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Evaluation of immune system function in neonatal pigs born vaginally or by Cesarean section☆

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Abstract

Full term crossbred sows were selected to study the interaction of the immune system, hypothalamus-pituitary-adrenal axis, and growth in pigs born by Cesarean section (c-section; $n=4$ sows) or vaginal birth ($n=4$ sows). Gestation length and birth weight did not differ between vaginal birth and c-section pigs ($P=0.34$ and 0.62, respectively). Blood and tissue samples were collected from 44 pigs at birth. Forty-five pigs were weaned at 13 d. On d 14, pigs received an i.p. injection of lipopolysaccaride (LPS; 150 μg/kg) or saline at min 0, and blood samples were collected at −20, −10, 0, 5, 10, 20, 40, 60, 90, and 120 min. Vaginal birth pigs had 21% greater average daily gain than c-section pigs on d 14 ($P<0.01$). Basal serum concentrations of adrenocorticotrophin (ACTH) and cortisol were greater in c-section than vaginal birth pigs at birth ($P<0.01$) but were not different at 14 d ($P=0.99$ and 0.80, respectively). LPS increased serum concentrations of ACTH, cortisol, interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α; $P<0.01$) but the response was not different between c-section and vaginal birth ($P>0.22$). Basal serum concentrations of TNF-α tended to be greater in c-section vs vaginal birth pigs at 14 d ($P=0.0967$); however, basal serum concentrations of IFN-γ tended to be lower in c-section pigs vs vaginal birth pigs at 14 d ($P=0.0787$). Expression of interleukin (IL)-6, IL-6 receptor, IL-1β, and TNF-α mRNA did not differ between vaginal birth and c-section pigs but changed in an age and tissue dependent manner. Thus, reduced growth rate of c-section pigs is associated with altered immune system function.

Keywords: C-section; Immune; Growth; Stress; Pigs

1. Introduction

In previous research, pigs born by Cesarian section (c-section) have been reported to have elevated basal serum concentrations of cortisol (CS) relative to vaginal birth pigs at 2 wk of age [1]. We have also demonstrated that pigs born by c-section have reduced growth rates and reduced circulating concentrations of insulin-like growth factor-I (IGF-I) despite having elevated circulating concentrations of growth hormone (GH) compared to vaginally delivered pigs [2].

Previous investigations with humans have provided evidence that the type of birth delivery can affect postnatal function of the immune system. Bessler et al. [3] reported that human infants born by c-section had decreased interleukin-2 (IL-2) at 24 h of age, increased

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ability to secrete interleukin-6 (IL-6) at 24 and 48 h of age, and higher tumor necrosis factor-α (TNF-α) at 48 h of age compared to vaginal birth infants. However, the investigators speculated that the general anesthesia administered to the mother during c-section may have been responsible for some or all of the effects on the immune system. Additional work in the human has provided evidence that c-section delivered infants have elevated levels of interleukin-13 (IL-13) and interferon-γ (IFN-γ; [4]). Vaginally delivered infants exhibited higher median concentrations of blood leukocytes, neutrophils, monocytes, and natural killer cells compared to c-section delivered infants at birth [5]. Furthermore, the survival of neutrophils was increased in vaginally delivered infants at birth [6]. Thus, the type of birth may influence development and function of the immune system.

In pigs, CS has been reported to suppress cell-mediated immune function [7]. Neonatal pigs with a high response of CS to a restraint stress have been reported to have a lower mitogen-induced lymphocyte proliferation response than did pigs with a low response of CS to a restraint stress at 12, 19, and 26 d of age [8]. Additionally, the immune system is not fully developed at birth in pigs [9]. Collectively, these results provide evidence that factors associated with postnatal development and the type of birth experienced may alter function of the immune system in neonatal pigs.

Bi-directional communication between the growth and immune systems influences the function of both systems in domestic livestock [10]. In the pig, challenge with lipopolysaccharide (LPS) stimulates activation of the acute phase immune response with increased circulating concentrations of the proinflammatory cytokines including interleukin-1β (IL-1β), TNF-α, IL-6, and IFN-γ. Additionally, LPS-induced stimulation of the acute phase immune response has been associated with an uncoupling of the GH/IGF-I axis in several domestic species including sheep, cattle, and swine [10]. Given this bi-directional communication between the growth and immune axes, we speculated that an altered immune function in c-section born pigs may be partially responsible for the reduction in growth rate previously observed [2].

