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Microstructure of lymphoid tissue in lymph nodes of different localization in young, mature and old rats

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ABSTRACT

Aging is a natural biological process of gradual degradation of parts and systems of the human body and the consequences of this process. These changes occur in the human genotype, both under the influence of environmental factors and as a result of endogenous processes, namely, a deficiency of antioxidant enzymes, changes in the immune system and the development of chronic inflammation. The lymphatic system is the internal environment of the body. In recent years, we and other scientists have determined general and regional signs of aging of the lymphatic system, reflecting a decrease in drainage-detoxification and immune functions, caused by a deficiency of trace elements in the lymph nodes. Considering the important role of the lymphatic system in the body, its participation in tissue drainage and its compensatory and adaptive functions, it is of interest to develop a program of restorative methods of lymphatic system functions on the processes occurring in the aging organism, and thereby contribute to the prolongation of life. This study aimed to determine the ratio of functional zones and microstructures of lymphoid tissue in nodes of different locations in young, mature, and senile rats. Lymph nodes were analyzed using Avtandilov's morphometry, and the samples were fixed in 10% neutral buffered solution formalin or Telesnitsky's fluid, processed, and embedded in paraffin for histological sectioning with various dyes, including hematoxylin and eosin. This study presents data on aging affects the lymph node microstructure and functional area. In young and mature animals, no significant differences were observed. However, aging leads to thickening of the capsule and increased connective tissue around blood vessels, disrupting the lymphoid lobule structure and causing lymph drainage issues. In older animals, the medulla remains unchanged as long as lymph flow is maintained. Compared to younger animals, older ones showed more connective tissue and fewer lymphoid nodules. There is a decreased in certain immune cell types, indicating lower immunoreactivity. Conclusion: In young animals, no significant changes were observed compared to mature animals. Ageing in lymph nodes showed increased connective tissue and reduced lymphoid nodules, with a decrease in certain cell types, and an elevation in the number of reticular cells, indicating lower immunoreactivity.

Keywords: Aging, Lymphatic system, Lymphoid tissue, Microstructure, Cells.
Article type: Research Article.

INTRODUCTION

Aging is a natural biological process of gradual degradation of systems of the human body and the consequences of this process. These changes occur in the human genotype, both under the influence of environmental factors and as a result of endogenous processes, namely, a deficiency of antioxidant enzymes, changes in the immune system and the development of chronic inflammation (Åberg *et al.* 2020; Cakala-Jakimowicz *et al.* 2021; Krupina *et al.* 2024). Aging is a process of decreasing functional reserves of all tissues and systems of the body, a progressive, and irreversible biological process associated with the processes of inflammation and thrombus

