



THE STRUCTURAL CHANGES IN THE MEDIASTINAL LYMPHATIC NODES IN THE TEST ANIMALS UNDER THE EXPERIMENTAL CONDITIONS

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ABSTRACT

Background: In the structure of a complex approach to study the effects of environmental influences on the health of the population a certain place belongs to immunomorphological research. The reactive changes in the lymphatic nodes are one of the early and informative signs of adverse effect of different chemical factors on the organism. We have studied the effect of the exhaust gases (EG) on the cellular composition of the mediastinal lymphatic nodes of rats after acute, sub-acute and chronic influence of exhaust gases.

KEYWORDS: LC - lethal concentration, EG - exhaust gases

INTRODUCTION Nowadays the reduction of air pollution by toxic substances emitted by industrial plants and motor vehicles, is one of the major problems facing humanity. Air pollution is harmful to humans and to the environment.^[15,17] During the intense urbanization and the growth of megacities road transport has become the most adverse environmental factor in the protection of human health and the environment in the city.^[1,2,13] A car, absorbing much needed oxygen to the flow of life, at the same time intensely pollutes the air environment with toxic components, causing considerable damage to all living things and inanimate. Contribution to environmental pollution mainly to atmosphere is 60-90%. The main toxic vehicle emissions include exhaust gases (EG), crankcase gases and fuel vapors. The exhaust gases or exhaust emitted by the engine contains carbon monoxide (CO), hydrocarbons, nitrogen oxides, benzo (a) pyrene, aldehydes and soot.^[15,17]

The problem of adaptation of the human organism and animals to the adverse effect of the environment is one of the most important in the morphology, physiology and immunology.^[11, 13, 20, 22]

In the structure of an integrated approach to study of the impacts of environmental pollutants on public health, a certain place belongs to immunomorphology. The reactive changes in the lymphatic nodes are one of the earliest and informative signs of adverse effects on the body of certain chemical factors. The lymphatic system plays a key role in maintaining the homeostasis of the internal environment of the body and one of the first to respond to exogenous and endogenous influence.^[4,5, 6, 9, 12,16] The lymphatic nodes are first involved in the response. Therefore, when analyzing the effects of

carcinogens in the organism requires a detailed study of general structural change and direction of cell responses in the lymph nodes. In addition, the lymphatic system is an important part of the immune system.^[18,21]

Lymphatic nodes are a biological filter, located on the way of the lymphatic vessels and are actively involved in phagocytosis, B-lymphocytes' formation, plasma cells and antibodies, as well as recycling of lymphocytes.^[3,18]

THE AIM OF THE STUDY: The aim of this study was to investigate morphological and functional features of the mediastinal lymphatic nodes of rats after the subacute and chronic exposure of exhaust gases.

MATERIALS AND METHODS: For the experimental analysis of the influence of the exhaust gas (EG) on the organs of the immune system 185 white rats of both gender weighing 160-180 g., 4-6 months aged were used. The poisoning of animals with EG was performed in a chamber volume of 50 liters for 4 hours. The change of the gas mixture in the chamber was performed every 15 minutes. Acute exposure was carried out two times the poisoning of animals for the past 2 and 5 days in a dose 1/50 of lethal concentration (LC) in terms of CO. The subacute exposure was performed by monthly poisoning of animals daily in a dose 1/100 of lethal concentration. Chronic exposure to animals carried with a four month poisoning in the lethal concentration dose of 1/200. All groups of animals were kept under standard vivarium conditions with free access to food and water.

We investigated the sections of organs. Extracted mediastinal lymphatic nodes of animals were fixed in 10% neutral formalin. The morphological entry of them

made by conventional techniques followed with pouring in paraffin. Paraffin blocks were cut into Sannomiya microtome, receiving sections of 4-6 mcm thickness. To study the general organs' structure preparations stained with hematoxylin-eosin. To identify the characteristics of the structure of the lymphatic nodes the part of the material stained with azure II eosin (by van Gieson). With the help of an ocular grid, comprising 64 squares and having an area of 64 mm³, increasing ocular × 7 and lens × 90 counted the total number of cells, all types of lymphocytes (small, medium, large), macrophages, the number of mitotically dividing cells, eosinophils structures lymph nodes. Counting was performed in 10 visual fields. The counting results were expressed as a percentage.

