10. *Shivanna N, Naika M, Khanum F, KaulVK*. Antioxidant, anti-diabetic and renal protective properties of Stevia rebaudiana. J Diabetes Complications. 2013;27(2):103-113
11. *Solin A.V., Korozin V., Lyashev Y.D.* Hepatoprotective effect of regulatory peptides during immobilization stress // Scientific Proceedings of Belgorod State University. 2012, N 22 (141), p. 123-126 (In Russian).
12. *Soufi S, D'Urso G, Pizza C, Rezgui S, Bettaieb T, Montoro P*. Steviolglycoside stargeted analysis in leaves of *Stevia rebaudiana* (Bertoni) from plants cultivated under chilling stress conditions // Food Chem 2016;190:572-58.

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17β-ESTRADIOL ATTENUATES THE LPS-INDUCED INFLAMMATORY RESPONSE IN WHOLE BLOOD CELLS CULTURE

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Estradiol belongs to a family of steroid hormones that primarily controls the reproductive system. 17β-estradiol (E2) is the predominant and most biologically active estrogen. Growing evidences suggest that E2 may have a regulatory effects on immune system and can directly influence the function if cell-mediated immunity. Nevertheless, the effect of E2 on innate immune cells, particularly monocytes and neutrophils, has been so far poorly investigated. This study was aimed to investigate *in vitro* production of TNF-α, IL-1β, MCP-1 and IL-8 by whole blood cells following short-term exposure (4 hours) to 17β-estradiol (E2) in the presence or absence of LPS. The impact of E2 on β2 integrin (CD11b/CD18) and L-selectin (CD62L) expression on the surface of human blood monocytes and neutrophils was also evaluated. We demonstrated an inhibitory effect of E2 on LPS-induced TNF-α production, the feature that could play a critical role in the regulation of inflammatory response. Expression of CD62L on neutrophils and monocytes was also decreased in the presence of E2. Thus, the results of our study indicate that E2 may have an immunomodulatory action on innate immune cells and modulate ongoing inflammatory response.

Neutrophils, monocytes, estradiol, gene expression, cytokines

Էստրադիոլը պատկանում է ստերոիդային հորմոնների ընտանիքին, որի առանցքային դերը վերարտադրողական համակարգի աշխատանքի կարգավորումն է: Էստրոգեն հորմոնի հիմնական և կենսաբանորեն առավել ակտիվ ձևը հանդիսանում է 17β էստրադիոլը (E2): Մի շարք հետազոտություններ ցույց են տվել, որ E2-ն կարող է ունենալ կարգավորիչ ազդեցություն իմունային համակարգի վրա և անմիջականորեն ազդել բջջային միջնորդավորված իմունային պատասխանի վրա: Այնուամենայնիվ, մինչ օրս, բնածին իմունային բջիջների՝ մասնավորապես մոնոցիտների և նեյտրոֆիլների վրա E2-ի ազդեցությունը բավականաչափ ուսումնասիրված չէ: Տվյալ հետազոտության նպատակն է ուսումնասիրել *in vitro* պայմաններում ամբողջական արյան բջիջների կողմից TNF-α, IL-1β, MCP-1 և IL-8 արտադրելու ունակությունը 17β-էստրադիոլի (E2) կարճատև ազդեցությունից հետո (4 ժ)՝ LPS-ի առկայության կամ բացակայության պայմաններում: Ինչպես նաև ուսումնասիրվել է E2-ի ազդեցությունը մոնոցիտների և նեյտրոֆիլների մակերեսային մոլեկուլներ β2 ինտեգրինի (CD11b/CD18) և L սելեկտինի (CD62L) էքսպրեսիայի վրա: Մեր կողմից ցույց է տրվել E2-ն ցուցաբերում է ճնշիչ ազդեցություն LPS-ով խթանված TNF-α-ի արտադրության վրա, ինչը, հնարավոր է, կարող է որոշիչ դեր ունենալ բորբոքային պատասխանի կարգավորման ժամանակ: E2-ի ազդեցությունից հետո նեյտրոֆիլներում և մոնոցիտներում նվազում է նաև CD62L-ի էքսպրեսիայի մակարդակը: Այսպիսով, ստացված արդյունքները ցույց են տալիս, որ բնածին իմունային բջիջներում E2-ն կարող է ունենալ իմունոկարգավորիչ ազդեցություն և modulate ընթացիկ իմունային պատասխանը:

Նեյտրոֆիլներ, մոնոցիտներ, Էստրադիոլ, գենային էքսպրեսիա, ցիտոկիններ

Эстрадиол относится к группе стероидных гормонов, которые главным образом контролируют репродуктивную систему. 17β-эстрадиол (E2) является одним из основных и наиболее биологически активных эстрогенов. Накопленные свидетельства указывают на то, что E2 способен оказывать регуляторный эффект на имунную систему и способен непосредственно воздействовать на функционирование клеточного звена иммунитета. Тем не менее, воздействие E2 на клетки врожденного иммунитета, в частности моноциты и нейтрофилы остается плохо изученным. Таким образом, целью данного исследования явилось изучение *in vitro* продукции TNF-α, IL-1β, MCP-1 и IL-8 клетками цельной крови прсле кротковременного (4 часа) воздействия 17β-эстрадиола (E2) в присутствии или отсутствии LPS. Кроме того была проведена оценка воздействия E2 на экспрессию β2 интегрина (CD11b/CD18) и L-селектина (CD62L) на поверхности моноцитов и нейтрофилов крови человека. Нами был продемонстрирован ингибиторный эффект E2 на LPS-индуцированную продукцию TNF-α, особенность/свойство которая может играть критическую роль в регуляции воспалительного ответа. Экпоессия CD62L на нейтрофилах и моноцитах так же была снижена в присутствии E2. Таким образом, результаты нашего исследования указывают на то, что E2 может иметь иммуномодуляторное воздействие на клетки врожденного иммунитета и способен модулировать развитие воспалительной реакции.

нейтрофилы, моноциты, эстрадиол, генная экспрессия, цитокиы

Hormones are chemical messengers secreted by endocrine glands that regulate numerous biologic processes, including growth and development, reproduction, immunity and homeostasis. The link between immune and endocrine systems is being increasingly recognized. The regulation of this well-balanced machinery occurs through interactions of hormones with receptors on immune cells which modulate immune cell response and function. Various observations suggest that sex hormones may not only simply affect the immune responses but may also predispose persons to allergies and autoimmune diseases [1]. It was suggested that high incidence and prevalence rates of autoimmune diseases in women could be associated with the hormonal fluctuations to which they are exposed throughout life [1]. Particularly, it was shown that estrogen (or 17β-estradiol) can broadly affect innate and adaptive immune processes through estrogen receptors expressed on most immune cells. Despite many known links between E2 and immune function, the exact role of this hormone in inflammation is controversial. While pro-inflammatory features of 17β-estradiol were previously reported [2], recent evidences suggested anti-inflammatory effect of E2 [3]. This study was aimed to analyze the production of TNF-α, IL-1β, MCP-1 and IL-8 by whole blood cells and determine the expression of β_2 integrin and L-selectin on monocytes and neutrophils from healthy subjects after E2 exposure in the presence or absence of LPS.

Materials and methods

Sampling. The study was approved by the Ethical Committee of the Institute of Molecular Biology of the NAS RA (IRB IORG0003427). Venous blood samples were obtained from 12 healthy women (mean age of 33 ± 3,4 years). None of the selected donors was taking any medication at the time of the experiments, or suffered from acute/chronic diseases. Blood of selected women was collected in the mid follicular phase to minimize diversity of estrogen levels in the blood.

In vitro stimulation of whole blood with E2 and LPS. Peripheral blood samples was collected in EDTA tubes, and diluted 1:10 with RPMI-1640 medium (Gibco) containing 10% fetal calf serum (FCS) and 2 mM L-glutamine (Sigma). The blood was distributed in 24-well plates and stimulated with E2 (100μM) in the presence or absence of LPS (100 ng/ml) for 4 hours at 37°C.

Cytokine measurements. Cytokine content in supernatants was measured with specific immunoassays according to the manufacturer's instructions. Cytokine production was analyzed using Human IL-1β, IL-8, TNF-α and MCP-1 ELISA MAX Deluxe kits

(Biolegend, UK). The samples were read at 450 nm in a 96-well plate reader (HumaReader HS, Human Diagnostics Worldwide, Germany).

Flow cytometric analysis. At the end of stimulations, cells were harvested and incubated with fluorescent mAb toward CD14, CD16, CD11b, CD18, and CD62L for 30 minutes. Labeled cells, after further washing, were resuspended in PBS supplemented with 1% BSA. Antigen expression was analyzed on a Partec CyFlow Space (Partec, Germany). 10 000 events were collected from each sample.

