LPS-induced chorioamnionitis and antenatal corticosteroids modulate Shh signaling in the ovine fetal lung

Jennifer J. P. Collins,1* Elke Kuypers,1* Ilias Nitsos,2 J. Jane Pillow,2 Graeme R. Polglase,2 Matthew W. Kemp,2 John P. Newnham,2 Jack P. Cleutjens,3 Suzanna G. M. Frints,4 Suhas G. Kallapur,2,5 Alan H. Jobe,2,5 and Boris W. Kramer1,5

Department of Pediatrics, School for Oncology and Developmental Biology, School for Mental Health and Neuroscience, Maastricht University Medical Center, Maastricht, The Netherlands; 2School of Women’s and Infants’ Health, The University of Western Australia, Perth, Western Australia, Australia; 3Department of Pathology, Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, The Netherlands; 4Department of Clinical Genetics, Prenatal Diagnosis and Therapy, School for Oncology and Developmental Biology, Maastricht University Medical Center, Maastricht, The Netherlands; and 5Cincinnati Children’s Hospital Medical Center, University of Cincinnati, Cincinnati, Ohio

Submitted 24 August 2011; accepted in final form 28 August 2012

BRONCHOPULMONARY DYSPLASIA (BPD), a disease of impaired lung development, is the most common adverse lung outcome of preterm birth (2, 30). BPD is associated with fetal lung inflammation, which can be initiated by chorioamnionitis, an intrauterine bacterial infection of the placental membranes and amniotic fluid that is often clinically silent (21). Chorioamnionitis can induce a potentially harmful inflammatory response in the immature fetal lungs that disrupts lung septation and vascular development, leading to a decreased lung surface area (16).

Antenatal corticosteroids are given to mothers at risk of imminent preterm birth to induce lung maturation in the fetus, which increases neonatal survival but does not decrease BPD (4, 14). Because the incidence of chorioamnionitis is ∼60% for very preterm babies, the administration of maternal antenatal corticosteroids in the presence of chorioamnionitis is common and standard of care (3). Although antenatal corticosteroids cause functional lung maturation, they also can inhibit lung development (54). As a result, a large number of premature infants are exposed in utero to both pro- and anti-inflammatory stimuli that each alter normal fetal lung development and might predispose the infants to the development of BPD (10). The molecular mechanisms by which chorioamnionitis and antenatal corticosteroids influence these lung developmental processes are largely unknown.

Sonic Hedgehog (Shh) signaling is critical for lung development since Shh-null mice have hypoplastic lungs and die from respiratory failure (32). During lung development, Shh expression is localized to the epithelium and activates Gli transcriptional activators Gli1, Gli2, and Gli3 (5). The Shh pathway regulates the expression of lung growth factors such as fibroblast growth factor 10 (FGF10) and bone morphogenetic protein 4 (BMP4), which both mediate branching and myofibroblast differentiation (52).

We hypothesized that chorioamnionitis and/or antenatal corticosteroids modulate Shh signaling to alter fetal lung structural development. We evaluated this signaling pathway by lipo-polysaccharide (LPS)-induced chorioamnionitis in a 120-day gestational age (GA) preterm lamb model during a stage of early alveolar septation. Fetal sheep were exposed in utero to intra-amniotic LPS from gram-negative bacteria and/or antenatal betamethasone (Beta), a corticosteroid used clinically to induce lung maturation (28, 41). We correlated Shh signaling with markers for lung damage [heat shock protein 70 (HSP70)], cell proliferation (Ki67), and changes in the lung structural proteins elastin and collagen, which are crucial for alveolar septation (9, 25, 49).

