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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Regulation of Recombinase Rag-1 Expression by Female Sex Steroids in Treg and Th17 Lymphocytes: Role of Oncostatin M

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Abstract—The effect of estradiol (E_2), progesterone (P_4), and oncostatin M (OSM) on the differentiation of $CD4^+$ T cells to T regulatory (Treg) lymphocytes and T helpers 17 (Th17) was investigated. The possibility of revision of the T cell receptor in these subpopulations by evaluating the expression of RAG-1 recombinase was also studied. E_2 at concentrations characteristic of pregnancy trimester I, but no P_4 or OSM, increased the Treg level. Combination of sex steroids with OSM increased the percent of $CD4^+FOXP3^+$ cells and enhanced RAG-1 expression in these cells, thus promoting the development of immune tolerance during pregnancy. In the study of Th17, such effect of the hormones and OSM was not detected.

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Pregnancy is a phenomenon of semiallogenic transplantation, the mechanisms of which are still unknown. It is known that sex steroid hormones—estradiol (E_2) and progesterone (P_4)—play an important role in the formation of the maternal immune system tolerance to the semiallogenic fetus [1]. Throughout pregnancy, the placenta produces a number of cytokines that affect both the growth and development of the fetoplacental complex as well as the formation of tolerogenic decidual microenvironment and modifications of systemic responses of maternal leukocytes [1, 2]. One of the key mediators determining the survival processes of implanted blastocyst is oncostatin M (OSM) [3]. Under different conditions, this cytokine may exhibit both proinflammatory and antiinflammatory activity [4]. It cannot be ruled out that reproductive hormones may serve as factors that polarize the immunoregulatory effect of OSM. It was also shown [5] that this cytokine can induce extrathymic differentiation of T cells. It is known that the extrathymic differentiation of T cells is activated during physiological pregnancy [6]. Since it is believed that T cell receptor (TCR) revision is one of the mechanisms of tolerance development in the periphery [7], this may create additional conditions for the survival of semiallogenic fetus [2].

The aim of this study was to investigate the influence of E_2 and P_4 on the background of the effect of OSM on the differentiation of T helpers (Th) to Treg or Th17 and on the expression of RAG-1 recombinase in these cells in vitro.

In experiments, we used peripheral venous blood of healthy nonpregnant women of reproductive age ($n = 10$). A suspension of peripheral blood mononuclear cells (PBMC) was obtained by centrifugation in a ficoll–verografin density gradient ($d = 1.077$ g/cm^3). Then, $CD4^+$ T cells were isolated from the suspension of PBMC by negative immunomagnetic separation using the R & D Systems reagent kit (United States). The isolated cells (5×10^6/mL) were then incubated with OSM and hormones in complete RPMI 1640 medium (Gibco®, ThermoFisher Scientific, United States) supplemented with 10% fetal calf serum, 1 mM HEPES, 2 mM L-glutamine, and 1 mg/mL gentamycin at 37°C for 48 h in a 5% CO_2 atmosphere. Oncostatin M (R & D Systems) was used at a concentration of 2 ng/mL, which was determined by extrapolating the level of its secretion in decidual tissue [8]. Hormones were used at concentrations that correspond to their blood concentrations in I and III trimesters of pregnancy [9]: E_2 (Sigma-Aldrich, United States) at concentrations of 1 and 10 ng/mL and P_4 (Sigma-Aldrich) at concentrations of 20 and 100 ng/mL, respectively. Officinal solvents of the hormones were used as a control.

After 48-h incubation of $CD4^+$ T cells with hormones and/or OSM, the phenotype of T cells was determined by flow cytometry (Becton Dickinson,

