Placental Inflammation and Fetal Hemodynamics in a Rat Model of Chorioamnionitis

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ABSTRACT: The relative contributions of inflammation and ischemia to the pathogenesis of periventricular leukomalacia (PVL) have not been elucidated. We hypothesized that fetal cardiovascular function and cerebral blood flow velocity (BFV) would be decreased in a rat model of chorioamnionitis. We also tested whether placental inflammation was related to proximity to the cervix in our model of chorioamnionitis [intracervical lipopolysaccharide (LPS) or vehicle (PBS) injection]. On embryonic d 15, Sprague-Dawley rats underwent baseline maternal and fetal echocardiography, followed by LPS or PBS injection, then serial echocardiographic evaluations until embryonic day (ED) 21. One hour after birth, pups had middle cerebral artery (MCA) BFV measured. Placentas of LPS-exposed pups exhibited uniform, higher inflammation grades ($p < 0.001$). All fetal BFVs increased with advancing GA ($p < 0.001$) whereas resistance index (RI) decreased ($p < 0.001$). There was no difference in fetal BFV between the groups other than a reduced gestation-related increase in descending aorta BFV in LPS-exposed rats ($p < 0.05$). Newborn pups exposed to LPS had lower heart rate ($p = 0.006$) and MCA BFV ($p = 0.024$) and higher RI ($p = 0.003$) and pulsatility index (PI; $p = 0.004$). In conclusion, intracervical LPS injection produces an inflammation independent of placental position, a blunted increase in gestation-related aortic BFV, and a decrease in MCA BFV in newborn pups. (*Pediatr Res* **68**: 513–518, 2010)

Periventricular leukomalacia (PVL) describes necrosis of the white matter adjacent to the lateral cerebral ventricles, which occurs primarily among premature infants and is strongly associated with the development of cerebral palsy (1,2). Historically, the pathogenesis of PVL was attributed to hypoxia/ischemia of developing oligodendroglia during their most vulnerable period in the late mid-trimester (3). Recently, several epidemiological and experimental studies have implicated chorioamnionitis in the pathogenesis of PVL (1–8). This association suggests that inflammation, rather than hypoxia/ischemia, may be a primary cause of white matter injury. However, because chorioamnionitis is also associated with hemodynamic alterations in the newborn premature infant (9,10), it remains possible that inflammation and ischemia both contribute to the pathogenesis of PVL in chorioamnionitis-associated preterm brain injury.

Several animal models have been developed to study the pathogenesis of chorioamnionitis-associated PVL (11–14). We have shown that intracervical injection of lipopolysaccharide (LPS) to a gravid rat produces PVL-like lesions in the brains of surviving LPS-exposed pups (13,14). The lesions are characterized by apoptosis (measured by TUNEL staining) and inflammation (TNF-α staining) (15). Our model (13) allows the investigation of fetal hemodynamics during and after LPS exposure because a majority of the pups survive. Yet, the contribution of ischemia to white matter injury had not been studied previously in this model.

The primary objective of our study was to determine whether maternal and fetal cardiovascular function and fetal and newborn cerebral blood flow velocities (BFV) are altered in our rat model of LPS-induced chorioamnionitis. As a secondary objective, we evaluated placental histology to investigate the relationships between the degree of placental inflammation and both fetal uterine position (relative to the cervix) and time after LPS injection.

MATERIALS AND METHODS

This protocol was approved by the Animal Care Committee of the University of Pittsburgh. Timed-pregnant Sprague-Dawley rats (Harlan Sprague Dawley, Inc., Indianapolis, IN) obtained at embryonic day (ED) 14 were allowed to acclimate to our animal facility for 24 h. On ED 15, rats were randomly assigned to LPS or PBS. Intracervical injection of LPS or PBS was performed as previously described (13). Briefly, each gravid rat was anesthetized in a transparent plastic holding box containing 4% isoflurane blended with 100% oxygen at 2 to 3 L/min flow. The rat was transferred to a table prepared with a custom-made warming pad. Anesthesia was continued *via* a snug-fitting nose cone with 1.5 to 2% isoflurane blended with 100% oxygen at 2 L/min flow. Adequacy of anesthesia was defined as cessation of spontaneous movement, absence of startle reflex, and nonlabored breathing. Animals underwent baseline maternal and fetal echocardiography as detailed below, followed by injection of 0.1 mL LPS [*Escherichia coli* (*E. coli*) serotype 0111:B4; Sigma Chemical, Co., St. Louis, MO] or PBS into the cervix. Serial echocardiographic evaluations were performed as described below. Pups were delivered by cesarean (C)-section so that the placentas could be harvested. Cerebral BFV was measured *via* ultrasound in the newborn pups before killing.