*J. J. P. Collins and E. Kuypers contributed equally to this work.
Address for reprint requests and other correspondence: B. W. Kramer, Dept. of Pediatrics, Maastricht Univ. Medical Center, Postbus 5800, 6202 AZ Maastricht, The Netherlands (e-mail: b.kramer@umc.nl).
METHODS

Animal model and sampling protocol. All studies were approved by the Animal Ethics Committees at The University of Western Australia and Cincinnati Children’s Hospital Medical Center (animal ethics protocol RA/3/100/830). The experimental design of this study was published previously (29). Time-mated Merino ewes with singleton fetuses were randomly assigned to one of six treatment groups to receive an intra-amniotic injection of LPS (10 mg Escherichia coli 055:B5, Sigma Chemical, St. Louis, MO) and/or an intramuscular injection of Beta [Celestone Soluspan, Schering-Plough, North Ryde, New South Wales (NSW), Australia], Australia) at 100 days GA to prevent preterm birth induced by Betax treatment. Lambs were surgically delivered at 120 days GA (term = 150 days GA) and euthanized after birth. Lung tissue from the right lower lobe (RLL) was snap frozen, and the right upper lobe (RUL) was inflation fixed in 10% buffered formalin for 24 h.

RNA extraction and real-time PCR. Total RNA was extracted from frozen lung tissue of the RLL by using the SV Total RNA Isolation system (Z3100, Promega, Madison, WI) according to the manufacturer’s instructions. HSP70 protein concentration was determined using a bicinchoninic acid (BCA) protein assay. Total RNA was reverse transcribed for 30 s. Total RNA was used as a template. PCR reactions were performed with the First Strand cDNA synthesis kit (4379012001, Roche Applied Science, Mannheim, Germany) according to manufacturer’s instructions by using anchored oligo primers. Primers for real-time PCR (RT-PCR) were constructed based on published ovine or bovine cDNA sequences (Table 1). Dilution experiments were performed to ensure similar PCR amplification efficiency of the primers. RT-PCR reactions were performed in duplicate with the LightCycler 480 SYBR Green I Master mix (4707516001, Roche-Applied) on a LightCycler 480 Instrument according to the manufacturer’s instructions. RT-PCR results were normalized to cyclophilin A, a housekeeping gene, and mean fold changes in mRNA expression were calculated by the ΔΔCt method (33).

Protein extraction and ELISA of HSP70. Frozen RLL lung tissue was homogenized (PRO Quick Connect Generators part no. 02-07095; PRO Scientific, Oxford, CT) in ice-cold RIPA buffer (R0278, Sigma Aldrich) containing 0.1% protease inhibitors (p9599, Sigma Aldrich) and subsequently centrifuged at 12 × relative centrifugal force for 5 min at 4°C (31). Heat shock protein 70 (HSP70) was measured with an R&D DuoSet ELISA development kit (human/mouse/rat total HSP70: DYC1663, R&D Systems, Minneapolis, MN) according to manufacturer’s instructions. HSP70 protein concentrations were calculated per kilogram body weight.

Immunohistochemistry. Paraffin-embedded RUL lung sections (4 μm, transverse) were stained for Ki67 (M7240, DAKO, Glostrup, Denmark), Gli1 (ab49314, Abcam, Cambridge, UK) and BMP4 (sc-6896, Santa Cruz Biotechnology, Santa Cruz, CA). Briefly, the sections were deparaffinized in an ethanol series and endogenous peroxidase activity was blocked by incubation with 0.5% H2O2 in 1× PBS (pH 7.4). Antigen retrieval was performed by incubating the sections in heated citrate buffer (10 mM, pH 6.0) for 30 min. To block specific binding, the slides were incubated with 20% normal goat serum in PBS. Sections were incubated overnight at 4°C with the diluted primary antibody (Ki67 1:50, Gli1 1:500, BMP4 1:500). After incubation with the appropriate secondary antibody, immunostaining was performed with the appropriate secondary antibody, immunostaining was

