Iranian Biomedical Journal 3 (1 & 2): 41-45 (April 1999)

Prostaglandin E2 in Soluble Factor-Mediated Immune Suppression by First Trimester Decidual Cells

Mehri Ghafourian Boroujerdnia[*1] and Rahim Chinipardaz[2]

[1] *Department of Immunology, Ahwaz Medical Science University;* [2] *Department of Statistics, Shahid Chamran University, Ahwaz, I.R.Iran*

ABSTRACT

Several studies have shown that decidual tissue produces soluble factors which can mediate a variety of immunosuppressive activities. In order to assess whether prostaglandin E2 (PGE2) was present in supernatants produced by explant culture of 10 human specimens of first trimester decidua. The effect of the supernatants from the two different series (with and without indomethacin treatment) of explant culture incubated for 24, 48 and 72 hours on the suppression of PHA-induced lymphoproliferation assay was investigated. The percentage of suppression was evaluated using Student`s t-test. The immunosuppressive activity of decidual explant supernatants was significantly reduced in the presence of indomethacin only in 24 hour culture period. The present study suggests the production of PGE2 by early pregnancy decidua does exist. Whether this molecule is critical for survival of the feto-placental unit requires more investigations, including comparison with nonpregnant endometrium in different cycle phases and investigation of pathological pregnancy. *Iran. Biomed. J. 3(1 & 2): 41-45, 1999*

Keywords: Prostaglandin E2, decidua, immunosuppression, Student`s t-test

INTRODUCTION

Local immunosuppression may be one explanation for progressive development of the embryo in a hostile maternal immunologic environment created by the semi-allogeneic nature of fetal cells. It has been proposed that cells in the pregnant uterus are locally activated to suppress immune responses, and that suppression is mediated by soluble factors produced by the local cells. The advantage of localized cellular activity is that the mother remains in a generally immunocompetent state. Support for this hypothesis comes from several studies where cells with suppressive activity have been isolated from decidual tissue surrounding the developing embryo [1-9]. Further support was derived from studies demonstrating that skin grafts, which are rejected in a rapid manner when transplanted systematically, enjoy prolonged survival in the pregnant uterus [10].

Several investigations have been aimed at defining the nature of the soluble suppressor factors responsible for the immunosuppression activity. Study of the nature of this decidual immunosup pressor factor showed that inhibitory activity of the culture supernatants was associated with both high (>1500 kDa) and low (<1500 kDa) molecular weight fractions [11]. By fractionation the MLR inhibitory activity of decidual supernatants was associated with three different molecular size fractions, specifically <1.5 KDa, 60 KDa and 1000 KDa; however, only the low (<1.5 KDa) molecular weight fraction was able to inhibit both the MLR and spontaneous neonatal thymocyte proliferation [2].

The non-specific immunosuppressive characteristics of culture supernatants of murine decidual tissue showed a major contribution from a low molecular weight soluble factor [12]. The immunosuppressive activity of culture supernatants was assessed in several assays of T cell lymphoproliferation including the MLR, thymocyte proliferation assay, CTL generation, and mitogen- and antigen-induced proliferation assays. These immunosuppressive activities were totally abolished by removing low molecular weight components from the supernatants by dialysis. In addition, treatment of culture supernatants with indomethacin, which prevents prostaglandin synthesis, led to a significant loss of suppressive activity compared with the paired untreated cultures. PGE2 mediated suppressor activity during pregnancy [13]. PGE2-mediated immunosuppression of NK cells in decidua may be necessary for fetal survival [14].

[*] Corresponding Author.

[DOR: 20.1001.1.1028852.1999.3.1.3.3]

The effect of the different soluble suppressor factors produced by murine decidual cells in culture was dependent on whether the murine decidual cells were mechanically or enzymatically dispersed [5, 15]. Suppressor activity was completely abolished in indomethacin-treated cultures of enzyme-prepared cells, while the level of suppression by cells cultured after mechanical dispersal was not influenced by indomethacin. Enzymatically dispersed cells produced very high levels of the prostaglandin PGE2 compared with the insignificant PGE2 levels produced when cells were dispersed mechanically reported by Searle [16]. This author has also suggested that the trauma of collection of decidual tissue and its manipulation prior to culture may cause the artificial and rapid release of PGE2 and hence induce artificially high levels of suppressor activity.

