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# Immunophenotype of the Decidual Lymphocytes in Early Pregnancy

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The antigenic phenotype of the lymphocytes in early human pregnancy deciduas was investigated. Decidual tissue was obtained from 13 healthy women undergoing therapeutic abortion of normally progressing pregnancy at 4–11 weeks' gestation. Decidual lymphocytes were examined for the expression of various cell surface markers by flow cytometry, using different monoclonal antibodies directly conjugated with FITC or phycoerythrin (PE): CD3FITC/CD4PE, CD3FITC/CD8PE, CD3FITC/CD19PE, CD3FITC/CD56PE. We have demonstrated that immunocompetent cells, predominantly lymphocytes, infiltrate the decidual tissue in the early pregnancy. The main subpopulation of lymphocytes in the decidua was CD3-CD56<sup>+</sup> NK cells. We found a similar count of the CD4<sup>+</sup> Th and CD8<sup>+</sup> Tc/s cells in the decidual tissue. During pregnancy the CD4 : CD8 ratio decreased. A study of decidual B lymphocytes showed that they usually represented only a small fraction of decidual cells. We have shown that inflammatory cells, neutrophils and macrophages, also infiltrate the decidual tissue. Uterine hematopoietic cells are equipped to perform certain immunological and non-immunological functions within their microenvironments that can have a major influence on the course of pregnancy.

**Key words:** decidual lymphocyte, immunophenotype, early pregnancy

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## INTRODUCTION

Although it has been over 50 years since Medawar pointed out that the self-nonsel model of the immune system predicted that mothers should reject their fetuses, it is not yet clear how the semiallogeneic fetus is protected from attack by its mother's immune system.

Two central functions of the immune system in the female reproductive tract are the perpetuation of the species and protection against potential pathogens. Thus, the mucosal immune system has evolved to be responsive to exposure to bacterial and viral pathogens and to the constraints of pregnancy.

Human endometrium contains several leukocyte populations that vary with menstrual cycle phase. Leukocytes account for approximately 5% of the total stromal cell population in proliferative endometrium, but increase in number to comprise approximately 25% of

stromal cells in the late secretory phase; this altered leukocyte profile is due to the presence of phenotypically unusual CD56<sup>+</sup>CD16<sup>-</sup>CD3<sup>-</sup> large granular lymphocytes, termed endometrial granulated lymphocytes (eGLs) [1].

Recently it has been reported that eGLs in premenstrual endometrium show a high bcl-2 and Ki67 expression with no evidence of apoptosis, suggesting active proliferation *in situ* [2].

Endometrial GLs may be important for defence against genital tract infection at ovulation, when sperm and pathogenic organisms are most likely to enter the reproductive tract. Although the genital tract is generally held to be protected from infection by the "hostile" vaginal and cervical environment, high levels of estrogen at ovulation mediate changes that facilitate sperm passage and may leave the reproductive tract vulnerable to infection. The increased proliferative capacity of eGLs in the secretory phase of the menstrual cycle therefore would be highly advantageous, especially as endometrial CD8<sup>+</sup> T lymphocyte cytotoxicity is downregulated during this period [3].

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The pregnant uterine mucosa, the decidua, is a transient tissue in which controlled invasion of fetal extravillous trophoblast cells occurs and where, therefore, maternal immunocompetent cells closely contact with cells of fetal origin.

The decidua is rich in lymphoid cells. Immunomorphometry of human decidua from early pregnancy showed that CD45<sup>+</sup> cells comprise as much as 10 to 15% of all decidual cells. They are scattered as single cells in the stroma or are located sub- or intraepithelially at the endometrial glands [4]. The immune cells in the decidua do not form lymphoid follicles, but clusters of closely packed activated lymphoid cells, lymphoid cell clusters, most frequently located adjacent to endometrial glands and blood vessels.

To clarify the immunologic role of endometrial leukocytes in early pregnancy decidua, we have undertaken an analysis of the immunophenotypic characteristics of immunocompetent cells in the decidual tissue.

## MATERIALS AND METHODS

**Decidual tissue and cells.** Vacuum-extracted decidua specimens were donated by healthy women undergoing elective termination of pregnancy at 4 to 11 wk of gestation (n = 13) at the Department of Gynaecology, Clinic of General Surgery, Vilnius University Emergency Hospital. The method of extraction has been described elsewhere (5). Briefly, fragments of decidual tissue were identified macroscopically, detached from fragments of trophoblast and fetal membranes and washed several times in cold PBS in order to remove blood coagulas.

