Antenatal Inflammation Reduces Expression of Caveolin-1 and Influences Multiple Signaling Pathways in Preterm Fetal Lungs

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Bronchopulmonary dysplasia (BPD), associated with chorioamnionitis, results from the simultaneous effects of disrupted lung development, lung injury, and repair superimposed on the developing lung. Caveolins (Cavs) are implicated as major modulators of lung injury and remodeling by multiple signaling pathways, although Cavs have been minimally studied in the injured developing lung. We hypothesized that chorioamnionitis-associated antenatal lung inflammation would decrease the expression of Cav-1 in preterm fetal lungs. We tested whether changes occurred in the transcription factors Smad2/3, Smad1/5, Stat3, and Stat1, and we also studied the activation of acid-sphingomyelinase (a-SMase) with the generation of ceramide, along with changes in the expression of heme oxygenase-1 (HO-1) as indicators of possible Cav-1-mediated effects. Fetal sheep were exposed to 10 mg of intra-amniotic endotoxin or saline for 2, 7, or 2 + 7 days before preterm delivery at 124 days of gestation. The expression of Cav-1 and HO-1 and the phosphorylation of Smad and Stat were evaluated by real-time PCR, Western blotting, and/or immunohistochemistry. The activity of a-SMase and the concentrations of ceramide were measured. Intra-amniotic endotoxin decreased Cav-1 mRNA and protein expression in the lungs, with a maximum reduction of Cav-1 mRNA to 50% $\pm$ 7% of the control value ($P < 0.05$), and of Cav-1 protein expression to 20% $\pm$ 5% of the control value ($P < 0.05$). Decreased concentrations of Cav-1 were associated with the elevated phosphorylation of Smad2/3, Stat3, and Stat1, but not of Smad1/5. The expression of HO-1, a-SMase activity, and ceramide increased. Antenatal inflammation decreased the expression of Cav-1 in the preterm fetal lung. The decreased expression of Cav-1 was associated with the activation of the Smad2/3, Stat, and a-SMase/ceramide pathways, and with the increased expression of HO-1. The decreased concentrations of Cav-1 and changes in other signaling pathways may contribute to BPD.

CLINICAL RELEVANCE

The study supports a role for Cav-1 in lung remodeling induced by antenatal inflammation in BPD.

Keywords: bronchopulmonary dysplasia; TGF- β ; a-SMase; ceramide; chorioamnionitis

Lung inflammation is a major contributor to the impaired development of alveoli and the microvasculature, resulting in bronchopulmonary dysplasia (BPD) (1). However, the mechanisms that link inflammation to alveolar and microvascular simplification are unclear (2). For many very preterm infants, pulmonary inflammation begins *in utero* with chorioamnionitis. Chorioamnionitis is defined as a microbial infection of the amnion and chorion, and is an important risk factor for preterm delivery in approximately 60% of very preterm deliveries (3). Chorioamnionitis increases proinflammatory cytokines in human amniotic fluid and fetal cord blood, presumably by fetal responses to bacterial products and injury (4). These proinflammatory cytokines may be important mediators that recruit activated inflammatory cells to the fetal lung (2). Fetal sheep develop chorioamnionitis after injections of LPS into the amniotic fluid, which initiates a sequence of lung injury (inflammation, apoptosis, and remodeling) that results in both lung maturation and decreased alveolar septation with microvascular injury (5). These changes in the fetal lung may initiate the progression to BPD (6).

Caveolin-1 (Cav-1) may be central in this pathophysiological sequence as a component of caveolae, which are 50–100-nm-wide omega-shaped plasma membrane invaginations (7). Caveolae and caveolins are present at high concentrations in the airway epithelium, smooth muscle, fibroblasts, inflammatory cells, and pulmonary vasculature (8). Caveolae function in protein trafficking, signal transduction, and sphingolipid biology (9). The down-regulation of Cav-1 occurs in diverse lung diseases such as asthma, chronic obstructive pulmonary disease, and idiopathic pulmonary fibrosis (10). Cav-1 is also a major modulator of LPS-induced lung injury in animal models (10).

At the molecular level, Cav-1 is fundamental in organizing multiple signaling pathways, including the TGF- β -induced Smad (11, 12), Stat (13–15), and acid-sphingomyelinase (a-SMase)/ceramide pathways (16, 17). These signaling pathways participate in airway inflammation and remodeling (18–22). Cav-1 also regulates inducible heme oxygenase-1 (HO-1) (23), which modulates oxidative and inflammatory defenses in the lung (24). Changes in Cav-1 or associated changes in these signaling pathways have not been evaluated in the preterm fetal lung exposed to inflammation.