It is therefore important to determine whether decidua produces PGE2 in pregnancy or rather only when decidua is cultured. The present study has been aimed to investigate the PGE2 activity in supernatants produced by intact decidual tissue as explant to define the true levels of prostaglandin production by human decidua during pregnancy.

MATERIALS AND METHODS

Explant medium: HAM's F10 (Sigma) medium was supplemented with 10% FCS, 2 mM L-glutamine, 1000 U/ml penicillin and 1 mg/ml streptomycin (Sigma). Phytohaemagglutinin (PHA) (Sigma) as a sterile powder was reconstituted in 1000 µl PBS, aliquoted and stored at -20^{O}C. Indomethacin. A 1×10^{-2} M solution of indomethacin (Sigma) was prepared in pure ethanol, passed through a 0.2 µm syringe filter and stored at 4^{O}C until use. Cultures were maintained in a CO_2 incubator at 37^{O}C in a humidified atmosphere of 5% CO_2 in air.

Tissues and tissue culture technique: Ten specimens of normal first trimester pregnancy decidua were obtained from the elective termination of apparently healthy pregnancy at 8-12 weeks' gestational age. Decidual explant fragments (1-2 mm^3 in size) for each specimen divided into two series: one series (three petri dishes) was cultured in 1×10^{-6} M indomethacin in HAM's F10 medium with 10% fetal calf serum to block PGE2 synthesis and the other series (three petri dishes) was cultured in only HAM's F10 medium. Cultures were incubated for 24, 48 and 72 hours. As a control, explant medium alone was incubated in the same way as the explant cultures themselves. At each time point the supernatants filtered and stored at -70˚C until use. The effect of supernatants from the two different series (with and without indomethacin treatment) on the suppression of PHA-induced lymphoproliferation assay was investigated. In order to check the morphology and viability of the tissue after culture, the tissue cubes of explants after the 24, 48 and 72 hour culture periods were fixed in 10% buffered formalin. After routine processing and embedding in paraffin wax 4 µm sections were cut, stained (according to the method of Haematoxylin and Eosin (H & E) [17]) and finally investigated microscopically.

Peripheral blood lymphocyte isolation technique: Heparinised (20 units/ml) venous blood from healthy male and female volunteers was used to isolate mononuclear peripheral blood cells by density gradient centrifugation. Heparinised blood was diluted with an equal volume of saline, layered over Lymphoprep (Nycomed Ltd., UK.) with a 2:1 ratio of diluted blood to Lymphoprep and centrifuged at 800g for 20 minutes at room temperature. The erythrocytes and polymorphonuclear leukocytes settled to the bottom of the tube, leaving the mononuclear cells at the lymphoprep/plasma interface. The mononuclear cells were then carefully harvested, washed and finally resuspended in complete medium for cell counting.

Cell counting. Cells were counted using an "improved Neubauer" haemocytometer and a light microscope (eye piece x10, objective x20). Cell viability was determined using a dye exclusion method with nigrosin (Sigma).

Suppression of PHA-induced lymphoproliferation assay: The assay was performed in U-bottomed sterile 96-well plates according to the method of lymphocyte transformation [18]. 50 µl decidual supernatants from each specimen were added to wells in triplicate. A 50 µl aliquot of PHA (2 µg/ml) and PBL (2 $x10^6$ cells/ml) were added to each well. The volume was made up to 200 µl with complete medium. Positive controls were set up in at least three wells without any supernatant, and the negative control wells included PBL without PHA or supernatant. The plates were cultured for 72 hours. Four hours prior to the end of incubation, the plates were pulsed with 0.4 Ci per well tritiated thymidine. The cultures were harvested, counted and calculated.

