Effects of age and pregnancy on the circulatory activin response of sheep to acute inflammatory challenge by lipopolysaccharide

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Abstract

The release of activin A in response to intravenous injection of the bacterial cell-wall component lipopolysaccharide (LPS) was investigated in an ovine model of acute inflammatory challenge in newborn and adult sheep, and in non-pregnant and pregnant ewes. Neonatal lambs (<20 days of age) showed a quantitatively similar response in terms of circulating concentrations of activin A, its binding protein follistatin and the cytokine interleukin-6 compared with adult ewes challenged with an equivalent dose (300 ng/kg bodyweight) of LPS. The fever response and plasma tumour necrosis factor- α release in response to LPS, however, were significantly ($P < 0.01$) less in lambs than in the adult group. Pregnant ewes in the last trimester of gestation had similar responses to LPS, in all aspects measured, compared with their non-pregnant counterparts, apart from an ablated fever response. Although the adult and neonatal sheep responded to LPS, a similar response was not apparent in the fetal circulation, possibly

due to a protective effect of the placenta. A 10-fold increase in the dose of LPS (from 300 ng to 3 μ g/kg bodyweight) given to neonatal lambs elicited an increase in several cytokine responses measured, with a significant ($P < 0.05$) increase in follistatin release. In contrast, the amount of activin released by the increased dose of LPS was similar to that invoked by the lower dose. The effect of tolerance to LPS was investigated by giving a second challenge of LPS 5 days after the initial injection. In all animals studied, there was an ablated ($P < 0.05$) response to the subsequent LPS injection, apart from a similar temperature-response profile. These data provide further evidence that activin A concentrations in the bloodstream are acutely responsive to inflammatory challenge in post-natal life and suggest that the response forms a significant component of the innate immune system.

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Introduction

The innate immune response is one of the most profound biological processes, as it affects almost all multicellular organisms at some stage of their development (Medzhitov 1998). Not only is it phylogenetically conserved, it is also of fundamental relevance during development and has received widespread attention based on its relevance to premature labour during human pregnancy (Gonçalves *et al.* 2002). Recently the growth factor, activin A, a member of the transforming growth factor- β (TGF- β) superfamily of proteins (Chang *et al.* 2002), has been characterized as a new component in inflammatory processes (Jones *et al.* 2000, 2004). This protein was originally isolated as a non-steroidal feedback regulator of follicle-stimulating hormone secretion, but is now known to have multiple functions besides the regulation of gonadal activity (Woodruff 1998).

The release of activin A into the bloodstream in response to an acute inflammatory challenge occurs rapidly, within minutes of exposure to agents such as the bacterial cell-wall component lipopolysaccharide (LPS; Jones *et al.* 2004). The response is more rapid than that seen for some of the key pro-inflammatory cytokines, such as tumour necrosis factor- α (TNF- α) or interleukin-6 (IL-6), and appears distinctly earlier than the release of follistatin, the high-affinity binding protein for activin that has neutralizing properties (Jones *et al.* 2000, 2004). However, the function of the activin release has not been delineated, except that activin A has both pro- and anti-inflammatory properties depending on the temporal and functional context (Phillips 2003). Given that the activin response to inflammatory mediators has only been investigated using *in vitro* approaches or in mature animals, it is not known if this mechanism pertains only in adult life or is indeed

a component of an innate immune response present in immature or fetal animals.

This series of studies explored the hypothesis that the release of activin A into the bloodstream following acute inflammatory challenge is a core component of the innate immune response. As much of the previous work in this area has been carried out in a sheep model of systemic LPS exposure (Jones *et al.* 2000, 2004), the response in adult ewes was compared with that in neonatal lambs and in fetuses where LPS was administered into the maternal circulation. Additionally, the response was characterized in neonates where the dose of LPS was increased 10-fold. Two other aspects of the activin response were explored. For some time now, pregnancy has been thought of as a state in which the maternal adaptive immune responses, particularly at the maternal–fetal interface, are suppressed, yet paradoxically fever and the innate immune response are activated (Sacks *et al.* 1999). Of relevance is that the fetus appears protected by the placenta in some aspects of the inflammatory response when the mother is challenged with LPS (Grigsby *et al.* 2003). Therefore the activin response in pregnant versus non-pregnant ewes was compared. Finally, whether the activin response was subject to tolerance, whereby there is a hyporesponsive state to subsequent challenge by LPS following an initial exposure (Cross 2002), was also investigated.

