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Expression of inhibitory receptors PD-1, CTLA-4, and Tim-3 by peripheral T cells during pregnancy

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ABSTRACT

Background. Inhibitory receptors and their ligands (also called checkpoint molecules) are important feedback regulators of the immune response. However, their role in immunological adaptation during pregnancy remains poorly understood.

The aim of the study was to evaluate the level of checkpoint molecule (PD-1, CTLA-4, Tim-3) expression in peripheral T cells in pregnant women compared with fertile non-pregnant women.

Materials and methods. The study included 36 women in the second half of pregnancy without pregnancy complications, 12 of whom had extragenital pathology. The control group consisted of 28 age-matched fertile non-pregnant women. The proportion of $CD8^+PD\text{-}1^+$, $CD8^+TIM\text{-}3^+$, $CD8^+PD\text{-}1^+TIM\text{-}3^+$, $CD4^+PD\text{-}1^+$, $CD4^+TIM\text{-}3^+$, and $CD4^+PD\text{-}1^+TIM\text{-}3^+$ was evaluated by flow cytometry using the corresponding monoclonal antibodies (BD Biosciences, USA).

Results. The proportion of CD4+Tim-3+ and CD8+PD-1+ T cells and CD4+ and CD8+ T lymphocytes co-expressing PD-1 and Tim-3 in the peripheral blood of pregnant women was statistically significantly higher than in non-pregnant women. An increase in CD4+Tim-3+ and CD8+PD-1+ T cells was observed both in pregnant women with and without extragenital pathology. However, pregnant women with extragenital pathology were characterized by a higher CD8+PD-1+ count and a smaller number of CD8+Tim-3+ cells, as well as by a lack of an increase in PD-1+Tim-3+ T cells typical of pregnant women. The number of comorbidities was directly correlated with the proportion of CD8+PD-1+ lymphocytes and inversely correlated with the proportion of CD8+Tim-3+ and CD4+ PD-1+Tim-3+ cells. In addition, the expression of checkpoint molecules was associated with gestational age (a direct correlation was found with the proportion of CD8+Tim-3+, CD4+PD-1+Tim-3+, and CD8+PD-1+Tim-3+ cells) and to a lesser extent – with the age of pregnant women (an inverse relationship was found with the proportion of CD8+Tim-3+ cells).

Conclusion. Pregnant women in the second half of pregnancy are characterized by increased expression of PD-1 and Tim-3 molecules in peripheral T cells. At the same time, concomitant extragenital pathology affects the expression of these molecules.

Keywords: T cells, pregnancy, inhibitory PD-1, TIM-3, CTLA-4 checkpoint molecules

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Экспрессия ингибиторных рецепторов PD-1, CTLA-4 и Tim-3 периферическими Т-клетками при беременности

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РЕЗЮМЕ

Актуальность. Ингибиторные рецепторы и их лиганды (чек-поинт молекулы) являются негативными регуляторами иммунного ответа. Однако их роль в иммунной адаптации при беременности остается малоизученной.

Цель исследования – оценить уровень экспрессии чек-поинт молекул (PD-1, CTLA-4, Tim-3) на периферических Т-клетках у беременных в сравнении с фертильными небеременными женщинами.

Материалы и методы. В исследование были включены 36 женщин во второй половине беременности без гестационных осложнений, у 12 из которых имелась экстрагенитальная патология. Контрольную группу составили 28 сопоставимых по возрасту фертильных небеременных. Относительное содержание $CD8^+PD\text{-}1^+$, $CD8^+TIM\text{-}3^+$, $CD8^+PD\text{-}1^+TIM\text{-}3^+$, $CD4^+PD\text{-}1^+$, $CD4^+TIM\text{-}3^+$, $CD4^+PD\text{-}1^+TIM\text{-}3^+$ Т-клеток крови оценивали методом проточной цитометрии с использованием соответствующих моноклональных антител (BDBiosciences, США).

