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IMMUNOSUPPRESSION IN HUMAN UTERINE TISSUES THROUGHOUT THE REPRODUCTIVE CYCLE

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ABSTRACT

The purpose of this study was to investigate and compare whether the explant supernatants prepared from endometrial tissues during the proliferative phase, secretory phase and early pregnancy differ in immunosuppressive activity. Specimens of nonpregnant endometrium from hysterectomies and normal first trimester decidua following elective aspiration termination of pregnancy were obtained. Supernatants from culture of endometrium or decidual fragments were prepared after 24, 48 and 72 hours. The effect of the supernatants on mitogen-induced lymphoproliferation *in vitro* was assessed. The percentage of suppression was calculated and then data were evaluated using Student's t-test. The results confirmed that endometrial immunosuppressive activity exists throughout the reproductive cycle and confirm that early pregnancy decidua is a source of immunoregulatory factors. Immunosuppressive activity was increased in early pregnancy decidua compared with secretory phase endometrium but the results did not reach statistical significance. In contrast, the difference in immunosuppressive activity between proliferative endometrium and early pregnancy decidua was highly significant. In conclusion, the high frequency of early embryonic loss before implantation and the low success rate of *in vitro* fertilization (IVF), embryo transfer and pathological pregnancy in humans can be explained in part by lack of suppressor activity in the endometrium.

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Keywords: Immunosuppression, Pregnancy, Endometrium, Menstrual cycle.

INTRODUCTION

The immunological mechanisms that protect the mammalian feto-placental unit from rejection have been the subject of many investigations. Reports demonstrating that the uterus is a favorable site for implantation have focused attention on the decidualized endometrium as the main locus for immunosuppression. Several studies of murine and first trimester human decidua have shown that supernatants produced by explant and cell suspension cultures suppress lymphocyte responses to mitogens and in the MLR. Golander et al.[12] reported that supernatants from 24 hour cultures of normal early pregnancy decidua suppressed lymphocyte proliferation both in response to lectins and in the MLR.

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Nakayama et al.[23] also demonstrated suppression of the MLR by single cell suspension supernatants prepared from normal human first trimester pregnancy. Wang et al.[32] investigated suppressor activity in supernatants from explant cultures of human endometrium and reported increased immunosuppression of PBL responses to PHA and in an MLR by secretory phase endometrium compared with proliferative phase endometrium. Johnson et al.[17] also observed greater suppressor activity by epithelial cell cultures prepared from secretory phase endometrial glands. However, Bulmer et al.[3] failed to detect any significant differences in the immunosuppressive activity between proliferative and secretory phase endometrial explant cultures. Immunosuppressive activity in early pregnancy decidua has also been reported in further researches,[5,6,13] and it appears that deficiency in this activity leads to abortion.[21] The composition, structure and function of human endometrium differs in the proliferative and secretory phases of the menstrual cycle and in early pregnancy; these changes include alteration in endometrial leucocyte populations, stromal cells, epithelial cells, endothelial cells, hormone-regulated factors, adhesion molecules and cytokine products. Despite this, few studies have attempted to compare immunosuppressive activity between the nonpregnant and pregnant states. In the present study, immunosuppressive activity was examined and compared in explant supernatants during the reproductive cycle, a term which will be used to include proliferative endometrium, secretory phase endometrium and early pregnancy decidua.

MATERIALS AND METHODS

Cell culture media

RPMI 1640 (Sigma) was supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine (Sigma), 1000 U/mL penicillin (Sigma) and 1 mg/mL streptomycin (Sigma), and will be referred to as complete medium.

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).

Incubation

Cultures were maintained in a CO_2 incubator at 37°C in a humidified atmosphere of 5% CO_2 in air.

Phytohemagglutinin (PHA)

PHA (Sigma) as a sterile powder was reconstituted in 1000 µL PBS, aliquoted and stored at -20° C.

