Effects of pregnancy-specific β-1-glycoprotein on the helper T-cell response

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Abstract: Pregnancy-specific β-1-glycoproteins (PSGs) are capable of regulating innate and adaptive immunity. As fetal antigens circulate in the blood of pregnant women, it is of particular interest to reveal the effects of PSGs on the differentiation of memory T cells in the context of maternal-fetal tolerance formation. We studied if, native PSG preparation affects helper T-cell proliferation, the frequencies of CD4⁺CD45R0⁺, CD4⁺CD45RA⁺, CD4⁺CD45RA⁺CD45R0⁻ cells, naive CD45RA⁺CD45R0⁻CD62L⁺ cells (NAIVE), central memory CD45RA⁺CD45R0⁻CD62L⁺ cells (TCM), effector memory helper T cells (CD45RA⁻CD45R0⁻CD62L⁻ (TEM) and CD45RA⁺CD45R0⁻CD62L⁻ cells (TEMRA), together with IL-4 and IFN-γ production by all CD4⁺ T cells. The suppressive effect of PSG on helper T-cell proliferation was established. It was found that PSG does not influence the frequencies of CD45RA⁺ and CD45R0⁻ cells, while it decreased the percentage of CD45RA⁺CD45R0⁻ cells. PSG increased the percentage of NAIVE cells in culture, and prevented the conversion of these cells into TEMRA, without affecting the levels of TCM and TEM. In addition, PSG lowered the amount of IL-4 and IFN-γ in the supernatants of helper T-cell cultures. As TEMRA exhibit cytotoxic activity that is unfavorable during pregnancy, the revealed PSG effects may play a fetoprotective role in vivo.

Keywords: pregnancy-specific β-1-glycoprotein; naive CD4⁺ T cells; helper T cells; effector memory T cells; RA⁺ effector memory T cells

Abbreviations: pregnancy-specific β-1-glycoprotein (PSG); CD4⁺CD45RA⁺CD45R0⁻CD62L⁺ naive helper T cells (NAIVE); CD4⁺CD45RA⁺CD45R0⁻CD62L⁺ central memory helper T cells (TCM); CD4⁺CD45RA⁺CD45R0⁻CD62L⁺ effector memory helper T cells (TEM); CD4⁺CD45RA⁺CD45R0⁻CD62L⁺ effector memory helper T cells (TEMRA)

INTRODUCTION

Pregnancy-specific β-1-glycoproteins (PSGs) are produced during pregnancy by cells of the syncytiotrophoblast [1]. PSGs are the product of the expression of psg genes and are members of the protein family with more than 30 isoforms [2,3]. The serum levels of PSGs in pregnancy gradually reach a concentration of 200-400 µg/mL in the III trimester, which significantly exceeds the content of other known placental proteins [4-6]. PSGs have different functions: they participate in implantation and placentation; regulate vasculogenesis and angiogenesis [7,8]. PSGs are involved in the regulation of both the innate and adaptive immune responses [9-11]. Our previous studies using native human PSG preparations showed their ability to regulate the differentiation of regulatory T (Treg) lymphocytes [12], indoleamine 2,3-dioxygenase (IDO) expression by human monocytes [13], and the cytokine/chemokine profile of immunocompetent cells [14]. However, the effect of PSGs on the human immune system has not yet been adequately studied. In particular, it is still not known how PSGs affect the differentiation of helper T cells, in particular, the formation of their central memory and effector memory subsets.
The expression of different CD45 isoforms is directly associated with T-cell status. CD45RA and CD45R0 surface molecules are commonly used to identify naive and memory T cells. CD45⁺CCR0⁻ T lymphocytes are considered to be naive and CD45R0⁺CDRA⁻ are memory T cells [15-17]. Sallusto et al. established the heterogeneity of the CD45R0⁺CDRA⁻ T-cell population according to different levels of expression of CCR7 and CD62L lymph node homing molecules. The authors described two main subsets: CCR7⁺CD62L⁺ central memory T cells (TCM) that have confined effector functions, and CCR7⁻CD62L⁻ effector memory T (TEM) cells, which preferentially move to peripheral tissues and realize rapid effector functions [18,19]. Subsequently it was established that highly differentiated effector T cells can re-express CD45RA on the cell surface [20, 21]. Some reports have suggested that these cells have characteristics of end-stage differentiation, and while they were called “terminally differentiated effector memory T cells”, it was later shown that they proliferated when provided with suitable stimulatory signals [22,23]. Therefore, it is probably more reasonable to call them CD4⁺ effector memory T cells re-expressing CD45RA (TEMRA).