Результаты. Относительное содержание CD4+Tim-3+ и CD8+PD-1+ Т-клеток, а также CD4+ и CD8+ Т-лимфоцитов, коэкспрессирующих PD-1 и Tim-3 в периферической крови беременных, статистически значимо превышало аналогичные показатели у небеременных. Возрастание CD4+Tim-3+ и CD8+PD-1+ Т-клеток регистрировалось как у беременных с наличием, так и отсутствием экстрагенитальной патологии. Однако беременные с экстрагенитальной патологией отличались более высоким содержанием CD8+PD-1+ и меньшим количеством CD8+Tim-3+ клеток, а также отсутствием (характерного для беременных) возрастания PD-1+Tim-3+ Т-клеток. Количество сопутствующих патологий прямо коррелировало с долей CD8+PD-1+ лимфоцитов и обратно – с долей CD8+Tim-3+ и CD4+PD-1+Tim-3+ клеток. Кроме того, экспрессия чек-поинт молекул ассоциировалась со сроком гестации (прямая корреляция выявлялась с содержанием CD8+Tim-3+, CD4+PD-1+Tim-3+ и CD8+PD-1+Tim-3+ клеток) и в меньшей степени с возрастом беременных (обратная зависимость с долей CD8+Tim-3+ клеток).

Заключение. Беременные во второй половине гестации характеризуются повышенной экспрессией молекул PD-1 и Tim-3 на периферических Т-клетках. При этом сопутствующая экстрагенитальная патология влияет на характер экспрессии указанных молекул.

Ключевые слова: Т-клетки, беременность, ингибиторные PD-1, TIM-3, CTLA-4 чек-поинт молекулы

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Inhibitory receptors and their ligands, collectively referred to as inhibitory checkpoint molecules, belong to the category of signaling molecules. They mediate various immunosuppressive mechanisms and play an important role in suppressing the immune response and forming tolerance to autoantigens, and in pathology, they inhibit the immune response against tumors and viral infections [1]. The most studied inhibitory T cell receptors are cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death protein-1 (PD-1), and T cell immunoglobulin and mucin-domain containing-3 (TIM-3). Triggering signaling pathways during the interaction of these receptors with the corresponding ligands (CTLA4 / CD80/86, PD-1 / PD-L1, TIM-3 / Gal-9) leads to suppression of effector T cells, skews the Th1 / Th2 cytokine balance toward Th2 [2, 3], and, on the other hand, increases activity and expansion of regulatory T cells (Treg) [4]. In addition, increased expression and co-expression of checkpoint molecules characterize a dysfunctional state known as T cell exhaustion, reflecting a progressive decrease in the functional activity of T lymphocytes during the transition of effector T cells to memory cells [5, 6].

The study of checkpoint molecule expression during pregnancy is of particular interest, since successful bearing of a semi-allogeneic fetus requires significant restructuring of the immune system aimed at inducing tolerance to fetal antigens [7]. The molecular mechanisms of such rearrangement remain understudied. However, dynamic changes in the Th1 / Th2 cytokine balance, limited cytotoxic potential of CD8+ T cells, Treg induction, and the recently described signs of T cell depletion in uncomplicated pregnancy suggest an important role of checkpoint molecules in the immunological adaptation during gestation [8]. Recent studies in experimental animals and humans have shown that decidual T cells during pregnancy are characterized by increased expression and co-expression of CTLA-4, PD-1, and Tim-3, while antigen-presenting and stromal cells localized in the decidua highly express ligands of these receptors [8].

It has been shown that increased expression of inhibitory receptors by decidual T cells can be induced by HLA-C and HLA-G molecules on trophoblasts [9]. At the same time, data on the expression of inhibitory molecules on peripheral T cells and their role in pregnancy remain poorly understood and ambiguous. Meanwhile, it should be noted that fetal antigens that enter the lymph nodes and spleen with the bloodstream can activate T cells, so tolerance should be maintained not only at the local, but also at the systemic level.

Inducers of the expression of inhibitory receptors on circulating T cells can be interleukin (IL)-10, progesterone, and vascular endothelial growth factor (VEGF) [3, 10]. Our studies also showed that one of the modulators of the functions of activated T cells, which can enhance the expression of checkpoint molecules, is placental growth factor (PlGF), the concentration of which drastically increases during pregnancy [11].