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5′-3′)</th>
<th>Amplicon Size, bp</th>
<th>Tm</th>
<th>Accession Code (RefSeq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ColIA1</td>
<td>GAAGAGAGGACATGCACCAG</td>
<td>125</td>
<td>60°C</td>
<td>NM_001034039.1</td>
</tr>
<tr>
<td></td>
<td>GTCTTTAGCTGTGCAAGG</td>
<td>150</td>
<td>59°C</td>
<td>EF144225.1</td>
</tr>
<tr>
<td></td>
<td>GCCGCGGAGTTGCAGCAAAG</td>
<td>144</td>
<td>60°C</td>
<td>NM_175772.1</td>
</tr>
<tr>
<td></td>
<td>ACTCCAGAGCCGGGAGAGC</td>
<td>82</td>
<td>68°C</td>
<td>XM_614193.3</td>
</tr>
<tr>
<td></td>
<td>AGAACCCCTCAGCCACACAC</td>
<td>137</td>
<td>60°C</td>
<td>NM_001099000.1</td>
</tr>
<tr>
<td></td>
<td>GTGCGGCTTCTGATGAG</td>
<td>147</td>
<td>60°C</td>
<td>NM_001192250.1</td>
</tr>
<tr>
<td></td>
<td>AGGACCTCAGGAGGACAG</td>
<td>197</td>
<td>60°C</td>
<td>XM_002686986.1</td>
</tr>
<tr>
<td></td>
<td>ACCATTTAGTTCCGCTGGG</td>
<td>220</td>
<td>60°C</td>
<td>NM_001099230.1</td>
</tr>
<tr>
<td></td>
<td>TGGGGCTCATGAGAGAGAGAG</td>
<td>173</td>
<td>61°C</td>
<td>NM_001110277.1</td>
</tr>
<tr>
<td></td>
<td>TTATAAGGATTGCTCCTTCA</td>
<td>93</td>
<td>60°C</td>
<td>NM_178320.2</td>
</tr>
</tbody>
</table>

Tm, melt temperature; RefSeq, reference sequence.

Table 1. Primers used for RT-PCR

AJP-Lung Cell Mol Physiol • doi:10.1152/ajplung.00280.2011 • www.ajplung.org
enhanced with Vectastain ABC Peroxidase Elite kit (PK-6200, Vector Laboratories, Burlingame, CA) and stained with nickel sulfate-diaminobenzidine. Subsequently, the sections were rinsed in Tris-saline and incubated with Tris-cobalt. After being counterstained with 0.1% Nuclear Fast Red, the sections were washed, dehydrated, and coverslipped. All slides were stained at the same time under the same conditions.

Evaluation was performed by light microscopy (Axioskop 40, Zeiss, Germany) with LeicaQWin Pro v.3.4.0 software (Leica Microsystems, Germany). Alveolar Ki67 and Gli1 staining was scored by blinded observers with a semiquantitative scoring system: 1, little staining; 2, some staining; and 3, heavy staining. BMP4 staining was semiquantitatively scored in three representative bronchioli using Image J software (W. S. Rasband, Image J, US National Institutes of Health, Bethesda, MD) and represented as a percentage of the entire bronchiole surface area.

Elastin and collagen staining. Elastin and collagen stainings were performed each on four paraffin sections of the RUL per animal (4/4 H9262, transverse). For the visualization of elastin, the sections were deparaffinized in an ethanol series and incubated in Hart’s staining solution [70% ethanol, 10% Weigerts Resorcine-Fuchsine (2E 030, Fig. 1. Lung injury and cell proliferation. A: protein levels of heat shock protein 70 (HSP70) did not change in homogenates of LPS and/or betamethasone (Beta) exposed fetal lungs. B: the number of Ki67-positive cells in the alveoli increased after LPS exposure. Pretreatment and particularly posttreatment with Beta partially prevented this increase. Alveolar Ki67 expression in controls (C) and 7-day (7d) LPS-exposed animals (D). BW, body weight; 14d, 14-day. *P < 0.05 vs. controls by 1-way ANOVA with Tukey’s post hoc test.