RESULTS AND DISCUSSION: After the application of the exhaust gases in a dose 1/50 of LC on the second day in the mediastinal lymph nodes is marked as follows: clearly distinguishable connective tissue capsule and trabeculae. There are amount of moderate of lymphoid nodules (follicles) with germinal centers in the cortex. In the medulla strands and sinuses are distinguishable. There are a lot of many small lymphocytes in the lymphoid nodules. In the paracortical zone individual eosinophils are found. Plasma cells are a little. The congestion of vessels is marked.

When slaughtering animals on the 5th day after acute exposure with EG: lymph node capsule compact, moving away from her connective trabeculae. Well distinguishable cortical plateau paracortical zone pulpy strands and lymphoid nodules with breeding centers. In all structures macrophages and eosinophils are found. There are cells with mitosis. The table № 1 shows the content of cells in the mediastinal lymphatic nodes structures in control rats after acute exposure to the exhaust gas on the 2nd and 5th days.

When slaughtering animals on the 2nd day after acute exposure in the cortical plateau established the following: small lymphocytes vary slightly, the average lymphocytes was significantly increased and amount to 13,1 ± 0,64%. The large lymphocytes are decreased to 1,4 ± 0,06%.

It is noted the presence of a small amount of plasma cells. The macrophages are reduced to 0,1 ± 0,01%. However eosinophils are increased to 0,3 ± 0,02%. Reticular cells tend to decrease. The cells with mitosis are also significantly reduced.

As can be noted in the paracortical zone the relative content of small lymphocytes tends to increase, wherein the large and medium lymphocytes tend to decrease. It is marked reduction of plasma cells and macrophages. The relative content of cells with mitosis significantly reduced.

In the pulpy strands the small lymphocytes increases slightly. The downward trend is observed in relation to the average of lymphocytes, with large lymphocytes decreased significantly and make up 3,9 ± 0,18%. Along with some decrease in plasma cells, macrophages content remains virtually unchanged and the number of eosinophils was significantly reduced. Noteworthy is the sharp decrease in the number of the cells with mitosis.

In the germinal center of the lymphatic node the indicator of small lymphocytes increases. Significantly reduces the performance of medium and large lymphocytes, plasma cells. The content of macrophages remains virtually unchanged. There has been a reduction of cells with mitosis.

Table 1 THE CONTENT OF CELLS IN THE STRUCTURES OF MEDIASTINAL LYMPHATIC NODES IN RATS OF CONTROL GROUP AFTER ACUTE INFLUENCE OF THE EXHAUST GASES (%)

Cells	Cortical Plateau	paracortical zone	pulpy strands	germinal center of lymph node
Lymphocytes:	Control			
Small	79,7 ± 3,98	72,5 ± 3,62	54,5 ± 2,72	47,6 ± 2,34
Medium	9,9 ± 0,48	13,1 ± 0,64	18,3 ± 0,90	23,1 ± 1,12
Large	2,4 ± 0,12	2,3 ± 0,12	5,7 ± 0,27	9,0 ± 0,44
Plasmacells	-	3,3 ± 0,15	9,1 ± 0,44	9,5 ± 0,46
Macrophages	0,3 ± 0,02	0,3 ± 0,02	0,1 ± 0,01	0,2 ± 0,01
Eosinophils	0,1 ± 0,01	0,2 ± 0,01	0,2 ± 0,01	0,1 ± 0,01
Reticularcells	7,3 ± 0,37	7,9 ± 0,38	11,4 ± 0,55	10,1 ± 0,49
Cellswithmitoses	0,3 ± 0,02	0,4 ± 0,02	0,7 ± 0,04	0,4 ± 0,03
Lymphocytes:	Slaughter on the 2nd day			
Small	78,4 ± 3,86	74,7 ± 3,52	59,0 ± 2,71	56,9 ± 2,80*
Medium	13,1 ± 0,64*	12,2 ± 0,54	17,8 ± 0,87	18,2 ± 0,89*
Large	1,4 ± 0,06*	2,1 ± 0,1	3,9 ± 0,18*	7,3 ± 0,35*
Plasmacells	0,1 ± 0,01	2,9 ± 0,13	8,0 ± 0,38	7,4 ± 0,35*
Macrophages	0,1 ± 0,01*	0,2 ± 0,01*	0,1 ± 0,01	0,2 ± 0,01
Eosinophils	0,3 ± 0,02*	0,2 ± 0,01	0,1 ± 0,01*	0,2 ± 0,01*
Reticularcells	6,5 ± 0,31	7,6 ± 0,37	10,9 ± 0,50	9,5 ± 0,45

