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THE ROLE OF RIG-I, AIM2 AND IFI16 RECEPTORS FOR VIRAL RNA AND DNA IN THE PATHOGENESIS OF SPONTANEOUS AND EARLY MISSED MISCARRIAGE

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Aim *To identify the features of the expression of mRNA of intracellular RIG-I, IFI16 and AIM2 receptors for viral RNA and DNA, and their signaling pathway proteins in the decidual tissue of patients with spontaneous and early missed miscarriages.*

Material and methods *The study comprised 68 patients with sporadic miscarriages, including 34 women with missed and 34 with spontaneous miscarriages at 6 to10 weeks of gestation. The control group included 57 patients who underwent surgical termination of pregnancy at the same gestational age. Patients with severe non-obstetric comorbidities, antiphospholipid syndrome, endocrine causes of miscarriage, and confirmed fetal chromosomal abnormalities were excluded from the study. Decidual tissues were collected by uterine curettage. Expression of RIG-I, IFI16 and AIM2 mRNA and their signaling pathway proteins (ASC, TBK1, STING, caspase-1, interleukin-1β) was analyzed using quantitative polymerase chain reaction.*

Results *Patients with missed miscarriages had a seven-fold increase in the expression of decidual AIM2 mRNA protein detecting intracellular viral and Listeria's DNA. This elevation was accompanied by an increase in the caspase-1 and IL-1β mRNA expression resulting in the initiation of an inflammatory response and pyroptotic cell death. Patients with missed miscarriages did not have significant changes in the expression of RIG-I and IFI16 mRNA, and their signaling pathway proteins. No changes in AIM2, RIG-I, and IFI16 mRNA expression and their signaling pathway proteins were observed in patients with spontaneous miscarriage.*

Conclusion *Patients with missed miscarriages have an increased decidual expression of the AIM2 receptor for viral and Listeria's DNA. This increase is accompanied by an increase in the caspase-1 and IL-1β mRNA expression, leading to the initiation of a pro-inflammatory response and cell death.*

Keywords: *undeveloped pregnancy, spontaneous miscarriage, missed miscarriage, AIM2, caspase, interleukin-1β.*

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Inflammatory diseases are one of the leading causes of early spontaneous miscarriage [1]. A large systematic review demonstrated an association of cytomegalovirus and influenza virus infections with an increased risk of miscarriage, while the effects papillomavirus, parvovirus B19, and herpes simplex virus infections remain controversial [2]. At the same time, not all patients with the above viral infections experience miscarriage, which indicates the role of the immune system in the miscarriage pathogenesis.

Cell receptors of the innate immune system are known to detect the conserved structures of viruses, bacteria, fungi, and protozoa and trigger an immune response, including activation of acquired immunity cells (T and B lymphocytes) [3-6].

Among receptors detecting foreign DNAs, including viral DNAs, are Toll-like receptors (TLRs) 9 located on endosomal membranes and cytosolic IFI16 and AIM2 receptors. Receptors that recognize viral RNA are TLR3 (ligand-double-stranded viral RNA), TLR7 and TLR8 (ligand-single-stranded viral RNA). Also, the double-stranded viral RNA also recognizes the RIG-I receptor.

Some studies reported a role of TLR, activated by viral ligands, in the pathogenesis of early pregnancy loss [7-10]. However, current literature is lacking studies on the features of the expression of other intracellular receptors capable of recognizing viral DNA and RNA - AIM2, IFI16, and RIG-I.

AIM2, IFI16, and RIG-I are intracellular receptors that detect viral nucleic acids and their own damaged DNAs [11] (Figure). The cytosolic DNA stimulates the AIM2 receptor, which triggers the caspase-1- and ASC — dependent activation of the inflammasome, leading to the production of interleukin-1β (IL-1β), and initiation of the inflammatory cascade. A distinctive feature of all inflammasomes is their ability to exist even after the cause of its activation is no longer present. This contributes to the prolonging and maintaining the inflammatory process. The IFI16 protein is also capable of recognizing cytosolic DNAs, followed by inflammasome activation. Also, it induces the synthesis of type I interferons (interferon-α and interferon-β) by activating the stimulator of interferon genes (STING),

and TBK-1. RIG-I recognizes double-stranded viral RNA and then drives type I interferons production through the TBK1 pathway.