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formation with subsequent replacement by connective tissue (Xu *et al.* 2017; Harinath *et al.* 2024; Garcia-Dominguez 2025). Cellular aging is characterized by the progressive accumulation of products that are removed by the autophagy system (Maiborodin *et al.* 2016; Budamagunta *et al.* 2021; Zhang *et al.* 2022), and the development of many types of diseases (Martinez-Cue & Rueda 2020; Sun *et al.* 2022). Changes in the microcirculation system are identified as one of the causes of aging. With aging, there is a slow achievement of the maximum reaction and a prolonged recovery period. When the cardiovascular system goes out of balance, as a result of which less pronounced shifts in hemodynamics with altered neurohumoral regulation can be the cause of frequent disruption of their activity (Tsyplenkova 2012, Jakica *et al.* 2020, Lee & Kim 2022). With age, the threshold of blood supply to various organs and systems decreases. A significant decrease in renal circulation has been shown with aging, which is directly associated with a decrease in microvascularization (Wang *et al.* 2014; Denic *et al.* 2016). During aging, changes occur with the manifestation of fading and disturbances of regulatory influences, important adaptive shifts are noted: the increased sensitivity of the heart and blood vessels to humoral factors and mediators in conditions of destruction of nerve endings, as well as weakening of the synthesis of mediators (Demchenko *et al.* 2019, Hu *et al.* 2022, Wang *et al.* 2025). In defining the mechanisms of aging of the organism associated with changes in the lymphatic system, we have determined biochemical, immunological and rheological changes in the lymph and blood of old animals (Koubasova *et al.* 2014, Thompson *et al.* 2017, Masters *et al.* 2019). These changes in the extracellular matrix reduce the homeostatic capabilities of the body and open the way to the development of diseases of old age (Lobov *et al.* 2015, Hagen & Derudder 2020). In old animals, a decrease in the amplitude and an elevation in the frequency of spontaneous contractions were revealed: A decrease in the sensitivity of receptors to vasoactive substances and an increase in the irritation threshold to them were noted (von der Weid & Zawieja 2004; Demchenko *et al.* 2021). This is due to changes in the innervation of the lymph nodes (Vanhoutte 1978, Abdreshov *et al.* 2019). The lymphatic system is the internal environment of the body (Chen *et al.* 2024; Rui-Cheng 2024), the authors have shown that aging of the body is a general and regional decrease in the drainage-detoxification and immune functions of the lymphatic system (Hagen & Derudder 2020, Abdreshov *et al.* 2024) and also caused by a deficiency of trace elements in the lymph nodes (Shang *et al.* 2019, Stritt *et al.* 2021, Li *et al.* 2022). The lymphatic system is an important component that supports the homeostasis of the milieu interieur during aging. Regional lymph nodes, which are peripheral lymphoid organs, form part of the body's protection system. These cells generate an immune response to external affecting factors (Olszewski 2003, Thangaswamy *et al.* 2012, Hsu & Itkin 2016). Lymph nodes play a special role among other lymphoid organs, since they are responsible for both drainage of interstitial fluid and immune function (Bontumasi *et al.* 2014, Thompson *et al.* 2019). Assessment of the state of regional lymph nodes in their relationship with the organs being drained remains the focus of morphological studies. The lack of a single opinion about the prognostic significance of lymphatic system immunomorphological restructuring during aging determines the need for anatomical and histological assessment of individual compartments and cellular composition. Age-related and pathological shifts in the lymphatic system have not been distinguished (Premier & Meeusen 1998, Kataru *et al.* 2022, Silva *et al.* 2024). The microstructure of lymph nodes of different locations in young, mature, and senile animals is of interest. This study aimed to determine the ratio of functional zones and microstructure of lymphoid tissue in the nodes of different locations in young, mature, and senile rats.

MATERIALS AND METHODS

Ethics statement

Animal studies were conducted in accordance with the principles of bioethics and the rules of Good Laboratory Practice. The study design was approved by the Local Ethics Committee of the Institute of Genetics and Physiology, Protocol No. 13-354 dated September 19, 2024, as well as the rules of bioethics approved by the European Convention for the Protection of Vertebrates (Strasbourg, 1986) and the guidelines outlined in the European Union Directive 2010/63/EU of 22 September 2010, titled "On the protection of animals used for scientific purposes".

Animals and Experiment Design

The experiments involved 42 white Spraque Dawley laboratory rats of different ages. The animals were kept in the vivarium of the Institute of Genetics and Physiology SC MSHE RK (Almaty, Kazakhstan) under standard food and water conditions. The animals were divided into three groups: group 1, young animals aged 1-2 months; group 2, mature rats aged 10-12 months; and group 3, senile rats aged 20-24 months. The lifespan of the animals was 36 months. They become sexually mature as early as 2-3 months, and the age of 24 months corresponds to 60-70 years in humans (Andreollo *et al.* 2012; Sengupta 2013). The release from the experiment and painful manipulations were performed under general anesthesia. The object of the study was the mesenteric lymph nodes, which were taken for morphological examination. The assessment of the structural and functional zones of the lymph nodes was carried out by histological, cytological and morphometric methods.

Morphological research

The lymph nodes were preserved in 10% neutral formalin for histological evaluation using light microscopy. The materials were cleared in xylene before being histologically examined in alcohols of increasing concentration, then embedded in paraffin. Using a Thermo Scientific HM325 microtome, histological sections of the lymph nodes with 4-5 μm thick were obtained. Histological sections of lymph nodes were stained with hematoxylin-eosin, azure, and eosin and embedded in polystyrene (Merkulov 1969; Ham & Cormack 1979). We used PC-connected Leica DM 750 and Mikmed-2 microscopes equipped with a ScanMicro scanning console. There were 120 preparations of cervical and mesenteric lymph nodes that were examined.