Therefore, the present study was designed to determine if the type of birth influences subsequent development and (or) function of the immune system in neonatal pigs. Specifically, we examined serum basal concentrations and LPS-stimulated concentrations of proinflammatory cytokines, as well as selected gene expression in various tissues, associated with the acute phase immune response in pigs born vaginally or by c-section.

2. Experimental procedures

2.1. Experimental animals

Male and female pigs were obtained by c-section ($n = 19$ boars and 24 gilts) or vaginal birth ($n = 22$ boars and 24 gilts) and randomly assigned to one of the following three treatment groups: (1) sacrificed for tissue collection 0.5–1 h after birth (d 0; $n = 10$ boars and 12 gilts for the c-section group and $n = 10$ boars and 12 gilts for the vaginal birth group), (2) assigned to the 2 wk (d 14) saline group (control; $n = 5$ boars and 6 gilts for the c-section group and $n = 5$ boars and 6 gilts for the vaginal birth group), or (3) assigned to the 2 wk LPS group ($n = 5$ boars and 6 gilts for the c-section group and $n = 6$ boars and 6 gilts for the vaginal birth group). Experimental procedures for all animals were reviewed and approved by the University of Missouri Animal Care and Use Committee.

The c-sections were performed on four multiparous sows at the University of Missouri Swine complex as described previously [1]. Briefly, sows were stunned with a penetrating captive bolt and immediately restrained with ropes to prevent excessive movement during the c-sections. Pigs were immediately removed, and then the sow was exsanguinated. Sows were chosen based upon their expected parturition dates to correspond to sows in the vaginal delivery group. Pigs were cross-fostered onto the vaginal birth sows of the present study that had farrowed within the previous 12 h. During the time that c-section delivered pigs were introduced to the vaginal birth sows for cross-fostering, the remaining vaginally delivered pigs were removed for approximately 1 h to allow the cross-fostered c-section pigs the opportunity to suckle without competition from the vaginally delivered pigs. All cross-fostered pigs were monitored for vigor and receipt of colostrum. Forty-four pigs were sacrificed by cervical dislocation by captive bolt followed by exsanguination between 0.5 and 1 h of birth (d 0). Blood samples were collected at sacrifice. Hypothalamus, pituitary, thymus, spleen, and adrenal glands were collected and immediately placed on dry ice and stored at −80 °C for RNA extraction. Serum was harvested and stored at −80 °C for subsequent hormone and cytokine analysis.

The c-section ($n = 22$) and vaginally delivered ($n = 23$) pigs that were not sacrificed at birth were weaned at 13 d of age and fitted nonsurgically with an indwelling jugular angiocatheter as described previously [11]. Briefly, an angiocatheter was inserted into the jugular vein while the pigs were immobilized with halothane for approximately 10 min. The angiocatheter was held in place with tissue

glue and two simple interrupted stitches. Pigs recovered rapidly (within 15 min) from the cannulation procedure. Approximately 24 h following cannulation, pigs were subjected to an i.p. injection of LPS (150 μg/kg) or saline (control). Blood samples were collected at −20, −10, 0, 5, 10, 20, 40, 60, 90, and 120 min by a remote cannula. Lipopolysaccharide or saline was administered following the 0 min sample. Blood samples were stored on ice until further processing. Immediately following the 120 min blood collection, pigs were sacrificed by captive bolt followed by exsanguination. Hypothalamus, pituitary, thymus, spleen, and adrenal glands were collected and immediately placed on dry ice. Tissue samples were then stored at −80 °C until extracted for RNA analysis. Serum was harvested and stored at −80 °C for subsequent hormone and cytokine analysis.

2.2. Centrifuged clot to blood ratio (CCB)

Centrifuged clot to blood ratio was determined as described previously [1]. Briefly, blood samples were collected in 4.5 ml Luer Monovette® serum tubes (Sarstedt, Newton, NC) for harvest of serum. Immediately following collection, blood samples were stored on ice until further processing. Following centrifugation, a ratio of the packed clot to the whole sample was recorded as the CCB and expressed as a percentage.

2.3. Serum analysis

2.3.1. Adrenocorticotropic hormone

Serum concentrations of ACTH were determined by radioimmunoassay using an ACTH double antibody assay kit for human ACTH as per the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA). This commercial assay has been used in our laboratory, as well as in other laboratories, for quantifying ACTH concentrations in the pig [1,12]. All serum samples were evaluated for ACTH in a single assay with a minimum detectability of 8 pg/ml and 2% intra-assay coefficient of variation.

2.3.2. Cortisol

Serum concentrations of CS were determined using a Coat-a-Count® assay kit as per the manufacturer's instructions (Diagnostic Products Corporation). All serum samples were also evaluated for CS in a single assay with a minimum detectability of 2 ng/ml and 2% intra-assay coefficient of variation.

