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The Effects of Testosterone and β-Estradiol on Activation of Lymphocytes Associated with IL-2 Production and Expression of CD25 (IL-2Rα)

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Abstract—We carried out an in vitro study to examine the effects of testosterone and β-estradiol on activation of naive ($CD45RA^+$) and primed ($CD45RO^+$) T cells based on the production of IL-2 and expression of the CD25 (IL-2Rα) molecule. We determined that, overall, testosterone exerts an inhibitory effect on the functional activity of both naive and primed T cells. Naive cells were more sensitive to the testosterone effects, than primed lymphocytes. We identified a dose-dependent suppressive effect of β-estradiol on production of IL-2 by the activated $CD45RA^+$ and $CD45RO^+$ lymphocytes in the course of a uniform decrease in the number of CD25-positive T cells.

Keywords: testosterone, β-estradiol, naive T lymphocytes, primed T memory cells, activation of cells

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Naive and primed T cells (cells of the immune memory) can be distinguished by determining the expression of cell surface markers and performing an analysis of the structural and functional cellular properties (Laurie et al., 2008). Selective expansion and differentiation of antigen-specific clones of the T memory cells determines the effectiveness of immune responses to antigens of different natures (Kaech et al., 2002; Elyaman et al., 2008; Gutsol et al., 2013). Presentation of the cell surface molecule $CD45RO^+$, in place of the $CD45RA^+$ isoform, is considered as a definitive phenotypic marker of differentiation of naive human T lymphocytes into memory T cells (Michie et al., 1992).

Disruptions in the formation of immune memory, mediated either by hyper- or hypoactivation of T cells, underlie the development of a number of diseases (Mizutani et al., 1995; Crotty, Ahmed, 2004; Learn et al., 2006; Gutsol et al., 2013; Litvinova et al., 2013). Therefore, it is essential to understand the physiological mechanisms underlying regulation of the primary and secondary immune responses. Sex hormones are key regulators of immune responses. Several reports indicate that sex hormones affect the ability of a mature effector cell to execute the immune response (Grossman et al., 1994; Seledtsov et al., 2010; Litvinova et al., 2013). Activation of T lymphocytes (naive and primed) is inextricably linked with production of IL-2 and expression of an early marker of lymphocyte activation, the CD25 molecule (IL-2Rα) (Johannisson, Festin, 995). The molecular mechanisms of deregulation of the IL-2–IL-2R system are not well studied, and the data published on this subject are contradictory.

The aim of this study was to determine the dose-dependent effects of sex hormones (testosterone and β-estradiol) on the expression of CD25 (IL-2Rα) and production of IL-2 in populations of naive ($CD45RA^+$) and primed ($CD45RO^+$) T lymphocytes in vitro.

MATERIALS AND METHODS

The tissue samples used in this study were venous blood from 22 conditionally healthy donors: 13 men and 9 women from 19 to 39 years of age.

Isolation of mononuclear blood fractions was performed by carrying out density centrifugation at 1.077 g/cm^3 in Ficoll-Urografin gradient (Schering, Spain; Pharmacia, Sweden).

Populations of naive $CD45RA^+$ and primed $CD45RO^+$ T lymphocytes were isolated from mononuclear blood fractions, obtained by immunomagnetic separation (MidiMACS Separator, LS Columns, Miltenyi Biotec, Germany), using paramagnetic particles (MicroBeads human, Miltenyi Biotec, Ger-

Abbreviations: mAb—monoclonal antibodies, Ac/Exp—Human T Cell Activation/Expansion Kit (activator of T lymphocytes), CD—cluster of differentiation, Est—estradiol, IL—interleukin, Test—testosterone.

The effect of testosterone (Test) and β-estradiol (Est) on the levels of IL-2 in supernatants of $CD45RA^+$ and $CD45RO^+$ lymphocytes

Cultivation conditions	Levels of IL-2 in lymphocyte supernatant, pg/mL	
	$CD45RA^+$	$CD45RO^+$
Control	12.72 ± 3.12	82.87 ± 10.13
1) +Ac/Exp	684.9 ± 121.43 ($p_0 < 0.05$)	715.19 ± 98.34 ($p_0 < 0.05$)
2) Ac/Exp + Est (10^{-5} M)	41.13 ± 12.61 ($p_1 < 0.05$)	234.45 ± 42.15 ($p_1 < 0.05$)
3) Ac/Exp + Est (10^{-6} M)	338.12 ± 89.01 ($p_1 < 0.05$; $p_2 < 0.05$)	353.12 ± 62.71 ($p_1 < 0.05$; $p_2 < 0.05$)
4) Ac/Exp + Est (10^{-7} M)	480.67 ± 56.83 ($p_1 < 0.05$; $p_2 < 0.001$, ($p_3 < 0.05$)	415.34 ± 45.03 ($p_1 < 0.05$; $p_2 < 0.05$)
5) Ac/Exp + Test (10^{-5} M)	464.3 ± 106.1 ($p_1 < 0.05$)	565.21 ± 87.48 ($p_1 < 0.05$)
6) Ac/Exp + Test (10^{-6} M)	472.0 ± 98.5 ($p_1 < 0.05$)	679.83 ± 89.11 ($p_1 > 0.05$)
7) Ac/Exp + Test (10^{-7} M)	456.0 ± 112.8 ($p_1 < 0.05$)	681.64 ± 68.61 ($p_1 > 0.05$)

Ac/Exp—Human T-Cell Activation/Expansion Kit. Significance of differences: p_0, compared with control; p_1, in comparison with conditions 1; p_2, in comparison with conditions 2 or 5; and p_3, in comparison with conditions 3 or 6.