Morphometric analysis

The lymph nodes were analyzed morphometric fully, as described by Avtandilov (1990). The collected pieces of regional lymph nodes were fixed in 10% neutral buffered formalin or Tellesnitsky's liquid, with a classical scheme of sample processing, embedding the material in paraffin, and preparing histological sections. Lymph node samples were embedded in epoxy resin to prepare semi-thin sections. The lymph node sections were stained with hematoxylin and eosin, azure and eosin, toluidine blue, and trichrome dye, according to Masson's method. Morphometric analysis of the structural components of the lymph nodes and determination of their specific area were carried out using a morphometric grid (Reid 1980) imposed on the lymph node sections. The cross-sectional areas of nodes or intersections of the grid were calculated for the slice in total and each structural component separately, as a percentage. The specific cross-sectional area of objects on the cut area corresponded to the specific volume of the object in the sample according to the fundamental Cavalieri-Ackera-Glagolev principle of stereology (Rogers 2006). In the structural and functional zones of the lymph nodes, the absolute number of cells was counted on a standard area of 2025 μm^2 at a microscope magnification of ×900. According to the International Histological Nomenclature, in the cytological picture of the lymph node, we differentiated the reticular cells that formed the node carcasses, the lymphopoietic cells – blasts, medium and small lymphocytes, and plasmocytes– including free macrophages (histiocytes), neutrophils (microphages), eosinophils (acidophilic leukocytes), and others. The cells are grouped into lymphoid tissue cells, cells that perform supporting and phagocytic functions, and peripheral blood cells (Tanasiychuk 2004; Suttie 2018).

Statistical Analysis

The experimental results were statistically processed in Microsoft Excel StatPlus Pro 2009 program (Analyst Soft, Inc.) using unpaired Fisher's and Student's tests. The arithmetic mean (M) ± and mean error (± m) are used to present the data. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Cervical lymph node microstructure

The morphofunctional state of the cervical lymph nodes is closely related to the drainage of the head and neck organs; therefore, a change in the function of these organs causes a certain restructuring of the lymph nodes.

The proportion of structures such as lymphoid nodules, the internodal part of the cortex, paracortex, and medullary cords was quite high in the anatomical structure of the cervical lymph nodes. The developed structural and functional zones in the lymph nodes indicated a fairly high immune potential in young animals (shown in Table 1). The prevalence of lymphoid nodules in the cortex indicates active lymphoproliferative processes in young animals (shown in Fig. 1). The sinus system of the lymph nodes was small and represented mainly by the subcapsular and medullary lymphatic sinuses. Cervical lymph nodes are compact. The cortex/medulla ratio (C/M ratio) for the cervical lymph nodes of the young animals was 2.32 ± 2.2. The microanatomical organization of cervical lymph nodes is characteristic of immune function, thus determining their functionality in the lymphatic region. Examination of cervical lymph nodes at the mature ontogenesis stage did not reveal a morphological picture of the decrease in immune function. The lymph node section area was unchanged compared to that in young animals. Cork-brain index – 2.3 ± 0.06. The cortex prevailed over the medium in the same proportion as in the young animals (shown in Fig. 1). The number of lymphoid nodules was the same as that in young animals. In senile animals, the lymph node section area was less than that in young animals, reflecting the involution of lymphoid tissue during aging. The lymph node tissue became more compact, with the cork-brain index reaching 2.5 ± 0.08. The cortex prevailed over the medulla, while the area occupied by the lymphatic sinuses in the lymph nodes decreased.

Table 1. The area of the structural and functional zones of the cervical lymph node in young, mature, and senile animals (μm^2).

