STUDY OF EXPRESSION OF TLR2, TLR4 AND TRANSCKRIPTION FACTOR NF-kB STRUCTURES OF GALT OF RATS IN THE CONDITIONS OF THE CHRONIC SOCIAL STRESS AND MODULATION OF STRUCTURE OF INTESTINAL MICROFLORA

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Stress-induced immune dysregulation results in significant health consequences for immune related disorders including viral infections, chronic autoimmune and inflammatory disease. Chronic social stress (CSS) impacts many physiological and pathological disease outcomes, including type 1 diabetes mellitus and IBD [14].

By using mouse and rats models of stress, significant progress has been made in determining the mechanisms behind stress-induced alterations in inflammatory immune status. For example, studies using a mouse model of repeated social defeat, termed social disruption (SDR) stress, have shown that stress alone can trigger the generation, egress, and trafficking of immature, inflammatory myeloid derived-cells that are glucocorticoid (GC) insensitive [6]. In addition, these GC insensitive cells produce high levels of IL-6 and other inflammatory cytokines and chemokines [12]. As a consequence, these stress-induced changes at the cellular level translate to significant immune (enhanced inflammatory responses and immunity to microbial, viral, and allergen challenge) and behavioral (prolonged anxiety-like behavior) changes [9,13]. Several reports have also described significant social stress-induced changes in immune organs, including the spleen and bone marrow, and in blood. Stress increases the size and the cellular composition of the spleen, primarily due to a significant increase in infiltrating $CD11b^+$ bone marrow-derived myeloid cells [14]. Along with an increase in number, CSSimpacts the GC sensitivity and effector function of bone marrow-derived myeloid cells, including differentiated macrophages and dendritic cells, and immature myeloid cells alike [12]. The important aspect of these findings is that the experience of repeated social defeat ramps up the production of ''primed'' immature myeloid populations in the bone marrow, which egress and traffic to peripheral and central tissues. Myeloid cells derived from CSS-treated mice display increased numbers of Toll-like receptors and co-stimulatory molecules and are resistant to the anti-apoptotic effects of high levels of GC, indicative of a primed state [12].

Pattern recognition receptors, such as Toll-like receptors (TLRs), are well known for their role in the recognition of infectious microbes and play a prominent role in initiating the expression of genes encoding for pro-inflammatory cytokines.

Despite that influence of CSS TLR on antigen-representative cells expression is shown in many works, analogous data for lymphoid GALT population is absent. TLR cells are actively expressed by adaptive immune system, T and B lymphocytes [7]. The level of expression can affect directly on the functional state of lymphocytes, affecting the level of differentiation, survival and proliferation [15].

In addition, the development of CSS accompanies with the changes of the intestinal microflora, which affects the level of signaling through PRR and leads to activation of nuclear factor NF-κB (nuclear factor kappa-B). Despite that classic AIC differ by the most intense expression of NF-kB, it is also always actively expressed in T-lymphocytes [19]. Moreover, changes in the level of NF-kB expression cells by T lymphocytes has a direct impact on the process of maturation, differentiation and activation has been shown to all major subpopulations T-helpers: Th1, Th2, Th9, Th17, T follicular helpers (Tfh) and T-regulatory lymphocytes [10]. Therefore, the aim of the research was to study the CSS influence on and modulation of intestine microflora content on the features of $TLR\text{-}2^+$ -, $TLR\text{-}4^+$ - and $NF\text{-}kB^+$-expressive cells in GALT line Wistar rats.

Material and methods. We studied 84 female Wistar rats dividing them into seven experimental groups: control rats were orally given 0,5 ml of physiological salt solution intragastrically per three weeks (group 1); rats whom CSS1 was modeled via 3 week social isolation and prolonged psychoemotional influence (PEI) implying permanent living of female in «aggressive environment» due to perforated partition in the cage with aggressive male confronting another planted male every day (group 2); rats, whom CSS2 was modeled via maintenance in overpopulated cage (20 rats in a cage) during 3 weeks with every day group change and the female under experiment was every day placed into a new balanced and overpopulated colony (group 3); rats with CSS1 and CSS2, whom the modulation of intestinal microflora composition was performed with intragastric daily administrations of aminoglycoside antibiotics Canamycine (Can) during 7 days beginning with the 3d week of modeling CSS in a dose of 15 mg/kg (group 4 and 5, respectively); rats with CSS1 and CSS2 whom modulation of intestinal microflora composition was performed via intragastric daily administrations

of *Lactobacterin* (*Lb*, a mixture of live lyophilically dried lactobacteria of *L.plantarum strain 8P-A3* and *L.fermentum strain 90T-C4*) during 3 weeks in a dose of 410^8CFU (group 6 and 7, respectively). The level of emotional-behavioral activity was established in the test «open field» accordingly the expression of experimental activity, the level of depression in animals was established in Porsalt test («enforced swimming», ES). The rats were removed from experiment with the method of decapitation under narcosis.

