



EXPERIMENTAL ARTICLE

IMMUNOMORPHOLOGICAL CHARACTERISTICS OF
THE EXPERIMENTAL ANIMALS' IMMUNOGENESIS ORGANS
UNDER PROLONGED EXPOSURE OF NOISE

MELKONYAN M.M.^{1*}, MANUKYAN A.L.¹, AVAGYAN S.A.², HUNANYAN L.S.¹, ZILFYAN A.V.²

¹ Department of Medical Chemistry, Yerevan State Medical University, Yerevan, Armenia

² Scientific Research Center of Yerevan State Medical University, Yerevan, Armenia

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ABSTRACT

Study of biochemical mechanisms of acoustic stress development as well as development of prevention techniques and treatment of its consequences is one of the actual problems of modernity due to permanent growth of noise in the environment in modern conditions of economically developed countries. Depending on the characteristics of the influencing noise, an increase is observed in morbidity, development of a number of pathologies, including hypertension, lesions of the cardiovascular system, ischemic heart disease, atherosclerosis, development of myocardial infarction, cognitive processes reduction, different degrees of hearing loss, gastric and duodenum ulcer, diabetes, congenital anomalies and pathologies, sexual dysfunction, Basedow disease.

Previous experimental studies have revealed the development of oxidative stress under the influence of noise with pronounced shifts in biomembranes structure, proinflammatory cytokines (IL-1, IL-2, IL-6) level and a series of other indices in rats. Morphological changes characteristic for the aplastic processes development in immunogenesis organs (thymus, spleen and lymph nodes) of rats in acute acoustic stress conditions. This paper presents the results of a study of structural and immunomorphological shifts in thymus and spleen of rats in conditions of prolonged exposure of noise.

The animals were exposed to broadband noise of 91 dBA level with maximal energy in the region of average and high frequency within 30 days, daily for 8 hours. Paraffin sections of thymus and spleen stained with hematoxylin-eosin as well as fresh frozen cryostat sections from rat spleen tissues were subjected to morphological analysis for the determination of IgG containing antibody-forming cells. Immunofluorescence studies were carried out using FITC-labeled antirat IgG serum. Quantitative analysis of antibody-forming cells was also performed using histostereometric grid of Avtandilov. Statistical analysis was carried out with Student t-test using standard programs.

The obtained results testify to impairments development in the thymus and spleen in experimental rats exposed to the prolonged exposure of noise. In our opinion, the revealed comparably deep hypoplastic processes in red and white pulps of spleen of the experimental animals testify to inhibition of regional reactions responsible for the cellular and, especially, the humoral immunity formation. A significant decrease of the antibodies-forming cells in the representative zones of follicles and the red pulp's spleen cords testify to suppression of reactions responsible for the process of antibody production in the spleen of rats subjected to prolonged exposure of noise (30x8h). At the same time, structural shifts were revealed in single lymphoid follicles on the background of the developed hypoplastic processes in spleen, which testify to the compensatory-adaptive mechanisms formation, where the lymphoid tissue of spleen follicles appears as an object.

KEYWORDS: noise, morphology, thymus, spleen.

INTRODUCTION

Multiple studies, conducted over the last years, testify that not only impairment of hearing organ

of various degrees is being developed, but also an increase of morbidity, development of a series of pathologies, including hypertensive disease and atherosclerosis are detected depending on exposure duration, level and other characteristics of influencing noise [Nichkov S, Krivitskaya G, 1969; Andreeva-Galanina E et al., 1972; Ising H,

ADDRESS FOR CORRESPONDENCE:

Department of Medical Chemistry
Yerevan State Medical University after M. Heratsi
2 Koryun Street, Yerevan 0025, Armenia
Tel.: (+060) 62-13-26
E-mail: magda.melkonyan@meduni.am

Kruppa B, 2004; Boman E et al., 2005]. The study of mechanisms on pathologic exposure of noise on the organisms of cellular and molecular level is also necessary for the development of possible preventive ways of its effect.

