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MICROSTRUCTURAL ALTERATIONS IN THE LYMPHOID ORGANS OF THE PERIADOLESCENT RATS AFTER EXPERIMENTAL EXPOSURE TO THE REPEATED AND VARIABLE STRESSORS.

Kapitonova M.[1], Ahmad A.[2], Nor-Ashikin M.N.K.[2], Fuad S.B.[2], Kuznetsov S.L.[3], Dydykin S.S.[3]

[1]Faculty of Medicine and Health Sciences, UNIMAS, Kota Samarahan, Sarawak, Malaysia, [2]Faculty of Medicine, UiTM, Sungai Buloh, Selangor, Malaysia, [3]Sechenov First Moscow State Medical University, Moscow, Russia.

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Abstract:

Recent investigations demonstrated that chronic variable stressors exhibit more severe effect on the endocrine and immune systems of the body compared the repeating stressors. During adolescent period of life lymphoid organs undergo fast developmental changes which may overlap with stress-induced immunomodulation. The influence of different chronically applied stressors on the lymphoid organs during various phases of periadolescence requires further investigation.

The objective of this research is to evaluate immunomodulatory effect of variable vs. repeating stressors on the morphology of the lymphoid organs in periadolescent experimental animals of different age.

Repeating or variable stressors were applied for 9 days continuously to the infant and early pubertal rats. Central (thymus) and peripheral (spleen) lymphoid organs were sampled and evaluated using morphometry of the immunologically stained histological sections.

Chronic variable stressors exposure showed more significant immunosuppressive effect in the thymus and spleen of the infant rats compared to the early pubertal ones, as demonstrated by more significant decrease of the volume density of different populations of immunocytes in the thymus and the spleen, and an increase of the density of caspase-3-positive cells in the spleen. Comparative evaluation of the repeating stressor exposure revealed significant reduction of the volume density of the CD20+ lymphocytes and increased apoptotic rate in the spleen of both age groups; CD45RC+ lymphocytes in the thymus and CD4+, CD8+ and CD90+cells in the spleen were reduced in the infant rats only, while in early pubertal rats reduction was not significant compared to the control animals.

This microscopic study shows that different neuroendocrine consequences of chronically applied variable vs. repeating stressors during various periods of early age induce complex age-dependent immunomodulation due to the overlapping of the developmental changes and post-stress alterations in the central and peripheral lymphoid organs in the immature body of the experimental animals.

Key words: *immune organs, stress, adolescence, immunohistochemistry, morphometry*

Corresponding author:

Kapitonova M,

Faculty of Medicine and Health Sciences, UNIMAS,
Kota Samarahan, Sarawak, Malaysia.

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INTRODUCTION:

In recent years, immunomodulatory effect of stress was shown in numerous papers [1-10]. Stress-responsiveness is determined by the interplay of the neuroendocrine and sympathetic nervous systems [11]. Stress-induced immunity changes are regulated by different mechanisms, among which the most significant is the effect of the hypothalamo-pituitary-adrenal axis hormones on the receptors of the immune cells [12,5,13]**.** Many researchers demonstrate that chronic stress may suppress immune function of the body and initiate accelerated immunological aging, while short-term stress is usually immuno-enhancing [14,15,10,5,6,8,9].

On the contrary, other investigators provide evidence of immu**nos**uppression after a short-term stress and absence of immu**nos**uppression or even immuno-enhancement in chronic stress [16,17,18,2,4,12,18,19]. For example, chronic psychosocial stress was shown to induce activation and differentiation of T cells into Th1, Th2, and Th17 effector cells in peripheral lymph nodes; enhance the frequency of regulatory T cells in the CD4 population, the suppressive activity of bone marrow-derived myeloid-derived suppressor cells towards proliferating T cells in the spleen and create inflammatory immune status of the body [16,17]. These controversies may be explained by the complexity of the effect of stress hormones on the immune function of the body and demonstrate that the problem of stress-related immunomodulation is far from being resolved.

