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Effects of pregnancy on the fever response of sheep to a Gram-negative pyrogen

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ABSTRACT

Ewes commonly display a reduction of immunity to gastrointestinal parasites during the periparturient period, so it is possible that other components of immunity change at this time. To determine whether the reported absence of fever in full-term-pregnant ewes occurs in a breed farmed commercially in New Zealand, 12 pregnant and 9 non-pregnant Coopworth ewes were treated with injections of *Escherichia coli* lipopolysaccharide (LPS) at five-weekly intervals for 3.5 months. An initial dose-response study, conducted in a separate group of ewes, determined that a dose of 200 ng/kg LPS induced fever (≥ 0.7°C increase in rectal temperature) in our sheep. Rectal temperature and plasma glucose concentration were monitored at the time of LPS injection and 4 h later. The magnitudes of both the mean increase in rectal temperature (range 0.4 to 2.1°C) and the mean reduction of plasma glucose concentration (range 2.9 to 17.7 mg/dl) appeared to vary throughout the study but they were not affected by pregnancy, nor was there any difference in these parameters between the two groups of sheep at the periparturient period. However, there was a reduction in the incidence of fever generated by LPS in the post-lambing period, lactating ewes appearing to have smaller increases in rectal temperature (mean increase 0.4°C, n = 11) than their non-lactating counterparts (mean increase 0.8°C, n = 8). We conclude that at least part of the immune response to LPS, i.e., fever and the reduction of plasma glucose concentration, is maintained in our Coopworth ewes at full-term of pregnancy.

Keywords: sheep; fever; lipopolysaccharide; pregnancy.

INTRODUCTION

In sheep, the periparturient increase in susceptibility to infection by gastrointestinal parasites indicates a major change in activity of the immune system during this period. Also, it has been reported that one component of immune function, the febrile response to exogenous pyrogens, is absent or suppressed in sheep during late pregnancy. For instance, a Canadian study (Kasting *et al*., 1978) recorded a loss of febrile response in pregnant ewes treated i.v. with a pyrogen from *Salmonella abortus equi* (Gram-negative bacterium) starting from 4 days before parturition and culminating with a total absence of response one day prior to parturition. At day 5 postpartum, some response had returned. Likewise a South African study (Goelst *et al*., 1992) showed that although periparturient ewes can display fever (e.g., from Gram-positive bacteria) they have a diminished response to endotoxin (Gram-negative pyrogen). In contrast, in an English study (Heap *et al*., 1981) pyrogens successfully induced fever in late-pregnant ewes, but the vasomotor response was attenuated. This group also studied sex steroid (oestrogen or oestrogen plus progesterone)-treated ovariectomized ewes and found no evidence of change in febrile response in these animals. Finally, Hales's group in Australia (Blatteis *et al*., 1988) recorded normal fevers in Merino ewes within 1-3 days prepartum after LPS challenge. These latter studies indicate that the absence of fever in full-term-pregnant sheep reported by other workers could not be regarded as a general phenomenon. The present study was conducted to determine whether Coopworth sheep in New Zealand lose the fever response to endotoxin during late pregnancy.

MATERIALS AND METHODS

Animals and experimental procedures

Animals used in these studies were Coopworth ewes (mean live weight 60.5 ± 4.7 kg) grazed on a ryegrass/white clover pasture at the Lincoln University Research Farm. They were weighed immediately after mustering from pasture to determine their non-fasted live weight (LW) and then held overnight in a woolshed prior to each endotoxin challenge. Separate animals were used in the two studies. Approval for the procedures was provided by the Lincoln University Animal Ethics Committee.

Dose-response study

Ewes were allocated randomly to four treatment groups (n = 4) which received 0, 200, 400 or 800 ng/kg LW of endotoxin (*Escherichia coli* 055:B5 lipopolysaccharide, LPS, Sigma, U.S.A.). Immediately prior to injection of endotoxin, 10 ml of venous blood was collected by jugular venipuncture to provide a plasma sample from each ewe and rectal temperature was measured using a digital clinical thermometer. Endotoxin, from a stock solution of 5 mg/ml in sterile 0.9% saline solution which had been freshly prepared on the day of challenge, was injected i.v. in a single bolus (T = 0 h) to give the appropriate dose. Ewes in the 0 ng/kg LW dose group received 2.5 ml of the saline solution i.v. Thereafter, rectal temperature was measured every 30 minutes for 5 h and a second blood sample was taken at 4 h (T = 4 h) post injection. Twenty one days later, the ewes were re-allocated to the four treatment groups and the procedure was repeated, thus providing n = 8 for this study.