Peripheral blood lymphocyte (PBL) isolation technique

Heparinized (20 units/mL) venous blood from healthy male and female volunteers was used to isolate mononuclear peripheral blood cells by density gradient centrifugation. Heparinized 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. The mononuclear cells at the lymphoprep/plasma interface were carefully harvested, washed twice in saline or PBS and finally resuspended in complete medium for cell counting.

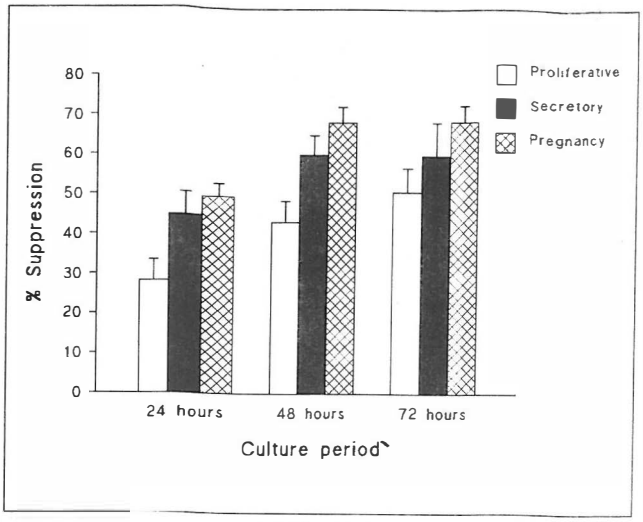

Fig. 1. Immunosuppressive activity of explant supernatants at 50% concentration on PBL proliferation in response to PHA.

Tissues

Endometrium: Specimens of nonpregnant endometrium (18 proliferative; 15 secretory) were obtained from hysterectomies performed for nonendometrial pathology in premenopausal women. Hysterectomy specimens were collected fresh from the operating theaters, Royal Victoria Infirmary, Newcastle upon Tyne. Specimens were opened in the laboratory within one hour of operative removal. Only specimens showing histologically normal endometrium in accordance with the menstrual dates were used.

Decidua: Eighteen specimens of normal first trimester pregnancy tissues were obtained following elective aspiration termination of apparently healthy pregnancy at 8-12 weeks' gestational age. Each tissue was collected into a sterile sealed bag which was opened aseptically in the laboratory.

Tissue culture techniques

Explant cultures: Fragments of endometrium or decidua were cut into cubes 1-2 mm^3 in size. Six cubes of tissue were cultured in 2 mL explant medium in each of three small petri dishes (35×10mm). Cultures were incubated at 37°C for 24, 48 and 72 hours. As a control, explant medium alone was

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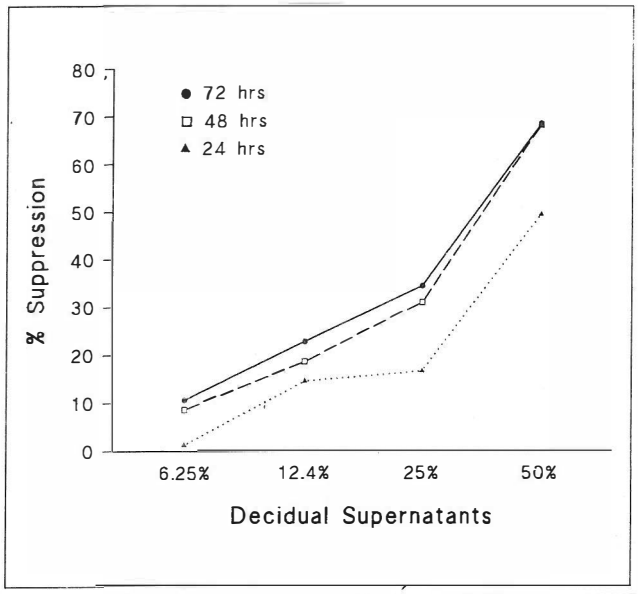

Fig. 2. The dose-related suppression of the PBL response to PHA by increasing concentrations of first trimester decidual explant supernatants.

incubated in the same way as the explant cultures themselves. The supernatants were removed from one of the petri dishes, centrifuged, filtered, aliquoted and stored at -70°C until required.