In our study, we used CD45RA, CD45R0 and CD62L molecules to determine memory cell sub-populations. Although the heterogeneous expression of CD62L on TEM was described, this is more important for CD8⁺ T cells [24]. Besides, it is unclear whether CCR7 expression unambiguously distinguishes between memory and effector CD4⁺ T cells [25]. It is known that myeloid-derived suppressor cells (MDSC) modulate naive T cells using ADAM 17-CD 62L interactions, and it was of particular interest to analyze the expression of L-selectin [26]. One more argument in support of our choice is that in the complex and multistep lymphocyte traffic to lymphoid organs, L-selectin turns on at the first step of the process leading to tethering and rolling of lymphocytes on the endothelium. Chemokine receptors (in particular CCR7) are involved in homing L-selectin, integrins and Ig superfamily adhesion molecules and in mediating further cell movement along the density gradient of the corresponding ligands [27,28]. Therefore, the expression of CD62L in relation to the expression of CCR7 in our opinion can be referred to as primary.

Thus, some naive CD45RA⁺CD45R0⁺CD62L⁺ T cells (NAIVE) after contact with the antigen undergo conversion to central memory CD45RA⁺CD45R0⁺CD62L⁺ T cells (TCM), which do not demonstrate effector functions but can respond quickly upon repeated stimulation with antigen. A portion of memory cells is transformed into CD45RA⁺CD45R0⁺CD62L⁻ effector memory T cells (TEM) and CD45RA⁺CD45R0⁻CD62L⁻ effector memory T cells (TEMRA) [20,29-33]. Both TEM and TEMRA secrete cytokines, primarily IL-4 and IFN-γ, as well as other biologically active molecules [31].

In 2017, T.E. Kieffer et al. [34] showed that physiological pregnancy does not affect the activation status of peripheral cytotoxic memory lymphocytes (CD45R0⁺CD8⁺), but increases the proportions of CD4⁺ TEM, CD4⁺ TCM and activated memory T cells. Thus, the aim of this study was to examine the role of PSG in the helper T cell response regulation. Our objectives were: to study the PSG effect on helper T cell proliferation; to evaluate the role of PSG in regulating helper T cell CD45RA/CD45R0 expression; to assess its effect on the conversion of naive T helpers to TCM, TEM and TEMRA, and on total IL-4 and IFN-γ production by these cells.

MATERIALS AND METHODS

The research was performed according to the World Medical Association's Declaration of Helsinki and Council of Europe Protocol of the Convention on Human Rights and Biomedicine and approved by the Ethics Committee of the Institute of Ecology and Genetics of Microorganisms, Ural Branch of the Russian Academy of Sciences (IRB00010009) on 12.06.2016. Written informed consent was obtained from all the participants.

Study groups

Venous blood samples were collected from healthy donors (non-pregnant women, n=12, 21-39 years-of-age) by venipuncture with vacuum tubes (BD Vacutainer™, Greiner-bio-one, Austria). Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Urografin (Pharmacia, Sweden, Bayer Pharma AG, Germany) density gradient centrifugation (ρ=1.077 g/cm³).
Anti-PSG antibody production and isolation

BAP3 hybridoma ("Genovac", Germany) was cultured in DCCM-1 medium (Biological Industries, Israel). Biospecific affinity chromatography was carried out to isolate antibodies from conditioned media. Thirty mL of antibodies containing medium (1 mg/mL) were applied to the 5-mL HiTrap™ Protein G HP column (GE Healthcare) at a rate of 1 mL/min, and the column was washed successively with 0.15 M phosphate buffer pH 7.0, 0.02 M phosphate buffer pH 7.0 and 0.15 M sodium chloride to zero optical density. Elution of the bound protein was carried out with 0.1 M glycine-HCl buffer, pH 2.7, elution rate of 1 mL/min. The eluate was immediately neutralized. The protein concentration was determined by the Bradford assay [35]. Resulting antibodies were dialyzed against PBS. The homogeneity of the antibody preparation was analyzed by sodium dodecyl sulfate (SDS) polyacrylamide-gel electrophoresis (PAGE).

