated with either 250 μg/kg of LPS or saline at P21 for 2 hours. (A) saline+ saline: (B) LPS + saline: (C) saline+ LPS: (D) LPS + LPS. Scale bar = 50μm.

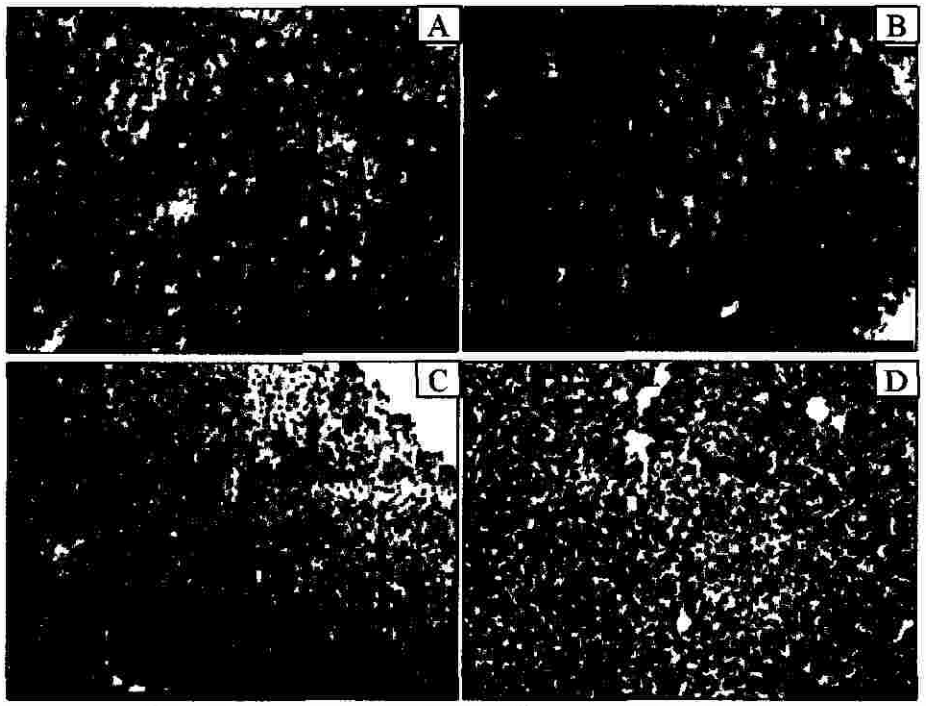

Figure 9. Representative images of p-p38 MAPK immunohistochemistry. Pregnant dams were treated with either 500 μg/kg of LPS or saline on day 18 of pregnancy, pups were then treated with either 250 μg/kg of LPS or saline at P21 for 2 hours. (A) saline + saline: (B) LPS + saline: (C) saline + LPS: (D) LPS + LPS. Scale bar = 50 μm.

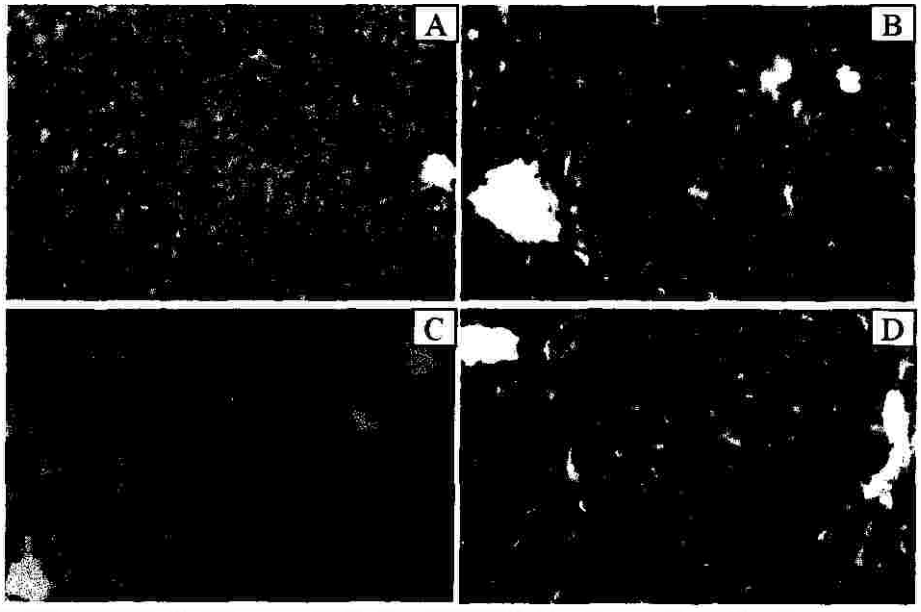

Figure 10. Representative images of p-JNK MAPK immunohistochemistry. Pregnant dams were treated with either 500 μg/kg of LPS or saline on day 18 of pregnancy, pups were then treated with either 250 μg/kg of LPS or saline at P21 for 2 hours. (A) saline+ saline: (B) LPS + saline: (C) saline+ LPS: (D) LPS + LPS. Scale bar = 25μm.