The morphology and viability of all explant tissues after the 24, 48 and 72 hour culture periods were assessed by H&E staining. Any explant culture which showed necrosis was discarded.

Suppression of mitogen-induced lymphoproliferation: The effect of decidual and endometrial supernatants on mitogen-induced lymphoproliferation *in vitro* was investigated using PHA as the mitogen. The assay was performed in U-bottomed sterile 96-well plates. The endometrial and decidual supernatants were added to wells in triplicate by doubling dilutions in complete medium to give a final supernatant concentration ranging from 50% v/v to 6.25% v/v. A 50 µL aliquot of PHA (2 µg/mL) and PBL (2×10^6 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 µCi per well tritiated thymidine. The cultures were harvested, counted and calculated with a Matrix 96 direct beta counter (Canberra Packard). 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:

$$\%\text{suppression} = \left(1 - \frac{\text{mean count per minute in the presence of the supernatant}}{\text{mean count per minute in the absence of the supernatant}}\right) \times 100$$

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 $p \leq 0.05$.

RESULTS

The results showed that immunosuppressive activity existed in explant supernatants of proliferative and secretory phase endometrium and early pregnancy and increased as the cycle progressed from proliferative phase to secretory phase and then into early pregnancy decidua (Table I). In some dilutions supernatants showed a stimulatory effect which increased the PBL proliferation response to PHA.

Comparison of immunosuppressive activity in explant supernatants from different cycle stages

The immunosuppressive activity of a 50% concentration of supernatants harvested from proliferative endometrium, secretory phase endometrium and first trimester decidua at 24, 48 and 72 hours (Fig. 1) was compared using the Student's t-test. The immunosuppressive activity of explant supernatants was increased significantly in the secretory phase compared with proliferative endometrium for 24 and 48 hour supernatants ($p= 0.041$ and $p= 0.025$, respectively), but was unchanged at 72 hours ($p= 0.29$). The suppressive activity of explant supernatants was increased significantly in the first trimester of pregnancy compared with proliferative endometrium after all three culture periods, particularly at 24 ($p= 0.0018$) and 48 ($p= 0.0008$) hours ($p= 0.022$ at 72 hours). The suppressive activity was increased in the first trimester of pregnancy compared with the secretory phase at all stages, but the results did not reach significance ($p= 0.47$ at 24 hours; $p= 0.32$ at 48 hours; $p= 0.58$ at 72 hours).

Dose response of immunosuppressive activity

The degree of suppression of the PBL response to PHA caused by both explant and cell suspension supernatants was dose related for supernatants harvested after 24, 48, and 72 hours from cultures of proliferative endometrium, secretory phase endometrium and first trimester decidua. Figure 2 shows the relationship between the concentration of explant supernatant and the percentage of suppression for first trimester decidua.

DISCUSSION

The results indicate that endometrial immunosuppressive activity exists throughout the reproductive cycle and confirm that early pregnancy decidua is a source of immunoregulatory factors. The possibility that endometrium has a local immunoregulatory function even in the proliferative phase was unexpected. It appears that at least part of this suppressor activity is independent of the occurrence of pregnancy and

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Table I. The percentage of immunosuppressive activity in explant supernatants harvested at 24, 48 and 72 hours on PBL proliferation in response to PHA during the reproductive cycle

Supernatant		Proliferative phase	Secretory phase	Early pregnancy decidua
24 hours	50%	28.14±5.23	44.92±5.54	49.47±3.23
	25%	8.09±6.90	26.56±5.12	16.68±4.59
	12.5%	4.14±4.47	17.23±4.46	14.50±4.24
	6.25%	- 4.20±5.44	11.02±7.11	1.30±4.32
48 hours	50%	43.13±5.35	60.28±4.81	68.28±4.03
	25%	25.41±6.61	37.32±8.94	31.04±7.3
	12.5%	14.40±4.68	29.60±8.20	18.57±5.13
	6.25%	- 3.57±7.0	22.39±7.77	8.61±4.27
72 hours	50%	50.74±6.08	60:06±8.35	68.57±4.14
	25%	25.56±7.39	35.6±13.0	34.5±6.62
	12.5%	10.16±6.40	26.8±12.9	22.76±6.41
	6.25%	- 11.1±9.4	11.3±10.5	10.66±7.75

may be relevant for local intra-uterine responses to spermatozoa, microbes and viruses, amongst others.

