

ОСОБЕННОСТИ ПОЛЯРИЗАЦИИ МОНОЦИТОВ ПРИ РАЗЛИЧНЫХ ИСХОДАХ БЕРЕМЕННОСТИ У ЖЕНЩИН С ПРИВЫЧНЫМ НЕВЫНАШИВАНИЕМ

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Резюме. В настоящее время в патогенезе привычного невынашивания особую роль уделяют иммунологическим факторам, в частности роли врожденного иммунитета. Цель исследования – оценка относительного содержания моноцитов в периферической крови, продуцирующих ИЛ-4, ИЛ-6, ИЛ-10, IFN γ , а также выявление новых критериев прогнозирования исхода беременности у женщин с угрозой прерывания в ранние сроки и привычным невынашиванием. Обследовано 88 беременных в сроке гестации 5-12 недель, основную группу составили 59 женщин с привычным невынашиванием и угрожающим выкидышем на момент исследования, группу контроля – 29 женщин с неосложненным течением беременности без привычного невынашивания. Основная группа в зависимости от исходов беременности была подразделена на подгруппы: I подгруппа – 42 женщины, чья беременность завершилась своевременными родами, II подгруппа – 8 женщин с преждевременными родами, III подгруппа – 9 женщин с прерыванием беременности в сроке до 22 недель (самопроизвольный выкидыш и неразвивающаяся беременность). В группе контроля у всех женщин произошли своевременные роды. Материал исследования – периферическая венозная кровь. На проточном цитофлуориметре FACSCanto II с использованием моноклональных антител оценивали относительное содержание ИЛ-4⁺, ИЛ-6⁺, ИЛ-10⁺, IFN γ ⁺ моноцитов. Статистическая обработка данных проводилась с помощью пакета стандартных прикладных программ.

В группе женщин с привычным невынашиванием и угрожающим выкидышем было снижено относительное содержание ИЛ-10⁺ и ИЛ-4⁺ моноцитов и повышено содержание ИЛ-6⁺ моноцитов по сравнению с группой контроля ($p = 0,0001$ во всех случаях). Статистически значимых различий в содержании IFN γ ⁺ моноцитов в сравниваемых группах не выявлено ($p = 0,069$). При относительном содержании ИЛ-4⁺ моноцитов равном 26,7% или менее прогнозируют преждевременные роды. При относительном содержании ИЛ-10⁺ моноцитов равном 27,0% или менее прогнозируют прерывание беременности (самопроизвольный выкидыш или неразвивающуюся беременность) в сроке геста-

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ции до 22 недель. Было выявлено повышение соотношения $IFN\gamma^+/IL-4^+$, $IFN\gamma^+/IL-10^+$, $IL-6^+/IL-4^+$, $IL-6^+/IL-10^+$ моноцитов в основной группе ($p < 0,0001$ во всех случаях).

У женщин с привычным невынашиванием во всех подгруппах уровень M1-моноцитов преобладал над уровнем M2-моноцитов. Полученные данные позволили разработать новые прогностические критерии прерывания беременности до 22 недель и преждевременных родов.