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We previously reported increased TGF- β 1 activity in fetal lungs after antenatal exposure to inflammation (25). TGF- β 1 was identified in lung fibroblasts and endothelial cells as a negative regulator for Cav-1 (26–28). We therefore hypothesized that antenatal inflammation would decrease the expression and function of Cav-1 in preterm lungs, thereby affecting the Smad, Stat, and α -SMase/ceramide pathways and the expression of HO-1. We used a well-characterized sheep model of LPS-induced chorioamnionitis to cause inflammation in fetal lungs (29). A better understanding of the signal transduction pathways in fetal lung inflammation may provide new therapeutic approaches to the treatment of postnatal lung injury.

MATERIALS AND METHODS

Animals

All animal experiments were performed in Western Australia with the approval of the Ethics Committees of the Department of Agriculture of Western Australia and the Children's Hospital Research Foundation in Cincinnati, Ohio. Time-mated ewes with singletons were assigned to groups of six or seven animals for ultrasound-guided intra-amniotic injections of LPS (10 mg, *Escherichia coli* O55:B5; Sigma Chemicals, St. Louis, MO) in 2 ml saline, 2, 7, or 2 + 7 days before delivery (Figure 1). Control animals received a 2-ml intra-amniotic injection of saline. No differences were evident among the control animals that received saline injections at different time points before delivery. Therefore, the control animals were combined into one group. All animals were operatively delivered at the same gestational age of 124 days (term, 150 days). Lung tissue was used for multiple assessments. Results in terms of lung inflammation and maturation were previously reported for these animals (30).

Immunohistochemistry and Histologic Analysis

The immunostaining methods were previously described (31) (please see the online supplement for details).

Measurements of α -SMase and Ceramide

The activity of α -SMase was determined with 14 C-labeled sphingomyelin (32). Powdered lung was mixed with α -SMase buffer (250 mM Na-acetate, 1 mM EDTA, and 0.1% Triton X-100, pH 5.0) and

homogenized. Samples were centrifuged at 4°C and $20,000 \times g$ for 20 minutes, and the protein content of the supernatant was determined. Samples were incubated at 37°C for 2 hours with 14 C-labeled sphingomyelin substrate. Samples were separated by chloroform/methanol extraction, scintillation liquid was added, and radioactivity was measured. Concentrations of ceramide in lung tissue and serum were determined as described elsewhere (33). In brief, powdered lung tissue was mixed in a methanol/chloroform water emulsion, sonicated, and centrifuged at 4°C and $4,000 \times g$ for 10 minutes to extract the lipids. Lipids were separated from other membrane components by chloroform/methanol extraction and dried with N_2 . Subsequently, lipids were dissolved in chloroform/methanol (9:1) and spotted on high-performance thin-layer chromatography plates (Silica Gel 60 Precoated Plates; Merck, Darmstadt, Germany). Ceramide was resolved with dichloromethane/methanol/acetate (100:2:5). Thin-layer chromatography plates were dried at 180°C, cooled, and immersed in a solution of 10% cupric sulfate and 8% phosphoric acid. After heating for 2 minutes at 110°C, lipid bands became visible and were measured with a Fujix-1000 Bioimager (Raytest, Straubenhardt, Germany).

For the extraction of RNA, PCR and Western blotting conditions, and statistical analyses, please see the online supplement.

RESULTS

Pulmonary Expression of Cav-1 Is Reduced by LPS-Induced Chorioamnionitis

The results of PCR, expressed as Cav-1 mRNA transcripts by PCR normalized to ovine ribosomal protein S15, showed that LPS reduced Cav-1 mRNA by 50% in the 2-day, 7-day, and 2 + 7-day LPS groups relative to the control group ($P < 0.05$) (Figure 2A). To confirm that the decreased expression of Cav-1 mRNA corresponded with reduced protein concentrations, Cav-1 was quantified by Western blot (Figures 2B and 2C) and immunohistochemistry (Figures 2D–2F) analysis. Cav-1 decreased similarly at 2 days and 7 days with these measurements, and a second exposure to LPS exerted no further effect on the expression of Cav-1 protein.