DISCUSSION

This study investigated how MAPKs were modulated in the liver by postnatal development as well as maternal immune activation. First, LPS-induced time course activation of MAPKs were performed on the liver of pups. P-p42/44 MAPK showed significant increase in the liver of LPS-treated pups at 1 h and 2 h. On the other hand, p-p38 MAPK levels decreased at 1 h and 2 h, although this was not significant. Previous studies have also shown that MAPKs are activated differently at various points in development. The pattern shows an increase in phosphorylation of MAPKs as development progresses. The activation of p42/44 MAPK exhibited age-dependency in pups treated with LPS at postnatal (P) day 1, P21 and P70. However, p38 MAPK activation was not affected by postnatal LPS treatments since no significant increase in phosphorylation were observed. Western blot results also showed that LPS-induced activation of p42/44 MAPK in the liver of P21 pups is attenuated by maternal exposure to LPS. Images from immunohistochemistry verified these results but show that the attenuation is region-dependent rather than cell-specific. This suggests that liver cells use p42/44 MAPK as a mediator for LPS intracellular signaling during postnatal development.

It has been reported that IL-6 expression is also diminished in the liver LPS-treated pups born to dams immunologically challenged by LPS without affecting IL-1β and TNF-α expression (Surriga et al., 2009). One study has shown that LPS-induced chemokine secretion in synctiotrophoblast (ST) cells, which are found in human placenta

and are in direct contact with maternal blood, is partly dependent on p42/44 MAPK (ERK1/2) activation but not p38 MAPK and JNK (Lucchi and Moore, 2006). Moreover, activation of p42/44 MAPK has been implicated in the sphingosylphosphorylcholine-induced production of IL-6 in human dermal fibroblast cells (Kwon et al., 2007) and the increased expression of IL-6 by Leptin in blood monocytes (Najib and Sanchez-Margalet, 2002). It also has been shown that IL-6 attenuation due to maternal exposure to LPS corresponds to p42/44 MAPK reduction liver tissue (Surriga et al., 2009). Furthermore, previous studies have shown that p38 MAPK is more involved in TNF-α and IL-1β expression in monocytes and macrophages (Chung et al., 2003: Jiang and Ulevitch, 1999: Kitazawa et al., 2007). This suggests that the attenuation due to maternal exposure to LPS of the inflammatory response in the offspring, through IL-6 production, depends, at least in part, on p42/44 MAPK activity, not p38 MAPK and JNK.

In the future, it will be worthwhile to see which transcription factors that p38 MAPK, p42/44 MAPK and JNK act on are also attenuated by maternal exposure to LPS. These transcription factors include SRE, AP-1, CRE and NF-κB (Guha and Mackman, 2000: Schwabe et al., 2006). It will also be interesting to see whether the activation of phosphatases that regulate kinase activity is increased in LPS-treated pups born to maternally challenged dams. MAP kinase phosphatase MKP-1 has been shown to inhibit p38 MAPK phosphorylation in macrophages (Bhattacharyya et al., 2007) and Hela cells (Lasa et al., 2002) though MKP-1 has been shown to more effectively inactivate JNK and p38 MAPK *in vitro* than p42/44 MAPK, PAC-1 specifically desphophorylates p42/44 MAPK and p38 MAPK, while MKP-2 regulates p42/44 MAPK and JNK (Kondoh and

Nishida, 2006). Future studies may show that these phosphatases can be implicated in the attenuation of p42/44 MAPK due to maternal exposure to LPS if there is an increase in their activity.

It has been shown that Kupffer cells and hepatocytes use different mechanisms in activating proinflammatory cytokines in response to LPS. On one hand, LPS-induced cytokine production in Kupffer cells was shown to be mediated by p38 MAPK and JNK (Shen et al., 2005 and Bode et al., 1998), on the other, hepatocytes used p42/44 MAPK as mediators (Jawan et al., 2008). However, our immunohistochemical results have indicated that phosphorylation of p42/44 MAPK is more region-dependent than cell-specific. It has yet to be determined what contributes to the region-dependent activation of p42/44 MAPK. It will also be interesting to compare the activation of p42/44 MAPK in Kupffer cells and hepatocyte, determining which type is more affected by LPS.

In summary, LPS-induced phosphorylation of p42/44 MAPK in the liver is age-dependent with p42/44 MAPK being significantly activated at P21 and P70 but not at P1 compared to their saline controls. Also, LPS-induced p42/44 MAPK activation was attenuated by maternal treatment with LPS in the liver of P21 rats. On other hand, p38 MAPK and JNK activation in the liver was not affected by age or maternal exposure to LPS. This suggests that p42/44 MAPK plays a key role in hepatic inflammatory function modulated by age and maternal LPS exposure.

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