Lymph node structure[1] and indices	**Young animals (1-2 mths.)**	**Mature animals (10-12 mths.)**	**Senile animals (22-24 mths.)**	***p*-value[2]**
Capsule	1.43 ± 0.13 (5.11 ± 0.21%)	1.58 ± 0.14 (5.53 ± 0.28%)	2.53 ± 0.24 (9.97 ± 0.41%)	$p < 0.05$
Supcapsulars sinus	1.25 ± 0.12 (4.46 ± 0.21%)	1.22 ± 0.13 (4.27 ± 0.24%)	0.79 ± 0.07 (3.11 ± 0.26%)	
Intermodal part of the cortex	3.97 ± 0.21 (14.17 ± 0.34%)	4.10 ± 0.24 (14.35 ± 0.48%)	4.35 ± 0.11 (17.53 ± 0.24%)	$p < 0.01$
Lymphoid nodule without germinal center (F_1)	2.16 ± 0.14 (7.71 ± 0.26%)	1.55 ± 0.15 (5.43 ± 0.21%)	1.99 ± 0.14 (7.84 ± 0.28%)	$p < 0.01$
Lymphoid nodule with germinal center (F_2)	2.90 ± 0.18 (10.35 ± 0.38%)	2.71 ± 0.21 (9.49 ± 0.41%)	1.73 ± 0.14 (6.81 ± 0.31%)	$p < 0.05$
Paracortex	7.63 ± 0.36 (27.24 ± 0.46%)	8.59 ± 0.34 (30.07 ± 0.42%)	6.63 ± 0.24 (26.12 ± 0.34%)	$p < 0.05$
Medullary cords	7.03 ± 0.36 (25.10 ± 0.29%)	7.06 ± 0.24 (24.71 ± 0.32%)	5.76 ± 0.24 (22.7 ± 0.36%)	$p < 0.01$
Medullary lymphatic sinus	1.65 ± 0.21 (5.88 ± 0.27%)	1.76 ± 0.14 (6.16 ± 0.24%)	1.5 ± 0.18 (5.9 ± 0.28%)	$p < 0.05$
Total area	28.02 ± 1.29 (100%)	28.57 ± 1.77 (100%)	25.38 ± 2.21 (100%)	
Cortex/medulla ratio (C/M ratio), %	28.02 ± 1.29 (100%)	28.57 ± 1.77 (100%)	25.38 ± 2.21 (100%)	$p < 0.05$
F_2/F_1 index	1.34 ± 0.06	1.75 ± 0.09	0.87 ± 0.07	

Note: [1]Lymph node structures are expressed in abs. figures and %; [1]Indices are expressed in %; [1]The value is reliable when compared with young + senile

The proportion of connective tissue in lymph nodes increased by age. This was mainly manifested by a 2.5-fold thickening of the capsule and the development of connective tissue around the blood vessels (shown in Table 1). Part of the lymph node was replaced by adipose tissue (shown in Fig. 2). Differentiation of the cortex and medulla in such lymph nodes is difficult. Fibrosis and replacement of lymphoid tissue with adipose tissue can promote the development of immune deficiency in old age. This is facilitated by the long-term contact of the oral cavity of animals with the external environment reflected in the anatomical structure of the regional cervical lymph node. The internodal and paracortex regions are related to the thymus-dependent zone. At a later stage of ontogenesis, the involutive changes in cortex structures reduced the areas of the internodal part of the cortex (by 1.4 times), lymphoid nodules with a germinal center (by 2.7 times), and the paracortex (by 1.3 times). Lymphoid nodules of the preserved lymph nodes showed some damaged cells. Structures of the medulla, such as medullary cords and medullary lymphatic sinus, also decreased in size with age. Extreme age-related transformation was manifested by significant lymphoid infiltration at the site of the former lymph node (shown in Fig. 3). The analysis of ordering of structures inside the lymph node showed a decreased size of most of the structural and functional zones in senile animals compared to young and mature animals, and an increased proportion of the connective tissue component. A decrease in the area of the lymphoid parenchyma of the cervical lymph node was associated with a drop in immunoreactivity at the late stage of ontogenesis.

