Depression of Maternal Immune Response during the Period of Implantation in Rabbits

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ABSTRACT

We determined the maternal peripheral lymphocyte response to mitogen during the period of implantation and evaluated the effects of hormones, which are known to be involved in the process of implantation, on the lymphocyte activity in rabbits. As compared with peripheral lymphocyte activity in non-pregnant rabbits, lymphocyte activity was significantly depressed on days 6, 7 and 9 of pregnancy. Although concentrations of serum progesterone were gradually increased during the implantation period, progesterone did not inhibit lymphocyte activity at physiological concentration. Serum $PGF_{2\alpha}$ was significantly increased on day 7 while PGE was slightly increased. $PGF_{2\alpha}$ did not modify lymphocyte activity even with greater concentrations than physiological level. However, lymphocyte activity was significantly inhibited by PGE even with physiological doses. The treatment of indomethacin at doses of 0.1 or 1.0 $\mu g/ml$ tended to enhance lymphocyte response, which was depressed on day 8 of pregnancy, 28% or 23% respectively. Although in non-pregnant rabbit, enhancement of lymphocyte response was also shown after the treatment of indomethacin, this enhancement was much less than that in pregnant rabbits. These results strongly suggest that maternal immune response was depressed during the process of implantation and PGE might be one of factors for immunomodulation during this period.

Key Words: Implantation, Immune response, Prostaglandin E

INTRODUCTION

The exact mechanism by which blastocysts implant to the uterine endometrium has not been clearly understood although it is well established that the morphological, endocrinological and biochemical changes occur during the period of implantation.

During the process of implantation, the trophoblast cells of the embryo invade the endometrium of uterus. Although the histocompatibility genes of paternal and maternal origin are expressed as antigens on the trophoblast cells of the embryo (Searle *et al.*, 1976; Sellens, 1977), maternal immune system does not reject the embryo as an allograft. One of the hypotheses to explain the survival of fetal allograft is that

maternal cell-mediated immunity might be depressed during pregnancy.

Maternal vulnerability to infections (Thong *et al.*, 1971) and prolonged survival time of skin allograft (Finn *et al.*, 1972) during pregnancy indicate a significant alteration in the immunological competence of pregnant women. Furthermore, it was demonstrated that cellular immunity as measured by mitogen-induced lymphocyte transformation was inhibited by sera from pregnant women (Fizet *et al.*, 1983; Wajner *et al.*, 1985). Various hormones including estradiol, progesterone and prostaglandins (PGs) are increased during pregnancy and are known to play an important role in the process of implantation as well as maintenance of pregnancy. Some protein and steroid hormones have been demonstrated to inhibit lymphocyte activity in vitro (Cerni *et al.*, 1977; Wyle & Kent, 1977; Holdstock *et al.*, 1982). The suppressive effect of pregnant serum on lymphocyte activity was progressively augmented

toward the end of gestation (Papiha et al., 1983). Then, it is postulated that hormones may modulate the maternal immune response.

To our knowledge, no study has been done on maternal immune response when uterine endometrium first interacts with blastocysts during the period of implantation.

In the present study we, therefore, determined the maternal peripheral lymphocyte response to mitogen during the period of implantation and evaluated the effects of hormones which are known to play an important role in the process of implantation on the peripheral lymphocyte activity in rabbits.

MATERIALS AND METHODS

Female rabbits weighing 2-3 kg were mated with fertile males. The day of mating was designated as day 0 of pregnancy.

Blood samples were collected from ear vein on days 0 to 10 of pregnancy throughout this study. For the determination of concentrations of progesterone, prostaglandin E (PGE) and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), blood was centrifuged at 1000 xg and serum was stored at $-20°C$ before being assayed. Rabbits were sacrificed on day 7 of pregnancy and uterus was removed to measure PGE and $PGF_{2\alpha}$ concentrations.

Peripheral blood mononuclear cells were separated from heparinized blood (20 IU/ml) on Ficoll-Hypaque density gradient. The cells were washed three times with RPMI 1640 medium containing L-glutamin, penicilline (100 IU/ml) and streptomycin (100 μg/ml) and resuspended in RPMI 1640 medium which was supplemented with 20% fetal calf serum.

