

Endometrial Granulated Lymphocytes in Women Suffering Spontaneous Early Pregnancy Loss

Mehri Ghafourian Boroujerdnia^{*1} and Rahim Chinipardaz²

¹Dept. of Immunology, Ahwaz Medical Science University, Ahwaz, Iran, ²Dept. of Statistics, Shahid Chamran University, Ahwaz, Iran

ABSTRACT

Spontaneous abortion is the most common complication of pregnancy. Several lines of evidence indicate that immunologic effector cells may play a role in the pathogenesis of idiopathic repetitive abortions. Leukocytes form a substantial proportion of stromal cells in decidua and the endometrial granulated lymphocytes (eGL) is the predominant decidual leukocyte population in the first trimester of normal human pregnancy. To investigate the involvement of eGL population in repetitive abortion of unknown etiology, a comparative analysis was performed on first-trimester decidual tissues obtained from thirty patients with recurrent spontaneous abortion and thirty samples at therapeutic abortion. The eGL in paraffin-embedded sections of all samples were demonstrated with phloxine-tartrazine staining. The results showed the presence of many eGL that scattered individually throughout the stroma and formed some aggregates around glands and some vessels. The number of positive cells was increased in the recurrent aborted decidua compared with normal pregnancy decidua, but the difference was not significant ($p>0.05$). *Iran. Biomed. J. 6 (2 & 3): 89-92, 2002*

Keywords: Spontaneous abortion, Endometrial granulated lymphocytes, First-trimester decidua, Phloxine-tartrazine stain

INTRODUCTION

A clinically detectable spontaneous abortion occurs in 10% to 20% of all pregnancies [1]. The etiology is uncertain and is likely to be diverse [2], but for a large proportion of cases the cause is unknown.

Immunological factors have been proposed as a cause of unexplained miscarriage; both fetus and trophoblast express paternally inherited gene products and tissue-specific differentiation antigens which could serve as immunological targets [3]. Studies in murine models have demonstrated that decidual cytotoxic effector cells like NK cells, macrophages, and T cells could be implicated in fetal resorption [4]. The soluble products of the activated immune cells could play a role in pregnancy loss. A deficiency of local immunosuppression may result in spontaneous abortion [3]. A lack of decidual suppressor cells has been detected in mice with spontaneous abortion [5].

Different subpopulations of leukocytes were detected among human decidual tissues that mediate different immunological functions. Approximately, 35% to 50% of the women with unexplained recurrent pregnancy loss, immune and inflammatory

cell responsiveness to trophoblast is activated as evidenced by the increased proliferation and secretion of embryotoxic factors such as IFN- γ [6]. Leukocytes form a substantial component of human decidua. An unusual population of granulated lymphocytes, the endometrial granulated lymphocytes (eGL), form the predominant stromal leukocyte population in normal first trimester decidua. The eGL increase in number dramatically in the late secretory phase of menstrual cycle, with further increase in early pregnancy [7].

The granules that contain the cytolytic molecules perforin, TIA-1 and granzyme-A [8], can be detected in formalin-fixed paraffin-embedded sections by using specific stains such as phloxine tartrazine, alician blue, Geimsa and toluidine blue [7]. The eGL have unusual natural killer cell-like phenotype. These cells are strongly positive for CD56, a marker of MHC-non-restricted cytotoxic or NK cells. However, most of these cells lack other "classical" NK cell antigens such as CD16 and CD57 [9]. eGL numbers increase dramatically in the late secretory phase and the first trimester of pregnancy. However, eGL numbers have a rapid decline subsequently at term. Similar, intrauterine granulated cells are present in other species,

^{*}Correspondence Author; Tel: (98-611) 336 0245, Mobile: 0911 618 4882

granulated metrial gland cells [10]. Therefore, it seems that these cells have a role in implantation and placentation.

In order to investigate the involvement of eGL population in repetitive abortion of unknown etiology, a comparative analysis was performed in the first-trimester decidual tissues of the patients with recurrent spontaneous abortion. Also, to compare these findings with those of gestational age-matched decidua tissues from women undergoing elective pregnancy termination.