Ключевые слова: беременность, привычное невынашивание, моноциты

FEATURES OF MONOCYTE POLARIZATION AT DIFFERENT OUTCOMES IN WOMEN WITH RECURRENT MISCARRIAGE

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Abstract. Currently, in the pathogenesis of recurrent miscarriage, a special role is given to immunological factors, in particular the role of innate immunity. The aim of the study was to assess the relative content of monocytes in the peripheral blood producing IL-4, IL-6, IL-10, $IFN\gamma$, as well as to identify new criteria for predicting the outcome of pregnancy in women with the threat of early termination and recurrent miscarriage. Materials and methods. 88 pregnant women at 5-12 weeks' gestation were examined, the main group consisted of 59 women with recurrent miscarriage and threatened miscarriage at the time of the study, the control group — 29 women with uncomplicated pregnancy without recurrent miscarriage. The main group, depending on the outcomes of pregnancy, was subdivided into subgroups: subgroup I — 42 women whose pregnancy ended in timely delivery, subgroup II — 8 women with preterm labor, subgroup III — 9 women with abortion up to 22 weeks (spontaneous miscarriage and non-developing pregnancy). In the control group, all women had a timely delivery. Research material — peripheral venous blood. The relative content of $IL-4^+$, $IL-6^+$, $IL-10^+$, $IFN\gamma^+$ monocytes was assessed on a FACSCanto II flow cytometer using monoclonal antibodies. Statistical data processing was carried out using a package of standard applied programs. Results. In the group of women with recurrent miscarriage and threatened miscarriage, the relative content of $IL-10^+$ and $IL-4^+$ monocytes was reduced and the content of $IL-6^+$ monocytes was increased compared to the control group ($p = 0.0001$ in all cases). There were no statistically significant differences in the content of $IFN\gamma^+$ monocytes in the compared groups ($p = 0.069$). With a relative content of $IL-4^+$ monocytes equal to 26.7% or less, preterm labor is predicted. With a relative content of $IL-10^+$ monocytes equal to 27.0% or less, abortion (spontaneous miscarriage or miscarriage) is predicted in gestational age up to 22 weeks. An increase in the ratio of $IFN\gamma^+/IL-4^+$, $IFN\gamma^+/IL-10^+$, $IL-6^+/IL-4^+$, $IL-6^+/IL-10^+$ monocytes was found in the main group ($p < 0.0001$ in all cases). Conclusions. In women with recurrent miscarriage in all subgroups, the level of M1 monocytes prevailed over the level of M2 monocytes. The data obtained allowed the development of new prognostic criteria for termination of pregnancy up to 22 weeks and premature birth.

Keywords: pregnancy, recurrent miscarriage, monocytes

Introduction

Recurrent miscarriage (RM) is a complication of early pregnancy, which occurs in married couples with a frequency of 2-5% [6, 8]. The etiology of recurrent miscarriage is diverse and not fully understood. Currently, special attention is paid to immunological factors that play an important role in the pathogenesis of this complication of pregnancy [2]. It is known that from the earliest stages of pregnancy various immune cells create a special microenvironment

to support a semi-allogeneic fetus [7]. The innate cells play a special role in these mechanisms [4, 7]. Monocytes are an integral part of the innate immune system [3] exerting high plasticity and, depending on the microenvironment, can functionally differentiate into a population of classically activated (M1) or alternatively activated (M2) cells [7, 9]. Each of the monocyte populations is characterized by different functions and cytokine production. Studies show that during normal pregnancy, monocyte differentiation

shifts towards an anti-inflammatory immune response with produced corresponding cytokines typical to the M2 population [10]. Moreover, an imbalance towards classically activated monocytes (M1) and their production of pro-inflammatory cytokines significantly increases the risk of early pregnancy loss or complications such as premature birth [2, 10]. Despite the available data on the participation of monocytes in immunological processes during pregnancy, it is necessary to identify a role of imbalance in their polarization in developing recurrent miscarriage.

The aim of the study was to assess the percentage of monocytes in the peripheral blood producing IL-4, IL-6, IL-10, IFN γ , as well as to identify new criteria for predicting outcome of pregnancy in women with threatened early termination and recurrent miscarriage in history.