Vulnerability of lymphoid tissue to stress depends not only on the type of stress (acute or chronic with various stress exposure length, physical or psychological, mild or severe, escapable or non-escapable, predictable or non-predictable), but also on the other features which affect responsiveness of the hypothalamo-pituitary-adre**n**al axis, such as age, sex, strain of the experimental animal, history of previous exposure to stressors, as well as the initial immune status of the body**.** On the contrary to the acute stress, the chronic one is more likely to adversely affect the neuroimmunoendocrine system [1,15,10,5,7].

In spite of all research performed to understand the complications of stress exposure of the body and attempts undertaken to find proper measures of defense, further searches are necessary for clearer understanding of the mechanisms of post-stress changes in the central nervous system, endocrine glands and lymphoid organs. Quite often stress-related impairment of the immunity is assessed using molecular or immunological methods or even the weight of the immune organs (thymus, spleen) [20,21,2,22,23,8,24**]**. These methods may evaluate total cell count of lymphoid cells in blood or lymphoid organs [1,3]. As thymus, lymph nodes and spleen are strongly compartmentalized organs, the distribution of different types of target cells between their compartments is very important for the adequate immune response. Very few microstructural research works have been done evaluating alterations in the immune organs after stress exposure. Some researchers assess various compartments in the lymphoid organs without evaluation of distribution of the immune cells among them and consideration of the crosstalk between different types of lymphocytes [25,4,9,26].

Early life stress may have severe consequences for the body, including disregulation of the hypothalamo-pituitary-adrenal axis and increased overall risk on psychopathology [27]. Although various aspects of post-stress immunomodulation has been studied widely in different age groups [12,13,26,19], detailed stress-induced alterations in the lymphoid organs during early life need to be further elaborated. In few papers on stress-induced morphological changes in the immune system of the immature body, the correlation between developmental and stress-induced alterations in the lymphoid organs is not emphasized [20,4,23,9,24].

Recently, some papers described consequences of exposure to the changing stressors, which become more common these days [20,28,29,9,24]. Changes to the microarchitecture of both central and peripheral lymphoid organs in the immature body exposed to variable stressors at some age deserve more attention for deeper comprehension of their mechanisms and development of the defensive measures.

The objective of this study is to assess the influence of repeating and variable stressors on the microstructure of lymphoid tissue in the primary and secondary immune organs of the periadolescent rats of different age.

MATERIAL AND METHODS:

Periadolescent Sprague Dawley male rats aged 30 (infant period) and 60 (early pubertal period) days [30] were involved in this study. The design of the project was approved by the ethical committee of the Faculty of Medicine, UiTM, Selangor, Malaysia, protocol ACUC 4-11, 14.04.11.

Each age group included three subgroups of six rats each, making a total of 36 rats. Group 1 served as an age-matched control, Group 2 animals were exposed

to the repeating stressors and Group 3 animals were exposed to variable stressors [31]. Exposure lasted for 9 days with daily 2-hour stress sessions. Control animals were not in contact with the treated animals. After the last stress session, animals were euthanized under anaesthesia. Their thymus and spleen were sampled, processed, embedded in paraffin, sectioned and stained by haematoxylin and eosin. Immunohistochemistry was performed using antibodies against CD4 (clone W3/25, AbDSerotec, US); CD8 (clone MRC OX8, AbDSerotec); CD90 (clone HIS51, BD Biosciences); caspase-3, (AbDSerotec, US); CD20 (clone R1N-**9D3,** **A**bDSerotec) and CD45RC (clone OX22, AbDSerotec, US). Streptavidin-biotin-peroxydase was used to identify the end product, according to manufacturer's recommendations. Quantitative data on the distribution of immunoreactivity were obtained using the Image Pro+ 8.0 (Media Cybernetics, US) software.

All data are presented as the mean ± S.E.M, (n = 6) group. The results were analyzed by one-way analysis of variance (ANOVA) followed by Student–Newman–Keuls multiple comparison test; $p<0.05$ values were considered as statistically significant.

RESULTS:

Both repeating and variable stressors caused microscopic changes in the spleen which included reduction of the volume of the T- and B-zones in the white pulp, marginal zone (MZ), expanded marginal sinus (MS), decreased cellular density in the periarterial lymphoid sheathes (PALS) around the central arteries (CA) and arteries of the white pulp (AWP), and in the lymphoid nodules (LN), increased number of macrophages and megakaryocytes (Mb) in the red pulp (RP) (Fig.1). Microarchitecture of the thymus in both experimental groups was also disrupted, with reduced thickness of the thymic cortex (C), indistinct border between cortex and medulla (M), increased number of ting**i**ble body macrophages (TbM) in the cortex and Hassal's corpuscles in the medulla (Fig.2).