Materials and Methods

Animals and catheterization details

All studies were performed in Border Leicester–Merino cross-bred animals and were approved by the Animal Ethics Committee of the Department of Physiology, Monash University, Australia. Adult ewes were housed in individual metabolism cages, fed a diet of chopped lucerne hay once daily and had free access to tap water. Lambs between 12 and 15 days of age were housed with their mothers in lambing pens. To provide a similar endocrine background between individuals, adult non-pregnant ewes underwent ovariectomy approximately 1 week before the initial exposure to LPS. At surgery, they also had sampling catheters inserted into the external jugular vein and the carotid artery. Patency of catheters was maintained with 0.9% saline solution containing 37 mM EDTA (BDH Laboratory Supplies, Poole, Dorset, UK). This anticoagulant does not affect circulating concentrations of activin A or follistatin, unlike heparin (Klein *et al.* 1996). Lambs had an indwelling jugular catheter inserted at surgery as described previously (Billiards *et al.* 2002). Pregnant ewes had surgery at 124–126 days of gestation (day 145=term) for the placement of catheters essentially as described previously (Grigsby *et al.* 2003). Briefly, maternal jugular vein, carotid artery, intra-amniotic fluid, fetal carotid artery and jugular vein catheters were inserted. A thermocouple temperature

probe was placed in the fetal neck next to the carotid artery. The calcium-chelating properties of EDTA make it unsuitable for *in vivo* use in fetuses and young lambs. Therefore, sterile saline solution (0.9% NaCl) containing 5000 IU heparin/ml (Pharmacia, Perth, Australia) was used to keep the catheters patent. At least 12 h before the commencement of an experiment, however, fetal and neonatal catheters were flushed with non-heparinized saline solution to avoid any artifactual increases in plasma activin or follistatin concentrations.

Experimental details

All animals, apart from fetuses, were treated i.v. with LPS (*Escherichia coli* serotype 0127:B8; Sigma, St Louis, MO, USA). The adult ewes and lambs were weighed the day before the experiments commenced. Non-pregnant adult ewes ($n=7$), pregnant ewes ($n=5$) and lambs ($n=5$) were treated with a bolus dose of 300 ng LPS/kg bodyweight. A further group of lambs ($n=4$) and two pregnant ewes were given a higher dose of 3 µg/kg LPS. Body temperature was recorded using a rectal digital thermometer for the ewes and lambs, and fetal temperature recorded using a thermistor probe implanted next to the carotid artery at surgery. Blood samples from fetuses, lambs or adults and the amniotic fluid of some pregnant ewes were collected from the indwelling catheters 1 h before and at the time of LPS injection. Samples were collected at half-hourly intervals up to 5 h after the LPS injection, then at 6, 9, 12 and 24 h thereafter. Blood gases (PO_2 , PCO_2) and pH were measured in freshly drawn arterial blood samples using an ABL5 blood gas analyzer and OSM2 Hemoximeter (Radiometer, Copenhagen, Denmark; data not shown). Five days after the initial LPS challenge, animals received a second LPS injection with an identical dose and blood-sampling regime to that of the first. In all instances, blood or amniotic fluid samples were spun on a benchtop centrifuge for 5 min at 3000 g. The resultant plasma or amniotic fluid supernatants were stored at $-20\text{ }^{\circ}\text{C}$ until assayed.

