Available online at www.sciencedirect.com
ScienceDirect
Toxicology Letters 176 (2008) 13–19
Toxicology Letters
www.elsevier.com/locate/toxlet

Maternally-administered lipopolysaccharide (LPS) increases tumor necrosis factor alpha in fetal liver and fetal brain: Its suppression by low-dose LPS pretreatment

Huan Ning [a], Hua Wang [a], Lei Zhao [a], Cheng Zhang [a], Xiang-Yun Li [a], Yuan-Hua Chen [a], De-Xiang Xu [a,b,*]

[a] *Department of Toxicology, Anhui Medical University, Hefei 230032, PR China*
[b] *Department of Microbiology and Parasitology, Anhui Medical University, Hefei 230032, PR China*

Received 6 April 2007; received in revised form 4 July 2007; accepted 2 August 2007
Available online 7 August 2007

Abstract

Lipopolysaccharide (LPS) has been associated with adverse developmental outcome, including intra-uterine fetal death (IUFD), intra-uterine growth retardation (IUGR) and neurological injury. In the LPS model, tumor necrosis factor alpha (TNF-α) is the major mediator leading to IUFD, IUGR and neurological injury. In the present study, we investigated the effect of maternally-administered LPS on TNF-α in maternal serum, amniotic fluid, fetal liver and fetal brain. The timed pregnant mice were intraperitoneally (i.p.) injected with a single dose of LPS (500 μg/kg) on gestational day 17. As expected, TNF-α was obviously increased in maternal serum and amniotic fluid in response to LPS. Although maternally-administered LPS also increased the level of TNF-α protein in fetal liver and brain, no significant difference in TNF-α mRNA level in fetal liver and brain was observed among different groups, suggesting that the increased TNF-α protein in fetal liver and brain may be transferred from either the maternal circulation or amniotic fluid or placenta. When the pregnant mice were pretreated with a low-dose LPS (10 μg/kg, i.p.) at 4, 12, 24 or 48 h before LPS (500 μg/kg, i.p.), LPS-evoked TNF-α in maternal serum and amniotic fluid was significantly inhibited. Importantly, low-dose LPS pretreatment also greatly attenuated LPS-induced increases in TNF-α protein in fetal liver and fetal brain. Taken together, these results indicate that perinatal exposure to low-dose LPS induces a reduced sensitivity to subsequent LPS challenge.

Keywords: Lipopolysaccharide; Tumor necrosis factor alpha; Fetal liver; Fetal brain

1. Introduction

Lipopolysaccharide (LPS) is a toxic component of cell walls of gram-negative bacteria and is widely present in the digestive tracts of humans and animals (Jacob et al., 1997). Humans are constantly exposed to low levels of LPS through infection. Gastrointestinal distress and alcohol consumption often increase the uptake of LPS from gastrointestinal tract into blood (Fukui et al., 1991). LPS induces a severe inflammatory response by initiating multiple intracellular signaling events, including the activation of NF-κB, which ultimately leads to the synthesis and release of cytokines, such as proinflammatory cytokine tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-16, and anti-inflammatory cytokine IL-10.

Maternal LPS exposure has been associated with adverse developmental outcome, including embryonic

* Corresponding author.
E-mail address: xudex@mail.hf.ah.cn (D.-X. Xu).

0378-4274/$ – see front matter © 2007 Published by Elsevier Ireland Ltd.
doi:10.1016/j.toxlet.2007.08.002

resorption, intra-uterine fetal death (IUFD), intra-uterine growth restriction (IUGR), and preterm labor and delivery in rodents (Collins et al., 1994; Gross et al., 2000; Ogando et al., 2003; Silver et al., 1995). A variety of experimental evidence indicates that maternal LPS exposure in pregnancy is associated with fetal neurological injury (Bell and Hallenbeck, 2002; Golan et al., 2005; Rousset et al., 2006; Yan et al., 2004). In the LPS model, TNF-α is one of the major mediators leading to embryonic resorption, IUFD, IUGR and preterm delivery (Silver et al., 1995; Xu et al., 2006). In addition, TNF-α contributes to LPS-induced fetal neurodevelopmental abnormality (Bell and Hallenbeck, 2002). However, it remains controversial whether maternally-administered LPS stimulates TNF-α in fetal liver and brain.