Cellswithmitoses	0,1 ±0,01*	0,1 ±0,01*	0,2 ± 0,02*	0,3 ± 0,02*
Lymphocytes:	Slaughter on the 5 th day			
Small	76,5 ± 3,74	74,2 ± 3,64	53,6 ±2,58	45,4 ±2,17
Medium	10,8 ± 0,49	12,5 ± 0,58	19,1 ±0,93	22,7 ± 1,12
Large	3,4 ±0,16*	2,0 ± 0,09	6,0 ± 0,28	10,3 ± 0,50
Plasmacells	0,1 ±0,01	3,1 ±0,13	8,8 ± 0,43	9,2 ± 0,46
Macrophages	0,4 ±0,03*	0,3 ± 0,02	-	0,3 ± 0,02*
Eosinophils	0,2 ±0,01*	0,1 ±0,01*	0,2 ± 0,01	0,1 ±0,01
Reticularcells	8,3 ± 0,40	7,5 ± 0,37	11,8 ±0,60	11,7 ±0,56
Cellswithmitoses	0,3 ± 0,02	0,3 ± 0,02*	0,5 ±0,03*	0,3 ± 0,02*

Note: The authenticity was calculated relative to the control, * p <0.05.

When slaughtering animals on the 5th day small lymphocytes in the cortical plateau decreased significantly compared with the control group. The medium lymphocytes are close to that of the control. There has been a significant increase in the relative content of large lymphocytes, which amounted to 3,4 ± 0,16%. There are revealed the individual plasma cells. The relative abundance of macrophages and eosinophils significantly increased. There is a tendency to an increase in reticular cells. The cells with mitosis are reached to the level of the control group.

The number of small, medium and large lymphocytes in paracortical zone varies slightly. There has been a slight increase in plasma cells compared with those on the 2nd day after effect. The macrophages' Indicator recovered and corresponds to the control group. The content of eosinophils decreases. The Indicator of cells with mitosis, increasing in comparison with the data of the second day of observation, still remains reduced in comparison with the control group.

In the pulpy strands the small lymphocytes are close to the parameters of the control group. The medium and large cells increase as compared with the second day of observation. There are a lot of plasma cells, they are close to the data of the control group. The macrophages virtually can not be detected. The eosinophils constitute 0, 2 ± 0, 01%, which is consistent with the control group. The Indicator of cells with mitosis is still reduced compared to controls.

In the germinal center of the lymphatic node the medium and large lymphocytes continued to increase compared with the second day of observation. The Indicator of plasma cells almost recovered and the rate of macrophages even exceeds the data of the control group. The indicator of eosinophils corresponds to the data of control. The content of cell with mitosis remains lower than in the control group.

Thus, on the second day after the acute effect of exhaust gases the small lymphocytes in the structures of lymph nodes tended to increase. The medium lymphocytes mainly reduced except the cortical plateau. The relative content of large lymphocytes decreased. The plasma cells decreased in all structures except the cortical plateau. The macrophages are reduced in the cortex and

paracortical plateau area. It is noted the eosinophils' redistribution on zones. The reticular cells tend to decrease. the relative abundance of cells with mitosis significantly decreased.

On the fifth day marked the restoration or the trend towards recovery of the parameters of all studied structures. The individual indicators exceed the observation data of the control group.