Echocardiogram study. Serial transabdominal echocardiograms were performed as follows: ED 15 (baseline and before injection), ED 15 (3 h
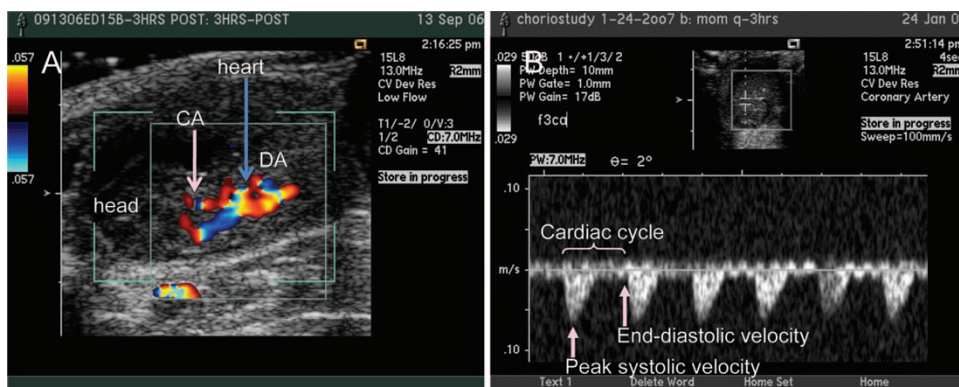
Figure 1. Fetal carotid artery identification and Doppler flow velocity wave forms. Doppler flow mapping of fetal heart and major vessels. *A*, Color flow mapping is used to identify the heart, carotid artery (CA), and descending aorta (DA). *B*, The cursor is placed over the CA to obtain the Doppler BFV waves. Mean velocity is obtained as area under the curve for each cardiac cycle.

postinjection), ED 16 (24 h postinjection), and ED 21 (6 d postinjection). All echocardiography studies were performed using an Acuson Sequoia C256 with a linear-phased array transducer 15L8 (Siemens Medical Solutions) by an investigator who was unaware of treatment group assignments.

The abdomen of each supine, anesthetized gravid rat was shaved and ultrasound transmission gel applied. Standard parasternal long-axis and short-axis images were recorded to calculate maternal heart rate, left ventricular dimensions, and left ventricular contractility. Then, an abdominal ultrasound screen, starting from the right lower quadrant of the gravid abdomen and advancing clockwise, was performed to identify the number and location of fetuses. BFVs in the umbilical artery and vein, descending aorta, and carotid artery (Fig. 1) were measured in individual fetuses. For each vessel, color flow mapping was used to identify the vessels and pulse wave Doppler velocimetry used to capture waveforms. Seven to 10 sequential waveforms were recorded from each artery. The three consecutive flow waveforms with the highest systolic velocities were selected for analysis. Resistance index [RI: (peak systolic BFV − end-diastolic BFV)/peak systolic BFV] and the pulsatility index [PI: (peak systolic BFV − end-diastolic BFV)/mean BFV] were calculated.

Inflammation protocol. LPS or PBS were injected immediately after baseline hemodynamic study while the rat was still anesthetized. The LPS was freshly prepared at a dilution of 1.25 mg LPS/mL PBS. By using the mean maternal weight on ED 15 of 250 g, a technician not involved in the hemodynamic evaluations drew up 0.1 mL of the LPS solution (0.5 mg/kg) and 0.1 mL of sterile PBS into separate sterile insulin syringes labeled only with the rat's code number. The vagina of the gravid rat was gently dilated using a nontooth forceps and kept open with a clean self-retaining speculum. The cervix was identified and gently held with a nontooth forceps. A total of 0.1 mL of the prepared LPS or PBS was injected into the cervical wall at the 12 o'clock position. The vaginal speculum was removed and anesthesia discontinued. The gravid rat was returned to its cage to recover.

Hysterotomy (C-Section). Hysterotomy was done immediately after the ED 21 echocardiography. After anesthesia was deepened (4% isoflurane blended with 100% oxygen), a midline vertical incision was made on the abdomen, and the gravid uterine horns were sequentially exposed. The uterine position of each pup and placenta relative to the cervix were recorded. By using a scalpel, a small incision was made on the uterine wall that resulted in delivery of the amniotic sac containing an individual pup and its placenta. The placenta was immediately separated from the pup and preserved for later analysis. After delivery of all pups, the gravid mother was killed while under anesthesia.