On the other hand, pretreatment with LPS has been shown to induce a reduced sensitivity to subsequent challenge of LPS. This phenomenon is termed LPS tolerance or LPS hyporesponsiveness. LPS tolerance is associated with downregulation of toll-like receptor 4 (TLR4) in macrophages and a reduced production of inflammatory cytokines in response to a secondary stimulation with LPS (Erroi et al., 1993; Fan et al., 2002; Medvedev et al., 2000; Nomura et al., 2000). Thus, it is especially interesting whether pre-exposure to low-dose LPS during pregnancy produces a tolerance to subsequent high-dose LPS challenge.

The purpose of the present study was to determine whether maternally-administered LPS stimulates the release of TNF-α in fetal liver and fetal brain, and to assess the effect of low-dose LPS pretreatment on LPS-induced TNF-α in maternal serum, amniotic fluid, fetal liver and fetal brain.

2. Materials and methods

2.1. Chemicals

Lipopolysaccharide (*Escherichia coli* LPS, serotype 0127:B8) was purchased from Sigma Chemical Co. (St. Louis, MO). All the other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and treatments

The ICR (CD-1) mice (8–10 week-old; male mice: 28–30 g; female mice: 24–26 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. The animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment for a period of 1 week before use. For mating purposes, four females were housed overnight with two males starting at 21:00 h. Females were checked by 7:00 h the next morning. The presence of a vaginal plug was designated as gestational day (gd) 0.

The pregnant mice were divided randomly into four groups. All pregnant mice except controls (saline) received an intraperitoneal (i.p.) injection of LPS (500 μg/kg) on gd 17. Some pregnant mice were pretreated with low-dose LPS (10 μg /kg, i.p.) at different time point (4, 12, 24 or 48 h) before LPS (500 μg/kg, i.p.). The control mice received saline. All pregnant mice were sacrificed at 1.5 h after LPS. Maternal serum and amniotic fluid were collected for measurement of cytokines. The fetal brain and fetal liver tissue (100 mg) was placed in 1 mL of iced lysis buffer containing 50 mM Tris–HCl, 150 mM NaCl, 1 % NP-40, 0.25% Na-deoxycholate, 1 mM EDTA, and 0.1 mM PMSF (recipe from Upstate, Charlottesville, VA). Samples were homogenized and centrifuged at 12000 rpm for 20 min at 4 °C. Supernatants were aliquoted for measurement of TNF-α. Protein concentrations of supernatant samples were measured according to the m**et**hod of Lowry et al. (1951), using bovine serum albumin as a standard.

All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Measurement for cytokine concentration

Commercial ELISA (R&D Systems) kits were used to determine levels of TNF-α in maternal serum, amniotic fluid, fetal liver and fetal brain according to the manufacturer's protocol. Briefly, samples were pipetted in wells precoated with specific antibody for mouse TNF-α and allowed to incubate for 2 h. After wells were rinsed to remove all unbound substance, an enzyme-linked antibody specific for mouse TNF-α was added to wells for 2 h. After wells were rinsed to remove all unbound enzyme-linked antibody, a substrate solution was added to wells for 30 min to yield a colored product that was quantified by optical density readings at 490 nm. The reaction was stopped and the optical density was measured at 490 nm using a Universal microplate reader (Bio-Tek Instruments, Inc.). For all kits used, the minimum detectable limit was less than 10 pg/ml. The mean intra-assay and inter-assay variations for TNF-α were less than 10%.