MATERIALS AND METHODS

Tissues. Thirty samples of uteroplacental tissues of presumably normal pregnancies at 8-12 week (control cases) were obtained from first-trimester elective pregnancy termination performed by vaginal curettage at women surgical ward in Ahwaz Imam Khomeini Hospital. Thirty samples of uteroplacental tissues (pathological cases) were also obtained randomly from women having recurrent spontaneous abortion of unknown etiology during three years. Decidual tissues were identified macroscopically by its opaque, grey-white solid appearance and were separated from the fetal and placental tissues. All samples were fixed in 10% buffered formalin and then embedded in paraffin wax and sectioned at 3 μ m by microtome.

Haematoxylin and eosin (H & E) staining. H&E staining was performed on all of the specimens in order to assess the morphology and viability of decidual. Paraffin sections (3 μ m) from each sample were dewaxed in xylene for 10 minutes and rehydrated through a series of descending concentrations of alcohol to water. The sections were then stained with haematoxylin and eosin and washed in tap water, dehydrated through a series of ascending concentrations of alcohol, cleared in xylene, mounted and finally investigated microscopically. The cases were carefully chosen to exclude specimens with decidual inflammation or necrosis that alter leukocyte populations.

Phloxine tartrazine staining. This procedure was performed in order to identify the eGL in paraffin embedded sections of decidual tissues because there was no commercially available anti-CD56 monoclonal antibody that performed reliably on formalin-fixed, paraffin-embedded tissues. The sections were deparaffinized in xylene for 10 minutes and rehydrated through graded alcohol to

water. The slides were then incubated with haematoxylin. After a brief wash in tap water, the slides were stained in phloxine solution (0.5% w/v phloxine in 0.5% w/v calcium chloride) for 25-30 minutes. After washing in water and then in cellosolve (2-ethoxyethanol), the slides were treated with a saturated solution of tartrazine in cellosolve for 10 minutes. The degree of differentiation was monitored microscopically. After dehydration through a series of ascending alcohols and clearing in xylene, the slides were mounted.

Quantification and analysis of results. The eGL positive cells were counted in all control and pathological cases in eight high-power fields at $\times 400$ magnification ($\times 10$ eyepiece, $\times 40$ objective). The fields were chosen randomly and no attempt was made to select fields rich or poor of positive cells. The results were analyzed by calculating the mean and the standard error mean (SEM) for each cell population. Z-test was used to compare the decidual eGL and determine any significant differences in their numbers between normal human pregnancy and spontaneous early pregnancy loss. The conventional level of $p < 0.05$ was taken as the limit of significance.

RESULTS AND DISCUSSION

The results of phloxine tartrazine staining showed many eGL in early pregnancy normal decidua. These cells were scattered individually throughout the stroma and formed some aggregates within stroma. The eGL was also aggregated around vessels and adjacent to some glands. Adhesion molecules may play a role in the distribution of eGL in early pregnancy decidua [11]. The number of eGL-positive cells in pathological decidua cases was increased (146.00 ± 5.40) in comparison with normal decidua cases (139.3 ± 4.9), however the difference was not significant ($p = 0.36$, Fig. 1). The same results were obtained by other histological studies [12]. But, by flow cytometry method the difference between two groups was significant [13]. This discrepancy between the results may be related to the methods that used and required further investigations. Apart from the number of eGL in decidua, several proposals have been made for the role of the eGL during pregnancy.

Immunosuppressive activity has been demonstrated in the first trimester of human decidua and it is

