ROLE OF GENE POLYMORPHISM OF IL-4 AND IL-17 IN RECURRENT MISCARRIAGE, CAME IN IVF CYCLES

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INTRODUCTION

Recurrent miscarriage (RM) is a genetically heterogeneous condition as a result of the co-existence of the two governing factors: maternal and embryonic [1, 2]. The embryo expresses antigens inherited from both parents, and survival of hemiallotransplant fetus to term is one of the most challenging processes associated with pregnancy. Mother's immune system plays an important role in a successful pregnancy by controlling the fertilization, implantation, development and maintenance of the pregnancy itself [3–5].

ANALYSIS OF PUBLISHED DATA AND RESEARCH TASKS

Successful maintenance of pregnancy is dependent on the delicate balance between Th-1, Th-2, Th-17 and Treg-cells of the immune system as well as between specific cytokines inherent [6, 7]. Cytokines are cellular signaling proteins, mainly secreted by immune cells and mediate cell-cell communication [8]. It was revealed that the cytokine polymorphisms may be associated with RM. It is known that one of the major cytokines involved in the gestation are IL-4 and IL-17. Human cells Th17, produce IL-17, may play a role in the rejection conceptus antigens and consequently may be harmful to the maintenance of pregnancy [9]. It is shown that IL-17-decidual, producing by CD4+T-cells, are not harmful to human pregnancy, as they also produce IL-4 [10]. Therefore, a special interest is the study of the characteristics of polymorphisms of IL-4 and IL-17 in women with RM.

IL-4 regulates the growth and differentiation of B-lymphocytes, as well as processes of biosynthesis and secretion of antibodies. It is produced by activated CD4+T-lymphocytes (Th2), mast cells and eosinophils, generally – by activated Th2 cells. IL-4 stimulates the differentiation of Th0 to Th2, inhibits the generation of cytotoxic lymphocytes, natural killer cells and the production of interferon-γ and antitumor activity of macrophages, reduces the production of proinflammatory cytokines IL-1 and TNFα [11, 12]. In the presence of genetically determined disorders of the immune system excessive synthesis of pro-inflammatory interleukins occurs, resulting in the rapid development of inflammation in the tissues even in the absence of pathogens in the hearth. Some evidence suggests that Th2-cytokine IL-4 and its receptor may be of particular interest for controlling a Th17-induced inflammation. In humans, decreased response to IL-4 is believed to contribute to autoimmune inflammation.

In humans, the gene, coding for IL-4, is located on chromosome 5 in the 5q31-q33, which is a so-called cytokine cluster, where they were found and other genes that are important for the immune system, such as IL-5, IL-9, IL-12, IL-13 and granulocyte-macrophage colony stimulating factor. IL-4 gene contains four exons and exon 2 is the shortest (48 bp encoding 16 AA); exon 3 is the longest. The full length protein IL-4, which is encoded all four exons, is an option that is commonly referred to simplest IL-4, and it is certainly the most studied isoform structurally and functionally [12].

It revealed more than 50 allelic variants of gene polymorphisms IL-4, including -590C/T (rs2243250), -33C/T (rs2070874), +3437C/G (rs2227282) and 2979G/T (rs2227284) and oth-

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ers. It was installed large variability of occurrence of abnormal IL-4 alleles in different populations [13]. Different ethnic groups may have especially immunopathogenesis that are both theoretical and practical interest [13].

A single nucleotide polymorphism (SNP), in IL-4R coding region, I50V [rs1805010] detects the presence of isoleucine (I) according to valine (V) at position 50 in the aminoacid sequences. This polymorphism in the IL-4R is functionally important, since it affects the signal force receptor and may be able to regulate the production of IL-17 involved in the gestation [14, 15]. At the same time, [15] found no significant difference in IL-4 VNTR genotype frequencies between RM and control (OR 0.91; 95% CI 0.58-1.45); [16] showed that IL-4R I50V [rs1805010] plays a causal role in the RM. The contradictory literature data requires further research.