Earlier conducted researches have revealed the development of acoustic stress under the influence of noise [Melkonyan M, 1988; Melkonyan M et al., 2010], wherein stationary balance is being disrupted, and prooxidant processes, creating prerequisites of organic lesions, are being predominated. Pronounced shifts have been discovered in the content of proinflammatory cytokines (IL-1, IL-2, IL-6) and intensity of induced lipid peroxidation under stress, testifies the existence of particular interdependence in the mechanisms of action of selected systems [Melkonyan M et al., 2006; 2008].

Available data also revealed the activation of immunity under acute stress and suppression of both cellular and humoral immunity under stressors chronic exposure [Lange K et al., 1989; McCarthy D et al., 1992; Zheng K, Ariizumi M, 2007; Prasher D, 2009; Kavita G et al., 2012]. At the same time, there are only fragmentary data on structural changes in the organs of immunity under acoustic stress of various degree, frequency characteristic, exposure duration related to the characteristics of processes' formation, which are responsible for the implementation of reactions of cellular and humoral immunity [Nichkov S, Krivitskaya G, 1969; Andreeva-Galanina E et al., 1972; Korneva E et al., 1978; Ising H, Kruppa B, 2004; Boman E et al., 2005]. The study of structural and immunomorphological shifts in thymus, spleen and lymphatic nodes under acute acoustic stress (single exposure of various duration), revealed an image of morphological shifts, characteristic to the development of aplastic processes at 6-hour exposure [Zilfyan A et al., 2011]. The study results of structural and immunomorphological shifts in thymus, spleen and lymphatic nodes of rats under prolonged systematic exposure of noise are presented.

MATERIAL AND METHODS

Experiments were carried out on 32 white rats weighing 150-200 g, which were divided into two groups: experimental and control. The rats of experimental group were exposed to broadband noise of 91 dBA level with maximal energy in the region of average and high frequency (63-16000 Hz)

within 30 days, daily for 8 hours. The animals of both groups stayed in the same conditions of drinking and feeding schedule in vivarium.

The animals of control and experimental groups were exposed to Nembutal narcosis and were derived from the experiment by means of decapitation immediately after the noise exposure. All necessary conditions were observed by Institutional Committee on Bioethics of YSMU on humanitarian appeal and euthanasia of experimental animals.

Pieces of thymus and spleen after fixing and wiring in alcohols of increasing concentration were embedded in paraffin and were stained with hematoxylin-eosin. Fresh frozen cryostat sections subjected to morphological analysis for the determination of IgG containing antibody-forming cells were prepared in a separate group of experiments, from tissues of rats' spleen of both control and experimental groups. Immunofluorescence studies were carried out using FITC-labeled antirat IgG serum, produced by Sigma (USA). Preparations were examined under a fluorescence microscope, manufactured by "Boeco", Germany. Quantitative analysis of antibody-forming cells was performed using histostereometric grid of Avtandilov. The results were treated statistically using standard programs. Statistical analysis was carried out with Student t-test using computer program of SPSS, ANOVA version.

RESULTS AND DISCUSSION

The obtained results testify to the development of impairments in cytoarchitectonics of central and peripheral immunogenesis organs in experimental rats exposed to chronic noise exposure [Lange K et al., 1989; McCarthy D et al., 1992].

It was revealed, that lobulation of thymus gland is preserved only in discrete parts in the thymus of rats exposed to prolonged noise (30x8 h). Boundary between the cortical and medullar layers is focused slightly. In the majority of lobules the cortical layer appears to be significantly slimmed down. The lymphoid cells disarrangement is taking place including both superficial and deep parts of the given layer. In this way the lymphocytes lose their compact arrangement and become loosely organized in reticular stroma of cortical layer (Fig. 1a).

