Antenatal inflammation induced TGF-β1 but suppressed CTGF in preterm lungs

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Kunzmann, Steffen, Christian P. Speer, Alan H. Jobe, and Boris W. Kramer. Antenatal inflammation induced TGF-β1 but suppressed CTGF in preterm lungs. Am J Physiol Lung Cell Mol Physiol 292: L223–L231, 2007. First published August 26, 2006; doi:10.1152/ajplung.00159.2006.—Chorioamnionitis is frequently associated with preterm birth and increases the risk of adverse outcomes such as bronchopulmonary dysplasia (BPD). Transforming growth factor (TGF)-β1 is a key regulator of lung development, airway remodeling, lung fibrosis, and regulation of inflammation, and all these processes contribute to the development of BPD. Connective tissue growth factor (CTGF) is a downstream mediator of some of the profibrotic effects of TGF-β1, vascular remodeling, and angiogenesis. TGF-β1-induced CTGF expression can be blocked by TNF-α. We asked whether chorioamnionitis-associated antenatal inflammation would regulate TGF-β1, the TGF-β1 signaling pathway, and CTGF in preterm lamb lungs. Fetal sheep were exposed to 4 mg of intra-amniotic endotoxin or saline for 5 h, 24 h, 72 h, or 7 days before preterm delivery at 125 days gestation (full term = 150 days). Intra-amniotic endotoxin increased lung TGF-β1 mRNA and protein expression. Elevated TGF-β1 levels were associated with TGF-β1-induced phosphorylation of Smad2. CTGF was selectively expressed in lung endothelial cells in control lungs, and intra-amniotic endotoxin caused CTGF expression to decrease to 30% of control values and TNF-α protein to increase. The antenatal inflammation-induced TGF-β1 expression and Smad signaling in the fetal lamb may contribute to impaired lung alveolarization and reduced lung inflammation. Decreased CTGF expression may inhibit vascular development or remodeling and limit lung fibrosis during remodeling. These effects may contribute to the impaired alveolar and pulmonary vascular development that is the hallmark of the new form of BPD. Chorioamnionitis; bronchopulmonary dysplasia; Smad signaling; fibrosis

LUNG INJURY IN THE MATURE lung often resolves with fibrosis, which is prominent in larger infants who developed bronchopulmonary dysplasia (BPD) after prolonged exposure to oxygen and mechanical ventilation (1, 22, 25, 27). In contrast, the lungs of preterm infants who most frequently develop BPD have less fibrosis but striking inhibition of alveolar septation and microvascular development (14, 22). This altered lung development occurs in preterm ventilated baboons and sheep in association with persistent inflammation. The alterations in lung development resulting in BPD also have been associated with chorioamnionitis in very low-birthweight infants (48). Chorioamnionitis results in increased concentrations of proinflammatory cytokines in human amniotic fluid and fetal cord blood, presumably in response to bacterial products and injury (18, 55). These proinflammatory cytokines may be important mediators in the early inflammatory response that recruits activated inflammatory cells to the fetal lung (12). Inflammation-induced tissue injury is normally followed by a phase of repair (39, 40, 44). The endotoxin-induced chorioamnionitis in preterm lambs initiated a sequence of lung injury, inflammation, apoptosis, and remodeling that resulted in a phenotype of clinical lung maturation with increased surfactant and improved gas exchange, but with microvascular injury and decreased alveolar septation (27, 30, 36). Within 7 days of intra-amniotic endotoxin exposure, the histological changes were similar to those in fetal sheep with mild BPD (52). The fetal lungs did not develop fibrosis, even with prolonged exposure to intra-amniotic endotoxin (30).