The increased immunosuppressive activity in the secretory phase and early pregnancy decidua may depend on several factors including hormonal changes, the presence of placental trophoblast in a fertile cycle, the altered cellular composition of the endometrium and/or altered cytokine secretion. Apart from two major cell components including stromal cell and epithelial cells, a significant leucocytic component has also been shown within endometrial stroma throughout the reproductive cycle which accounts for 8% of stromal cells in proliferative endometrium, 23% in the late secretory phase and 36% in normal early pregnancy decidua,[1,18] which falls into three major populations: mature T cells, class II MHC-positive macrophages and large granulated lymphoid cells. Dramatic changes occur in the number and comparative proportions of these cells during the different stages of the menstrual cycle and early pregnancy.[2,14,28]

Human mid-secretory phase endometrial lymphocytes were activated after incubation with trophoblast membranes and produced immunosuppressive factors similar to those released by the suppressor cells of normal early pregnancy decidua.[11] This activation appeared to be locally restricted since no activation of immunosuppressive activity was observed in endometrial cells obtained from women with ectopic fallopian tube pregnancy.[10] It is likely that this activation is related to cytokine secretion; several cytokines produced by human endometrium have been shown to vary according to menstrual cycle stage, presumably in response to hormonal changes.[31] TNFα has also been reported to be produced by endometrial stromal cells and secretion may vary according to cycle stage.[15] In the present study lower concentrations of explant supernatants of nonpregnant endometrium often stimulated rather than suppressed the PBL response to PHA. This stimulatory effect of supernatants at lower concentrations may reflect differing concentrations of various cytokines altering the balance between suppressive and stimulatory effects.

It has been reported that PP14 (placenta protein 14) has immunosupppressive properties and may play a part in the fertilization process by inhibiting binding of spermatozoa to the zona.[29] Epithelial cells from endometrium in the late secretory phase produced significantly greater amounts of PP14 compared with that produced by cells from early secretory and proliferative endometrium.[20] PP14 is produced by glandular and surface epithelial cells in early pregnancy decidua and is thought to be immunosuppressive and important for the maintenance of pregnancy.[4,26]

Decidual contact with trophoblast appeared to be necessary for the full activation of trophoblast-dependent suppressor cells since non-adherent intra-uterine decidual cells stimulated by hormones or other substances produced by trophoblasts situated outside the uterus-such as occurring in ectopic tubal pregnancy-showed very low suppressor activity.[25] It has been reported that the trophoblast can produce a variety of cytokines including GM-CSF, CSF-1, G-CSF, IL-1, IL-6, TNFα and TGFα; receptors for several cytokines have also been detected.[16] Therefore, apart from the endometrial hormones, trophoblast is likely to play a role, either directly or indirectly, in the production of local immunosuppressive activity during pregnancy.

Production of prostaglandin as a suppressor factor has been shown in proliferative and secretory phase endometrium.[30] It has been suggested that the production of

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PGE_2 in decidua is a major suppressor factor and may be necessary for fetal survival *in vivo*.[24,27] Matthiesen et al.[21] also reported that PGE_2 mediated suppressor activity during pregnancy. On the other hand, Clark et al.[6,7] reported that the predominant suppressive molecule at the murine feto-maternal interface is an active form of TGFβ which can suppress the cytotoxic activity of the natural effector system including macrophages and NK/LAK type cells. Production of $TGF\beta_2$ by decidual large granular lymphocytes in early human pregnancy has also been reported.[9] Large granulated lymphocytes increase during the menstrual cycle and account for more than 70% of the leucocyte population in early pregnancy decidua,[2,18] hence it is possible that the increase in immunosuppressive activity is related to this population and their releasing factor.

