Suppression of T-cell function in the pregnant ewe

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ABSTRACT

Levels of cell mediated immunity (CMI) were monitored in pregnant and non-pregnant control ewes to assess the impact of pregnancy on CMI in sheep. Pregnant plasma caused a significant depression in lymphocyte culture reactivity with mitogens (PHA, Con A and PWM) or with antigen (BSA) in primed animals. No defect in transformation was evident in pregnant lymphocytes *per se*, which gave normal transformation when cultured in control plasma. Delayed-type hypersensitivity (DTH) responses to specific antigens were significantly lower during pregnancy in response to 1^{O} (BSA) and 2^{O} (clostridial) sensitisation. The induction of the primary reaction was significantly impaired. Secondary sensitisation induced normal levels of reactivity, with the function, as measured by DTH, significantly impaired throughout pregnancy but recovering post partum.

Keywords Ewe; pregnancy; cellular-immunity; suppression.

INTRODUCTION

Pregnancy represents a state of maternal tolerance of incompatible foetoplacental tissues in defiance of all the accepted laws of graft rejection. Though the pregnant female recognises the antigens produced by the foetus, the effector response produced is different from a conventional immunisation and serves to protect rather than damage the foetus. Altered immune function during pregnancy, resulting from the production of immunomodulating hormones and proteins, has been identified in many species, including rodents (Smith *et al.*, 1982), cows (Newman and Hines, 1980), mares (Allen, 1979) and women (Terasaki *et al.*, 1970).

Pregnancy associated changes in immune function are most evident in the suppression of T-lymphocyte function (Stimson, 1980). This pathway is vital for cellular regulation of immunity and provides the effector cells for cell-mediated immunity (CMI), both facets of which are essential for the rejection of incompatible grafts. Alterations in immunity in the pregnant female have implications not only for the health of the mother but also for the acquisition of passive immunity by the foetus or neonate (Smith and Little, 1922). Immunisation of pregnant females is used widely in animal management. In 1984 a survey of 57 sheep farmers in South Otago showed that 81% vaccinated their ewes late in pregnancy against clostridial diseases (Q.B. Whithell, personal communication). A proper understanding of alterations in immunity due to pregnancy would facilitate optimal exploitation of vaccination regimes in the breeding female.

The present study examines the immune status of the pregnant ewe to determine if special consideration should be given to the use of vaccines in the management of breeding females. Different aspects of cell-mediated immune function were assessed in experimental ewes using standard *in vitro* laboratory culture assays (Bloom, 1971) involving both mitogens and antigens. *In vivo* functional assays for specific cellular reactivity to antigens were tested for by the measurement of delayed-type hypersensitivity (DTH) responses in immune animals re-exposed to test antigens intradermally.

MATERIALS AND METHODS

Sixteen non-pregnant and 14 pregnant Romney ewes were used in this study. The pregnant ewes had been synchronised with progestagen impregnated sponges (Veramix (60 MAP)-Upjohn), so that all were mated within a 24 hour period. Pregnant and non-pregnant ewes were always treated and sampled on the same day under identical conditions. At 100 days gestation all animals were immunised (1^{O}) with 500 μg bovine serum albumin (BSA-Sigma-V) emulsified in Freund's complete adjuvant (FCA-Difco), given as a 2 ml dose intraperitoneally. At 120 days gestation each animal was immunised (2^{O}) with a composite clostridial vaccine (Triptet-Wellcome), given as a 2 ml dose subcutaneously into the neck. All these ewes had been exposed to this vaccine at least once prior to pregnancy. Blood sampling was carried out at 130 days gestation and 28 days following parturition.

Whole Blood Microculture

Leucocytes were cultured and processed as 1/10 whole blood cell suspensions in microculture (Griffin

and Banwell, 1978) to test for mitogenic responses with the polyclonal T-cell stimulants; phyto-haemagglutinin (PHA- 1 μl-Gibco), conconavalin A (Con A- 2 μg-Sigma) and pokeweed mitogen (PWM-2 μl-Gibco). After culturing for 96 hours in a humidified CO_2 (5%) incubator at 37°C, a radioactive tracer of 0.5 μCi ^{3}H-thymidine (Amersham Sp. Act.≃6 Ci/m mol) was added to each well. The cells were cultured in triplicate for a further 18 hours and harvested to recover the radiolabelled nucleotides from dividing cells. Results were expressed as mean counts per minute (cpm), and the cellular activity estimated by comparison with control cultures devoid of mitogen.

