Maternal glucocorticoids increase endotoxin-induced lung inflammation in preterm lambs

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Kallapur, Suhas G., Boris W. Kramer, Timothy J. M. Moss, John P. Newnham, Alan H. Jobe, Machiko Ikegami, and Cindy J. Bachurski. Maternal glucocorticoids increase endotoxin-induced lung inflammation in preterm lambs. Am J Physiol Lung Cell Mol Physiol 284: L633–L642, 2003. First published December 6, 2002; 10.1152/ajplung.00344.2002.—Antenatal betamethasone (Beta) is widely used in women with asymptomatic chorioamnionitis at risk for preterm delivery, but its effects on fetal inflammation are unstudied. Groups of ewes at 109 ± 1 days of gestation received the following treatments: intra-amniotic (IA) saline (control), 0.5 mg/kg intramuscular Beta, 10 mg IA endotoxin (Endo), and Beta + 2 h later Endo (Beta + Endo). Beta suppressed Endo-induced lung inflammation at 1 day. However, compared with Endo 5 days after treatment, Beta + Endo lambs had increased alveolar neutrophils, proinflammatory cytokine mRNA expression, and serum amyloid A3 (SAA3) mRNA expression. IL-1β mRNA expression was localized to the inflammatory cells, whereas SAA3 mRNA expression was induced in the bronchial epithelium and the inflammatory cells. Compared with Endo, Beta + Endo lambs had increased lung inflammation but equivalent lung volumes 15 days after treatment. The large increase in inflammation in the Beta + Endo animals suggests that glucocorticoids impair the ability of the preterm lung to downregulate Endo-induced inflammation after fetal clearance of the glucocorticoids. These results have implications for lung inflammation and bronchopulmonary dysplasia in preterm infants exposed to chorioamnionitis and maternal glucocorticoids. Bronchopulmonary dysplasia; acute-phase reactant; proinflammatory cytokines; endotoxin tolerance; chorioamnionitis

CHORIOAMNIONITIS AND FETAL INFLAMMATION are associated with preterm birth and the development of bronchopulmonary dysplasia (BPD) (13, 14). About 75% of women in preterm labor receive glucocorticoids to enhance fetal lung maturation (25). Preterm rupture of membranes and histological chorioamnionitis are not contraindications to maternal glucocorticoids (12, 15). Thus a large number of infants delivered prematurely are exposed to the combined effects of antenatal glucocorticoids and a proinflammatory stimulus. The effects of antenatal glucocorticoids on fetal lung inflamma-

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tion (term = 150 days): 10 mg IA saline (control) or IA Endo (Escherichia coli 055:B5, Sigma, St. Louis, MO) or 0.5 mg/kg intramuscular (IM) maternal Beta (Celestone chronodose; Schering-Plough, New South Wales, Australia) or IM Beta given 2 h before IA Endo (Beta + Endo). The 2-h time interval between Beta and Endo was used to achieve a blood level of Beta in the fetus before the Endo was given (8).

Delivery and sample collection, inflammatory markers, and physiological measurements. The ewes were preanesthetized with ketamine and xylazine and received spinal anesthesia for cesarean section delivery of preterm lambs. Groups of three to seven lambs per treatment modality and time were studied (IA saline, IA Endo, IM Beta, and IM Beta + IA Endo were used at 2 and 5 h, and 1, 5, and 15 days after treatments). After cesarean section, the lambs that were evaluated from 2 h to 5 days after the treatments were given a lethal dose of 100 mg/kg pentobarbital by injection via an umbilical vein. For the animals that were evaluated 15 days after treatments, a 4.5-mm endotracheal tube was placed in the trachea at delivery, and the lambs were ventilated for 40 min to evaluate lung function as described previously (20). With time, we measured lung compliance by determining lung gas volumes upon inflation to evaluate lung function as described previously (20).

