Maternal exposure to endotoxin delays alveolarization during postnatal rat lung development

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Cao L, Wang J, Tseu I, Luo D, Post M. Maternal exposure to endotoxin delays alveolarization during postnatal development. Am J Physiol Lung Cell Mol Physiol 296: L726–L737, 2009. First published February 13, 2009; doi:10.1152/ajplung.90405.2008.—Maternal bacterial infections adversely affect lung development by crossing the placental barrier and infecting the developing fetus. The underlying mechanism negatively affecting pulmonary development remains unknown. Herein, we investigated whether a systemic maternal infection affects postnatal inflammation and alveolar development. Pregnant rats were injected with 2.5 mg/kg LPS on day 20 and 21 (term = 22 days). Postnatal (PN0–21) mRNA and protein expression of cytokines (IL-1β, IL-6, IL-10, CXCL1/2, TNFα) and genes implicated in alveologenesis [tropoelastin, lysyl oxidase (LOX), lysyl oxidase-like (LOXL)1, tenascin-C (TNC), fibulin 5, vascular endothelial growth factor (VEGF-A), VEGF receptor (VEGFR)2, VEGFR1, platelet-derived growth factor (PDGF)A, PDGFB, and PDGFRα] were quantified by real-time PCR and beadlyte technology. Lung transcript and protein levels of IL-1β, IL-6, and CXCL1/2 were significantly greater in LPS-exposed pups than those of control pups at PN0, 2, 6, 10, and 14. Bronchoalveolar lavage fluid (BALF) of LPS-exposed animals contained significantly more macrophages at PN2 and 14 than BALF of control pups. Morphometric analysis revealed that LPS-exposed animals had fewer and larger alveoli, fewer secondary septa, and decreased peripheral vessel density when compared with control pups. This morphological delay in alveolar development was significantly greater in LPS-exposed pups than those of control animals in PN2–14 lungs. TNC, LOX, fibulin 5, VEGFR1, PDGFα, and PDGFB expression was not affected by maternal LPS exposure. Together, the data demonstrate that maternal exposure to endotoxin results in a prolonged pulmonary inflammation postnatally, altered gene expression of molecules implicated in alveologenesis, and delayed morphological maturation of the lung.

antenatal endotoxin; pulmonary inflammation

MATERNAL INFECTIONS can adversely influence fetal development by crossing the feto-placental barrier and infecting the developing fetus (36, 47). Vaginal, cervical, and intrauterine infections are commonly associated with preterm deliveries (16), and preterm birth is a major contributor to neonatal lung injury (22). Chorioamnionitis has been shown to increase the risk for bronchopulmonary dysplasia (BPD) (19, 26) likely via a negative effect of inflammation on lung development (22). Maternal urinary tract (48), periodontal (2, 55), and respiratory infections (15) have also been associated with adverse pregnancy outcomes. Whether systemic maternal infections directly affect the newborn lung is unknown. Maternal infections can trigger an intrauterine immune response (1, 45) leading to the production of proinflammatory mediators (45, 56). Intra-amniotic fluid injection of endotoxin in sheep caused fetal lung inflammation (20, 39), which was associated with BPD-like morphological alterations, namely reduced vascularization and alveolar septation (21). Emerging evidence suggests that prenatal endotoxin exposure also adversely affects lung development in rodents. Amniotic injections of endotoxin in pregnant mice have been reported to disrupt distal lung development of the fetus (41). Unfortunately, the effect on alveolar formation was not investigated as mouse lungs alveolarize after birth. Another report showed that intra-amniotic injections of endotoxin in pregnant rats resulted in fewer and larger alveoli postnatally (52). Endotoxin-exposed alveoli had fewer secondary septa, suggesting an arrest of alveologenesis (52). The relationship between inflammatory mediators and alterations in lung morphology was, however, not investigated. In the present study, pulmonary inflammation and postnatal lung development were investigated in a rat model of systemic maternal infection during late pregnancy. Maternal exposure to bacterial endotoxin triggered a prolonged pulmonary inflammation and delayed alveolar development, which was associated with altered expression of a number of genes implicated in alveologenesis.

