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MORPHOLOGICAL FEATURES OF POPLITEAL LYMPH NODES IN WHITE OUTBRED RATS WITH RHEUMATOID ARTHRITIS

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Abstract. The study investigates age-dependent histochemical and morphometric changes in peripheral lymph nodes of white outbred rats with experimental rheumatoid arthritis. Alsian blue staining revealed reactive-proliferative and reparative processes in young (3-month-old) rats, moderate compensatory activity in middle-aged (12-month-old) rats, and predominance of fibrotic and involutional changes in older (18-month-old) rats. Age-related accumulation of glycosaminoglycans in stroma, trabeculae, and sinus walls reflects chronic inflammation and tissue remodeling. The findings highlight dynamic structural and functional adaptations of lymph nodes in autoimmune pathology, providing insights into immunoregulatory mechanisms and potential therapeutic interventions.

Keywords: rheumatoid arthritis, peripheral lymph nodes, Alsian blue, histochemistry, morphometry, glycosaminoglycans, immune response, tissue remodeling.

Introduction. The lymphatic system is a complex morphofunctional network that plays a crucial role in the immune defense mechanisms of the body. It is actively involved in removing excess tissue fluid, detecting antigens, and orchestrating immune responses. As peripheral immune organs, lymph nodes function as biological filters, capturing microorganisms, foreign antigens, and cellular debris transported via lymph flow, while providing a microenvironment that supports lymphocyte proliferation and differentiation [1]. Within lymph nodes, antigen presentation occurs, initiating the

adaptive immune response, which ensures effective defense against infectious and inflammatory agents [2].

Peripheral lymph nodes are key components of immune surveillance, particularly serving as the primary sites for immune responses to antigens derived from tissues in direct contact with the external environment. They facilitate antigen recognition, clonal expansion of lymphocytes, and formation of effector cells, thereby maintaining homeostasis [2]. Histologically, lymph nodes are encapsulated and consist of cortical, paracortical, and medullary zones, each with distinct cellular compositions and specialized functions that support various stages of the immune response [3]. The cortical zone is rich in B-lymphocyte-containing lymphoid follicles, where germinal centers develop and antibody-producing cells mature. The paracortical zone is predominantly occupied by T-lymphocytes and serves as the central hub for cellular immune responses. The medullary zone contains plasma cells, macrophages, and a reticular stroma, participating in the final phases of immune reactions [4]. The structural and functional balance among these zones is essential for normal immunological activity.

With advancing age, involutional changes occur in lymphoid organs. Lymphocyte proliferative activity declines, renewal of immune cells slows, and the relative proportion of stromal components increases [5]. This process, known as immunosenescence, limits the functional capacity of the immune system and reduces responsiveness to antigens [6]. Consequently, the organism becomes more susceptible to infections, chronic inflammation, and autoimmune reactions [7]. Experimental morphological studies have shown age-related structural remodeling of lymph nodes, including reduced numbers of lymphoid follicles, smaller germinal centers, and altered ratios of parenchymal and stromal components [8]. These changes are important morphometric indicators reflecting the functional state of the immune system. Therefore, comprehensive assessment of lymph node morphometric parameters provides insights into adaptive and compensatory processes within the immune system.

Recent studies indicate that pathological conditions and corrective interventions can significantly influence structural remodeling of lymphoid tissues. Under the influence of corrective factors, lymphoid tissue may exhibit enhanced proliferative activity, restoration of germinal centers, and a relative decrease in stromal components

[9]. Some investigations have also reported that immunomodulatory treatments can stimulate compensatory-regenerative processes within lymph nodes [10].

Inguinal lymph nodes are a critical part of the peripheral lymphatic system, filtering lymph from the lower extremities, capturing antigens, and ensuring regional immune surveillance. These lymph nodes are considered sensitive indicators of the overall immune reactivity of the organism [11]. Hence, the morphological assessment of inguinal lymph nodes holds significant scientific value in experimental immunomorphological studies.

A review of the literature reveals that morphometric parameters of lymph nodes across different ages and under corrective interventions remain insufficiently studied. In particular, structural remodeling mechanisms in older experimental animals have not been fully elucidated, making this an important unresolved scientific issue [12]. Therefore, investigating the morphological characteristics of inguinal lymph nodes in 18-month-old male outbred white rats under corrective conditions is of considerable scientific and practical relevance.

Objective. The aim of this study was to investigate age-dependent histochemical and morphometric changes in peripheral lymph nodes of white outbred rats with experimentally induced rheumatoid arthritis, using Alsian blue staining. The study focused on assessing reactive-proliferative, reparative, and fibrotic processes in lymphoid and stromal compartments, as well as evaluating glycosaminoglycan accumulation, in order to understand the dynamics of immune responses and tissue remodeling across different age groups.