Bronchoalveolar lavage (BAL) repeated at 2 and 5 h, and 1, 5, and 15 days after treatments, a 4.5-mm endotracheal tube was placed in the trachea at delivery, and the lambs were ventilated for 40 min to evaluate lung function as described previously (20). With time, we measured lung compliance by determining lung gas volumes upon inflation to evaluate lung function as described previously (20). After treatments, BAL was collected at 2 h and 5 days after treatments. BAL fluid (BALF) was pooled, the volume was measured, and 30 ml of BALF were centrifuged at 170 g for 5 min. The cell pellet was resuspended in 1 ml of PBS, and the aliquots were used for cytospin and a total cell count determined by hemocytometer using trypan blue exclusion to identify live cells. The cytospin slides were stained with Leishman’s stain (BDH, Poole, UK) for a differential cell count of 200 cells per field. The cytospin slides were stained with Leishman’s stain (BDH, Poole, UK) for a differential cell count of 200 cells per field. An aliquot of the cell pellet also was used for a hydrogen peroxide assay (Bioxy tech H2O2-560 assay; OSI International, Portland, OR). Pieces of the right lower lobe of the lung were snap frozen for RNA analysis. The right upper lobe of the lung was inflation fixed in 10% buffered formalin at 30 cmH2O and used for in situ hybridization.

RNA extraction, RNase protection assay. Total RNA was isolated using a modified Chomczynski method (2), and 10 µg of total lung RNA or RNA extracted from the BALF cell pellet were used for RNase protection assays as described (22). The sheep-specific riboprobes for IL-1β, IL-6, IL-8, TNF-α, and L32 were generated as described (22). Briefly, solution hybridization was performed in 80% deionized formamide, 0.4 M NaCl, 2 mM EDTA, and 0.04 M PIPES, pH 6.6, using a molar excess of [α-32P]UTP-labeled probes for 16 h at 55°C. Single-stranded RNA was digested with RNase A/T1 (Pharmingen, San Diego, CA). RNase was inactivated, and the protected RNA was precipitated using RNase inactivation buffer (Ambion, Austin, TX). L32 (ribosomal protein mRNA) was used as an internal control for loading (22). The protected fragments were resolved on 6% polyacrylamide 8 mol/l urea gels, visualized by autoradiography, and quantified on a PhosphorImager using ImageQuant version 1.2 software (Molecular Dynamics, Sunnyvale, CA).

Serum amyloid A3 Northern blot. We generated Northern blots using 15 µg of total RNA per sample and electrophoresed them in a 1.2% formaldehyde agarose gel. We cloned the sheep serum amyloid A3 (SAA3) cDNA in a subtractive hybridization screen using pooled mRNA from 119-day preterm lamb lungs treated with 20 mg of IA Endo vs. saline control (21). The cDNA pGEMT-sSAA3(8C3), a 347-bp clone homologous to the bovine SAA3 cDNA encoding amino acids 18–115 (GenBank accession no. AF160867), was used to generate a Northern probe (Decaprime II kit; Ambion). The probe was mixed in a concentration of 1.1 × 106 counts per min (cpm/ml) in a hybridization buffer containing 33% formamide (2), incubated at 45°C overnight, and subsequently washed to a final stringency of 0.1× SSC with 0.1% SDS at 55°C (2). The Northern bands were visualized by autoradiography and quantified using PhosphorImager using ImageQuant version 1.2 software.

In situ hybridization. In situ hybridization of paraffin-embedded sections was performed as described previously using [35S]UTP (35). The plasmid pGEM-7z IL-8β was digested with BamHI for antisense probe (T7 polymerase; Promega, Madison WI) and with XbaI for sense probe (SP6 polymerase; Promega). The plasmid pSAA3(8C3) was digested with NotI for antisense probe (T7 polymerase; Promega) and with NcoI for sense probe (SP6 polymerase; Promega). For hybridization, the sense and antisense probes were diluted in hybridization buffer to a final concentration of 3 × 104 cpm/µl and incubated at 50°C for IL-1β and 57°C for SAA3. After hybridization, the sections with IL-1β riboprobes were washed to a stringency of 0.5× SSC, 20 mM DTT for 60 min at 60°C. Because of the higher GC content of the SAA3 riboprobe, these sections were washed at a higher stringency using a wash buffer containing 50% formamide, 2× SSC, and 10 mM DTT for 30 min at 65°C. The sections were then treated with RNase A/T1 to reduce nonspecific binding and subsequently washed in a descending series of SSC solutions ending with a 15-min wash at room temperature in 0.1× SSC/1 mM DTT. Tissue sections were examined and photographed under dark-field illumination and then counterstained with hematoxylin for morphological analysis. Controls for specificity of riboprobe binding included use of lung tissues obtained from lambs exposed to IA saline and the use of homologous (sense) probe.