MATERIALS AND METHODS

Animals. Timed pregnant Wistar rats (Charles River, Oakville, Quebec, Canada) were randomly assigned to two different treatment strategies consisting of an intraperitoneal injection on fetal days 20 and 21 (term = 22 days) of either 2.5 mg/kg body wt of lipopolysaccharide (LPS) from Escherichia coli serotype 026:B6 (Sigma-Aldrich, St. Louis, MO) or the same volume of saline. On day 22 of pregnancy, pregnant rats from each group were killed by ether overdose, and lungs and liver of mother, placenta, fetal membranes, and lungs of postnatal days (PN)0 rat pups were recovered and processed for analysis. Other pregnant rats were allowed to deliver, and lungs were collected and processed at PN2, 6, 14, and 21. Postnatal pups also were killed by ether overdose. The weight of the pups was measured every day after delivery.

Institutional review. Approval for the study was obtained from the Animal Care Review Committee of the Hospital for Sick Children Research Institute. All animal procedures were conducted according to the guidelines of the Canadian Council for Animal Care.

RNA isolation and real-time RT-PCR. Placental and lung tissue were homogenized in TRIzol (Invitrogen Canada, Burlington, ON, Canada), and total RNA was extracted according to the manufacturer’s protocol. Total RNA was treated with DNase I (Invitrogen Canada) at 37°C to remove residual genomic DNA. Total RNA was reverse transcribed using Superscript II reverse transcriptase (Invitrogen Canada) and random hexamers (Applied Biosystems, Foster City, CA). Complementary DNA was quantified in a 7700 Sequence Detector.

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Amplification was performed with AmpliTaq Gold polymerase (Applied Biosystems) using TaqMan primers and probes (Applied Biosystems) for the target genes [IL-6, IL-1β, IL-10, CXCL2 (GRO2/MIP-2, macrophage inflammatory protein-2)], TNFα, tropoelastin (TE), and tenasin-C (TNC), as recently described (Ref. 10). (See Supplemental Table S1; supplemental data for this article is available online at the AJP-Lung web site.) Vascular endothelial growth factor (VEGF-A), VEGF receptor 2 (VEGFR2), lysyl oxidase (LOX), lysyl oxidase-like 1 (LOXL1), fibulin 5, and 18S were Assays on Demand from Applied Biosystems (Foster City, CA). VEGFR1, platelet-derived growth factor (PDGF)α, PDGFB, and PDGF receptor β (PDGFRβ) cDNA (see Supplemental Table S2) were quantified using SYBRGREEN (Applied Biosystems). For each probe, a dilution series determined the efficiency of amplification of each primer-probe set, allowing the relative quantification method to be employed (33). For the relative quantitation, PCR signals were compared among groups after normalization using 18S as an internal reference.

Western blot analysis. Lungs were homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS), and homogenates were centrifuged for 10 min at 10,000 g at 4°C. Supernatants were collected and stored at −80°C. Total protein content was measured according to Bradford (6). Samples (50 μg of protein) were subjected to 10% SDS-PAGE and transferred to PVDF membranes. Membranes were washed in TBST buffer (20 mM Tris, 137 mM NaCl, 0.1% Tween 20), blocked with 5% skim milk in TBST, and then incubated with anti-VEGF (dilution of 1:500, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. The next day, the membranes were washed in TBST and incubated for 1 h with goat anti-rabbit horseradish peroxidase-conjugated IgG (dilution of 1:1,000, Santa Cruz Biotechnology). After several washes with TBST, protein bands were visualized using an enhanced chemiluminescence detection kit (Amersham, Piscataway, NJ). Band densities were quantified using Scion Image software (version 1.6; National Institutes of Health, Bethesda, MD). Equal

Fig. 1. Dose-response of maternal endotoxin on IL-6 and CXCL2 mRNA expression in lungs of rat pups at birth. IL-6 and CXCL2 mRNA expression was quantified by real-time PCR. Data are means ± SD, n = 3 animals per control (●) and LPS (■) group. *P > 0.05 vs. control (saline-treated) group.

Fig. 2. Effect of endotoxin administration on cytokine/chemokine mRNA expression in maternal lung and liver at birth. IL-6, CXCL2, IL-1β, TNFα, and IL-10 mRNA expression was quantified by real-time PCR. Data are means ± SD, n = 6 animals per control and LPS group. *P > 0.05 vs. control (saline-treated) group.