Materials and methods

A survey of 88 pregnant women at a gestational age of 5-12 weeks was carried out at the gynecological clinic of the V. Gorodkov Ivanovo Research Institute of Maternity and Childhood. The main group included 59 women with RM and threatened miscarriage at the time of the study. By retrospective analysis, this group of women was divided into subgroups depending on the pregnancy outcome. Subgroup I consisted of 42 women in whom pregnancy ended in timely delivery, subgroup II – 8 women with preterm labor, subgroup III – 9 women whose pregnancy ended in abortion at age up to 22 weeks of gestation, including spontaneous miscarriage and undeveloped pregnancy. The control group included 29 women with uncomplicated pregnancy and lacking recurrent miscarriage in history, who had a timely delivery. The groups are recruited uniformly. Exclusion criteria: spontaneous miscarriage in progress; multiple pregnancy; pregnancy resulting from the use of assisted reproductive technologies; anembryonia or other reliable signs of an unviable uterine pregnancy; abnormalities in the uterus development; structural changes in the karyotypes of spouses, established at the pre-gravid stage; active infection and presence of severe female extragenital pathology at the time of examination; severe allergic reactions at the time of the examination. The study complied with ethical standards. All women enrolled to the study signed an informed consent. The biological material was presented by whole peripheral venous blood collected before treatment, in a volume of 10 ml into a test tube added with heparin. FACSCanto II flow cytometer (Becton Dickinson, USA) was used with monoclonal antibodies anti-IL-10, anti-IL-6 (eBioscience), anti-IL-4, anti-IFN γ (Beckman Coulter) to assess percentage of IL-4⁺, IL-6⁺, IL-10⁺, IFN γ ⁺ monocytes. Statistical data processing was carried out by using “Statistica 13.0”, “MedCalc” software.

Before calculations, all parameters were tested for normality. The normal distribution was checked using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Taking into account the normality of the distribution, the data were presented as arithmetic mean and standard error of the mean ($M \pm m$). The threshold significance level of differences was set at $p < 0.05$. The search for the separation point, the calculation of sensitivity and specificity was carried out based on the construction of the receiver operating characteristic curve (ROC analysis).

Results and discussion

While analyzing the studied parameters, it was revealed that the relative percentage of IL-4⁺ and IL-10⁺ monocytes in the peripheral blood was lower in the main group with threatened termination in the first trimester with RM compared with those in uncomplicated pregnancy ($p = 0, 0001$ in both cases), whereas the level of IL-6⁺ monocytes in the main group was higher than in the control group ($p = 0.0001$). No significant differences in the percentage of IFN γ ⁺ monocytes in pregnant women from both groups were found ($p = 0.069$) (Table 1).

The population of M2 monocytes is characterized by the production of anti-inflammatory cytokines, which we assessed by the intracellular production of IL-4⁺ and IL-10⁺. A comparative analysis of percentages of IL-4⁺ M2 monocytes in the peripheral blood in women with threatened interruption with RM revealed that in all subgroups it was significantly decreased compared to control group ($p < 0.05$). Our retrospective analysis showed that the relative content of IL-4⁺ M2 monocytes in the group of women with RM differed depending on the outcome of pregnancy. In the subgroups of women whose pregnancy ended in abortion at age of up to 22 weeks and premature birth, it was lower than in the subgroup of women with timely birth ($p < 0.05$ in all cases). In subgroup I, the level of IL-4⁺ monocytes was $35.47 \pm 2.0\%$, subgroup II – $23.56 \pm 2.12\%$, subgroup III – $23.87 \pm 3.66\%$, and in the control group – $53, 23 \pm 2.3\%$. However, it should be noted that the level of IL-4⁺ monocytes was lower in the subgroup of women with threatened miscarriage with RM and timely delivery compared to the control group ($p < 0.05$).

According to our data, we have identified new prognostic criteria for preterm birth in women with threatened miscarriage and a history of recurrent miscarriage. So, with a relative content of IL-4⁺ monocytes comprising at least 26.7%, preterm labor may be predicted. According to the results of the ROC analysis, the area under the ROC curve (AUC) was 0.885, the sensitivity of the method was 77.8%, the specificity – 95.7%, and the accuracy – 90.6%. Based on these data, a “Method for predicting premature

TABLE 1. CONTENT OF IFN γ ⁺, IL-4⁺, IL-10⁺ AND IL-6⁺ MONOCYTES IN WOMEN WITH THE THREAT OF TERMINATION AND RECURRENT MISCARRIAGE

Indicator, %	Control group (n = 29)	Main group (n = 59)	
IL-4 ⁺	53.04±2.36	30.12±1.47	p = 0.0001
IL-6 ⁺	29.45±1.57	71.69±1.29	p = 0.0001
IL-10 ⁺	65.61±2.27	30.45±1.51	p = 0.0001
IFN γ ⁺	50.50±4.57	59.90±2.06	p = 0.069

birth in women with threatened early miscarriage with RM in history” was proposed.