2.3.3. Interferon-γ

Serum concentrations of IFN-γ were determined by ELISA in 15 assays as described previously [13]. The dynamic range of the assay was 12.3–1000 pg/ml with a minimum detectability of 5 pg/ml. Intra-assay coefficients of variation were less than 10% and the inter-assay coefficient of variation was 13%.

2.3.4. Tumor necrosis factor-α

Serum concentrations of TNF-α were determined utilizing a pig TNF-α ELISA kit as per the instructions of the manufacturer (Endogen Inc., Woburn, MA). The assay provided recovery rates of 79 ± 8% at 296 pg/ml and 76 ± 14% at 129 pg/ml from serum. The dynamic range of the assay was 38.4–1500 pg/ml with a minimum sensitivity of 5.7 pg/ml. The assay does not cross-react with human TNF-α, human TNF-β, or mouse TNF-α. The inter-assay coefficient of variation was less than 15%, and the intra-assay coefficient of variation was less than 10%.

2.4. Quantitation of mRNA

Total RNA was extracted from hypothalamus, pituitary, thymus, spleen, liver, and adrenal glands (Tri-Reagent, Molecular Research Center, Inc., Cincinnati, OH) and transferred to a nylon membrane with a slot-blot apparatus (Bio-Dot SF, Bio-Rad Laboratories, Hercules, CA). Hybridization and detection were carried out with a commercially available kit according to the manufacturer's instructions (BrightStar System, Ambion Inc., Austin, TX). Hybridization signal intensities were quantitated by densitometry, with target mRNA values expressed relative to 28S ribosomal (r) RNA for each sample.

Hypothalamus, pituitary, thymus, spleen, liver, and adrenal RNA were probed for IL-1β, IL-6, IL-6 receptor, and TNF-α mRNA. Polymerase chain reaction (PCR) was used to amplify IL-1β, IL-6, IL-6 receptor, and TNF-α cDNA (RNA-PCR kit, PerkinElmer Corp., Foster City, CA). The up- and down-stream oligonucleotide primers for PCR amplification were 5′ CAA CGT GCA GTC TAT GGA GT 3′ and 5′ GAG GTG CTG ATG TAC CAG TT 3′ for IL-1β (372 bp GenBank accession #M86725), 5′ GGA CGC CTG GAA GAA GAT 3′ and 5′ TCT TCA TCC ACT CGT TCT GT 3′ for IL-6 (474 bp; GenBank accession #M80258), 5′ AGC CCC AGC TCT CCT GCT TC 3′ and 5′ GGC GAC GCA CAT GGA CAC TA 3′ for IL-6 receptor (239 bp; GenBank accession #AF015116), and 5′ ACC ACG CTC TTC TGC CTA CT 3′ and 5′ AGA TAG TCG GGC AGG TTG AT 3′ for TNF-α (518 bp; GenBank accession #X57321). The PCR products were

subsequently cloned into a T-cloning vector (PCR-II, Invitrogen Corp., San Diego, CA). The identities of the cDNA clones were confirmed by dideoxy termination sequencing. Biotinylated riboprobes were synthesized from these clones for use in chemiluminescence-based detection (BrightStar System, Ambion Inc., Austin, TX)

2.5. Statistical analysis

Messenger RNA levels of IL-1β, IL-6, IL-6 receptor, and TNF-α and basal serum concentrations of TNF-α and IFN-γ were analyzed by analysis of variance and mean comparisons using Fisher's Protected Least Significant Differences [14]. Serum concentrations of ACTH, CS, TNF-α, and IFN-γ collected from the serial blood sampling were analyzed by analysis of variance for repeated measures.

3. Results

Gestation length and birth weight did not differ between vaginal birth and c-section pigs ($P = 0.34$ and 0.62, respectively). Vaginal birth pigs had 21% greater average daily gain (ADG) than c-section pigs (172 ± 10 g/d vs 153 ± 12 g/d, respectively; $P < 0.01$). Centrifuge clot to blood ratio was greater among vaginal birth than c-section pigs at birth ($49 \pm 2\%$ vs $37 \pm 2\%$, respectively; $P < 0.01$) but did not differ at 14 d ($40 \pm 2\%$ vs $37 \pm 2\%$, respectively; $P = 0.37$).