This work was aimed to identify the features of the expression of mRNA of intracellular RIG-I, IFI16 and AIM2 receptors for viral RNA and DNA, and their signaling pathway proteins in the decidual tissue of patients with spontaneous and early missed miscarriages.

Material and methods

The study comprised 68 patients with sporadic miscarriage, including 34 women with missed and 34 with spontaneous miscarriage at 6 to10 weeks of gestation. The control group included 57 patients who underwent surgical termination of pregnancy at the same gestational age. Patients with severe non-obstetric comorbidities, antiphospholipid syndrome, endocrine causes of miscarriage, and confirmed fetal chromosomal abnormalities were excluded from the study.

The material was decidual tissues collected by uterine curettage. The nature of the tissue was confirmed histologically. The decidual tissue was placed in RNAlater RNA-stabilizing solution (Ambion, USA). RNA extraction and reverse transcription were performed as described previously [10].

Expression of mRNA of RIG-I, IFI16 and AIM2 receptors and their signaling pathway proteins (ASC, TBK1, STING, caspase-1, interleukin-1β) was analyzed using quantitative polymerase chain reaction (PCR) according to the MIQE guidelines [12]. The gene specific primers were designed using the BLAST software (www.ncbi.nlm.nih.gov). Quantitative real-time-PCR (qRT-PCR) was performed using a qPCRmix-HS SYBR mixture (Eurogen, Russia). The most stably expressed peptidylprolyl isomerase A (PPIA) and β-actin were used as housekeeping genes for real-time PCR.

Amplification was performed on a CFX96 BioRad machine (Biorad, USA) as described previously [10]. The primer sequences and the annealing temperatures are shown in Table. 1.

The results were expressed as a ratio by using the following equation: $R = 2 - ^{(Cq\ target - (Cq\ ref1+Cqref2)/2))}$, where R is a normalized mRNA expression of genes under investigation; Cq ref1, and Cq ref2 - Cq of housekeeping genes; Cq target -Cq of the gene under investigation [13].

Statistical analysis was performed using the Statistica 13.1 software (Statsoft, USA). The normality of the dis-

Table 1. Primer sequences used for analysis of mRNA expression

Gene	Forward primer	Reverse primer	Annealing temperature, °C
RIG-I	CCGGCCTCATTTCCTCAAAAA	CCCCTTTTGTCCTTGTGGGA	55
IFI16	AACTAAGTGACTCAACCAAGGC	CACTGTTTTCGGGTTCTGAGC	57
AIM2	TGGGGGTGAAGGGAAGTGTTT	AACTTTGGGATCAGCCTCCTG	56
TBK1	AGCCGGAAGTGTCCTGAGTC	CCCACCACATCTCGCAAAAC	57
STING	CCTGCATCCATCCATCCCGT	GCGGCAGTTGTTCTGAGACT	59
ASC	ATCCAGGCCCCTCCTCAG	AGAGCTTCCGCATCTTGCTT	56
IL-1β	TGAGCTCGCCAGTGAAATGA	AACACGCAGGACAGGTACAG	57
Caspase -1	GGGAGTGTGGGAAGGTTGAG	GTGCCAATAGCCAGTTTGGG	55
β- actin	CAGGCACCAGGGCGTGATGG	GATGGAGGGGCCGGACTCGT	64
PPIA	CCGCCGAGGAAAACCGTGTACT	TGGACAAGATGCCAGGACCCGT	64

Table 2. Expression of RIG-I, IFI16, and AIM2 mRNA and their signaling pathway proteins in women with missed miscarriage and early spontaneous miscarriage, ratio, Me (25%;75%)