Immunofluorescence analysis: Population structure of $TLR2^+$-, $TLR4^+$- and $NF\text{-}kB^+$ cells GALT was studied basing on analysis of serial histological sections and findings of their morphometric and densitometric descriptions. To carry out this investigation on with rotary microtom MICROM HR-360 (Microm, Germany), 5-micron serial sections of iliac fixed according to Buen were performed, which were then deparaffined in xilol, rehydrated in decreasing concentrations of ethanol (100%, 96%, 70%), washed off in 0,1 M phosphate buffer (pH = 7,4) and painted with monoclonal anti-TLR-2, anti-TLR-4 FITC -conjugated (HycultBiotech) and primary anti- NF-kB (SantaCruzBiotechnology) antibodies during 18 hours in a moist chamber in t = 4 C. After washing off the rest of primary antibodies in 0,1 M phosphate buffer, the sections were incubated for 60 minutes (T = 37 C) with secondary antibodies in solution 1:64. As secondary antibodies, goat's ones were used to full molecule IgG of rabbit, conjugated with FITC (Santa Cruz Biotechnology). The processed histological sections were studied with the help of computer program ImageJ (NIH, USA). Images obtained on the microscope PrimoStar (ZEISS, Germany) in ultraviolet spectrum of stimulation 390 nm (FITC) with the help of highly sensitive chamber AxioCam 5c (ZEISS, Germany) and program package for obtaining, archiving and preparation of the images for publication AxioVision 4.7.2 (ZEISS, Germany) were immediately introduced into computer. At the same time, the areas with statistically significant fluorescence distinctive for lymphoid tissues expressing TLR2, TLR4 and NF-kB were determined in automatic regime. The lymphoid follicles (Lf) and subepithelial (sub) zone of Peyer's patches (PP) and lymphocyte-filled villi (LFV) were studied.

The results were statistically processed by means of software Statistica v. 6.0 (StatSoft, USA) using the Student's t-criterion. The resultssignificantly differed in statistics at $p<0,05$.

Results and their discussion. CSS development is accompanied by one way directed trend on increase in of total quantity of TLR^+ -lymphocytes in lymphoid structures of ileum of rats. The most expressed in LFV ($TLR2^+$ -lymphocytes) and in PP LFs ($TLR4^+$ - lymphocytes) (Fig. 1 AB). So, summary density of $TLR2^+$ - cells in the LFV has increased in 3.2 times ($p<0.05$) in CSS in 2.4 times ($p<0.05$) in CSS c PP Sub - 2.1 times ($p<0.05$) in CSS and 86% ($p<0.05$) in CSS2, in PP LFs - 2.2 times ($p<0.05$) in CSS1 and by 56% ($p<0.05$) in comparison with CSS2 control (Fig. 1 A-B).

Multitude of $TLR4^+$-cells in PP LFs has increased on 66% ($p<0.05$) in CSS and 44% ($p<0.05$) in CSS2 c PP Sub - 50% ($p<0.05$) only in the event CSS1 in LFV - on 59% ($p<0.05$) in CSS1 and on 41% ($p<0.05$) in CSS2 in comparison with the control (Fig. 1 AB).This increase in the total number of $TLR2^+$ - and $TLR4^+$ - cells was due to increase in all classes of lymphocytes, with the most intense increase in all areas of population density lymphoblasts.

The intensity of fluorescence TLR^+-lymphocytes, reflecting the density of TLR2 and TLR4 surface immunopositive cells showed a significant increase in this parameter also predominantly in lymphoblasts. In particular, the density in TLR2 $TLR2^+$- lymphoblasts in LFV increased on 26% (CSS2) -41% (CSS1) in PP sub 19% (CSS2) - 28% (CSS1) and PP LFs 20% (CSS1) and the density of TLR4 with $TLR4^+$ lymphoblasts in LFV increased on 24% (CSS1) -39% (CSS2) in the PP LFs 25% (CSS2) -39% (CSS1) PP sub in 15% (CSS1) - 21% (CSS2) in comparison with the control.

