

IMMUNOPATHOLOGICAL ASPECTS OF DECIDUAL TISSUES IN WOMEN WITH FIRST-TRIMESTER RECURRENT SPONTANEOUS ABORTION

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ABSTRACT

Background/objective: Studies of the decidual leukocyte populations in women suffering from spontaneous early pregnancy loss may provide insight into immunopathological aspects and even cast light on the etiology of recurrent spontaneous abortion.

Methods: In order to clarify the immunological role of endometrial leukocytes in repetitive abortion of unknown etiology, a comparative analysis of phenotypes of leukocyte sub-populations was performed on first-trimester decidual tissues obtained from thirty patients with recurrent spontaneous abortion and thirty samples at therapeutic abortion. Paraffin-embedded sections of these samples were labeled with a panel of monoclonal antibodies against leukocyte markers, CD68, CD3, CD45RA, CD57 using streptavidin-conjugated peroxidase technique. The numbers of positive cells for each monoclonal antibody for all control and pathological cases were counted microscopically at $\times 400$ magnification.

Results: Many macrophages were observed in pathological decidual tissues compared with normal early pregnancy decidua, however the difference was not significant ($p=0.36$). There was no significant difference in the number of T cells and B cells despite a slight decrease in recurrent spontaneous aborters ($p=0.36$; $p=0.15$, respectively). Scantly CD57-positive classical NK cells were in normal pregnancy decidua. Although the number of classical CD57-positive natural killer cells varies in pathological cases compared with normal pregnancy cases, the difference between the two groups reached significant level ($p=0.003$).

Conclusion: These findings suggest that classical NK cells possibly play a role together with other activated decidual cell populations in the control of trophoblast growth and placental development.

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Key Words • Abortion, spontaneous • leukocyte • trimester, first • decidua • immunohistochemistry

Introduction

Spontaneous abortion is the most common complication of pregnancy. Abortion occurs in the first-trimester much more frequently than in the second-trimester. Historically, recurrent pregnancy loss has been defined as

three or more spontaneous abortions prior to 20 weeks gestation, and occurs in 0.3% of pregnant women. Diverse possible causes for recurrent spontaneous abortion (RSA) have been proposed including chromosomal aberration, anatomic anomalies, endocrine disorders and infectious factors.¹ Nevertheless, in a large proportion of couples, the etiology of repetitive abortions remains unexplained.

During the last decade, research has been focused on the decidualized endometrium to

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elucidate maternal-fetal interaction. The development of monoclonal antibodies (mAbs) has improved our knowledge about the populations that exist in human decidua and have given us clues as to their *in vivo* role. Flow cytometric analysis has indicated that CD45-positive leukocytes account for 75% of cells in enzymatically dispersed cell suspensions prepared from first-trimester decidua.² At the decidua, maternal leukocyte sub-populations may come into intimate contact with the placenta and are capable of responding to allogenic stimulation, such as that of the fetus. Therefore, to maintain pregnancy, the fetus is normally protected from immunologic assaults through mechanisms that are not fully understood. Several studies have shown that murine and human decidual tissues produce soluble factors which can regulate a variety of immunosuppressive activities *in vivo*.³ A deficiency of local immunosuppression may result in spontaneous abortion.⁴ A small granulated non-T non-B lymphocyte causes immunosuppression in murine decidua by secretion of a $TGF\beta_2$ which blocks lymphocyte responses to IL-2.⁵ A lack of decidual suppressor cells has been detected in mice with spontaneous abortion. It has been reported that the decidua-associated suppressor cells in abortion-prone DBA/2-mated CBA/J mice that release bioactive $TGF\beta_2$ -related immunosuppressive molecules, express a bone marrow-derived natural suppressor cell marker and $\gamma\delta$ T cell receptor.⁶ A subset of women with RSA are deficient in $TGF\beta_2$ producing suppressor cells in uterine tissue near the placental attachment site.⁷ Studies in murine models have demonstrated that decidual leukocyte cells like NK cells, macrophages, and T cells might be implicated in fetal resorption. In approximately 35% to 50% of women with unexplained RSA, immune and inflammatory cell responsiveness to trophoblast is activated as evidenced by increased proliferation and secretion of

embryotoxic factors such as $IFN\gamma$.⁸ In biopsies from some women with a history of recurrent abortion, extremely high helper/suppressor cells ratios were found, suggesting that immunologic mechanisms may be operative in spontaneous abortion.⁹ Although in humans controversy persists, few data are available on the involvement of leukocytes in the abortion process.