Lung injury in adult animals induces transforming growth factor (TGF)-β1 as part of the tissue repair-and-healing process ("airway remodeling"). TGF-β1 is present in an latent state, which prevents interaction with its receptor (32). The biological effects of TGF-β1 may be controlled at the level of conversion of latent TGF-β1 to active TGF-β1 (33). If the repair processes are repeatedly triggered and not adequately controlled, fibrosis will ensue (4). TGF-β1 also contributes to normal lung development. Overexpression of TGF-β1 during the critical period of lung alveolarization in mice causes morphological changes similar to those in human BPD (16, 50). Downstream of TGF-β1, connective tissue growth factor (CTGF) and other proteins modulate the cellular response to TGF-β1 and can prolong wound healing and promote the fibrotic response. CTGF mediates the profibrotic effects of TGF-β1 by stimulating fibroblast growth and extracellular matrix synthesis (20). In addition, CTGF increases the affinity of TGF-β1 for its receptors (3) and influences the regulation of endothelial cell function, angiogenesis, and vascular remodeling (7, 41). TGF-β1 normally induces CTGF expression in fibroblasts and endothelial cells (6), and CTGF expression can be inhibited by proinflammatory cytokines such as TNF-α (2). Because of its involvement in airway and vascular remodeling processes, CTGF might have a role in mediating the pathogenesis of BPD. However, there is no information about CTGF in BPD or in preterm animals. Therefore, we used lung tissue from preterm fetal sheep exposed to endotoxin-induced chorioamnionitis to measure TGF-β1 signaling and CTGF expression. Our hypothesis was that chorioamnionitis induced by endotoxin would increase TGF-β1 in the fetal lung but that CTGF might not be induced.

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MATERIALS AND METHODS

Tissue for study. The animal studies were performed in Western Australia, as approved by the Animal Care and Use Committees of the Cincinnati Children’s Hospital and the Western Australian Department of Agriculture. Date-bred Merino ewes were randomized to receive 4 mg of endotoxin (Escherichia coli, serotype 055:B5; Sigma Chemical, St. Louis, MO) at 5 h, 24 h, 72 h, or 7 days before delivery at 125 days gestational age. Control animals received an intraamniotic saline injection under ultrasound guidance at the same time points. Preterm lambs were delivered by cesarean section, and the tissues used for the measurements reported here were from previous studies (35, 36).

RNA extraction and RT-PCR. Total RNA was isolated from tissue pieces from the right lower lung lobe by guanidinium thiocyanate-phenol-chloroform extraction, as described elsewhere (31). RT-PCR were performed with Taq polymerase (TaqPCR Master Mix Kit, Qiagen, Hilden, Germany). Primers and optimized PCR conditions for all the molecules have been reported previously (Table 1) (17, 31, 54). A negative control of non-reverse-transcribed total RNA did not yield a PCR product for any of the primers, indicating there was no detectable genomic DNA contamination. The PCR product for CTGF was confirmed by sequencing. PCR products were loaded to a standard and analyzed on 1% agarose gels. Image analysis was performed using a fluorescence image analyzer (model FLA 3000, Fuji, Dielsdorf, Switzerland) and quantified using AIDA software (Raytest, Urdorf, Switzerland).

Immunohistochemistry and histological analyses. The immunostaining methods were performed as previously described (35). Lung tissue was fixed in formalin, embedded in paraffin, and cut into 5-μm sections. Endogeneous peroxidase activity was removed by incubation with hydrogen peroxide. Nonspecific binding sites were blocked with serum. Sections were incubated at 4°C overnight with a rabbit polyclonal antibody for bioactive TGF-β1 (catalog no. sc-146, Santa Cruz Biotechnology, Santa Cruz, CA), a goat polyclonal antibody for CTGF (catalog no. sc-14939, Santa Cruz Biotechnology), or a rabbit anti-phosphorylated Smad2 (generous gift from Dr. C.-H. Heldin, Ludwig Institute for Cancer Research, Uppsala, Sweden). Unbound antibody was removed with PBS, and the slides were incubated with the secondary biotinylated antibody against rabbit or goat IgG (Vector Laboratories, Burlingame, CA) for 1 h at room temperature. Immunostaining was visualized by the Vectastain ABC peroxidase Elite kit for detection of the antigen-antibody complexes (Vector Laboratories). For analysis of immunohistochemistry, we used a previously described five-step semiquantitative scale (15). Two independent observers who were unaware of the identity of the samples performed the analysis at ×20 and ×40 magnification. Four to five tissue sections of each lung from different regions were analyzed. The average count of all results was calculated for each lung.