Assays

Plasma activin A levels were determined as described previously using an enzyme-linked immunosorbent assay (ELISA; Knight *et al.* 1996). The assay measures ‘total’ activin A – that is, both free and bound – and has been validated extensively for sheep plasma and amniotic fluid (Jenkin *et al.* 2001a). The assay standard was human recombinant activin A (Biotech Australia Pty. Ltd; Robertson *et al.* 1992). The mean assay sensitivity was 0.01 ng/ml, and the mean intra- and inter-assay coefficients of variation (CVs) were both <9%.

Follistatin was measured by a RIA as described previously (Phillips *et al.* 1996), which also measures both free and bound forms. The assay uses purified

heterologous bovine follistatin as standard and iodinated bovine follistatin as tracer. The assay sensitivity was 2.3 ng/ml and the intra- and inter-assay CVs were both <8%.

TNF- α was measured by an ELISA as described previously (Jones *et al.* 2000) with minor modifications. The standard was ovine recombinant TNF- α (Centre for Animal Biotechnology, University of Melbourne, Australia), the assay sensitivity was 0.03 ng/ml, and the intra- and inter-assay CVs were both <9%.

IL-6 concentrations were measured by ELISA as described previously (Jones *et al.* 2000), using antibodies specific to ovine IL-6 (Epitope Technologies, Melbourne, Australia). The standard was ovine recombinant IL-6 (Centre for Animal Biotechnology), the sensitivity was 0.02 ng/ml and the intra- and inter-assay CVs were both <14%.

Data analysis

Data were analysed statistically using GraphPad version 2 (GraphPad Software, San Diego, CA, USA). This package was also used to calculate the area under the curve (AUC) function. Where appropriate, data were log-transformed before analysis to correct for heterogeneity of variances. Parameters between groups of animals were analysed by one-way analysis of variance (ANOVA), followed by the Newman-Keuls *post-hoc* test, where appropriate. Comparisons between two different groups were analysed using Student's *t*-test and for comparisons within the same group of animals paired Student's *t*-test was used.

Results

Response to LPS in adult non-pregnant versus neonatal lambs

Adult ovariectomized ewes exhibited a classical fever response to 300 ng/kg LPS (Fig. 1A). The body temperature was significantly ($P<0.05$) elevated for 6 h, after which time it returned to pre-treatment levels. Measurement of plasma concentrations of activin A, follistatin, IL-6 and TNF- α showed typical responses to LPS challenge (Fig. 1B–E). When an identical per-bodyweight dose of LPS was administered to neonatal lambs, they too showed an increase in body temperature and an increase in the various blood factors measured (Fig. 1A–E). The response was similar to that of the adult ewes, except that the increases of temperature ($P<0.01$), plasma follistatin ($P<0.05$) and TNF- α ($P<0.05$) responses were less than for the ewes, as assessed by AUC (Table 1). Of interest was that the basal plasma concentrations of activin A differed between adults and neonates before LPS treatment, with the lambs having significantly ($P<0.001$) higher concentrations (0.137 ± 0.007 ng/ml, mean $\pm$ S.E.M.) compared with the adult ewes (0.015 ± 0.002 ng/ml), respectively.

Response to LPS in adult non-pregnant versus pregnant ewes

The effect of pregnancy on the response to LPS administration was assessed in pregnant ewes at 130–135 days of gestation, with comparison against the ovariectomized group of ewes. Both groups of animals had a qualitatively similar response to LPS in terms of fever and plasma concentrations of activin A, follistatin, IL-6 and TNF- α (Fig. 2), but the pyrexia was significantly ($P<0.01$) less in the pregnant animals (Table 1). All other parameters measured were not statistically different between the two groups in response to LPS challenge. Similar to the neonatal lambs, however, pregnant adult ewes had significantly ($P<0.001$) higher concentrations of activin A before LPS administration compared with their non-pregnant counterparts (0.05 ± 0.007 and 0.015 ± 0.002 ng/ml, respectively).