The pups. After delivery, the pups were kept warm in a forced draft incubator. At 1 h of life, surviving pups were transferred onto a customized warming pad for cranial ultrasound imaging using the Acuson system as described earlier. BFV was recorded in a middle cerebral artery (MCA) of each pup using light anesthesia (1.5% isoflurane blended with 100% oxygen) administered *via* face mask. At the completion of cranial imaging, anesthesia was increased to 4% isoflurane, and pups were killed.

Placental processing. Once the placenta was separated from the pup, a linear incision was made on the fetal surface of the placenta extending about [1/3]-[1/2] the thickness of the placental plate. The placenta was then immersed in 4%-buffered paraformaldehyde fixative for 3–7 d. Each placenta was then bisected with a transverse cut from the fetal to the maternal surface and embedded in paraffin block from which 4-μm sections were cut. Six sections were placed on an individual glass slide, and the slides were baked at 56°C for 30 min. Slides were subsequently stained with hematoxylin and eosin (H&E).

Table 1. *Placenta pathology score*

| Score	Placenta pathology
0	No pathology seen
1	Acute decidual necrosis at edge with mild infarction, gestational sac intact, and parenchyma intact
2	Acute decidual necrosis along basal plate, gestational sac intact, and parenchyma intact
3	2 + local gestational sac necrosis
4	Extensive decidual necrosis with inflammation and microabscesses, gestational sac necrosis, and parenchyma necrosis
5	Findings of stage 4 plus variable edema
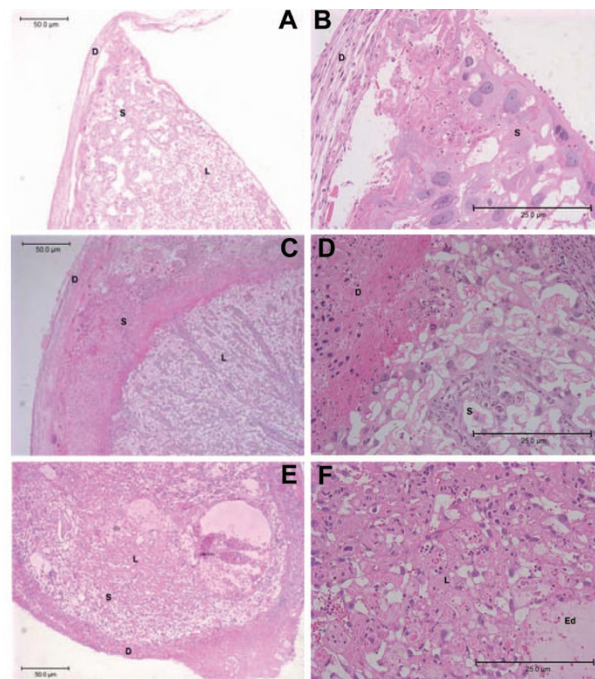
Figure 2. Placental H&E at ×4 (*A, C, E*) and ×20 (*B, D, F*) magnifications. Representative samples of inflammation scores. Score 0 (*A, B*), no inflammation; score 4 (*C, D*), high-grade inflammation; and score 5 (*E, F*), severe inflammation. *D*, decidua; *S*, spongiotrophoblast; *L*, labyrinth; *Ed*, edema.

H&E scoring system. A grading system was developed and applied to the placental H&E slides (Table 1). Scores ranged from 0 to 5 (Fig. 2) and were based on the severity and extent of placenta inflammation and necrosis. The placental slides were reviewed and scored by a pathologist who was unaware of experimental group assignment or uterine position.