Native PSG isolation

Granules of Sepharose CL 4B (GE Healthcare) were conjugated with isolated anti-PSG antibodies according to the manufacturer’s instructions and further used as a biospecific sorbent. Sera of pregnant women (at a gestation period of more than 36 weeks) were pooled and centrifuged at 25000 g, mixed with the sorbent and incubated for 36-48 h at 4°C. The sorbent was washed with PBS on an affinity column, pH 7.25, to zero value of optical density. Elution was performed with 0.1 M glycine-HCl buffer, pH 2.6. Protein-containing fractions were combined and immediately exposed to concentration and diafiltration against a normal saline solution, followed by negative chromatography in a HiTrap™ Protein G HP column (GE Healthcare). PSG concentration in the preparation was determined using ELISA (Vector-Best, Russia). The molecular weight of the obtained preparation according to SDS-PAGE [36] was 50-75 kDa, and was in agreement with existing data [37,38]. Liquid chromatography-tandem mass spectrometry assay (LC/MS) was performed at the Israel Institute of Technology, using LTQ-Orbitrap (Thermo Fisher, USA). Several molecular forms of the PSG were detected (Fig. 1).

CD4+ cell isolation and cultivation

The experiment included several steps: isolation of CD4+ T cells, further cultivation in the presence of PSG and T-cell activator and subsequent flow cytometric detection of proliferation and T-cell marker expression. CD4+ cells were obtained from PBMCs by immunomagnetic separation with MACS® MicroBeads and MS Columns (Miltenyi Biotec, Gmbh, Germany). Isolated cells (1x10^6 cells/mL, 200 µL) were cultured in 96-well plates in complete medium (CM): RPMI-1640 (Sigma-Aldrich, USA) supplemented with 10% FBS (Sigma, USA), 10 mM HEPES, 2 mM L-glutamine (both from ICN Pharmaceuticals, USA), IL-2 (10 ng/mL), and 30 µg/mL of gentamycin (KRKA, Slovenia) in a humidified CO₂ incubator at 37°C and 5% of CO₂ for 48 h. T Cell Activation/Expansion Kit human, (Anti-Biotin MACSiBead™ particles loaded with biotinylated antibodies against human CD2, CD3 and CD28, Miltenyi Biotec, Germany) was used to activate T cells via the T-cell receptor (TCR stimulation). Physiological concentrations of PSG were used (1, 10 and 100 µg/mL) that correspond to first, first-second and second-third trimesters of pregnancy, respectively [5,39]. The sample with CM instead of hormone served as a negative control. After the incubation, the viability of cells was evaluated (0.4% trypan blue, Invitrogen, USA), and was 95-98%.

![Fig. 1. A – SDS-PAGE of the PSG preparation; 1 – molecular weight markers (Bio-Rad, USA), 2 – pooled pregnant women serum, 3 – the sample after ammonium sulfate precipitation and ultrafiltration, 4 – the sample after affinity chromatography. B – Identified proteins of the PSG family in the obtained PSG preparation (Fig. 1A, column 4, molecular weight range from 50-75 kDa) according to LC/MS analysis and http://www.uniprot.org.](image-url)
Proliferation analysis

The differential gating method [40] according to the modification [41] was used to determine the proliferative status of cells. This method is a flow cytometric variant of "classic" microscopic evaluation of blast transformation by determining the proportion of large cells. Flow cytometry has made this method more rapid, objective and reliable. The method is based on changes in the light scatter parameters of proliferating cells. The advantages are that it does not require the use of fluorescent antibodies and allows retrospective analysis of existing flow cytometry data files. Gating strategy was as follows: three regions were determined at the FSC-H/SSC-H dot plots of CD4⁺-cell cultures in accordance with their light scatter characteristics as follows: the 1st were non-proliferating living lymphocytes (small size and low granularity; NP); the 2nd were proliferating living lymphocytes (larger in size and higher granularity; P); the 3rd were dead and apoptotic (smaller size, different granularity; A). The percentages of cells in each gate from the total CD4⁺ cell number were determined (Fig. 2). Data were acquired on the CytoFLEX S flow cytometer and analyzed in CytExpert 2.0 software (Beckman Coulter, USA).