Western blot. Frozen lung samples were homogenized in ice-cold buffer containing 50 mM Tris·HCl, pH 7.5, 1 mM EGTA, 1 mM EDTA, and protease inhibitor cocktail (complete mini, Roche, Mannheim, Germany) supplemented with 1 mM phenylmethylsulfonyl fluoride (Sigma Chemical). The samples were sonicated and then centrifuged at 500 g for 20 min at 4°C for removal of cellular debris. Protein content in the supernatant was determined by the bicinchoninic acid method, with BSA as the standard. Protein samples (50 μg/lane) were boiled and loaded onto a molecular weight marker (Invitrogen, Karlsruhe, Germany) on NuPAGE 4–12% Bis-Tris gel (Invitrogen) under nonreducing conditions. The proteins were electroblotted onto a Hybond-P polyvinylidene difluoride membrane (Amersham Life Science, Freiburg, Germany), and the blots were blocked for 1 h in 5% nonfat dry milk in TBS with 0.1% Tween 20. Activated TGF-β1 was detected with an anti-TGF-β1 antibody (catalog no. TS05, BD Pharmingen, Heidelberg, Germany) and with a secondary antibody (goat anti-mouse IgG1 horseradish peroxidase-conjugated antibody; Amersham Life Science). The reaction was visualized on X-ray medical film (Konica Minolta, Untersöding, Germany) after incubation of membranes with lumino-based chemiluminescence reagent (Pierce Biotechnology, Rockford, IL) for 1 min. For normalization of the experiments, membranes were stripped, as recommended by the manufacturer (Pierce Biotechnology), and re-probed with an antibody against β-actin (Santa Cruz Biotechnology). The autoradiographs were scanned at high resolution, and the images were acquired using Adobe Photoshop software. The densitometric quantitation was performed using AIDA software.

Measurement of TGF-β1 and TNF-α protein in lung tissue by ELISA. Pieces of the right lower lobe were sonicated as described above. Supernatants were diluted 1:10. Levels of bioactive TGF-β1 were determined using a specific TGF-β1 enzyme-liked immunosorbent assay (ELISA) kit (R & D Systems, Minneapolis, MN) that cross-reacts with porcine, rodent, and sheep TGF-β1, but not with TGF-β2 or TGF-β3. Total active TGF-β1 levels were measured using acid-activating samples, as recommended by the manufacturer (32). TNF-α levels were measured with an ELISA kit to detect ovine TNF-α (ENDOGEN, Rockford, IL). TGF-β1 and TNF-α protein concentrations were calculated per kilogram of body weight.

Statistical analysis. Values are means ± SE. Analysis of variance was used for comparisons between endotoxin groups at each gestational age; Student-Newman-Keuls tests were used for post hoc analyses. Significance was accepted at P < 0.05.

RESULTS

TGF-β1 expression in response to endotoxin-induced chorioamnionitis. We evaluated the expression of TGF-β1 mRNA in fetal lung tissue by RT-PCR. Signal intensity of TGF-β1 mRNA was expressed relative to that of the ribosomal gene L32. The ratio of TGF-β1 mRNA to L32 mRNA increased with time in the endotoxin-exposed groups compared with the control group (Fig. 1). To confirm that increased TGF-β1

Table 1. Primer sequences and PCR conditions

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Temperature/Time</th>
<th>No. of Cycles</th>
<th>PCR Product, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β1</td>
<td>5′ GGC GAC CCA CAG AGA GGA AAT AG 3′</td>
<td>94°C/40 s 58°C/40 s 72°C/40 s</td>
<td>30</td>
<td>349</td>
</tr>
<tr>
<td>Reverse</td>
<td>5′ AGG CAG AAA TGG GCG TGG TAG C 3′</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTGF</td>
<td>5′ AGG CAG AAA TGG GCG TGG TAG C 3′</td>
<td>94°C/40 s 58°C/40 s 72°C/40 s</td>
<td>30</td>
<td>349</td>
</tr>
<tr>
<td>L32</td>
<td>5′ AAG TTC ATC CGG CAT CAG TC 3′</td>
<td>94°C/40 s 58°C/40 s 72°C/40 s</td>
<td>30</td>
<td>349</td>
</tr>
</tbody>
</table>

TGF-β1, transforming growth factor-β1; CTGF, connective tissue growth factor.
mRNA corresponded with increased protein levels, bioactive TGF-β1 was quantified by Western blot and ELISA analysis of whole lung homogenates and by immunohistochemistry in lung tissue. Bioactive TGF-β1 protein in lung tissue increased by 300% in Western blot analysis (Fig. 2A) and by 350% in ELISA analysis (Fig. 2B). TGF-β1 immunostaining was weakly detected in the control lungs. Staining for TGF-β1 was more apparent in lung stroma of the endotoxin-exposed group (Fig. 3). Immunostaining for bioactive TGF-β1 was increased about twofold at all times after endotoxin exposure.