2.4. Isolation of total RNA and RT

50 mg of tissue was collected on each mouse. Total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. RNase-free DNase (Promega) was used to remove genomic DNA. The integrity and concentration of RNA was determined by measuring absorbance at 260 nm followed by electrophoresis on agarose gels. Total RNA was stored at −80 °C. For the synthesis of cDNA, 2.0 μg of total RNA from each sample was resuspended in a 20-μl final volume of reaction buffer, which contained 25 mM Tris–HCl, pH 8.3, 37.5 mM KCl,

10 mM dithiothreitol, 1.5 mM $MgCl_2$, 10 mM of each dNTP and 0.5 mg oligo$(dT)_{15}$ primer (Promega). After the reaction mixture reached 38 °C, 400 units of RT (Promega) was added to each tube and the sample was incubated for 60 min at 38 °C. Reverse transcription was stopped by denaturing the enzyme at 95 °C.

2.5. PCR amplification

The final PCR mixture contained 2.5 μl of cDNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM dNTP mixture, 1 U of Taq DNA polymerase, 1 μM sense and antisense primers, and sterile water to 50 μl. The reaction mixture was covered with mineral oil. PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was performed on each individual sample as an internal positive-control standard. Following primers were synthesized by Shanghai Sangon Biological Engineering Technology and Service Company (Shanghai, China), according to sequence designs previously described by others (Chen et al., 2005). GAPDH, 5′-GAG GGG CCA TCC ACA GTC TTC-3′ and 5′-CAT CAC CAT CTT CCA GGA GCG-3′; TNF-α, 5′-GGC AGG TCT ACT TTG GAG TCA TTG C-3′ and 5′-ACA TTC GAG GCT CCA GTG AAT TCG G-3′. The sizes of amplified PCR products were 340 bp for GAPDH and 307 bp for TNF-α. Number of cycles and annealing temperature were optimized for each primer pair. For GAPDH, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 30 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min. For TNF-α, amplification was initiated by 3 min of denaturation at 94 °C for 1 cycle, followed by 44 cycles each of denaturation at 94 °C for 45 s, annealing of primer and fragment at 60 °C for 45 s, and primer extension at 72 °C for 1 min. A final extension of 72 °C for 10 min was included. The amplified PCR products were subjected to electrophoresis at 75 V through 1.5 % agarose gels (Sigma, St. Louis, MO) for 45 min. The pBR322 DNA digested with Alu I was used for molecular markers (MBI Fermentas). Agarose gels were stained with 0.5 μg/ml ethidium bromide (Sigma, St. Louis, MO) TBE buffer.

2.6. Statistical analysis

The TNF-α mRNA level was normalized to GAPDH mRNA level in the same samples. The TNF-α mRNA level