Serum concentrations of ACTH, CS, IFN-γ, and TNF-α increased in response to LPS challenge (Fig. 1; $P < 0.01$) but were not different between c-section and vaginal birth pigs ($P > 0.22$). Basal serum concentrations of ACTH and CS were greater in c-section than vaginal birth pigs at birth (Fig. 2; $P < 0.01$) but were not different at 14 d ($P = 0.99$ and 0.80, respectively). Basal serum concentrations of TNF-α tended to be greater in c-section vs vaginal birth pigs at 14 d (Fig. 2; $P < 0.0967$); however, basal serum concentrations of IFN-γ tended to be lower in c-section pigs vs vaginal birth pigs at 14 d (Fig. 2; $P < 0.0787$).

Relative expression of IL-6 mRNA also did not differ between c-section and vaginal birth pigs but decreased from birth to 14 d of age in the adrenal gland and thymus (Table 1; $P < 0.02$) and increased from birth to 14 d of age in the liver, pituitary, and hypothalamus (Table 1; $P < 0.001$). As with IL-6, relative expression of IL-6 receptor mRNA did not differ between c-section and vaginal birth pigs but decreased from birth to 14 d of age in the adrenal gland, spleen, and hypothalamus (Table 1; $P < 0.03$) and increased from birth to 14 d of age in the pituitary and thymus (Table 1; $P < 0.0001$). Relative

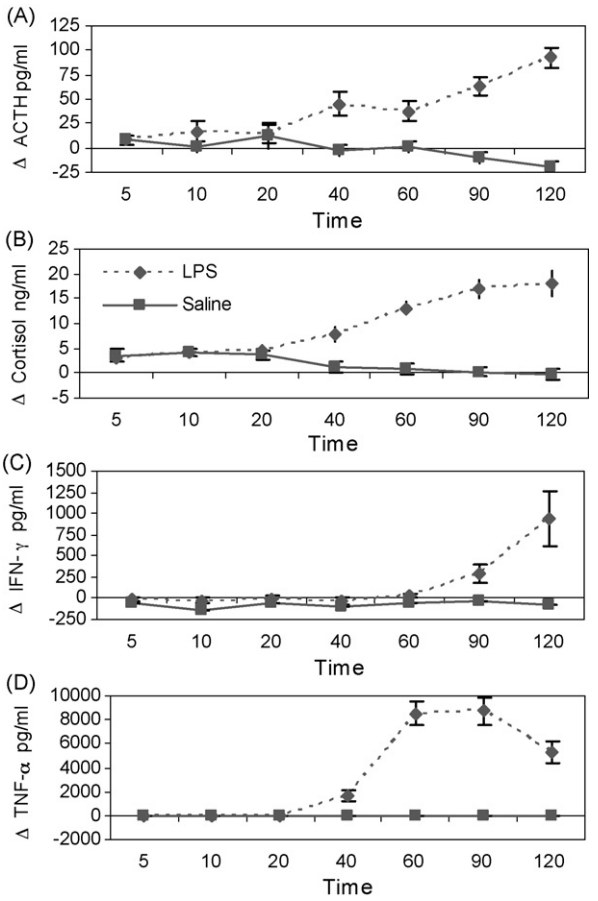

Fig. 1. Pigs' response to lipopolysaccaride (LPS) or saline challenge at 2 wk of age. Challenge was administered i.p. at time 0, and the *y*-axis represents the response to challenge relative to basal levels observed prior to challenge. There was no difference in response to challenge between c-section and vaginal delivery pigs. Serum concentrations of ACTH (A), cortisol (B), interferon-γ (C), and tumor necrosis factor-α (D) were all significantly increased by LPS challenge relative to saline ($P < 0.01$).

expression of IL-1β did not differ between c-section and vaginal birth pigs but decreased from birth to 14 d of age in the adrenal gland, liver, spleen, and thymus (Table 1; $P < 0.0001$) and increased from birth to 14 d of age in the hypothalamus and pituitary (Table 1; $P < 0.0001$). Relative expression of TNF-α also did not differ between c-section and vaginal birth pigs but increased from birth to 14 d of age in the hypothalamus, pituitary, adrenal gland, liver, and spleen and decreased from birth to 14 d of age in the thymus (Table 1; $P < 0.05$).