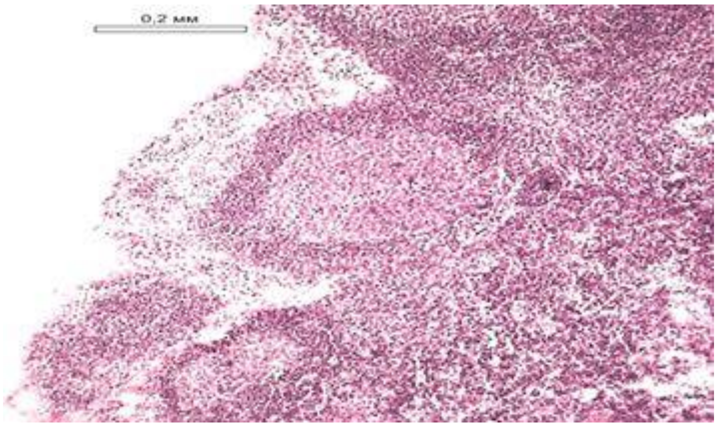

Fig. 1. Dilated germinal centers in the cortical lymphoid nodules in front of the dilated part of the subcapsular sinus, cervical lymph node. Young animals. Staining with hematoxylin and eosin. Magnification x120.

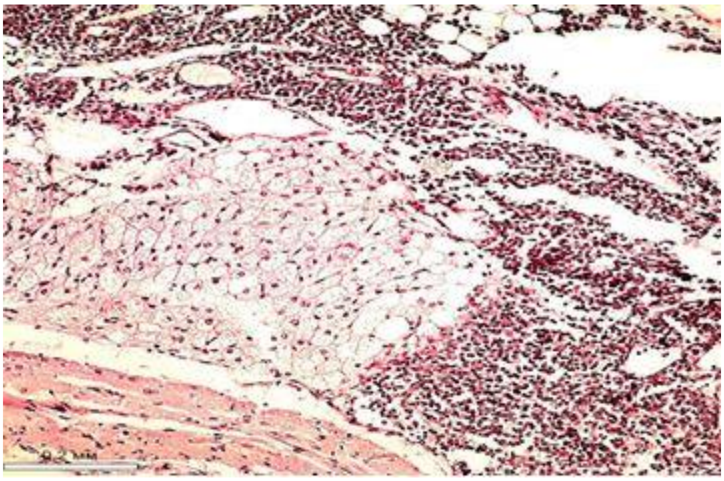

Fig. 2. Fat replacement of lymphoid tissue of a part of the cervical lymph node. Senile animals. Staining with hematoxylin and eosin. Magnification x120.

Mesenteric lymph node microstructure

The mesenteric lymph node is a peripheral lymphoid organ with high immune activity, owing to the constant operation of the digestive tract. The mesenteric lymph node has a thin surrounding capsule, from which the trabecule extend into the node (shown in Fig. 4). In young animals, the structure of the lymph node was dominated by the cortex; the C/M ratio was equal to 2.14 ± 0.09 (shown in Table 2). The presence of lymphoid nodules, with and without a germinal center (Fig. 4), in the cortex in the ratio of 1.36 ± 0.11 (Table 2) indicated active lymphopoietic functioning of the lymph node. The medulla was well-developed and represented by large medullary cords and wide lymphatic sinuses. The Structural and functional zones were evenly distributed in the cortex and medulla. Such a mixed morphological structure of the lymph node, with a sufficiently spread sinus system, indicates the simultaneous performance of immune and drainage functions. The changes involved the structures of the cortex and medulla in the lymph node and reflected the age-related involution of the lymphoid tissue, albeit to varying degrees. The peripheral lymphoid cortex was narrowed and concentrated closer to the capsule and the deep part of the cortex was divided into the penetrating medulla. Moreover, the lymph node structure was dominated by reticular stroma.