Measurement of serum cortisol levels. Cortisol levels were measured in 50 µl of lamb serum by radioimmunoassay using the manufacturer’s instructions (RIA kit; ICN Biomedicals, Costa Mesa, CA).

Statistics. All values were expressed as means ± SE, and comparisons between treated animals and controls were made with two-tailed Mann-Whitney nonparametric test, Welch t-test, or two-way Kruskal-Wallis nonparametric ANOVA as appropriate. Significance was accepted at P < 0.05.

RESULTS

BALF inflammatory cell influx. Cell counts on BALF were performed to assess inflammation in the fetal lungs. In control animals and in animals exposed to Beta alone (not shown), no neutrophils were detected in the BALF. After Endo, neutrophil counts in the BALF were the highest at 2 days (mean value 26 × 106/kg body wt) with a 75% decrease at 5 days after treatment (mean value 7 × 106/kg) (Fig. 1A). Beta significantly decreased Endo-induced neutrophil influx in the fetal airways at 2 and 5 h following treatment. At 1 and 2 days after treatment, the neutrophil cell count was no longer significantly decreased in Beta + Endo lambs compared with Endo-exposed lambs. However, at 5 days, Beta + Endo-exposed lambs had significantly more neutrophils (66 × 106/kg vs. 7 × 106/kg) compared with those exposed to Endo alone. Endo increased monocyte and lymphocyte counts in the BALF of fetal lambs from 1 to 5 days after treatment.
Thus maternal glucocorticoids caused an early suppression but a later increase in neutrophil influx induced by IA Endo in the fetal airways.

Inflammation in fetal airways. We assessed inflammation in the fetal airways by measuring hydrogen peroxide and cytokine mRNA in the cell pellets from BALF. Control preterm lambs had no detectable hydrogen peroxide (Fig. 2). At 5 h and 1 day after treatment, Beta significantly decreased Endo-induced hydrogen peroxide in BALF cells. However, 5 days after treatment, lambs exposed to Beta + Endo had higher BALF cell hydrogen peroxide compared with lambs exposed to Endo alone. IL-1β and IL-8 mRNA also were increased at 5 days in these cells from Beta + Endo animals (not shown). Therefore maternal glucocorticoids caused an early suppression but a later increase in BALF hydrogen peroxide in lungs exposed to IA Endo.

Cytokine mRNA expression in fetal lung. We previously showed that proinflammatory cytokine mRNA expression in the fetal lung was maximally induced at 1–2 days after IA Endo (22). We evaluated the effects of maternal glucocorticoids on the IA Endo-induced cytokine mRNA expression measured in total RNA from the fetal lung. Control animals and lambs exposed to Beta alone (not shown) had minimal expression of IL-1β, IL-6, IL-8, and TNF-α mRNA (Fig. 3, A–D). Compared with controls, preterm lambs exposed to IA Endo alone had maximum increases in IL-1β (50-fold), IL-6 (fourfold), IL-8 (eightfold), and TNF-α (14-fold) mRNA at 1–2 days with a decreased expression 5 days after treatment. Beta decreased proinflammatory cytokine mRNA induced by Endo in fetal lung 1 day after treatment (Fig. 3A, IL-1β mRNA increased 24-fold with Endo at 1 day, whereas the increase was twofold with Beta + Endo). At 2 days after treatment, the Beta suppression of Endo-induced proinflammatory cytokine mRNA expression in fetal lung was less pronounced. Five days after treatment, lambs exposed to Beta + Endo had higher proinflammatory cytokine

(Fig. 1, B and C). Beta alone modestly increased monocyte counts but not lymphocyte counts in the BALF of preterm lambs from 5 h to 5 days after treatment. Lambs exposed to Beta + Endo did not have significantly different monocyte and lymphocyte counts in the BALF compared with those exposed to Endo alone.
mRNAs compared with preterm lambs exposed to Endo alone (Fig. 3A). IL-1β mRNA increased fivefold in Endo exposed lambs at 5 days, whereas the increase was 52-fold with Beta + Endo).