Treatment with LPS decreased expression of IL-6 receptor mRNA in the adrenal (1.35 ± 0.03 vs 1.45 ± 0.03 arbitrary units relative to expression of 28S rRNA in LPS vs saline treated pigs, respectively) and increased expression of IL-1β mRNA in

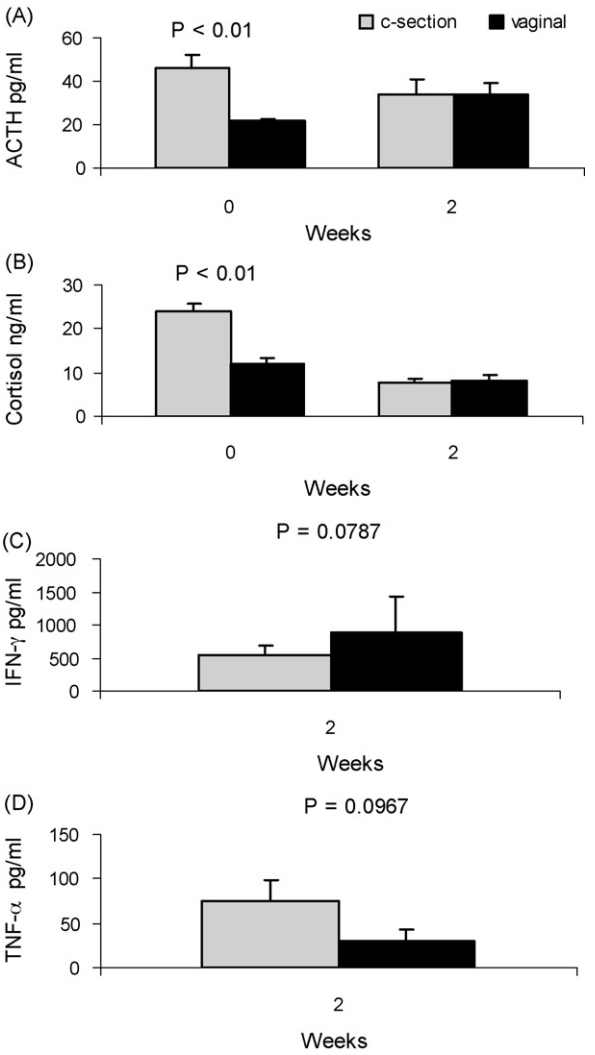

Fig. 2. Basal serum concentrations: the panels represent the basal serum concentrations of ACTH (A) and cortisol (B) at 0 and 2 wk of age and interferon-γ (C) and tumor necrosis factor-α (D) at 2 wk in c-section and vaginal delivery pigs. At birth, serum concentrations of interferon-γ and tumor necrosis factor-α were below assay minimum standards.

the spleen (0.25 ± 0.03 vs 0.13 ± 0.02 arbitrary units relative to expression of 28S rRNA in LPS vs saline treated pigs, respectively; $P < 0.02$). Treatment with LPS tended to decrease TNF-α expression in the hypothalamus (1.13 ± 0.06 vs 1.23 ± 0.09 arbitrary units relative to expression of 28S rRNA in LPS vs saline treated pigs, respectively; $P = 0.0826$) and ACTH receptor expression in the adrenal gland ($0.65 \pm .02$ vs 0.75 ± 0.02 arbitrary units relative to expression of 28S rRNA in LPS vs saline treated pigs, respectively; $P = 0.0873$).

4. Discussion

The advantage in ADG for the vaginal birth pigs demonstrates a growth advantage. In agreement with previous research conducted in our lab, vaginal birth pigs were heavier at 2 wk of age than c-section birth pigs [1,2]. In concordance with previous work in our laboratory [1], serum concentrations of ACTH in the present study were significantly greater in c-section than vaginal birth pigs at birth and did not differ between birth types at 2 wk of age.

Interestingly, serum concentrations of CS were greater in c-section pigs than vaginal birth pigs at birth in the present study. Previous studies have demonstrated lower serum concentration of CS in c-section than vaginal birth pigs at birth [15,16] or no difference in serum concentration of CS between c-section or vaginal birth pigs [1]. However, in those studies the c-section pigs were of a younger gestational age than the c-section pigs in the present study (111–113 d vs 116 d of gestational age). Although, sows were showing no obvious signs of labor, c-section pigs in the present study were likely very close to the time of vaginal birth. A sharp rise in circulating concentrations of corticosteroids has been observed in fetal pigs 8 h prior to birth [17]. Perhaps, the c-section pigs in the present study were within the 8 h prior to birth during which cortisol concentrations sharply increase, and thus had higher circulating concentrations of cortisol than vaginal birth pigs. Alternatively, as the c-sections in the present study were performed immediately following stunning, an increase in cortisol, and other stress hormones, from the sow could have elevated the circulating concentrations of cortisol in the c-section pigs. However, in our previous study, c-sections were also performed immediately following stunning, but circulating concentrations of cortisol were not different between c-section and vaginal birth pigs at birth [1]. In the present study, serum concentrations of CS were not different at 2 wk of age between c-section and vaginal birth pigs. In previous work in our laboratory, c-section pigs had greater basal levels of CS than vaginal birth pigs at 2 wk of age [1].