The questions of morphological reorganization in the lymphatic nodes and other organs of the immune system in response to antigens and stimuli of non-antigenic nature is of particular interest.^[5, 6, 9, 14, 19] The processes of proliferation and differentiation of lymphoid tissue in maintaining provide constant and changes the cellular composition of immunocompetent lymphocytes types in accordance with a changing range of antigens entering to the organism.^[1,4,12,18,21]

After the influence of exhaust gas in a dose 1/100 of LC over one month the alteration of structural components of the lymph nodes was marked. It is noticeable the decreasing in the area of the brain substance due to expansion of the sinuses. The boundaries between the structures are not always clearly differentiated. The pulpy strands mainly concentrated in the gate of the node. There are lymphoid nodules with breeding centers in the cortex. The connective capsule is often hydropic and infiltrated with fat cells. There are areas of proliferation of connective tissue. The vessels are dramatically expanded and full-blooded. There some areas of delymphatization with exposing of reticular stroma and fat cells are identified. There the effacement of layers and the areas of hydropic fluid accumulation are noted.

In the Table №2 the content of cells in the structures of mediastinal lymphatic nodes after subacute influence of exhaust gases is reflected. In the cortical plateau is observed the increasing in the content of small lymphocytes in comparison with the control group. Medium and large lymphocytes are reduced to 7,1 ± 0,40% and 1,8 ± 0,11% respectively. Macrophages are significantly reduced and make up 0,1 ± 0,01%. It is noted the increasing of eosinophils. The relative content of reticulocytes decreases. It is sharply reduced the content of cells with mitosis.

In the paracortical zone the small lymphocytes are increased, medium and large are reduced. The relative content of plasma cells was significantly reduced and amounts to $2,5 \pm 0,12\%$. Macrophages are reduced to $0,1 \pm 0,01\%$.

It is noteworthy the reduction of eosinophils. The relative content of reticulocytes significantly increased and

amounts to $10,3 \pm 0,54\%$. The content of cells with mitosis is significantly reduced.

In the pulpy strands detected increase in the rate of small lymphocytes and a significant decline in medium and large. The content of plasma cells is reduced to $6,8 \pm 0,35\%$. There are some separated eosinophils. The reticulocytes are somewhat increased. The content of cells with mitosis is sharply reduced to $0,2 \pm 0,01\%$.

Table 2 THE CONTENT OF CELLS IN THE STRUCTURES OF MEDIASTINAL LYMPHATIC NODES IN THE RATS OF CONTROL GROUP AND AFTER SUBCHRONIC INFLUENCE OF EXHAUST GASES (%)

Cells	Cortical Plateau	paracortical zone	pulp strands	germinal center of lymph node
Lymphocytes:	Control			
Small	$79,6 \pm 3,72$	$72,5 \pm 3,78$	$56,7 \pm 2,86$	$47,9 \pm 2,40$
Medium	$9,6 \pm 0,48$	$12,8 \pm 0,64$	$17,5 \pm 0,90$	$22,6 \pm 1,19$
Large	$2,5 \pm 0,14$	$2,4 \pm 0,14$	$5,5 \pm 0,29$	$9,3 \pm 0,47$
Plasmacells	-	$3,2 \pm 0,18$	$8,7 \pm 0,45$	$9,8 \pm 0,50$
Macrophages	$0,3 \pm 0,02$	$0,3 \pm 0,02$	-	$0,2 \pm 0,01$
Eosinophils	$0,1 \pm 0,01$	$0,2 \pm 0,01$	-	$0,1 \pm 0,01$
Reticularcells	$7,6 \pm 0,41$	$8,2 \pm 0,43$	$10,9 \pm 0,57$	$9,7 \pm 0,48$
Cellswithmitoses	$0,3 \pm 0,02$	$0,4 \pm 0,02$	$0,7 \pm 0,03$	$0,4 \pm 0,02$
Lymphocytes:	Sub acute influence of exhaust gas			
Small	$84,7 \pm 4,23$	$74,8 \pm 3,61$	$63,0 \pm 3,33$	$56,0 \pm 2,82$
Medium	$7,1 \pm 0,40^*$	$10,2 \pm 0,52^*$	$14,0 \pm 0,72^*$	$18,5 \pm 0,95^*$
Large	$1,8 \pm 0,11^*$	$1,9 \pm 0,11^*$	$4,2 \pm 0,23^*$	$7,3 \pm 0,37^*$
Plasmacells	-	$2,5 \pm 0,12^*$	$6,8 \pm 0,35^*$	$7,5 \pm 0,38^*$
Macrophages	$0,1 \pm 0,01^*$	$0,1 \pm 0,01^*$	-	$0,1 \pm 0,01^*$
Eosinophils	$0,2 \pm 0,02^*$	$0,1 \pm 0,01^*$	$0,1 \pm 0,01$	$0,1 \pm 0,01$
Reticularcells	$6,0 \pm 0,32^*$	$10,3 \pm 0,54^*$	$11,7 \pm 0,61$	$10,4 \pm 0,54$
Cellswithmitoses	$0,1 \pm 0,01^*$	$0,1 \pm 0,01^*$	$0,2 \pm 0,01^*$	$0,1 \pm 0,01^*$