[DOR: 20.1001.1.1028852.1999.3.1.3.3]

The results were expressed as mean counts per minute (CPM) with standard error of the mean (SEM) in triplicate wells. The percentage of suppression was calculated using the following equation:

$$\% \text{ Suppression} = \left(1 - \frac{\text{Mean cpm in the presence of the supernatant}}{\text{Mean cpm in the absence of the supernatant}}\right) \times 100$$

Statistical evaluation: The significance of differences between the mean values was evaluated using Student`s t-test. A difference was considered significant at the conventional level of *P 0.05*.

RESULT AND DISCUSSION

Prostaglandins are known to regulate T cell lymphoproliferative responses by inhibiting the production of IL-2 and by modifying the expression of receptors for transferrin and IL-2 which are necessary to promote the initiation of cytotoxic immune responses [5, 19]. In the present study the effect of indomethacin, which blocks the prostaglandin production, was examined. Only explant culture supernatants were used since it has been proposed that enzymatic dispersion of decidual tissues, followed by washing, removes the suppressive effect of inhibitory proteins, thereby allowing the decidual cells to metabolize arachidonic acid and produce PGE2 *in vitro* [20]. In the present study, the immunosuppressive activity of decidual explant supernatants was significantly reduced in the presence of indomethacin after 24 hours of culture (*P=0.024*) but the results with 48 and 72 hour culture supernatants were not significant (Figure 1). There was not any necrosis and abnormal morphology in all the tissue slides with H & E staining prepared from explant samples even after the 72 hour culture period (Figure 2). Therefore, it can be concluded from this study that PGE2 is present in decidual explant supernatants.

In agreement with the present study several studies in both mouse and human have shown those decidual cells, or their supernatants, abrogated lymphocytes proliferation in the mixed lymphocyte reaction or mitogen-induced proliferation and that this suppressive activity is related to the presence of PGE2. However, the cell type (s) which plays a major role in secretion of this molecule and indeed whether PGE2 is an essential molecule for the survival of the semi-allogenic conceptus without maternal rejection remains a subject of dispute.

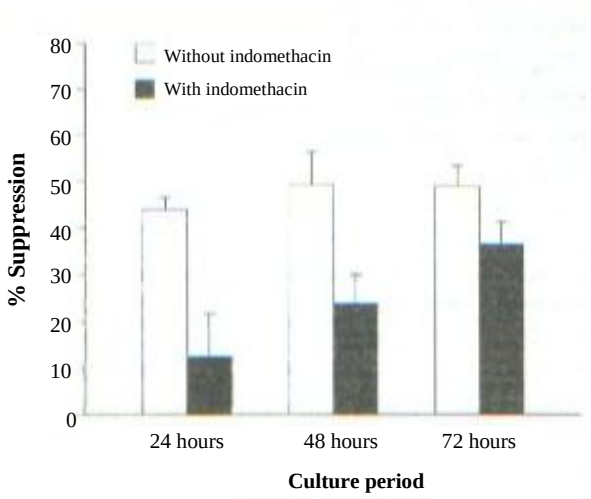

Fig. 1. Immunosuppressive activity of decidual explant supernatants harvested at 24, 48 and 72 hours with and without addition of indomethacin.

Fig. 2. Paraffin embedded section of a 72 hour explant culture of human first trimester decidua stained with H& E. Note there is no necrosis in the explant tissue even after the 72 hour culture period. Magnification ×400.

The immunosuppressive activity of supernatants from short-term cultures of murine decidual tissue was abrogated after dialysis of a low molecule weight (14KDa) molecule [2, 13]. These authors maintained that it was unlikely that prostaglandin was secreted as an *in vitro* artifact of cell behavior in their cultures since decidual suspensions were prepared after extensive washing to remove metabolites released into the medium during the initial 3-5 hour period *in vitro.* Short-term cultures of decidual tissue contained two distinct cell populations: macrophages and typical decidualised stromal cells [21]. Murine decidual macrophages have also been shown to mediate immunosuppressive activity by secretion of