As expected, LPS stimulated an increase in serum concentrations of ACTH, CS, IFN-γ, and TNF-α. Growing older pigs treated with LPS had increased serum concentrations of TNF-α at 2 and 4 h post LPS, increased serum concentrations of IL-6 at 2 h post LPS which peaked at 4 h post LPS, and elevated serum concentrations CS at 2, 4, and 8 h post LPS [18]. Treatment of pigs with LPS increased serum concentrations of IL-6 and TNF-α in both 1 and 28 d old pigs, 3 h post treatment [19,20]. Treatment with LPS also increased serum concentrations of CS in 28 d old pigs, 3 h post treatment [19].

Table 1
Expression of IL-6, IL-6 receptor, IL-1β, and TNF-α mRNA in various tissues of pigs born by c-section or vaginal delivery at 0 and 2 wk of age

mRNA	Age	Type of birth	Hypothalamus	Pituitary	Adrenal	Liver	Spleen	Thymus
IL-6	0	c-section	0.49 ± 0.05	0.87 ± 0.05	0.69 ± 0.09	1.80 ± 0.13	1.44 ± 0.12	1.20 ± 0.08
		Vaginal	0.52 ± 0.04	0.92 ± 0.04	0.67 ± 0.07	2.03 ± 0.08	1.49 ± 0.10	1.23 ± 0.08
	2	c-section	0.68 ± 0.05	1.38 ± 0.07	0.51 ± 0.06	3.32 ± 0.42	1.38 ± 0.09	0.74 ± 0.06
		Vaginal	0.70 ± 0.05	1.38 ± 0.08	0.50 ± 0.06	3.96 ± 0.29	1.38 ± 0.06	0.82 ± 0.05
IL-6 receptor	0	c-section	0.61 ± 0.04	0.30 ± 0.02	1.95 ± 0.04	1.34 ± 0.10	1.82 ± 0.07	0.67 ± 0.03
		Vaginal	0.63 ± 0.16	0.34 ± 0.03	1.90 ± 0.04	1.58 ± 0.08	2.02 ± 0.07	0.65 ± 0.04
	2	c-section	0.49 ± 0.03	0.67 ± 0.07	1.39 ± 0.03	1.35 ± 0.16	1.37 ± 0.03	0.97 ± 0.07
		Vaginal	0.54 ± 0.03	0.60 ± 0.59	1.42 ± 0.03	1.24 ± 0.09	1.32 ± 0.04	0.88 ± 0.07
IL-1β	0	c-section	1.83 ± 0.04	0.92 ± 0.07	0.93 ± 0.04	0.73 ± 0.06	0.46 ± 0.04	1.10 ± 0.03
		Vaginal	1.80 ± 0.04	0.93 ± 0.06	0.79 ± 0.05	0.80 ± 0.05	0.43 ± 0.03	1.05 ± 0.04
	2	c-section	2.13 ± 0.08	1.41 ± 0.08	0.48 ± 0.06	0.41 ± 0.04	0.18 ± 0.02	0.84 ± 0.03
		Vaginal	2.09 ± 0.07	1.22 ± 0.13	0.33 ± 0.05	0.44 ± 0.04	0.17 ± 0.02	0.87 ± 0.03
TNF-α	0	c-section	0.81 ± 0.08	0.82 ± 0.08	0.72 ± 0.04	0.78 ± 0.05	1.15 ± 0.05	0.9 ± 0.04
		Vaginal	0.94 ± 0.06	0.92 ± 0.07	0.74 ± 0.05	0.83 ± 0.03	1.16 ± 0.05	0.91 ± 0.03
	2	c-section	1.40 ± 0.11	1.20 ± 0.16	0.97 ± 0.07	1.03 ± 0.08	1.21 ± 0.08	0.72 ± 0.03
		Vaginal	1.20 ± 0.09	0.95 ± 0.10	0.99 ± 0.06	1.07 ± 0.10	1.39 ± 0.09	0.71 ± 0.03

Data are expressed as mean ± SEM in arbitrary units relative to the expression of 28S rRNA in the same tissue. Effect of type of birth on level of mRNA expression was not significant. Effect of age on level of mRNA expression was significant ($P < 0.05$) in all tissues except for IL-6 mRNA expression in the spleen and IL-6 receptor mRNA expression in the liver.

