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INVOLUTIONAL CHANGES OF SPLENIC LYMPHOID TISSUE IN 18-MONTH-OLD WHITE OUTBRED RATS

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Abstract
Aging is a complex biological process characterized by progressive structural and functional alterations in organs of the immune system. The spleen, as a major peripheral lymphoid organ, plays a key role in immunological surveillance, antigen presentation, lymphocyte proliferation, and hematopoietic regulation. Age-related involution of splenic lymphoid tissue is associated with decreased immune responsiveness and remodeling of stromal and vascular components; however, detailed morphometric characterization of these changes remains insufficiently described in experimental models.

Keywords: Aging; spleen; lymphoid tissue; involution; morphometry; immunosenescence; immunomorphology; white outbred rats.

Introduction

The spleen is the largest secondary lymphoid organ in rodents, playing a central role in initiating immune responses to blood-borne antigens and filtering aged or damaged erythrocytes, with a distinct organization into white and red pulp regions that support specialized immune and hematological functions [1]. During postnatal development in white rats, the spleen undergoes significant morphological and morphometric changes, reflecting maturation and functional adaptation of lymphoid structures over time [2]. The white pulp typically increases until approximately 9 weeks of age and then stabilizes, whereas advanced age is associated with gradual blurring of structural boundaries and changes in pulp composition [3]. At birth, the rat spleen lacks well-defined histological features; by one month, splenic sinuses and marginal zones become distinguishable, and by two months, the main splenic compartments are clearly formed [4].

The reticular connective tissue framework provides essential structural support for the red and white pulp and facilitates lymphocyte trafficking and antigen presentation [5]. Within the white pulp, periarteriolar lymphoid sheaths (PALS) and lymphoid follicles support T- and B-cell interactions critical for adaptive immunity [6]. The red pulp, in turn, functions in blood filtration and erythrocyte turnover, containing venous sinuses and splenic cords where macrophages phagocytose senescent red blood cells [7]. Spleen development in healthy rodents is characterized by continuous growth

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and maturation of lymphoid compartments during early postnatal life, reaching functional maturity before full adulthood [8].

Structural components such as the splenic capsule and trabeculae, composed of collagen and reticular fibers, provide mechanical support while allowing expansion of the parenchyma during immune responses [9]. The marginal zone between the white and red pulp serves as a critical interface for antigen capture and initiation of both innate and adaptive immune reactions [10]. Morphometric analysis further demonstrates that proportions of white and red pulp relative to total organ area vary with age, yet structural boundaries remain distinct, indicative of proper immunological function in non-diseased animals [11].

Objective

The aim of this study was to investigate age-related involutional changes in the splenic lymphoid tissue of 18-month-old white outbred rats through comprehensive organometric, morphological, and morphometric analyses in order to assess structural remodeling of the spleen associated with aging and decreased immune functional activity.

Materials and Methods

This scientific research was carried out during 2024–2025 at the Research Laboratory of the Bukhara State Medical Institute named after Abu Ali Ibn Sino. A total of 212 white outbred rats maintained under vivarium conditions were selected for experimental investigations. Laboratory animals belonging to the 18-month age group were included in the study. All animals were housed under standard vivarium conditions with identical microclimate parameters, lighting regimen, and dietary supply.

In the second stage of the research, carcinogenic substances and chemotherapeutic agents were administered to experimental groups of white outbred rats, followed by appropriate corrective interventions. To evaluate tissue alterations in the spleen and selected organs of the immune system, morphological, morphometric, and immunohistochemical research methods were employed.

All experimental procedures were conducted with prior approval of the institutional ethics committee and in full compliance with bioethical and biosafety regulations. Each stage of the study was properly documented according to established research protocols.

At the final stage, the obtained morphological, morphometric, and immunohistochemical data were systematized and prepared for statistical analysis. Particular attention was paid to ensuring the reliability and adequacy of the results, as well as to the development of scientifically grounded conclusions and practical recommendations.

All experimental results were personally obtained and analyzed by the dissertation author under the supervision of the scientific advisor.

Results

The examination of the spleen in 18-month-old white outbred rats of the control group revealed pronounced age-related structural and morphometric alterations reflecting involutional changes of lymphoid tissue.

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The body weight of experimental animals ranged from 290 to 320 g, with an average value of 312.26±3.68 g. The absolute spleen weight varied between 1.3 and 1.7 g, averaging 1.52±0.037 g. The spleen index ranged from 0.333% to 0.552%, with a mean value of 0.455±0.024%. Organometric measurements demonstrated that spleen length ranged from 29.4 to 35.2 mm (33.78±0.63 mm on average), width from 4.6 to 7.4 mm (6.52±0.26 mm), and thickness from 2.8 to 3.9 mm (2.92±0.29 mm).

Macroscopically, the spleen had an elongated shape and a dark-red coloration due to pronounced blood filling. The organ was externally covered by a serous membrane and a dense fibrous capsule. Trabeculae extending from the capsule into the splenic parenchyma were clearly visible. Histological examination revealed thickening of trabeculae and vascular walls, with signs of fibrotic changes and sclerosis, which were more evident after Van Gieson staining.