The relationship between immune suppression and Th1/Th2 populations and their cytokines in early pregnancy decidua has been investigated by Chaouat et al.[5] who suggested that natural killer cells could be in fact regulatory cells pushing the maternal immune response towards Th2 cytokines, beneficial fetal survival, or towards Th1 cytokines which act in synergy.

In conclusion, local immunosuppressive activity may be necessary for the endometrium to provide a suitable environment after ovulation to accept the implanting blastocyst. The high frequency of early embryonic loss before implantation and the low success rate of *in vitro* fertilization (IVF), embryo transfer and pathological pregnancy in the human could be explained in part by lack of suppressor activity in the endometrium. Various cell populations and several factors mediate immunosuppressive activity during the reproductive cycle but the critical cell type and molecule responsible is not yet clearly understood. Study of pathological pregnancy, such as spontaneous abortion and failed IVF, may be worthwhile in humans in order to enhance the understanding of the mechanisms controlling implantation and early placental development.

REFERENCES

1. Bulmer JN, Morrison L, Longfellow M, Ritson A: Leukocytes in human decidua: investigation of surface markers and function. Colloque INSERM 212: 189-91,1991.
2. Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D: Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. Hum Reprod 6: 791-798, 1991.
3. Bulmer JN, Longfellow M, Ritson A: Leucocytes and resident blood cells in endometrium. Ann NY Acad Sci 622: 57-68, 1991.
4. Chard T, Olajide F: Endometrial protein PP14: a new test of endometrial function. Reprod Med Revi 3: 43-52, 1994.
5. Chaouat G, Tranchot-Diallo J, Volumenie JL, Menu E, Gras G, Delage G: Immune suppression and Th1/Th2 balance in pregnancy revised. Am J Reprod Immunol 37(6): 427-34, 1997.
6. Clark DA, Drake B, Head JR, Stedronska-Clark J, Banwatt D: Decidua-associated suppressor activity and viability of the individual implantation sites of allopregnant C3H mice. J Reprod Immunol 17: 253-64, 1990.
7. Clark DA, Lea RG, Poder T, Daya S, Banwatt D, Harley C: Cytokines determining the success or failure of pregnancy. Ann NY Acad Sci 626: 524-536, 1991.
8. Clark DA, Merali FS, Hoskin DW, Steel-Norwood D, Arck PC, Croitoru K, Murgita RA: Decidua associated suppressor cells in abortion-Pron DBA/mated CBA/2mated CBA/J mice that release bioactive transforming growth factor-beta2-related immunosuppressive molecules which express a bone marrow-derived natural suppressor cell marker and gamma delta T-cell receptor. Biol Reprod 56(5): 1351-60, 1997.
9. Clark DA, Vince G, Flanders KC, Hirte H, Starkey P: CD56 lymphoid cells in human first trimester pregnancy decidua as a source of novel transforming growth factor-β2-related immunosuppressive factors. Hum Reprod 9: 2270-2277, 1994.
10. Daya S, Clark DA, Devlin C, Jarrell J: Preliminary characterization of two types of suppressor cells in the human uterus. Fertil Steril 44: 778-785, 1985.
11. Daya S, Johnson PM, Clark DA: Trophoblast induction of suppressor type cell activity in human endometrial tissue. Am J Reprod Immunol Microbiol 19: 65-72, 1989.
12. Golander A, Zakuth V, Shechter Y, Spirei Z: Suppression of lymphocyte reactivity *in vitro* by a soluble factor secreted by explants of human decidua. Eur J Immunol 11: 849-851, 1981.
13. Haller H, Gudelj L, Rubesa G, Petrovic O, Rukavina D: Suppressive effect of decidual leucocytes. Periodicum Biologorum 95: 327-333, 1993.
14. Haller H, Radillo O, Rukavina D, Tedesco F, Candussi G, Petrovic O, Randic L: An immunohistochemical study of leukocytes in human endometrium, first and third trimester basal decidua. J Reprod Immunol 23: 41-49, 1993.
15. Hunt JS, Chen HL, Hu XL, Tabibzadeh S: Tumour necrosis factor-α messenger ribonucleic acid and protein in human endometrium. Biol Reprod 47: 141-147, 1992.
16. Johnson PM: Immunobiology of the human placental trophoblast. Exp Clin Immunogenet 10: 118-122, 1993.
17. Johnson P M, Risk J M, Bulmer J N, Niewola Z, Kimber I: Antigen expression at human materno-fetal interfaces, In: Gill TJ III, Wegmann TG, (eds.), Maternal Immunoregulation and Fetal Survival. Chapter 13, New York: Oxford University Press, p. 181-196, 1987.
18. King A, Loke YW: On the nature and function of human uterine granular lymphocytes. Immunol Today 12: 432-435, 1991.
19. Klentzeris LD, Bulmer JN, Warren A, Morrison L, Li TC, Cooke ID: Endometrial lymphoid tissue in the timed