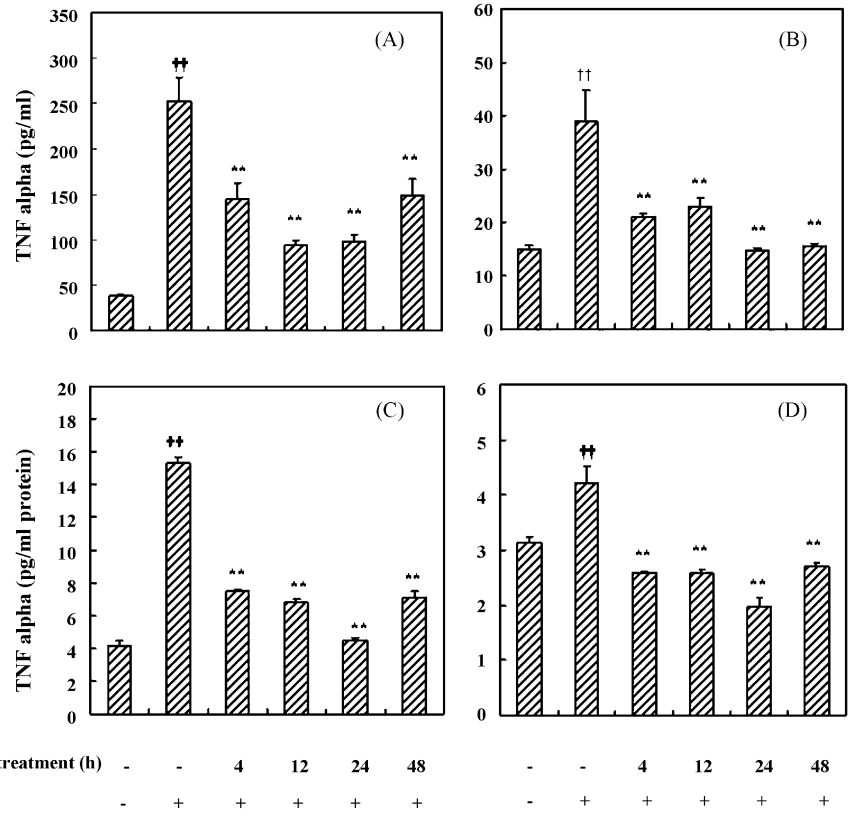

Fig. 1. The effects of low-dose LPS pretreatment on LPS-evoked TNF-α in maternal serum, amniotic fluid, fetal liver and brain. The pregnant mice were pretreated with low-dose LPS (10 μg/kg, i.p.) at different time point (4, 12, 24, 48 h) before LPS (500 μg/kg, i.p.). TNF-α was measured in maternal serum, amniotic fluid, fetal liver and brain at 1.5 h after LPS. (A) TNF-α in maternal serum; (B) TNF-α in amniotic fluid; (C) TNF-α in fetal liver; (D) TNF-α in fetal brain. Data were expressed as means ± S.E.M. ($n = 12$). (††) $P < 0.01$ as compared with control group. (**) $P < 0.01$ as compared with LPS group.

of the control was assigned as 100%. Quantified data were expressed as means ± S.E.M. at each point. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences between the treated animals and the control. Differences were considered to be significant only for $P < 0.05$.

3. Results

3.1. Effects of LPS on TNF-α in maternal serum

The effects of LPS on TNF-α in maternal serum are analyzed. In response to LPS challenge, TNF-α in maternal serum were obviously increased 1.5 h after LPS administration (38.58 ± 1.28 pg/ml versus 252.62 ± 25.82 pg/ml, $P < 0.01$). When administered 4, 12, 24 or 48 h before high-dose LPS, low-dose LPS pretreatment significantly attenuated LPS-evoked elevation of TNF-α in maternal serum (Fig. 1A).

3.2. Effects of LPS on TNF-α in amniotic fluid

The effects of LPS on TNF-α in amniotic fluid are analyzed. Results showed that TNF-α in amniotic fluid were obviously increased in response to LPS (14.72 ± 0.87 pg/ml versus 38.73 ± 5.93 pg/ml, $P < 0.01$). Low-dose LPS pretreatment significantly attenuated LPS-evoked elevation of TNF-α in amniotic fluid (Fig. 1B).

3.3. Effects of LPS on TNF-α in fetal liver

The effects of LPS on TNF-α in fetal liver were analyzed. As shown in Fig. 1C, maternally-administered LPS significantly increased the levels of TNF-α in fetal liver (4.17 ± 0.31 pg/mg protein versus 15.29 ± 0.36 pg/mg protein, $P < 0.01$). However, no significant difference in TNF-α mRNA level in fetal liver was observed between LPS-treated mice and the control (Fig. 2). The effects of low-dose LPS pretreatment on TNF-α in fetal liver were analyzed. When administered 4, 12, 24 or 48 h before high-dose LPS, low-dose LPS pretreatment significantly attenuated LPS-induced increase in TNF-α in fetal liver (Fig. 1C). However, no significant difference in TNF-α mRNA level in fetal liver was observed among different groups (Fig. 2).

3.4. Effects of LPS on TNF-α in fetal brain

The effects of LPS on TNF-α in fetal brain are presented in Fig. 1D. Results showed that maternally-administered LPS slightly increased TNF-α in fetal brain (3.14 ± 0.11 pg/mg protein versus 4.22 ± 0.30 pg/mg

