Endometrial Granulated Lymphocytes in Women Suffering Spontaneous Early Pregnancy Loss

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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 ($p > 0.05$).

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].

Keywords: Spontaneous abortion, Endometrial granulated lymphocytes, First-trimester decidua, Phloxine-tartrazine stain
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 µm 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 µm) 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 × 400 magnification (× 10 eyepiece, × 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
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]. TGFβ2 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: immunosuppression activity, natural killer activity, and cytokine analysis in spontaneous early pregnancy loss.

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