Maternal versus fetal response to LPS when administered to the mother

To determine if LPS given maternally had effects on the fetal-placental environment, LPS was administered i.v. at 300 ng/kg bodyweight to pregnant ewes. As mentioned above, the adult ewes had a profound fever response and changes in plasma concentrations of activin A, follistatin, IL-6 and TNF- α (Fig. 3). Fetal body temperature (Fig. 3A) was also elevated in response to maternal LPS challenge and the profile was similar to that observed in the mother. Nevertheless, there was little effect on fetal plasma concentrations of activin A, follistatin, IL-6 or TNF- α following LPS (Fig. 3B–E). This was despite significantly ($P<0.05$) higher pre-treatment concentrations of activin A in the fetuses compared with maternal plasma concentrations (0.101 ± 0.019 versus 0.05 ± 0.007 ng/ml). There was a minor but not statistically significant increase in fetal plasma follistatin concentrations (Fig. 3C) and three out of five fetuses exhibited elevated activin A concentrations at the 24-h timepoint (Fig. 3B). In general, the same fetuses that had an elevated activin level at 24 h were those that also had elevated follistatin concentrations. Similar to the fetal circulating concentrations, there was little change in amniotic fluid concentrations of either activin A or follistatin with maternal i.v. LPS challenge. The mean ($\pm$ S.E.M.) concentration of activin A in amniotic fluid before LPS challenge was 2.93 ± 1.30 ng/ml and for follistatin 10.5 ± 1.6 ng/ml. These concentrations did not alter by more than 25% over the 24-h period following LPS administration. To ascertain if a response in the fetal or amniotic fluid compartments was apparent if the dose of LPS was increased, two pregnant ewes were injected i.v. with 3 μ g/kg LPS. Similar to the 300 ng/kg dose, there were no apparent effects on fetal plasma activin A, IL-6 or TNF- α concentrations, nor on amniotic fluid activin A or follistatin levels (data not shown). In both fetuses, however, there were

Figure 1 Changes in (A) body temperature and plasma concentrations of (B) activin A, (C) follistatin, (D) IL-6 and (E) TNF- α in non-pregnant ewes ($n=7$, $\square$) or neonatal lambs ($n=5$, $\bullet$) following i.v. injection of 300 ng/kg LPS. Values are means $\pm$ S.E.M.

elevations in fetal plasma follistatin concentrations similar to those seen with the lower dose of LPS.

Response to LPS in neonatal lambs comparing two different doses

To determine if the response to LPS was dependent on the dose used, groups of neonatal lambs were challenged with either 300 ng/kg LPS or a 10-fold-higher dose of 3 μ g/kg LPS (Fig. 4 and Table 1). Both doses induced a fever response and increases in plasma concentrations of the factors measured. While statistically significant

($P<0.05$) only for follistatin (Table 1), the other factors had increased AUC responses to the higher dose of LPS, with the exception of a reduced response for activin A.

Differing response to LPS following an initial versus a subsequent challenge

Whether the response to LPS was altered by repetition of the challenge was assessed when animals were treated with identical doses of LPS after an interval of 5 days. A consistent effect in all groups (non-pregnant adults, pregnant adults and neonatal lambs) was that the overall

Table 1 Responses to LPS at the doses indicated, measured as AUC, for various parameters for non-pregnant ewes, pregnant ewes and neonatal lambs. Data are means $\pm$ S.E.M., analysed by one-way ANOVA with differences between groups detected by the Newman-Keuls *post hoc* test

Parameter	AUC			
	Non-pregnant ewes (n=7) 300 ng/kg LPS	Pregnant ewes (n=5) 300 ng/kg LPS	Neonatal lambs (n=5) 300 ng/kg LPS	Neonatal lambs (n=4) 3 μ g/kg LPS
Temperature ($^{\circ}$ C/h)	12.93 $\pm$ 1.89	4.05 $\pm$ 0.68**	4.63 $\pm$ 1.05**	7.96 $\pm$ 1.62
Activin A (ng/ml/h)	0.42 $\pm$ 0.13	0.38 $\pm$ 0.09	0.67 $\pm$ 0.21	0.34 $\pm$ 0.10
Follistatin (ng/ml/h)	295.7 $\pm$ 72.8*	235.6 $\pm$ 19.6	93.6 $\pm$ 18.9	221.5 $\pm$ 41.3*
IL-6 (ng/ml/h)	69.6 $\pm$ 24.0	41.8 $\pm$ 10.1	42.4 $\pm$ 21.6	120.5 $\pm$ 35.9
TNF- α (ng/ml/h)	145.3 $\pm$ 21.1*	130.9 $\pm$ 13.1*	71.5 $\pm$ 24.9	133.5 $\pm$ 11.3