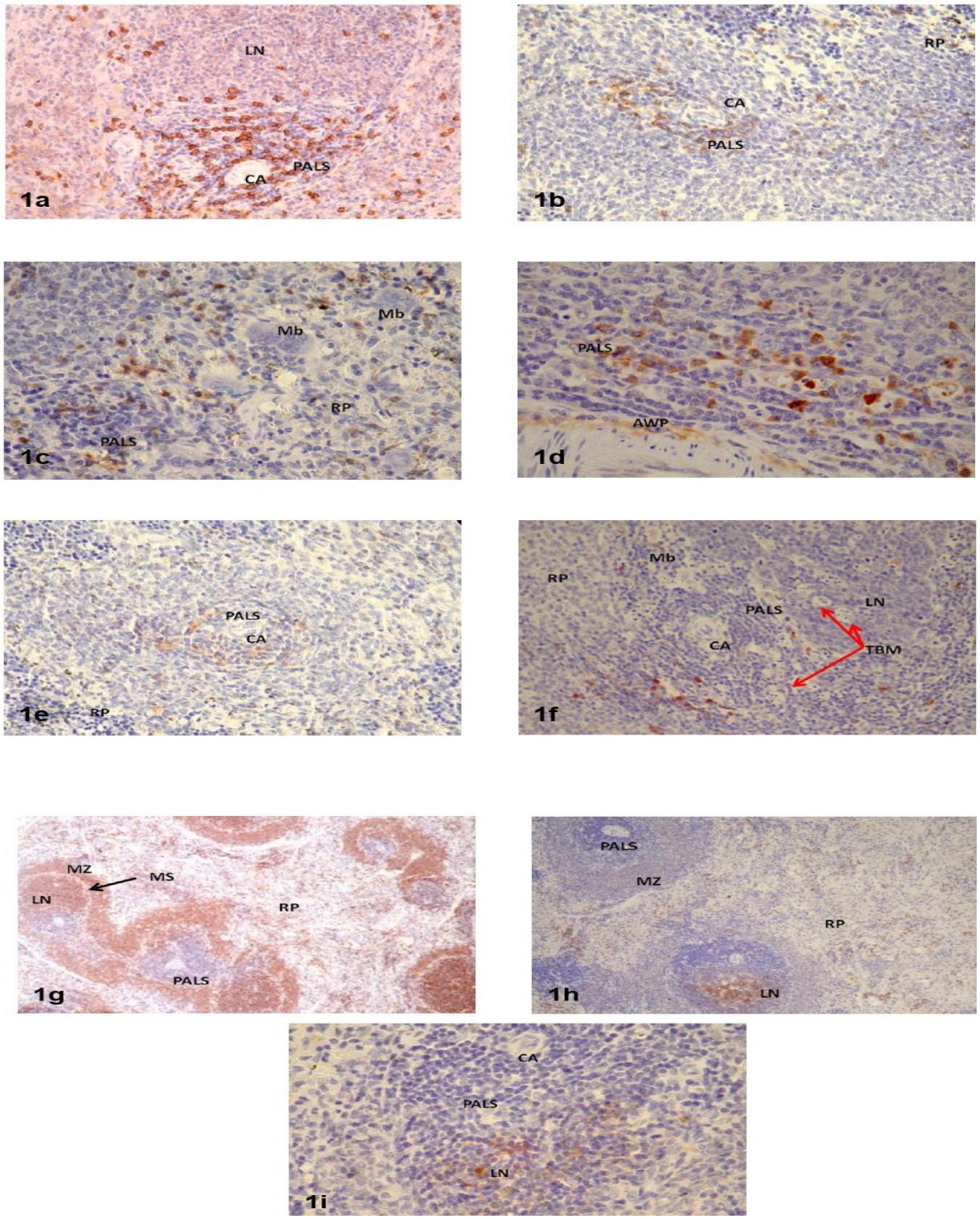


Fig.1. Microphotographs of the spleen of the control (1a,d,g) and experimental (repeating-1b,e,h; variable-1c,f,i)groups of rats; 1a,b,c – infant rats, staining for CD8; 1d,e,f – early pubertal rats, staining for CD90; 1g,h,I infant rats, staining for CD20. Magnification x400 (1a,b,c,d,e,f,i) and x200 (g,h). Legends: MZ – marginal zone, MS – marginal sinus, PALS – periarterial lymphoid sheath, CA – central artery, AWP – artery of the white pulp, LN – lymphoid nodule, Mb – megakaryocyte.