Given these facts, we hypothesized that the expression of checkpoint molecules on peripheral T cells during pregnancy may also change, reflecting the restructuring of the immune system at the systemic level. The aim of this study was to test this hypothesis.

MATERIALS AND METHODS

The study included 36 pregnant women without pregnancy complications, who were examined at City Clinical Hospital No. 1, and 28 fertile non-pregnant women (Table 1). The study was carried out in accordance with the ethical principles of the Declaration of Helsinki developed by the World Medical Association “Ethical Principles for Medical Research Involving Human Subjects” amended in 2000 and “Rules of Clinical Practice in the Russian Federation” approved by the order of the Ministry of Health of the Russian Federation No. 266 of 19.06.2003.

The age of women in the group with uncomplicated pregnancy ranged from 18 to 45 years (median (*Me*) 27 years) and did not differ significantly from that in the group of fertile non-pregnant women (22 to 45 years, *Me* 31 years; $P_U = 0.11$). Gestational age ranged from 26 to 40 weeks with a median of 36 weeks. In 20 (55.6%) women, the pregnancy was the first, 16 (44.4%) women had repeat pregnancy. Concomitant extragenital pathology (arterial hypertension, including gestational hypertension; swelling during pregnancy; diabetes mellitus, including gestational diabetes; obesity; pathology of hemostasis;

thyroid disease, history of hypothyroidism; chronic kidney disease without exacerbations) was detected in 12 out of 36 (33 .3%) pregnant women. Diabetes mellitus was registered in 58.3% (7 / 12) of cases, arterial hypertension and obesity were observed in 33.3% (4 / 12) of women, pathology of hemostasis – in 25% (3 / 12) of patients, and a history of thyroid disease – in 16 .6% (3/12) of cases. Swelling was observed in 33.3% (4 / 12) of women and chronic pyelonephritis – in 8.3% (1/12) of cases. The presence of four pathologies simultaneously was recorded in one pregnant woman (8.3%), three pathologies – in four patients (33.3%), and two pathologies – in two pregnant women (16.7%). In the remaining 5 cases, the presence of one extragenital pathology was noted. At the time of the examination, concomitant chronic diseases were in remission. All pregnancies in this study were singleton gestations. None of the participants had active labor at the time of enrollment and blood sampling.

Peripheral blood mononuclear cells (MNCs) were isolated by standard centrifugation of heparinized venous whole blood in the ficoll – verografin density gradient (ρ = 1.078). Erythrocyte lysis was performed with the VersaLyse lysing solution (Beckman Coulter, France) according to the instructions. Relative counts of CD8+CTLA-4+, CD8+PD-1+, CD8+TIM-3+, CD8+PD-1+TIM-3+, CD4+CTLA-4+, CD4+PD-1+, CD4+TIM-3+, and CD4+PD-1+TIM-3+ were estimated by flow cytometry using anti-CD8 (FITC), anti-CD4 (FITC, PerCP), anti-CTLA-4 (PE), anti-PD-1 (APC), and anti-TIM-3 (PE, PerCP / Cy 5.5) monoclonal antibodies (BD Biosciences, USA). The study was carried out according to the generally accepted method using the parameters of forward and side light scatter and fluorescence in the FL-1 (FITC), FL-2 (PE), FL-3 (PerCP, PerCP/Cy 5.5, PE-Cy 5), and FL-4 (APC) channels (BD FACSCalibur, CellQuest Software, USA). We focused on the evaluation of PD-1-expressing T cells, their counts in CD4+ and CD8+ cell subsets were studied in all 36 pregnant and 28 fertile non-pregnant controls. In addition, the expression of CTLA-4 (18 pregnant women and 20 non-pregnant women) and Tim-3 (19 pregnant women and 26 non-pregnant women), as well as the co-expression of PD-1 and Tim-3 (14 pregnant women and 16 non-pregnant women) were evaluated.