Flow cytometry

Stained samples were analyzed by running a three-color flow cytometry with CytoFLEX S (Beckman Coulter, USA). Mathematical processing of the flow cytometry data was performed by CytExpert 2.0 software (Beckman Coulter, USA). The purity of the isolated T-helper population was checked using the two-color BD Simultest™ IMK-Lymphocyte kit (Becton, Dickinson and Company, BD Biosciences, USA) and CytoFLEX S Flow Cytometer (Beckman Coulter, USA). The percentage of B-cells (CD3⁻CD19⁺) did not exceed 0.02%, of monocytes (CD45⁺CD14⁺) 0.2%, of CTLs (CD3⁺CD8⁺) 1%, and of NK-cells (CD3⁻CD16/56⁺) 0.05%. The proportion of CD3⁺CD4⁺ cells from all events varied within 97-99%. CD45RA⁺CD45R0⁻ and CD45R0⁺CD45RA⁺ gates within the lymphocyte gate. C, D – Subsets of interest are identified according to CD62L expression in CD45RA CD45R0⁻ and CD45R0 CD45RA⁺ gates.

Fig. 2. Determination of the proliferative status of cells by the differential gating method. Representative light scatter dot plots of one experiment are shown. A – control without TCR-stimulation beads; B – control with TCR-stimulation beads. Abbreviations on the dot plots: A – dead and apoptotic cell gate, NP – gate of non-proliferating cells, P – gate of proliferating cells. The numbers indicate the percentage of cells in the corresponding gate of the total number of cells.

Fig. 3. Gating strategy example. A – Gating on living lymphocytes. B – CD45RA CD45R0⁻ and CD45R0⁺CD45RA⁺ gates within the lymphocyte gate. C, D – Subsets of interest are identified according to CD62L expression in CD45RA CD45R0⁻ and CD45R0⁺CD45RA⁺ gates.
used were CD62L-APC (clone 145/15), CD45RA-FITC (clone HI100) and CD45R0-PE (clone UCHL1) (Bio-Legend, USA). The data are presented as percentages of naive T cells, TCM, TEM, TEMRA, CD45RA-CD45R0-, CD45RA CD45R0+, CD45RA-CD45R0· cells from the number of cells in the living lymphocyte gate, made according to FSC and SSC properties (Fig. 3).

Evaluation of cytokine concentrations

IL-4 and IFN-γ concentrations in the supernatants were evaluated by enzyme-linked immunosorbent assay (ELISA) kits (Vector-Best, Russia) according to the manufacturer’s instructions using multichannel spectrophotometer Biohit BP 800 (Finland).

Statistical procedures

Data were analyzed using paired Student’s T-test (proliferation) and paired Wilcoxon’s test (surface markers) in Statistica 8.0 (Dell, USA) and are presented as arithmetic means and standard errors of the mean (M±m) and median, first and third quartile values (Me(Q1-Q3)). Differences were considered significant when P<0.05.

RESULTS

The effect of TCR stimulation on the proliferative status of CD4+ lymphocytes

TCR stimulation led to a significant change in the proliferative status of CD4+ cells. The percentage of proliferating cells significantly increased, and the percentage of non-proliferating cells decreased. This indicated adequate activation of cells under these experimental conditions. With regard to dead and apoptotic cells, their percentages significantly decreased in the presence of the activating particles (Table 1).

The effect of PSG on the proliferative status of activated CD4+ cells

All PSG concentrations reduced the frequency of proliferating CD4+ cells while simultaneously increasing the percentage of non-proliferating lymphocytes in culture.

Table 1. The effect of TCR stimulation on the proliferative status of CD4+ lymphocytes (n=12).

<table>
<thead>
<tr>
<th></th>
<th>Control (M±m)</th>
<th>TCR stimulation (M±m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-proliferating (%)</td>
<td>70.51±0.96</td>
<td>14.71±1.48</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Proliferating (%)</td>
<td>9.54±0.79</td>
<td>74.98±1.25</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Dead and apoptotic (%)</td>
<td>19.23±1.27</td>
<td>9.58±0.74</td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Control – T cell culture without TCR stimulation. The arithmetic means and standard errors of the mean (M±m) of cell percentages in corresponding gates from all CD4+ lymphocytes are shown. P values according to Student’s t-test are shown (compared with the control).

Fig. 4. PSG effects on the proliferative status of CD4+ cells. X-axis – PSG concentrations; Y-axis – percentage of cells in the corresponding gate. Control – helper T cell culture with the activator (TCR stimulation) alone. * – P<0.05, ** – P<0.01, *** – P<0.001, paired Student’s T-test.

At concentrations of 10 and 100 µg/mL, PSG changed the percentage of cells in the dead and apoptotic gate (Fig. 4). We observed a decrease in the proliferative activity of helper T cells under the influence of PSGs.