[DOR: 20.1001.1.1028852.1999.3.1.3.3]

soluble mediators, including PGE2 [5]. The important suppressor cells in human decidua are the typical decidual stromal cells identified on the basis of their morphology and distinguished from other cell types by numerous phenotypic markers [22]. Dispersed uterine cell suspensions from pregnant mice containing 28% macrophages suppressed the responses of normal murine spleen cells to PHA; the suppressor cells showed macrophage characteristics [23]. Typical stromal cells in murine decidua have also been reported to release prostaglandin PGE2 *in vitro* [24]. On the other hand, Smith and Kelly [25] reported that the main source of PGE2 in both nonpregnant endometrium and early pregnancy decidua is glandular epithelial cells rather than the stromal cells, and that the level of PGE2 secretion from the glandular epithelium declines after ovulation.

The *in vivo* importance of PGE2 remains to be established with certainty. Decidua-derived prostaglandins could protect the fetus from a variety of potential maternal effector mechanisms by regulating maternal lymphocyte proliferation, CTL generation and NK cell activity at the materno-fetal interface [12]. It has been reported that women with arthritis who were under treatment with prostaglandin inhibitors had a high rate of pregnancy loss [13]. PGE2 production in decidua in early pregnancy is suppressed rather than increased "as would be expected if nature had intended PGE2 to act as a major inhibitor of rejection during early pregnancy" [26]. Furthermore, the effect of enzymatic dispersion of decidual tissue, which leads to artificial secretion of PGE2, was emphasized. These authors also stated that pregnant patients who have been on long term treatment with acetylsalicylic acid or other prostaglandin synthetase inhibitors do not appear to have a high incidence of spontaneous abortions as would be expected if PGE2 was an essential local immunosuppressive agent during early pregnancy.

The present study suggests that production of PGE2 by early pregnancy decidua does indeed exist since it was detected in supernatants of explant cultures rather than supernatants of enzymatically dispersed cells. Macrophages, epithelial cells and stromal cells can act as suppressor cells by secretion of PGE2. Whether this molecule is critical for survival of the feto-placental unit requires more investigations, including comparison with nonpregnant endometrium in different cycle phases and investigation of pathological pregnancy.

REFERENCES

1. Golander, A., Zakuth, V., Shechter, Y. and Spirei, Z. (1981) Suppression of lymphocyte reactivity *in vitro* by a soluble factor secreted by explants of human decidua. *Eur. J. Immunol. 11: 849-851*.
2. Badet, M. T., Bell, S. C. and Billington, W. D. (1983) Partial characterization of immunosuppressive factors short-term cultures of murine decidual tissue. *Ann. Immunol., 1343: 321-329.*
3. Hunt, J. S., Manning, L.S. and Wood, G. W. (1984) Macrophages in murine uterus are immunosuppressive. *Cell. Immunol. 85: 499-510*.
4. Nakayama, E., Asano, S., Kodo and Miwa, S. J. (1985) Suppression of the mixed lymphocyte reaction by cells of human first pregnancy endometrium. *J. Reprod. Immunol. 8: 25-31*.
5. Tawfik, O. W. Hunt, J. S. and Wood, G. W. (1986) Implication of prostaglandin E2 in soluble factor-mediated immune suppression by murine decidual cells. *Am. J. Reprod. Immunol. Microbiol. 12: 111-117*.
6. Daya, S., Rosenthal, K. L. and Clark, D. (1987) A immunosuppressor factor(s) produced by decidua-associated suppressor cells: a proposed mechanism for fetal allograft survival. *Am. J. Obstet. Gynecol. 156: 344-350*.
7. Clark, D. A., Drake, B., Head, J. R., *et al.* (1990) Decidua-associated suppressor activity and viability of the individual implantation sites of allopregnant C3H mice. *J. Reprod. Immunol. 17: 253-64*.
8. Haller, H., Gudelj, L., Rubesa, G. Petrovic, O. and Rukavina, D. (1993) Suppressive effect of decidual leukocytes. *Periodicum Biologorum 95: 327-333.*
9. Chaouat, G., Tranchot-Diallo, J., Volumenie, J. L., Menu, E. (1997) Immune suppression and Th1/Th2 balance in pregnancy revised. *Am. J. Reprod. Immunol. 37(6): 427-34.*
10. Glass,R., and Colbus, M., (1978) Habitual abortion. *Fertil. Steril. 29: 257-265.*
11. Kirkwood, K. J. and Bell, S. C. (1981) Inhibitory activity of supernatants from murine decidual cell cultures on the mixed lymphocyte reaction. *J. Reprod. Immunol. 63: 243-252.*
12. Mathews, C. J. and Searle, R. F. (1988) Antigen presenting capacity of murine decidual tissue *in vivo. J. Reprod. Immunol. 12: 287-295.*
13. Matthiesen, L., Berg, G., Erenerudu, J., and Haskansson, L. (1996) Lymphocyte subsets and mitogen stimulation of blood lymphocytes in normal pregnancy. *Am. J. Reprod. Immunol. 32 (2): 70-79.*
14. Lala, P. K., Scodras, J. M., Graham, C. H., Lysiak, J. J., and Parhar, R. S., (1990) Activation of maternal killer cells in the pregnant uterus with