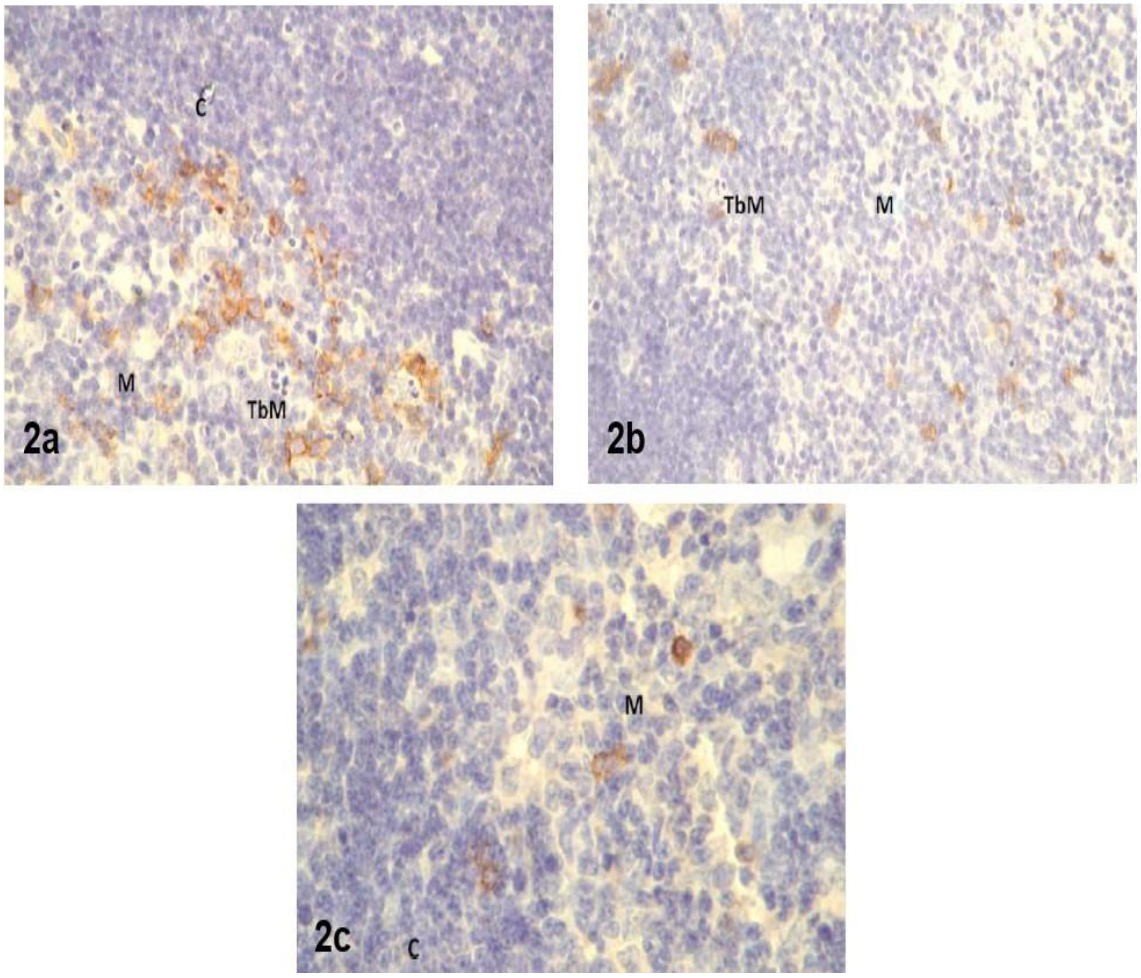


Fig.2. Microphotographs of the thymus of the control (2a) and experimental (repeating – 2b, variable – 2c) groups of infant rats; staining for CD45RC; magnification x400. Legends: C – cortex, M - medulla, TbM -tingible body macrophages

Immunohistochemical staining revealed changes in the distribution of the immunocytes between different compartments of the spleen and thymus. The data of morphometric analysis are presented in Figures 3 to 8.

