Intra-Amniotic Administration of *E coli* Lipopolysaccharides Causes Sustained Inflammation of the Fetal Skin in Sheep

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Abstract

Preterm birth is associated with in utero infection and inflammation. Although the fetal membranes and fetus contribute to the intra-amniotic inflammatory profile, the relationships between a proinflammatory exposure to the fetal compartment and cytokine expression in the fetal skin are unknown. Using an ovine model, we asked whether the fetal skin would generate an extended response to inflammatory stimuli. Relative to control, intra-amniotic lipopolysaccharide (LPS) induced significant increases in cytokine/chemokine (interleukin 1 β , IL-8, tumor necrosis factor- α , and monocyte chemoattractant protein 1) expression in skin that lasted for at least 15 days. Histological analysis demonstrated inflammatory cell infiltration in skin between 2 days and 15 days post-LPS exposure. In contrast to the fetal lung, the fetal skin continues to express proinflammatory cytokines for at least 15 days after exposure to LPS. These novel data suggest that the fetal skin may cause prolonged in utero inflammatory response causally associated with preterm birth.

Keywords

preterm birth, fetal skin, *E coli* LPS, inflammation

Introduction

Despite advances in our understanding of the risk factors and mechanisms related to preterm labor, the rate of preterm birth in many communities remains high, ranging from 5% to 13% across the developed world.¹ In recent years, the survival rates for very early preterm births have increased due to improvements in the clinical management of the newborn. The consequences of preterm birth, however, which may include both short-term mortality and long-term disabilities, are a significant burden to many survivors, their families, and the community at large.²

The causes of early preterm birth include pathophysiological processes such as intrauterine infection/inflammation; antepartum hemorrhage; preeclampsia; uterine overdistension; and placental insufficiency.^{3,4} The earlier the preterm birth, the more likely intrauterine inflammation/infection is the major contributor. Infection of the amniotic cavity accounts for up to 70% of preterm deliveries before 32 weeks of gestation.⁵

Microorganisms that invade amniotic environment cause preterm labor by activation of the innate immune system. Pathogen-associated microbial components such as lipopolysaccharides (LPS) are primarily recognized by pattern-recognition receptors such as Toll-like receptor 4 (TLR-4), which in turn activates signaling cascades resulting in the release of proinflammatory chemokines and cytokines including interleukin (IL)-8, IL-1 β , and tumor necrosis factor (TNF)- α .^{3,6-8}

Numerous clinical and experimental studies have associated chorioamnionitis with adverse neonatal outcomes in newborn infants. The most pronounced effects are identified for infants having “fetal inflammatory response syndrome” (FIRS), a systemic fetal inflammatory response to intra-amniotic (IA) infection characterized by elevated levels of IL-6 in cord blood.⁹ The FIRS is likely a multiorgan fetal response to IA infection, involving the brain, lungs, cardiovascular system, bowel, and skin; FIRS is a risk factor for short-term perinatal morbidity and mortality as well as the development of long-term disability.^{10,11}

The fetal skin is in direct contact with amniotic fluid and, accordingly, is exposed to any microorganisms and

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inflammatory product in the amniotic environment.^{12,13} The skin functions not only as a mechanical barrier but also as an active immune organ that participates in protection against microbial invasion. The skin produces antimicrobial peptides, bioactive lipids, and can activate the innate immune system.¹⁴ The skin from embryonic and newborn mice and human newborn foreskin has robust expression of cathelicidin and β -defensin antimicrobial peptides.¹⁵ The TLRs, a key component of the innate immune system, are expressed by a number of epidermal cell types including keratinocytes, melanocytes, and Langerhans cells.¹⁶⁻¹⁹

The relative contributions of fetal and maternal tissues to the IA inflammation leading to fetal injury and preterm birth remain to be identified. The fetal lung responds to an IA exposure to LPS with a quick inflammatory response that is quickly extinguished.²⁰ The time course for inflammatory responses in the skin has not been evaluated. Therefore, we used our established ovine model of in utero inflammation to investigate the response of fetal skin to inflammatory stimulation over a 15-day period in this study.

Materials and Methods

Animals and IA Administration of LPS

All procedures with animals were performed in Western Australia and were approved by the animal care and use committees of the Cincinnati Children's Hospital (Cincinnati, Ohio) and The University of Western Australia. Date-mated Australian merino ewes (term = 148 ± 2 days) were bred to carry single pregnancies.