The effect of TCR-mediated activation on helper T cell percentage

TCR stimulation of CD4+ cells led to a significant decrease in NAIVE, from 55.64% to 46.77%, Me and central memory T cells (TCM; from 29.81% to 23.62% Me, and to an increase in the percentage of TEM: 1.4-fold (from 7.42% to 11.10%, Me and TEMRA: 6.3-fold (from 0.88% to 5.56%, Me).
The effect of PSG on the regulation of CD45 isoform expression on helper T cells

Activation of CD4+ cells with TCR stimulation beads did not change the frequencies of CD45RA+CD45R0 and CD45R0*CD45RA- cells in culture (Table 2). PSG produced no significant effect on CD45 isoform expression in the T-helper culture, but at the concentrations of 10 and 100 µg/mL reduced the frequency of CD45RA+CD45R0- cells (Table 2).

**Table 2.** The effect of PSG on the regulation of CD45 isoform expression on T helpers (n=12).

<table>
<thead>
<tr>
<th></th>
<th>CD45RA CD45R0 (%)</th>
<th>CD45RA CD45R0 (%)</th>
<th>CD45RA CD45R0 (%)</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>56.00 (48.37-60.54)</td>
<td>36.54 (27.49-39.03)</td>
<td>4.81 (3.55-8.48)</td>
</tr>
<tr>
<td>Control+TCR stimulation</td>
<td>51.77 (46.27-61.19)</td>
<td>28.15 (25.57-40.87)</td>
<td>8.69 (7.22-13.63)</td>
</tr>
<tr>
<td>PSG 1 µg/mL +TCR stimulation</td>
<td>53.89 (50.88-59.15)</td>
<td>33.50 (25.80-37.08)</td>
<td>7.98 (6.22-11.88)</td>
</tr>
<tr>
<td>PSG 10 µg/mL +TCR stimulation</td>
<td>54.12 (51.09-60.62)</td>
<td>34.47 (27.70-37.73)</td>
<td>7.51* (5.27-8.60)</td>
</tr>
<tr>
<td>PSG 100 µg/mL +TCR stimulation</td>
<td>53.09 (51.23-59.30)</td>
<td>34.30 (26.92-35.31)</td>
<td>6.11* (5.27-8.60)</td>
</tr>
</tbody>
</table>

Control – T cell culture without both TCR stimulation and PSG. Me, Q1 and Q3 are shown. * – P<0.05 as compared to control+TCR stimulation, paired Wilcoxon test.

**Table 3.** The effect of PSG on IL-4 and IFN-γ levels in T-helper culture supernatants (n = 12).

<table>
<thead>
<tr>
<th></th>
<th>IL-4 (pg/mL)</th>
<th>IFN-γ (ng/mL)</th>
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<tbody>
<tr>
<td>Control</td>
<td>3.42 (3.36-3.62)</td>
<td>0.5 (0.4-0.8)</td>
</tr>
<tr>
<td>Control+TCR stimulation</td>
<td>22.16* (15.63-40.45)</td>
<td>6.35* (5.29-7.24)</td>
</tr>
<tr>
<td>PSG 1 µg/mL +TCR stimulation</td>
<td>23.26 (17.49-32.82)</td>
<td>6.84 (5.89-7.94)</td>
</tr>
<tr>
<td>PSG 10 µg/mL +TCR stimulation</td>
<td>20.62** (12.84-32.73)</td>
<td>6.83 (5.56-7.43)</td>
</tr>
<tr>
<td>PSG 100 µg/mL +TCR stimulation</td>
<td>12.71** (9.62-24.30)</td>
<td>4.30** (3.68-5.84)</td>
</tr>
</tbody>
</table>

Control – T cell culture without both TCR stimulation and PSG. Me, Q1 and Q3 are shown. * – P<0.05 as compared with control, paired Wilcoxon test. **– P<0.05 as compared to control+TCR stimulation, paired Wilcoxon test.

**PSG effects on the frequencies of NAIVE, TCM, TEM and TEMRA in CD4+ cell culture**

Introduction of 1, 10 and 100 µg/mL of PSG into the activated lymphocyte cultures did not affect the conversion of naive T helpers to TCM and TEM. However, the medium and the high concentrations of PSG (10 and 100 µg/mL) significantly reduced the percentage of TEMRA and decreased the percentage of NAIVE (Fig. 5).