Elastin and collagen staining. Elastin and collagen stainings were performed each on four paraffin sections of the RUL per animal (4 μm, transverse). For the visualization of elastin, the sections were deparaffinized in an ethanol series and incubated in Hart’s staining solution [70% ethanol, 10% Weigerts Resorcine-Fuchsine (2E 030,
Chroma, Münster, Germany) and 2% hydrochloric acid) overnight at room temperature. After rinse with water, the sections were incubated in 0.25% acetic acid for 3 min at room temperature. Subsequently the sections were washed and dehydrated. For the detection of collagen fibers, the sections were deparaffinized and incubated in 0.2% phosphomolybdic acid for 5 min. Sections were placed in a Sirius red solution for 90 min in the dark. After rinse with 0.01 M HCl for 3 min, the sections were washed, dehydrated, and coverslipped. Evaluation was performed by light microscopy (Zeiss, Axioskop 40) with LeicaQWin Pro v.3.4.0 software. The number of elastin foci and the percentage of collagen fibers in the total lung surface area were quantified by using four paraffin sections per animal, 12 representative images per section across septa at ×200 magnification by a blinded observer using specialized LeicaQWin Pro v.3.4.0 software.

**Data analysis.** Results are given as means ± SE. The groups were compared by one-way ANOVA with Dunnett’s or Tukey’s test for post hoc analysis as appropriate. Statistical analysis was performed by GraphPad Prism v5.0. Significance was accepted at P < 0.05.

**RESULTS**

**Lung damage and cell proliferation.** Characteristics of the animals and the pulmonary inflammatory and maturation response to LPS-induced chorioamnionitis and/or antenatal corticosteroids were reported previously (29). Lung injury due to the exposure to LPS was assessed by measurement of HSP70 in the lung tissue. HSP70 protein expression was not increased in any of the experimental groups compared with control (Fig. 1A). To assess cell proliferation, lung tissue was stained for Ki67, a marker of mitotic cells. There were increased proliferating cells, which by morphological evaluation could be discerned as immune cells, 7 and 14 days after the exposure to LPS (Fig. 1B). Representative images are shown for controls (Fig. 1C) and 7-day LPS-exposed lungs (Fig. 1D).

**Changes in Shh signaling after intrauterine LPS exposure.** Shh mRNA levels decreased to less than 25% of control value after 7 and 14 days of LPS exposure (Fig. 2E). Beta pre- or posttreatment prevented the decrease in Shh mRNA. In addition, we analyzed the expression of Gli1 and Gli2, which are components of the Shh pathway. Gli1 mRNA expression had a similar decreased expression at 7 and 14 days following LPS exposure (Fig. 2F). Gli1 protein expression was mainly detected in the bronchiar and alveolar epithelium in controls (Fig. 2A). Exposure to LPS for 7 or 14 days selectively decreased Gli1 protein expression in the alveolar epithelium (Fig. 2B). Beta pre- or posttreatment again prevented this decline (Fig. 2G). Representative images are shown for controls (Fig. 2A), 14-day LPS-exposed lungs (Fig. 2B), 7-day Beta-exposed lungs (Fig. 2C), and 14-day LPS and 7-day Beta-exposed lungs (Fig. 2D). Gli2 mRNA expression had similar trends toward declines after LPS exposure (Fig. 2H).

Levels of FGF10 and BMP4, which are two important Shh-regulated mediators of lung development, were also assessed. Both FGF10 and BMP4 mRNA increased 14 days after LPS exposure, by 2-fold and 3.5-fold, respectively (Fig. 3A and B). Exposure to Beta after LPS exposure lowered FGF10 and BMP4 mRNA. BMP4 protein expression was mainly localized in the bronchial epithelial cells, which corresponds with recent data obtained in adult lung tissue (35, 42). Immunohistochemical analysis of BMP4 expression in bronchioli revealed that BMP4 was decreased 7 days after LPS exposure and showed a trend toward increased expression at 14 days after LPS exposure (Fig. 3C). Treatment with Beta before LPS exposure prevented the decrease in BMP4 levels seen after 7-day LPS exposure only. Treatment with Beta 7 days after the LPS exposure decreased BMP4 levels.