To clarify the immunologic role of decidual leukocyte sub-populations in repetitive abortions of unknown etiology, this study was carried out on analysis of the immunophenotypic characteristics of macrophages, T cells, B cells and classical NK cells by immunohistochemistry in the first-trimester decidual tissues of patients with RSA and compared with those of gestational age-matched decidua tissues from women undergoing elective pregnancy termination.

Materials and Methods

Thirty samples of uteroplacental tissues of therapeutic abortion (control cases) were obtained from elective pregnancy terminations at 8-12 weeks performed by vaginal curettage at the female surgical ward in Imam Khomeini Hospital, Ahwaz. Thirty samples of uteroplacental tissues (pathological cases) were also obtained randomly from women with RSA of unknown etiology with no prior full-term pregnancy. These were matched with the control cases during a three-year period. Decidual tissue was identified macroscopically by its opaque, grey-white solid appearance and separated from the fetal and placental tissues. Specimens of tonsil tissues were obtained from the surgical ward. All samples were fixed in 10% buffered formalin and then embedded in paraffin wax and sectioned at 3 μ m by microtome. Sections from each sample were stained with H&E method in order to assess the morphology and viability of decidual tissues. Four murine mAbs were used to stain immunohistochemically different leukocyte

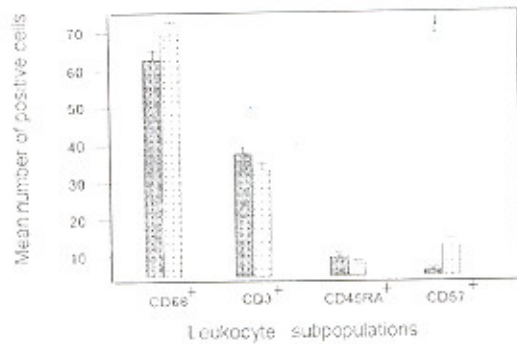


Figure 1: Leukocyte sub-populations in electively aborted normal decidua (column 1) and in decidua of patients with recurrent spontaneous abortion (column 2) in early pregnancy. Each column represents the mean number of positive cells in eight high power fields \pm SEM (magnification $\times 400$). A significant difference was detected in the number of NK classical cells (CD57-positive cells) between normal pregnancy and recurrent spontaneous early pregnancy loss ($p = 0.003$).

markers in all samples. All were suitable for use on formalin-fixed paraffin-embedded tissue sections. These were: monoclonal anti-human T cell, CD3, which detect the CD3 chain immunogen on T lymphocyte; monoclonal mouse anti-human macrophages, CD68, which

stains macrophage in a variety of human tissues; monoclonal mouse anti-human B cell, CD45RA, which react with most B cells in peripheral blood and in tissue sections; monoclonal mouse anti-human NK cell line which detect the CD57 NK cell marker on the surface of classical NK cells. All monoclonal antibodies were supplied from DAKO Ltd.

Immunohistochemical staining:

Serial sections prepared from all samples were stained with a streptavidin-conjugated peroxidase technique. The sections were deparaffinized, hydrated, incubated with hydrogen peroxide and then trypsinized if required. The tissue sections were sequentially incubated with normal rabbit serum, the specific mAbs, biotinylated rabbit anti-mouse immunoglobulin and streptavidin-conjugated horseradish peroxidase reagent were employed at appropriate times. The reaction was developed with 3,3'-diaminobenzidine tetrahydrochloride chromogen solution. The sections were then lightly counter-stained with Mayer's hematoxylin, blued in Scott's tap water solution, dehydrated, cleared and mounted. Sections of tonsil as positive control were included in each run, and a negative

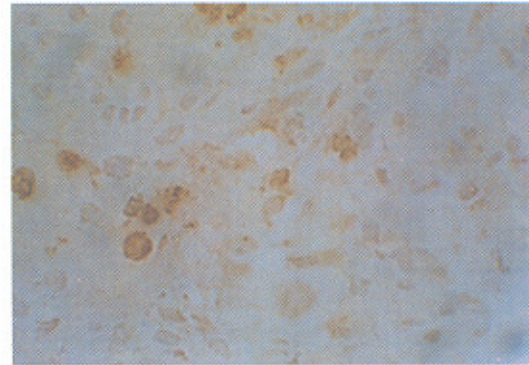
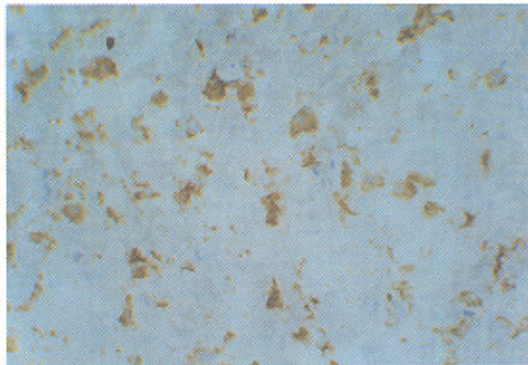