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endometrial biopsy: morphometric and immunohistochemical aspects. Am J Obstet Gynecol 167: 667-674, 1992.

20. Laird SM, Li TC, Bolton AE: The production of placental protein 14 and interleukin 6 by human endometrial cells in culture. Hum Reprod 8: 793-798, 1993.
21. Lea RG, Underwood J, Flanders KC, Hirte H, Banwatt D, Finotto S, Ohno I, Daya S, Harley C, Michel M, Mowbray JF, Clark DA: A subset of patients with recurrent spontaneous abortion is deficient in transforming growth factor β2-production by "suppressor cells" in uterine tissue near the placental attachment site. Am J Reprod Immunol 34: 52-64, 1995.
22. Matthiesen L, Berg G, Erenerudu J, Haskansson L: Lymphocyte subsets and mitogen stimulation of blood lymphocytes in normal pregnancy. Am J Reprod Immunol 32(2): 70-9, 1996.
23. Nakayama E, Asano S, Miwa SJ: Suppression of the mixed lymphocyte reaction by cells of human first pregnancy endometrium. J Reprod Immunol 8: 25-31, 1985.
24. Parhar RS, Kennedy TG, Lala PK: Suppression of lymphocyte alloreactivity by early gestational human decidua. (I). Characterization of suppressor cells and suppressor molecules. Cell Immunol 116: 392-410, 1988.
25. Petrovic O, Gudelj L, Rubesa G, Haller H, Beer AE, Rukavina D: Decidual-trophoblast interaction: decidual lymphoid cell function in normal, anembryonic, missed abortion and ectopic human pregnancy. J Reprod Immunol 26: 217-231, 1994.
26. Pockley AG: Suppression of *in vitro* lymphocyte reactivity to phytohemagglutinin by placenta protein 14. J Reprod Immunol 13: 31-39, 1988.
27. Searle RF: Decidua-associated immunoregulatory factors, In: Chaouat G, (ed), Immunology of Pregnancy, Chapter 7, New Jersey: CRC Press, pp. 93-113, 1993.
28. Starkey P M, Clover L M, Rees M C P: Variation during the menstrual cycle of immune cell populations in human endometrium. Eur J Obstet Gynecol Reprod Biol 39: 203-207, 1991.
29. Sepala M, Koistinen R, Rutanen E M: Uterine endocrinology and paracrinology: insulin-like growth factor binding protein-1 and placental protein 14. Human Reprod 9(5): 917-925, 1994.
30. Smith S K, Kelly R W: The release of PGF2α and PGE2 from separated cells of human endometrium and decidua. Prostagl Leukotr Ess Fatty Acids 33: 91-96, 1988.
31. Tabibzadeh S: Cytokine and the hypothalamic-pituitary-ovarian-endometrial axis. Human Reprod 9(5): 947-967, 1994.
32. Wang H S, Kanzaki H, Yoshida M, Sato S, Tokushinge M, Mori T: Suppression of lymphocyte reactivity *in vitro* by supernatants of explants of human endometrium. Am J Obstet Gynecol 157: 956-963, 1987.
33. Wegmann T G, Lin H, Guilbert L, Mossman T R: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a T_H2 phenomenon? Immunol Today 14: 353-356, 1993.

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