**Expression of lung structural proteins.** Elastin foci were quantified in lung sections as an assessment of secondary septa. Representative images are shown for control (Fig. 4A), 7-day LPS (Fig. 4B), 14-day LPS (Fig. 4C), 7-day Beta (Fig. 4D), 14-day Beta + 7-day LPS (Fig. 4E), and 14-day LPS + 7-day Beta (Fig. 4F) lambs. The number of elastin foci decreased in the lungs of LPS-exposed groups lambs (Fig. 4G). Pretreatment with Beta minimized the decrease in elastin foci. Post-treatment with Beta after LPS exposure increased elastin foci in the fetal lung. ELN mRNA first decreased by 50% 7 days after LPS exposure followed by a 50% increase 14 days after LPS exposure compared with controls (Fig. 4H). Beta pretreatment followed by 7-day LPS exposure increased ELN mRNA by 50% compared with controls. No change in ELN mRNA was detected in the 14-day LPS + 7-day Beta animals.

Representative images of the collagen deposition in the fetal lungs are shown for controls (Fig. 5A), 7-day LPS (Fig. 5B), 14-day LPS (Fig. 5C), 7-day Beta (Fig. 5D), 14-day Beta + 7-day LPS (Fig. 5E), and 14-day LPS + 7-day Beta (Fig. 5F) lambs. Col1A1 mRNA increased more than twofold after combined 14-day Beta and 7-day LPS exposure (Fig. 5H). Collagen type I deposition increased after 14 days of LPS exposure (Fig. 5G). In contrast, 14-day LPS exposure followed by Beta posttreatment resulted in a significant decrease of collagen type I deposition. Beta pretreatment followed by 7-day LPS exposure increased collagen type I deposition in the fetal lung. In contrast, 14-day LPS exposure followed by Beta posttreatment significantly decreased mRNA levels of Coll1A1 similar to the collagen deposition in the lung. mRNA levels of collagen type I gene Coll1A2 decreased to 50% after 14 days of LPS exposure irrespective of Beta posttreatment (Fig. 5I). Interestingly, 7-day Beta exposure only also decreased Coll1A2 mRNA by ~40%. Further computerized morphometric analyses are reported elsewhere and did not show differences (29).

**DISCUSSION**

**LPS exposure leads to changes in Shh signaling in the fetal lung.** In the context of developmental biology research, less is known about later fetal lung development than early organogenesis. Later lung development is, however, an area of human biology where clinical care interfaces with development since survival after very preterm birth at 60% of gestation is now frequent. We used an animal model with similarities to late-gestation human lung development to test two very common clinical exposures, chorioamnionitis and antenatal steroids. We used 7- and 14-day intrauterine periods of exposure based on our previous findings of a delay in alveolar development after 7 and 14 days of LPS-induced inflammation (25, 54). Here we show that fetal lung exposure to LPS-induced inflammation (29) is accompanied by changes in the Shh pathway, which is crucial for early lung development. In addition, we demonstrated that a maternal intramuscular injection of Beta attenuated the effects of LPS on this developmental pathway. We therefore provide some molecular insights into the observational data from clinical practice that maternal corticosteroids are beneficial despite the inflammation of chorioamnionitis (20).
Exposure to intra-amniotic LPS has been shown to cause severe lung inflammation and damage leading to structural changes in the fetal lung that mimic pulmonary changes seen in BPD patients (26). Although in this study we did not measure an increased expression of HSP70, an indicator of oxidative-stress mediated lung damage, previous work from our group showed that LPS-induced chorioamnionitis causes fetal lung injury as early as 5 h after the exposure (26). Tissue remodeling was further characterized by increased proliferation seen up to 14 days after the LPS exposure and maturation of alveolar type II cells (26, 29).