Increased of TLR2 and TLR4 GALT lymphocytes expression in the development of stress naturally leads to an increase in the number and $Nf\text{-}kB^+$- cells: in LFV in 1.8-2 times ($p<0.05$) in the PP sub - by 52-91% in PP LFs - to 89-92% ($p<0.05$), followed by increasing concentrations of Nf-kB in $Nf\text{-}kB^+$- macrophages by 15% ($p<0.05$) in PP sub at CSS1 and $Nf\text{-}kB^+$- lymphocytes 11% ($p<0.05$) in the LFV CSS1 in comparison with the control (Fig. 2 A,D).

Noteworthy is the fact that the Can injection to the experimental animals there is a general downward trend in the total population density $TLR2^+$ - and $TLR4^+$ - lymphocytes was significantly expressed for $TLR2^+$ - cells in the PP LFs at CSS1 (22%, $p<0.05$) in LFV and in case PP sub CSS2 (31% and 37%, respectively) and $TLR4^+$-lymphocytes in PP sub (29%, $p<0.05$, CSS1 in exception of PP LFs with CSS2, wherein the reaction of stressed rats Can expressed in administering increasing of total $TLR2^+$ - and $TLR4^+$ - 75% of the cells in 2.1-fold ($p<0,05$) (Fig. 1 C-D).

In this case, changes in the density of TLR2 and TLR4 with the Can injection had multi directional nature and depended on the type of stress. Furthermore, experimental animals administration of Can are accompanied by a reduction of the total PP $Nf\text{-}kB^+$-cells in CSS1 (LFV to 26%, $p<0.05$; PP sub to 33%, $p<0.05$ in PP LFs 27% $p<0.05$) and increased in their number PP sub in CSS2 (54%, $p<0.05$) and led to an increase in the concentration of nuclear factor $Nf\text{-}kB^+$-macrophages and $Nf\text{-}kB^+$-dendritic cell sin PP sub and PP LFs CSS2 (Fig.2B, E).