Along with the whole cortical layer the number of small lymphocytes is dramatically decreased, due to which the organ's reticular stroma becomes bare

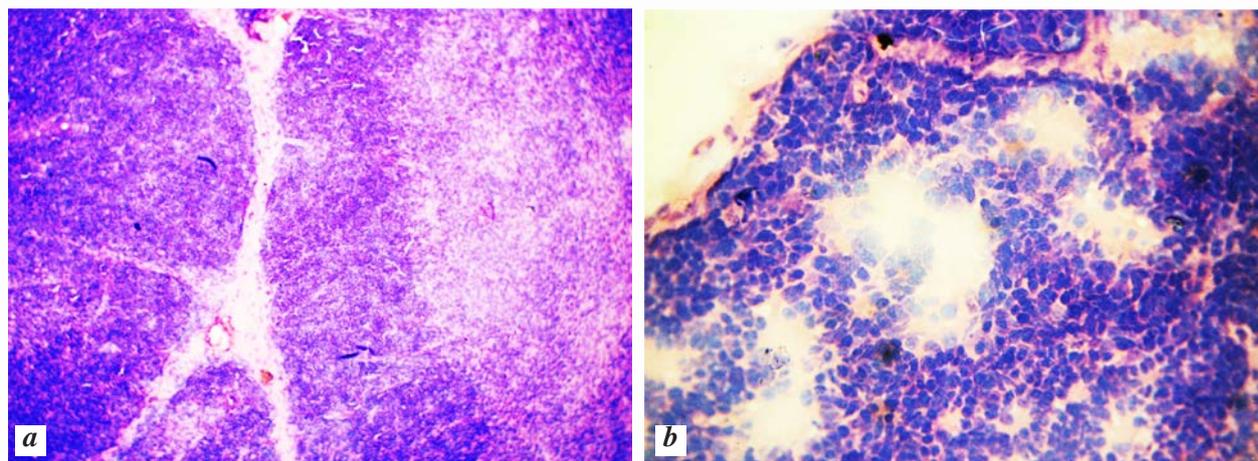


FIGURE 1. Structural changes in the thymus of rats subjected to prolonged exposure of noise (30x8h). Staining is by hematoxylin-eosin.

- a)** Thymus of intact rat. Lobular structure of the organ and boundaries between the medullar and cortical layers are clearly seen. Lymphocytes are of strict homogenous compact arrangement along with the whole perimeter of cortical layer. Ob. 10, oc. 7.
- b)** Signs of irregularity and rarefaction in the lymphoid tissue with big and optically light parts appearance in the cortical layer. "Accidental involution" process in the cortical layer of thymus. Ob. 40, oc. 15.

and optically light big parts appeared because of the expressed hypoplastic processes. The medullar part of thymus along with its whole length looks narrowed with unclear medullar sinuses. In discrete structurally regular sinuses the lumen is somehow expanded, the endotheliocytes are exposed to dystrophic changes till their ultimate decomposition and exfoliation into the micro-vessel's lumen. On this background, the number of layers of lymphocyte series and reticulocytes is dramatically decreased both in medullar and cortical cells (Fig. 1b).

In the spleen of experimental animals, which were exposed to the prolonged noise exposure (30x8h) an activation of the catabolic reactions is also observed. Relatively severe hypoplastic processes serve as a morphological manifestation of these reactions, which equally are observed both in white and red pulps of the spleen.

The white pulp is represented mainly by small imperforate follicles, in majority of which the reactive centers are absent. Cytoarchitectonics of layers and zones is impaired in some preserved average-sized follicles. Herein, there are single small lymphocytes circularly arranged around the central artery in the periarterial zone. Medullary area of the mentioned follicles is characterized by scarce content of the cells of lymphocytic and reticular series. There are only single small lymphocytes and reticulocytes in this layer on the background of moderate edema and reticular stroma bareness.

Boundaries between separate zones of the lymphoid follicles (periarterial, mantle and marginal) are not contoured. There is significant decrease of the cells of lymphocytic series, which is fully represented by small lymphocytes in the region of these zones, on the background of moderate edema (Fig. 2a).