Fragments of decidual tissue were cut into small pieces. Cell suspension was filtered through a 40 µm pore size nylon mesh (Becton Dickinson, Mountain View, CA) in order to generate a leukocyte-en-

riched fraction and spun at 400 g for 5 min. The pellet was resuspended in PBS, layered onto Histopaque-1077 (Sigma, St. Louis, USA), and centrifuged at 400 g for 20 min. (Fig. 1). The band of cells at the interface was collected and washed. Viability was assessed using 0.4% trypan blue solution and was always above 94%.

The differential cell count was performed as May-Grünwald-Giemsa stained smears of decidual cell samples.

**Immunophenotyping of the decidual lymphocytes by two-colour-flow cytometry.** Decidual lymphocytes were examined for the expression of various cell surface markers by flow cytometry, using different monoclonal antibodies (MoAbs) directly conjugated with FITC or phycoerythrin (PE): CD3FITC/CD4PE, CD3FITC/CD8PE, CD3FITC/CD19PE, CD3FITC/CD56PE. Briefly, 50 µl of washed decidual cells (about  $5 \times 10^5$  cells) were incubated with the appropriate MoAbs for 15 min at room temperature in the dark. To lyse erythrocytes, the stained lymphoid cells were suspended in 2 ml of FACS lysing solution at room temperature and in the dark. These samples were then centrifuged, washed in PBS containing 0.1% sodium azide, fixed with 0.5% paraformaldehyde and stored at 4 °C till the analysis. Within 20 h after staining the samples were analysed by two-colour flow cytometry using a FACSCalibur. Irrelevant mouse IgG antibodies of the same isotype and concentration were used as controls. Ten thousand events were measured. Lymphocytes and mononuclear phagocytes were electronically gated on the basis of their forward scatter *versus* 90° scatter criteria. Analysis was performed using CellQuest software.

**Statistical analysis.** Mean  $\pm$  standard deviations are indicated. Correlation was examined by square linear regression analysis.

## RESULTS

### Leukocyte population

As shown in Fig. 2, the major leukocyte population detected in pregnant endometrium consisted of lymphocytes ( $79.82 \pm 8.16\%$ ). We also found macrophages ( $3.91 \pm 2.66\%$ ), neutrophils ( $15.36 \pm 9.31\%$ ), mast cells ( $0.79 \pm 0.73\%$ ) and eosinophils ( $0.4 \pm 0.18\%$ ).

### Lymphocyte subpopulation

Using two-colour flow cytometric analysis, we verified that the majority of CD56<sup>+</sup> cells were also CD3<sup>-</sup>, and could thus be clearly identified as NK cells ( $76.71 \pm$

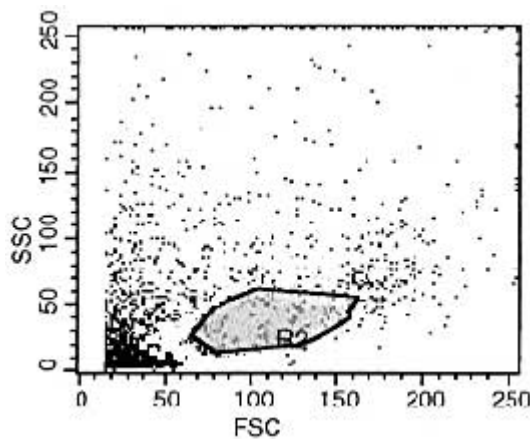


Fig. 1. Mononuclear cells isolated from decidual tissue samples by Histopaque-1077 density centrifugation

± 5.85%) (Figs. 3, 4). In the pregnant uterine endometrium we found also CD3<sup>-</sup>CD8<sup>+</sup> NK cell populations (5.42 ± 3.19%).