Fig. 3. Maternal endotoxin exposure does not affect postnatal lung/body weight (L/BW) ratios. Data are means ± SD, n = 6 pups per control (●) and LPS (■) group.
protein loading was confirmed by β-actin immunoblotting of same membrane.

Cytokine protein measurement in lung tissue. Lungs were homogenized in RIPA cell lysis buffer (100 ml of PBS containing 0.05% wt/vol sodium azide, 2 mM phenylmethylsulfonyl fluoride, and 1 μg/ml aprotinin, leupeptin, and pepstatin A). Homogenates were centrifuged for 1 h at 23,000 g at 15°C. Supernatants were collected and stored at −80°C. Total protein content was measured according to Bradford (6). Cytokines/chemokines were measured in the tissue lysates using multiplex immunoassays for Luminex technology from Linco Research (St. Charles, MO) as previously described (43, 50).

Inflammatory cells in bronchoalveolar lavage. Bronchoalveolar lavage was performed through a catheter inserted into the trachea by a tracheotomy as recently described (50) and adapted for newborn animals (8). Briefly, the lungs were lavaged three times with 1 ml of PBS. Lavage fluid was centrifuged (400 g at 4°C for 10 min), the cell pellet was resuspended in 0.5 ml of PBS, and total cell numbers were determined using a cell counter (Beckman). To differentiate inflammatory cell types, cytospin smears were prepared and stained with Giemsa (Sigma, St. Louis, MO). At least 400 cells from each smear were counted under a light microscope. The proportion of each cell population was expressed as a percentage of total cells, and this ratio together with the total cell count was used to calculate the number of each cell type.

Histology. Whole lungs were infused in situ with 4% (wt/vol) paraformaldehyde (PFA) in PBS with a constant pressure of 20 cmH2O over 2 min to have equalized filling pressure over the whole lung. Under these constant pressure conditions, the cannula was
removed, and the trachea was immediately ligated. The lungs were excised and immersed in 4% PFA in PBS overnight, dehydrated in an ethanol/xylene series, and embedded in paraffin. Sections of 5 μm were stained with hematoxylin and eosin or stained for elastin using Accustain atrazine solution (Sigma, St. Louis, MO).

**Immunohistochemistry.** Immunostaining was performed using the avidin-biotin (ABC) immunoperoxidase method. Following sectioning, antigen retrieval, and endogenous peroxidase quenching and blocking with NGS/BSA, sections were incubated with 1:100 diluted rabbit anti-CD68 (Santa Cruz Biotechnology) or 1:100 diluted rabbit anti-MPO antibody (Thermo Lab Vision, Fremont, CA). Biotinylated goat anti-rabbit IgG (1:2,000) was used as secondary antibody. All sections were counterstained with hematoxylin.

**Morphometric analyses.** Digital images were captured from H&E-stained slides using a Leica digital imaging system at ×20 magnification with random sampling of all tissue in an unbiased fashion. Images were captured randomly from 10 non-overlapping fields from each slide, with 3 slides per animal and 6 animals per group. Morphometric assessments were performed on code images to mask the treatments. Mean linear intercepts (Lm) were measured and calculated as described by Dunnill (12). Ln values were used to calculate alveolar surface area per unit of lung volume (24). Tissue fraction and secondary crest volume density was measured as described previously (40). Total alveolar numbers were calculated as described by Weibel and Gomez (53). Slides were stained for elastin to enhance recognition of vessels, and number of small vessels (diameter range 20–65 μm) per field was assessed. Average values were calculated for 10 images per slide.

**Statistical analysis.** All data are expressed as means ± SD. For comparison between two groups, we used Student’s t-test. For comparison of the percentage of different inflammatory cells in bronchoalveolar lavage fluid (BALF), we used the Chi-square test. Statistical analyses were carried out using JMP statistical software, and significance was accepted at P < 0.05.