Statistics. Statistical analyses were carried out using the Statsoft Statistica package (http://www.statsoft.com). All values are given as means ± standard errors of the means. For continuous variables, groups were compared using the paired Student's t-test. *P* values ≤ 0.05 were considered as significant.

Results

Effect of E2 and LPS on surface expression of CD11b, CD18, and CD62L. In neutrophils, LPS significantly enhanced membrane expression of CD11b/CD18 (P <0.01). In monocytes, LPS exposure resulted in increased CD11b surface expression (P <0.05). Expression of CD62L on neutrophils were suppressed by LPS (P <0.05), while in monocytes the level of CD62L remained unchanged after exposure to LPS. Presence of E2 did not substantially affected LPS-induced expression of surface CD11b, CD18 or CD62L on both cell populations. In absence of LPS, E2 administration significantly suppressed cell-surface expression of CD62L on both monocytes and neutrophils (P <0.05) (Figure 1).

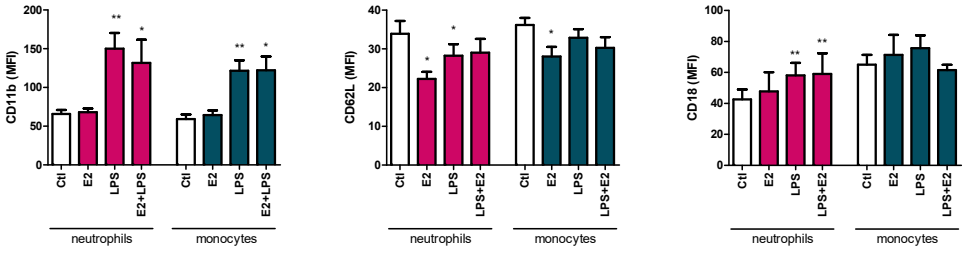

Figure 1. Effect of E2 (100 μM) and LPS (100 ng/ml) on expression CD11b, CD62L, and CD18 surface markers in neutrophils and monocytes from lysed whole blood. Data represented as mean fluorescence intensity (MFI) ± S.E.M. Neutrophils were identified according to FSC/SSC gate and showing $CD16^{bright}$ and $CD14^{-}$ staining. Monocytees were identified according to FSC/SSC gate and showing $CD14^{+}$ staining. *P < 0.05, **P < 0.01 compared with the untreated control group (Ctl).

Cytokine production by whole blood cells in the presence of E2 and LPS. A low level of constitutive IL-1β, IL-8, TNF-α and MCP-1 was detected in supernatants from unstimulated control cells. Administration of endotoxin increased the levels of all tested cytokines in whole blood culture supernatants in comparison to unstimulated group (Figure 2).

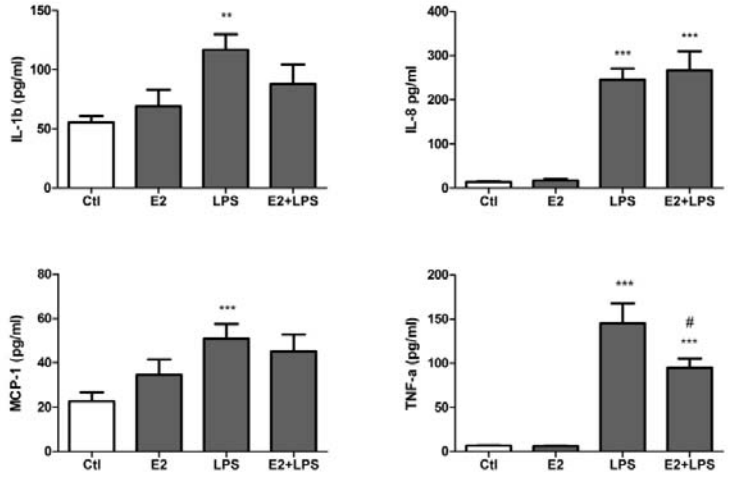

Figure 2. Effect of E2 (100 μM) and LPS (100 ng/ml) on the production of IL-1β, IL-8, TNF-α and MCP-1. Data are presented as means ± S.E.M. **P < 0.01, ***P < 0.001 compared with the untreated control group (Ctl), $^{\#}$P < 0.05 compared with the LPS exposure.

Treatment of cells with E2 had no significant effect on production of all studied cytokines. E2 exposure had an inhibitory effect on LPS-induced production of TNF-α (P <0.05) (Figure 2).