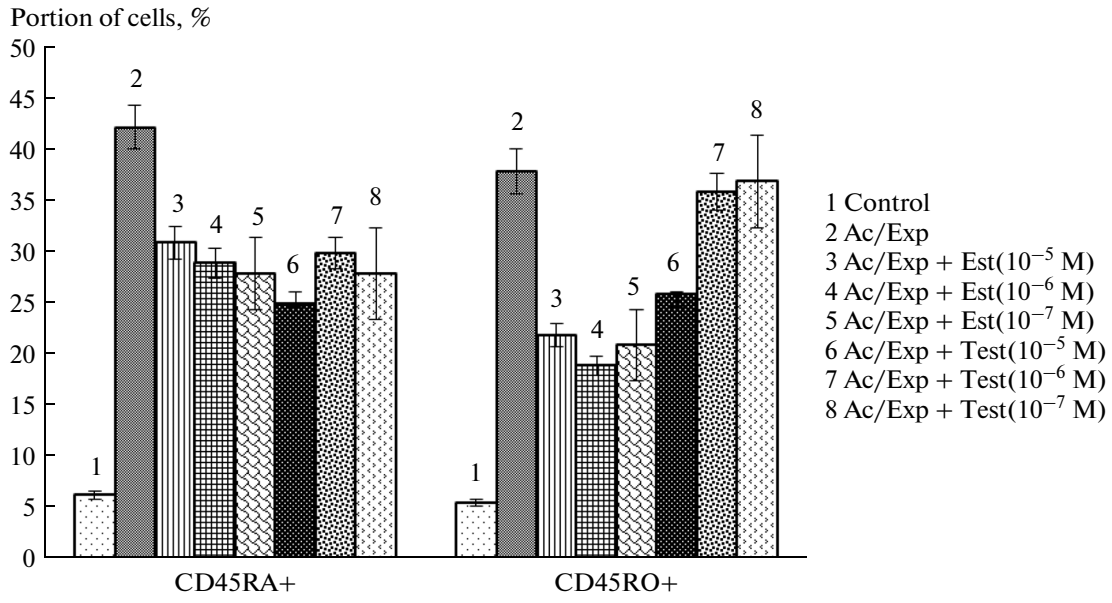
many), and saturated with monoclonal antibodies (MAbs) to $CD45RA^+$ and $CD45RO^+$ in accordance with the manufacturers' instructions. The portions of the desired $CD45RA^+$ and $CD45RO^+$ lymphocyte fractions in the study samples constituted no less than 95%. Cells (1 mln cells/mL) were then cultured for 48 h in Iscove's medium (Sigma, United States) containing 5×10^{-5} M mercaptoethanol (Acros Organics, United States) and 30 μmL gentamycin, in the presence of varying concentrations of testosterone (Test) and β-estradiol (Est) (Sigma, United States). T lymphocytes were activated using a Human T Cell Activation/Expansion Kit (Ac/Exp) (Miltenyi Biotec, Germany) containing antibiotin MACSiBead™ particles with biotinylated antibodies against $CD2^+$, $CD3^+$, and $CD28^+$. Ac/Exp reagent was added to the samples in a volume of 5 mL containing 0.5×10^6 antibiotin MACSiBead™ particles. The ratio of cells to activating particles was 1 : 2. We employed the following cultivation conditions: (1) control, (2) in the presence of Ac/Exp, (3) in the presence of Ac/Exp and Test (10^{-5}, 10^{-6} and 10^{-7} M), and (4) in the presence of Ac/Exp and Est (10^{-5}, 10^{-6} and 10^{-7} M).

Concentrations of $CD25^+$ lymphocytes in both populations were measured by flow cytometry on a GuavaEasyCyte Plus device (Millipore, United States), using MAbs, labeled with fluorescent dyes (Sorbent Russia). Concentrations of IL-2 in the culture supernatant were determined using the ELISA test system in accordance with the manufacturer's instructions (Vector-Best, Russia).

Statistical analysis of the data was performed using the software package Statistika 7.0. We calculated the values of the mean and standard deviation. Significance of differences was assessed using the U-Mann–Whitney test. Differences were considered significant at a reliability level of $p < 0.05$.