Fig.3 Volume density of the CD8+cells in the spleen of the control and experimental rats (%), M±m

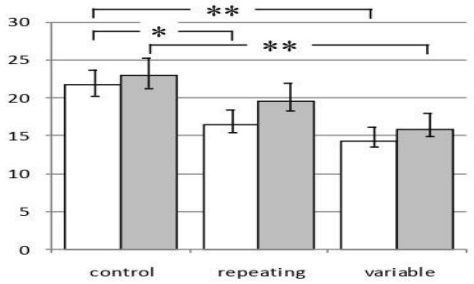


Fig.4 Volume density of the CD90+cells in the spleen of the control and experimental rats (%), M±m

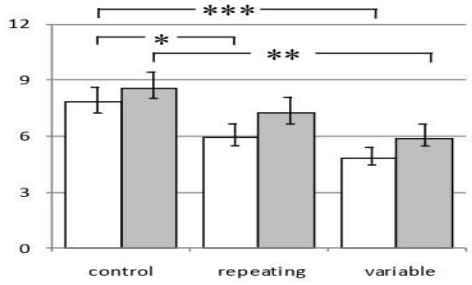


Fig.5 Volume density of the CD4+cells in the spleen of the control and experimental rats (%), M±m

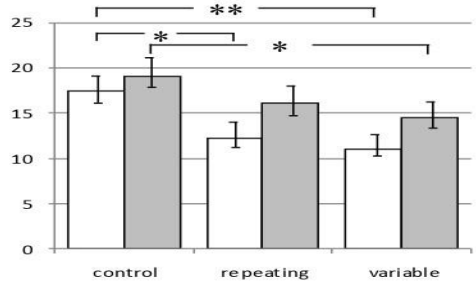


Fig.6 Volume density of the CD20+cells in the spleen of the control and experimental prepubertal rats (%), M±m

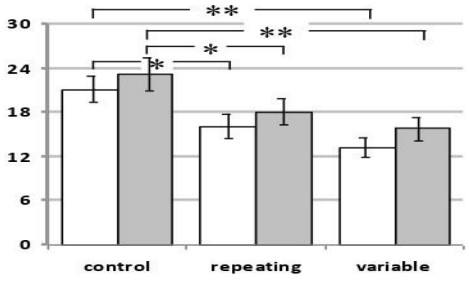


Fig.7. Volume density of the caspase-3+cells in the spleen of the control and experimental rats (%), M±m

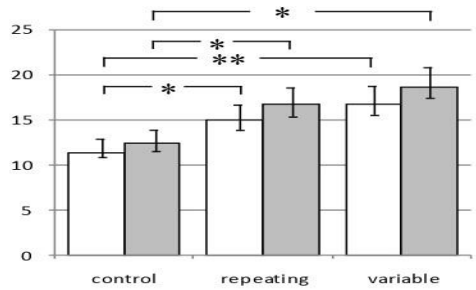
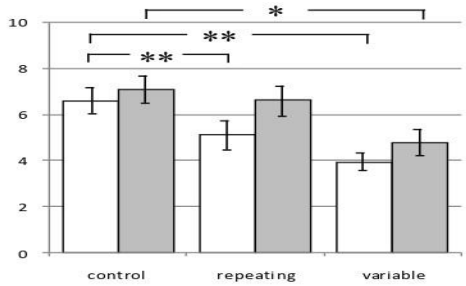


Fig.8. Volume density of the CD45RC+cells in the thymus of the control and experimental rats (%), M±m