**RESULTS**

**LPS dosage.** To find an effective LPS dose able to trigger a systemic maternal inflammation without inducing premature delivery, we injected pregnant rats intraperitoneally (ip) with either 1, 2, 2.5, 3, or 5 mg/kg body wt of LPS (n = 3 animals per dose) on days 20 and 21 of pregnancy. Cytokine (IL-6) and chemokine (CXCL2) mRNA expression in placental and lung tissues was measured 24 h after final injection. Maternal injection of 2.5 mg/kg body wt of LPS resulted in a significant increase (2-fold) of IL-6 and CXCL2 mRNA expression in lungs of rat pups at birth (PN0) compared with control tissues of rats treated with lower dosages of LPS or saline (Fig. 1). This dosage of LPS neither induced premature delivery nor increased the number of stillborns; outcomes noted with larger dosages of LPS. Clinical signs of distress including rapid breathing, listlessness, and ruffled fur were noted in most pregnant rats injected with 2.5 mg/kg body wt of LPS. These signs disappeared 24–36 h after the second injection. This dosage of LPS also increased IL-6, IL-1β, TNFα, and CXCL2 mRNA expression in the liver and lungs of the mother at term (Fig. 2), in agreement with LPS triggering a systemic maternal inflammation. Thus, to simulate a systemic maternal infection, we administered 2.5 mg/kg body wt of LPS on days 20 and 21 of pregnancy in all the following experiments.

**Placental, lung, and body weights.** Placental weights were similar between control (saline injected) and LPS-treated animals (data not shown). No structural abnormalities were observed between placentae from LPS-treated and control animals. Although postnatal (PN0–21) lung and body weights were slightly decreased in LPS-exposed rat pups, lung/body
weight ratios were not different between LPS-exposed and control animals (Fig. 3). Similar results were obtained when the LPS-exposed pups were nursed by control (saline treated) dams (data not shown). Thus, maternal exposure to LPS did not significantly affect placental weight/morphology and postnatal lung growth.

**Cytokine/chemokine mRNA expression in placenta and lung.** Maternal exposure to LPS significantly increased placental mRNA expression of IL-6, CXCL2, IL-1β, and TNFα, but not IL-10, at term (Fig. 4). LPS exposure also increased the message levels of IL-6, CXCL2, IL-1β, and TNFα of fetal placental membranes (not shown). Pulmonary levels of IL-6, CXCL2, and IL-1β mRNA of LPS-exposed animals were significantly greater than those of control (saline) pups at PN0, 2, 6, and 14 (Fig. 4). Maternal LPS exposure slightly increased TNFα mRNA expression at PN6 and PN14, whereas pulmonary IL-10 gene expression was unaffected by maternal LPS treatment. No discernable differences in gene expression of all tested cytokines/chemokines were observed between lungs of maternal LPS or saline-exposed pups at PN21 (Fig. 4).

**Cytokine/chemokine protein expression in placenta and lung tissue.** Because of a lack of a good rat CXCL2 (GRO2/MIP2) detection kit for the Luminex system, a closely related chemokine, CXCL1 (GRO1/KC), was measured. CXCL1 belongs to the glutamyl-leucyl-arginine (ELR) motif containing CXC chemokine subfamily and also has a chemoattractant effect on monocytes and neutrophils. Placental IL-6 and CXCL1 protein levels were significantly increased in LPS-exposed animals compared with saline-injected controls (Fig. 5). Similarly, IL-6, CXCL1, and IL-1β proteins were increased in lungs of LPS-exposed animals at PN0, 2, and 14 (Fig. 5). TNFα and IL-10 protein levels were not altered by maternal LPS exposure.

**Table 1. Inflammatory cells in bronchoalveolar lavage fluid**

<table>
<thead>
<tr>
<th>Gestation</th>
<th>Treatment</th>
<th>Total Cell (10^6 cells/ml)</th>
<th>%Macrophages</th>
<th>%Lymphocytes</th>
<th>%PMNs</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN2</td>
<td>Saline</td>
<td>0.15±0.01</td>
<td>32.6</td>
<td>65.4</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>0.23±0.02*</td>
<td>62.6*</td>
<td>36*</td>
<td>0.76</td>
</tr>
<tr>
<td>PN14</td>
<td>Saline</td>
<td>0.16±0.03</td>
<td>35.5</td>
<td>60.3</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>0.22±0.04*</td>
<td>74.3*</td>
<td>23.3*</td>
<td>0.38</td>
</tr>
<tr>
<td>PN21</td>
<td>Saline</td>
<td>0.13±0.02</td>
<td>92.51</td>
<td>7.35</td>
<td>0.019</td>
</tr>
<tr>
<td></td>
<td>LPS</td>
<td>0.16±0.03</td>
<td>91.47</td>
<td>8.33</td>
<td>0.020</td>
</tr>
</tbody>
</table>