Morphometric analysis demonstrated a reduction in lymphoid tissue components characteristic of age-related involution. The relative area of white pulp ranged from 24.6% to 28.2%, averaging 20.54±0.69%. In contrast, connective tissue elements occupied 5.6–6.7% of the splenic section area, with a mean value of 6.22±0.22%, indicating stromal expansion. The predominance of red pulp was observed, accompanied by dilatation of sinusoids, increased numbers of macrophages, and focal hemosiderin accumulation (figure 1).

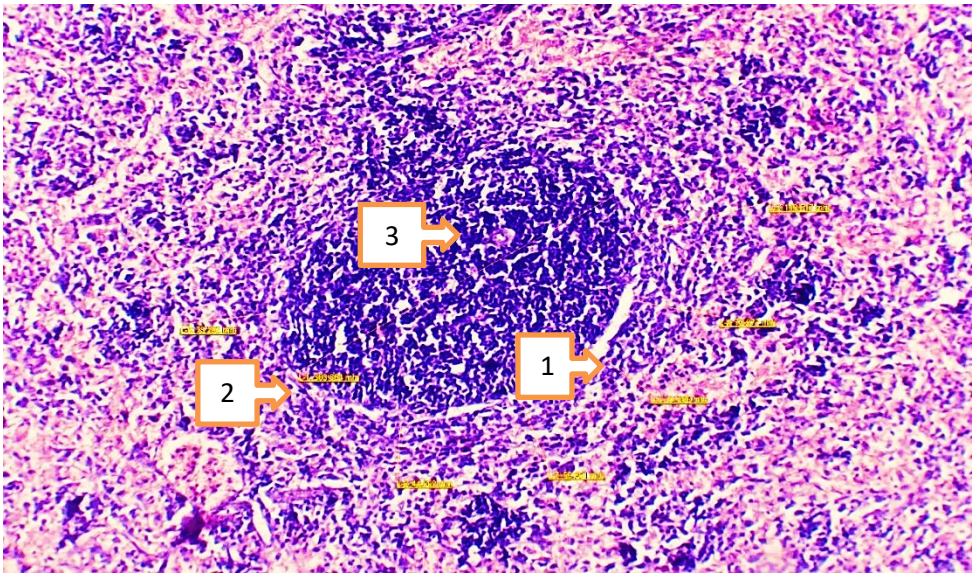

Figure 1. Morphological structure of the spleen tissue in 18-month-old albino rats. Staining: (G–E). Objective: 4×20. 1 – ed pulp shows signs of atrophy, with increased hemolysis in the perisinusoidal area; 2 – White pulp area is markedly reduced in volume: mature lymphocytes in the follicles are decreased, while the number of blast cells is increased; 3 –the mantle and marginal zones are diminished.

The diameter of periarterial lymphatic sheaths (PALS) ranged from 228.2 to 242.6 µm, averaging 236.22±2.55 µm. Lymphoid follicle diameter varied between 380.8 and 477.05 µm, with a mean value of 420.96±20.44 µm. The proportion of primary and secondary lymphoid follicles constituted 34% and 66%, respectively. Germinal center diameter ranged from 222.4 to 247.7 µm, averaging 235.08±2.73 µm.

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The mantle zone thickness ranged from 40.5 to 50.4 μm (46.56±2.06 μm), while the marginal zone measured 74.5–86.2 μm (80.72±2.26 μm). The periarterial zone width varied from 84.9 to 94.7 μm, with an average value of 89.42±2.06 μm.

Quantitative cellular analysis revealed that lymphoid follicles lacking germinal centers contained 52–62 lymphocytes, with an average of 57.2±0.97 cells. The total number of lymphocytes in periarterial lymphatic sheaths ranged from 53 to 62 cells, averaging 58.4±0.86 cells.

Overall, the obtained data indicate age-associated involution characterized by reduction of white pulp components, increased connective tissue content, vascular remodeling, and decreased lymphoid cellularity, reflecting diminished immunological activity of the spleen in aged animals.

Conclusions

The present study demonstrated significant age-related involutional changes in the spleen of 18-month-old white outbred rats. Organometric analysis revealed stable dimensional parameters of the organ accompanied by structural remodeling of splenic tissue. Histological and morphometric findings showed a reduction in the relative area of white pulp, decreased lymphoid follicle activity, and poorly expressed germinal centers, indicating suppression of proliferative processes within lymphoid compartments.

Age-associated thickening and sclerosis of the capsule and trabeculae, together with an increased proportion of connective tissue elements, reflect progressive stromal remodeling of the spleen. The predominance of red pulp, dilation of sinusoids, accumulation of macrophages, and presence of hemosiderin deposits suggest intensified erythrocyte degradation and altered hematological function in aging animals.

Quantitative analysis of lymphoid structures demonstrated reduced lymphocyte density, confirming a decline in immunological reactivity characteristic of immunosenescence. Overall, the obtained data indicate that aging leads to structural reorganization and functional attenuation of splenic lymphoid tissue.

These findings provide morphometric evidence of age-related immune system decline and may serve as a morphological baseline for further experimental studies investigating immune dysfunction, chronic diseases, and therapeutic interventions associated with aging.

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