There was a highly significant reduction of volume density of the CD8+ ($p<0.01$) and CD90+ ($p<0.01$ and $p<0.001$) cells in the variable stress groups of younger and older age subgroups. In the repeating stress group, it was significantly reduced in the younger age subgroup only ($p<0.05$), while in the older subgroup the difference from control group did not reach the level of significance (Figures 3 and 4). In the variable stress group, CD4+ cells in the spleen showed highly significant ($p<0.01$) and significant ($p<0.05$) reductions of volume densities in the younger and older groups respectively. In the repeating stress group, this reduction was significant only in younger rats ($p<0.05$) (Fig.5). CD20-positive cells were significantly reduced in both experimental groups and age subgroups with different levels of significance ($p<0.05$ in the repeating stress group and $p<0.01$ in the variable stress group) (Fig. 6). Caspase-3 positive cells displayed significantly increased volume density ($p<0.05$) in both age subgroups of the repeating stress group. In the heterotypic variable stress group, the difference was significant for the older subgroup ($p<0.05$) and highly significant ($p<0.01$) for the younger age group (Fig.7).

In the thymus, volume density of the CD45RC groups showed highly significant reductions in both younger age stress subgroups ($p<0.01$), while in the older group this difference was significant only in the variable stress group ($p<0.05$) (Fig.8).

DISCUSSION:

Our data are in agreement with the results of other investigators on the immunosuppressive effect of chronic stress with reduced lymphocyte population and antibody production due to stress hormones in the periadolescent animals [20,23,8,24,19], and provide additional information on the apoptotic rate of splenocytes and distribution of different populations of lymphocytes between the compartments of the central and peripheral lymphoid organs in the growing body of the stressed experimental animals. Earlier we reported our data on the changes in the splenic white pulp of the infantile and adolescent rats induced by the homo- and heterotypic stressors, which showed that stress exposure, particularly during the most sensitive period of immunological maturation (preweaning age) causes lymphocyte depletion in the T- and B-zones of the organ, reduction of the number of follicular dendritic cells, inhibition of proliferation of splenocytes and increased apoptotic rate, the severity of which depended on the type of stressors applied [32].

Our current research demonstrated the effect of the repeating and variable stressors on the microarchitecture and intensity of apoptoses in the central (thymus) and peripheral (spleen) organs of the immune system in the infant and early pubertal rats, and altered distribution of different subpopulations of immunocytes between thymic cortex/medulla and splenic white pulp/red pulp/marginal zone.

It has been shown [4,18] that chronic stress increases the mass of the white pulp and its subcompartments and enhances immune reactivity in the animals with the previous early life stress history. Our results show that early life stress has immediate immunosuppressive effect which makes the growing body vulnerable to infections and other adverse events, but once overcome, we presume, it may result in enhanced immunity during stress exposure in adulthood.

On the contrary to the other researchers, who were mainly using immunological and biochemical/molecular methods for the evaluation of the post-stress immune status of the body, we applied histological and immunohistochemical methods which allowed us not only to observe the microscopic alterations in the subcompartments of the central and peripheral immune organs, but also to assess distribution of different population of immunocytes between them. Our results showed that **s**ensitivity to the different types of stress (repeating vs. variable) depends on the particular age within periadolescent period of early life. Different subpopulation of T-cells (CD4+, CD8+, CD90+) were significantly reduced in the younger group of animals subjected to both types of stress exposures. In older rats, this was true only in the variable stress group. Similar patterns of changes were observed in the recirculation pool of lymphocytes, as demonstrated by image analysis of the CD45RC+ cells in the thymic medulla. These changes correspond to the hypothalamo-hypopheseo-adrenal system activation patterns in different stress paradigms at early stages of postnatal development [**33,34**]. On the contrary, B-cell density was significantly reduced in the older rats of both stress groups. This finding indicates different sensitivity to various types of stress of the cells in the B-zones of the spleen in the different age groups. Similarly, the rate of apoptosis was increased in both types of chronic stress in younger and older rats as evidenced by image analysis of the caspase-3+cells. Thus, variable and repeating stress-related immunomodulation in the central and peripheral immune organs shows diverse scopes and ranges at different stages of early postnatal life and proves to

be a result of complex correlations of developmental and post-stress alterations in the body.