Supplemental experiments. Two supplemental experiments were performed. Supplemental experiment I was done to evaluate early placental
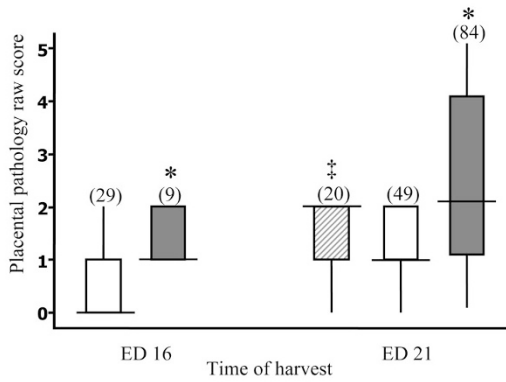
Figure 3. Placental pathology raw scores and time of harvest. Scores at ED 16 and ED 21 in rats exposed to PBS (□), LPS (▩), and those not injected (▨). LPS-exposed rats exhibited a higher degree of inflammation than those exposed to PBS at 24 h and 6 d postexposure (*p = 0.000), whereas rats not injected have a higher degree of inflammation than those exposed to PBS (‡p = 0.036). Median and Q1–Q3 plotted.
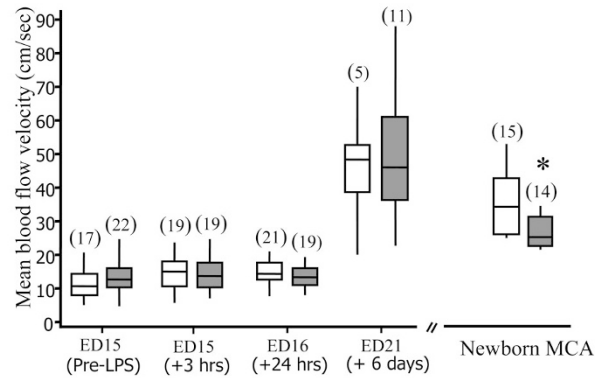

Figure 4. Mean BFV in fetal carotid artery and newborn MCA. Mean BFV increased with advancing GA in both PBS-exposed (□) and LPS-exposed (▩) rats, with no difference between the groups. In contrast, newborn pups exposed to LPS had significantly lower mean BFV in the MCA compared with pups exposed to PBS (Tukey's test *p = 0.024). Median and Q1–Q3 plotted.

inflammation. On ED 15, gravid rats underwent intracervical injection of LPS (n = 1) or PBS (n = 2). Hysterotomy occurred 24 h later. Placentas were harvested and processed as described earlier. Placenta slides were scored as described earlier by a single pathologist who was unaware of treatment assignment. The pups were killed after delivery. No hemodynamic studies were conducted in this group.

Supplemental experiment II was done to define the normal range of placental pathology scores at term. On ED 21, hysterotomy was performed on two gravid rats. Placentas were harvested and processed as described earlier. Placenta slides were scored as described earlier by a single pathologist. These noninjected placentas were intermixed with the experimental placentas (LPS and PBS) so that the pathologist remained blinded to the injection status of each placenta during scoring. Pups were killed after delivery. No hemodynamic studies were done in this group.

Statistical analysis. Hemodynamic parameters were analyzed using two-way ANOVA with Tukey's post hoc test (fetal data; Sigma Stat version 3.0) and Mann-Whitney U test (Minitab 15, pups data). The pathology scores were compared between the groups using Mann-Whitney U test. The p values of <0.05 were considered significant.

RESULTS

Twenty gravid rats had intracervical injections (LPS, n = 11 and PBS, n = 9). No maternal morbidity or mortality occurred in either study group. All fetuses from three LPS-exposed gravid rats and eight fetuses total from two other LPS-exposed gravid rats died *in utero*, yielding a 41% fetal mortality rate for LPS-exposed fetuses. No fetal mortality occurred in either the PBS-exposed or the noninjected (n = 2) controls.

Placenta pathology. One hundred ninety-one placentas were harvested from 16 mothers. The placentas of LPS-exposed pups (n = 93) exhibited significantly higher grades of inflammation than the placentas of PBS-exposed pups (n = 78) at 24 h and 6 d postexposure (Fig. 3, *p = 0.000). The degree of inflammation did not correlate with the proximity of the placenta (fetus) to the cervix. Placentas from pups not exposed to any injection (n = 20) had higher inflammation scores than placentas of pups exposed to PBS injection (Fig. 3, ‡p = 0.036), although both remained in the mild range.