Acute-phase reactant expression in the fetal lung. SAA3 is a class of acute-phase reactant proteins whose transcription is robustly induced by inflammation (17). To further evaluate the Beta effects on acute-phase response to inflammation, we measured SAA3 mRNA induction in the total lung RNA. Control animals had minimal expression of SAA3 mRNA (Fig. 4). Compared with controls, preterm lambs exposed to Endo alone had a maximum increase of SAA3 mRNA at 2 days (191-fold) with a decreased expression 5 days after treatment. Beta decreased Endo-induced SAA3 mRNA expression in the fetal lungs at 1 day (90- vs. 5-fold). At 2 days after treatment, Beta suppression of Endo-induced SAA3 mRNA expression in the fetal lung was less pronounced. However, 5 days after treatment, lambs exposed to Beta + Endo had higher SAA3 mRNA values compared with lambs exposed to Endo alone (335- vs. 55-fold). Thus, as demonstrated by mRNA expression patterns of proinflammatory cytokine mRNAs and the acute-phase reactant gene, maternal glucocorticoids caused an early suppression and delayed increases in IA Endo-induced acute-phase fetal lung inflammatory responses.

Expression pattern of IL-1β and SAA3 mRNA in the lung. We evaluated the Beta effect on IA Endo-induced fetal lung inflammation by determining the cellular localization of IL-1β and SAA3 mRNA in the fetal lung at 1 and 5 days after treatment. The IL-1β or SAA3 mRNA expression was not detected by in situ hybridization.
ization in control animals (Fig. 5A) or in lung sections from Endo-treated animals by the sense IL-1β and SAA3 probes (not shown). One day after Endo, intense focal IL-1β mRNA expression was detected in the infiltrating inflammatory cells (Fig. 5, B–D). Most of the inflammatory cells expressing IL-1β were morphologically identified as neutrophils, and the remainder were monocytes and macrophages. In contrast to the exclusive inflammatory cell expression of IL-1β, SAA3 mRNA expression was detected both in the inflammatory cells, predominantly neutrophils (Fig. 5, E and F), the bronchial epithelium, and the bronchial glands (Fig. 5, G and H). The IL-1β and SAA3 mRNA signals were barely detectable in preterm lambs exposed to Beta + Endo at 1 day and were indistinguishable from controls (not shown).

In contrast to the expression pattern at 1 day after treatment, minimal IL-1β mRNA expression was detected in the fetal lungs 5 days after Endo (Fig. 6A). SAA3 mRNA expression also decreased 5 days after

Fig. 5. IL-1β or SAA3 mRNA expression in preterm lung 1 d after Endo exposure. In situ hybridization was performed with 35S-labeled antisense sheep IL-1β or SAA3 riboprobes on lung sections from control and IA Endo-exposed preterm lambs. A: control preterm lambs had no detectable IL-1β expression or SAA3 expression (not shown). B: intense focal expression of IL-1β was seen in animals exposed to Endo. C and D: higher magnification shows IL-1β expression in inflammatory cells in the airway and inflammatory cells infiltrating the lung parenchyma. E and F: SAA3 mRNA expression in the inflammatory cells. G and H: SAA3 mRNA expression in the bronchial epithelium and the bronchial glands. A–C, E, and G are dark-field and D, F, and H are bright-field pictures. Br, bronchus or bronchiole; arrows, inflammatory cells. Bar represents 50 μm.
Endo, with expression restricted to bronchial epithelium and a few inflammatory cells (Fig. 6B). However, in preterm lambs exposed to Beta + Endo at 5 days, intense focal expression of IL-1β mRNA was detected in the neutrophils, monocytes, and macrophages (Fig. 6, C, G, and H). Compared with the lambs exposed to Endo at 5 days, SAA3 mRNA expression also increased in lambs exposed to Beta + Endo with predominant expression in the inflammatory cells and some expression in the bronchial epithelium and peribronchial interstitial cells (Fig. 6, D–F). Morphologically, most of the inflammatory cells expressing SAA3 mRNA were neutrophils and monocytes/macrophages. Thus preterm lambs exposed to Beta + Endo had an early suppression but later (5 days) increased IL-1β mRNA and SAA3 expression compared with preterm lambs exposed to Endo alone. The increased expression of IL-1β mRNA and SAA3 mRNA 5 days after Beta + Endo compared with Endo alone occurred primarily in the inflammatory cells.