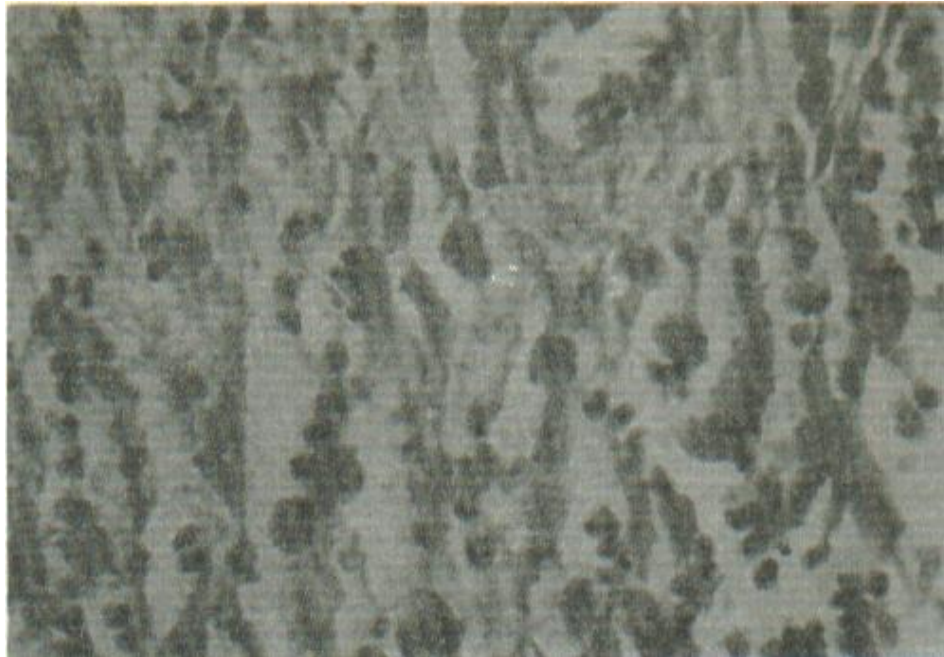


Fig. 1. Section of human decidual tissue in spontaneous early pregnancy loss labelled with phloxine tartrazine technique. Granules are seen in eGL-positive cells in the decidual stroma. Magnification $\times 400$.

possible to play a role in preventing maternal immunologic attack on the allogeneic embryo. Thereby, preventing spontaneous abortion [3], hence eGL may act as a suppressor cell. Supernatants produced by decidual explants and unfractionated decidual cell suspensions consistently showed higher levels of suppression of mitogen-induced lymphocyte proliferation than supernatants from purified eGL [14]. The immunosuppressive activity of eGL in spontaneous abortion needs more investigation to clarify whether this role is critical for maintenance of pregnancy.

In view of their phenotype and cytoplasmic granularity, it is possible that eGL act as a type of NK cell and hence a prime candidate to exert harmful effects on placental trophoblast. The eGL from normal first trimester of human decidua have consistently exhibited lytic activity against the NK cell-sensitive target K562 in a standard chromium release assay [15]. Fresh eGL are unable to lyse first-trimester placental trophoblast cells [15], although they have been reported to do lysis after activation with IL-2 [16]. Cytokines such as IFN- γ and IL-2 are present in the decidua of women suffering spontaneous abortion [3]. This could induce activation of eGL to mediate lytic effects on the fetoplacental unit. In this study, eGL granules were demonstrated by staining with the phloxine

stain. Staining with a monoclonal antibody reactive to the CD56 antigen may have given more prominent differences in the numbers between normal and spontaneous early pregnancy loss. It is possible that eGL or their soluble products play a role in the outcome of human pregnancy because their numbers appeared to be increased in spontaneous abortion. The eGL freshly isolated from normal first trimester decidua produced M-CSF, GM-CSF, G-CSF, TNF- α , IFN- γ and LIF [17]. TGF β 2 has also been detected by CD56⁺ CD16⁻ CD3⁻ decidual large granulated lymphocytes [18]. The production of soluble products from eGL deserves more attention in future investigations in spontaneous abortion.

In conclusion, the immunological mechanisms that contribute to the maintenance of normal human pregnancy and determine the failure of a pathological pregnancy remain poorly understood. Comparison of the cell populations within decidua and characterization of their soluble products in both normal human pregnancy and spontaneous abortion will help us to define the critical factors that determine pregnancy outcome. Our study has provided eGL, that form the predominant leukocyte population in normal human pregnancy, appeared to be increased in decidua associated with spontaneous abortion. Further studies is required to more clarify

the role of eGL such as the expression of activation markers, cytokine receptors as well as more detailed characterization of their function: immuno-suppression activity, natural killer activity, and cytokine analysis in spontaneous early pregnancy loss.

ACKNOWLEDGMENTS

The authors are thankful to Ahwaz Medical Science University for financial support of this project. Also, we thank Dr. Iran Rashidi from Department of pathology for her help in pathological work during the project.