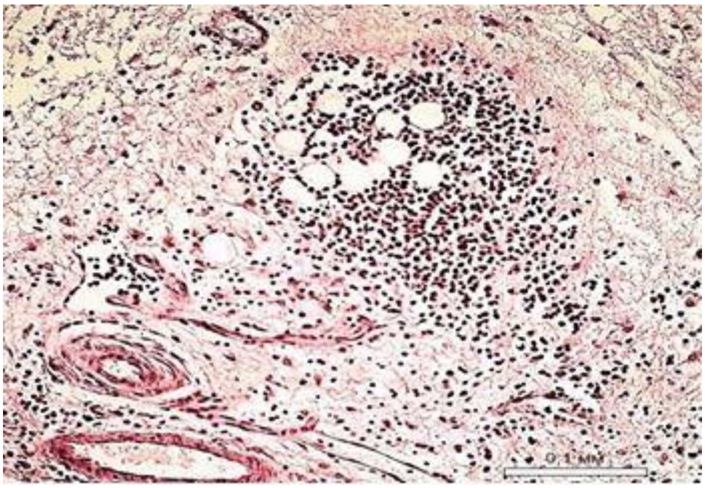

Fig. 3. Old animals. Lymphoid infiltrates at the location of the cervical lymph node. Stained with hematoxylin and eosin. The total magnification is x240.

Table 2. The area of the structural and functional zones of the mesenteric lymph node in young, mature, and senile animals, μm².

Lymph node structure[1] and indices	Young animals (1-2 mths.	Mature animals (10-12 mths.)	Senile animals (22-24 mths.)	*p*-value [2]
Capsule	5.72 ± 0.19 (9.26%)	5.95 ± 0.22 (9.38%)	9.43 ± 0.45 (14.93%)	$p < 0.01$
Subcapsular sinus	4.57 ± 0.17 (7.40%)	4.55 ± 0.2 (7.35%)	3.38 ± 0.27 (5.35%)	$p < 0.05$
Intermodal part of the cortex	7.74 ± 0.31 (12.53%)	7.84 ± 0.25 (12.7%)	3.78 ± 0.24 (5.98%)	$p < 0.01$
Lymphoid nodule without germinal center (F_1)	4.18 ± 0.17 (6.77%)	4.12 ± 0.19 (6.57%)	3.32 ± 0.22 (5.26%)	$p < 0.05$
Lymphoid nodule with germinal center (F_2)	5.69 ± 0.19 (9.21%)	5.52 ± 0.2 (9.05%)	3.03 ± 0.26 (4.80%)	$p < 0.01$
Paracortex	16.02 ± 0.56 (25.93%)	16.15 ± 0.43 (27.4%)	14.29 ± 0.54 (22.63%)	$p < 0.05$
Medullary cords	10.55 ± 0.24 (17.07%)	11.3 ± 0.35 (19.4%)	22.03 ± 0.72 (34.88%)	$p < 0.001$
Medullary lymphatic sinus	7.31 ± 0.29 (11.83%)	7.4 ± 0.25 (12.9%)	3.89 ± 0.35 (6.16%)	$p < 0.01$
Total area	61.78 ± 1.91 (100%)	62.83 ± 2.9 (100%)	63.16 ± 1.59 (100%)	$p < 0.01$
Cortex/medulla ratio (C/M ratio; %)	2.14 ± 0.09	2.35 ± 0.1	1.07 ± 0.11	$p < 0.01$
F_2/F_1 index	1.36 ± 0.11	1.34 ± 1.05	0.91 ± 0.08	$p < 0.01$

Note: [1]Lymph node structures are expressed in abs. figures and %; [1]Indices are expressed in %; [1]The value is reliable when compared with young + senile

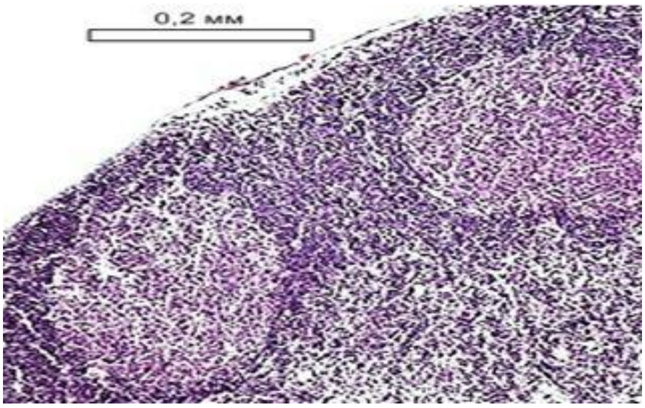

Fig. 4. Young animals. Expanded germinal centers in the lymphoid nodules of the mesenteric lymph node. Stained with hematoxylin and eosin. The total magnification is x120.