Statistical data processing was performed using the Statistica 6.0 (StatSoft) and GraphPad Prism 5 (GraphPad Software, Inc.) software packages. The Mann – Whitney U test was used to assess the significance of differences between two independent groups. Statistical differences between dependent groups were analyzed by the paired Wilcoxon signed rank test. The Spearman's rank correlation coefficient (Rs) was used to evaluate correlations. The data were presented as the median and the interquartile range Me (Q_1–Q_3). The differences were considered statistically significant at $p < 0.05$ (two-tailed).

RESULTS

A comparative analysis of the relative counts of PD-1+ cells in CD4+ T lymphocytes did not reveal significant differences between the groups of pregnant and fertile non-pregnant women (Fig.). At the same time, the proportion of PD-1+ cells in CD8+ T lymphocytes in pregnant women was 2.4 times higher than in the group of non-pregnant women. The proportion of PD-1+ cells in CD4+ lymphocytes was significantly higher than in CD8+ lymphocytes. This was manifested through a pronounced trend in the non-pregnant group ($p_w = 0.09$) and a statistically significant difference in the pregnant group ($p_w = 0.004$). The study of CTLA-4 expression did not reveal any differences in the content of CD4+CTLA-4+ and CD8+CTLA-4+ T lymphocytes in the compared groups ($p_w = 0.16$ and $p_w = 0.19$; respectively). At the same time, evaluation of Tim-3+ cells revealed a 5-fold increase in the proportion of Tim-3+ cells in CD4+ T lymphocytes in pregnant women compared with the non-pregnant group.

The percentage of Tim-3+ cells among CD8+ T lymphocytes in the compared groups did not differ (Fig.). Given the increased expression of PD-1+ and Tim-3+ on T cells, it seemed important to study the co-expression of these molecules. As seen from the data in the Figure, the proportion of PD-1+Tim-3+ cells in CD4+ T lymphocytes in pregnant women was 5 times higher than in the control group. Similarly, the relative count of CD8+ cells co-expressing PD-1 and Tim-3 in pregnant women was 2.3 times higher than in non-pregnant women.

It should be noted that, despite the absence of late pregnancy complications, especially preeclampsia, women with uncomplicated pregnancy differed in the presence of concomitant extragenital pathologies, which were detected in every third pregnant woman (see Materials and methods section). To find out whether the increased expression of inhibitory receptors on T cells had been associated with comorbidity, at the next stage we compared the counts of T cells expressing checkpoint molecules in the groups of pregnant women with and without extragenital pathology (Table 1).

Table 1

Expression of inhibitory receptors in subsets of T cells in pregnant women with and without comorbidities, *Me* (Q_1–Q_3)

Parameter	Fertile non-pregnant women	Pregnant women without extragenital pathology	Pregnant women with extragenital pathology
Clinical parameters		*n* = 24	*n* = 12
Age, years		26 (20–32)	29 (26–33)
Gestational age, weeks		34 (32–37)	35 (32–37)
Gravidity, times		1 (1–3)	1.5 (1–2)
Parity, times		1 (1–1)	1 (1–2)
T cell subsets, %			
$CD4^+PD\text{-}1^+$	4.1 (3.0–5.9) *n* = 28	4.8 (3.0–7.4) *n* = 24	6.6 (2.5–9.6) *n* = 12
$CD4^+CTLA\text{-}4^+$	2.6 (1.3–4.0) *n* = 20	3.8 (2.0–4.0) *n* = 10	2.4 (2.1–2.8) *n* = 8
$CD4^+Tim\text{-}3^+$	1.4 (1.0–2.9) *n* = 24	6.5 (1.6–8.6)* *n* = 14	9.7 (6.9–13)* *n* = 5
$CD8^+PD\text{-}1^+$	2.9 (1.1–5.9) *n* = 26	5.8 (4.7–8.3)* *n* = 24	10 (6.9–15)*# *n* = 12
$CD8^+CTLA4^+$	2.0 (0.9–3.4) *n* = 20	1.2 (0.5–4.0) *n* = 10	3.4 (1.3–5.5) *n* = 8
$CD8^+Tim\text{-}3^+$	5.3 (2.5–9.2) *n* = 26	5.8 (2.3–19) *n* = 14	1.1 (0.6–1.9)*# *n* = 5
$CD4^+PD1^+Tim3^+$	0.35 (0.19–0.5) *n* = 16	2.1 (1.6–2.8)* *n* = 10	0.27 (0.18–1.5) *n* = 4
$CD8^+PD1^+Tim3^+$	1.5 (0.7–2.3) *n* = 16	3.9 (2.2–4.5)* *n* = 10	0.8 (0.6–3.5) *n* = 4