Effect of pregnancy study

On 9 March, 2001 intravaginal progesterone-releasing devices (CIDR type G, Carter Holt Harvey Plastics, containing 300 mg progesterone) were administered to 17 ewes for 11 days. Mating by two crayon-harnessed Dorset Down rams was monitored daily for four days and ewes were subjected to ultrasonic scan approximately

60 days later to determine their pregnancy status. With the addition of four non-pregnant ewes, two groups were established comprising 12 pregnant and nine non-pregnant animals. Subsequently these sheep received four challenges of 200 ng/kg LW endotoxin. These were: on 6 June and 11 July, corresponding to mid and late gestation for the pregnant ewes; about 18 August, corresponding to the periparturient period for the pregnant ewes (i.e. ± 2 days from lambing); and 18 September, during lactation for the pregnant ewes. When each pregnant ewe received her periparturient challenge (based on predicted date of lambing or if she had lambed in the previous 1 or 2 days) a non-pregnant ewe was selected at random to undergo a challenge at the same time. The procedure for each challenge was as for the dose-response study (above), except that blood samples were collected and rectal temperatures were measured at 0 and 4 h only. Because of misdiagnosis of non-pregnancy and two deaths not associated with the experimental procedures, the number of animals per group changed slightly throughout the study.

Plasma glucose assay and statistical analyses

Glucose concentration of the plasma samples was measured using the glucose oxidase method (GOD-PAP kit, Roche Diagnostics Limited, Auckland). Mean and standard error of the mean (S.E.M.) were calculated for rectal temperature, plasma glucose concentration and the change in these values, where the change was calculated as the T = 4 h value minus the T = 0 h value. Data for rectal temperature and plasma glucose concentration were subjected to analysis of covariance (ANCOVA, using Genstat 5 release 4.1) using the T = 0 h value as the covariate. Where necessary the Greenhouse-Geisser correction factor was used. Areas under the curve were calculated for data from the dose-response study and subjected to linear regression analysis.

RESULTS

Dose-response study

LPS treatment caused a significant increase in rectal temperature at all doses (P<0.001, Figure 1). The rectal temperature response was directly related to the dose of LPS, as indicated by a significant linear relationship between dose of LPS and the area under the response curve (P<0.001). There was no significant change in rectal temperature of control sheep during the five-hour study period (Figure 1), whereas LPS-treated groups appeared to have a biphasic rise in rectal temperature with an initial peak between 1 and 2 h post injection and a major peak around 4 h post LPS injection (Figure 1). Rectal temperatures of LPS-treated sheep at 5 h post-injection were lower than the peak values around 4 h post-injection but had not returned to the pre-injection level at this time. There appeared to be a direct linear relationship between the dose of LPS and the reduction in plasma glucose concentration, but this was not significant.

Effect of pregnancy study

Pregnancy status of the ewe had no effect on the increase in rectal temperature caused by the LPS injection.

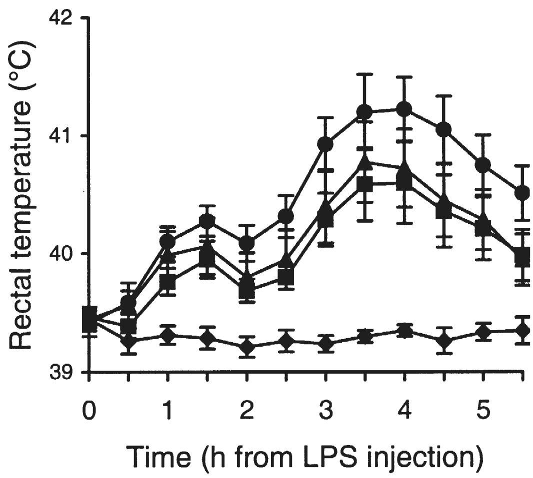

FIGURE 1. Changes in mean (± SEM) rectal temperature of sheep following an injection of LPS (n = 8) at a dose of 0 (◆), 200 (■), 400 (▲), or 800 (●) ng/kg live weight.

However, the magnitude of the elevation in rectal temperature caused by LPS injection altered with successive LPS challenges (P<0.001, Table 1). At the lactation-period challenge with LPS (on 18 September), many of the sheep (6/11 lactating, 3/8 non-pregnant) had a rectal temperature increase below 0.7°C, i.e., within the normal range of variability (95% fiducial limit) in temperature elevation for these sheep. This reduction in fever response was most severe in the lactating ewes, such that their mean change in rectal temperature was only 0.4°C (Table 1). However, the mean rectal temperature in the pregnant group was not significantly different from that of the non-pregnant group (Table 1). At the time of the late-pregnancy LPS challenge (11 July), pregnant ewes had a lower than usual plasma glucose concentration at both T = 0 h and T = 4 h (mean significantly lower, P<0.05, than that of non-pregnant sheep on this occasion, Table 1), but they experienced a similar magnitude in reduction of plasma glucose concentration following the LPS challenge. Mean plasma glucose concentration and the reduction following LPS challenge appeared to vary throughout the study periods (Table 1).