Smad2/3 but Not Smad1/5 Phosphorylation Increase with LPS-Induced Chorioamnionitis

Cytoplasmic staining for phosphorylated Smad2/3 was weak in bronchial epithelial cells in control lungs (Figure 3A). In contrast, exposure to LPS resulted in intense, phosphorylated Smad2/3 staining (Figure 3B). The phosphorylated Smad2/3 staining increased 2 days after exposure to LPS, and increased further by approximately threefold, 7 days after exposure to LPS (Figure 3C). The majority of cells exhibited nuclear staining (Figure 3B, inset and arrow), consistent with TGF- β 1 signaling and the nuclear translocation of phosphorylated Smad2/3. Only weak staining was detected for phosphorylated Smad1/5 in LPS-treated and control animals (Figure 3D).

Phosphorylation of Stat3 and Stat1 by LPS-Induced Chorioamnionitis

Concentrations of phosphorylated Stat3 increased in the 2-day, 7-day, and 2 + 7-day LPS-exposed animals according to Western blot analysis, compared with control animals (Figure 4A). A semiquantitative analysis of immunoblots demonstrated that phosphorylated Stat3 was induced 355% ($P < 0.05$) in the 2 + 7-day LPS-exposed group, compared with the control group (Figure 4B). The phosphorylation of Stat3 was also detected through immunohistochemistry with anti-phosphorylated Stat3-specific antibodies (Figures 4C–4E). Staining for phosphorylated Stat3 was weak in control lungs (Figure 4C). In contrast, exposure to LPS resulted in the intense staining of

Figure 1. Study design. Six to seven animals per group received ultrasound-guided intraamniotic injections with 10 mg LPS or NaCl 0.9% (control), 2 days, 7 days, or 2 + 7 days before delivery at the same gestational age of 124 days. d, days.

phosphorylated Stat3 (Figure 4D). A semiquantitative analysis of the immunohistochemistry demonstrated that phosphorylated Stat3 increased approximately fourfold ($P < 0.05$) in the 7-day and 2 + 7-day LPS-exposed group, compared with the control group (Figure 4E).

The phosphorylation of Stat1 increased to 210% of the control value, according to Western blot analysis, with 2 days of exposure to LPS (Figures 5A and 5B). According to immunohistochemical analysis, a 4.8-fold increase (Figures 5C–5E) was also measured after 2 days of exposure to LPS, relative to control values ($P < 0.05$). For Stat3 and Stat1, the majority of bronchial epithelial cells

Figure 2. Intra-amniotic exposure to LPS decreases expression of caveolin-1 (Cav-1). (A) Real-time PCR measurements of Cav-1 mRNA expression in whole-lung homogenates. Mean fold change in lung mRNA expression of Cav-1 normalized for ovine ribosomal protein S15 by the $\Delta\Delta C_t$ method. (B) Western blot measurements of Cav-1 protein expression with an anti-Cav-1 antibody. The same membrane was analyzed with anti- β -actin antibody. (C) Concentrations of Cav-1 and β -actin protein were semiquantified by densitometry. Optical density of Cav-1 protein band was corrected to β -actin, and results are expressed as ratio (%) of endotoxin-exposed animals to control animals. (D–F) Immunohistochemical evaluation of Cav-1 expression in preterm lung tissue. Sections are representative of a control animal (D) and an animal exposed to LPS-induced chorioamnionitis for 2 days (E), stained for Cav-1. Magnification, $\times 50$. (F) Immunostaining for Cav-1 was graded on a scale from 0–3. Values are means $\pm$ SE. * $P < 0.05$, versus control group.

exhibited nuclear staining, consistent with translocation to the nucleus (Figures 4D and 5D, *insets* and *arrows*).

Activity of a-SMase and Concentrations of Ceramide Increase with LPS-Induced Chorioamnionitis

In the LPS-exposed groups, both the activity of a-SMase and concentrations of ceramide were elevated, compared with control values (Figure 6). The activity of a-SMase increased 18-fold in the 7-day LPS-exposed group (Figure 6A; $P < 0.05$), and concentrations of ceramide increased 38-fold in the 7-day LPS-exposed group (Figure 6B; $P < 0.05$).

Induction of HO-1 Expression by LPS-Induced Chorioamnionitis

According to Western blot analysis, the ratio of HO-1 protein to β -actin protein increased 4.5-fold in the 7-day LPS-exposed group compared with the control group ($P < 0.05$) (Figures 7A and 7B). HO-1 protein was increased in all three experimental groups according to immunohistochemical analysis, with a maximum 12-fold increase in the 7-day LPS-exposed group, compared with the control group ($P < 0.05$) (Figures 7C–7E).