Intra-amniotic administration of LPS from *Escherichia coli* (055:B5; Sigma Aldrich, St Louis, Missouri) was performed under ultrasound guidance with successful IA targeting verified by electrolyte analysis of amniotic fluid.²¹ To investigate the proinflammatory effects of LPS on the fetal skin, 5 to 7 animals were randomly assigned to receive either 10 mg of LPS in 2 mL of sterile saline or 2 mL saline as a control. Animals were randomized to 1 of 8 groups with time as the interval between the IA exposure and tissue collection. Gestational age (GA) is the gestation to get IA exposure. (1) 5 hours of IA LPS (124 days of GA, $n = 6$); (2) 12 hours of IA LPS (123 days of GA, $n = 7$); (3) 24 hours of IA LPS (123 days of GA, $n = 7$); (4) 2 days of IA LPS (122 days of GA, $n = 6$); (5) 4 days of IA LPS (120 days of GA, $n = 6$); (6) 8 days of IA LPS (116 days of GA, $n = 7$); (7) 15 days of IA LPS (109 days of GA, $n = 7$); or (8) control group, 2 mL sterile saline at 124 days, 123 days, 122 days, 116 days, or 109 days ($n = 1$ per time point, pooled to give a control group $n = 5$).

At 124 ± 2 days of GA, the animals were heavily sedated with intravenous metomidine (0.12 mg/kg) and ketamine (12 mg/kg) (Provet, Perth, Australia). The fetus was then surgically delivered and the lamb was euthanized with pentobarbital (100 mg/kg). Skin samples for RNA, protein, or histological analyses were immediately collected from the left groin of each newborn, for cryofixation in Optimal Cutting

Temperature compound (OCT) or snap frozen in liquid nitrogen and stored at -80°C .

RNA Isolation and Complementary DNA Generation

Total RNA was isolated from frozen tissue using Trizol (Invitrogen, Carlsbad, California). Briefly, frozen tissue was finely milled in 1 mL of Trizol and processed in keeping with manufacturer's instructions. Air-dried RNA pellets were resuspended in nuclease free water. RNA yield and purity were assessed using 260/280 nm absorbance readings. Complementary DNA (cDNA) was generated from 500 ng RNA using BIOSCRIPT MMLV reverse transcriptase (BioLine, London, UK), according to manufacturer's instructions.

Quantitative Polymerase Chain Reaction

Quantitative polymerase chain reaction (qPCR) analysis (cycling conditions: 1×300 seconds initial denaturation [95°C], 35×30 seconds denaturation [95°C], 30 seconds annealing [60°C], 30 seconds extension [60°C], and $1 \times$ final 0.5°C stepped temperature ramp [60°C - 95°C]) of cytokine and chemokine expression were performed on a Corbett Robotics Rotorgene 3000 using $2 \times$ power SYBR master mix (Qiagen, Hilden, Germany) in a final volume of 20 μL . All reactions were performed in triplicate. Primer specificity was confirmed by the presence of a stable, single peak in melt curve analysis and an absence of amplification in no-template controls. Averaged Cq values for each target amplicon were normalized against averaged glyceraldehyde 3-phosphate dehydrogenase (GAPDH) Cq values. A Mann-Whitney *U* test on the Cq values for saline and LPS GAPDH values was employed to confirm LPS did not systematically alter GAPDH expression. One-way analysis of variance (ANOVA) was used to compare individual replicate efficiencies for each amplicon as appropriate. No significant differences ($P > .05$) were detected in GAPDH expressions and amplicon efficiencies; accordingly, data were processed to generate fold change estimates using a $2^{-\text{ddCq}}$ method. Primer pairs used in this study are shown in the following table as published previously (Table 1).²²⁻²⁴

Immunofluorescence

The OCT-embedded skin was transversely orientated and serially sectioned with a Leica CM1900 cryostat to a thickness of 9 μm . Skin sections were snap fixed in 500 μL of a 1:1 solution of ice-cold acetone: methanol, then blocked for 2 hours at room temperature in 1% newborn calf serum (FCS) in phosphate-buffered saline (PBS) containing 0.1% triton X-100. Three sections were analyzed for each animal. For immunofluorescence analysis, primary antibodies against IL-1 β , IL-8, TNF- α , monocyte chemoattractant protein (MCP)-1 (1:250, MCA1658, AHP425, AHP852Z, and AAM43 respectively; ABDSerotec, Kidlington, UK), and CD-3 (1:2000, A0452; DakoCytomation, California) were diluted in PBS containing 1% FCS, 0.1% triton X-100 and applied to fixed sections. After overnight (16 hours)