Mitogen assay: Mononuclear cells at a concentration of 1×10^6 cells/ml were cultured with or without concanavalin A (Con A, 45μg/ml) in CO_2 incubator for 72 hrs and cells were exposed to 1 μ Ci ^{3}H-thymidine/well for 18 hrs prior to harvesting on glass fiber filters with a Titer cell harvester. The degree of ^{3}H-thymidine incorporation into the cells was determined by counting radioactivity on dried filters in liquid scintillation counter and the stimulation index was calculated as follows;

Stimulation index (SI)
$$= \frac{\text{cpm of } ^3\text{H-thymidine incorporation with Con A}}{\text{cpm of } ^3\text{H-thymidine incorporation without Con A}}$$

Hormone treatment; The stock solutions of progesterone (5 mg/ml), PGE (1 mg/ml) and $PGF_{2\alpha}$ (1 mg/ml) were prepared in absolute ethanol and final concentration of ethanol was adjusted to less than 0.04% so as not to affect cell cultures. Different concentrations of hormones were added directly to the wells with Con A to determine their effects on lymphocyte activity. Indomethacin (ID, 0.1 or 1.0 μg/ml) was also added to cultures in order to inhibit PGs synthesis. Percent inhibition or % enhancement of ^{3}H-thymidine incorporation was calculated as follows;

% inhibition
$$= \left(1 - \frac{\begin{array}{c}\text{cpm of } ^3\text{H-thymidine incorporation} \\ \text{with Con A and hormone}\end{array}}{\begin{array}{c}\text{cpm of } ^3\text{H-thymidine incorporation} \\ \text{with Con A}\end{array}}\right) \times 100$$

% enhancement
$$= \frac{\begin{array}{c}\text{cpm of } ^3\text{H-thymidine incorporation} \\ \text{with Con A and ID}\end{array}}{\begin{array}{c}\text{cpm of } ^3\text{H-thymidine incorporation} \\ \text{with Con A}\end{array}} \times 100$$

Concentration of progesterone was determined by solid-phase radioimmunoassay (Diagnostic Product Corporation, U.S.A.) and PGs concentrations by double-antibody radioimmunoassay (Clinical Assay, U.S.A.). Uterine PGs were extracted by method previously described (Lee et al., 1985).

RESULTS

Concentrations of progesterone and prostaglandins during early pregnancy

Concentrations of serum progesterone were gradually increased during the implantation period, reaching the highest, 11.56 ± 0.71 ng/ml on day 7 and maintaining similar levels thereafter (Fig. 1). $PGF_{2\alpha}$ was significantly increased to 1.07 ± 0.06 ng/ml on day 7 ($P < 0.01$) while concentration of PGE was slightly increased on day 7 (Table 1). Concentrations of $PGF_{2\alpha}$ and PGE in uterine endometrium on day 7 were 6.10 ± 1.10 and 4.35 ± 0.57 ng/g tissue respectively.

Maternal peripheral lymphocyte activity during the period of implantation

To demonstrate whether maternal immune

response was inhibited during the implantation period when endometrium first accepted the blastocyst, peripheral lymphocyte response to Con A was observed during the implantation period. Activity of peripheral lymphocytes was presented as stimulation index. In rabbits, implantation occurs on day 7 post coitum. As compared with lymphocyte activity of non-pregnant rabbits, peripheral lymphocyte activity was significantly depressed on days 6, 7 and 9 of pregnancy. Stimulation index was 17.00 ± 3.69 in non-pregnant rabbits, but stimulation index was significantly dropped to 3.20 ± 2.10 on day 6, 3.30 ± 1.39 on day 7 and 1.70 ± 0.69 on day 9 of pregnancy ($P < 0.01$), indicating a marked depression in lymphocyte activity during the implantation period (Fig. 2).

Effects of hormones on the lymphocyte response to Con A
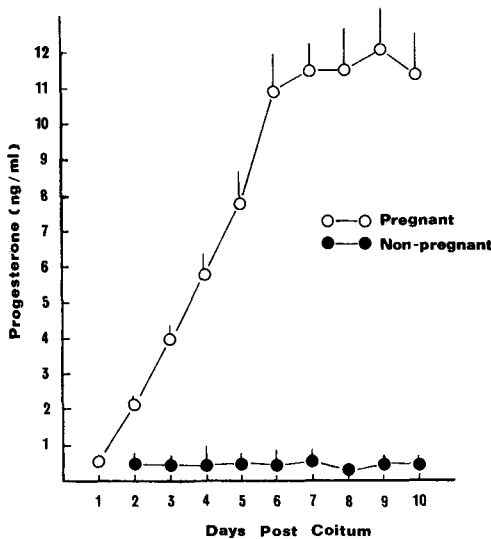
To determine immunomodulatory factors

involved in the process of implantation the effects of hormones on peripheral lymphocyte response to Con A were studied.