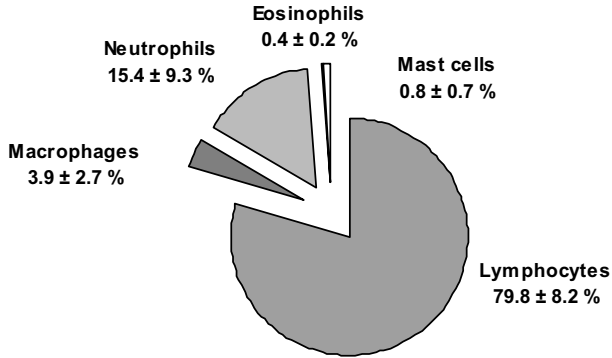


Fig. 2. Leukocyte populations in the decidua in the early pregnancy

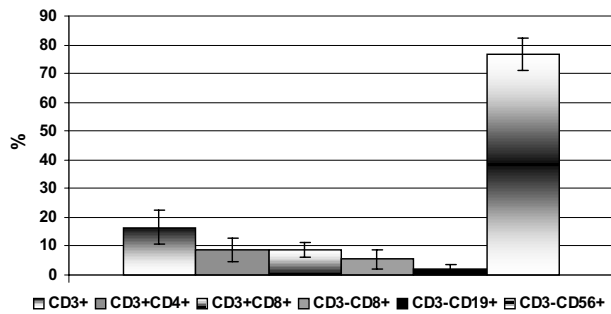


Fig. 3. Lymphocyte subpopulations in the decidua in the early pregnancy

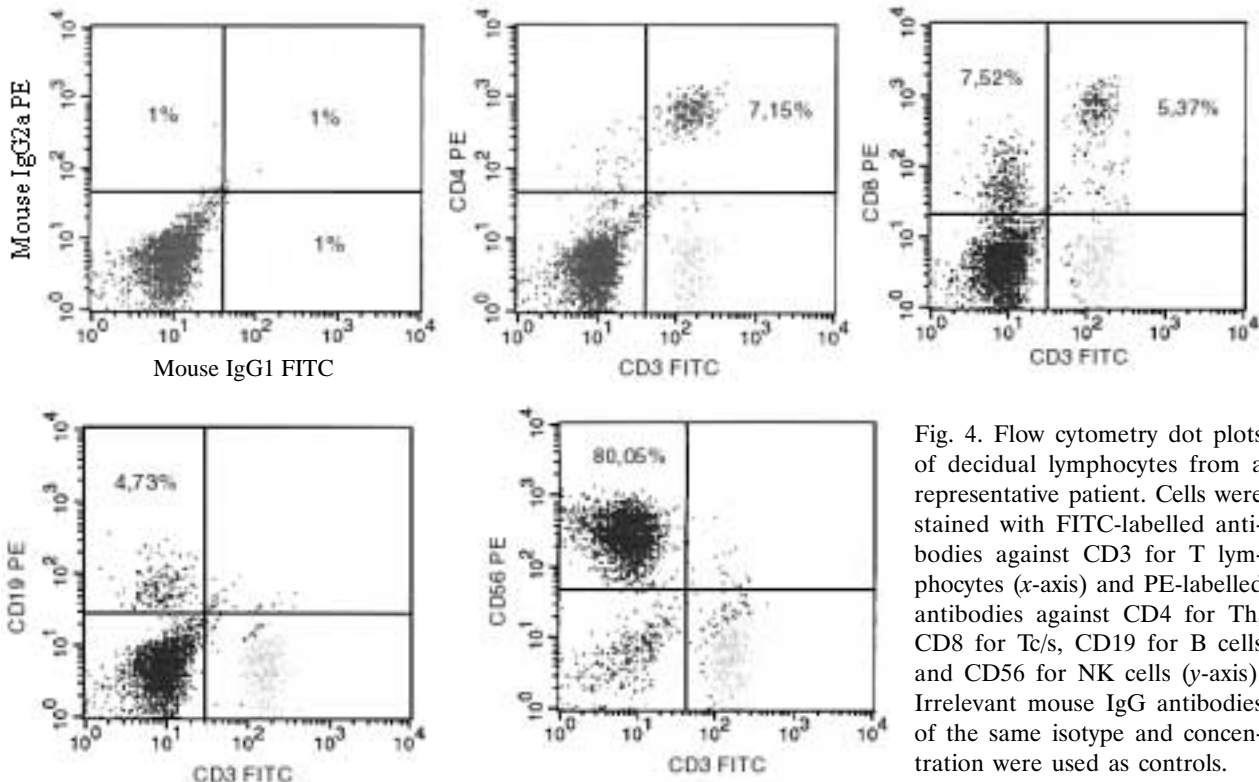


Fig. 4. Flow cytometry dot plots of decidual lymphocytes from a representative patient. Cells were stained with FITC-labelled antibodies against CD3 for T lymphocytes (x-axis) and PE-labelled antibodies against CD4 for Th, CD8 for Tc/s, CD19 for B cells and CD56 for NK cells (y-axis). Irrelevant mouse IgG antibodies of the same isotype and concentration were used as controls.