Note: The authenticity was calculated relative to the control, * $p < 0,05$.

In the germinal centers of the lymphatic nodules measure small lymphocytes increases, medium and large are reduced. This large lymphocytes accounted for $7,3 \pm 0,37\%$. The plasma cells and macrophages are reduced. The index of reticulocytes varies slightly. The percentages content of cells with mitosis is reduced to $0,1 \pm 0,01\%$.

Thus, the structures of mediastinal lymphatic nodes after subchronic influence of the exhaust gases there is an increase of the relative content of small lymphocytes, eosinophils and reticulocytes. The relative content of medium and large lymphocytes, plasma cells, macrophages and cells with mitosis is significantly reduced.

In the chronic influence, animals were exposed to exhaust gases over 4 months in a dose of a lethal concentration of 1/200. The histological study of lymphatic nodes showed the following: the capsule is peeled and hydropic in some parts. There are centers of sclerotic changes in the capsule and trabeculae. The paracortical zone is uneven, thinned, identify local areas of its hyperplasia, in which a plurality of immunoblast among mature lymphocytes is observed. There are macrophages found with signs of phagocytosis in the expanded sinuses. The number of follicles is reduced. It is noted the presence of a large number of thin-walled, sanguineous vessels. The Table 3 reflects the number of cells in the structures of content mediastinal lymphatic nodes after chronic exposure to EG.

Table 3 THE CONTENT OF CELLS IN THE STRUCTURES OF MEDIASTINAL LYMPHATIC NODES IN THE RATS OF CONTROL GROUP AFTER THE CHRONIC INFLUENCE OF EXHAUST GASES (%)

Cells	Cortical Plateau	paracortical area	pulpy strands	germinal center of lymph node
Lymphocytes:	Control			
Small	$79,7 \pm 3,77$	$72,6 \pm 3,72$	$53,8 \pm 2,55$	$48,2 \pm 2,42$
Medium	$10,2 \pm 0,52$	$13,3 \pm 0,70$	$18,7 \pm 0,95$	$22,9 \pm 1,27$
Large	$2,3 \pm 0,13$	$2,2 \pm 0,12$	$5,8 \pm 0,30$	$8,8 \pm 0,46$
Plasmacells	-	$3,4 \pm 0,16$	$9,3 \pm 0,47$	$9,2 \pm 0,47$
Macrophages	$0,3 \pm 0,02$	$0,3 \pm 0,02$	$0,1 \pm 0,01$	$0,2 \pm 0,01$
Eosinophils	$0,1 \pm 0,01$	$0,2 \pm 0,01$	$0,1 \pm 0,01$	$0,1 \pm 0,01$

Reticularcells	7,1 ± 0,34	7,6 ± 0,41	11,6 ± 0,58	10,2 ± 0,52
Cellswithmitoses	0,3 ± 0,02	0,4 ± 0,02	0,6 ± 0,03	0,4 ± 0,02
Lymphocytes:	Chronic influence of exhaust gases			
Small	81,0 ± 4,0	73,0 ± 3,65	56,7 ± 2,77	51,0 ± 2,57
Medium	8,8 ± 0,45	12,5 ± 0,64	16,3 ± 0,84	21,2 ± 1,04
Big	2,5 ± 0,14	2,4 ± 0,13	5,1 ± 0,27	9,1 ± 0,45
Plasmacells	0,1 ± 0,01	2,9 ± 0,16*	9,2 ± 0,48	8,6 ± 0,42
Macrophages	0,2 ± 0,01*	0,2 ± 0,01*	0,2 ± 0,01*	0,1 ± 0,01*
Eosinophils	0,2 ± 0,01*	0,1 ± 0,01*	0,1 ± 0,01	-
Reticularcells	6,9 ± 0,33	8,7 ± 0,45	11,9 ± 0,55	9,7 ± 0,47
Cellswithmitoses	0,3 ± 0,02	0,2 ± 0,01*	0,5 ± 0,03	0,3 ± 0,02*

Note: The authenticity was calculated relative to the control, * p <0.05.