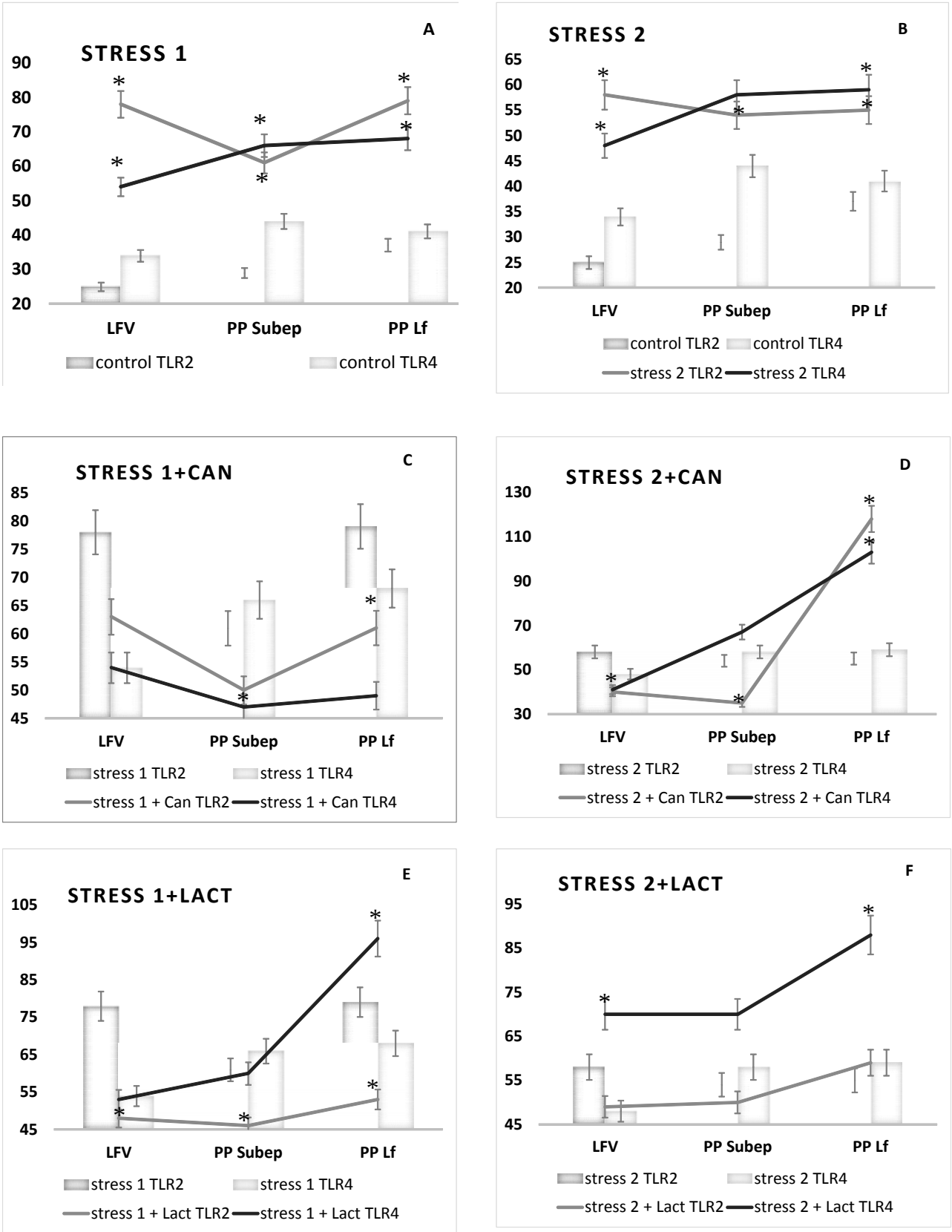

Fig. 1. The number of TLR2⁺- and TLR4⁺-cells
*note: * - p<0,05*

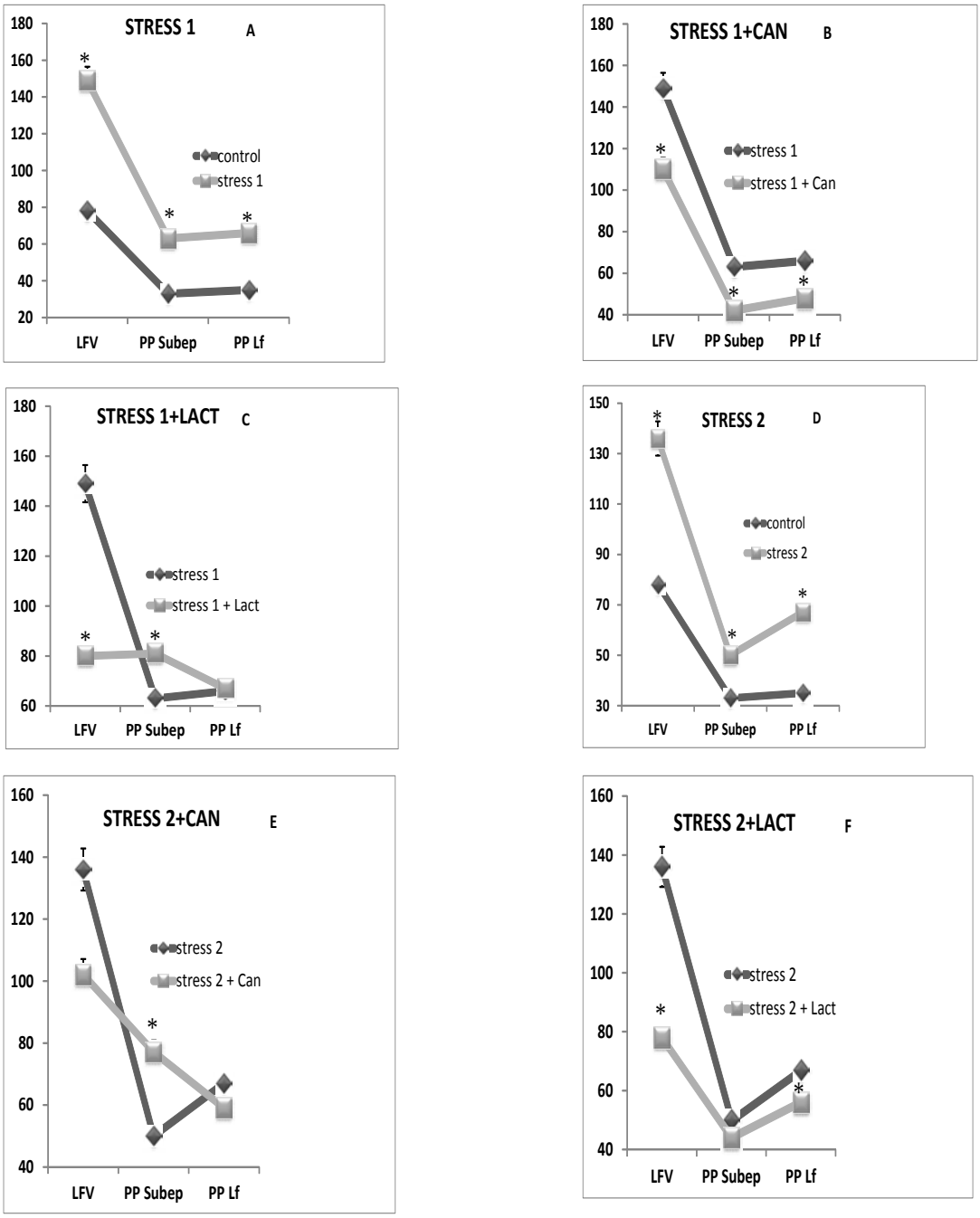

Fig. 2.The number of Nf-kB⁺-cells
*note: * - p<0,05*

Injections of Lb to the stressed animals led to a decrease in the total PP TLR2⁺ - lymphocytes in the development of CSS1 (in LFV -by 37%, p <0.05, in PP sub - by 23%, p <0.05, in the PP LFs on 33%, p <0.05) and had no effect on their numbers in the case of CSS2, while the total number of TLR4⁺ -lymphocytes is mainly increased (in the LFV -by 46%, p <0.05 in the case of CSS2; LFs in the PP on 43%, p <0.05, CSS1 and 49%, p <0.05 CSS2) (Fig. 1 E-F).

These changes were accompanied by a significant attenuation of the density of TLR2- and TLR4 - receptors inTLR2⁺- lymphoblasts (in LFV by 27% and PP LFs by 21% at CSS1), TLR4⁺-lymphoblasts (in the LFV, CSS2 and PP LFs, CSS1) and a small TLR2⁺- lymphocytes (the PP LFs 9% at CSS1 LFV and 11% at CSS2). At the same time, Lb injection advantageously is reduced total population density of Nf-kB⁺- cells (LFV - 46%, p <0.05 CSS1 and 50%, p<0.05 in the case CSS2; LFs in the PP 17%, p<0.05 CSS2) except PP sub where their number at CSS1 significantly is increased (on 29%, p <0.05) and had a mixed effect on the concentration of Nf-kB in immunopositive cells (Fig. 2C, F). (Fig. 2 C, F).

Our results coincide with the data of other researchers.

Thus, Powell N. et al. (2009) showed that exposure to SDR also increased the expression of TLR2 and TLR4. The mean fluorescence intensity of anti-TLR2 antibody staining on splenic CD11b+ monocytes/macrophages was significantly higher in cells from mice exposed to SDR than in cells from HCC mice. In addition to the SDR-induced increase in anti-TLR2 staining, the mean fluorescence intensity of anti-TLR4 antibody staining on splenic CD11b+ monocytes/macrophages was also significantly higher for mice