* $p_U < 0.05$ – statistically significant differences compared with non-pregnant women; # $p_U < 0.05$ – statistically significant differences between pregnant women with and without extragenital pathology.

There were no significant differences between the studied groups in the age, gestational age, gravidity, and parity. It can also be seen that an increase in the number of CD4+Tim3+ and CD8+PD-1+ cells typical of pregnancy was revealed in both groups. However, it was more pronounced in the group of pregnant women with extragenital pathology, as indicated by a higher count of CD8+PD-1+ cells ($p_U = 0.03$) and a trend toward a higher count of CD4+Tim-3+ cells ($p_U = 0.1$) compared with pregnant women without concomitant pathologies. At the same time, an increase in the count of PD-1+Tim3+ T cells was observed only in pregnant women without a concomitant disease and was not detected in the group of pregnant women with a comorbidity. Another feature of pregnant women with extragenital pathology was a decrease in the count of CD4+Tim-3+ cells, both in non-pregnant women and in pregnant women without a concomitant disease.

Considering that the number of extragenital pathologies in different women varied from 0 to 4, a relationship between the expression of checkpoint molecules and the number of concomitant pathologies per pregnant woman was studied (Table 2). The relative count of CD8+PD-1+ cells was directly correlated with the number of extragenital diseases, while the proportion of CD8+3Tim-3+ cells was inversely correlated with the number of comorbidities. Besides, there were inverse correlations between the proportion of PD-1+Tim-3+Tcells and the number of extragenital diseases (statistically significant for CD4+PD-1+Tim-3+ T cells and as a trend for CD8+PD -1+Tim-3+ T cells).

Table 2

Correlations (Rs) between the expression of checkpoint molecules and the number of concomitant diseases, age, and gestational age

Subset	Number of comorbidities	Age	Gestational age
CD4+PD-1+, *n* = 36	0.25 (0.14)	–0.08 (0.64)	0.19 (0.26)
CD4+CTLA-4+, *n* = 18	–0.19 (0.44)	0.04 (0.86)	0.06 (0.81)
CD4+Tim-3+, *n* = 19	0.34 (0.15)	–0.23 (0.33)	–0.34 (0.14)
CD8+PD-1+, *n* = 36	0.37(0.02)	–0.004 (0.86)	–0.23 (0.18)
CD8+CTLA-4+, *n* = 18	0.2 (0.42)	–0.04 (0.86)	–0.01 (0.95)
CD8+Tim-3+, *n* = 19	–0.58 (0.01)	–0.43 (0.06)	0.5 (0.03)
CD4+ PD-1+Tim-3+, *n* = 14	–0.57 (0.03)	0.05 (0.87)	0.54 (0.04)
CD8+ PD-1+Tim-3+, *n* = 14	–0.51 (0.06)	–0.42 (0.10)	0.61(0.02)

Note: statistical significance of the correlation is presented in the parentheses.

Since the pregnant women included in the study were characterized by a fairly wide age range (from 18 to 45 years), and the gestational age varied from 26 to 40 weeks, we also studied the association of these factors with the expression of checkpoint molecules by T cells. As can be seen from the data presented in Table 2, there were no significant correlations between the relative counts of T cells expressing PD-1, CTLA-4, and Tim-3 and age. However, there were strong trends toward inverse correlations between age and both CD8+ Tim-3+ cells and CD8+PD-1+Tim-3+ cells. At the same time, the relative counts of CD8+-Tim-3+ cells, CD4+PD-1+Tim-3+, and CD8+PD-1+Tim-3+ cells were directly correlated with gestational age.