Within a parameter, * P <0.05 compared with neonates given 300 ng/kg LPS, ** P <0.01 compared with non-pregnant ewes.

response to LPS was attenuated with the second challenge. Profiles from the non-pregnant group are presented in Fig. 5 and the peak responses and AUC values are shown for all groups in Table 2. While there was no significant decrease in the fever response, in all other parameters assessed there was a decrease in the peak plasma concentrations (with the exception of activin A) and in the AUC profiles determined for activin A, follistatin, TNF- α and IL-6.

Discussion

The data presented in this study provide significant new insights into the rapid release of activin A into the circulation following immune activation and lend further support to the concept that activin A is an important component of the innate immune response, at least in higher mammals. The experiments show that the activin response is functional at least as early as the first days of post-natal life and that while there is a maternal response during pregnancy, the fetus appears protected from many of the inflammatory changes because of the placental barrier. Over the limited dose range tested, the substantial release of activin in response to LPS inflammatory stimulus did not appear to be dose-dependent, although the activin response to subsequent challenge appeared to be reduced, consistent with the development of tolerance to this endotoxin.

While the release of activin A into the circulation following inflammatory challenge by agents such as LPS has been documented by us previously (Jones *et al.* 2000, 2004), this has been characterized only in adult subjects. A major aim of the present study was to establish if this response mechanism was operant only in mature individuals and, as such, developed in post-natal life. When post-natal lambs were challenged with LPS, however, they displayed a typical pyrogenic response and importantly a qualitatively similar release of activin A, follistatin and pro-inflammatory cytokines to that of adults. On this basis, it appears that, similar to other aspects of innate

immunity, the activin component is fully functional in the immature animal. When administered at the same per-bodyweight dose to immature animals, the response to LPS was less in most of the parameters measured compared with adults. This is consistent with previous studies suggesting that early neonates are less responsive to LPS compared with older immature animals or in adults (Bech-Jansen *et al.* 1972, Goelst *et al.* 1992, Cocci *et al.* 1995). Of interest was that the basal circulating concentrations of activin A were significantly (P <0.001) higher in the neonate group compared with the adults. As shown in Fig. 1, the levels of activin following the initial 'surge' after LPS treatment then became lower than pre-treatment concentrations, but had returned to pre-LPS levels after 24 h. The significance of this biphasic effect for the neonate is not known and nor, as far as we can ascertain, have the concentrations of activin A been reported previously for neonates and found to be higher than in adults.

The effect of pregnancy on the release of activin A following LPS challenge was explored in sheep during the last trimester. This is particularly relevant given the large body of literature suggesting that pregnancy is an endocrine state in which the adaptive immune response is suppressed to avoid maternal rejection of the developing fetus, but where aspects of the innate immune response are sensitized (Sacks *et al.* 1999). Pregnant animals had a similar response to LPS challenge compared with their non-pregnant counterparts in all aspects measured, except for a reduced fever response. Therefore, there appeared to be little effect of pregnancy on the quantitative response of activin A, follistatin and the pro-inflammatory cytokines measured following immune activation. Nevertheless, the basal concentrations of activin A were higher in pregnant ewes than in the non-pregnant group, consistent with observations that circulating maternal activin levels increase during pregnancy and particularly in the last trimester (Fowler *et al.* 1998, Schneider-Kolsky *et al.* 2000, Muttukrishna 2001). On balance, while the current study was not explicitly formulated to address the question of

Figure 2 Changes in (A) body temperature and plasma concentrations of (B) activin A, (C) follistatin, (D) IL-6 and (E) TNF- α in non-pregnant ewes ($n=7$, $\square$) or pregnant ewes ($n=5$, $\blacksquare$) following i.v. injection of 300 ng/kg LPS. Values are means $\pm$ S.E.M.

heightened innate immunity or otherwise during pregnancy, it therefore suggests that the activin response to inflammatory challenge is manifested regardless of the pregnancy status of an individual.