Gene	1. Patients with missed miscarriage (n=34)	2. Patients with spontaneous miscarriage (n=34)	3. Control group (medical abortion) (n=57)	P_{1-3}	P_{2-3}
RIG-I	0.20 (0.09; 0.66)	0.34 (0.03; 0.68)	0.40 (0.21; 1.07)	>0.05	>0.05
IFI16	0.17 (0.06; 1.16)	0.28 (0.038; 0.60)	0.17 (0.05; 0.57)	>0.05	>0.05
TBK1	0.002 (0.0002; 0.006)	0.0008 (0.000006; 0.027)	0.0002 (0.000004; 0.0009)	>0.05	>0.05
STING	0.08 (0.02; 0.21)	0.16 (0.011; 0.36)	0.05 (0.006; 0.36)	>0.05	>0.05
AIM2	0.14 (0.02; 0.67)	0.11 (0.011; 0.75)	0.02 (0.006; 0.12)	<0.05	>0.05
ASC	0.02 (0.001; 0.10)	0.03 (0.006; 0.13)	0.01 (0.001; 0.06)	>0.05	>0.05
IL-1β	0.008 (0.0001; 0.033)	0.0003 (0.000001; 0.03)	0.00005 (0.000001; 0.003)	<0.01	>0.05
Каспаза-1	0.04 (0.006; 0.12)	0.01 (0.005; 0.105)	0.013 (0.004; 0.027)	<0.05	>0.05

tribution was tested by the Kolmogorov-Smirnov test. Variables showing normal distribution were compared by the Student's test for two independent samples and the results were presented as M ± m. If the distribution of the data was not normal, the nonparametric Mann-Whitney U-test was used and the results were presented as a median (lower quartile, upper quartile). Differences with $p < 0.05$ were considered statistically significant.

Results and discussion

Clinical evaluation of the study participants included an analysis of general health history, pre-pregnancy health status, age, social and economic well-being, occupational hazards, menstrual function characteristics, reproductive history, and morphometric parameters.

No significant differences between study groups were observed concerning age and gestational age. The mean age of patients with missed miscarriage, spontaneous miscarriage, and the patients of the control group was 27.88 ± 2.31, 27.05 ± 2.01, and 26.46 ± 1.98 years, respectively. All women were residents of the city of Belgorod and had secondary and higher education. No occupational hazards were identified. There were no significant differences in the age of menarche and menstrual cycle duration. The number of past pregnancies and births in patients of all study groups was similar. However, the number of miscarriages was significantly higher among patients with missed miscarriage (0.72 ± 0.48) and spontaneous miscarriage (0.81 ± 0.59) compared with the control group (0.20 ± 0.16) ($p < 0.05$).