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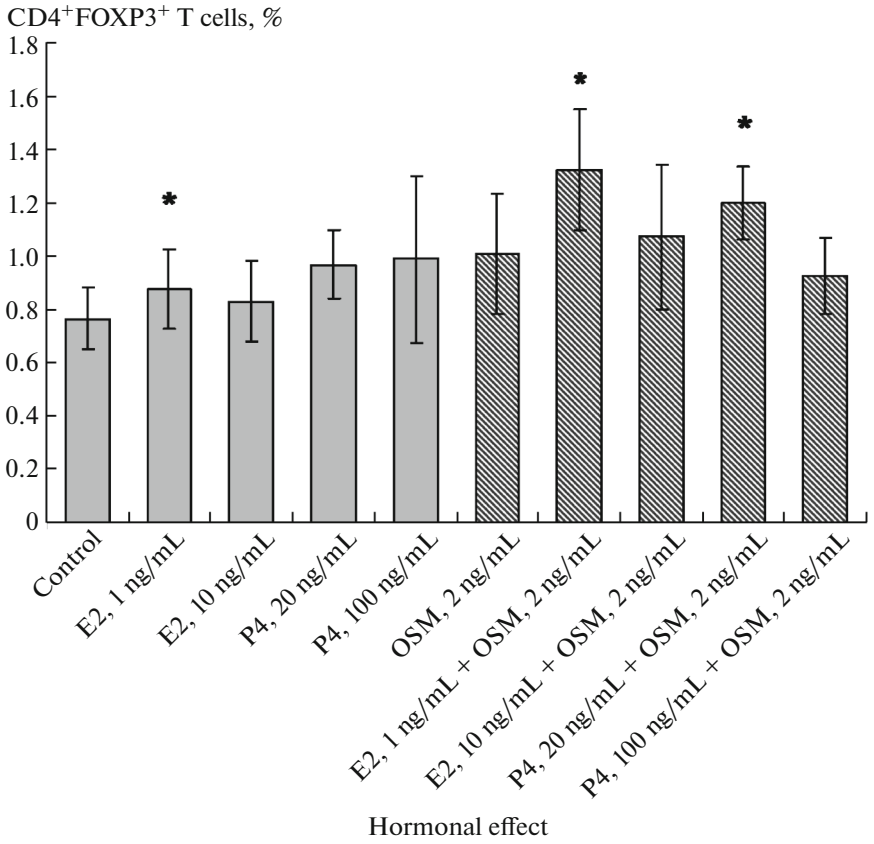

Fig. 1. Effect of E_2, P_4, and OSM on the content of $CD4^+FOXP3^+$ T cells. Here, as well as in Figs. 2–4, data are represented as $M \pm SE$, $n = 10$, $^* p < 0.05$ as compared to control.

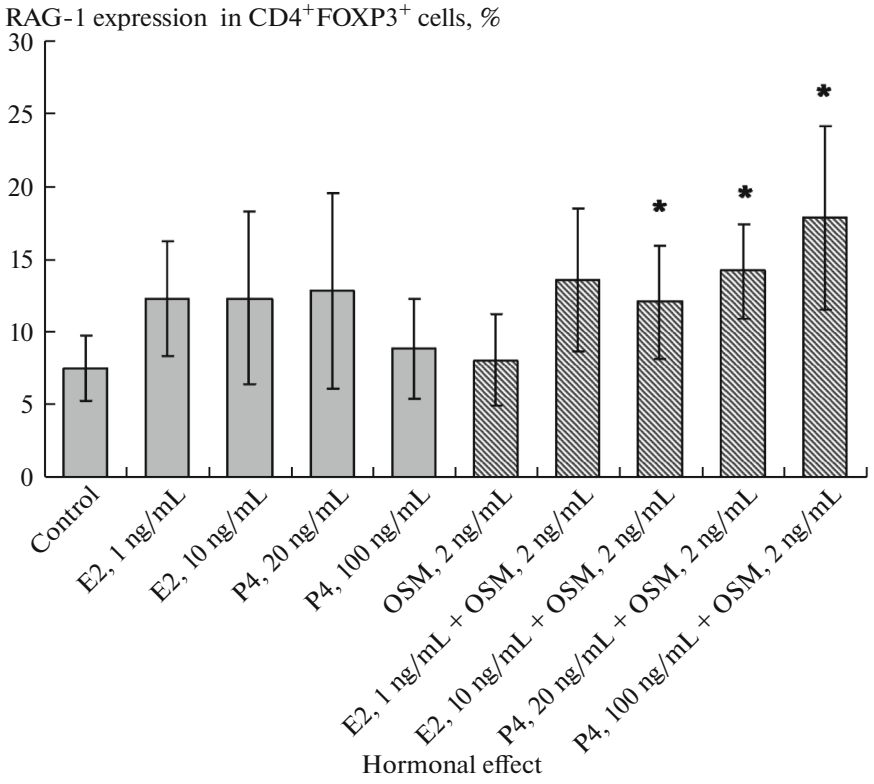

Fig. 2. Effect of E_2, P_4, and OSM on the RAG-1 expression in $CD4^+FOXP3^+$ T cells.