Means ± SD; n = 6 pups per group. *P < 0.05. PMNs, polymorphonuclear granulocytes; PN, postnatal day.
Inflammatory cells in lung tissue. Lung sections were stained for macrophages (CD68 antigen) and neutrophils (MPO). The number of CD68- and MPO-positive cells was increased in lungs of LPS-exposed animals compared with saline-injected controls at PN2 and 14 (Fig. 6).

Inflammatory cells in BALF. The majority of inflammatory cells in BALF of control pups at PN2 and PN14 were lymphocytes (>60%) and alveolar macrophages (>30%). Less than 1% were polymorphonuclear cells (PMNs) (Table 1). Maternal exposure to LPS increased the total number of inflammatory cells in the BALF by 50 to 37% at PN2 and PN14, respectively. LPS exposure significantly increased the number of macrophages in BALF at PN2 and PN14 compared with BALF of control pups. Surprisingly, no significant differences in the number of PMNs between LPS-exposed and control animals were observed. At PN21, the amount of total inflammatory cells was similar in BALF of LPS-exposed and control animals, and 92% of the cells were alveolar macrophages (Table 1).
Morphometric analyses of postnatal alveolar development. Histologically, postnatal (PN2-PN14) lungs of rats exposed to maternal LPS appeared less mature with fewer and larger alveoli (Fig. 7). There was no obvious evidence of alveolar destruction, cell infiltration of alveoli, intra-alveolar edema, or fibrosis. Morphometric analyses revealed that maternal LPS exposure decreased the number of alveoli per unit area at PN2, 6, and 14 compared with control pups (Table 2). To quantitatively assess alveolar development, we calculated the secondary crest number/field and the secondary crest/tissue ratio. Secondary crest counts per unit area were significantly lower in prenatal LPS-exposed pups vs. control pups on PN2, 6, and 14, even when corrected per tissue fraction. These data suggest diminished secondary crest formation. To further evaluate alveolar development, we measured mean linear intercept and alveolar surface area per unit of lung volume. As anticipated, the Lm decreased with advancing postnatal gestation in both LPS-exposed and control animals (Table 2). However, pups exposed to maternal LPS had a greater Lm at PN2, 6, and 14 compared with control pups. Also, prenatal LPS exposure resulted in a significantly reduced alveolar surface density compared with control pups at PN2, 6, and 14. These findings are consistent with a delayed alveolar formation in LPS-exposed animals. Interestingly, the density of small vessels (diameter range 20–65 µm) in the lungs of animals exposed to prenatal LPS was also significantly lower than that of control pups at PN2, 6, and 14 (Table 2). No alveolar morphometric differences were noted between LPS-exposed and control pups at PN21.

Expression of factors implicated in alveolar development. To evaluate the effect of the maternal LPS exposure on postnatal lung development, we also analyzed the mRNA expression of factors known to have an impact on alveolar development (43). Expression of TE, LOXL1, VEGF-A, VEGFR2, and PDGFRα mRNA was significantly greater in lungs of animals exposed to prenatal LPS than those of control animals at PN2, 6, and 14, but not at PN21 (Figs. 8 and 9). Western blot analysis revealed increases in VEGF protein content in lungs of maternal LPS-exposed pups at PN0, 2, and 14, in agreement with elevated mRNA expression (Fig. 10). No differences in gene expression were noted for TNC, fibulin 5, LOX, VEGFR1, PDGFA, and PDGFB (Figs. 8 and 9). Elastin staining did reveal minor changes in elastin deposition and arrangement in the lungs of LPS- vs. saline-exposed pups at PN14 (Fig. 11). Elastin content appeared to be increased in the lungs of LPS-exposed rat pups, and elastin fibers displayed a wider alveolar distribution, i.e., not only at the septal tips as seen in the saline (control) pups.