exposed to SDR. Mice lacking functional TLR4 (i.e., C3H/HeJ mice) did not show the same SDR-induced increase in clearance of bacteria from the spleen as did stressed mice containing functional TLR4 (i.e., CD-1 and C3H/HeN mice exposed to SDR) [12]. In addition to the ability of CSS to induce the activation of TLR CSS, our results coincide with a number of studies that have shown increased activity of the transcription factor NF-kB in this kind of stress. So, Pace T. (2006) showed that psychosocial stress induces the transcription factor NF-κB in peripheral blood mononuclear cells in healthy volunteers undergoing the Trier social stress test (TSST). In addition, TSST induced increases in IL-6 and NF-κB DNA-binding were greater in major depression patients with increased early life stress and independently correlated with depression severity, but not early life stress [11]. Male major depression patients with increased early life stress exhibit enhanced inflammatory responsiveness to psychosocial stress, providing preliminary indication of a link between major depression, early life stress and adverse health outcomes in diseases associated with inflammation.

At present, convincing clinical efficacy of probiotic lactobacilli applications has been documented for various conditions, including prevention of antibiotic-associated diarrhea, prevention of severe necrotizing enterocolitis in preterm infants, symptom alleviation in irritable bowel syndrome, and reduction of respiratory tract infection incidence [2]. Despite the large number of studies on anti-inflammatory effects of LB [8,18], a number of questions are remained open: 1) To what extent can we speak about common responses to different probiotic lactobacilli? 2) Can the reception of LB stimulate inflammation in the gut? 3) What are the nuances of answer the congenital and adaptive immune system to LB under stress?