Although concentrations of progesterone were much greater than normal physiological levels, progesterone significantly depressed lymphocyte reactivity at concentrations between 1.1 and 22
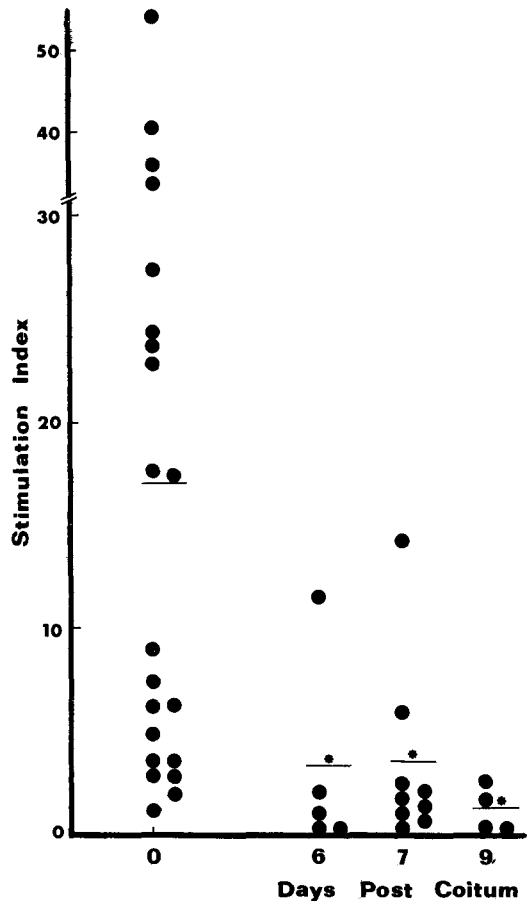

Fig. 1. Peripheral serum progesterone concentrations during early pregnancy in rabbits.

Fig. 2. Peripheral lymphocyte response to Con A during the period of implantation in rabbits. * P < 0.01.

Table 1. PGE and $PGF_{2\alpha}$ concentrations in serum and uterine endometrium on day 7 of pregnancy

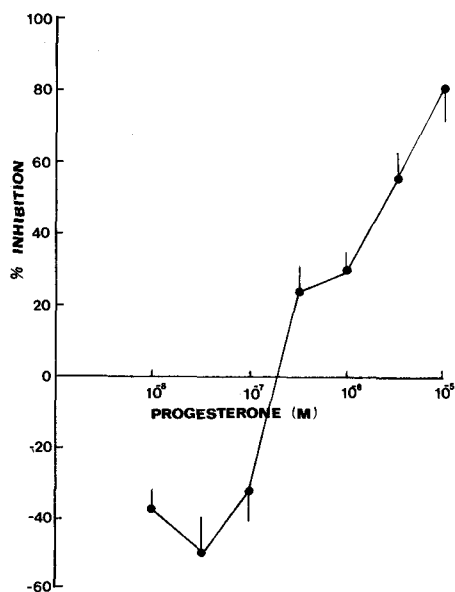|	Serum (ng/ml)		Endometrium (ng/g tissue)	
	PGE	$PGF_{2\alpha}$	PGE	$PGF_{2\alpha}$
Day 7	1.09 ± 0.13	1.07 ± 0.06*	4.35 ± 0.57	6.10 ± 1.10
Non-pregnant	0.82 ± 0.12	0.59 ± 0.07	—	—

Values are mean ± S.E.   * P < 0.01

Fig. 3. Effect of progesterone on lymphocyte response to Con A in rabbits.
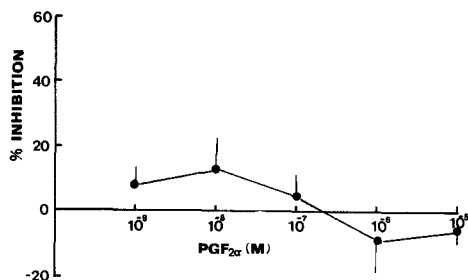

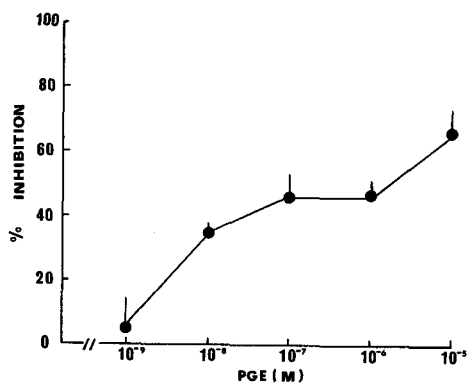Fig. 4. Effect of PGF$_{2\alpha}$ on lymphocyte response to Con A in rabbits.