Hemodynamic studies. Hemodynamic studies were performed on 12 gravid rats (LPS = 7 and PBS = 5) and 29 newborn pups (LPS = 14 and PBS = 15). The numbers of fetuses examined differ at each time point (Fig. 4). There was

Table 2. *Maternal hemodynamic parameters*

Experimental group	Baseline	3 h Postinjection	24 h Postinjection	6 d Postinjection (ED 21)
End-diastolic diameter (EDD, mm)				
PBS	81.4 (3.97)	80.0 (3.97)	78.6 (3.97)	73.9 (5.12)
LPS	79.4 (3.35)	83.2 (3.35)	76.2 (3.35)	73.0 (3.97)
End-systolic diameter (ESD, mm)				
PBS	41.3 (2.9)	42.6 (2.9)	40.6 (2.9)	41.0 (3.8)
LPS	41.7 (2.47)	45.1 (2.47)	37.7 (2.47)	41.0 (2.9)
Fractional shortening (EDD − ESD/EDD)				
PBS	0.49	0.47	0.48	0.46
LPS	0.47	0.46	0.51	0.44

Except for fractional shortening, values represent mean (±SEM).

Table 3. *Resistance index and pulsatility index in the fetal carotid artery*

Experimental group	Baseline	3 h Postinjection	24 h Postinjection	6 d Postinjection (ED 21)
RI				
PBS	1.0 (0.004)	0.99 (0.005)	1.0 (0.005)	0.86 (0.007)*†
LPS	1.0 (0.004)	1.0 (0.004)	1.0 (0.005)	0.87 (0.005)*†
PI				
PBS	2.97 (0.14)	3.59 (0.15)	2.83 (0.14)	1.88 (0.2)*†
LPS	2.99 (0.12)	2.97 (0.12)	2.87 (0.14)	1.9 (0.15)*†

Values represent mean (±SEM).
* ANOVA (time) p < 0.001.
† Tukey's test p < 0.001 (ED 21 *vs* all previous time points).

no change in maternal heart rate or left ventricular function with advancing gestation nor any difference between mothers exposed to LPS *versus* PBS (Table 2).

Fetal carotid artery. With advancing GA, heart rate, carotid artery peak systolic, end-diastolic, and mean (Fig. 4) BFVs all increased (ANOVA, p < 0.001), whereas RI and PI decreased (Table 3, ANOVA, p < 0.001) in both the LPS- and

Table 4. *Fetal descending aorta doppler indices*

Experimental group	Baseline	3 h Postinjection	24 h Postinjection	6 d Postinjection (ED 21)
Peak velocity (cm/s)				
PBS	15.1 (0.69)	19.1 (0.70)	18.3 (0.69)	25.4 (0.92)*†‡
LPS	15.5 (0.56)	17.5 (0.58)	16.3 (0.64)	22.6 (0.74)*‡
Minimum velocity (cm/s)				
PBS	4.5 (0.4)	0.1 (0.04)	4.5 (0.04)	0.3 (0.05)*
LPS	3.8 (0.03)	4.3 (0.03)	3.8 (0.03)	0.3 (0.04)*
Mean velocity (cm/s)				
PBS	4.9 (0.25)	6.5 (0.26)	6.3 (0.25)	8.8 (0.33)*§‡
LPS	5.2 (0.20)	5.9 (0.21)	5.7 (0.23)	8.1 (0.27)*‡
RI				
PBS	1.0 (0.002)	1.0 (0.002)	1.0 (0.002)	0.99 (0.003)*‡
LPS	1.0 (0.002)	1.0 (0.002)	1.0 (0.002)	0.99 (0.002)*‡
PI				
PBS	3.22 (0.08)	2.95 (0.08)	2.91 (0.08)‖	2.85 (0.11)*¶
LPS	3.01 (0.07)	3.01 (0.07)	2.88 (0.08)	2.79 (0.09)

* ANOVA (time) $p < 0.004$.
† ANOVA (group) $p = 0.003$.
‡ Tukey's test $p < 0.001$ (d 21 *vs* all previous time points).
§ ANOVA (group) $p = 0.022$.
‖ Tukey's test $p = 0.041$ (24 h *vs* baseline).
¶ Tukey's test $p = 0.032$ (ED 21 *vs* baseline).