As evidence is accumulating that the Shh pathway is involved during aberrant lung development and disease (24, 51), we asked whether intra-amniotic LPS exposure altered Shh signaling in the fetal lung. Recently, LPS was shown to downregulate Shh in vitro in pulmonary microvascular endothelial cells (55). In our study, LPS strongly downregulated mRNA levels of Shh and its signaling components Gli1 and Gli2 in the ovine fetal lung. LPS exposure also selectively decreased Gli1 protein expression in the distal epithelial tips where Shh signaling in the lung is mainly localized (57). Shh expression can be induced by retinoic acid (40), which is decreased in babies who develop BPD (44). The downregulation of Shh is in line with a previous study from our group, in which LPS-induced chorioamnionitis decreased retinoic acid in fetal sheep lungs (25). Therefore, the LPS-induced decrease of retinoic acid may have decreased Shh mRNA.

The decreases in mRNA and protein expression of Shh signaling pathway components were accompanied by two- to threefold increases in FGF10 and BMP4 expression 14 days after LPS exposure, following a slight decrease 7 days after LPS exposure. The initial decrease in FGF10 expression, which has also been measured in the lung tissue of infants with BPD (8), might be due to the activation of TLR2 and 4 by LPS, which can suppress FGF10 through binding of NF-κB to the FGF10 promoter (7). Since FGF10 induces BMP4 expression in the developing lung (52), the inhibition of FGF10 may indirectly decrease BMP4 expression. The continuous suppression of Shh measured at 7 and 14 days after LPS exposure, which normally downregulates FGF10 (6), may have caused FGF10 and BMP4 levels to rise.

The changes in these pivotal developmental pathways were accompanied by changes in mRNA levels and deposition of structural proteins that are known to direct alveolar septation (9, 25, 49). In a normally developing lung, focal expression of elastin identifies sites for alveolar budding (13). In the lungs of the 7-day LPS-exposed lambs, mRNA levels of ELN and the numbers of elastin foci decreased. Persistent exposure to LPS did not only result in less elastin foci but also increased collagen deposition along the alveolar wall. These observations of dysregulated elastin and collagen deposition in the fetal lung are consistent with ventilation-induced (1, 9, 12) and inflammation-induced (25, 27) animal models of BPD and histology reports of BPD patients (46, 48). Although Shh signaling has been implicated in the activation of fibroblasts and production of fibroblast growth factor 10 (FGF10) and bone morphogenetic protein 4 (BMP4). A: mRNA levels of FGF10 were increased 2-fold 14 days after LPS exposure. Posttreatment with Beta normalized FGF10 levels compared with controls. B: mRNA levels of BMP4 were increased 3.5-fold 14 days after LPS exposure. Posttreatment with Beta normalized BMP4 levels compared with controls. C: immunohistochemical analysis of BMP4 expression in bronchioli decreased after 7 days of LPS exposure but showed a recovery of BMP4 14 days after LPS exposure. Pretreatment with Beta before LPS exposure prevented a drop in BMP4 levels. *P < 0.05 vs. controls and §P < 0.05 between experimental groups by 1-way ANOVA with Tukey’s post hoc test.
of extracellular matrix (ECM) proteins such as collagen (19, 23, 51), Shh seems to act mainly through regulation of FGF10 to direct ECM deposition in the developing lung. Shh expression at the pulmonary epithelial tips controls FGF10 expression, which in turn controls bud size and shape (37). Both overexpression and inhibition of FGFs lead to inhibition of lung branching and alterations in ECM protein expression (15, 38, 45).

Betamethasone and lung development. Antenatal corticosteroids are routinely administered to mothers who are at risk of
preterm birth to mature the fetal organs (4). A secondary benefit may be suppression of inflammation (4). Antenatal steroids also reduce adverse neonatal outcome after preterm birth associated with chorioamnionitis (20), which constitute the majority of early-gestational preterm births (4). The effect of these combined pro- and anti-inflammatory stimuli on pathways that are crucial for the developing lung are, however, unknown.