Table 1. Primers of target genes

Gene	Forward Primer	Reverse Primer
IL-1 β	ACGAACATGTCTTCCGTGAT	ACCAGGGATTTTGCTCTCT
IL-6	ACCTGGACTTCCTCCAGAAC	TTGAGGACTGCATCTTCTCC
IL-8	TTCCAAGCTGGCTGTTGCT	TTGACAGAACTGCAGCTTCACA
MCP-1	GCTGTGATTTTCAAGACCATCCT	GGCGTCCTGGACCCATTT
TNF- α	CAACCTGGGACACCCAGAAT	TCTCAAGGAACGTTGCGAAGT
IL-10	GTCGGAAATGATCCAGTTTACCT	GTCAGGCCCATGGTTCTCA
TLR-4	TGGATTTATCCAGATGCGAAA	GGCCACCAGCTTCTGTAAAC
GAPDH	GTCCGTTGTGGATCTGACCT	TGCTGTAGCCGAATTCATTG

incubation at 4°C, sections were rinsed repeatedly in PBS followed by incubation with secondary antibody: Alexa fluor 488 antimouse/rabbit immunoglobulin G (IgG; Biolegend, London, UK) diluted 1:600 in PBS containing 1% FCS, 0.1% triton X-100, and incubated for 2 hours at room temperature. Sections were rinsed repeatedly in PBS before being mounted using an aqueous mounting medium containing 4',6-diamidino-2-phenylindole, dihydrochloride (DAPI; H-1200, Vector Laboratories, Burlingame, California) and sealed with hard-set mounting medium. Staining specificity was demonstrated by the absence of staining in sections prepared without primary antibody. Sections were imaged using a Nikon Eclipse Ti inverted fluorescent microscope set to a 2-second exposure time at a constant aperture.

Representative images were made following the analysis of 3 sections (per target/per animal) by an investigator blinded to sample groups. Representative images were assessed for staining distribution (greater/less) and staining intensity (greater/less) relative to control tissues. Sections for hemotoxylin and eosin staining were processed and imaged using a standard protocol using an Olympus BX-51 brightfield microscope.

Enzyme-Linked Immunosorbent Assay

Quantification of IL-8 protein concentrations in frozen skin tissue was performed using an in-house enzyme-linked immunosorbent assay (ELISA) as follows: Plate wells were coated overnight at 4°C with 4 μ g/mL capture antibody (MCA1660; ABD Serotech, Kidlington, UK) diluted in 100 μ L coating buffer (4 g/L Na₂HPO₄, buffered to pH 9.0 with Na₂CO₃). Wells were washed 3 times in PBS containing 0.1% Tween-20 (Sigma Aldrich) and then blocked with blocking buffer (coating buffer with 50 g/L hydrolysed casein) for 1 hour at room temperature. Protein standards, 100 μ L (6, 1:3 dilutions of 8000 pg/ml RP0023B; Kingfisher Biotech, St Paul, Missouri), and protein samples in a total volume of 100 μ L were added to wells and incubated for 2 hours at room temperature with gentle shaking. Wells were washed 4 times in PBS containing 0.1% Tween-20. Detection antibody (AHP425; ABD Serotech) diluted 1:1000 in assay buffer (16 g/L NaCl, 4 g/L Na₂HPO₄, 1.2 g/L NaH₂PO₄, 0.5 ml/L Tween 20, 50 g/L hydrolyzed casein, buffered to pH 7.2) was added to wells and the plate incubated at room temperature for 2 hours with gentle shaking. Wells were washed 3 times in PBS containing 0.1% Tween-20. Anti-rabbit Horseradish Peroxidase (HRPO)-conjugated IgG

(Sigma Aldrich) diluted 1:2000 in 100 μ L assay buffer was added to each well and the plate incubated for 1 hour at room temperature with gentle shaking. Wells were washed 3 times in PBS containing 0.1% Tween-20. TMB substrate, 100 μ L (T8865; Sigma Aldrich), was added to each well and the plate incubated in the dark at room temperature for 15 minutes. The chromogenic reaction was halted by the addition of 100 μ L 2% sulfuric acid solution in PBS to each well. The plate was immediately read (λ = 450 nm) on a Spectramax plate reader (Molecular Devices LLC, Sunnyvale, California). The IL-8 concentration was normalized against the concentration of total skin protein analyzed. All samples and standards were assayed in duplicate.