DISCUSSION

Chorioamnionitis caused LPS-induced lung inflammation and airway remodeling (34, 35). This fetal inflammatory response also down-regulated Cav-1, activated Stat, Smad, and a-SMase, and up-regulated the expression of ceramide and HO-1 in the fetal lung. These findings support the hypothesis that the decreased expression of pulmonary Cav-1 and effects on downstream signaling pathways may contribute to remodeling and functional impairment in the developing lung.

Animal models of LPS-induced chorioamnionitis were extensively used to evaluate the effects of inflammation on lungs and other organs (36–38) and potential mediators (39–41). Intra-amniotic LPS can reliably reproduce the fetal inflammatory response in chorioamnionitis, but with the limitation that chorioamnionitis is induced with only one proinflammatory Toll-like receptor ligand and not with living bacterial infections, which are often polymicrobial in chorioamnionitis (42).

TGF- β 1 can be a negative regulator of Cav-1 expression in the lung, because in human pulmonary fibroblasts, TGF- β 1 can markedly decrease the expression of Cav-1 (26, 28). Furthermore, the treatment of cultured bovine aortic endothelial cells with TGF- β 1 decreased the expression of Cav-1 (27). We also detected

Figure 3. Effect of intra-amniotic exposure to LPS on Smad signaling. The phosphorylation of Smad2/3 (A–C) and Smad1/5 (D) was evaluated in lung tissue by immunohistochemistry. Sections are representative of a control animal (A) and an animal exposed to LPS-induced chorioamnionitis for 7 days (B), stained for phosphorylated Smad2/3. Magnification, $\times 50$. Inset shows higher magnification, and arrow identifies nuclear staining for phosphorylated Smad2/3. (C) Immunohistochemical semiquantification of phosphorylated Smad2/3 in lung sections. (D) Immunohistochemical semiquantification of phosphorylated Smad1/5 in lung sections. Immunostaining for phosphorylated (P) Smad2/3 or Smad1/5 was graded on a scale from 0–3. Values represent means $\pm$ SE. * $P < 0.05$, versus control group.

the down-regulation of Cav-1 mRNA expression by TGF- β 1 in human pulmonary microvascular endothelial cell-ST1.6R cells, in A549 cells (an adenocarcinoma human alveolar epithelial cell line), and in H441 cells (a human lung adenocarcinoma cell line with characteristics of bronchiolar Clara epithelial cells) (data not shown). Antenatal inflammation induced the expression of TGF- β 1 in the lungs of fetal lambs (25), and concentrations of TGF- β 1 were increased in airway samples from preterm infants developing BPD (43, 44). Therefore, the reduction of Cav-1 expression by chorioamnionitis-induced inflammation may be mediated by increased concentrations of TGF- β 1 in the lung (25). Although the

Figure 4. Effect of intra-amniotic exposure to LPS on Stat3 signaling. (A) Western blot measurements of Stat3 phosphorylation with an anti-phosphorylated Stat3 antibody. The same membrane was analyzed with anti- β -actin antibody. Phosphorylated Stat3 and β -actin protein concentrations were semiquantified by densitometry. (B) Optical density of phosphorylated Stat3 protein bands was corrected to β -actin, and results are expressed as the ratio (%) of LPS-exposed animals to control animals. (C–E) Evaluation of Stat3 phosphorylation in lung tissue by immunohistochemistry. Sections are representative of a control animal (C) and an animal exposed to LPS-induced chorioamnionitis for 7 days (D), stained for phosphorylated Stat3. Magnification, $\times 50$. Inset shows higher magnification, and arrow identifies nuclear staining for phosphorylated Stat3. (E) Immunohistochemical semiquantification of phosphorylated Stat3 in lung sections. Values represent means $\pm$ SE. * $P < 0.05$, versus control group.

Figure 5. Effect of intra-amniotic exposure to LPS on Stat1 signaling. (A) Western blot measurements of Stat1 phosphorylation with an anti-phosphorylated Stat1 antibody. The same membrane was analyzed with anti- β -actin antibody. Concentrations of phosphorylated Stat1 and β -actin protein were semiquantified by densitometry. (B) Optical density of phosphorylated Stat1 protein band was corrected for β -actin, and results are expressed as the ratio (%) of LPS-exposed animals to control animals. Values represent means $\pm$ SE. * $P < 0.05$, versus control group. (C–E) Evaluation of Stat1 phosphorylation in lung tissue by immunohistochemistry. Representative sections from a control animal (C) and an animal exposed to LPS-induced chorioamnionitis for 2 days (D), stained for phosphorylated Stat1. Magnification, $\times 50$. Inset shows higher magnification, and arrow indicates nuclear staining for phosphorylated Stat1. (E) Immunohistochemical semiquantification of phosphorylated Stat1 in lung sections. Values represent means $\pm$ SE. * $P < 0.05$, versus control group.