Figure 2: Sections of human decidua in normal early pregnancy labeled with: **Left)** anti-CD68 monoclonal antibody; **Right)** anti-CD57 monoclonal antibody using Dako Streptavidin-Biotin-Peroxidase technique. Macrophages and classical NK cells are seen scattered in the decidua stroma. (Magnification $\times 400$)

Table 1: Mean number (\pm SEM) of positive cells in eight high-power fields ($\times 400$) in paraffin-embedded sections of normal pregnancy decidua and spontaneous pregnancy loss decidua sections reactive with anti-leukocyte monoclonal antibodies

Antigen	Mean number (\pm SEM) of positive cells in decidua in normal pregnancy	Mean number (\pm SEM) of positive cells in decidua in spontaneous pregnancy loss
CD68	63.00 \pm 2.50	69.30 \pm 3.30
CD3	37.40 \pm 1.90	33.07 \pm 1.80
CD45RA	9.63 \pm 1.00	7.80 \pm 0.76
CD57	5.80 \pm 0.73	12.60 \pm 2.10

control was performed for each sample with no primary antibody.

Results

The results of immunohistochemical staining showed that all leukocyte sub-populations in tonsil tissue and decidual tissues from either elective or spontaneous abortion were present in varying amounts (Table 1) and with no difference in their distribution. Negative control with tonsil and decidual sections showed no staining. Many macrophages were observed in decidual tissues which were scattered within decidual stroma, sometimes forming aggregates around glands (Fig. 2, right). The number of macrophages increased

slightly in pathological decidua compared with normal early pregnancy decidua, however, the difference was not significant ($p=0.36$). Scantly CD57-positive classical NK cells were seen scattered within the stromal cells in normal pregnancy decidua (Fig. 2, left). Although the number of classical CD57-positive NK cells varies in pathological cases compared with normal pregnancy cases, the difference between the two groups reached a significant level ($p=0.003$). T cells were scattered diffusely throughout the stroma and formed small aggregates within the stroma (Fig. 3, right and left). There was no significant difference in the number of T cells, despite a slight decrease in recurrent spontaneous aborters ($p=0.36$). Few B cells were seen scattered diffusely in decidual

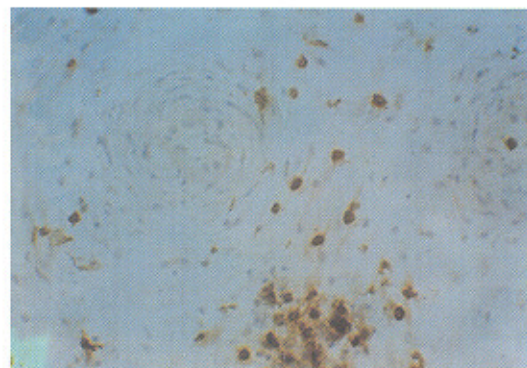
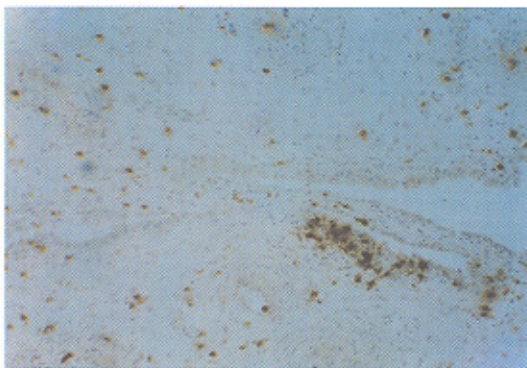


Figure 3: A section of human decidua in normal early pregnancy labeled with anti-CD3 monoclonal antibody using Dako Streptavidin-Biotin-Peroxidase technique. Positive T cells are seen scattered in the decidual stroma. Aggregates near gland and vessel are visible. **(Left)** Magnification $\times 100$; **(Right)** Magnification $\times 200$.

tissues. The number of B cells was decreased in pathological pregnancy decidua compared with normal pregnancy decidua, but the difference was not significant ($p=0.15$). The proportion of leukocyte sub-population is shown in Figure 1.