In the current study, treatment with LPS did not affect expression of IL-1β, IL-6, IL-6 receptor, or TNF-α mRNA in most tissues. Exceptions were expression of IL-1β mRNA in the spleen was increased following LPS treatment. Expression of IL-6 receptor and ACTH receptor mRNA in the adrenal gland was decreased in LPS treated pigs, and TNF-α mRNA in the hypothalamus tended to decrease in LPS treated pigs. Matteri et al. observed increased relative mRNA for IL-6 and TNF-α in the pituitary, hypothalamus, spleen, thymus, and liver 3 h following LPS challenge in both 1 and 28 d old pigs [20]. Failure to observe differences in mRNA expression in response to LPS in the present study could be due to time of collection of tissue (2 h following LPS) relative to LPS challenge. Alternatively, our failure to observe differences in mRNA expression in response to LPS in the present study could be due to the age of the pigs. The pigs in the present study were 14 d old whereas Matteri et al. observed increased IL-6 and TNF-α mRNA following LPS in younger (1 d old) and older (28 d old) pigs [20]. Perhaps at 14 d of age development of the immune system or passive immunity from the colostrum results in reduced ability to respond to LPS in the tissues were examined.

In pigs, the immune system is not fully developed at birth [9]. Interestingly, expression of the mRNA associated with the acute phase response of the immune system provided evidence of tissue-specific increases or decreases from birth to 2 wk of age in the present study. Expression of IL-6 mRNA from birth to 2 wk of age decreased in the adrenal gland and thymus and increased in the liver, pituitary, and hypothalamus. In agreement with the present study, previous research demonstrated that hypothalamic and pituitary expression of IL-6 mRNA increased from 1 to 28 d of age in pigs [20]. However, Matteri et al. [20] did not observe any developmental changes in IL-6 mRNA expression in the spleen, thymus, or liver. Expression of IL-6 receptor mRNA from birth to 2 wk of age decreased in the adrenal gland, spleen, and hypothalamus but increased in the pituitary and thymus. Expression of IL-1β mRNA from birth to 2 wk of age decreased in the adrenal gland, liver, spleen, and thymus and increased in the hypothalamus and pituitary. Expression of TNF-α mRNA from birth to 2 wk of age increased in the hypothalamus, pituitary, adrenal gland, liver, and spleen but decreased in the thymus. In the study by Matteri et al. [20], 28 d old pigs had higher levels of TNF-α mRNA in the thymus than the 1 d old pigs; mRNA levels did not differ by age in the hypothalamus, pituitary, spleen, or liver. The results of the present study provide evidence that expression of these mRNAs associated with the acute phase response occur in an age and tissue specific manner in the first 2 wk of life.

In the present study, pigs born by c-section tended to have higher basal serum concentrations of TNF-α than vaginal birth pigs at 2 wk of age. Infants born by c-section had higher serum concentrations of TNF-α at 48 h of age than vaginal birth infants [3]. However, the investigators speculated that the drugs administered to

the mother may have been responsible for some or all of the effects on the immune system [3]. In the present study, anesthesia and analgesia were not used, suggesting that the birth process may alter immune system function. Alternatively, as the c-section in the present study was performed immediately following stunning, the c-sections pigs may have experienced changes in cortisol, or other stress hormones, from the mother which could have potentially altered immune system function. In the pig, an LPS challenge results in elevated circulating concentration of TNF-α and uncoupling of the GH/IGF-I axis [21]. Perhaps, the tendency for basal circulating concentrations of TNF-α to be elevated in c-section pigs contributes to the reduction in growth observed relative to vaginal birth pigs.

Basal serum concentrations of IFN-γ tended to be lower in c-section than vaginal birth pigs at 2 wk of age. In contrast, human infants delivered by c-section were observed to have greater concentrations of IFN-γ in cord blood than vaginally delivered infants [4]. Age or species differences could explain the observed difference in IFN-γ. Additionally, Ly et al. speculated increased levels of IFN-γ in c-section born infants could be due to reduced exposure to gram-positive anaerobes [4]. The piglets in the current study likely had been exposed to a variety of bacteria by 2 wk of age.

In conclusion, these data provide evidence that altered immune system function may be partially responsible for the reduction in growth observed in c-section compared to vaginally delivered pigs. Expression of IL-1β, IL-6, IL-6 receptor, and TNF-α appears to be controlled in an age and tissue specific manner in the neonatal pig. The possibility that the type of birth delivery may alter the function of the immune system is an important consideration for animals born by c-section. Further research is warranted to determine if the type of birth negatively affects the future development and function of the immune system, and if any effect is detrimental to the animal's overall health.