The lymphoid tissue was replaced by connective tissue that expanded perivascularly and in the lymph node parenchyma. Minimization of the main structural and functional zones, especially lymphoid nodules with germinal centers, indicated a decrease in cell proliferation and, consequently, the reduced immune potential of the lymph node. The lymphatic sinus system was narrow in the specimens, indicating a drop in lymph node drainage function. The predominance of the medulla in old animals formed an intermediate morphological structure of the lymph node, with a C/M ratio of 1.07 ± 0.11 and a humoral-type immune response. Such a morphotype would be optimal for a lymph node, but it was not properly represented owing to age-induced changes. Senile changes are accompanied by decreased functional activity of the lymph nodes.

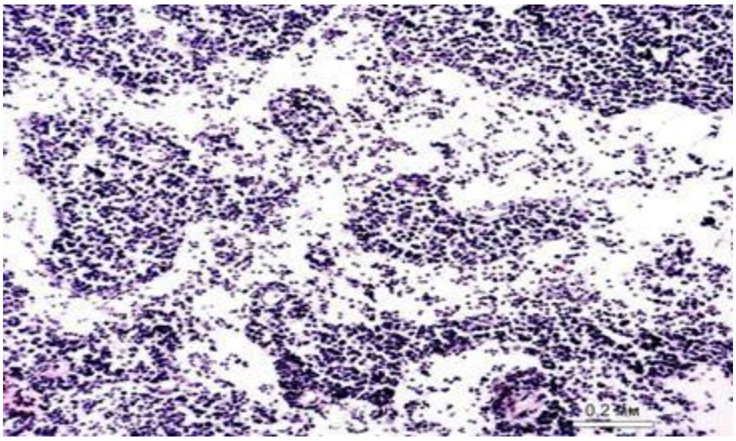

Fig. 5. Mature animals. Stained with hematoxylin and eosin. Fragment of a mesenteric lymph node with a wide lumen of the cerebral lymphatic sinus and large cerebral cords. The total magnification is x120.

In 30% of senile animals, the area of the cortex structures in the mesenteric lymph node was increased due to paracortex hyperplasia. The sizes of the paracortex and medullary cords were 28.92 ± 1.27% and 8.76 ± 0.51% respectively. The germinal centers of lymphoid nodules were wide in this group of animals. The medullary lymphatic sinuses remained narrow and constituted 2.52 ± 0.26%. The morphometry of the mesenteric lymph nodes differed from the data in the group of animals with a predominance of the medulla (Table 2). The data obtained were typical for a lymph node with a compact morphotype and the development of a thymus-dependent zone that ensures the cellular part of the immune response.