DISCUSSION

These results show that the febrile response to LPS in Coopworth ewes is maintained in late pregnancy and support the contention of Blatteis *et al.* (1988) that absence of the fever response in periparturient ewes, which has been described by other workers, is not a general phenomenon in sheep. A corollary of this is that the periparturient rise in susceptibility to gastrointestinal parasitism that occurs in Coopworth ewes (Leyva *et al.*, 1982) must be attributable to a change in immune function which is separate from at least some of the components involved in the endotoxin-fever pathway.

It is clear from the dose-response study that the dose of LPS used in the pregnancy study (200 ng/kg) was effective in producing a fever in these sheep. Features of this response are the biphasic nature of the increase in rectal temperature, as reported by Kluger (1991), and its

TABLE 1. Mean (± S.E.M.) rectal temperature (T_{re}; °C) and plasma glucose concentration (Gluc; mg/dl) of pregnant (P) and non-pregnant (NP) sheep before (0 h) and four hours after (4 h) LPS injection at four challenges from mid pregnancy to lactation.

Group		Mid-pregnancy (6 June)			Late-pregnancy (11 July)			Periparturient (18 August ± 2 days)			Lactation (18 September)		
		0 h	**4 h**	**Change**	**0 h**	**4 h**	**Change**	**0 h**	**4 h**	**Change**	**0 h**	**4 h**	**Change**
P	T_{re}	38.6	40.8	**+2.2*** **	39.3	40.3	**+1.0**	38.9	40.2	**+1.3**	39.5	39.9	**+0.4**
		± 0.05	± 0.09	± 0.10	± 0.05	± 0.15	±0.14	± 0.09	±0.16	±0.16	±0.10	±0.12	±0.13
P	Gluc	42.2	38.9	**-3.3**	27.4	22.1	**-5.3**	69.9	52.2	**-17.7**	63.6	54.6	**-9.0**
		±1.39	±1.59	±1.06	±1.78	±2.40	±1.11	±6.75	±5.41	±6.04	±1.21	±2.05	±1.89
	(n)	(12)			(12)			(11)			(11)		
NP	T_{re}	38.5	40.4	**+1.9*** **	39.1	40.1	**+1.0**	38.7	39.5	**+0.8**	39.2	40.0	**0.8**
		±0.11	±0.25	±0.26	±0.05	±0.16	±0.15	±0.08	±0.27	±0.30	±0.10	±0.23	±0.23
NP	Gluc	51.8	49.0	**-2.9**	49.0*	43.6 *	**-5.4**	61.4	44.7	**-16.7**	64.2	52.4	**-11.8**
		±2.43	±2.35	±2.74	±0.16	±2.96	±2.08	±2.70	±1.85	±3.03	2.85	±2.45	±2.35
	(n)	(9)			(9)			(9)			(8)		

*** P<0.001 different to all other challenge periods
* P<0.05 difference between pregnant and non-pregnant groups

amplitude. The mean increase in rectal temperature at 4 h post challenge for all doses of LPS exceeded 0.7°C, a value which our data has determined as the threshold for a significant elevation from normal fluctuations. Other authors (Kasting *et al*., 1978; Heap *et al*., 1981) seemed to have classified sheep as being febrile if rectal temperature increased by 0.4 °C.

One concern is the possibility that the immune response may diminish with repeated challenges. In fact, the data show large variability throughout the study, but this is probably due to random effects such as changes in weather and metabolic status of the ewes. The four LPS challenges were conducted approximately 35 days apart, which is well outside the 14-day period that is likely to be required for sheep to maintain immunological 'memory' of a LPS challenge (Prof F. J. Karsch, personal communication). Although changes in nutritional status may have affected some of the results recorded here, the responses to LPS were not affected at the late-pregnancy challenge when the pregnant ewes had low plasma glucose concentrations (Table 1).

At all of the LPS challenges there were individual ewes that failed to have a fever. This lack of response was random and may be due to inadequate intravenous delivery of LPS or to other unknown factors. It is noteworthy that a large incidence of such failure occurred at the September challenge and was particularly marked, but not significantly so, in the lactating group. This may warrant further investigation.

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