Discussion

In the present study we investigated immunomodulatory properties of primary female hormone 17β-estradiol and its role in LPS-mediated innate immune response. We observed a modulatory effect of E2 on neutrophils and monocyte-derived molecules which are involved in the regulation of inflammation. E2 did not affect cytokine production in cultures of whole blood cells, but notably suppressed LPS-induced TNF-α production. Besides, E2 administration diminished production of MCP-1 and IL-1β in LPS-treated cells (albeit data is not significant). Available data in the literature concerning effect of estradiol on endotoxin-stimulated cells is highly controversial. Different research groups demonstrated that production of cytokine such as IL-1β and IL-8 could be inhibited or even potentiated by E2, depending on experimental condition [2; 4; 5]. Our results suggest activation of adhesion properties of the cells by E2 as reflected by the decreased expression of CD62L (decrease in the expression of CD62L is due to shedding effect). Together these results indicate that under inflammatory condition E2 is capable of attenuating cytokine-mediated inflammatory responses of innate immune cells, presenting promising way to prevent excessive inflammation.

In conclusion, our experimental findings have provided additional evidence of the immunomodulatory properties of 17β-estradiol which is of special importance for regulating aberrant immune responses during steady state and inflammation.

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References

1. *Marriott I, Huet-Hudson YM.* Sexual dimorphism in innate immune responses to infectious organisms. Immunol. Res. 2006; 34: 177-192.

2. *Bengtsson AK, Ryan EJ, Giordano D, Magaletti DM, Clark EA*. 17beta-estradiol (E2) modulates cytokine and chemokine expression in human monocyte-derived dendritic cells. Blood 2004; 104: 1404-1410.
3. *Tyagi AM, Srivastava K, Mansoori MN, Trivedi R, Chattopadhyay N, Singh D*. Estrogen deficiency induces the differentiation of IL-17 secreting Th17 cells: a new candidate in the pathogenesis of osteoporosis. PLoS One 2012; 7: 44552.
4. *Pioli PA, Weaver LK, Schaefer TM, Wright JA, Wira CR, Guyre PM*. Lipopolysaccharide-induced IL-1 beta production by human uterine macrophages up-regulates uterine epithelial cell expression of human beta-defensin 2. J. Immunol. 2006; 176: 6647-6655.
5. *Pioli PA, Jensen AL, Weaver LK, Amiel E, Shen Z, Shen L, Wira CR, Guyre PM*. Estradiol attenuates lipopolysaccharide-induced CXC chemokine ligand 8 production by human peripheral blood monocytes. J. Immunol. 2007; 179: 6284-6290.
6. *Miller AP, Feng W, Xing D, Weathington NM, Blalock JE, Chen YF, Oparil S.* Estrogen modulates inflammatory mediator expression and neutrophil chemotaxis in injured arteries. Circulation 2004; 110: 1664-1669.

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CRONIC ACOUSTIC STRESS AND A_2- ADRENOBLOCKERS EFFECT ON OPEN FIELD ACTIVITY OF THE RAT

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Open field, noise, α_2-–adrenoblockers,behavioral activity, stress
«Բաց դաշտ», աղմուկ, α_2–ադրենաբլոկատորներ, վարքային ակտիվություն, սթրես
Открытое поле, шум, α_2– адрноблокаторы, поведенческая активность, стресс

It is shown that among hazardous factors of the environment the high level of industrial, transport and community ambient noise are extremely dangerous, which drastically decrease the resistibility of an organism, promote different diseases development and particularly lead to the **Cognitive disorders**, affect learning, memory, perception, and the problem solving [20,15,17]. The latteris much likely to be associated with an increase of the stress hormone release and the oxidative stress (OS) development [1]. Epidemiological research provides the possibility of an integral risk estimation of community noise based directly on the empirical data gained under the genuine conditions of exposure, considering factors potent to amplify or attenuate the noise exerted effects [21].

The data obtained serve as evidence of the structural reorganization and the functional change of bio-membranes in experimental animals under the noise action due to lipid peroxidation (LPO) process activation and α-Tocopherol exhaustion in tissues and significant protective effects of antioxidants and the stress –limiting compounds, among which we consider adrenoblockers [14]. Our investigations have shown a harmful action of chronic noise on the protein oxidative modification processes intensity both in plasma and Erythrocyte Membranes and the modifying effects of α_2-adrenoblockers on oxidative intensity, as well [14,22].

Considering all the above mentioned it is necessary to study effects related only to the noise influence in experiments, eliminating possibility of side effects.

Open field activity enables scientists evaluating locomotor behavioral activity, including the Total distance travelled, Average speed, Number of line crossings, Total