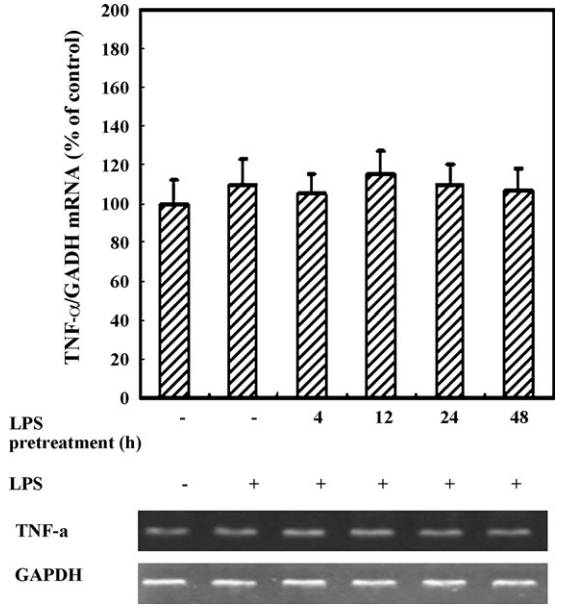

Fig. 2. The effects of maternally-administered LPS on TNF-α mRNA in fetal liver. The pregnant mice were administered with LPS (500 μg/kg, i.p.). Some pregnant mice were pretreated with low-dose LPS (10 μg/kg, i.p.) at different time point (4, 12, 24, 48 h) before LPS. TNF-α mRNA was measured at 1.5 h after LPS using RT-PCR. The TNF-α mRNA was normalized to GAPDH mRNA level in the same samples. The TNF-α mRNA level of the control was assigned as 100%. Data were expressed as means ± S.E.M. ($n = 4$).

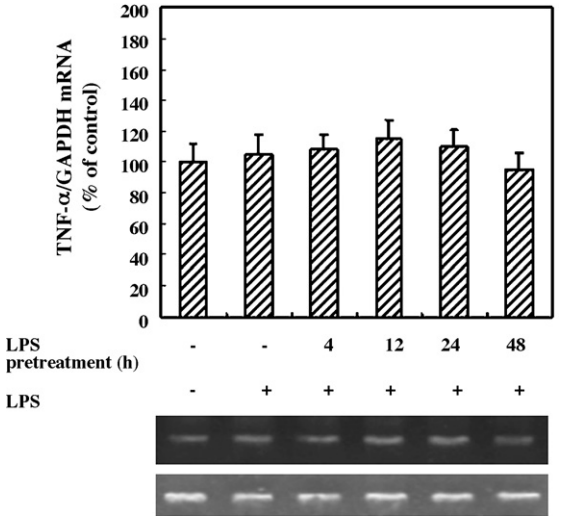

Fig. 3. The effects of maternally-administered LPS on TNF-α mRNA in fetal brain. The pregnant mice were administered with LPS (500 μg/kg, i.p.). Some pregnant mice were pretreated with low-dose LPS (10 μg/kg, i.p.) at different time point (4, 12, 24, 48 h) before LPS. TNF-α mRNA was measured at 1.5 h after LPS using RT-PCR. The TNF-α mRNA was normalized to GAPDH mRNA level in the same samples. The TNF-α mRNA level of the control was assigned as 100%. Data were expressed as means ± S.E.M. ($n = 4$).

protein, $P < 0.01$). However, no significant difference in TNF-α mRNA level in fetal brain was observed between LPS-treated mice and the control (Fig. 3). Furthermore, low-dose LPS pretreatment significantly attenuated LPS-induced elevation of TNF-α in fetal brain (Fig. 1D). However, low-dose LPS pretreatment had little effect on TNF-α mRNA in fetal brain (Fig. 3).

4. Discussion

In the LPS model, TNF-α is one of the major mediators leading to embryonic resorption, IUFD, IUGR and preterm delivery (Silver et al., 1995; Xu et al., 2006). The present study showed that an intraperitoneal injection of a single dose LPS on gd 17 obviously increased releases of TNF-α in maternal serum. TNF-α in amniotic fluid was also increased in response to maternally-administered LPS, whereas the magnitude of induction in amniotic fluid was lower than that observed in maternal serum. These results are in agreement with earlier work of Gayle et al. (2004), in which TNF-α were increased in maternal serum and amniotic fluid of LPS-treated pregnant rats. Several in vitro studies have demonstrated that LPS pretreatment inhibits subsequent LPS-evoked releases of proinflammatory cytokines in macrophage (Nomura et al., 2000; Martin et al., 2001). Therefore, it is especially interesting whether low-dose LPS pretreatment inhibits subsequent high-dose LPS-induced release of TNF-α in maternal serum and amniotic fluid. In the present study, the pregnant mice were pretreated with low-dose LPS (10 μg /kg, i.p.) at 4, 12, 24 or 48 h before high-dose LPS (500 μg/kg, i.p.). Interestingly, low-dose LPS pretreatment at different time point significantly attenuated LPS-evoked elevation of TNF-α in maternal serum and amniotic fluid.