RESULTS AND DISCUSSION

The addition of a T-cell activator that simulates the function of antigen-presenting cells to the cultured



Portions of $CD25^+$; $CD45RA^+$ and $CD25^+$; $CD45RO^+$ T cells when cultured in the presence of β-estradiol (Est) or testosterone (Test) at various hormone concentrations. Ac/Exp—Human T-Cell Activation/Expansion Kit (for activation of T-lymphocytes).

naive and primed lymphocytes resulted in a significant increase in the levels of IL-2 in the culture medium: 53-fold ($p_0 < 0.05$) in the population of $CD45RA^+$ lymphocytes and by 8.6 times ($p_0 < 0.01$) in cultured $CD45RO^+$ T memory cells (table). An increase in the number of $CD25^+$ T lymphocytes in the presence of activator in both $CD45RA^+$ and $CD45RO^+$ fractions was expected, as CD25 expression is associated with the IL-2-dependent stage of immune response in activated lymphocytes (Létourneau et al., 2009).

The key function of IL-2 is to enable the transition of antigen-activated $CD4^+$ and $CD8^+$ T lymphocytes from the G1 to S-phase of the cell cycle, which ultimately leads to their proliferation (Ke et al., 1998; Saparov et al., 1999). The CD25 molecule represents the α-chain of the IL-2 receptor. Appearance of the α-chain in the composition of the $\beta\gamma_c$ receptor to IL-2, on the membrane of resting lymphocytes, leads to a several-fold increase in its affinity to IL-2, which mediates the unfolding of the signaling events, directly regulating the entry of resting T lymphocytes into the cell cycle (Ellery, Nicholls, 2002; Benczik, Gaffen, 2004).

After 48 h of culturing of $CD45RA^+$ and $CD45RO^+$ lymphocytes under control conditions, the IL-2 content in cell culture supernatants was, respectively, 12.72 ± 3.12 and 82.87 ± 10.13 pg/mL. The corresponding portions of $CD45RA^+$ and $CD45RO^+$ lymphocytes expressing CD25 were approximately equal (on average, 6.21 ± 3.37%) (figure).

Comparative analysis of the effects of the sex hormones on activation of naive and primed T cells associated with production of IL-2 and expression of CD25 in apparently healthy individuals and in groups differentiated by gender showed no significant differences?in the test parameters.

Based on published data, androgens have the capability of mild suppression of immune system. Androgen receptors on thymocytes and lymphocytes are present in large quantities (Litvinova et al., 2011, 2013). We found that testosterone uniformly inhibited IL-2 production in $CD45RA^+$ T lymphocytes, regardless of concentration (on average, by 1.4 times) ($p_1 < 0.05$). At the same time, testosterone at a concentration of from 10^{-5} to 10^{-7} M promoted reduction in the portion of CD25-positive $CD45RA^+$ lymphocytes (figure). During culturing of $CD45RO^+$ lymphocytes with testosterone, a reduction in the concentration of IL-2 in the supernatant and decrease in the number of $CD25^+$ cells, was detected only at a maximum concentration (10^{-5}M) of the male sex hormone ($p_1 < 0.05$) (table, figure). The inhibitory effect of testosterone on the level of $CD45RO^+$ and $CD25^+$ cells and the production of IL-2 can be attributed to its antiproliferative effects (Litvinova et al., 2011, 2013).

In summarizing the obtained data on the effects of estrogen on development of immune response, first, the dose-dependent effect of estrogen on T and B lymphocytes, and immune system in general, should be noted. The common view is that estrogen can enhance the immune response (Grossman et al., 1994). In our

study, β-estradiol, at concentrations of from 10^{-5} to 10^{-7}M, exerted a pronounced suppressive effect on the level IL-2 production in both populations of T lymphocytes (Table I). This may be explained by the previously identified apoptotic action of the β-estradiol on T lymphocytes (Litvinova et al., 2013). We found that the action of β-estradiol on IL-2 production in naive and primed T cells was dose-dependent (Table I). Interestingly, we detected that β-estradiol uniformly (irrespective of concentration) inhibited the expression of the activation marker CD25 in $CD45RA^+$ and $CD45RO^+$ populations of lymphocytes (figure). The effect was most pronounced in the case of primed ($CD45RO^+$) lymphocytes. We suggest that this may be due to a higher density of the high-affinity estrogen receptors on the membrane of "mature" lymphocytes (Anderson, 2000; Chernyshov et al., 2001).

Thus, we obtained new data describing the effects of testosterone and β-estradiol on activation of T-lymphocytes at different stages of differentiation associated with expression of CD25 and production of IL-2. Overall, testosterone exerted an inhibitory effect on the functional activity of both naive and primed T-cells. The $CD45RA^+$ lymphocytes appeared to be more sensitive to the effects of testosterone than $CD45RO^+$ cells. The effect of testosterone on production of IL-2 and expression of the activation marker CD25 by the T cells was independent of the concentration of the hormone. In turn, we identified a dose-dependent inhibitory function of the β-estradiol on production of IL-2 by the activated $CD45RA^+$ and $CD45RO^+$ T lymphocytes. The decrease in the number of CD25-positive T lymphocytes (naive and primed) under the effect of β-estradiol did not depend on the dose of the hormone and had a uniform character that was most pronounced in the case of $CD45RO^+$ T cells.

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