It is known that in some cases the receiving of LB can increase the pro-inflammatory signaling and increase risk of AID, development rheumatoid arthritis experimental autoimmune encephalitis. Thus, monocolonization of IL-1 receptor antagonist-knockout ($Il1rn^{-/-}$) mice, which spontaneously develop an autoimmune T-cell-mediated arthritis, with the commensal *Lactobacillus bifidus* resulted in rapid disease onset, of comparable severity and incidence to the arthritis observed in non-germ-free mice. L. bifidus-triggered arthritis in this model is driven by an imbalance in TREG–TH17 cell homeostasis and mediated through TLR2–TLR4 signaling [1]. Our data show that oral administration of LB in the CSS may increase in some areas of the expression of TLR, and TLR-4, which are not direct sensors of PAMP lactobacilli. This does not contradict the other work that demonstrated that oral administration of LB by experimental animals can activate the expression of TLR2 and TLR4 types, decrease the level of Treg-cells and increase the amount of proinflammatory Th17- and Th1-cells and also IL-12 [17]. Таκ, Purified LTA from *L. plantarum*str. WCFS1, L. plantarum str. KCTC10887, Lactobacillus casei str. YIT9029 and Lactobacillus fermentum str. YIT0159, as well as whole cells of *L. acidophilus str. NCFM*, modulates tumour necrosis factor (TNF) levels through a TLR2-dependent mechanism [4], and according to Chiba Y. (2010) In spleen cells, probiotic Lactobacillus casei induced interleukin (IL)-12 production by CD11b+ cells more strongly than pathogenic Gram-positive and Gram-negative bacteria and effectively promoted the development of T helper (Th) type 1 cells followed by high levels of secretion of interferon (IFN)-γ [5].

Our data on the change in the expression of NF-kB after the application of LB in the CSS coincide with a number of works that have demonstrated in volves inhibition of the inhibitor kB /nuclear factor kB (IkB/NF-kB) pathway by block of IkB-a degradation, which prevents nuclear translocation of active NF-kB dimmer and subsequent relevant gene expression [3]. At the same time, the subepithelial zone of PP is observed increase of total NF-kB^+-cells after receiving LB (CSS1) in comparison with the stressed animals.

A number of studies have shown that depletion of the microbiota as a consequence of antibiotic treatment results in reduced TLR signaling and downstream regulation of innate defences [16]. Nevertheless, our results showed that the AB use although the general trend is accompanied by a decrease of $TLR2^+$ -, $TLR4^+$ -and Nf-kB^+-cells may in certain areas to increase their number and affect the density of the receptors.

CONCLUSION

1. Development of CSS is associated with an increase of the total number of lymphocytes expressing TLR2 and 4 type GALT rats with the most pronounced in LFV ($TLR2^+$-lymphocytes) and PP LFs ($TLR4^+$- cells) led to an increase in the number of Nf-kB^+-cells: in LFV 12,8 - fold ($p < 0.05$) in the PP sub - by 52-91% ($p < 0.05$) in PP LFs – by 89-92% ($p < 0.05$), and affect the density of TLR2, TLR4, and the concentration of Nf-kB in immunopositive cells.
2. Changes in the content of the intestinal flora by administering Can injection to experimental animals followed by a general trend to reduce the number of $TLR2^+$ -, $TLR4^+$ - and Nf-kB^+- cells except PP LFs with CSS2 wherein the total number of $TLR2^+$ - and $TLR4^+$ - lymphocytes is increased on 75% - 2.1 times ($p<0.05$) and CSS2 PP sub in where the number of Nf-kB^+-cells is increased on 54% ($p < 0.05$).
3. Lb injections to the stressed animals led to a decrease in

the total PP TLR2+ - lymphocytes in the development of CSS1 had no effect on their numbers in the case of CSS2, while the total number of TLR4+ - lymphocytes mainly is increased (in LFV – on 46%, CSS2, in LFs-PP on 43% CSS1 and 49%, CSS2). Lb injections reduced the number of Nf-kB+-cells (LFV - 46%, CSS1 and 50% CSS2; the PP LFs-17%, CSS2) except PP sub where their number in CSS1 significantly is increased (on 29%) and had a mixed influenced on the concentration of Nf-kB in immunopositive cells.