In the present study, we investigated the effect of maternally-administered LPS on TNF-α in fetal liver. We showed that the levels of TNF-α in fetal liver were significantly increased 1.5 h after maternal LPS exposure. Indeed, Kupffer cells are present in fetal liver. A recent study found that murine fetal liver expresses high levels of TLR-4 mRNA (Harju et al., 2001). In vitro studies have demonstrated that fetal Kupffer cells secret TNF-α in response to LPS (Kutteh et al., 1991). However, it remains controversial whether LPS injected into mothers can pass through placenta to fetuses. An earlier study showed that an injection with 125I-labeled LPS into the pregnant mice resulted in considerable levels of radioactivity in fetuses (Kohmura et al., 2000). By contrast, a recent study found that maternally-administered LPS could not pass through rat placenta to fetuses (Ashdown et al., 2006). To determine whether TNF-α are stimulated directly by LPS or indirectly by cytokines produced from the maternal side or are transferred from the maternal circulation or amniotic fluid or placenta, we measured TNF-α mRNA level in fetal liver. Results showed that no significant difference in TNF-α mRNA level in fetal liver was observed among different groups. These results suggest that maternally-administered LPS can not stimulate directly the expression of TNF-α mRNA in fetal liver. The increased TNF-α protein in fetal liver may be transferred from either the maternal circulation or amniotic fluid or placenta.

Maternal infections with bacterial or viral agents during pregnancy are associated with an increased incidence of schizophrenia in the offspring at adulthood. Previous studies have demonstrated that TNF-α contributes to LPS-induced fetal neurodevelopmental abnormality (Bell and Hallenbeck, 2002). An in vitro study showed that LPS-activated microglia-derived TNF-α inhibited the neuronal differentiation and induced neuronal cell death in the embryonic neural progenitor culture (Liu et al., 2005). However, it remains controversial whether maternally-administered LPS stimulates the release of TNF-α in fetal brain. Several studies showed that maternal LPS exposure did not increase the expression of TNF-α in fetal brain (Fan et al., 2002; Urakubo et al., 2001). In the present study, we investigated the effect of maternally-administered LPS on TNF-α in fetal brain. We showed that TNF-α in fetal brain were significantly increased in response to maternal LPS challenge. These results are in agreement with a recent study, in which TNF-α was increased five-fold in fetal brain in response to maternally administered LPS (Bell et al., 2004). To determine whether TNF-α are stimulated directly by LPS or indirectly by cytokines produced from the maternal side or are transferred from the maternal circulation or amniotic fluid or placenta, we measured TNF-α mRNA level in fetal brain. Results showed that no significant difference in TNF-α mRNA level in fetal brain was observed among different groups. These results suggest that maternally-administered LPS can not stimulate directly the expression of TNF-α mRNA in fetal brain. The increased TNF-α protein in fetal brain may be transferred from either the maternal circulation or amniotic fluid or placenta.

In the present study, we also investigated the effect of low-dose LPS pretreatment on high-dose LPS-induced TNF-α in fetal liver and fetal brain. When the pregnant mice were intraperitoneally injected with a low-dose LPS 4, 12, 24 or 48 h before high-dose LPS, LPS-induced elevation of TNF-α in fetal brain was significantly attenuated. Furthermore, low-dose LPS pretreatment also

significantly attenuated LPS-induced elevation of TNF-α in fetal liver. Interestingly, we found that low-dose LPS pretreatment had little effect on TNF-α mRNA expression in fetal liver and brain. These results suggest that the effect of low-dose LPS pretreatment on high-dose LPS-induced TNF-α in fetal liver and fetal brain may be due to the decreased TNF-α level in the maternal circulation or amniotic fluid or placenta.