Table 5. *Fetal umbilical artery Doppler indices*

Experimental group	Baseline	3 h Postinjection	24 h Postinjection	6 d Postinjection ED 21
Peak velocity (cm/s)				
PBS	7.7 (0.61)	10.0 (0.63)	9.0 (0.62)	20.1 (0.83)*†
LPS	8.4 (0.52)	8.9 (0.53)	9.5 (0.57)	18.7 (0.68)*†
Minimum velocity (cm/s)				
PBS	0.0 (0.04)	0.0 (0.04)	0.0 (0.04)	0.4 (0.05)*
LPS	2.8 (0.03)	2.8 (0.03)	2.8 (0.03)	0.3 (0.04)*
Mean velocity (cm/s)				
PBS	2.6 (0.22)	3.3 (0.22)	3.2 (0.22)	7.2 (0.3)*†
LPS	2.8 (0.18)	3.0 (0.19)	3.4 (0.2)	6.7 (0.24)*†
RI				
PBS	1.0 (0.002)	1.0 (0.002)	1.0 (0.002)	0.97 (0.003)*†
LPS	1.0 (0.002)	1.0 (0.002)	1.0 (0.002)	0.98 (0.003)*†
PI				
PBS	2.96 (0.11)	3.23 (0.11)	2.85 (0.11)	2.76 (0.15)*‡
LPS	3.03 (0.09)	2.97 (0.09)	2.82 (0.1)	2.83 (0.12)

* ANOVA (time) $p < 0.05$.
† Tukey's test $p < 0.001$ (d 21 *vs* all previous time points).
‡ Tukey's test $p = 0.051$ (ED 21 *vs* 3 h postinjection).

the PBS-exposed groups. On post hoc analysis, the ED 21 values for all carotid artery variables differed from that of all previous study times (Tukey's test $p < 0.05$). There was no significant difference between the two experimental groups over time.

Fetal descending aorta. With advancing GA, descending aortic peak systolic, end-diastolic, and mean BFVs increased (ANOVA $p < 0.001$) in both groups. However, peak and mean systolic BFVs increased less over time in fetuses exposed to LPS compared with those exposed to PBS (Table 4, ANOVA $p = 0.003$ and $p = 0.022$, respectively). The RI and PI decreased with advancing GA in both groups (Table 4, ANOVA, $p < 0.001$ and $p = 0.004$, respectively). On post hoc analysis, the ED 21 values for all descending aorta variables except for PI differed from that of all previous study times (Tukey's test $p < 0.05$). Post hoc analysis for PI revealed significant differences between values at 24 h postinjection and ED 21 compared with baseline values in PBS-exposed fetuses only (Tukey's test $p = 0.04$ and 0.032). Neither RI nor PI differed between the groups.

Umbilical artery and vein. With advancing GA, umbilical artery and vein peak systolic, end-diastolic, and mean BFVs increased and the RI and PI decreased (Table 5, ANOVA, $p < 0.05$) in both groups. On post hoc analysis, the ED 21 peak systolic and mean BFVs and RI values differed from all previous time points ($p < 0.001$). Post hoc analysis for PI revealed significant differences between values at ED 21 compared with 3 h postinjection ($p = 0.05$). No significant difference was seen between the LPS and PBS groups.

Newborn pup MCA. Both heart rate (225 *versus* 278 bpm; $p = 0.006$) and MCA BFV (Fig. 4, $p = 0.024$) were lower in LPS-exposed newborn pups compared with PBS-exposed

pups. The RI and PI were higher in the LPS-exposed pups compared with those exposed to PBS ($p = 0.003$ and $p = 0.004$, respectively).

DISCUSSION

This is the first study to describe the placental findings and hemodynamic changes that occur in a rat model of chorioamnionitis induced *via* LPS injection into the cervix. We found that placentas of LPS-exposed fetuses exhibited significantly higher grades of inflammation than placentas of PBS-exposed fetuses at 24 h and 6 d postexposure. The fetal heart rate and peak systolic, end-diastolic, and mean BFV increased, whereas the RI decreased with advancing GA in all vessels studied in both experimental groups. In the fetal descending aorta, peak systolic and mean BFVs increased less over time in the LPS-exposed fetuses than in the PBS-exposed fetuses. Umbilical and cerebral BFVs did not differ between LPS- and PBS-exposed fetuses. However, mean BFV was lower, and the RI and PI higher, in the MCA of LPS-exposed newborn pups compared with PBS-exposed pups.

We chose to use the cervical LPS injection model to induce chorioamnionitis (13) rather than i.v., i.p., intrauterine, or intracranial routes of LPS injection to facilitate the study of chorioamnionitis-associated fetal hemodynamic alterations because of 1) a relative close resemblance to how human chorioamnionitis may evolve and 2) a relatively lower risk for maternal morbidity and mortality, fetal mortality, and preterm delivery, which allows for repeated measurements over time.