Recently, we showed that Beta treatment before the LPS exposure suppressed fetal lung inflammation by an unknown priming or conditioning mechanism of the fetal immune system (29). As such little pulmonary damage was inflicted by the exposure to LPS and no changes in the developmental pathways that we studied were detected. Very little is known about the effect of maternal corticosteroids on Shh, FGF10, and BMP4. Corticosteroids can inhibit Shh-mediated neural development and as such can have a detrimental effect on the neonatal developing brain (22). We found that maternal corticosteroids alone did not change the expression of these factors in the fetal lung compared with controls. Moreover, the effects of LPS on these factors were neutralized by maternal Beta, irrespective of whether Beta was given 7 days before or after LPS.
At the lung structural level, corticosteroid treatment before LPS exposure could mitigate the decrease in elastin foci and increase in ELN mRNA levels. Corticosteroid posttreatment even increased elastin foci, which is in line with previous reports showing that corticosteroids can stimulate tropoelastin production in a dose-dependent manner (34, 39), most likely through a TGF-β-mediated mechanism (56). Here we show that corticosteroid treatment can counteract the negative effects of the LPS exposure on elastin deposition.

Furthermore, treatment with only Beta 7 days before delivery decreased CollA2 mRNA levels. Whether this resulted in a decreased collagen deposition in a later stage remains to be investigated. However, combined exposure to 7-day Beta treatment with 14-day LPS exposure did decrease collagen deposition. Several studies have shown beneficial effects of corticosteroid treatment on attenuating fibrotic processes following lung injury (17, 36, 50). Preterm infants at risk of BPD patients showed a significant reduction in markers of collagen synthesis after prenatal corticosteroid treatment up to 7 days after administration (18, 43). Surprisingly, corticosteroid treatment before the inflammatory stimulus stimulated collagen deposition although little pulmonary inflammation was noticed in these animals (29). Brenner et al. (11) demonstrated in vitro that corticosteroids do not uniformly suppress the fibrotic activity of lung fibroblasts in vitro. This could explain our contradictory results and the diverse outcomes of lung morphology and function of BPD patients after corticosteroid treatment.

In this study, we modeled a common clinical exposure to provide new insights into the effects of exposures on fetal lung development (47, 54). However, there are limitations, such as exposures at different time points, for different intervals and to a single dose or repeated doses of corticosteroids during fetal development may have different outcomes. The effects of profound anti-inflammatory stimuli on the developing lung are clearly complex. It remains to be determined whether the observed changes are the result of direct or indirect effects of LPS and Beta with these developmental pathways. On the basis of these results, intervention studies using inhibitors of the Shh pathway [e.g., cycloamine (53)] would be helpful. The developing lung is plastic in that it is continually changing over gestation. Because choioamnionitis is often clinically silent, the duration of exposure of the lung to choioamnionitis and the subsequent inflammatory response are unknown. It is generally unknown whether antenatal maternal corticosteroids are administered before or after the onset of choioamnionitis (4).

In conclusion, this report shows that LPS-induced choioamnionitis can disturb Shh signaling during early alveolar lung development, which is partially mitigated by Beta exposure. Therefore, this report provides some insights into the complicated interactions that can alter lung structure during the maturation phase of lung development where clinical intervention may already occur.

ACKNOWLEDGMENTS

We thank Monique Willems, Dennis Kruk, Nynke van den Hoogen, Richard Dalton, Joe Derwort, Masatoshi Saito, Clare Berry, Caryn McLean, Shaofu Li, and Jennifer Henderson for excellent technical support.

Present address of I. Nitsos and G. R. Polglase: The Ritchie Centre, Monash Institute of Medical Research, Melbourne, NSW, Australia.

GRANTS

This study was supported by NIH HD-57869 (S. G. Kallapur) from the National Institutes of Health, the National Health and Medical Research Council of Australia, the Women and Infants Research Foundation, Western Australia. Yego BKW, 016.096.141 from the Dutch Scientific Research Organization and the Research School for Oncology and Developmental Biology (GROW), Maastricht University. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

DISCLOSURES

No conflicts of interest, financial or otherwise are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