Discussion

Leukocytes form a substantial component of human decidua. Different sub-populations of leukocytes have been detected in decidual tissue. One of the major leukocyte populations in stroma of first-trimester decidua is macrophage.¹⁰ In the present study, macrophages were found to comprise a greater proportion of the decidual leukocytes in the recurrent aborted decidua compared with normal pregnancy decidua, but the difference just failed to reach a significant level. Similar observations were also reported in other studies.¹¹ Data from animal models have suggested that macrophages could play a role in pregnancy decidua. Decidual macrophages have been implicated as suppressor cells through secretion of prostaglandin E_2 .¹² Prostaglandin E_2 mediates immunosuppression by human first-trimester decidual cells.¹² Such immunosuppression has been reported to block the lytic activity of decidual lymphocytes with potential antitrophoblast activity. A drastic increase in prostaglandin levels after spontaneous abortion at a gestational age of 8 to 10 weeks have been detected compared with normal pregnancy termination. Decidual macrophages could be activated by spermatozoa, trophoblasts or microbial factors, and stimulate the secretion of cytokines that may be detrimental to pregnancy. A low affinity receptor for TNF on human placental syncytiotrophoblast was demonstrated.¹³ It has been proposed that high rates of resorption spontaneous abortion may result from interaction in the decidua of TNF α producing macrophages.¹⁴

In contrast to B cells in normal human pregnancy, many T lymphocytes exist in decidual tissues.¹⁰ The present study showed that the number of T as well as B cells is decreased in spontaneous abortion in comparison with normal pregnancy. However, the results are not considered significant. B lymphocytes involved in autoimmune mechanisms have been proposed to be the cause of some recurrent pregnancy loss such as antiphospholipid syndrome.¹ A significant increased number of CD5-positive B cells has been reported in RSA. Over 75% of stromal T cells are CD8-positive suppressor/cytotoxic cells, in contrast with peripheral blood where CD4-positive T cells predominate.¹⁰ Bondarenko et al.¹⁵ found that CD4-positive cells rather than CD8-positive cells predominated in spontaneous abortion decidua. However, in other studies all the decidual T cell subsets, numbers and proportions in normal early pregnancy were similar to spontaneous abortion.^{11,16} In endometrial biopsy of non-pregnant recurrent aborters during their secretory phase, the percentage of CD8-positive cells was significantly decreased and their CD4:CD8 ratio was increased. In contrast, the proportion of CD20-positive B cells was strikingly increased. Repetitive aborters who had normal CD8-positive and CD20-positive cell numbers and a normal CD4:CD8 ratio subsequently underwent successful pregnancies.⁹ T helper cell 1 (Th1) and Th2 cytokine responses to trophoblast or other antigens are proposed to be involved in pregnancy loss and success, respectively. The Th1 cytokines IL-2, TNF and IFN γ are reported to affect many reproductive processes adversely. The Th1 initiating cytokine IL-12 has been found in the decidua of aborting women with Th1 immunity to trophoblast while the Th2 type cytokine IL-10 was detected in reproduced normal women.⁸

The contribution of NK cells to fetal resorption has been a controversial issue. Indeed, several

studies suggest that NK cells are a prerequisite for maintaining pregnancy, while others suggest that NK cells display detrimental effects on fetal development, resulting in spontaneous abortion in mice or humans¹⁷. Although NK cells are phenotypically and functionally heterogeneous cells in the decidua,^{10,18} the present study investigates the CD57-positive classical NK cells which are very few in normal early pregnancy decidua. CD57-positive NK cells were increased significantly in spontaneous aborted decidua in comparison with therapeutic aborters. All specimens used in this study were subjected to detailed histological examination. Those cases with obviously inflamed decidua or necrotic areas were excluded because these factors could affect the numbers and proportions of the decidual leukocyte populations. Elevated numbers of classical NK cells have not been detected in all cases of spontaneous aborted decidua. This may explain why the standard error of mean is high in comparison with normal pregnancy decidua. Nevertheless, the difference is significant. Therefore, it is possible that a proportion of unexplained spontaneous abortions occur as a result of increased numbers of classical CD57-positive NK cells in human decidua, which may become activated by local cytokines to attack the trophoblast. Interestingly, abortion-prone mating combinations stimulate production of TNF α , IFN γ and IL-2 in mixed lymphocyte placenta reaction.¹⁹ TNF α polymorphism associated with recurrent abortion in women with Th1 immunity to trophoblast cells was detected.⁸ Significantly increased levels of IFN γ in serum of women with RSA were also reported.²⁰

In conclusion, abnormalities of the delicate cytokine balance within first-trimester decidua may lead to the activated cells which are capable of killing the trophoblast. Thus, NK cells within decidua may act together with other activated decidual cell populations to

attack the trophoblast population, resulting in subsequent pregnancy loss.

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