**Induction of the Smad signaling pathway.** TGF-β1 signaling from the cell membrane to the nucleus is mediated through Smad proteins (47). Early events for TGF-β1 signal transduction are the phosphorylation of TGF-β type I and type II receptors and the subsequent phosphorylation and translocation of the intracellular effectors Smad2 and Smad3 to the nucleus, where they regulate gene transcription. Therefore, the effect of intra-amniotic endotoxin on the subcellular distribution of phosphorylated Smad2 was evaluated in the lung of preterm lambs. Smad2 phosphorylation was detected by immunostaining with anti-phosphorylated Smad2-specific antibodies. Cytoplasmic staining for phosphorylated Smad 2 was weak in bronchial epithelial cells in the control lungs (Fig. 4A). In contrast, endotoxin exposure resulted in intense staining of phosphorylated Smad2 in almost all bronchial epithelial cells and some staining in vascular endothelial and fibroblast-like cells (Fig. 4B). The Smad2 staining increased 5 h after intra-amniotic exposure to endotoxin (Fig. 4C). The majority of the cells had nuclear staining, consistent with TGF-β1 signaling and translocation of phosphorylated Smad2 to the nucleus.

**CTGF expression in response to endotoxin-induced chorioamnionitis.** We evaluated the steady-state levels of mRNA of CTGF mRNA in sheep lung homogenates by RT-PCR. The ratio of CTGF mRNA to L32 mRNA decreased to <33% of control levels in the endotoxin-exposed groups (Fig. 5). Expression of CTGF protein was evaluated by immunohistochemistry (Fig. 6). CTGF was exclusively detected in lung endothelial cells in control lungs, with decreased immunostaining in endotoxin-exposed animals (Fig. 6, A and B). A semiquantitative analysis of the immunohistochemistry demonstrated that CTGF protein was reduced to <50% of control values by 5 h of endotoxin exposure and remained low at later times (Fig. 6).

*Intra-amniotic endotoxin induced TNF-α expression in the lung.* We measured the TNF-α concentration in whole lung homogenates by ELISA, because TNF-α is a known inhibitor of TGF-β1-induced CTGF expression (2). TNF-α protein increased in lung tissue of the endotoxin-exposed groups >20-fold relative to the control group (Fig. 7).

**DISCUSSION**

Chorioamnionitis is a complex disease that has multiple and contrasting effects on preterm babies. Although the incidence of respiratory distress syndrome may be decreased, the incidence of BPD is increased (51). We and others have developed models to study this disease, which occurs in the context of a developing fetus (8). Bry and colleagues (10) were the first to describe an increase in surfactant pool size and compliance after intrauterine exposure to the proinflammatory cytokine IL-1 in the preterm rabbit lung. This phenotypic lung maturation, however, was different from lung maturation induced by maternal corticosteroid treatment, since the systemic cortisol levels were unchanged (26). The phenotypic lung maturation was induced in fetal sheep within 7 days of intrauterine exposure to inflammation; however, an injury response resulting in interference with alveolar and microvascular development also occurred (52). Moreover, the response of the fetal lung to a secondary inflammatory stimulus, such as mechanical ventilation, also was increased by intrauterine exposure to inflammation (23).

Pulmonary inflammation could be induced in utero with IL-1α, IL-1β, endotoxin from periodontal organisms, or endo-
toxin from *E. coli*, all of which resulted in a phenotype of maturation of the fetal lung with increased surfactant pool sizes in sheep (26, 42, 53). The injury and developmental responses of the fetal lung seem to be generalizable. Perinatal expression of IL-1β in a transgenic mouse model resulted in a phenotype similar to BPD, with alveolar simplification and impaired microvascular development (9).