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REFERENCES:

1. Monteiro S, Roque S, de Sá-Calçada D, Sousa N, Correia-Neves M, Cerqueira JJ. An efficient chronic unpredictable stress protocol to induce stress-related responses in C57BL/6 mice. Front Psychiatry, 2015;6:6.
2. Pertsov SS, Grigorchuk OS, Koplik EV, Abramova AY, Chekmareva NY, Chekhlov VV. State of Stress-Marker Organs in Rats with Various Behavioral Characteristics during Repeated Stress Exposures. Bull Exp Biol Med, 2015;160(1):20-3.
3. Sarapultsev PA, Chupakhin ON, Medvedeva SU, Mukhlynina EA, Brilliant SA, Sidorova LP, Danilova IG, Sarapultsev AP.The impact of immunomodulator compound from the group of substituted thiadiazines on the course of stress reaction. Int Immunopharmacol, 2015;25(2):440-9.
4. Vásquez B, Sandoval C, Smith RL, del Sol M. Effects of early and late adverse experiences on morpho-quantitative characteristics of Sprague-Dawley rat spleen subjected to stress during adulthood. Int J Clin Exp Pathol, 2015;8(4):3624-35.
5. Harlé G, Kaminski S, Dubayle D, Frippiat JP, Ropars A. Murine splenic B cells express corticotropin-releasing hormone receptor 2 that affect their viability during a stress response. Sci Rep, 2018;8(1):143.
6. Prather AA, Epel ES, Portela Parra E, Coccia M, Puterman E, Aiello AE, Dhabhar FS. Associations between chronic caregiving stress and T cell markers implicated in immunosenescence. Brain Behav Immun, 2018;73:546-549.
7. Antoni MH, Dhabhar FS. The impact of psychosocial stress and stress management on immune responses in patients with cancer. Canver, 2019;125:1417-1431.
8. Cho J, Bing SJ, Kim A, Herath KHINM, Ahn G, Jee Y. Jeju ground water containing vanadium induces normal T cell development and immune activation in chronically stressed mice. Mol Biol Rep, 2019;46(4):4443-4452.
9. Miao M, Peng M, Chen H, Liu B. Effects of Baihe Dihuang powder on chronic stress depression rat models. Saudi J Biol Sci, 2019;26(3):582-588.
10. Dhabhar FS.The power of positive stress - a complementary commentary. Stress, 2019;22(5):526-529.
11. Russell AL, Tasker JG, Lucion AB, Fiedler J, Munhoz CD, Wu TJ, Deak T. Factors promoting vulnerability to dysregulated stress reactivity and stress-related disease. J Neuroendocrinol, 2018;30(10):e12641.
12. Wang LI, Liu F, Luo Y, Zhu L, Li G. Effect of acute heat stress on adrenocorticotropic hormone, cortisol, interleukin-2, interleukin-12 and apoptosis gene expression in rats. Biomed Rep, 2015;3(3):425-429
13. Rudak PT, Gangireddy R, Choi J, Burhan AM, Summers KL, Jackson DN, Inoue W, Haeryfar SMM. Stress-elicited glucocorticoid receptor signaling upregulates TIGIT in innate-like invariant T lymphocytes. Brain Behav Immun, 2019;80:793-804.
14. Eren S, Drugan RC, Hazi A, Flannery G, Kent S. Coping in an intermittent swim stress paradigm compromises natural killer cell activity in rats. Behav Brain Res, 2012; 227(1):291-4.
15. Dhabhar FS.The short-term stress response - Mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. Front Neuroendocrinol, 2018;49:175-192.
16. Schmidt D, Reber SO, Botteron C, Barth T, Peterlik D, Uschold N, et al. Chronic psychosocial stress promotes systemic immune activation and the development of inflammatory Th cell responses. Brain Behav Immun, 2010;24(7):1097–104.
17. Schmidt D, Peterlik D, Reber SO, Lechner A, Männel DN. Induction of Suppressor Cells and Increased Tumor Growth following Chronic Psychosocial Stress in Male Mice. PLoS One, 2016;11(7):e0159059. eCollection 2016.
18. Bekhbat M, Howell PA, Rowson SA, Kelly SD, Tansey MG, Neigh GN. Chronic adolescent stress sex-specifically alters central and peripheral neuro-immune reactivity in rats. Brain Behav Immun, 2019;76:248-257.
19. Zhang Y, Xu H, Wang J, Ren F, Shao F, Ellenbroek B, Lin W, Wang W. Transient upregulation of immune activity induc ed by adolescent social stress is involved in cognitive deficit in adult male mice and early intervention with minocycline. Behav Brain Res, 2019;374:112136.
20. Duarte JO, Cruz FC, Leão RM, Planeta CS,