DISCUSSION

Activation of immunosuppressive mechanisms can be observed from the moment of embryo implantation and throughout the entire pregnancy. However, the role of inhibitory receptors in the implementation of these mechanisms during pregnancy, especially at the systemic level, remains poorly understood.

The present study has shown that women in the second half of uncomplicated pregnancy are characterized by an increased count of circulating CD4+-Tim-3+ and CD8+PD-1+ T cells, as well as CD4+ and CD8+ T lymphocytes co-expressing PD-1 and Tim-3 compared with fertile non-pregnant women, which indicates an increase in the expression of inhibitory receptors on peripheral T cells during pregnancy. Increased expression of inhibitory receptors during pregnancy was described on decidual T cells. It was shown that CD4+PD-1+TIM-3+ T lymphocytes have Th2 phenotype [12], and activation of the PD-1/PD-L1 signaling pathway suppresses production of Th1 cytokines [13]. In turn, increased expression of checkpoint molecules on decidual CD8+ T cells is discussed as one of the mechanisms for reducing the cytotoxic potential of CD8+ lymphocytes [12, 14–16]. Thus, in *in vitro* studies, the interaction of TIM-3 and PD-1 with the corresponding ligands suppresses the cytotoxic activity of CD8+ T cells, which can provide tolerance to fetal antigens [17, 18]. R. Slutsky et al. described CD4+ and CD8+ T cells with the phenotype of effector memory T cells co-expressing PD-1 and Tim-3 in the decidua, and for the first time identified them as exhausted T cells [4]. However, earlier, T cells with a similar phenotype at the initial stages of pregnancy were described by a number of authors as T cells with regulatory (Th2) activity [2].

Few publications describe expression of checkpoint molecules on peripheral T cells. J. Zhao. et al. did not reveal an increase in Tim-3 in CD3+ T lymphocytes of pregnant women [19]. M. Meggyes et al. in their studies also found no differences between the count of CD8+Tim-3+ in pregnant and non-pregnant women, however, CD8+TIM-3+ cells in pregnant women in all trimesters produced less proinflammatory cytokines (TNF-α, IFN-γ) compared with CD8+TIM-3 cells in non-pregnant women [20].

Later, it was shown that the counts of CD4+ and CD8+ T cells expressing PD-1 in the third trimester of pregnancy also did not differ from those in non-pregnant women [21]. These results are consistent with our data on the absence of differences in the counts of CD4+PD-1+ and CD8+TIM-3+ cells between pregnant and non-pregnant women, however, these groups differed in the count of CD4+TIM-3+ and CD8+PD-1+ T cells, which was elevated during pregnancy, according to our data. At the same time, we studied for the first time T cells co-expressing TIM-3 and PD-1 and showed an increase in their relative counts in CD4+ and CD8+ T lymphocytes during pregnancy. The existing discrepancies with the data of the mentioned authors may be due to the differences in the cohorts of the examined pregnant women; additionally, the cytometric analysis in the previous studies was performed in cryopreserved cells.

The second important result of this study is the analysis of checkpoint molecule expression on T cells of pregnant women, depending on the presence or absence of concomitant extragenital pathology. The increase in the number of T cells expressing PD-1 and Tim-3 in both groups suggests that elevated expression of inhibitory receptors on peripheral T cells is a consequence of gestation rather than a comorbidity. On the one hand, this fragment of studies showed that the presence of concomitant pathology affects the pattern of inhibitory molecule expression, in particular, it is associated with a more pronounced increase in the number of CD8+PD-1+ cells (compared with pregnant women without concomitant pathology). On the other hand, comorbidity is associated with a lower content of CD8+Tim-3+ and the absence (characteristic of pregnant women without comorbidity) of an increase in the number of T cells co-expressing PD-1 and Tim-3 molecules. An additional confirmation of the association between comorbidity and the expression of checkpoint molecules is the revealed correlations between the number of comorbidities and the

expression of PD-1 and Tim-3 (a direct correlation with the proportion of CD8+PD-1+ and an inverse correlation with the proportion of CD8+Tim-3+ and CD4+PD-1+Tim-3+ cells).