We have identified increased expression of TGF-β1 and decreased expression of CTGF in fetal sheep exposed to endotoxin-induced chorioamnionitis. The BPD phenotype of extremely immature babies with gestations <28 wk (46), a patient group that generally did not survive when BPD was first described. BPD is defined as supplemental oxygen dependency of preterm babies at a postmenstrual age of 36 wk (25). The “new” BPD is frequently diagnosed in very preterm infants who did not initially have severe respiratory distress syndrome (13, 27). In clinical series and animal models, chorioamnionitis resulted in a lung maturation response, because surfactant increased and the volume of the potential gas space in the fetal lung increased. However, prenatal exposure to chorioamnionitis was associated with the development of BPD in these very preterm infants (19, 49, 51). The inflammation already present in the lungs may be sustained or aggravated by therapeutic interventions, such as resuscitation, oxygen, mechanical ventilation, or postnatal infection (45). Angiogenesis occurs with alveolarization during normal lung development, and injury to the developing pulmonary circulation during a critical period of growth can also contribute to development of BPD (1). Decreased concentrations of vascular endothelial growth factor and its receptors were measured in lung tissue of preterm babies who died of BPD and in the lungs of preterm lambs exposed to chorioamnionitis (5, 29, 37).

The activation of latent TGF-β1 to active TGF-β1 has been proposed to contribute to the pathogenesis of acute respiratory distress syndrome, causing a disruption of the alveolar epithelial barrier function (21). However, the role of TGF-β1 in the normal development and injury response of the lung in preterm infants remains unclear. The TGF-β family has been associated with the pathophysiology of BPD, because the levels of TGF-β1 protein increased in airway samples from preterm infants developing BPD (28, 34, 38). However, it is not known whether an elevated TGF-β1 concentration in the fetal lung induced activation of the Smad signaling pathway in vivo. In fetal sheep exposed to chorioamnionitis, the Smad signaling pathway was activated during the inflammation-triggered lung injury and remodeling processes.

TGF-β1 is important for wound healing and in the pathogenesis of fibrosis, but it is also involved in other critical biological activities, including suppression of immune reactions. Because of its multifunctional role, inhibition of TGF-β1...
Fig. 3. Immunohistochemical evaluation of TGF-β1 protein in lung tissue. A: representative sections from a control animal and an animal exposed to endotoxin-induced chorioamnionitis for 7 days stained with an antibody that detects TGF-β1. Magnification ×320. B: immunohistochemical quantification of TGF-β1 in lung sections. Immunostaining for TGF-β1 was graded on a scale from 0 to 5. Values are means ± SE; n = 4 animals in each group at each time point. *P < 0.05 vs. control.

Fig. 4. Intra-amniotic endotoxin exposure induces the Smad signaling pathway in lungs of preterm lambs. Smad2 phosphorylation was evaluated in lung tissue by immunohistochemistry. A: representative sections from a control animal and an animal exposed to endotoxin-induced chorioamnionitis for 7 days stained with an antibody that reacts specifically with phosphorylated Smad2. Magnification ×320. B: immunohistochemical quantification of phosphorylated Smad2 in lung sections. Immunostaining for phosphorylated Smad2 was graded on a scale from 0 to 5. Values are means ± SE; n = 4 animals in each group at each time point. *P < 0.05 vs. control.
would be expected to impact numerous other important functions, many in an adverse way. CTGF might play a role in targeting fibrotic processes. The exposure of fibroblasts and endothelial cells to TGF-β1 is sufficient to induce CTGF expression, which mediated some of the profibrotic effect of TGF-β1 (6). Recent reports suggest that CTGF may anchor vascular endothelial growth factor to the extracellular matrix to promote angiogenesis (11). We found that the CTGF production was reduced in endotoxin-exposed animals. Furthermore, CTGF expression was limited to endothelial cells of blood vessels in the lung. The limited expression of CTGF in endothelial lung cells and the downregulation of CTGF in these cells in the endotoxin-exposed animals support the vascular hypothesis of BPD. The selective expression of

Fig. 5. Intra-amniotic endotoxin exposure decreased steady-state levels of connective tissue growth factor (CTGF) mRNA in the lung. A: representative RT-PCR for CTGF and L32 from lung of control animal and animals exposed to endotoxin for 5 h, 24 h, 72 h, and 7 days. B: densitometric analysis of PCR results. CTGF was quantified at 5 h, 24 h, 72 h, and 7 days after normalization to L32 (ribosomal protein mRNA). Mean RNA value in control animals was given a value of 1, and levels at each time point are expressed relative to the mean control value. Values are means ± SE; n = 4 animals in each group at each time point. *P < 0.05 vs. control.