[DOR: 20.1001.1.1028852.1999.3.1.3.3]

chronic indomethacin therapy, IL-2 therapy or a combination of therapy is associated with embryonic demise. *Cell. Immunol. 127: 368-381*.

15. Wood, G., Kamel, S. and Smith, K. (1988) Immunoregulation and prostaglandin production by mechanically-derived and enzyme-derived murine decidual cells. *J. Reprod. Immunol. 13: 235-248*.
16. Searle, R. F. (1993) Decidua-associated immunoregulatory factors. In: *Immunology of pregnancy*. (Chaouat, G. ed), CRC press, New Jersey, USA, pp. 93-113.
17. Luna, L. G. (1960) *Manual of histologic staining methods of the armed forces institute of pathology*, Third edition, McGraw-Hill book company, New York, USA.
18. Miller, L.E., Ludke, H.R., Peacock, J.E., Tomar, R.H. (1991) *Manual of laboratory immunology*. Second edition, Lea & Febiger Publishers, Philadelphia, USA.
19. Lala, P. K., Kennedy, T. G. and Parhar, R. S. (1988) Suppression of lymphocyte alloreactivity by early gestational human decidua. II. Characterization of the suppressor mechanism. *Cell. Immunol. 116: 411-422*.
20. Korte, K., MacDonald, P. C., Johnston, J. M., Okita, J. R. and Casey, M. L. (1983) Metabolism of arachidonic acid and prostanoids in human endometrial cells in monolayer culture. *Biochim. Biophys. Acta. 752: 423-433*.
21. Searle, R. F., Bell, S. C. and Billington, W. D. (1983) Ia antigen bearing decidual cells and macrophages in cultures of mouse decidual tissue. *Placenta 4: 139-148.*
22. Parhar, R. S., Kennedy, T. G. and Lala, P. K. (1988) Suppression of lymphocyte alloreactivity by early gestational human decidua (I) Characterization of suppressor cells and suppressor molecules. *Cell. Immunol. 116: 392-410*.
23. Hunt, J. S., Manning, L.S. and Wood, G. W. (1984) Macrophages in murine uterus are immunosuppressive. *Cell. Immunol. 85: 499-510.*
24. Parhar, R. S. and Lala, P. K. (1985) Changes in the host natural killer cell population in mice during tumour development. The mechanism of suppression of NK activity. *Cell. Immunol. 93: 265-279*.
25. Smith, S. K. and Kelly, R. W. (1988) The release of PGF2 and PGE2 from separated cells of human endometrium and decidua. *Prostagl. Leukotr. Ess. Fatty Acids 33: 91-96.*
26. Daya, S. and Clark, D. A. (1990) Immunoregulation at the maternofetal interface. The role of suppressor cells in preventing fetal allograft rejection. *Immunol. Allergy Clinics North Am. 10: 49-64.*

[DOR: 20.1001.1.1028852.1999.3.1.3.3]