DISCUSSION

Clinical and experimental studies indicate that maternal infection can result in neonatal lung injury, which has led to the idea that prenatal inflammation may affect lung development. In the present study, we examined the effects of systemic maternal infection (maternal LPS exposure) on postnatal lung development in rats. Although it is disputed whether transplacental transfer of LPS occurs in rodents (3, 25), we observed that maternal LPS administration increased the mRNA levels of IL-6, CXCL2, IL-1β, and TNFα in the placenta and fetal placental membranes at term. Placental IL-6, IL-1β, and CXCL1 protein levels were also increased, suggesting that these cytokines are part of the inflammatory response in intrauterine tissues. In addition, LPS exposure significantly increased the number of macrophages in the placenta (not shown). At term, lung tissue of rat pups from LPS-treated mothers had significantly greater IL-6, CXCL1, CXCL2, and IL-1β transcript and protein levels compared with pups from saline-injected mothers. These data suggest that the onset of lung inflammation in the LPS-exposed offspring started before birth.

Most interestingly, we found that transcript and protein levels of IL-6, CXCL1, CXCL2, and IL-1β remained elevated in the lungs of maternal LPS-exposed pups until at least PN14. Moreover, maternal exposure to LPS resulted in substantially higher numbers of inflammatory cells at PN2 and PN14. Full-term rats are born with their lungs in the saccular phase of development, which corresponds to a human lung at 26–28 wk of gestation. Emerging evidence suggests that postnatal rat lung alveolarization takes place in two phases (46), phase one (days 4–21), lifting off of new septa from immature preexisting septa, and phase two (days 14–60), formation of septa from mature preexisting septa. The first phase corresponds to ∼35–40 wk of gestation in the human lung (5, 44, 46). Thus, inflammatory mediators and cells were increased in lungs of maternal LPS-exposed rat pups at the time of maximal alveolar formation, i.e., lifting off of new septa from immature preexisting septa. Studies using transgenic mice have shown that inflammatory cytokines can interfere with postnatal alveolar development. Perinatal expression of IL-1β in the airway epithelium resulted in disrupted alveolar septation and elastin deposition in the septa of distal air spaces (11). In the present...
study, we observed a delayed first phase alveolarization in maternal LPS-exposed pups up to the end of increased lung inflammation (PN14). We did not see any obvious (visible) signs of alveolar destruction. Instead, the lungs were morphologically immature, with fewer secondary septae and fewer and larger alveoli, implying that maternal LPS exposure reduced the rate of alveolarization. Although other studies have reported that intra-amniotic LPS administration inhibits postnatal lung development (21, 22, 41, 52), few of them have assessed the effect of maternal LPS exposure on lung inflammation and growth during the alveolarization period. Prolonged fetal exposure to intra-amniotic LPS has been shown to cause a mild persistent inflammation without major structural abnormalities in lungs of fetal sheep (23). Similarly, we observed that the inhibitory effect of systemic maternal inflammation on postnatal alveolarization in rats was transient and that there is catch-up alveolar growth.

Our longitudinal study enabled us to assess expression of factors implicated in alveolar development, including extracellular matrix-related and vascular genes. In the developing lung, elastic fibers are assembled in the extracellular matrix as the lung undergoes transition from the saccular to the alveolar stage of development. In the lung, the synthesis of the soluble precursor of elastin, the tropoelastin (TE) monomer, occurs primarily during late fetal and early postnatal life (i.e., at the time of secondary septa development), ceasing after maturation of the lung is complete (7). Indeed, young mice with the elastin-null mutation fail to form alveolar crests (54). In the present study, we found that prenatal LPS exposure upregulated TE mRNA expression in postnatal rat lung tissue up to PN14. The integrity of extracellular matrix (ECM) in the lung is largely dependent on the conversion of collagen and elastin to insoluble, cross-linked fibers, a process catalyzed by LOX. Mammalian genomes have up to five potential LOX family
members encoding the prototypic LOX and four LOX-like proteins (LOXL1, LOXL2, LOXL3, and LOXL4) (35). Their individual roles in elastogenesis remains unclear, but knock-out mice deficient in LOX (35) or LOXL1 (32) cannot properly crosslink collagen or elastin, which results in abnormal alveolar development. We found an increased mRNA expression for LOXL1, but not LOX, in maternal LPS-exposed pups starting at PN0 and persisting until PN14. Maternal LPS exposure had no effect on expression of fibulin 5, a scaffold molecule essential for elastin fiber assembly (27, 38), and tenascin-C, a multidomain ECM protein implicated in early lung branching (42) and the formation of new alveolar septae (38). Although we did not perform quantitative image analysis, some minor changes in elastin deposition in the alveolar region were noted. However, it is yet unclear whether aberrant TE and LOXL1 expression contribute to the delayed alveolarization seen in pups exposed to maternal LPS.