Materials and Methods. This study was conducted at the Research Laboratory of the Scientific and Experimental Biomedicine Center of Bukhara State Medical Institute. For the experimental work, 18-month-old male outbred white rats were selected, each weighing approximately 250–290 g. All animals were obtained from a single vivarium and maintained under standard conditions: relative humidity 50–60%, temperature 19–22°C, and a 12-hour light/12-hour dark cycle. Vivarium rooms were cleaned daily, strictly following sanitation and hygiene protocols.

Deceased animals were documented according to established regulations, disinfected with 20% chlorinated lime solution, and buried. All rats were fed a standard vivarium diet. In the correction group, 18-month-old rats (n=23) were maintained on

the standard diet and experimentally induced with rheumatoid arthritis. For this purpose, 0.1 ml of Freund's adjuvant was administered subcutaneously at the base of the tail in a single injection. During the first three days post-injection, local swelling and hyperalgesia were observed, and by days 7–10, signs of inflammation appeared in the distal joints.

At the end of the experiment, all animals were decapitated in the morning after overnight fasting. The popliteal lymph nodes were carefully excised, their macroscopic dimensions recorded, and fixed for morphological and morphometric analysis. Tissue sections were stained with hematoxylin-eosin and Van Gieson stains and examined under a microscope. All morphological and morphometric parameters were statistically analyzed, and the results were documented following scientific standards.

Results. germinal center activity and sustained chronic inflammation. Histochemical analysis of Alsian blue-stained popliteal lymph nodes in white outbred rats with experimental rheumatoid arthritis revealed significant age-dependent changes in both lymphoid and stromal compartments, reflecting the dynamic balance between reactive-proliferative, reparative, and fibrotic processes.

In 3-month-old rats, lymph nodes were in an active reactive-proliferative phase, showing intensive immunological activity and tissue remodeling. This stage was considered the reference (100%) for comparison. The cortical zone was expanded, germinal centers were highly active, and lymphoid follicles were numerous and of larger diameter. The medullary zone accounted for 20.1% of the lymph node volume, and stromal volume comprised 28.9%. The accumulation of glycosaminoglycans in the stroma, trabeculae, and sinus walls, indicated by metachromatic Alsian blue staining, reflected early-stage inflammation and high reparative activity.

In 12-month-old rats, lymph nodes exhibited moderate reactive and reparative activity. Relative to 3-month-old animals, the total lymph node diameter increased by 20.8%, cortical zone thickness by 8.8%, paracortical zone by 8.4%, and the number of lymphoid follicles per section by 5.3%. The average follicle diameter increased by 8.4%, and germinal center activity increased by 8.5%. The medullary zone volume decreased by 10.5%, while stromal volume increased slightly by 3.2%. These findings indicate a balanced compensatory response, with moderate proliferation of

lymphocytes and partial preservation of germinal center activity, alongside controlled stromal remodeling.

In 18-month-old rats, lymph nodes demonstrated predominance of fibrotic and involutional processes, characteristic of chronic rheumatoid changes in older animals. Compared to 3-month-old rats, the total lymph node diameter increased by 29.6%, cortical and paracortical zones increased by 3.9% and 4.2%, respectively. The number of lymphoid follicles decreased by 5.3%, while the average follicle diameter slightly increased by 4.2%. Germinal center activity decreased by 4.1%, medullary zone volume declined by 17.4%, and stromal volume significantly increased by 14.5%. Accumulation of glycosaminoglycans in the stroma, trabeculae, and sinus compartments was prominent, reflecting chronic inflammation, fibrotic remodeling, and partial loss of immune reactivity.

Overall, these results demonstrate a clear age-dependent pattern in peripheral lymph nodes under experimental rheumatoid arthritis: young rats exhibit maximal immune and reparative activity, middle-aged rats maintain balanced compensatory processes, and older rats show dominant fibrotic remodeling and partial involution of lymphoid structures, accompanied by reduced

Conclusions. The present study demonstrates that experimental rheumatoid arthritis induces pronounced age-dependent changes in peripheral lymph nodes of white outbred rats. In young (3-month-old) animals, lymph nodes exhibited high reactive-proliferative activity, intensive germinal center proliferation, and active stromal remodeling, reflecting a strong compensatory and reparative immune response. In middle-aged (12-month-old) rats, lymph nodes maintained balanced reactive and reparative processes with moderate lymphoid proliferation and partial preservation of germinal center activity, indicating sustained immune competence. In older (18-month-old) rats, lymph nodes showed predominance of fibrotic remodeling, partial involution of lymphoid follicles, decreased germinal center activity, and continued chronic inflammation, highlighting age-related decline in immune reactivity and reparative potential.

These findings suggest that the progression of rheumatoid arthritis is accompanied by dynamic structural and functional adaptations in peripheral lymph nodes, with younger animals exhibiting maximal compensatory capacity, while older animals show

predominance of degenerative and fibrotic changes. Understanding these age-dependent lymph node changes provides important insights into immune system dynamics in chronic autoimmune inflammation and may inform strategies for immunomodulatory interventions and therapeutic biocorrection.

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