Crestani CC. Stress vulnerability during adolescence. Psychosomatic Medicine, 2015; 77: 186–99.
21. Naryzhnaya NV, Maslov LN, Vychuzhanova EA, Sementsov AS, Podoksyonov YK, Portnichenko AG, Lishmanov YB. Effect of Hypoxic Preconditioning on Stress Reaction in Rats. Bull Exp Biol Med, 2015;159(4):450-2.
22. Shakya A, Soni UK, Rai G, Chatterjee SS, Kumar V.Gastro-protective and Anti-stress Efficacies of Monomethyl Fumarate and a Fumaria indica Extract in Chronically Stressed Rats. Cell Mol Neurobiol, 2016;36(4):621-35
23. Foertsch S, Haffner-Luntzer M, Kroner J, Gross F, Kaiser K, Erber M, Reber SO, Ignatius A.Chronic psychosocial stress disturbs long-bone growth in adolescent mice. Dis Model Mech, 2017;10(12):1399-409.
24. Umeoka EHL, Robinson EJ, Turimella SL, van Campen JS, Motta-Teixeira RLC, Sarabdjitsingh A, Garcia-Cairasco N, Braun K, de Graan PN, Joëls M. Hyperthermia-induced seizures followed by repetitive stress are associated with age-dependent changes in specific aspects of the mouse stress system. Journal of Neuroendocrinology, 2019; e12697.
25. Hernandez ME, Martinez-Mota L, Salinas C, Marquez-Velasco R, Hernandez-Chan NG, Morales-Montor J, Pérez-Tapia M, Streber ML, Granados-Camacho I, Becerril E, Javier BH, Pavón L. Chronic stress induces structural alterations
in splenic lymphoid tissue thatare associated with changes in corticosterone levels in Wistar-Kyoto rats. Biomed Res Int, 2013; 2013:868742.
26. Sarjan H.N., Yajurvedi H.N. Duration dependent effect of chronic stress on primary and secondary lymphoid organs and their reversibility in rats. Immunobiology, 2019;224(1):133-141.
27. Van Bodegom M, Homberg J R, Henckens M J A G. Modulation of the Hypothalamic-Pituitary-Adrenal Axis by Early Life Stress Exposure. Frontiers in Cellular Neuroscience, 2017;11:87. eCollection 2017.
28. Vieira, JO, Duarte, JO, Costa-Ferreira W, Morais-Silva G, Marin MT, Crestani CC. Sex differences in cardiovascular, neuroendocrine and behavioral changes evoked by chronic stressors in rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 2018; 81:426–437.
29. Costa-Ferreira W, Morais-Silva G, Gomes-de-Souza L, Marin MT, Crestani CC. The AT1 Receptor Antagonist Losartan Does Not Affect Depressive-Like State and Memory Impairment Evoked by Chronic Stressors in Rats. Front Pharmacol, 2019;10:705. eCollection 2019.
30. Sengupta P.The Laboratory Rat: Relating Its Age With Human's. Int J Prev Med,2013; 4(6): 624–630.
31. Choudhury BK, Shi XZ, Sarna SK. Norepinephrine mediates the transcriptional effects of heterotypic chronic stress on colonic motor function. Am J Physiol Gastrointest Liver Physiol, 2009;296:1238-47
32. Kapitonova MY, Fuad SB, Kuznetsov SL, Dydykin SS, Chernov DA, Nor-Ashikin MNK, Ahmad A. Age-related patterns of changes in the splenic white pulp in heterotypic stress: experimental morphological study. Morfologiia, 2017; 152:38-43.
33. Kapitonova MY, Kuznetsov SL, Khlebnikov VV, Zagrebin VL, Morozova ZCh, Degtyar YV. Immunohistochemical characteristics of the hypophysis in normal conditions and chronic stress. Neurosci Behav Physiol, 2010;40(1):97-102.
34. Walker SE, Sandi C. Long-term programing of psychopathology-like behaviors in male rats by peripubertal stress depends on individual's glucocorticoid responsiveness to stress. Stress,2018;21(5):433-442.