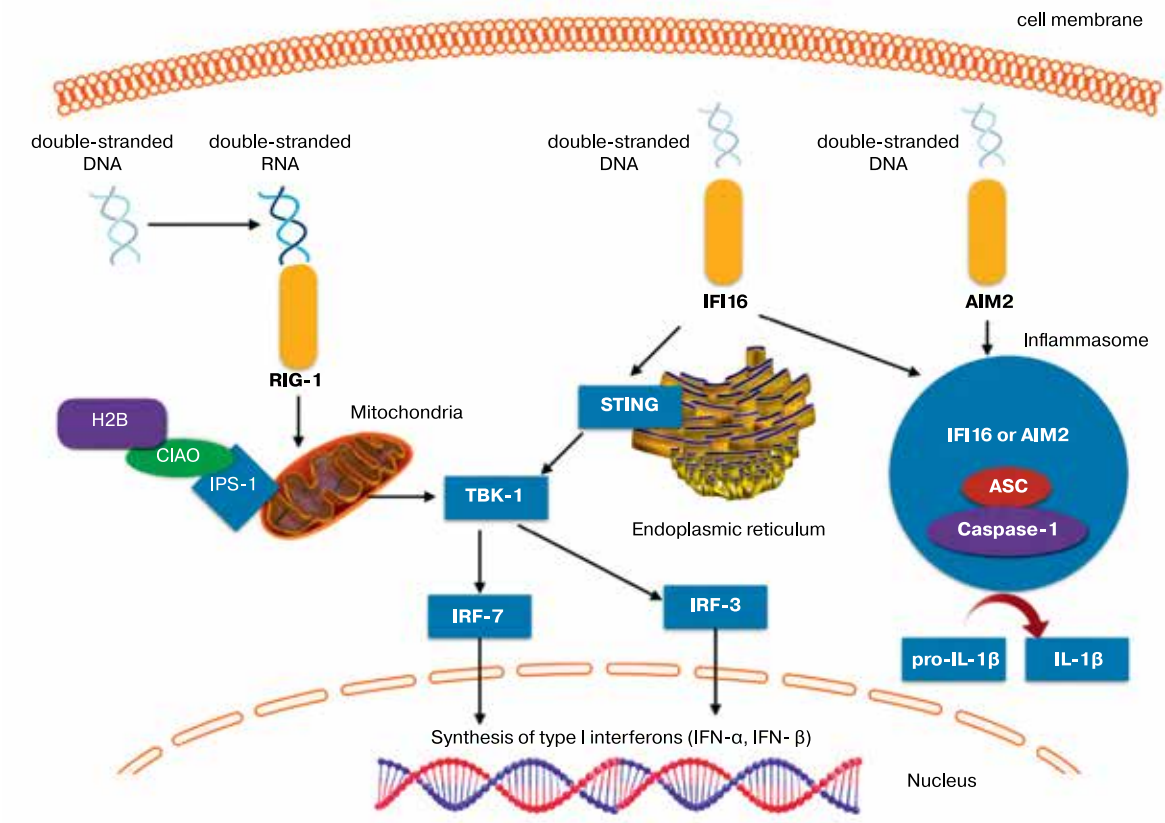
The morphometric parameters (height, weight, body mass index) showed no significant differences between the groups.

Patients with missed miscarriage had a seven-fold increase in the expression of AIM2 mRNA receptor compared with the control group, which was accompanied by an increase in the expression of caspase-1 and IL-1β (Table 2). The expression of decidual RIG-I and IFI16 mRNA, and their signaling pathway proteins in patients with missed miscarriage did not differ significantly from that in the control group.

Cytosolic AIM2 receptor recognizes both viral DNA and DNA of *Listeria*, which are persistent intracellular bacteria [14, 15]. AIM2 binds to corresponding ligands and forms an inflammasome to activate caspase-1[16]. In turn, activation of caspase-1 leads to an increase in IL-1β thus triggering an inflammatory response. IL-1β activates the innate immunity cells (neutrophils, macrophages, natural killers) and Th17 lymphocytes, which exerts a cytotoxic effect [17]. Another property of caspase-1 is the ability to induce pyroptotic cell death [18]. Apparently, this mechanism has a role in the pathogenesis of missed miscarriage.

The expression of AIM-2, IFI16, RIG-I mRNA and their signaling pathway proteins showed no significant differences in patients with spontaneous early miscarriage. This indicates that these receptors have no role in the pathogenesis of this pregnancy complication.

Figure. The intracellular RIG-I, AIM2 and IFI16 receptors for viral nucleic acids and their signaling pathways



Conclusion

Patients with missed miscarriages have an abnormally high decidual expression of the AIM2 receptor for viral and *Listeria's* DNA. This elevation is accompanied by an increase in the caspase-1 and IL-1β mRNA expression, triggering a pro-inflammatory response and pyroptotic cell death.

Patients with spontaneous miscarriage have no increase in the expression of AIM2, RIG-I, IFI16 mRNA and their signaling pathway proteins, indicating that these receptors do not have a significant role in spontaneous miscarriage pathogenesis.

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