The red pulp of spleen appears to be moderately edematous and hypertrophic. The structure of pulp cords along with the major extent of red pulp is disarranged. There are processes of disarrangement observed in the localization area of spleen cords, as a result of which the cells of lymphocytic-plasmatic series in reticular stroma are located haphazardly as single small lymphocytes and plasmatic cells and/or their separate groups.

The stroma of red pulp's reticular base is edematous. On this background the processes of angiomatosis of capillaries with signs of their plasmorrhagia and increased erythrodiapedesis are distinctly observed (Fig. 2b).

It's noteworthy, that some structural shifts of focal and local character, which testify to the formation of compensatory mechanisms in the spleen of experimental animals, were revealed based on the above-mentioned hypoplastic processes in spleen. Thus, single average-sized follicles (which are not infrequently "fused" with each other) were shown up, in which the active medullar centers were revealed and the appropriate zones were clearly contoured. Herein, the marginal zone looks

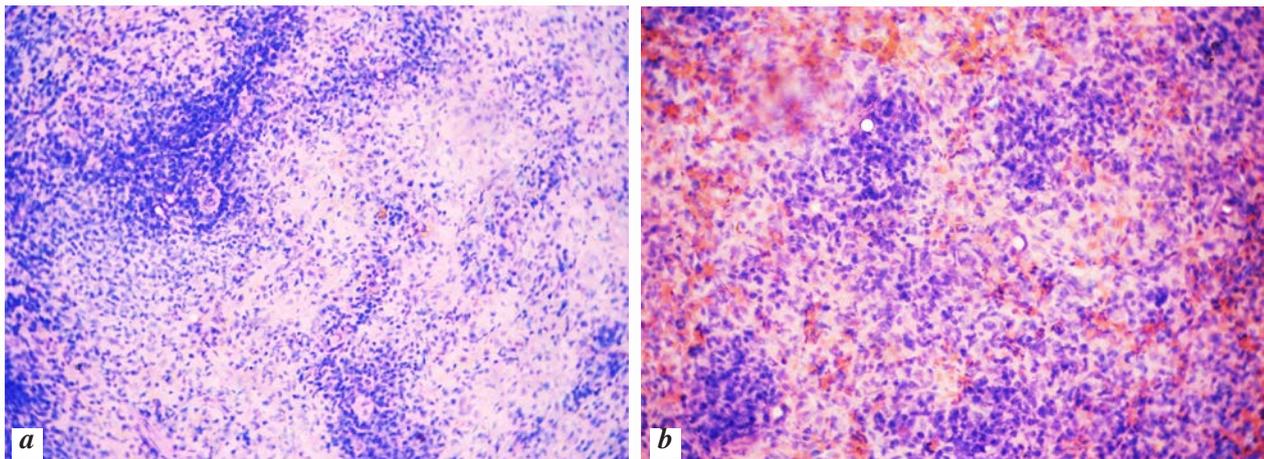


FIGURE 2. Structural changes in the spleen of rats subjected to prolonged noise exposure (30x8h). Staining is by hematoxylin-eosin.

- a)** Cytoarchitectonics of white and red pulps is disorganized. Hypoplastic processes are rather well observed in the reactive center area, in the lymphoid follicles' discrete zones, as well as in the red pulp spleen cords disarranged parts. Ob. 10, oc. 7.
- b)** Spleen cords highly expressed disarrangement together with angiolysis, plasmorrhagy and erythrodiapedesis in the red pulp. Ob. 20, oc. 15.

moderately expanded and is presented by small and blastic forms of lymphocytes, arranged in 3-5 layers circularly on the boundary between the red and white pulps of spleen.