Fig. 5. Effect of PGE on lymphocyte response to Con A in rabbits.

Table 2. Effect of indomethacin on depressed immune response during the implantation period in rabbits

	Indomethacin (μg/ml)	^{3}H-thymidine incorporation ($\times 10^{-3}$ cpm)	% increase
Pregnant	0	33.3 ± 11.2	—
	0.1	42.7 ± 13.9	28
	1.0	41.2 ± 14.5	23
Non-pregnant	0	72.4 ± 22.5	—
	0.1	75.8 ± 23.7	4
	1.0	81.3 ± 26.9	12

μg/ml (Fig. 3). PGF$_{2\alpha}$ did not modify lymphocyte activity at concentrations between 4.5 and 4,500 μg/ml (Fig. 4). However, Con A-stimulated lymphocyte transformation was significantly inhibited by PGE even with physiological concentrations (Fig. 5).

Effect of indomethacin on lymphocyte response to Con A during the implantation period

This experiment was done to determine if endogenous PGs increased during the implantation period induced PGE-producing lymphocytes, thereby contributing to immunosuppression since there is evidence that PGE itself can induce PGE-producing lymphocytes. The treatment of ID at doses of 0.1 or 1.0 μg/ml tended to enhance lymphocyte response of pregnant rabbits on day 8, 28% or 23% respectively although there was no statistical significance. In non-pregnant rabbits whose endogenous PG levels were not increased, enhancement of lymphocyte response was only 4 % or 11% after the treatment of 0.1 or 1.0 μg/ml ID (Table 2). This result suggests that endogenous PGs elevated during the implantation period might induce PGE-producing lymphocytes.

DISCUSSION

During pregnancy, maternal immune response is challenged by foreign histocompatibility antigens of fetus (Youtananukorn & Matangkasombut, 1972, 1974; Searle et al., 1976). Despite of a potentially hostile immune system, fetal allograft survives in utero for the period of gestation. Two hypotheses have been proposed to explain

the reason why the fetal allograft is exempted from immune rejection by the mother. The first suggested that placenta might act as a barrier shielding the fetus from passage of immunocompetent maternal lymphocytes and in this stage trophoblast cells might be lacking antigens (Taylor & Hancock, 1975; Taylor et al., 1976; Hunziker et al., 1984). The second hypothesis was that maternal immune response might be inhibited by hormones or other factors of maternal origin during pregnancy (Leikin, 1972; Lawrence et al., 1980; Fizet et al., 1983; Smart, 1984).

In the present study, lymphocyte activity to Con A was significantly depressed during the period of implantation in pregnant rabbits, suggesting that maternal immune suppression might partly be responsible for the implantation process. To our knowledge, this result is the first report concerned with maternal immunity during the period of implantation. In some pregnant rabbits, we found stimulation index less than 1 which seemed to be unexplainable. This phenomenon, however, might be due to the fact that Con A might predominantly activate suppressor cells or concentration of Con A (45 μg/ml) used in this study was too high, thereby inducing more suppressor cells. Previous findings that maternal lymphocyte response to PHA was depressed in most pregnant women (Purtilo et al., 1972) and plasma from pregnant women suppressed lymphocyte activity in non-pregnant women (Leikin, 1972; Fizet et al., 1983) were at least partly supportive for our findings. In our separate study, we also found that addition of serum from pregnant rat during the period of implantation significantly suppressed non-pregnant thymocyte response to Con A (manuscript in preparation). These results strongly suggested that peripheral components might be responsible for the immunomodulation during the implantation and pregnancy. Then, what kinds of factors are involved in immunomodulation during the period of implantation ?

During the period of implantation, concentration of serum progesterone and PG, especially uterine PGs are significantly increased (Hoffman, 1978) and the administration of indomethacin, a PG synthetase inhibitor, decreased uterine PG levels resulting in inhibition of implantation (Hoffman, 1978; Lee et al., 1985).