According to the literature, concomitant extragenital pathologies are usually associated with chronic inflammation and can significantly complicate pregnancy and, in some cases, lead to maternal mortality [22, 23]. Our results demonstrate that concomitant extragenital pathology makes a contribution to it by expressing checkpoint molecules on T cells. At the same time, the multidirectional relationship between the number of comorbidities and subsets of CD8+PD-1+ (positive relationship) and CD8+Tim3+ cells (negative relationship) may indicate a different role of PD-1 and Tim-3 molecules in the regulation of CD8+ cell functions during pregnancy.

Moreover, in this work, it was shown for the first time that the expression of inhibitory receptors on circulating T cells of pregnant women is associated with the age of women and gestational age. Of the three analyzed inhibitory receptors, the relationship with age and gestational age was detected only for Tim-3 and was the most pronounced in relation to its expression on CD8+ cells. According to the data obtained, a higher count of Tim-3+ T cells was noted in younger pregnant women and at longer gestational ages.

CONCLUSION

The data obtained indicate increased expression of a number of inhibitory receptors on peripheral T cells during pregnancy and substantiate the relevance of further research on checkpoint molecules as potential biomarkers in pregnancy with complications.

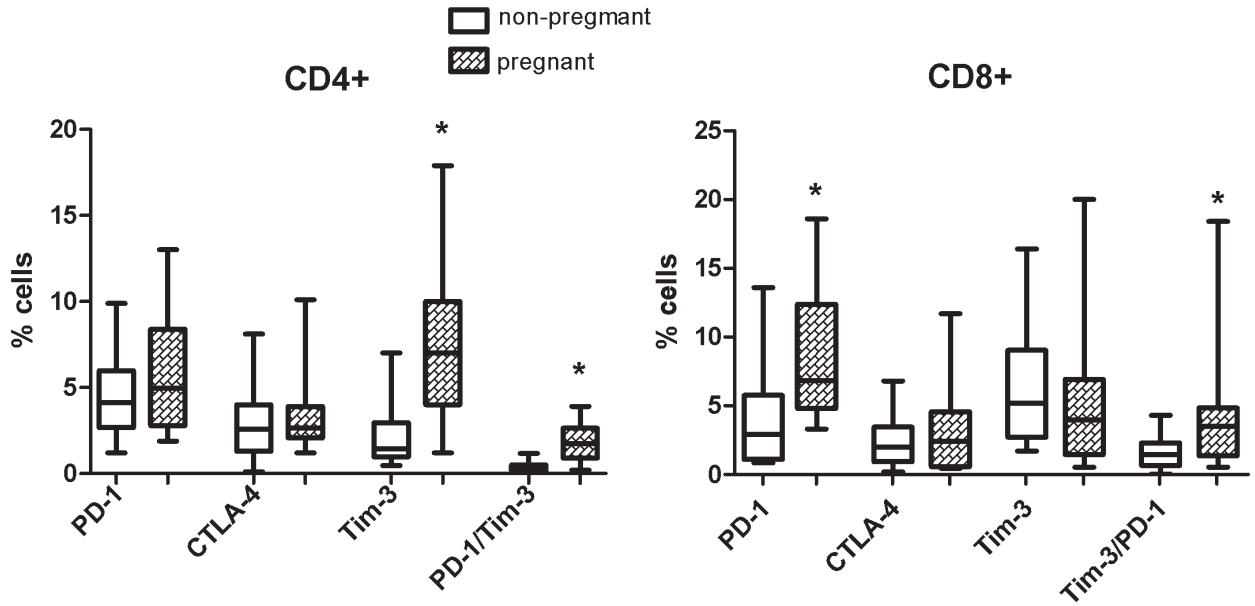

Figure. The content of T cells expressing inhibitory receptors in pregnant women with uncomplicated gestation and fertile non-pregnant women, *Me* (Q_1–Q_3)

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