In summary, the present study indicates that maternal infection increases the releases of TNF-α in maternal serum and amniotic fluid, which may contribute to LPS-induced IUFD, IUGR and preterm labor. Importantly, maternal LPS exposure increased TNF-α in fetal liver and fetal brain. However, no significant difference in TNF-α mRNA level in fetal liver and brain was observed among different groups, suggesting that maternally-administered LPS can not stimulate directly the expression of TNF-α mRNA in fetal liver and brain. The increased TNF-α protein in fetal liver and brain may be transferred from either the maternal circulation or amniotic fluid or placenta. Interestingly, we found that low-dose LPS pretreatment inhibits LPS-evoked release of TNF-α in maternal serum and amniotic fluid, fetal liver and fetal brain. These results suggest that perinatal exposure to low-dose LPS induces a reduced sensitivity to subsequent LPS challenge. Therefore, further studies are vital to explore whether pre-exposure to low-dose LPS attenuates subsequent LPS-induced developmental toxicity.

Acknowledgements

The project was supported by National Natural Science Foundation of China (30371667, 30572223, 30671786) and Anhui Provincial Natural Science Foundation (050430714).

References

Ashdown, H., Dumont, Y., Ng, M., Poole, S., Boksa, P., Luheshi, G.N., 2006. The role of cytokines in mediating effects of prenatal infection on the fetus: implications for schizophrenia. Mol. Psychiat. 11, 47–55.

Bell, M.J., Hallenbeck, J.M., 2002. Effects of intrauterine inflammation on developing rat brain. J. Neurosci. Res. 70, 570–579.

Bell, M.J., Hallenbeck, J.M., Gallo, V., 2004. Determining the fetal inflammatory response in an experimental model of intrauterine inflammation in rats. Pediatr. Res. 56, 541–546.

Chen, Y.H., Wang, J.P., Wang, H., Sun, M.F., Wei, L.Z., Wei, W., Xu, D.X., 2005. Lipopolysaccharide treatment downregulates the expression of the pregnane X receptor, cyp3a11 and mdr1a genes in mouse placenta. Toxicology 211, 242–252.

Collins, J.G., Smith, M.A., Arnold, R.R., Offenbacher, S., 1994. Effects of *Escherichia coli* and *Porphyromonas gingivalis* lipopolysaccharide on pregnancy outcome in the golden hamster. Infect. Immun. 62, 4652–6455.

Erroi, A., Fantuzzi, G., Mengozzi, M., Sironi, M., Orencole, S.F., Clark, B.D., Dinarello, C.A., Isetta, A., Gnocchi, P., Giovarelli, M., 1993. Differential regulation of cytokine production in lipopolysaccharide tolerance in mice. Infect. Immun. 61, 4356–4359.

Fan, J., Kapus, A., Marsden, P.A., Li, Y.H., Oreopoulos, G., Marshall, J.C., Frantz, S., Kelly, R.A., Medzhitov, R., Rotstein, O.D., 2002. Regulation of toll-like receptor 4 expression in the lung following hemorrhagic shock and lipopolysaccharide. J. Immunol. 168, 5252–5259.

Fukui, H., Brauner, B., Bode, J.C., Bode, C., 1991. Plasma endotoxin concentrations in patients with alcoholic and non-alcoholic liver disease: reevaluation with an improved chromogenic assay. J. Hepatol. 12, 162–169.

Gayle, D.A., Beloosesky, R., Desai, M., Amidi, F., Nunez, S.E., Ross, M.G., 2004. Maternal LPS induces cytokines in the amniotic fluid and corticotropin releasing hormone in the fetal rat brain. Am. J. Physiol. Regul. Integr. Comp. Physiol. 286, 1024–1029.

Golan, H.M., Lev, V., Hallak, M., Sorokin, Y., Huleihel, M., 2005. Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. Neuropharmacology 48, 903–917.