**The effect of PSG on the levels of IL-4 and IFN-γ in helper T cell culture supernatants**

At doses of 10 and 100 µg/mL, PSG reduced the production of IL-4, and at a concentration of 100 µg/mL reduced the production of IFN-γ in cultures of activated helper T cells (Table 3).

**DISCUSSION**

The effect of PSGs on lymphocyte proliferation is consistent with existing data [42,11]. According to these investigations, PSG1 and PSG9 suppressive effects are mediated through the TGF-β1 pathway [43,44]. This cytokine is secreted by many cells, including CD4+...
T lymphocytes. It carries out autocrine regulation of these cells’ functions [45]. It is likely that the observed effect of T-helper proliferation suppression by PSGs was realized via activation of the latent form of TGF-β1.

Naive T lymphocytes express a high molecular weight CD45 isoform, CD45RA, which after cell activation is replaced by a low molecular weight isoform, CD45R0 [46–48]. In vivo and in vitro human T cells can pass from CD45RA+ to CD45R0+ without antigen stimulation [49]. In our study, PSG had no significant effect on the expression of the CD45 isoform in T-helper culture, but the medium and its high concentrations (10 and 100 μg/mL) reduced the number of CD45RA+CD45R0+ cells that considered to be activated (Table 2) [50]. In general, the mechanisms of regulation of CD45 expression have been poorly studied, and there is no evidence of ligand-induced activation of CD45. It is possible that CD45 is permanently active, and that it causes dephosphorylation of Src family kinases, maintaining them in a working, non-phosphorylated state [51].

In our study, IFN-γ and IL-4, the central cytokines determining the direction of the immune response, were evaluated. There is a so-called Th1/Th2 paradigm or “Th2 bias” hypothesis in pregnancy [52,53], based on which it would be reasonable to expect that PSG, the most abundant fetal protein in maternal bloodstream, contributes towards the secretion of antiinflammatory cytokines and inhibition of the synthesis of proinflammatory ones. However, new data are inconsistent with this theory and an increasing number of researchers refers to this as an oversimplification [54–56]. For example, it was shown that CD4+ IFN-γ+ (Th1) and CD4+ IL-4+ (Th2) cells increase in equal amount in the second trimester of pregnancy, and in the third trimester a decrease is observed [57]. Because maternal PSG concentration peaks in the third trimester, these results overlap to some extent with our study that demonstrates the inhibitory effect of PSG at high doses on the production of both Th1 proinflammatory IFN-γ and Th2 antiinflammatory IL-4.

TEM and TEMRA make the main contribution to the production of IL-4 and IFN-γ [31,58], and the amount of IFN-γ-producing TEMRA moderately increases in pregnancy and strongly in preeclampsia [57]. Therefore, the decrease in the production of cytokines mentioned above under the influence of PSG could be associated with a decrease in TEMRA percentage. It is likely that a decrease in TEMRA percentage under the influence of PSG has a fetoprotective effect in vivo, as these cells are known for their cytotoxic activity, and for this reason they are even called CD4+ cytotoxic T lymphocytes (CTLs) [21].

The obtained data has an applied aspect. The therapeutic potential of PSGs was demonstrated relatively recently. In particular, in vivo expression of pregnancy-specific glycoproteins inhibits the symptoms of collagen-induced arthritis and prevents dextran sodium sulfate (DSS)-induced colitis in mice [59–61]. In these studies, the involvement primarily of regulatory T (Treg) cells was shown, while memory T cells are also involved in autoimmune processes [61].

**CONCLUSIONS**

We previously demonstrated that PSG affects immunity parameters associated with immune tolerance, IDO production, Treg proportion, etc. In this study, we found that PSG regulates the functional activity of circulating CD4+ memory T cells capable of carrying out antigen-specific responses to fetal antigens. PSG may be one of the factors preventing the immune response to fetoplacental antigens. Our findings broaden our understanding of the role of PSG in the modulation of human T-helper functions that are of particular importance in the context of immune tolerance during pregnancy.

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**Author contributions:** PSG isolation: Rayev, Bochkova, Khramtsov; cell culture: Bochkova, Kochurova, Timaganova; laboratory measurements of immunological markers: Timaganova, Bochkova, Kochurova; planning and performing of statistical analyses: Bochkova, Khramtsov; drafting the manuscript and data interpretation: Zamorina, Timaganova, Rayev; obtaining funding
and work guidance: Zamorina; final approval of the version to be published: all authors.

Conflict of interest disclosure: The authors certify that there is no actual or potential conflict of interest in relation to this article.

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