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SUMMARY

STUDY OF EXPRESSION OF TLR2, TLR4 AND TRANSCKRIPTION FACTOR NF-kB STRUCTURES OF GALT OF RATS IN THE CONDITIONS OF THE CHRONIC SOCIAL STRESS AND MODULATION OF STRUCTURE OF INTESTINAL MICROFLORA

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The present study was conducted to investigate of the influence of chronic social stress (CSS) and modulation of the composition of intestinal microflora on the distribution of TLR2+-, TLR4+- and Nf-kB+-cells in the GALT of ileum of the rats. Researchers have been conducted on 84 rats (female) of Wistar line, which were divided on 7 experimental groups: control rats (group 1); rats, which were modeled CSS1 by means of three weeks social isolation and prolong psychoemotional influence (group2); rats, which having CSS 2 modeling by means of keeping animals in over populated cages with every day change of grouping (group 3); rats with CSS1 and CSS2, which were made the modeling of intestinal microflora by means of administrations of aminoglycosed antibiotic kanamycin (group 4 and 5, accordingly); rats with CSS1 and CSS2, which were made the modeling of intestinal microflora by means of everyday administrations of lactobacterine (groups 6 and 7, accordingly). Structure of population of TLR2+-, TLR4+- and Nf-kB+-cells has been studied by the

analysis of serial histological sections using the method of direct and indirect immunofluorescense with monoclonal antibodies to TLR2, TLR4 and Nf-kB. CSS development is accompanied with increase in total lymphocytes expressing TLR2 and 4 type GALT rats with the most pronounced in LFV (TLR2$^+$-lymphocytes) and PP LFs (TLR4$^+$-cells) led to an increase in the number of Nf-kB$^+$- cells: in LFV a 1.8-2 fold ($p<0.05$) in PP at the sub - 52-91% ($p<0.05$) in PP LFs – for 89-92% ($p<0.05$), and it is also influenced on the density of TLR2, TLR4, and the concentration of Nf-kB in immunopositive cells. AB and PB injections were accompanied by a decrease in the number of studied cells, so in the separate zone GALT is increased to their number, changing the density of immune system receptors.

Keywords: chronic social stress, GALT, TLR2, TLR4, Nf-kB.

РЕЗЮМЕ

ИЗУЧЕНИЕ ЭКСПРЕССИИ TLR2, TLR4 И ТРАНСКРИПЦИОННОГО ФАКТОРА NF-kB В СРУКТУРАХ GALT КРЫС В УСЛОВИЯХ ХРОНИЧЕСКОГО СОЦИАЛЬНОГО СТРЕССА И МОДУЛЯЦИИ СОСТАВА КИШЕЧНОЙ МИКРОФЛОРЫ

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Исследование проведено с целью изучения влияния хронического социального стресса (ХСС) и модуляции состава кишечной микрофлоры на распределение TLR2$^+$-, TLR4$^+$- и Nf-kB$^+$- клеток в GALT подвздошной кишки крыс. Исследования проводились на 84 крысах (самки) линии Wistar, которые были разделены на 7 экспериментальных групп: контрольные крысы (I группа); II группа - крысы, которым был смоделирован ХСС1 с помощью трехнедельной социальной изоляции и длительного психоэмоционального воздействия; III группа - крысы, у которых был смоделирован ХСС2 методом содержания животных в перенаселенных клетках и с каждодневным изменением состава группировки; крысы с ХСС1 и ХСС2, которым с целью изменения состава кишечной микрофлоры был введен аминогликозидный антибиотик (АБ) канамицин (IV и V группы, соответственно); крысы с ХСС1 и ХСС2, которым моделировали состав кишечной микрофлоры посредством каждодневного введения пробиотика (ПБ) лактобактерина (VI и VII группы, соответственно). Структуру популяции TLR2$^+$-, TLR4$^+$- и Nf-kB$^+$- клеток изучали путем анализа серийных гистологических срезов методом прямой и непрямой иммунофлюоресценции с применением моноклональных антител к TLR2, TLR4 и NF-кВ. Развитие ХСС сопровождалось увеличением общего количества лимфоцитов, экспрессирующих TLR2 и TLR4 в GALT крыс, наиболее выраженным в LFV (TLR2$^+$-лимфоциты) и в PP LFs (TLR4$^+$-лимфоциты), приводило к возрастанию числа Nf-kB$^+$-клеток: в LFV в 1,8-2 раза ($p<0,05$); в PP sub– на 52-91% ($p<0,05$); в PP LFs – на 89-92% ($p<0,05$), а также влияло на плотность TLR2, TLR4 и концентрацию Nf-kB в иммунопозитивных клетках. Введение АБ и ПБ сопровождалось как снижением числа исследуемых клеток, так и, на фоне изменения плотности рецепторов, врожденного иммунитета увеличением их количества в отдельных зонах GALT.