Purified Leucocyte Culture

Leucocyte responses to a specific antigen (BSA-1-μg) were tested using mononuclear cells isolated from peripheral blood (Boyum, 1968). Cell suspensions were washed, and cultured in 100 μl volumes in medium (RPM1-1640) supplemented with 10% autologous plasma or a control batch of non-pregnant heterologous plasma. Antigen solutions were added and the cells cultured for 120 hours, before the addition of ^{3}H-thymidine under similar conditions to that of the whole blood cultures. Thereafter all procedures for harvesting and analysis were similar to the whole-blood microculture.

Skin Test Reactivity

All animals were skin tested by the introduction of 100 μl of different antigens intradermally into the shaved neck region. The antigens used were administered at separate sites and included BSA (10 μg), mycobacterial PPD (1000 i u), tetanus, perfringens and septicum toxoid (10 μl). The indurated weal was measured 48 hours post inoculation using calipers, and estimating the mean diameter of the reaction across 2 axes, at right angles. Composite mean values were obtained for each antigen within the respective groups.

RESULTS

Whole Blood Microculture

The results in Table 1 show a significant ($P<0.05$) reduction in mitogenesis in samples from pregnant ewes, cultured in autologous plasma by comparison with pregnant cells cultured in heterologous non-pregnant plasma. Non-pregnant animals had higher responses ($P<0.05$) than pregnant cells in autologous plasma, irrespective of whether they were cultured in autologous or heterologous control plasma. These figures identified pregnant sheep plasma as the source of suppressor factors which impair mitogenic responses in culture, showing that suppression of pregnant leucocytes in culture is not a property of the cells but due rather to plasma factors.

Leucocyte Culture

Measurement of antigen (BSA) transformation of purified leucocytes from animals following immunisation (Table 2) showed a similar trend to that obtained with mitogens. Leucocytes obtained from pregnant ewes were significantly ($P<0.01$) less responsive than control cells cultured in autologous plasma. Replacement of pregnant plasma by control (heterologous) plasma caused a significant recovery

TABLE 1 Response to mitogens in culture of whole blood from 14 pregnant and 12 non-pregnant ewes. (Results expressed as counts/min ± SE from radiolabel uptake in cells).

Mitogen	Pregnant ewes		Non-pregnant ewes	
	Autologous plasma	Heterologous plasma	Autologous plasma	Heterologous plasma
PHA	8190 ± 1460	17590 ± 2600	16350 ± 2080	15650 ± 2500
ConA	6830 ± 1100	12590 ± 1530	11330 ± 1400	10630 ± 1480
PWM	2630 ± 820	4040 ± 1040	5460 ± 1060	4860 ± 830
Control	710 ± 180	640 ± 130	540 ± 120	550 ± 140

TABLE 2 Ovine leucocyte transformation with bovine serum albumin antigen in 12 pregnant and 12 non-pregnant ewes using autologous or heterologous plasma from pregnant (P) or non-pregnant (NP) sheep. (Results expressed as counts/min ± SE).

Plasma	Pregnant ewes		Non-pregnant ewes	
	Autologous (P)	Heterologous (NP)	Autologous (NP)	Heterologous (NP)
Response to BSA	3410 ± 790	6430 ± 1240	8360 ± 980	6730 ± 850

TABLE 3 Skin test response 48 h after inoculation in immunised ewes. (Results expressed as mean (SE) diameter (mm))

Ewes	BSA	PPD	Stimulant *Cl. tetani*	*Cl. perfringens*	*Cl. septicum*
Non-pregnant (n = 16)	16.3 (0.8)	13.5 (1.1)	10.5 (0.9)	8.2 (0.9)	9.4 (0.3)
Pregnant (n = 14)	11.0 (1.1)	9.3 (0.5)	5.1 (0.9)	3.0 (0.6)	3.2 (0.4)
Post partum (n = 14)	9.6 (0.9)	10.8 (1.0)	11.3 (0.5)	6.8 (0.8)	10.3 (0.9)

of leucocytic responses to levels equivalent to control cells cultured in heterologous plasma ($P<0.01$). Again this specific transformation assay identified pregnancy plasma suppressor factors as the key component in the reduced leucocyte response. Functional impairment of leucocytes *per se* was not evident.