While it is clear that the maternal inflammatory system is able to respond to endotoxin challenge during pregnancy, the fetus appears to be protected from the maternal response. The experiments reported here showed that, despite robust temperature, activin A, follistatin and cytokine responses in the mother, with the exception of temperature concomitant increases in the fetal compartment were not seen. Fetal temperature did rise simul-

taneously with that of the mother, consistent with the idea that most heat is lost from the fetal compartment across the placenta into maternal blood (Schröder & Power 1997). However, in terms of TNF- α and IL-6 concentrations in the fetus, there were no significant changes in the 24-h period following LPS treatment. The data for activin A and follistatin were also statistically non-significant, although some of the fetuses had higher activin concentrations at the 24-h timepoint and circulating fetal follistatin concentrations increased in some animals between 8 and 24 h after LPS was administered to the mother. Whether this is a true effect is yet to be established and it

Figure 3 Changes in (A) body temperature and plasma concentrations of (B) activin A, (C) follistatin, (D) IL-6 and (E) TNF- α in pregnant ewes ($n=5$, ■) or their fetus ($n=5$, Δ) following i.v. injection of 300 ng/kg LPS to the mother. Values are means $\pm$ S.E.M. Note the differing axis scale for (B; right-hand side) at the 24 h timepoint.

is not known if direct stimulation of the fetus by inflammatory challenges such as LPS will result in activin A and follistatin being released into the fetal circulation. Overall, however, the current data suggest that neither LPS nor the cytokines are able to cross the placental barrier, and that the placenta acts to contain the maternal immune response and ensure that inappropriate and unnecessary activation of the fetal immune system is not a consequence. This is consistent with a recent study showing that maternal activation only results in certain components, such as cortisol and prostaglandin E₂, responding in the fetal compartment (Grigsby *et al.* 2003). Interestingly, the resting concentrations of activin A in the fetal and maternal circulations were significantly different, with fetal levels

being approximately double that in the mother. This is in contrast to published findings in human pregnancies where maternal activin A concentrations appear higher (Florio *et al.* 1999, Schneider-Kolsky *et al.* 2002), but consistent with those shown previously in the sheep (Jenkin *et al.* 2001b) where fetal levels are higher. The reasons behind this apparent dichotomy between the two species are unclear, but may involve the different placental types, with cotyledonary in the sheep versus discoid in the human affecting placental transfer of activin, as discussed by Loose and colleagues (Loose *et al.* 2004).

The magnitude of the activin response to LPS challenge was investigated in this study by evaluating the effects of a 10-fold difference in the dose of LPS used. In neonatal

Figure 4 Changes in (A) body temperature and plasma concentrations of (B) activin A, (C) follistatin, (D) IL-6 and (E) TNF- α in neonatal lambs following i.v. injection of 300 ng/kg LPS ($n=5$, $\bullet$) or 3 μ g/kg LPS ($n=4$, $\circ$). Values are means $\pm$ S.E.M.

lambs subjected to this paradigm, although there were increases in the response to the higher LPS dose in most parameters measured, the only statistically significant ($P<0.05$) increase was in the amount of follistatin released. An enhancement in cytokine release in response to increasing doses of LPS has been reported for larger mammalian species but this may be dependent on the cytokine assessed (LeMay *et al.* 1990, Gerros *et al.* 1993, Mullington *et al.* 2000, Haudek *et al.* 2003). The notable exception to the overall effect on cytokine responses was that at the higher dose of LPS employed (3 μ g/kg), there was less activin A released, although this was not statisti-

cally different from that of the lower dose of LPS used (300 ng/kg). Of relevance is that at an intermediate dose (1 μ g/kg; Jones *et al.* 2000, 2004) adult animals appear to have a qualitatively similar activin A release compared with the 300 ng/kg dose used in the present study for both the adults and neonatal animals. Therefore, whether the activin A release in response to graded doses of LPS challenge is part of an all-or-none effect, or is to some extent dose-dependent, should be the subject of future studies.