IgG- containing cells of the lymphocytic and plasmocytic series have been revealed both in the white and the red pulps of spleen in rats of the control group (intact animals). Just single lymphocytes have been found with detected IgG on their surfaces as a specific homogenous and/or fine-grained fluorescence in the active centers of lymphocytic follicles

Group accumulation of B-lymphocytes and

plasmatic cells with specific fluorescence on their surface and also in cytoplasm were found in the mantle and marginal zones of lymphoid follicles and in the pulp cords of spleen (Fig. 3a, b).

In spleen of rats subjected to prolonged exposure of noise (30x8h) antibody-forming cells are more rarely detected in white and red pulp. Thus, in white pulp the single lymphocytes and plasmatic cells having specific fluorescence on their surfaces and also in the cytoplasm were found just in discrete segments of the mantle and marginal zones. In red pulp the antibodies-forming cells are characterized by chaotic and irregular arrangement in

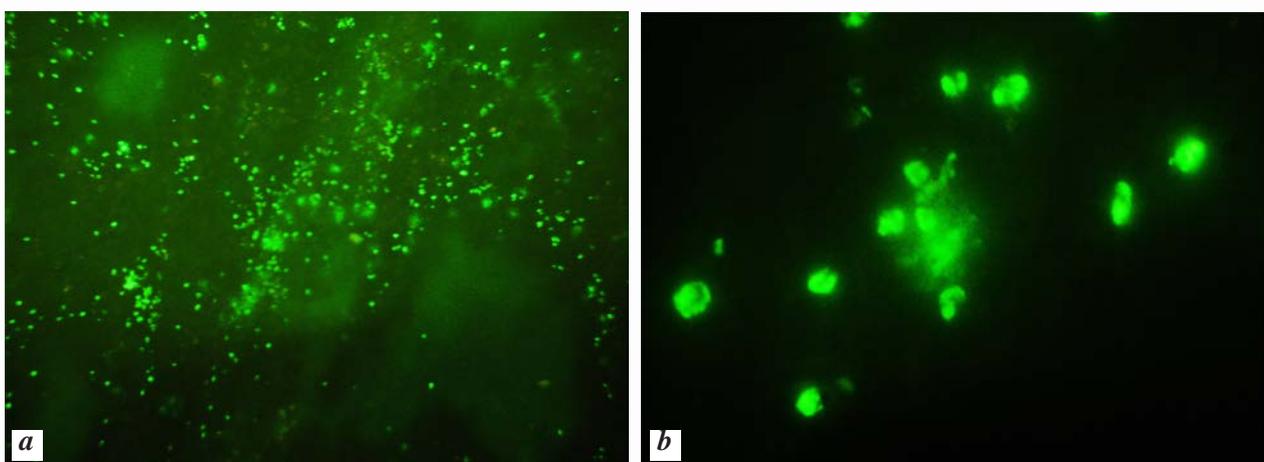


FIGURE 3. Topical peculiarities of the B-lymphocytes localization in the spleen of intact rats. Method of direct immunofluorescence:

- a)** Group accumulation of the antibodies-forming cells in marginal parts of adjacent lymphocytic follicles. Ob. 10, oc. 7.
- b)** Preparation details: IgG-containing plasmatic cells in the marginal zone. Ob. 100, oc. 7.

its reticular base due to the observed hypoplastic processes, manifested particularly in the spleen cords disarrangement (Fig. 4a, b).

A quantitative (planimetric) analysis was performed to find out IgG-containing immunocompetent cells in B-dependent zones of white and red pulp of spleen for more detailed characteristics of antibody-forming process in the spleen of intact rats and animals subjected to prolonged exposure of noise (30x8h). The results of immunomorphological studies are shown in the table.

ing cells in white and red pulps of spleen in the experimental group of animals was decreased in comparison with the analogical indices in the control one in 2.7 and 3 times, correspondingly.

Based on the performed investigation it can be concluded that the induced chronic acoustic stress in experimental rats' thymus and spleen is accompanied by catabolic reactions activation, structural manifestations of which are hypoplastic processes occurred in their lymphoid tissue.