References

[1] Daniel JA, Keisler DH, Sterle JA, Matteri RL, Carroll JA. Birth by Caesarian section alters postnatal function of the hypothalamic-pituitary-adrenal (HPA) axis in the young pig. J Anim Sci 1999;77:742–9.

[2] Carroll JA, Daniel JA, Keisler DH, Matteri RL. Postnatal function of the somatotrophic axis in pigs born naturally or by Caesarian section. Domest Anim Endocrinol 2000;19:39–52.

[3] Bessler H, Kuperman A, Beilin B, Klinger G, Gurary N, Mozes C, et al. Labor affects cytokine production in newborns. Am J Reprod Immunol 1998;39:27–32.

[4] Ly NP, Ruiz-Pérez B, Onderdonk AB, Tzianabos AO, Litonjua AA, Liang C, et al. Mode of delivery and cord blood cytokines: a birth cohort study. Clin Mol Allergy 2006;4:13–24.

[5] Thilaganathan B, Meher-Homji N, Nicolaides KH. Labor: an immunologically beneficial process for the neonate. Am J Obstet Gynecol 1994;171:1271–2.

[6] Molloy EJ, O'Neill AJ, Grantham JJ, Sheridan-Pereira M, Fitzpatrick JM, Webb DW, et al. Labor promotes neonatal neutrophil survival and lipopolysaccharide responsiveness. Pediatr Res 2004;56:99–103.

[7] Westly HJ, Kelley KW. Physiologic concentrations of cortisol suppress cell-mediated immune events in the domestic pig. Proc Soc Exp Biol Med 1984;177:156–64.

[8] Brown-Borg HM, Klemcke HG, Blecha F. Lymphocyte proliferation responses in neonatal pigs with high or low plasma cortisol concentration after stress induced by restraint. Am J Vet Res 1993;54:2015–20.

[9] Becker BA, Misfeldt ML. Evaluation of the mitogen-induced proliferation and cell surface differentiation antigens of lymphocytes from pigs 1 to 30 days of age. J Anim Sci 1993;71:2073–8.

[10] Carroll JA. Bi-Directional communication: growth and immunity in domestic livestock. J Anim Sci 2007; doi:10.2527/jas.2007-0480.

[11] Carroll JA, Daniel JA, Keisler DH, Matteri RL. Non-surgical catheterization of the jugular vein in young pigs. Lab Anim 1999;33:1–6.

[12] Eisenhauer CL, Matsuda LS, Uyehara CFT. Normal physiologic values of neonatal pigs and the effects of isoflurane and pentobarbital anesthesia. Lab Anim Sci 1994;44:245–52.

[13] Luchene LJ, Orencole SF, Beausang LA. Development of a sensitive, 2-site immunometric ELISA for the quantitation of porcine interferon γ. In: Conference of Research Workers in Animal Diseases—Proceedings, vol. 79. 1998. p. 52.

[14] StatView. 2nd ed. Cary, NC: SAS Inst. Inc.; 1998.

[15] Sangild PT. Stimulation of gastric proteases in the neonatal pig by a rise in adrenocortical secretion at parturition. Reprod Fertil Dev 1995;7:1293–8.

[16] Sangild PT, Diernæ L, Christiansen IJ, Skadhauge E. Intestinal transport of sodium, glucose and immunoglobulin in neonatal pigs. Effect of glucocorticoids. Exp Physiol 1993;78:485–97.

[17] Randall GCB. Changes in concentrations of corticosteroids in the blood of fetal pigs and their dams during late gestation and labor. Biol Reprod 1983;29:1077–84.

[18] Webel DM, Finck BN, Baker DH, Johnson RW. Time course of increased plasma cytokines, cortisol, and urea nitrogen in pigs following intraperitoneal injection of lipopolysaccharide. J Anim Sci 1997;75:1514–20.

[19] Klir JJ, Shahbazian LM, Matteri RL, Fritsche KL, Becker BA. Effects of thermal environment on response to acute peripheral lipopolysaccharide challenge exposure in neonatal pigs. Am J Vet Res 1997;58:364–9.

[20] Matteri RL, Klir JJ, Fink BN, Johnson RW. Neuroendocrine-immune interactions in the neonate. Domest Anim Endocrinol 1998;15(5):397–407.

[21] Hevener W, Almond GW, Armstrong JD, Richards RG. Effects of acute endotoxemia on serum somatotropin and insulin-like growth factor I concentrations in prepubertal gilts. Am J Vet Res 1997;58:1010–3.