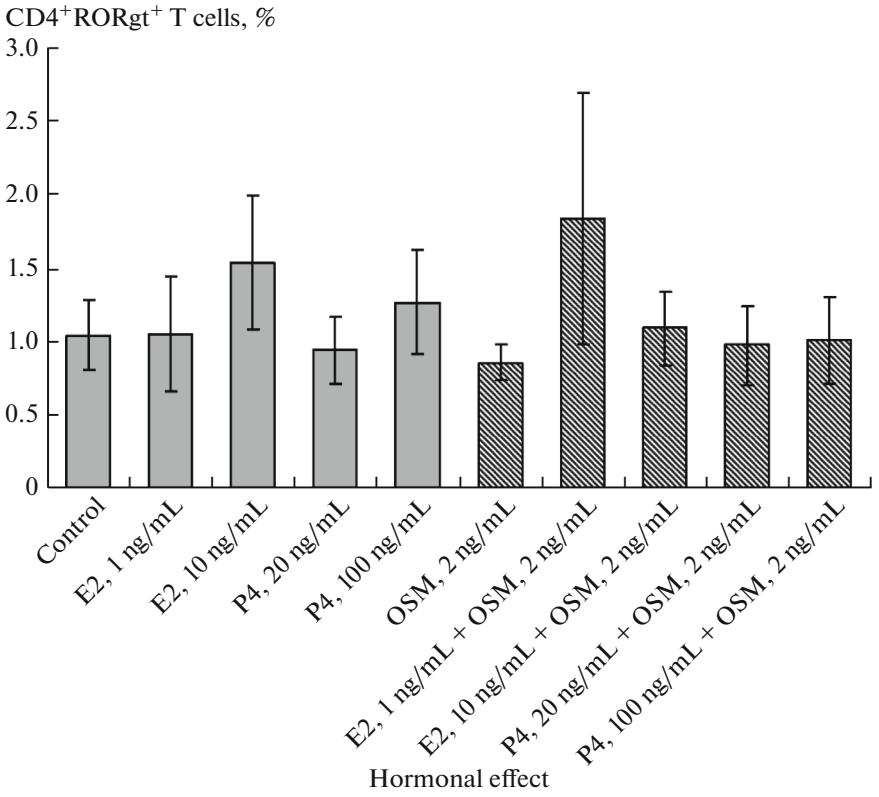

Fig. 3. Effect of E_2, P_4, and OSM on the content of $CD4^+ROR\gamma t^+$ T cells.

United States). Treg cells were identified as $CD4^+FOXP3^+$ cells (Novus Biologicals, United States), and Th17 cells were identified as $CD4^+ROR\gamma t^+$ cells (Novus Biologicals). The marker of rearrangement of antigen receptor genes was the RAG-1 recombinase (Recombination Activating Gene 1) [10], whose intracellular expression was determined in Treg and Th17 cells (Cell Signaling Technology, United States).

The study of the effect of E_2 on the induction of Treg ($FOXP3^+$) in $CD4^+$ T cells revealed a significant increase (in percent) in the content of $CD4^+FOXP3^+$ T cells (iTreg) only when the hormone concentration corresponded to that in pregnancy trimester I. Since we did not perform additional stimulation of Treg cells with the polarizing cytokines, it can be postulated that E_2 is an independent factor of differentiation $CD4^+$ T cells in the iTreg direction. Cytokine OSM and P_4, regardless of the concentration, had no effect on differentiation. A combined effect of OSM, E_2, and P_4 on $CD4^+$ T cells at concentrations extrapolated from the hormone levels in trimester I also resulted in a significant increase in the proportion of $CD4^+FOXP3^+$ T cells. The combination of OSM with the hormones at concentrations typical for trimester III did not possess such ability (Fig. 1). It is known that pregnancy trimesters I and II are the most vulnerable to immune-mediated abortions [11]. Therefore, the realization of the tolerogenic potential of E_2 and the combination of the hormones and the cytokine in this period is biologically justified.

The functional activity of iTreg is determined by the ability to recognize antigenic structures of the major histocompatibility complex (MHC) [12], and the possibility of revision of αβTCR of these cells may enhance their suppressive functions with respect to fetal antigenic structures. In view of this, we investigated the effect of the above-mentioned hormones and their combination with OSM, which induces the extrathymic differentiation during pregnancy [2], on the expression of RAG-1 recombinase in $CD4^+FOXP3^+$ T cells. The results of this series of experiments are shown in Fig. 2. As can be seen from this figure, the hormones per se had no effect on the studied parameters. However, after the addition of OSM, the RAG-1 expression in Treg cells increased under the influence of both P_4 concentrations and the E_2 concentration corresponding to pregnancy trimester III.