Structural changes in thymus, in general, corre-

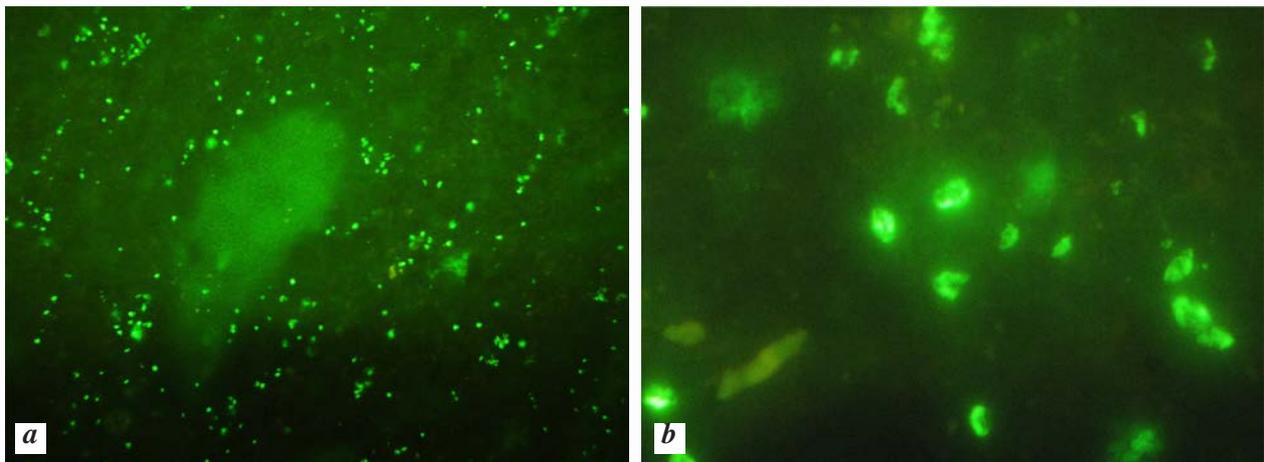


FIGURE 4. Topical peculiarities of B-lymphocytes reactions in the spleen of rats exposed to prolonged acoustic stress. Method of direct immunofluorescence:

- a) Presence of single antibodies-forming cells in the peripheral parts of lymphoid follicles and in red pulp. Ob. 10, oc. 7.
- b) Preparation details: IgG-containing plasmatic cells localized in the lymphocytic follicle's marginal zone. Ob. 100, oc. 7.

TABLE.

Number of IgG-producing cells in B-dependent zones of spleen of experimental rats exposed to prolonged exposure of noise (30x8h)

Study groups (n=15)	Number of antibody-forming cells	
	Lymphoid follicles of white pulp	Spleen cords of red pulp
Control (intact animals)	30.67 ±3.2	31.0 ±4.45
Experimental	11.42 ±1.13 p<0.0005	10.17 ±1.15 0.005>p>0.0005

NOTE: p – related to the ratio of the experimental group indices with those of the control group.

As it is obvious from the table a relative prolonged acoustic stress is accompanied by a significant inhibition of the antibodies-forming cells activity in spleen. Thus, the number of IgG-produc-

late with the functional state of organ in different extreme situations and are known as “accidental involution” of thymus cortical layer. In our opinion, the revealed comparably deep hypoplastic processes in the red and white pulps of spleen of the experimental animals testify to inhibition of regional reactions responsible for the cellular and, especially, the humoral immunity formation. A significant decrease of the antibodies-forming cells in the representative zones of follicles and the red pulp's spleen cords testify to suppression of reactions responsible for the process of antibody production in the spleen of rats subjected to prolonged exposure of noise (30x8h). At the same time, structural shifts were revealed in single lymphoid follicles on the background of the developed hypoplastic processes in spleen, which testify to the compensatory-adaptive mechanisms formation, where the lymphoid tissue of spleen follicles appears as an object.

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