**Inflammation and maturation 15 days after treatment.** Residual inflammation and lung maturation were evaluated 15 days after the treatments. Compared with controls, Endo- and Beta + Endo-treated animals had increased BALF neutrophil, monocyte, and lymphocyte cell counts 15 days after treatment (Fig. 7A). However, preterm lambs exposed to Beta + Endo had increased BALF neutrophil count compared with lambs exposed to Endo alone (96 × 10⁵ vs. 11 × 10⁵ neutrophils/kg body wt). This increased BALF neu-

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Fig. 6. IL-1β or SAA3 mRNA expression in preterm lung 5 d after Endo exposure. In situ hybridization was performed with 35S-labeled antisense sheep IL-1β or SAA3 riboprobes. A: after IA Endo lung IL-1β mRNA signal was no longer seen. B: however, SAA3 mRNA was detectable. C: exposure to B+E increased IL-1β mRNA signal in the lung. D: SAA3 mRNA expression increased in the lung parenchyma in B+E lambs. E and F: higher magnification shows SAA3 expression in inflammatory cells, bronchial epithelium, and peribronchial regions. G and H: IL-1β mRNA expression was localized to the inflammatory cells in the B+E lambs. A–E and G are dark-field and F and H are bright-field pictures. Arrows, inflammatory cells. Bar represents 50 μm.
trophil count was consistent with the increased hydrogen peroxide in the BALF cell pellet in preterm lambs exposed to Beta + Endo compared with those exposed to Endo alone (Fig. 7B). Although preterm lambs exposed to Beta + Endo had increased inflammation at 15 days, static lung compliance as evaluated by lung volumes at 40 cmH₂O of pressure was comparable with those exposed to Endo alone and significantly increased in both groups compared with controls (Fig. 7C). Preterm lambs exposed to Beta alone did not have significantly increased lung volumes compared with controls. Thus the increased inflammation in preterm lambs exposed to Beta + Endo did not decrease Endo-induced lung maturation 15 days after treatment.

Serum cortisol levels. Serum cortisol levels were measured to determine endogenous adrenocortical function after treatments (4–7 lambs/group). In control preterm lambs, the serum cortisol level was 0.52 ± 0.06 μg/dl. At 5 days after treatment, the serum cortisol levels were 0.79 ± 0.13 μg/dl for Endo-exposed animals and 0.82 ± 0.16 μg/dl for the Beta + Endo-exposed animals. Similarly at 15 days after treatment, the serum cortisol values in the Endo-exposed animals were 1.57 ± 0.25 vs. 1.13 ± 0.14 μg/dl in the Beta + Endo-exposed animals (not significantly different between Endo and Beta + Endo at 5 and 15 days).

DISCUSSION

We evaluated how maternally administered glucocorticoids would influence fetal lung inflammation caused by chorioamnionitis and whether this interaction affected the inflammation-induced lung maturation. We anticipated, 1 day after treatment, maternal glucocorticoids suppressed the inflammation-induced lung inflammation. However, 5 and 15 days after treatment, maternal glucocorticoids paradoxically amplified Endo-induced lung inflammation. Lung inflammation was indicated by increased influx of neutrophils, production of hydrogen peroxide by inflammatory cells, and transcription of proinflammatory cytokines and the acute-phase reactant SAA3. Although exposure to glucocorticoids increased the Endo-induced lung inflammation at 5 and 15 days, the lung maturation induced by either Endo or the combined Endo and glucocorticoid treatment was similar 15 days after exposure. These experiments are the first to show that glucocorticoids can augment lung inflammation in fetuses exposed to chorioamnionitis.