Ample studies have shown the importance of lung vascular growth for lung development. VEGF is a potent inducer of endothelial cell growth that acts on vascular endothelial cells through two different receptors, VEGFR1 and VEGFR2 (13). Inhibition of VEGF signaling has been shown to impair fetal (14, 17) and postnatal (28, 51) rat lung development. Moreover, VEGF gene therapy has been shown to improve vascular development, thereby preventing hyperoxia-induced alveolar arrest in newborn rats (51). A recent study demonstrated that antenatal lung inflammation inhibits endothelial cell protein expression, including VEGF and VEGFR2, followed by vascular remodeling changes in small pulmonary arteries (21). In contrast, we observed that VEGF-A and VEGFR2, but not VEGFR1, mRNA expression was significantly greater in lungs of pups exposed to prenatal endotoxin than that of control animals. Also, VEGF protein expression was increased in the lungs of maternal LPS-exposed pups. However, the density of small vessels (20–65 μm) was significantly decreased in lungs.

Fig. 9. VEGF, VEGFR2, and PDGFRα mRNA expression in neonatal lungs of antenatal endotoxin-exposed animals. Angiogenic molecules implicated in alveologenesis (25) were quantified by real-time PCR. Data are means ± SD, n = 6 animals per control (gray bars) and LPS (black bars) group. *P < 0.05 vs. control (saline-treated) group.
of maternal LPS-exposed pups compared with control animals. We did not investigate the expression of the individual VEGF-A isoforms (VEGF120, VEGF164, VEGF188). Both ECM-bound isoforms of VEGF (VEGF164 and 188) are important for the formation of distal lung vasculature (14), and it is possible that LPS treatment particularly induced the expression of the VEGF120 isoform while decreasing those of VEGF164 and 188. In addition, we did not investigate other crucial angiogenic factors such as angiopoietins, ephrins, and their receptors.

There is increasing evidence that PDGF-A/B and its receptors (PDGFRα, PDGFRβ) play a significant role in lung development and blood vessel formation (18). Specifically, it has been shown that loss of PDGF-A signaling results in failure of alveologenesis (4, 30). Surprisingly, overexpression of PDGF-A in the lung epithelium is lethal due to a delay in fetal lung development (29). Exposure of rat lung myofibroblast to LPS has been shown to upregulate PDGFRα expression (9), in agreement with our finding of increased PDGFRα mRNA expression in lungs of maternal LPS-exposed rats. The increase in PDGFRα mRNA expression may also be due to elevated IL-1β levels, since IL-1β has been shown to induce PDGF in lung myofibroblasts (31). The resulting alteration in PFGFA/PDGFRα signaling in LPS-exposed pups may affect proper alveolar formation.

In summary, a systemic maternal inflammation induced by endotoxin in rats results in a pulmonary inflammation postnatally, altered gene expression of matrix and angiogenic molecules implicated in alveologenesis, and delayed alveolar maturation.

GRANTS

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Fig. 10. Maternal endotoxin increases VEGF protein content in neonatal lungs. VEGF protein was quantified in PN0, 2, and 14 lungs by Western blotting. A: densitometric analysis of blots. Data are means ± SD, n = 4 animals per control (gray bars) and LPS (black bars) group. *P > 0.05 vs control (saline-treated) group. B: representative immunoblot of PN14 lungs.

Fig. 11. Effect of maternal endotoxin exposure on elastin deposition in the neonatal lung. Shown are representative photomicrographs of elastin-stained lung sections at PN14. Black color indicates elastin fibers. Arrows indicate elongated alveolar distribution; ×400 magnification.

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