Most T cells were of the usual CD3<sup>+</sup>CD56<sup>-</sup> phenotype: 16.44 ± 5.8%. CD19 was expressed by only 2.11 ± 1.66% of decidual lymphocytes. CD4<sup>+</sup>CD3<sup>+</sup> cells represented 8.67 ± 4.09% of CD45<sup>+</sup> and CD8<sup>+</sup>CD3<sup>+</sup> 8.64 ± 2.36% cells in pregnant endometrium. The ratio of CD4 : CD8 cells was 0.97 ± 0.32. We found no correlation between the CD4<sup>+</sup> cells, CD8<sup>+</sup> cells and the week of gestations (p > 0.1), but we found a negative correlation between the CD4 : CD8 ratio and the week of gestation (r = -0.59, p < 0.04).

**DISCUSSION**

One of the greatest mysteries yet to be solved by immunologists is the mechanism by which the fetal allograft is able to survive the immunologic defenses of the mother.

In the present study we investigated the antigenic phenotype of the lymphocytes in early human pregnancy decidua. We have demonstrated that immunocompetent cells, predominantly lymphocytes, infiltrate decidua in the early pregnancy. The main subpopulation of lymphocytes in the decidua was CD3<sup>-</sup>CD56<sup>+</sup> NK cells. They have the characteristic morphology of large granulated lymphocytes (LGL).

The contribution of NK cells to fetal engraftment has been a controversial issue. Indeed, several studies suggest that NK cells are a prerequisite for

maintaining pregnancy [1, 6], while others suggest that NK cells display deleterious effects on fetal development, resulting in spontaneous abortion in mice [7] or humans [8]. Their association with two HLA class I molecules, HLA-G and HLA-E, on the surface of extravillous trophoblast is well documented [9–11]. Indeed, these cells seem to accompany the invading trophoblast [11]. The syncytiotrophoblast is devoid of all HLA antigens, but there is no evidence of NK attack *in vivo*. The cytotrophoblast expresses HLA-G which appears to have a dual role, protecting the trophoblast from both NK and cytotoxic T cell activity.

Early findings that have received very little follow up demonstrate that peptides of HLA-G bind as adhesion molecules to CD8<sup>+</sup> LGLs [9]. In this way the trophoblast may select an army of suppressor cells that accompany it as it interfaces and interacts with the maternal host. In addition, CD3<sup>+</sup>CD8<sup>+</sup>CD56<sup>+</sup> granular lymphocyte clones are functionally a more inert subset of CD3<sup>+</sup>CD56<sup>+</sup> cells in respect of cytokine production.

We found the similar count of the CD4<sup>+</sup> Th and CD8<sup>+</sup> Tc/s cells in the decidual tissue. During pregnancy the CD4 : CD8 ratio decreased. To establish fetal tolerance, the endometrium maintains the proportion of CD8<sup>+</sup> T lymphocyte and CD4 : CD8 ratios. When this proportion is disrupted, a pregnancy suffers from autoimmune activation, this results in repetitive pregnancy losses [5, 8].

A study of decidual B lymphocytes has shown that they usually represent only a small fraction of decidual cells.

We have shown that inflammatory cells, neutrophils and macrophages, also infiltrate the decidual tissue. Activated neutrophils and macrophages are a rich source of inflammatory mediators. During pregnancy, elevated levels of cytokines secreted by uteroplacental macrophages, activated by either bacterial endotoxins or receptor-bound cytokines, may compromise the pregnancy [12].

We found a small count of mast cells and eosinophils in the deciduas. The function of mast cells in the pregnant uterus remains unclear, although it has been proposed that myometrial mast cells regulate uterine contractility during labour [13]. Further, these cells are considered to play a pivotal role in wound healing, fibrosis and tissue remodeling and might be involved in promoting collagen degradation and uterine involution in the postnatal period.

Activated uterine hematopoietic cells are equipped to perform certain immunological and non-immunological functions within their microenvironments that can have major influences on the course of pregnancy.

## CONCLUSIONS

- Lymphocytes are the main populations of leukocytes found in the decidua.
- The maternal lymphocytes present in the decidua during the early weeks of pregnancy are composed of CD3<sup>+</sup>CD56<sup>+</sup> NK cells, CD3<sup>+</sup> T cells and small population CD19<sup>+</sup> B cells.
- We found a negative correlation between the CD4 : CD8 ratio and the week of gestation.

## ACKNOWLEDGEMENT

We want to express our gratitude to the gynaecologists from the Department of Gynaecology of the General Surgery Clinic of the Vilnius University Emergency Hospital for the availability of the material for our investigations.