The treatment of progesterone inhibited lymphocyte activity at doses more than 1 μg/ml. Although these doses are much higher than physi-ological dose, possible immunosuppressive role of progesterone might not be ruled out during the implantation period. It has been reported that progesterone inhibited the initial activation of lymphocyte response to allogenic cells or mitogen (Clemens et al., 1979). It was also reported (Mendelsohn et al., 1977) that Con A generation of suppressor cells was enhanced by the addition of progesterone but not by testosterone or estradiol. Accordingly it is presumed that progesterone produced locally by trophoblasts as well as progesterone in ovarian origin (Borland et al., 1977) might block the initial activation process of lymphocytes to histocompatibility cells. Lymphocyte response to Con A was paradoxically stimulated by doses less than 1 μg/ml of progesterone but this phenomenon can not be explained at this moment. However, Wyle and Kent (1977) observed a similar results with 0.01-0.1 μg/ml of progesterone, estradiol and testosterone.

PGF$_{2\alpha}$ did not modulate lymphocyte activity even at much greater concentrations than physiological concentrations. On the other hand, PGE significantly inhibited Con A-induced lymphocyte transformation even with physiological concentrations and this inhibition was dose-dependent between 0.45 and 4,500 ng/ml. These results are consistent with the findings that 1-1000 ng/ml of PGE depressed lymphocyte activity of PHA in dose-responsive manner in human (Smith et al. 1971; Goodwin et al., 1977). Goodwin et al. (1977) suggested that PGE was produced by mitogen-stimulated human leukocytes and PGE itself in turn inhibited this stimulation, suggesting that PGE might act as an endogenous modulator of human immune reaction.

It has been reported that lymphocyte proliferative response to mitogen is depressed in patients with lung cancer owing to the presence of non-T suppressor cells (Han & Takita, 1980). The treatment of ID significantly enhanced mitogen-induced lymphocyte response of these patients (Han & Takita, 1980; Balch et al., 1982). Balch et al. (1982) found that polymorphonuclear cells from head and neck cancer patients produced more PGE than cells from normal individuals. Therefore, it is suggested that PGE synthesized by suppressor cells might play an immunomodulatory role in immunodepressed cancer patients. In our experiments, the treatment of ID could enhance lymphocyte activity which was depressed during the period of implantation. In non-pregnant rabbits whose endogenous PG levels were not

increased, much less enhancement was shown after ID treatment. PGE itself produced by uterine endometrium (Hoffman et al., 1984) and blastocyst (Dey et al., 1980; Pakrasi & Dey, 1983) might induce PGE-producing suppressor cells (Fulton & Levy, 1981), thereby contributing to immunomodulation during the period of implantation.

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== 국문초록 ==

토끼의 착상기간 중 모체의 면역 억제 현상에 관한 연구

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　본 실험에서는 가토에서 배반포가 자궁내막에 착상할 때, 모체의 면역기능의 변화를 알아보고, 착상시 관여하는 호르몬과 모체의 면역기능과의 연관성을 관찰하여 다음과 같은 결과를 얻었다.

1. 임신 7일째 가토의 말초혈관 림프구에 concanavalin A를 처리하면, 임신하지 않은 가토의 림프구보다 훨씬 낮은 자극지수를 나타냄으로써 면역억제 현상이 나타나는 것을 관찰할 수 있었다.
2. 임신 7일 때의 혈청 progesterone 농도인 11.56 ng/ml을 임신하지 않는 가토의 말초혈관 림프구에 처리하면, 억제현상을 나타내지 않았으나, progesterone의 농도가 1000 ng/ml 이상에서는 농도에 비례하여 림프구의 활성도가 감소되었다.
3. 4.5 ng/ml~4,500 ng/ml의 $PGF_{2\alpha}$는 concanavalin A처리시 림프구의 활성도에 별다른 영향을 끼치지 않았으나, PGE는 0.45 ng/ml~4,500 ng/ml에서 농도에 비례하여 림프구의 활성도를 유의하게 감소시켰다. 그러나 임신 제 8 일째의 말초혈관 림프구에 prostaglandin 합성 억제제인 indomethacin 0.1 또는 1.0 μg/ml을 처리하면 억제되었던 림프구 활성도가 각각 28%, 23% 증가되었다.

　이상의 결과로 보아 토끼의 착상기간중 모체의 면역기능이 저하되며 PGE가 착상기간중 면역기능을 저하시키는 요소중 하나인 것으로 생각된다.