Statistical Analysis

Analyses were performed using SigmaPlot 11.0 (Systat Software, Erkrath, Germany). Analysis of apparent changes in transcript expression across 3 or more groups was assessed using 1-way ANOVA. Least Significant Difference (LSD) test was used to perform post hoc analysis to investigate the apparent differences between specific groups for qPCR analysis in Figure 1. For non-parametric data in Figure 2, comparisons among groups were performed by Kruskal-Wallis 1-way ANOVA on Ranks and multiple comparisons versus control group were performed using Dunn method. Differences were considered significant at $P < .05$.

Results

Cytokine and Chemokine Expression in the Fetal Skin

Compared with the pooled control group, qPCR analysis of fetal skin from 5 hours to 15 days of LPS exposure animals demonstrated statistically significant differences between all the groups in MCP-1 ($P = .008$), IL-8 ($P < .001$), TNF- α ($P = .021$), and IL-1 β ($P = .022$; Figure 1). There was however no significant difference between all the groups detected in IL-6 ($P = .6$), IL-10 ($P = .7$), and TLR-4 ($P = .158$) expression (Figure 1).

Within groups, the expression of chemokine MCP-1 in the 12-hour LPS exposure group was significantly increased when compared with the control group ($P = .002$; Figure 1A). Interleukin 8 mRNA expression increased greatly; a significant increase in expression was identified in fetal skin after exposure from 24 hours to 15 days of LPS stimulation compared

Figure 1. Quantitative PCR analysis of cytokines and receptor expression in fetal ovine skin following intra-amniotic exposure to LPS for different time periods. Graphs represent mean fold change + SEM. *indicates a significant increase in transcript expression relative to control. The transverse line in each graph represents no change in expression relative to pooled control. PCR indicates polymerase chain reaction; LPS, lipopolysaccharide; SEM, standard error of the mean.

with the control group ($P < .05$). The relative ratio of IL-8 expression peaked in the 4-day LPS exposure group ($P = .002$; Figure 1B).

Compared with the pooled control group, the expression of TNF- α in 12-hour, 2-day, 8-day, and 15-day LPS exposure groups significantly increased ($P < .05$; Figure 1C). Expression of IL-1 β in fetal skin was significantly increased in fetal skin exposed to LPS for 2 days, 4 days, and 15 days compared with the pooled control group ($P < .05$; Figure 1D). Expression of IL-6 was significantly increased only in fetal skin exposed to 12-hour LPS ($P = .010$; Figure 1E). Compared with the control group, IL-10 mRNA expression in fetal skin was significantly increased after 12-hour, 2-day, and 15-day LPS exposure ($P < .05$; Figure 1F). The LPS receptor TLR-4 was

significantly increased only in the 12-hour LPS exposure group when compared with the control group ($P = .032$; Figure 1G).

Interleukin 8 protein quantification by ELISA further confirmed the increase in IL-8 mRNA expression. The IL-8 protein expression increased significantly after 2 days, 4 days, and 8 days of LPS exposure compared with the control group ($P < .05$; Figure 2).

Immunohistochemical analyses of fetal skin for the 4 inflammatory mediators that were upregulated at a transcript level revealed similar changes in the apparent intensity of protein staining (Figure 3). The staining for both TNF- α and IL-1 β was mainly located in the epidermis and the intensity of staining was much stronger in the 12-hour and 2-day group compared with the control group (Figure 3, TNF- α and IL-1 β),

Figure 2. Interleukin (IL)-8 protein expression in fetal ovine skin following intra-amniotic exposure to lipopolysaccharide (LPS) for different time periods. The scatter plot represents all the IL-8 protein values normalized against the concentration of total protein for each sample in each group. The transverse line represents the median value of each group. * indicates a significant increase in protein expression relative to control.

although the staining pattern observed in the 15-day group did not correlate as well with the qPCR results. The expression of MCP-1 was mainly in the apical layer of the epidermis in the control group (Figure 3, MCP-1), however, in the 12-hour group, the signal was much stronger in both epidermis and dermis and was distributed evenly across the entirety of the epidermis. The staining pattern for IL-8 was predominantly limited to the epidermis in control animals. Both staining intensity and distribution increased progressively after LPS stimulation; the signal in both epidermis and dermis peaked in the 8-day exposure group (Figure 3, IL-8).