Received 17 September 2001

Accepted 18 October 2001

## References

1. Searle RF, Jones RK, Bulmer JN. Phenotypic analysis and proliferative responses of human endometrial granulated lymphocytes during the menstrual cycle. *Biol Reprod* 1999; 60: 871–8.
2. Jones RK, Searle RF, Stewart JA, Turner S, Bulmer JN. Apoptosis, bcl-2 expression and proliferation activity in human endometrial stroma and endometrial granulated lymphocytes. *Biol Reprod* 1998; 58: 995–1002.
3. White HD, Crassi KM, Givan AL et al. CD3<sup>+</sup>CD8<sup>+</sup>+CTL activity within the human female reproductive tract. *J Immunol* 1997; 158: 3017–27.
4. Kletzeris LD, Bulmer JN, Warren A, Morrison L, Li TC, Cooke ID. Endometrial lymphoid tissue in the timed endometrial biopsy: morphometric and immunohistochemical aspects. *Am J Obstet Gynecol* 1992; 167: 67–74.
5. Rukavina D, Rubesa G, Gudelj L, Haller H, Podack ER. Characteristics of perforin expressing lymphocytes within the first trimester decidua of human pregnancy. *J Reprod Immunol* 1996; 31: 394–404.
6. Vince GS, Johnson PM. Reproductive immunology – conception, contraception and the consequences. *Immunologist* 1996; 4/5: 172–8.
7. Clark DA. Maternal aggression against placenta? *Am J Reprod Immunol* 1994; 31: 205–11.
8. Raghupathy R. Maternal anti-placental cell-mediated reactivity and spontaneous abortions. *Am J Reprod Immunol* 1997; 37: 478–84.
9. Carosella E, Rouas-Freiss N, Paul P, Dausset J. HLA-G: a tolerance molecule from the major histocompatibility complex. *Immunol Today* 1999; 20: 60–2.
10. Zingoni A, Palmieri G et al. CD69-triggered ERK activation and functions are negatively regulated by CD94/NK62-A inhibitory receptor. *Eur J Immunol* 2000; 30: 644–51.

11. Hammer A, Hutter H, Dohr G. HLA class I expression on the materno-fetal interface. *Am J Reprod Immunol* 1997; 38: 150–7.
12. Sacks G, Sargent I, Redman Ch. An innate view of human pregnancy. *Immunol Today* 1999; 20: 114–8.
13. Thomson AJ, Telfer JF, Young A et al. Leukocytes infiltrate the myometrium during human parturition: further evidence that labour is an inflammatory process. *Hum Reprod* 1999; 14: 229–36.

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#### **DECIDUALINIŲ LIMFOCITŲ IMUNOFENOTIPAS ANKSTYVAJAME NĖŠTUME**

##### **S a n t r a u k a**

Vaisius, paveldėdamas dalį genetinės informacijos iš motinos ir tėvo, visada yra pusiau svetimas savo motinos imuninei sistemai. Todėl viena didžiausių mįslių, kurią turi įminti imunologai, kaip vaisius sugeba apsiginti nuo

motinos imuninės sistemos apsauginių reakcijų. Šio darbo tikslas – nustatyti ankstyvojo nėštumo decidualinio audinio leukocitų populiacijas ir identifikuoti limfocitų antigeninį fenotipą. Decidualinis audinys gautas nutraukus normalų nėštumą 13-ai sveikų moterų 4–11 nėštumo savaitę. Decidualinių limfocitų paviršinių antigenų ekspresijai tirti tėkmės citometrijos metodu buvo naudojami įvairūs FITC ar fikoeritrinu (PE) žymėti monokloniniai antikūnai: CD3FITC/CD4PE, CD3FITC/CD8PE, CD3FITC/CD19PE, CD3FITC/CD56PE. Decidualinį audinį ankstyvajame nėštume daugiausia infiltravo limfocitai. CD3-CD56<sup>+</sup> NK ląstelėse vyravo limfocitų populiacija. Panašus kiekis CD4<sup>+</sup> Th ir CD8<sup>+</sup> Tc/s ląstelių buvo rastas decidualiniame audinyje. Nėštumo metu CD4 : CD8 santykis mažėjo. B limfocitai sudarė tik nedidelę decidualinių ląstelių frakciją. Buvo rasta ir uždegiminių ląstelių, neutrofilų ir makrofagų. Imunologinėse ir neimunologinėse funkcijose dalyvaujanti gimdos hematopoetinės ląstelės yra svarbios sėkmingai nėštumo raidai.

**Raktažodžiai:** decidualiniai limfocitai, imunofenotipas, ankstyvinis nėštumas