Skin Test Reactivity

The response to BSA during pregnancy (Table 3) was similar to that found from leucocyte cultures, showing that pregnant ewes were significantly less responsive than the non-pregnant controls ($P<0.01$). Surprisingly though, the reduced level of reactivity persisted after pregnancy and at 28 day post-partum lactating ewes still had a significantly reduced DTH response to BSA ($P<0.01$). Similar responses were found between BSA and PPD within pregnancy and in the post partum phase. In contrast to this was the response with clostridial toxoids, where although there was a significant ($P<0.01$) reduction in reactivity during pregnancy, the response recovered to control levels in the post partum period.

DISCUSSION

Non-specific mitogens such as were used in the present study have become established in diagnostic immunology as valid markers for measuring lymphocyte reactivity in peripheral blood (Schultz, 1982). Individuals suffering from immune deficiency states invariably have reduced lymphocyte transformation responses with polyclonal activators though it has not been established how such responses are reflected in the functional status of the immune response. The present findings show that plasma from pregnant sheep ("pregnant plasma"), contains suppressor factors for mitogenesis analogous to those found in other species (Gill and Repetti, 1979). No intrinsic defect was found in leucocytes *per se*, from pregnant sheep, as mitogenesis was normal in the absence of pregnant plasma, a finding observed earlier in humans (Griffin and Beck, 1983).

In an attempt to increase the discrimination of the assays of cellular immunity the major part of this study monitored functional responses in animals following specific sensitisation with antigens. Whereas the lymphocyte transformation assays with antigens did not add to the finding of plasma suppressors in the mitogen transformation assay, the *in vivo* DTH test pointed to more subtle perturbations in immunity following immunisation during pregnancy. Because it has been established that primary immune responses are more sensitive to suppression than secondary memory responses (Segal *et al.*, 1972) we have compared reactions in pregnant animals exposed to primary (BSA) and secondary (Cl. toxoids) immunogens.

The level of cellular immunity produced following immunisation is influenced by regulatory interactions in the inductive stages of cell activation, or at the effector stage of impairing the expression of the activated cells. The results obtained in the present study show that there was impaired induction of immunity in pregnant animals exposed to 1^{o} immunisation with BSA. This resulted in depressed functional immunity, which remained low throughout pregnancy and following parturition.

Immunisation of pregnant ewes with booster doses of immunogens (clostridial toxoids), induced normal levels of cellular immunity but the effector response was depressed. Reduced effector responses would result in increased disease susceptibility in the dam but more significantly cause a decrease in passive immune factors (antibody) available to the neonate. Recovery of effector responses in the ewe in the week post partum would allow for active protection in the post parturient dam. This would be of little consequence to the lamb where the humoral factors available to the neonate are produced by the dam during pregnancy. The major component of passive immunity is acquired via the gut in the neonate during the first 48 hours after birth (McCarty and McDougall, 1949). Reduced immunocompetence in the pregnant dam would be of greater significance in low responder animals which could become severely 'compromised' during

breeding. Another area of special relevance is with multiple offspring where passive immune factors available to individual lambs would have to be shared amongst siblings.

The results of the present study focus on the potential danger of extrapolating too widely from parameters which have been defined in normal animals. Pregnancy appears to result in a degree of immunomodulation, producing a 'compromised' host which would merit special consideration. Areas of particular relevance include potency of immunogen and timing of immunisation. The widespread practice of immunising ewes with booster doses of clostridial toxoids in late pregnancy requires re-evaluation. Reduced levels of immune reactivity during pregnancy may mitigate against the acquisition of adequate levels of passive immunity by the newborn.

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