The pharmacokinetic elimination of glucocorticoids after maternal treatment differs from that of Endo in the fetal compartment. In a human study, peak fetal serum Beta levels were measured 1–2 h after maternal Beta treatment with a return to baseline within 2 days of treatment (4). In fetal sheep, maternal Beta treatment resulted in peak fetal Beta levels 3 h after treatment with a decrease to 50% of the peak levels at 6 h (8). These plasma levels are consistent with suppression of fetal lung inflammation in lambs exposed to Beta + Endo for 1 day after treatment in this study. The estimated half-life of Endo was measured as ~30 h in the amniotic fluid of preterm lambs after an IA administration and exhibited first-order kinetics of elimination. Thus the fetal lung inflammation in the present study at 5 days after treatment is most likely due to residual Endo exposure after clearance of the Beta. Serum cortisol levels in Endo-exposed animals were similar to the combined Beta + Endo animals at 5 and 15 days after treatment, and, therefore, the increased fetal inflammation in the Beta + Endo-exposed lambs cannot be explained by adrenocortical suppression secondary to exogenous glucocorticoids.

We evaluated fetal lung inflammation 15 days after treatment after a 40-min exposure to mechanical ventilation. The amniotic fluid Endo level at 15 days was
below the threshold to elicit inflammation (24). The increased lung inflammation in ventilated Endo-exposed preterm lambs at 15 days compared with control gestation-matched ventilated lambs suggests that prior exposure to Endo primes the fetal lung to amplify ventilation-induced inflammation. This result is consistent with our demonstration that Endo priming amplifies ventilation-induced lung inflammation 30 days after exposure (16). Significantly, in this experiment, Beta + Endo animals had a further amplification of ventilation-induced fetal lung inflammation 15 days after treatment compared with the Endo-only group. The increased inflammation in the Beta + Endo-exposed lambs both at 5 and 15 days after treatment could be due to priming the fetal lung to induce an exaggerated response to Endo. Alternatively, prior exposure to glucocorticoids might decrease the ability of the fetal lung to downregulate the inflammatory response to Endo.

Although glucocorticoids are well known anti-inflammatory agents, some in vivo studies have shown opposite effects. Adult mice given high-dose intraperitoneal dexamethasone before intratracheal Endo had a paradoxical increase in lung inflammation compared with mice receiving Endo alone (32). In healthy adult humans, intravenous cortisol infusions given 12 h or 6 days before intravenous Endo increased systemic TNF-α, soluble TNF-α receptor, and IL-6 levels 2 h after infusion compared with volunteers receiving Endo alone (5, 6). In these same human studies, cortisol given at the same time as the Endo decreased indexes of systemic inflammation compared with Endo alone. Together, these results demonstrate that prior exposure to steroids can increase Endo-induced inflammation. Our study is unique in that the route of Endo was IA and lung inflammation was studied in preterm fetuses.

Variability of anti-inflammatory effects of glucocorticoids have also been shown in other animal models. Systemic glucocorticoids did not suppress inflammation in a guinea pig Endo model (37). In a rat Endo-induced lung inflammation model, dexamethasone decreased neutrophil influx and BALF TNF-α levels but did not decrease protein leak or BALF macrophage inflammatory protein-2 levels (31). In a rabbit model of endotoxemia, steroids decreased neutrophil but not monocyte influx in the lung (29). In our study, glucocorticoids alone slightly increased monocyte influx in the lung and did not significantly decrease Endo-induced BALF monocyte influx. Although glucocorticoids have potent anti-inflammatory effects in vitro, they did not improve survival or respiratory outcomes in several large trials of sepsis and acute respiratory distress syndrome in adults and BPD in preterm neonates (7, 10, 33). Thus the in vivo effects of glucocorticoids on inflammation are variable, and the interactions between these agents and inflammation in vivo are complex.