რეზიუმე

TLR2 და TLR4 ექსპრესიის და ტრანსკრიფციული ფაქტორის NF-kB შესწავლა ვირთხების GALT სტრუქტურებში ქრონიკული სოციალური სტრესის და ნაწლავების მიკროფლორის მოდულაციის პირობებში

ი. ტოპოლი, ა. კამიშნი

ზაპოროჟიეს სახელმწიფო სამედიცინო უნივერსიტეტი, მიკრობიოლოგიის, ვირუსოლოგიის და იმმუნოლოგიის დეპარტამენტი, უკრაინა

კვლევის მიზანს წარმოადგენდა ქრონიკული სოციალური სტრესის (ქსს) და ნაწლავის შემადგენელი მიკროფლორის მოდულაციის გავლენის შესწავლა ვირთხების GALT-ში TLR2$^+$-, TLR4$^+$ და Nf-kB$^+$-უჯრედების განაწილებაზე. გამოკვლევა ჩატარდა Wistar-ის მდედრობითი სქესის 84 ვირთხაზე, რომლებიც განაწილებული იყო 7 ექსპერიმენტულ ჯგუფში: I ჯგუფი – საკონტროლო, II ჯგუფი – ვირთხები, რომლებსაც სამკვირიანი სოციალური იზოლაციის და ხანგრძლივი ფსიქოემოციური ზემოქმედების მეშვეობით ჩამოუყალიბდა ქსს-1; III ჯგუფის ვირთხებში ქსს-2 გამოწვეული იყო გალიებში მათი დიდი რაოდენობით მოთავსებით და მათი შემადგენლობის ხშირი ცვლით; IV და V ჯგუფი მოთავსდნენ ქსს-1 და ქსს-2 ვირთხები, რომლების-თვისაც ნაწლავური მიკროფლორის ჩამოყა-ლიბების მიზნით შეყავდათ ამინოგლიკოზიდური ანტიბიოტიკი (აბ) – კანამიცინი; VI და VII ჯგუფი შეადგინა ქსს-1 და ქსს-2 ვირთხებმა, რომელთა ნაწლავური მიკროფლორის მოდელირება განხორციელდა პრობიოტიკის (პბ) – ლაქტობაქტერინის შეყვანით. TLR2$^+$-, TLR4$^+$ და Nf-kB$^+$-უჯრედების პოპულაციის შესწავლა ხორციელდებოდა სერიული ჰისტოლოგიური ანათლების გაანალიზების მეშვეობით პირდაპირი და არაპირდაპირი იმმუნოფლუორესცენციის მეთოდით TLR2, TLR4 და Nf-кВ მიმართ მონოკლონური ანტისხეულების გამოყენებით. ქსს-ის განვითარებას თან სდევდა ლიმფოციტე-

ბის საერთო რაოდენობის ზრდა, რაც ვირთხების GALT-ში იწვევდა $TLR2^+$- და $TLR4^+$- ტიპის ექსპრესიას, რომელიც განსაკუთრებით გამოხატული იყო LFV-ში ($TLR2^+$- ლიმფოციტები) და PP LFs ($TLR4^+$-ლიმფოციტები). ყოველივე ზემოაღნიშნული იწვევდა $Nf\text{-}kB^+$-უჯრედების რაოდენობის ზრდას: LFV-ში – 1,8-2-ჯერ ($p<0,05$), PP sub-ში – 52-91%-ით ($p<0,05$), PP LFs-ში – 89-92%-ით ($p<0,05$). აღნიშნული გავლენას ახდენდა TLR2 და TLR4 სიმკვრივეზე და იმმუნოპოზიტიურ უჯრედებში Nf-kB კონცენტრაციაზე. ვირთხების ორგანიზმში აბ-ს და პბ-ს შეყვანას თან სდევდა როგორც საკვლევ უჯრედების რიცხვის შემცირება, ასევე მათი რაოდენობის ზრდა GALT-ის ცალკეულ ზონებში, თანდაყოლილი იმუნიტეტის რეცეპტორების სიმკვრივის ცვალებადობის ფონზე.

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