A comparative analysis of percentage of IL-10⁺ monocytes in the peripheral blood in women with the threatened interruption with RM found that all subgroups had it significantly decreased *vs* control group ($p < 0.05$). In addition, we found that the level of IL-10⁺ monocytes in the group of women with RM was lower in the subgroups with terminated pregnancy at age of up to 22 weeks (23.7±1.42%) and preterm birth (24.12±2.33%) *vs* subgroup of women with timely delivery (34.45±1.67%) ($p = 0.0001$ and $p = 0.006$, respectively).

According to the data obtained, we have identified new prognostic criteria for termination of pregnancy before 22 weeks of gestation in women with threatened miscarriage with RM in history. Abortion (spontaneous miscarriage or miscarriage) is predicted at gestational age of up to 22 weeks in case of percentage of IL-10⁺ monocytes reaching at least 27.0%. Based on these data, a “Method for predicting abortion in women with threatened miscarriage and recurrent miscarriage in history” was proposed.

The level of M1 monocytes, which is characterized by the production of proinflammatory cytokines, was assessed by the intracellular production of IFN γ ⁺ and IL-6⁺. Analyzing the results obtained, we revealed no significant differences in the frequency of such cells in all subgroups of women with RM ($p > 0.05$ in all cases). However, the level of IL-6⁺ monocytes was higher in all subgroups of women with recurrent miscarriage compared with the control group ($p < 0.05$ in all cases), whereas the relative content of IFN γ ⁺ monocytes did not differ from that one in the control group and in all subgroups of women with RM ($p > 0.05$ in all cases).

We also found increased ratio for IFN γ ⁺/IL-4⁺, IFN γ ⁺/IL-10⁺, IL-6⁺/IL-4⁺, IL-6⁺/IL-10⁺ monocytes in the group of women with RM ($p < 0.0001$ in all cases), which indicates the predominance of inflammatory reactions in this pathology.

It is known that a successful pregnancy contributes to the maintenance of the immune balance, ensures the mother’s immunotolerance to the fetal antigens [3, 10]. IL-4 and IL-10 – producing monocytes are characterized by anti-inflammatory and immunosuppressive properties [1] being involved in

angiogenesis, placenta formation and maintenance of tolerance to semi-allogenic fetus, additionally regulating Treg function [1]. Local inflammation, mediated by cells of innate and acquired immunity, is necessary for successful implantation and tissue remodeling in the first trimester. However, an excessive inflammatory response leads to serious complications of pregnancy, including termination of pregnancy. According to the literature, unfavorable pregnancy outcome is coupled to higher level of proinflammatory cytokines [2]. IFN γ and IL-6 – producing monocytes are characterized by pro-inflammatory functions [5], but an excessive inflammatory response inhibits the anti-inflammatory response.

We found that women with recurrent miscarriage and threatened early miscarriage had reduced percentage of IL-10⁺ and IL-4⁺ monocytes with M2 functions, whereas frequency of IL-6⁺ monocytes was increased compared to the control group. According to our data, women with recurrent miscarriage in all subgroups had dominated M1 profile.

While assessing the study results, a similar dynamic of changes was found in women with RM, whose pregnancy ended in interruption at age of up to 22 weeks and premature birth. In this situation, it can be assumed that other mechanisms able to compensate for pathological processes in threatened termination might occur allowing to prolong pregnancy until some certain timepoint. According to the data obtained, it can be assumed that if pregnancy is terminated before 22 weeks, a persistent inflammatory reaction, impaired angiogenesis and placental development from early gestation develop most rapidly. Whereas a chronic inflammatory reaction in early pregnancy can probably lead to a decrease in the anti-infectious protection of the fetoplacental complex and amniotic fluid, which ultimately can lead to premature birth. Together, these data support the notion that the early absence of appropriate anti-inflammatory reactions and excessive inflammatory response can lead to unfavorable course of pregnancy. The data obtained allowed to identify new early predictive criteria for termination of pregnancy before 22 weeks and premature birth.

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