Our placental histology results provide evidence that our model is valid for studying chorioamnionitis in the rat. Placentas from LPS-exposed pups showed a substantially higher degree of wide-spread inflammation (chorioamnionitis) compared with placentas from PBS-exposed pups. Placental inflammation was evident by 24 h postinjection and was even

greater at 6 d postinjection. The degree of inflammation was not related to fetal position relative to the injection site (cervix). This suggests that LPS injected into the cervix may have either been absorbed in the gravid rat to induce systemic inflammation that spread to the fetuses (16) or induced local cervical inflammation, which then extended uniformly to the fetuses (12,16). It is interesting to note that placentas from naive (noninjected) pups showed baseline inflammation of a statistically higher degree than that of the PBS-injected control placentas. However, the extent of inflammation in both groups was no more than grade 2 and was restricted to the decidua (the maternal component of the placenta). Thus, this mild inflammation may not represent true chorioamnionitis and could be normal. In humans, it is not unusual to find foci of acute deciduitis in the absence of chorioamnionitis. In such cases, it is thought to represent ascending infection before development of true chorioamnionitis or to be the consequence of uterine contractions and mechanical shearing during labor. The rats used in this study are supposed to be pathogen free, and delivery was by planned C-section before the onset of labor, reducing the likelihood of infection. Still, we cannot exclude the possibility that one or more rats were in early labor before hysterotomy.

We conducted hemodynamic studies to evaluate the influence of advancing GA on cardiovascular function in a developing rat fetus and how this is modified in the presence of chorioamnionitis. Our primary measure was Doppler flow velocity, which is used as an index for changes in flow rather than as an absolute value. BFV has been shown to correlate linearly with cerebral (17) and mesenteric (18) blood flow measured by radionucleotide microspheres. The area under the curve of the Doppler flow velocity wave form is directly related to flow for vessels of fixed diameters. We did not measure vessel diameter (and volume flow) because a) there can be variations in vessel diameter between systole and diastole and b) lack of adequate resolution in the rat fetus limited accuracy. Indistinct vessel walls and small changes in fetal position may alter the appearance of vessel diameter. Because, by Poiseuille's equation, flow is proportional to the fourth power of the vessel radius, a small change in (or error in measurement of) radius will manifest as a 4-fold change in flow.

We are the first group to report maternal and fetal hemodynamic studies in this model of chorioamnionitis. Duncan *et al.* (2) had used repeated i.v. injection of LPS and showed decreased blood pressure and decreased arterial oxygen saturation of arterial blood in premature ovine fetus. Eklind *et al.* (19) used i.p. LPS injection in 7-d-old rat pups; they measured the cerebral blood flow using autoradiographic iodo $[^{14}C]$ antipyrine method adapted to immature rats. They found no differences in the cerebral blood flow between the LPS and the control groups. In our study, the increase in BFV and the decrease in RI with advancing GA seen in the carotid artery of both groups of rats most likely represent normal developmental changes.

With advancing GA, physiologic increase in BFVs and decrease in RI was also seen in descending aorta and umbilical artery and vein of both groups. However, there were some regional differences. The increases in peak systolic and mean velocity in the descending aorta were significantly less in the LPS-exposed fetuses compared with those exposed to PBS. This suggests that the presence of LPS had depressed the physiologic increase in peak systolic and mean velocities seen with advancing GA. We propose that this finding was limited to the descending aorta because of preferential tight regulation of blood flow to vital organs (*e.g.* brain) in a developing rat fetus.

We had hypothesized that the pathogenesis of chorioamnionitis-associated PVL may include fetal cerebral ischemia resulting from *in utero* decreases in cerebral blood flow. We were unable to prove this theory with this study design. However, we did find a significantly lower middle cerebral arterial BFV and higher RI and PI in LPS-exposed newborn pups compared with the PBS-exposed pups. This suggests that although the fetuses exposed to LPS were able to compensate *in utero*, the added stress of delivery may have unmasked inherent hemodynamic alterations. Light anesthesia was used in both groups of pups and, therefore, unlikely to account for the observed differences. The small decrease in neonatal cerebral blood flow alone may not result in PVL because of the presence of reserve brain oxygen extraction. However, in the face of cytokine-mediated damage, the brain may be less able to extract needed additional oxygen, and this level of blood flow may thus be inadequate for nutrient delivery and contribute to the development of PVL. Prenatal and perinatal imprinting to altered cerebral hemodynamics is likely very important; however, this study was not designed to assess imprinting (*via* epigenetic changes in chromatin, etc).