DISCUSSION

Our study demonstrated the importance of localization of the sclerotic process in the lymphoid parenchyma. Neoplasms or proliferation of connective tissue at the site of parenchymal cell atrophy indicates the development of sclerosis (Turner & Mabbott 2017; Priya 2023; Rodriguez-Mogeda *et al.* 2025). In lymph nodes, sclerosis can affect the medulla, cortex, and paracortex (Ahmadi *et al.* 2013, Richner *et al.* 2015, Takeda *et al.* 2023). We observed sclerotic alterations in the lymph node capsule of senile animals and proliferation of connective tissues along the blood vessels. This paper describes, for the first time, the localization of subcapsular sclerosis in the peripheral cortex parallel to the marginal sinus and the local thickening of the capsule. Such localization disrupts the structure of the lymphoid lobule and lymph flow through the lymph node, leading to the development of immune and drainage deficiencies. However, old rats do not lose their medulla as long as the lymph still flows through the lymph nodes. In contrast, the medulla was enlarged and even deposited due to dilated medullary lymphatic sinuses during subcapsular sclerosis. An increase in the volume of stromal elements reduces the lumen of the sinuses in the visceral lymph nodes. A decrease in lymph flow through visceral nodes is associated with a high content of toxic substances in the inflowing lymph (Randall 2010; Liao & Padera 2013). In senile animals, the cortex of the mesenteric lymph node undergoes the most pronounced changes and increase. Numerous studies have confirmed the important role of lymph nodes in humoral and cellular immune responses (Fletcher *et al.* 2015; Milutinovic *et al.* 2021; Nurmakhanova *et al.* 2023). The change in the lymph node compartments (Johnson 2021; Abdreshov *et al.* 2023), observed in senile animals could reflect antagonism between the humoral and cellular parts of immunity. The area occupied by the thymus-dependent zone in the lymph nodes of older animals shows that a reduction in resistance due to immune system disorders leads to immune deficiency (Qi *et al.* 2014; Demchenko *et al.* 2023). Age-related reorganization of lymph nodes can take different forms: (i) an increase in the cortex due to paracortex hyperplasia and a decrease in the medulla; and (ii) an increase in the medulla and atrophy of the cortex. This determines the humoral or cellular type of final immune response (Siegrist & Aspinall 2009, Cakala-Jakimowicz *et al.* 2021). We assumed the influence of environmental and nutritional factors on age-related morphological changes in the mesenteric lymph nodes. Age-related changes in the immune system include atrophy of lymphoid organs, a decrease in the number of peripheral T-cells (Chinn *et al.* 2012; Betterman & Harvey 2016), and an elevation in the number of immature lymphocytes owing to a delay in their differentiation (Hadamitzky *et al.* 2010; Lancaster 2023). Cyst composition reflects the stages of age-dependent transformation of the lymph node lymphoid parenchyma (Beregi *et al.* 1980; Cakala-Jakimowicz *et al.* 2021). Typical changes in senile animals include a drop in the number of blasts and small lymphocytes, an elevation in the number of medium lymphocytes in lymphoid nodules, a reduction in the number of small and medium lymphocytes, an upraise in the number of macrophages in the paracortex, a decrease in plasma cells as well as increases in the number of macrophages in the pulp cords, and also in the number of small lymphocytes in the medullary lymphatic sinus. The lymphoid cell composition provides morphological verification of the activation or deceleration of migration, proliferation, and differentiation of immunocompetent cells. The predominance of mature and immature plasma cells provides evidence of the active immune function of the lymph node (Morandi *et al.* 2014; Zhang *et al.* 2019). We observed a characteristic age-dependent decrease in the number of prolymphocytes and a corresponding elevation in the number of mature lymphoid cells. Blasts are transformed when the transition of a prolymphocyte (middle lymphocyte) to a lymphoblast is delayed. In senile and infant age, 20-30% fewer cells undergo transformation (Laribi *et al.* 2017; Masters *et al.* 2019). There is a notable inverse relationship between the numbers of mature lymphoid cells and lymphocytes (Sackstein 1993). Delay in blast formation is more evident in senile animals, primarily in lymphoid nodules.

CONCLUSION

In young animals, compared with mature animals, no significant changes in the studied indicators were observed. Thus, the lymph nodes, regardless of their location, have common signs of aging: (i) an increase in connective tissue and a decrease in the area of lymphoid nodules with germinal centers, with the preservation of the compact morph type; and (ii) unidirectional changes in cellular composition – a drop in the number of blasts and medium lymphocytes in lymphoid nodules, paracortex, and plasmocytes in the medullary cords, as well as an elevation in reticular cells, which indicates a reduction in the immunoreactivity of these structures.

Authors' Contributions

All authors participated in the planning of the experiment, interpretation of the results and review of the article; ZhL were involved in the experimentation and revised the work; ZY, LK and DKh was involved in the literature search and drafting of the manuscript; SNA and VG acted as an instructor for the experiment and wrote the article; VG and MB participated in the experiment, contributed to the discussion; SNA and GAD contributed to the concept the work and reviewed the work, were responsible for the design and verification of the work; SNA reviewed the work.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the European Convention for the Protection of Vertebrates and the guidelines outlined in the European Union Directive and Use Committee of the Institute of Genetics and Physiology (protocol No. 13-354 of September 19, 2024).

Informed Consent Statement

Not applicable.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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