A major mechanism of Endo-induced inflammation is activation and nuclear translocation of NF-κB and subsequent transactivation of NF-κB-inducible genes such as proinflammatory cytokine IL-1β and acute-phase reactant serum amyloid A (11) (27). To determine whether Beta affected the Endo cellular inflammatory response in the preterm lung, we performed in situ hybridization for the localization of IL-1β and SAA3 mRNAs. IL-1β expression was studied, because we previously showed large induction in response to IA Endo (22), and IA injection of IL-1α caused lung inflammation and maturation in preterm lambs (36). SAA3 is the only acute-phase serum amyloid A apoprotein with significant extravascular expression. We initially identified SAA3 mRNA induction by IA Endo in a subtraction hybridization screen using lung mRNA from control vs. lambs 1 day after IA Endo (21). Subsequently we demonstrated 200-fold increases in mRNA 2 days after IA Endo, a response similar to the 300-fold increase observed in hepatic serum amyloid A1, A2, and A3 transcription in adult mice 3 h after an Endo challenge (26). In the present study, IL-1β mRNA expression was predominantly in the inflammatory cells in the lung both in the 1-day Endo-exposed lambs and the 5-day Beta + Endo-exposed lambs. SAA3 transcription is known to be induced by IL-1 and Endo (34). In the present study, SAA3 mRNA expression was induced primarily in the bronchial epithelium, the bronchial glands, and the lung inflammatory cells in the 1-day Endo-exposed lambs. In the 5-day Beta + Endo-exposed lambs, SAA3 mRNA expression was detected primarily in the lung inflammatory cells with some expression in the bronchial epithelium and peribronchial interstitial cells. We previously reported that IA Endo induced expression of the heat shock protein 70 within 5 h in the bronchial epithelium (23). These results demonstrate that one of the major cell types responding to the Endo-induced chorioamnionitis is the bronchial epithelium, whereas, in Beta + Endo-exposed lambs, the major source of IL-1β and SAA3 is the pulmonary inflammatory cells.

The mechanism of the exaggerated inflammation in the Beta + Endo-exposed animals is not clear, although a number of possibilities exist. Glucocorticoids inhibit IL-10 production from adult peripheral blood monocytes (9) and may inhibit cellular responses that are induced to downregulate Endo-mediated inflammation. Glucocorticoids decrease neutrophil apoptosis (28) and may prolong survival of neutrophils. Glucocorticoids can exert anti-inflammatory effects by upregulating the IkB family, which are cytoplasmic inhibitors of NF-κB activation, and by direct antagonism between the glucocorticoid receptor and NF-κB, resulting in blocked transcription of responsive genes (1, 27). However, the timing of changes in cellular levels of mediators in the NF-κB pathway induced by Endo after prior exposure to glucocorticoids is not known.

We previously reported that IA Endo but not Beta improved lung compliance, ventilatory efficiency index, and the alveolar wash-saturated phosphatidylcholine pool sizes 15 days after treatment (3, 18, 22). In the present study, static compliance was measured as an indirect assessment of surfactant function. The findings of increased lung compliance in the Endo-exposed
but not the Beta-exposed preterm lambs 15 days after treatment in the present study is consistent with our previous published data. Despite the increased inflammation 15 days after treatment, exposure to Beta + Endo did not decrease the lung volumes compared with exposure to Endo alone.

In summary, the maternal glucocorticoids transiently decreased fetal lung inflammation induced by IA Endo but, at later times, increased fetal lung inflammation. The present standard of care for women with undiagnosed chorioamnionitis with preterm labor is to give glucocorticoids to enhance fetal lung maturation (12, 15). Clearly more studies and animal experiments are needed before this recommendation should be changed. However, our studies do suggest the possibility that, in a subgroup of preterm infants with chorioamnionitis, maternal glucocorticoids may increase lung inflammation, which could promote the subsequent development of BPD.

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