T-helper 17 (Th17) – the third type of the newly discovered T helper cells. T helper 17 cell characterized by the expression of IL-17A, IL17F, IL-6, TNF-α and IL-22. Six ligands of family IL-17 (IL-17A-F) and five receptors (IL-17RA-RD and SEF) were identificated. IL-17A and IL-17F are members of the cytokine IL-17 responsible for pathogenic cell activity Th17; they induce multiple proinflammatory mediators including chemokines, cytokines and metalloproteinases of epithelial cells and fibroblasts [17]. The genes IL-17A and IL-17F are located on chromosome 6P12. Development Th17-cells and cytokine secretion are reduced *in vitro* upon exposure IFN-γ and IL-4 produced by Th1 and Th2-cells, respectively. Polymorphisms IL-4R (I50V) may monitor the possibility of the immune systems control the amount of human IL-17 production.

The aim of the study was to study the incidence of genotypes and allelic variants of the IL-4 (S589T and S33T), IL-4R (Q576R), IL-17A (G197A) and IL-17F (488T/C) genes, depending on the reproductive status and evaluation of associative due to recurrent miscarriage in IVF cycles.

MATERIAL AND METHODS

Under supervision there were 240 patients of group R with habitual miscarriage, came in IVF cycles and 100 apparently healthy fertile women in the control group K with a history of at least one term delivery and lack of spontaneous abortion episodes. All the women were Eastern Slav.

Patients were taken peripheral whole blood into vacuum tube type plastic Vacuette volume of 4.0 ml added as anticoagulant disodium ethylenediaminetetraacetate salt in a final concentration of 2.0 mg/ml.

Molecular genetic studies carried out in the molecular genetics laboratory of "Diagnostic center 'Eugenics'" (Head Lab. PhD Makshaeva E.T.) in Odessa. DNA is isolated from nuclei of lymphocytes. The method is based on the destruction of lymphocytes using lysis buffer without affecting the integrity of the membranes of lymphocytes nuclei. For typing of gene SNPs immune response using PCR fusion reaction products in the presence of "adjacent" oligonucleotides (variant of the adjacent probes, kissing probes).

For genotyping polymorphisms IL-4 sequence using forward and reverse primers: For IL-4 –33C/T direct primer 5'GCCCCAAGT GACTGACAATC3' and reverse 5'TCACCTTCTGCTCTGTGTGAGG3'; for IL-4 -589C/T – respectively 5'AACACCTAAACTTGGG AGGA3' and 5'CTGTCATGGAAAAGCTGATCT3; for IL-4R Q576R – 5'-CTCTCTGAGCCAACCACTGT-3' and 5'-GCTCCACCGCAT GTACAAAC-3'. For genotyping of IL-17 polymorphisms were used: IL-17A to G-197A direct 5'-TCTCCATCTCCATCACCTTTG-3' and reverse 5'-GTCCAAATCAGCAAGAGCATC-3'; for IL-17F 7488 T/C: direct 5'-CACTGGTGCTCTGATGAGGA-3' and reverse 5'-CATTGTGCTTTGGCTTGCT-3'.

Amplification was performed using detection thermocycler DT-96 "DTprime" ("NPO DNA Technology" Ltd, Russia).

Statistical data processing carried out with the help of the electronic program Microsoft Office 2007 for Windows XP Professional, STATISTICA 6.0 (StatSoft Inc, US) with the definition of the differences were significant at a value of $p < 0.05$. For comparison, the frequency for a normal distribution of feature t-test was used. To estimate the nonparametric, unrelated metrics using the Mann-Whitney test related – Wilcoxon test. Compliance with the observed distributions of genotype frequencies, theoretically expected by the Hardy-Weinberg equation, evaluated using χ^2 test. To assess the association calculated the relative risk (OR). For OR calculated confidence interval (CI) at 95% significance level. If OR is equal to 1, it is considered that there is no association if OR was greater than 2 was considered a positive association. It was assessed preventive and etiological fraction.