Figure 6. Effects of intra-amniotic exposure to LPS on acid-sphingomyelinase (a-SMase) activity and ceramide. (A) Measurements of a-SMase activity in lung tissue by a modified micellar *in vitro* assay. (B) Measurements of ceramide concentrations by two-dimensional thin layer chromatography. Values represent means $\pm$ SE. * $P < 0.05$, versus control group.

down-regulation of Cav-1 was described previously in other lung diseases (10), the reduced expression of Cav-1 in preterm lung disease constitutes a new observation.

In this translational model of fetal sheep, we cannot directly demonstrate that TGF- β 1 was regulating Cav-1. Cav-1 also contributes to the regulation of TGF- β signaling by its participation in the internalization of T β R (TGF- β receptor) (45). T β R can be internalized by two different mechanisms, either by Cav-1 associated lipid rafts, or by early endosome antigen-1 nonlipid raft pathways. Whereas nonlipid raft-associated internalization increases TGF- β signaling, caveolin-associated internalization increases the degradation of T β R and decreases TGF- β signaling (46). The absence of one compartment or an imbalance in the densities of the two compartments may affect the level of TGF- β pathway activity, given the same amount of ligand binding. Because this process occurs at the level of internalization of the T β R immediately after ligand engagement, it likely represents an important mechanism for regulating TGF- β signaling. In human fetal pulmonary fibroblasts, experiments on both the gain and loss of function identified the regulatory role of Cav-1 in this process (28). The down-regulation of Cav-1 by siRNA transfection increased the phosphorylation of Smad-2 and of Smad2/3 nuclear translocation, whereas the overexpression of Cav-1 suppressed the phosphorylation of Smad2 and nuclear translocation (28). In addition, enhanced TGF- β signaling was measured in Cav-1-deficient mice (47) and in ovalbumin allergen-challenged Cav-1-deficient mice in a model of asthma (48). Furthermore, Razani and colleagues described an interaction between Cav-1 and the Type I TGF- β receptor, in which Cav-1 suppressed the TGF- β 1-mediated phosphorylation of

Figure 7. Intra-amniotic exposure to LPS induces expression of heme oxygenase-1 (HO-1). (A) Western blot measurements of HO-1 protein expression with an anti-HO-1 antibody. The same membrane was analyzed with anti- β -actin antibody. HO-1 and β -actin protein levels were semiquantified by densitometry. (B) Optical density of HO-1 protein bands was corrected for β -actin, and results are expressed as ratio (%) of endotoxin-exposed to control animals. (C–E) Evaluation of HO-1 expression in lung tissue by immunohistochemistry. Sections are representative of a control animal (C) and an animal exposed to endotoxin-induced chorioamnionitis for 7 days (D), stained for HO-1. Magnification, $\times 50$. (E) Immunohistochemical semiquantification of HO-1 in lung sections, on a scale from 0–3. Values represent means $\pm$ SE. * $P < 0.05$, versus control group.

Smad2 (11). We analyzed the phosphorylation of Smad2/3 and Smad1/5 in preterm lungs. Smads are downstream effectors of TGF- β , and provide an indication of the extent to which TGF- β signaling is activated. In concordance with other studies, the decreased expression of Cav-1 in our model was associated with a substantial increase in phosphorylated Smad2/3 in the lungs of animals exposed to LPS. The increase of Smad2/3 phosphorylation is in agreement with an earlier similar study with different intervals of exposure to LPS (25).

In addition to the Smad signaling pathway, Cav-1 can also regulate the Stat signaling pathway. Like Smad, Stat proteins exist in a latent form in the cytoplasm, and upon receptor activation by cytokines, Stats are phosphorylated on tyrosine residues by members of the Janus kinase (JAK) family (JAK1, JAK2, and JAK3) or tyrosine kinase 2 (Tyk2) (49). An activated Stat pathway is commonly observed in acute lung injury (e.g., in endotoxin-induced lung injury) (19). Caveolins associate with Stat proteins, and Stat3 was hyperphosphorylated in lungs from Cav-1-deficient (Cav-1 $^{-/-}$) mice (14). In addition, the Jak/Stat signaling cascade is hyperactivated in the mammary glands of Cav-1 $^{-/-}$ mice (50). Thus, the down-regulation of Cav-1 expression should hyperactivate the phosphorylation of Stat3. In our model of LPS-induced lung injury, the reduced expression of Cav-1 was associated with increased phosphorylation of Stat-3 and Stat-1.