A reduced responsiveness to subsequent LPS challenge, coined endotoxin tolerance, has been the subject of recent

Figure 5 Changes in (A) body temperature and plasma concentrations of (B) activin A, (C) follistatin, (D) IL-6 and (E) TNF- α in non-pregnant ewes ($n=5$) following i.v. injection of 300 ng/kg LPS as an initial challenge ($\square$) or an identical dose 5 days later ($\diamond$). Values are means $\pm$ S.E.M.

interest in that it resembles the situation that often arises in human patients with sepsis or non-infectious systemic inflammatory response syndrome (SIRS) brought about by surgery and acute trauma of various causes (Cavaillon *et al.* 2003). On this basis, it was investigated whether the release of activin A into the circulation was subject to a tolerance-like effect; that is, was reduced in response to a second LPS challenge. Similar to the ablated effect on TNF- α and IL-6, the response of both activin A and follistatin was reduced following LPS treatment of an identical dose administered 5 days after an initial challenge. The nature of how these proteins form part of the

tolerance mechanism has not been elucidated; tolerance is readily demonstrable in mature sheep (Whyte *et al.* 1989) and is also presumed to occur in immature animals. Of interest will be to establish if the diminished release of activin/follistatin is subject to a recovery in responsiveness, as in rats there is an initial refractory period to repeated LPS stimulation which disappears after some weeks and after which a full response to LPS is observed again (Sanchez-Cantu *et al.* 1989).

The role of activin A and its release during inflammatory challenge is still largely unclear. *In vitro* studies have shown that activin can have both pro- and anti-

Table 2 Responses to an initial (#1) or subsequent (#2) challenge to LPS, measured as peak temperature, peak concentration or AUC, for various parameters for non-pregnant ewes, pregnant ewes and neonatal lambs. Data are means ± S.E.M., analysed by paired Student's *t*-test

Parameter	Peak response (°C or ng/ml)		AUC (°C/h or ng/ml/h)	
	LPS challenge #1	LPS challenge #2	LPS challenge #1	LPS challenge #2
Temperature	41.2 ± 0.1	41.1 ± 0.1	7.89 ± 1.11	7.70 ± 1.27
Activin A	0.27 ± 0.04	0.20 ± 0.02	0.45 ± 0.07	0.25 ± 0.05*
Follistatin	23.6 ± 2.2	17.8 ± 1.4**	204.5 ± 31.4	113.2 ± 15.1***
IL-6	6.6 ± 1.1	3.3 ± 0.4***	69.5 ± 13.9	20.4 ± 3.5**
TNF-α	48.2 ± 2.4	40.4 ± 2.3**	122.0 ± 11.3	75.2 ± 7.5***

P*<0.05, *P*<0.01, ****P*<0.001 compared with the LPS challenge #1.

inflammatory functions (Phillips 2003), with its effects likely to be dependent on the cell types participating in the response and the inflammatory networks acting at that particular phase. The source of activin during inflammatory processes may be immune cell types or endothelial cells lining the vasculature, but further work is required to confirm this proposition. Nevertheless, the current studies highlight new aspects in delineating the activin response in a widely accepted model of acute inflammatory challenge. In summary, these findings demonstrate that activin forms part of a coordinated inflammatory reaction that is present in development from at least the first days of post-natal life. During pregnancy, the placenta appears to act as a barrier to protect the fetus from the effects of the maternal responses. Of interest was that, at least at the doses tested, the release of activin to LPS was not dose-dependent and possibly is part of an all-or-none response. Finally, the activin and follistatin components had ablated responses to subsequent LPS challenge, suggesting that they form part of the well-known hyporesponsive tolerance mechanism of repeated immune activation.

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