Fig. 6. Immunohistochemical evaluation of CTGF protein in lung tissue. Intra-amniotic endotoxin exposure decreased CTGF protein in the lung. A: representative sections from a control animal and an animal exposed to endotoxin-induced chorioamnionitis for 7 days stained with an antibody that reacts with CTGF. Arrow, brownish-stained endothelial cell layer. Magnification ×320. B: immunohistochemical quantification of CTGF in lung sections. Immunostaining for CTGF was graded on a scale from 0 to 5. Values are means ± SE; n = 4 animals in each group at each time point. *P < 0.05 vs. control.
CTGF in endothelial cells could play an important role during normal lung development and for the homeostasis of endothelial cells (7). The regulation of the CTGF promoter is well understood. TNF-α was found to suppress the TGF-β1-induced expression of CTGF protein in cultured normal fibroblasts at the transcriptional level (2). Interestingly, the sequences between –244 and –166 of the CTGF promoter were necessary for TGF-β1 and TNF-α modulation of CTGF expression. Taken together, TNF-α could influence two steps in the pathogenesis of BPD: 1) TNF-α initiates the inflammatory responses, and 2) TNF-α seems to inhibit the generation of fibrosis by suppressing CTGF synthesis (2). Increased TNF-α concentrations in preterm babies have been associated with the development of BPD (55). Surprisingly, fetal sheep do not respond to bioactive recombinant ovine TNF-α, irrespective of the route of administration (24). Intra-amniotic or intratracheal administration did not result in an inflammatory response by the pulmonary immune system. However, mechanical ventilation and chorioamnionitis induced some TNF-α mRNA (24). Although TNF-α may not be an important mediator of inflammation in the sheep fetus, it may modulate other signaling pathways.

Increased TGF-β1 concentration did not result in increased CTGF concentration in the fetal lung that was exposed to inflammation. The changed ratio of TGF-β1 to CTGF may explain, in part, the lack of prominent fibrosis in the new BPD. We summarized our results and interpretations with respect to the clinical findings in new BPD in Fig. 8. The first aspect is the physiological role of TGF-β1 in lung development. Increased concentrations of TGF-β1 reduce alveolarization (50). Furthermore, the anti-inflammatory effects of TGF-β1 reduce lung inflammation (4). TGF-β1 is also essential for the “wound remodeling or healing” after lung injury. These effects are mediated in part by TGF-β1 itself, but the fibrosis is particularly mediated by CTGF. We found that endotoxin-mediated chorioamnionitis induced TGF-β1 and TNF-α but decreased CTGF. The downregulation of CTGF could be mediated by TNF-α. Therefore, fibrosis was not initiated, which is typical for the new BPD. The reduced concentrations of CTGF might increase the impaired development of the microvasculature, since CTGF is involved in normal vascular development (7). Our findings support the notion that increased TGF-β1 and decreased CTGF concentrations in the fetal lung impaired alveologenesis (50). In addition, in this sheep model, chronic chorioamnionitis induced by 28 days of exposure to endotoxin did not induce lung fibrosis (30), demonstrating the resistance of the fetal lung to the development of fibrosis, despite the prolonged presence of proinflammatory agonists.

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Fig. 7. Intra-amniotic endotoxin exposure increased TNF-α protein in the fetal lung. TNF-α concentration was measured in whole lung homogenates by ELISA and standardized per kilogram of body weight. Values are means ± SE; n = 4 animals in each group at each time point. *P < 0.05 vs. control.

Fig. 8. Model of interaction between endotoxin, TNF-α, TGF-β1, and CTGF in the pathophysiology of “new” BPD. Some of the hallmarks of the new BPD may be explained by the cytokine profiles. TGF-β1 is essential for the “wound remodeling or healing” after lung injury, but increased TGF-β1 concentration impairs alveologenesis. These effects are mediated in part by TGF-β1 itself, but the fibrosis is primarily mediated by CTGF. Furthermore, TGF-β1 reduces lung inflammation. TGF-β1 and TNF-α were induced, but CTGF was decreased, in this model of endotoxin-induced chorioamnionitis. Downregulation of CTGF might be mediated by TNF-α. Therefore, the reduced concentrations of CTGF might have limited fibrosis but also impaired microvascular development, since CTGF is involved in normal vascular development. Taken together, the cytokine profile may explain, in an overly simplified way, the findings of new BPD.
ANTENATAL INFLAMMATION INDUCED TGF-β1 IN PRETERM LUNGS

REFERENCES