The a-SMase/ceramide pathway is critical in a variety of lung diseases (21), including acute neonatal inflammatory lung injury (22). Sphingolipids are structure-bearing elements of biological membranes that regulate key physiological processes such as apoptosis, innate and acquired immunity, vascular permeability, and smooth muscle tone (21). Ceramide is a structure-bearing lipid and probably also a second messenger that is generated by the hydrolysis of sphingomyelin by a-SMase during inflammation. Consistent with interactions between a-SMase and Cav-1 (16, 17, 51), we observed that the decreased pulmonary Cav-1 induced by antenatal inflammation was associated with increased pulmonary a-SMase activity and the production of ceramide, as observed in many models of acute lung injury (21). The decreased expression of a-SMase/ceramide after repetitive injections of endotoxin into the amniotic fluid may be part of the endotoxin tolerance that we previously reported in this model (30).

We further investigated the role of HO-1 in LPS-induced lung injury, because of the known association between the decreased expression of Cav-1 and increased expression of HO-1 in the lung (23, 52). Like the Smad and Stat signaling pathways, HO-1 can influence pulmonary remodeling (24). HO-1 is a mediator of tissue protection against a wide variety of injurious insults (24). The increased expression of HO-1 was described in premature infants with respiratory distress syndrome (53). In addition, Maroti and colleagues suggested that HO-1 plays a role in the early adaption process, with increased expression of HO-1 for 2–3 days after birth (54). A direct link between Cav-1 and HO-1 was characterized by Jin and colleagues (23) when they examined the underlying mechanisms by which Cav-1 $^{-/-}$ mice manifested prolonged survival and reduced lung injury after hyperoxia. The apparent resistance to hyperoxia in Cav-1 $^{-/-}$ pulmonary cells and tissues resulted from the increased expression of stress protein HO-1 (23). Furthermore, Kim and colleagues demonstrated that the activity of HO-1 dramatically increased in endothelial cells expressing Cav-1 antisense transcripts, suggesting a negative regulatory role for Cav-1 (52).

In parallel with these observations, the connections between Cav-1 and these signaling pathways are consistent with associations between Cav-1 and the signaling pathways in LPS-induced chorioamnionitis. This study contains the limitation that a direct mechanistic linkage between Cav-1 and these signaling pathways was not shown because of the nature of translational research

models in large animals. However, our results support the involvement of Cav-1 down-regulation and associated signaling pathways in the lung remodeling induced by LPS-induced chorioamnionitis. A next step involves testing therapies that increase the bioavailability of Cav-1 in the lungs of sheep. Razani and colleagues demonstrated that the interaction between Cav-1 and the TGF- β receptor was mediated by a small region within the Cav-1 protein, identified as the caveolin scaffolding domain (CSD), which specifically recognizes and binds to a short amino-acid sequence, termed the caveolin-binding motif, present in the TGF- β receptor (55). The function of Cav-1 may be mimicked by the delivery of a penetrating CSD fusion peptide, leading to an inhibition of TGF- β -induced Smad2/3 activation and collagen expression (56). This peptide might restore the function of Cav-1 in the setting of lung injury induced by LPS-induced chorioamnionitis.

In conclusion, our study supports a role for Cav-1 in the lung remodeling induced by antenatal inflammation (Fig. S1 in the online data supplement). The expression of Cav-1 mRNA and protein were low in lung tissues after antenatal inflammation. In contrast, TGF- β 1 increased considerably with antenatal inflammation-induced lung remodeling (25), providing a basis for the hypothesis that TGF- β 1 may be one of the negative regulators of pulmonary Cav-1 expression (26–28). In addition, the expression of Cav-1 is associated with the activation of other signaling pathways and enzymes in the lung. The Stat and a-SMase/ceramide pathways and the expression of HO-1 may contribute to inflammation and remodeling in the preterm injured lung. We do not know whether these events are the causes or effects of the loss of pulmonary Cav-1. Whether Cav-1 exerts positive or negative effects on airway remodeling also remains unknown (8).

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