RESULTS AND ITS DISCUSSION

The groups were representative of age. The average age of women surveyed group N was 29.80 ± 0.30 years and in the group K – 30.09 ± 0.30 ($p > 0.05$). Termination of pregnancy in Group H in the first trimester had 51.25% of the patients, in the second – 34.17%, in the third – 14.58%.

The presence of polymorphisms of IL-4, IL-4R, IL-17A and IL-17F, we have studied on the basis of the total (Table 1) and a multiplicative model of inheritance (Table 2).

As seen from Table 1, both the genotype CT^{-33} (OR 3.99; 95%

Table 1. Total inheritance model (χ^2 test, df = 2)

SNP	Genotype	Group R (n = 240)	Group K (n = 100)	χ^2	p	OR (95% CI)
IL-4 -33C/T rs2070874	CC	0.358	0.650	37.39	8.0E-9	0.30 (0.18–0.49)
	CT	0.642	0.310			3.99 (2.42–6.57)
	TT	0.000	0.040			0.04 (0.00–0.84)
IL-4 -589C/T rs 2243250	CC	0.358	0.650	37.39	8.0E-9	0.30 (0.18–0.49)
	CT	0.642	0.310			3.99 (2.42–6.57)
	TT	0.000	0.040			0.04 (0.00–0.84)
IL-4R Q576R rs 180275	AA	0.513	0.740	17.04	0.0002	0.37 (0.22–0.62)
	AG	0.379	0.240			1.93 (1.14–3.28)
	GG	0.108	0.020			5.95 (1.39–25.58)
IL-17A G197A rs227593	GG	0.613	0.540	5.31	0.07	1.35 (0.84–2.16)
	GA	0.304	0.420			0.60 (0.37–0.98)
	AA	0.083	0.040			2.18 (0.73–6.55)
IL-17F 488T/C rs763780	TT	0.450	0.260	15.84	0.0004	2.33 (1.39–3.91)
	CT	0.463	0.700			0.37 (0.22–0.61)
	CC	0.087	0.040			2.27 (0.76–6.84)

Table 2. The multiplicative model of inheritance (χ^2 test, df = 1)

SNP	Allele	Group R (n = 240)	Group K (n = 100)	χ^2	p	OR (95% CI)
IL-4 -33C/T rs2070874	C	0.679	0.805	11.00	0.0009	0.51 (0.34–0.76)
	T	0.321	0.195			1.95 (1.31–2.91)
IL-4 -589C/T rs 2243250	C	0.679	0.805	11.00	0.0009	0.51 (0.34–0.76)
	T	0.321	0.195			1.95 (1.31–2.91)
IL-4R Q576R rs 180275	A	0.702	0.860	18.70	2.0E-5	0.38 (0.25–0.60)
	G	0.298	0.140			2.61 (1.67–4.07)
IL-17A G197A rs227593	G	0.765	0.750	0.16	0.68	1.08 (0.74–1.59)
	A	0.235	0.250			0.92 (0.63–1.36)
IL-17F 488T/C rs763780	T	0.682	0.610	3.21	0.07	1.37 (0.97–1.93)
	C	0.318	0.390			0.73 (0.52–1.03)

CI 2.42–6.57) and CT^{-589} (OR 3.99; 95% CI 2.42–6.57) IL-4 gene; a genotype GG^{576} (OR 5.95; 95% CI 1.39–25.58) gene IL-4R and genotypes TT^{488} (OR 2.33; 95% CI 1.39–3.91) and the CC^{488} (OR 2.27; 95% CI 0.76–6.84) IL-17F gene were associated with a significant increase in the risk of miscarriage. On the other hand we found no significant differences between the study and control group in frequency of polymorphisms IL-17A-G197A (rs227593).