REFERENCES

1. Alberman, E. (1988) The epidemiology of repeated abortion. In: *Early pregnancy loss: Mechanisms and treatment*. (Beard, R.W. & Sharp, F. eds.), New York, Springer Verlag, pp. 9-17.
2. Ryan K.J., Berkowitz, R.S. and Barbieri, R.L. (1999) *Kistner's Gynecology & Women's Health*; 7th ed., St. Louis, Missouri, Mosby, pp. 396-416.
3. Hill, J.A. and Choi, B.C. (2000) Maternal immunological aspects of pregnancy success and failure. *J. Reprod. Fertil. Suppl.* 55: 91-97.
4. Ito, K., Karasawa, M., Kawano, T., Akasaka, T., Koseki, H. and Kondo, E. (2000) Involvement of decidual Valpha14 NKT cells in abortion. *Proc. Natl. Acad. Sci. USA* 97 (2): 740-744.
5. Clark, D.A., Chaput, A. and Tutton, D. (1986) Active suppression of host-vs-graft reaction in pregnant mice: VII. Spontaneous abortion of allogeneic CBA/J x DBA/2 fetuses in the uterus of CBA/J mice correlates its deficient non-T suppressor cell activity. *J. Immunol.* 136: 1668-1675.
6. Yamada, H., Polgar, K. and Hill, J.A. (1994) Cell-mediate immunity to trophoblast antigens in women with recurrent spontaneous abortion. *Am. J. Obstet.* 2: 1344-1339.
7. Bulmer, J.N., Morrison, L., Longfellow, M., Ritson, A. and Pace, D. (1991) Granulated lymphocytes in woman endometrium: histochemical and immuno-histochemical studies. *Hum. Reprod.* 6: 791-798.
8. King, A., Wooding, P., Gardner, L. and Loke, Y.W. (1993) Expression of perforin, granzyme A and TIA-1 by human uterine CD56⁺ NK cells implies they are activated and capable of effector functions. *Hum. Reprod.* 8: 2061-2067.
9. King, A., Balendran, N., Wooding, P., Carter, N.P. and Loke, Y.W. (1991) CD3-leukocytes present in the human uterus during early placentation: Phenotypic and morphologic characterization of the CD56⁺ population. *Dev. Immunol.* 1: 169-190.
10. Stewart, I.J. (1991) Granulated metrial gland cells: pregnancy specific leukocytes. *J. Leuk. Biol.* 50: 198-207.
11. Marzusch, K., Ruck, P., Geiselhart, A., Handgretinger, R., Dietl, J.A., Kaiserling, E., Horney, H., Harney, P., Vince, G. and Redman, C.W.G. (1993) Distribution of cell adhesion molecules on CD56⁺, CD3⁻, CD16⁻ large granular lymphocytes and endometrial cells in first trimester human decidua. *Hum. Reprod.* 8: 1203-1208.
12. Vassiliadou, N. and Bulmer, J.N. (1996) Immunohistochemical evidence for increased numbers of classic CD57⁺ natural killer cells in the endometrium of women suffering spontaneous early pregnancy loss. *Hum. Reprod.* 11 (7): 101-106.
13. Kodama, T., Hara, T., Okamoto, E., Kusunoki, Y. and Ohama, K. (1998) Characteristic changes of large granular lymphocytes that strongly express CD56 in endometrium during the menstrual cycle and early pregnancy. *Hum. Reprod.* 13 (4): 1036-1043.
14. Ghafourian Boroujerdnia, M. and Chinipradaz, R. (1998) Early pregnancy decidua as a source of TGFβ. *Iran. J. Med. Sci.* 23 (3&4): 99-104.
15. King, A., Birkby, C. and Loke, Y.W. (1989) Early human cells exhibit NK activity against the K562 cell line but not against first trimester trophoblast. *Cell. Immunol.* 118: 337-344.
16. King, A., Kalra, P. and Loke, Y.W. (1990) Human trophoblast cell resistance to decidual NK lysis due to lack of NK target structures. *Cell Immunol.* 127: 230-237.
17. Saito, S., Nishikawa, K., Morii, T., Enomoto, M., Narita, N., Motoyoshi, K. and Ichijo, M. (1993) Cytokine production by CD16- CD56 bright natural killer cells in the human early pregnant decidua. *Int. Immunol.* 5: 559-563.
18. Clark, D.A., Vince, G., Flanders, K.C., Hirte, H. and Starkey, P. (1994) CD56 lymphoid cells in human first trimester pregnancy decidua as a source of novel transforming growth factor-2-related immunosuppressive factors. *Hum. Reprod.* 9: 2270-2277.