Gross, G., Imamura, T., Vogt, S.K., Wozniak, D.F., Nelson, D.M., Sadovsky, Y., Muglia, L.J., 2000. Inhibition of cyclooxygenase-2 prevents inflammation-mediated preterm labor in the mouse. Am. J. Physiol. Regul. Integr. Comp. Physiol. 278, 1415–1423.

Harju, K., Glumoff, V., Hallman, M., 2001. Ontogeny of toll-like receptors Tlr2 and Tlr4 in mice. Pediatr. Res. 49, 81–83.

Jacob, A.L., Goldberg, P.K., Bloom, N., Degenshein, G.A., Kozinn, P.J., 1997. Endotoxin and bacteria in portal blood. Gastroenterology 72, 1268–1270.

Kohmura, Y., Kirikae, T., Kirikae, F., Nakano, M., Sato, I., 2000. Lipopolysaccharide (LPS)-induced intra-uterine fetal death (IUFD) in mice is principally due to maternal cause but not fetal sensitivity to LPS. Microbiol. Immunol. 44, 897–904.

Kutteh, W.H., Rainey, W.E., Carr, B.R., 1991. Glucocorticoids inhibit lipopolysaccharide-induced production of tumor necrosis factor-alpha by human fetal Kupffer cells. J. Clin. Endocrinol. Metab. 73, 296–301.

Liu, Y.P., Lin, H.I., Tzeng, S.F., 2005. Tumor necrosis factor-alpha and interleukin-18 modulate neuronal cell fate in embryonic neural progenitor culture. Brain Res. 1054, 152–158.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Martin, M., Katz, J., Vogel, S.N., Michalek, S.M., 2001. Differential induction of endotoxin tolerance by lipopolysaccharides derived from *Porphyromonas gingivalis* and *Escherichia coli*. J. Immunol. 167, 5278–5285.

Medvedev, A.E., Kopydlowski, K.M., Vogel, S.N., 2000. Inhibition of lipopolysaccharide-induced signal transduction in endotoxin-tolerized mouse macrophages: dysregulation of cytokine, chemokine, and toll-like receptor 2 and 4 gene expression. J. Immunol. 164, 5564–5574.

Nomura, F., Akashi, S., Sakao, Y., Sato, S., Kawai, T., Matsumoto, M., Nakanishi, K., Kimoto, M., Miyake, K., Takeda, K., Akira, S., 2000. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. J. Immunol. 164, 3476–3479.

Ogando, D.G., Paz, D., Cella, M., Franchi, A.M., 2003. The functional role of increased production of nitric oxide in lipopolysaccharide-induced embryonic resorption in mice. Reproduction 125, 95–110.

Rousset, C.I., Chalon, S., Cantagrel, S., Bodard, S., Andres, C., Gressens, P., Saliba, E., 2006. Maternal exposure to LPS induces hypomyelination in the internal capsule and programmed cell death in the deep gray matter in newborn rats. Pediatr. Res. 59, 428–433.

Silver, R.M., Edwin, S.S., Trautman, M.S., Simmons, D.L., Branch, D.W., Dudley, D.J., Mitchell, M.D., 1995. Bacterial lipopolysaccharide-mediated fetal death. Production of a newly recognized form of inducible cyclooxygenase (COX-2) in murine decidua in response to lipopolysaccharide. J. Clin. Invest. 95, 725–731.

Urakubo, A., Jarskog, L.F., Lieberman, J.A., Gilmore, J.H., 2001. Prenatal exposure to maternal infection alters cytokine expression in the placenta, amniotic fluid, and fetal brain. Schizophr. Res. 47, 27–36.

Xu, D.X., Chen, Y.H., Wang, H., Zhao, L., Wang, J.P., Wei, W., 2006. Tumor necrosis factor alpha partially contributes to lipopolysaccharide-induced intra-uterine fetal growth restriction and skeletal development retardation in mice. Toxicol. Lett. 163, 20–29.

Yan, E., Castillo-Melendez, M., Nicholls, T., Hirst, J., Walker, D., 2004. Cerebrovascular responses in the fetal sheep brain to low-dose endotoxin. Pediatr. Res. 55, 855–863.