In the analysis of the frequency of Alella in women with RM and physiological pregnancy was revealed statistic positive association with reproductive losses and alleles: T^{-33} (0.321 vs. 0.195; OR 1.95; 95% CI 1.31–2.91); T^{-589} (0.321 vs. 0.195; OR 1.95; 95% CI 1.31–2.91); G^{Q576R} (0.298 vs. 0.140; OR 2.61; 95% CI 1.67–4.07) (Table 2). The protective value of child bearing alleles were C^{-33} (0.805 vs. 0.679, p <0.0009), C^{-589} (0.805 vs. 0.679, p <0.0009) and G^{Q576R} (0.860 vs. 0.702, p <2.0E-5).

CONCLUSIONS

1. The results of the molecular-genetic typing of IL-4, IL-4R, IL-17A and Il-17F possible to identify markers in the Eastern Slavic population immunogenetic susceptibility/resistance to the development of recurrent miscarriage.

2. Typing of cytokine genes can be used as a method of early diagnosis and prediction of Pregravidar immune forms of reproductive loss in women.

РОЛЬ ПОЛИМОРФИЗМОВ ГЕНОВ IL-4 И IL-17 В ПРИВЫЧНОМ НЕВЫНАШИВАНИИ БЕРЕМЕННОСТИ, НАСТУПИВШЕЙ В ЦИКЛАХ ВРТ

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ВВЕДЕНИЕ

Большинство исследователей подтверждают, что свыше 50% беременных после ВРТ сталкиваются с проблемой невынашивания беременности, особенно в первом триместре. Привычное невынашивание беременности (ПНБ) является генетически гетерогенным состоянием в результате сосуществования двух регулирующих факторов: материнского и эмбрионального [1, 2]. Плод экспрессирует антигены, унаследованные от обоих родителей, и выживание полу-аллотрансплантантного плода до срока является одним из наиболее сложных процессов, связанных с беременностью. Иммунная система матери играет свою важную роль в успешной беременности, контролируя оплодотворение, имплантацию, развитие и поддержание самой беременности [3–5]. Успешная беременность зависит от поддержания тонкого баланса между Th-1, Th-2, Th-17 и Treg-клетками иммунной системы, а также между присутствующими в них специфическими цитокинами [6, 7].

АНАЛИЗ ЛИТЕРАТУРНЫХ ДАННЫХ И ПОСТАНОВКА ЗАДАЧИ ИССЛЕДОВАНИЯ

Цитокины являются клеточными сигнальными белками, в основном секретируемыми иммунными клетками и медиированными клеточно-клеточными коммуникациями [8]. Выявлено, что полиморфизмы генов цитокинов могут быть связаны с ПНБ.

Известно, что одними из основных цитокинов, участвующих в вынашивании беременности, являются интерлейкин-4 (IL-4) и интерлейкин-17 (IL-17). Клетки человека Th17, продуцирующие IL-17, могут играть важную роль в отвержении антигенов концептуса и, следовательно, могут вредить сохранению беременности [9]. Показано, что IL-17-продуцирующие децидуальные CD4+T-клетки не являются вредными для беременности женщины, когда они также продуцируют IL-4 [10]. Поэтому особый интерес представляет изучение особенностей полиморфизмов генов IL-4 и IL-17 у женщин с ПНБ.

IL-4 регулирует рост и дифференцировку В-лимфоцитов, а также процессы биосинтеза и секреции антител. Он продуцируется активированными CD4+T-лимфоцитами (Th2), тучными клетками и эозинофилами, но в основном – активированными Th2 клетками. IL-4 стимулирует дифференцировку Th0 в Th2, подавляет генерацию цитотоксических лимфоцитов, натуральных киллеров, а также продукцию интерферона-γ и противоопухолевую активность макрофагов, уменьшает выработку провоспалительных цитокинов IL-1 и TNFα [11, 12]. При наличии генетически обусловленных нарушений в иммунной системе происходит из-