Thus, E_2 in trimester I enhanced the iTreg generation, thus promoting the tolerogenic potential with respect to fetal antigens, but did not activate the RAG-1 expression. Conversely, in trimester III, E_2 had no effect on the Treg induction but functions as a coacti-

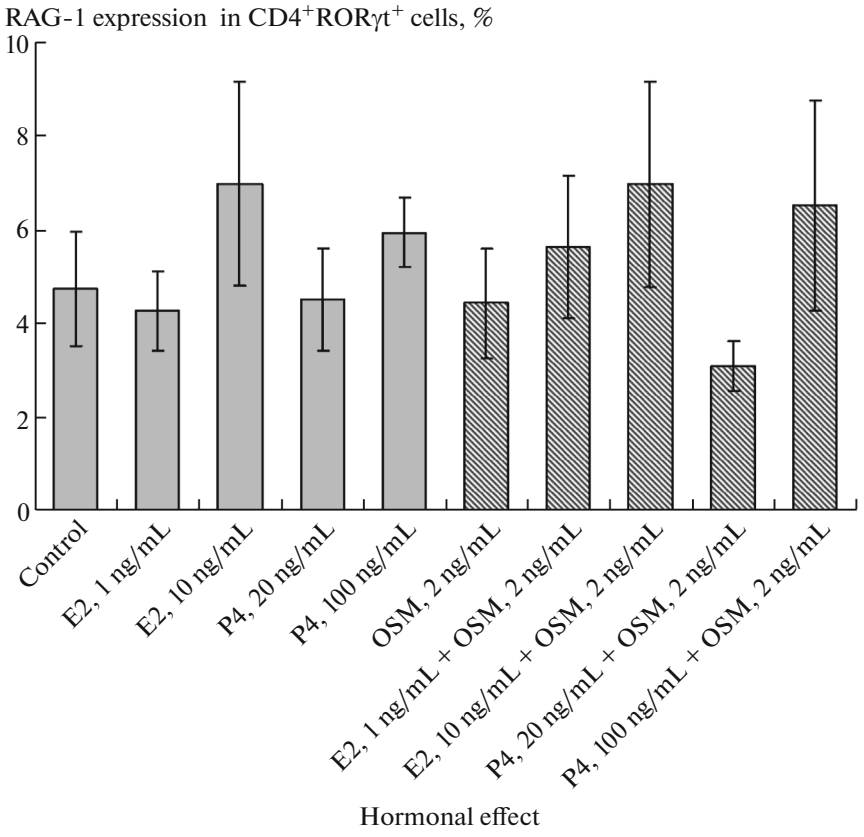

Fig. 4. Effect of E_2, P_4, and OSM on the RAG-1 expression in $CD4^+ROR\gamma t^+$ T cells.

vator of OSM, which resulted in an increased expression of RAG-1. Unlike E_2, P_4 did not induce Treg throughout pregnancy, but enhanced the influence of OSM on the αβTCR Treg rearrangement, thus functioning as an essential cofactor without which OSM itself cannot enhance the RAG-1 expression.

The studied hormones and their combination with OSM did not change significantly (compared to the control group) the percentage of $CD4^+RORgt^+$ T cells (Th17) and the level of RAG-1 expression in them (Figs. 3, 4). Thus, E_2 and P_4 may be physiological regulators guiding the effect of OSM towards the antiinflammatory action, thus enhancing the suppressive component of the maternal immune system during pregnancy.

Since pregnancy is accompanied by the thymus atrophy, the enhancement of the extrathymic differentiation in this period may be compensatory and represent a mechanism for correction of the antigen-recognition repertoire of $CD4^+\alpha\beta$ T cells [13]. Apparently, the simultaneous action of hormones and OSM promotes the αβTCR revision is the already mature Treg in the placental zone, which may be the basis for the development of a local Treg $CD4^+$ network. A change in the antigen-recognition ability of these cells may serve as a condition for the appearance of fundamentally new mechanisms of immunological tolerance to fetal antigens during physiological pregnancy (in particular, the mechanism of formation of the pool of lymphocytes that recognize fetal molecules as autoantigens).

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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