It is possible that cardiac function was depressed in the newborns, accounting for both the decrease in heart rate and the lower MCA in the LPS-treated newborns. This is supported by the lesser increase in descending aortic BFV with advancing gestation in the LPS-treated fetuses relative to controls. We were unable to measure blood pressure in the newborn because of their small size. We did not perform a detailed cardiac function analysis in this study because our focus was on cerebral blood flow. In newborn infants, cerebral blood flow decreases immediately after birth and then slowly begins to rise over the subsequent hours (20). To obtain reliable MCA measurements of all pups from the litter, it was vital to catch all newborns at approximately the same time point after birth, a process that was expedited by limiting measurements to the MCA BFV alone. Further study of newborns including sophisticated imaging of cardiac function and aortic blood flow after birth may help to sort out the contribution of impaired cardiac function to the decreased MCA BFV of LPS-exposed newborns.

This study used a higher dose of LPS than the dose we previously recommended (13) (0.5 mg/kg *versus* 0.1 mg/kg) because our goal was to assess the hemodynamic alterations that occur in association with chorioamnionitis. Eklind *et al.* (19) showed previously that 0.3 mg/kg of LPS given i.p. did not produce altered cerebral blood flow in 7-d-old rat. If LPS were to cause fetal cerebral blood flow disturbances, then we would have expected to see an effect at the 0.5 mg/kg dose, because this dose resulted in 41% fetal mortality. Thus, al-

though a lower dose may have increased the survivability of the fetuses, enabling more repeat measures of parameters, we doubt that BFV changes would have been evident at a lower LPS dose. It is possible that fetal cerebral BFV disturbances might have been observed among the LPS-treated group if we had studied more time points before the fetal demise. For example, we did not acquire hemodynamic data between 24 h and 6 d post-LPS injection. Thus, we may have missed a critical period of fetal hemodynamic instability, which may have resulted in fetal demise or resolved with advancing gestation and fetal adaptation.

Our study has several limitations. First, although it was our preference to measure flow velocity in the MCA, which supplies areas of the preterm infant's brain affected by intraventricular hemorrhage and PVL, imaging through the mother's abdominal wall and uterus limits vessel resolution. Thus, we elected to measure flow velocity in the larger carotid artery of the fetus. Unfortunately, because we measured two different vessels, our study cannot distinguish whether the effect of LPS was limited to the period after birth or to MCA flow. It is possible that the anatomical differences between the carotid artery and the MCA can explain our findings. The carotid artery supplies both intracerebral and extracerebral structures including the face whereas the MCA supplies only intracerebral structures.

Next, because of the growth and folding of the uterine horn as gestation advances, it was impossible to be certain that each fetus maintained the same intrauterine position between the hemodynamic studies. Thus, we were unable to use a repeated measures design, which would have enabled us to follow individual fetal hemodynamics rather than group hemodynamics. We did not use the repeated measures design with "litter" as variable because this would result in a much smaller *N*. In either case, individual hemodynamic changes may have indeed occurred and been masked by the grouped analysis.

We did not weigh the pups' brains. Rather than LPS causing reduced blood flow and, thus, cerebral ischemia, LPS exposure might decrease brain growth and brain weight with a secondary decrease in cerebral blood flow. If these were the case, we would have expected to see decreased cerebral BFV in the fetus approaching term. Finally, because we attempted to keep pups alive for the newborn hemodynamic study, our study designs precluded ideal evaluation for PVL. However, we previously confirmed that our chorioamnionitis model produces PVL in surviving LPS-exposed pups (13,14).

In conclusion, we found that LPS injection into the cervix of the gravid rat induces high-grade chorioamnionitis uniformly across all implantation sites as early as 24 h after injection. We found a blunted increase in descending aortic blood flow with advancing GA among LPS-exposed compared with PBS-exposed fetuses. No regional BFV differences were identified among surviving LPS-exposed compared with PBS-exposed fetuses. However, MCA BFV was lower and the PI and RI higher in the newborn pups exposed to LPS

compared with those exposed to PBS. We speculate that the rat fetuses exposed to LPS may have had borderline hemodynamic changes not detected by our study, which became apparent in the pups following the added stress of delivery. We suggest that this model be adapted to further assess the mechanism of and contribution of cerebral blood flow disturbances to chorioamnionitis-associated PVL and to evaluate the impact of *in utero* stresses, such as hypoxia, on regional hemodynamics in the LPS-exposed fetal rat.

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