Influx of Inflammatory Cells in Fetal Skin

Hematoxylin and eosin staining of the skin from fetuses exposed to LPS demonstrated a progressing basophilic infiltration of the epidermis and dermis (Figure 4). The epidermis demonstrated an apparent increase in thickness beginning at 12-hour post-LPS exposure and reached a peak in the skin of fetuses exposed to IA LPS for 2 days before appearing to abate. The basophilic infiltration was most striking in preparations from fetuses exposed to LPS for 12 hours to 4 days; however, dermal infiltration was still noticeable in the skin of fetuses exposed to LPS for 15 days.

To further characterize the inflammatory cell infiltration, we investigated the recruitment of CD-3+ T lymphocytes to the fetal skin after LPS exposure (Figure 5). CD-3+ T lymphocytes were uncommon in skin from animals exposed to saline or LPS for 5 hours or 12 hours (Figure 5A-C). Increased numbers were apparent in fetal skin exposed to LPS for 24 hours and 2 days

(Figure 5D and E). The lymphocyte infiltration persisted for 15 days after the LPS exposure (Figure 5H).

Discussion

The dynamic response of fetal skin to bacterial exposure is, to date, largely unstudied. The data in the current article constitute the first assessment of which we are aware of the nature and timing of the inflammatory response of the fetal skin to in utero LPS exposure over a 15-day period. These data suggest that, in contrast to other fetal organs such as the lung,^{20,25} the fetal skin continues to express proinflammatory cytokines for at least 15 days after initial exposure to IA LPS.

As the evidence demonstrating a causal association between in utero infection, the fetal inflammatory response and preterm labor (especially early preterm labor), so does the need to understand both the origins and dynamics of in utero inflammation.⁹ The inflammatory response mounted by the fetal lung,²⁵⁻²⁶ brain,²⁷ and intestine²⁸ toward in utero infection have been investigated widely. The fetal skin however, has until recently received little attention as a likely source of the in utero inflammation causally associated with preterm birth.^{24,29,30} It is well appreciated that the adult skin is an immunological response organ.¹⁴ The fetal skin recently was shown to possess the ability to mount an inflammatory response to in utero inflammation.^{13,15}

The expression of 2 chemokines, MCP-1 and IL-8 were assessed in this study. The expression of both targets was found to be significantly increased following LPS stimulation of the fetal skin; MCP-1 expression was significantly increased in fetal skin from animals exposed to LPS for 12 hours, with transcript expression returning to baseline for all other time points assessed. In contrast, IL-8 transcript expression remained significantly increased at all time points after 12 h, with the largest increase in expression seen following 4 days of LPS exposure. The ELISA analysis of IL-8 expression demonstrated a similar pattern, with the amount of IL-8 protein in the fetal skin significantly increased between 2 days and 8 days of LPS exposure.

Chemokines play a crucial role in the initiation of a robust immunological response to infection; with primary roles including the timely infiltration of leukocytes, differential activation of immunocytes and stimulation of proinflammatory cytokine expression. The MCP-1 is a member of the CC chemokine subfamily and a potent monocyte attractant. The MCP-1 acts through binding to G-protein-coupled receptors on the surface of leukocytes targeted for activation and migration.³¹ The IL-8 is a potent chemotactic and neutrophil-activating factor and a part of the response elicited in the host against a microbial invasion. The IL-8 is also responsible for the recruitment of neutrophils to the fetal membranes and the placenta during the development of an intrauterine infection.³²

The magnitude of specific transcript expression in the fetal skin was highly dependent on the duration of LPS stimulation. The IL-6 is a multifunctional cytokine that regulates immune response, inflammation, and hematopoiesis. Increases in IL-6 expression are frequently identified in association with preterm