In the cortical plateau the medium lymphocytes are reduced in comparison with the control group. The individual plasma cells are identified. The macrophage relative content reduced to 0,2 ± 0,01%. The relative content of eosinophils increases. In paracortical zone there is a tendency to an increase in large lymphocytes and decrease of plasma cells. The relative content of macrophages and eosinophils decreases. It is reduced the percentage of cells with mitosis.

In the pulpy strands medium and large cells tend to decrease. It is noted a slight increase in the percentage of macrophages.

In the germinal centers of the lymph nodules there is a tendency to lower plasma cells and a significant decrease in macrophages. The eosinophils practically are not identified. It is Marked decline in cells with mitosis.

Thus, after a chronic influence of the exhaust gases in the structures of the mediastinal lymphatic nodes is observed change in the content of a number of cellular elements, which showed a decrease of relative content of medium lymphocytes and macrophages, as well as cells with mitosis.

CONCLUSIONS

1. Conducted acute exposure with EG in a dose 1/50 of LC at slaughter animals on the 2nd day it possible to establish in certain mediastinal lymphatic nodes a dyscirculatory disorder. In the structures of lymphatic nodes tended to increase in small lymphocytes. The medium lymphocytes mainly reduced, with the exception of the cortical plateau. The relative content of large lymphocytes is decreased. The plasma cells decreased in all structures, except the cortical plateau. The macrophages are reduced in the cortex and paracortical plateau zone. There is a redistribution of eosinophils on zones. The reticular cells tend to decrease. Significantly decreased the relative abundance of cell mitosis. The changes revealed by us in the T and B zones of the lymphatic nodes is said to reduce the process of cell differentiation.
2. At slaughtering of animals on the 5th day after acute exposure to EG the mediastinal lymph nodes found to recovery or a trend towards recovery indicators in

T-dependent and T-independent zone. The individual performance data exceeds the control group observation. Thus, on the 5th day after acute exposure to EG a recovery processes and activation of immune cells in the peripheral organ were observed.

3. According to our study, subacute exposure to the exhaust gases leads to severe histological changes in the peripheral organs of the immune system. The lymph nodes of subacute exposure to EG reduces the area of the medulla through the expansion of the sinuses. The boundaries between the structures are not always clearly differentiated. The vessels are dramatically expanded. The areas of delymphatization with exposing of reticular stroma are identified. Is noted the effacement layers and plots of the accumulation of edema fluid.
4. It established a small increase of the relative content of lymphocytes, eosinophils and reticulocytes in the Lymphatic nodes. The relative content of medium and large lymphocytes, plasma cells, macrophages and cells with mitosis is significantly reduced. The reduction of plasma cells in the pulpy strands and germinal center of lymphatic node indicates the braking of localimmunogenesis.^[5, 8,18] The detection of lymph node changes is indicative of inhibition of cell differentiation processes, depletion and inhibition of cell division and differentiation in the organ.
5. After chronic exposure to EG in the lymph nodes paracortical area is uneven, thinning sections alternate with sections hyperplasia. Sinuses are expanded. Vessels are full-blooded. In the study of cell structures the content of mediastinal lymphatic nodes of rats observed reduction of medium lymphocytes and macrophages, as well as cells with mitosis. Thus, it was found that chronic exposure to exhaust gases causes certain changes in the peripheral organ of the immune system, suggesting that the relevant changes in immune status.
6. It follows from the above that the study of morphological and functional (histological, cytological) indicators in experimental studies can significantly broaden and deepen the interpretation of responses of the organism to the impact of environmental factors, which necessitates the use of

these indicators in immunological, genetic and epidemiological studies.^[2]

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