Figure 3. Immunohistochemical analysis of proinflammatory cytokines (TNF- α and IL-1 β) and chemokines (MCP-1 and IL-8) in the ovine fetal skin after different periods of in utero LPS stimulation. The last row are representative 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) staining images in each group. Staining for TNF- α and IL-1 β was increased in the epidermis of animals exposed to LPS for 12 hours and 2 days compared with saline-exposed control animals (TNF- α -c, IL-1 β -c). The expression of MCP-1 in the 12-hour LPS exposure group was markedly increased compared with control group (MCP-1-c). The staining intensity of IL-8 was stronger in the 8-day group than in the control group (IL-8-c). Scale bar: 50 μ m. TNF- α indicates tumor necrosis factor- α ; IL, interleukin; MCP-1, monocyte chemoattractant protein 1; LPS, lipopolysaccharide.

birth, and IL-6 is recognized as a marker of systemic FIRS (cord blood IL-6 >11 pg/mL).⁹ In the present study, IL-6 transcript expression in the fetal skin was found to be increased significantly only after 12 hours of LPS stimulation, suggesting an acute role for IL-6 in fetal skin inflammation in accordance with IL-6's biological role as an important mediator of the acute phase response to tissue injury.³³ The IL-6 transcript expression

was not significantly upregulated at later time points. These data suggest that, other than the initial phase of the inflammatory response, the fetal skin is not a key source of IL-6 expression in our ovine model of intrauterine inflammation.

The expression of TNF- α was significantly increased at 12 hours, 2 days, 8 days, and 15 days following LPS exposure; a similar expression pattern was identified for IL-1 β , which was

Figure 4. Histological features of ovine fetal skin following different periods of in utero LPS stimulation. A represents the IA saline control group. B to H represents exposure following 5 hours to 15 days intraamniotic LPS treatment. Scale bar represents 50 μ m. LPS indicates lipopolysaccharide; IA, intra-amniotic.

significantly upregulated 2 days, 4 days, and 15 days post-LPS administration. The proinflammatory cytokines IL-1 β and TNF- α are considered to be the central mediators of septic shock and play a central role in the mechanism of inflammation/infection-induced preterm parturition.³ The protracted expression of IL-1 β and TNF- α by the fetal skin in response to LPS stimulation may be of significance, given association of these cytokines with the induction of infection-associated preterm labor in response to infection.

The patterns of cytokine/chemokine expression in the skin are different from the responses described in the fetal lung. For example, our previous study showed higher cytokine mRNA 2 days after exposure in the fetal lung than skin. However, the cytokine decreased in the lung by 7 days.²⁵ Since the fetal skin has such a prolonged duration of proinflammatory cytokine expression and it is directly bathed in amniotic fluid, the increased cytokines in the epidermis might be released to the amniotic fluid contributing to a persistent inflammatory response.

Histological analysis of the fetal skin's response to LPS stimulation demonstrated a robust, progressive response with an accumulation of basophilic staining cells in epidermis and dermis between 2 days and 15 days post-LPS exposure. The pattern of basophilic accumulation in the dermis and epidermis was broadly consistent with the increases in cytokine/chemokine expression. Although an apparent increase in the frequency of CD3+ T-cells was identified, this increase was not as marked as had been anticipated. One potential explanation for this may stem from the structural immaturity of the developing fetal skin, allowing immunocytes to egress into the amniotic fluid. Indeed, our previous studies have suggested that immunocytes are recruited into the amniotic fluid.²⁰ Immunocytes isolated from the amniotic fluid were found to express proinflammatory cytokine mRNA for 7 days (with no cytokine mRNA detectable in the chorioamnion, lung, or spleen after 72 hours) after IA LPS administration.²⁰

In conclusion, the fetal skin can mount a vigorous and lengthy inflammatory response to direct exposure to the

Figure 5. Merged images of CD-3+ T lymphocytes and cell nuclei in fetal ovine skin following different periods of in utero LPS stimulation. A represents the IA saline control group. B to H represents following 5 hours to 15 days IA LPS treatment. Scale bar represents 100 μ m.

TLR-4 agonist LPS. The importance of these data is 2-fold: first, due to its significant mass, external aspect, and lengthy inflammatory response, the fetal skin may be an important source of the inflammation associated with fetal injury and pre-term birth. Second, we suggest that targeting the inflammatory response initiated by the fetal skin in response to in utero infection may serve, in conjunction with treatment of the infection itself, as a possible way of controlling the inflammation that mechanistically underpins the induction of preterm labor and fetal injury.

Declaration of Conflicting Interests

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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