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Comparative Features of CD-20 Marker Expression in the Thymus During Experimental Intestinal Fibrosis

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Abstract The intestinal cicatricial process is associated with complex immune and morphofunctional alterations that may affect the cellular composition of the thymus. Particular interest is given to the B-cell component of the thymic tissue, which can be characterized by immunohistochemical detection of the CD-20 marker. The aim of this study was to assess the features of CD-20 immunohistochemical expression in the thymus of white outbred rats during experimental intestinal cicatrization. Materials and methods. The study included white outbred rats of different age groups. An experimental intestinal cicatricial process was modeled, followed by morphological and immunohistochemical examination of thymic tissue. Histological sections were prepared and immunohistochemical staining for the CD-20 marker was performed. The number and distribution of CD-20-positive cells in the thymic tissue were evaluated, and the obtained quantitative data were subjected to statistical analysis. Results. The investigation demonstrated age-related differences in the representation and distribution of CD-20-positive cells in the thymus. Under conditions of experimental intestinal cicatrization, changes in CD-20 expression were observed, indicating a restructuring of the B-cell component of the thymic microenvironment in response to the pathological process. The character and degree of these changes differed depending on the age of the animals. Conclusion. Experimental intestinal cicatrization is accompanied by changes in CD-20 immunohistochemical expression in the thymus, reflecting age-dependent remodeling of its immune cellular composition. Assessment of CD-20 expression may serve as an informative additional approach for evaluating immunomorphological changes in the thymus during chronic intestinal pathology.

Keywords Thymus, CD-20, Immunohistochemistry, B-lymphocytes, Immunomorphology, Rats, Age-related changes, Experimental study

1. Introduction

Chronic inflammatory and fibrotic-scarring processes in the intestine can affect not only the body's local tissue structures but also the central organs of the immune system. Intestinal fibrosis is typically associated with long-term inflammatory and reparative processes, and its development involves a complex interplay between immune cells, cytokines, fibroblasts, and tissue remodeling [1,2]. Modern research has placed a special focus on the immunological link between the intestine and the thymus. In particular, it has been noted that chronic immune dysregulation in the intestine can affect the structure and function of the thymus [3].

As a central lymphoid organ, the thymus plays a key role in the development and selection of T-lymphocytes and the establishment of immunological tolerance [4]. However, the thymus is not a structure composed solely of T-lymphocytes. Scientific research has shown that a small number of B-lymphocytes are also present in the thymic medulla. They may participate in antigen presentation and the establishment of central immunological tolerance within the thymic microenvironment [5,6].

Thymic B-cells differ morphologically and functionally to some extent from peripheral B-lymphocytes [9]. It has been established that the majority are located in the medullary and perivascular regions of the thymus. Some studies have indicated that the number of B-lymphocytes in a normal thymus is relatively low, whereas in pathological conditions, their number and distribution can change significantly [7,8].

The development of immunohistochemical methods has expanded the ability to identify B-cells in the thymus and assess their distribution patterns. Specifically, CD20 is one of the most widely used markers for identifying B-lymphocytes. Immunohistochemical studies have shown that the localization of CD20-positive cells in the thymic medulla, as well as their quantity and distribution, can vary depending on pathological conditions [9,10].

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The significance of thymic B-lymphocytes in the body is not limited to antibody production. As antigen-presenting cells, they may participate in the central selection of T-lymphocytes and contribute to establishing immunological tolerance to self-antigens. Therefore, assessing the quantity and distribution of CD20-positive cells in the thymus serves as an additional important indicator for studying the morphofunctional state of the immune system [11].

Age-related factors also influence the B-cell population in the thymus. According to scientific data, the involutional processes of the thymus intensify with age, and its cellular composition is remodeled. The accumulation of B-cells in perivascular areas may also have age-related characteristics. Therefore, a comparative study of CD20 expression in animals of different ages allows for the assessment of age-related immunomorphological restructuring of the thymus [12,13].

Changes that occur in the immune system against the backdrop of chronic inflammatory and scarring processes of the intestine are one of the key manifestations of the "gut-immune system" axis. The contemporary view that intestinal immune dysregulation can affect the immunological function of the thymus and alter mechanisms of central immune tolerance underscores the importance of research in this area [14].

At the same time, age-related changes in the expression of CD20-positive cells in the thymus in the context of experimental intestinal scarring have not been sufficiently studied. In particular, an immunohistochemical assessment of the pathological scarring process's impact on the B-lymphocytic component of the thymus is crucial for determining the systemic immunomorphological consequences of this process.

2. Purpose of the Research

To study the characteristics of CD20-immunohistochemical expression in the thymus of white purebred rats during experimental intestinal scarring, as well as to evaluate the age dynamics of the identified changes and their morphological characteristics.

3. Materials and Methods

The study was conducted on 180 outbred white rats aged 4, 7, and 10 months, kept under standard vivarium conditions. The animals were divided into control and experimental groups based on age and the type of intervention.

The first group (control) consisted of 30 intact animals, with 10 rats in each of the 4-, 7-, and 10-month age groups.

The second group (experimental) consisted of 30 animals with an induced intestinal cicatricial process, with 10 rats from each age group.

The third group (experimental) consisted of 60 animals with experimental intestinal scarring that were treated with silymarin extract, with 20 rats in each of the 4-, 7-, and 10-month age groups.

Morphometric, cytomorphological, and immunohistochemical methods were used to assess the state of the thymus and bone marrow. In the thymus, key morphometric parameters were determined, and the expression of immunohistochemical markers CD-20 was evaluated.

4. Results

The findings of the study on the immunohistochemical expression levels in the thymus of 4-, 7-, and 10-month-old rats with an experimental intestinal cicatricial process demonstrate that chronic cicatricial and sclerotic changes in the intestinal tissues affect not only the local morphology of the damaged organ but also the systemic immune homeostasis, particularly the morphofunctional state of the thymus as a central immune organ.

The development of intestinal cicatricial changes is accompanied by a persistent inflammatory response, disturbances in immune regulation, alterations in reparative processes, and functional adaptation of the immune system. These systemic changes are reflected in the altered immunohistochemical expression patterns within the thymic tissue. The obtained results contribute to a better understanding of the morphofunctional relationship between chronic intestinal cicatricial changes and the thymus, the central organ of immunogenesis. The findings provide a theoretical basis for further investigation of the immunopathogenetic mechanisms underlying chronic intestinal disorders, assessment of systemic immune disturbances, and development of promising approaches for their prevention and correction.

Based on the results of immunohistochemical analysis, the characteristics of CD-20 marker expression were studied to assess the state of B-lymphocyte cells in the thymus of 4-month-old white outbred rats under conditions of cicatricial intestinal processes. During this age period, the body's immune system is formed, and response reactions to a pathological factor are primarily carried out through compensatory and adaptive mechanisms (Fig. 1).

Table 1. Quantitative analysis of CD20-positive and CD20-negative cells and CD20 expression in the thymus of 4-month-old outbred rats during experimental intestinal cicatricial processes

Total detected number of cells	271
Positive cells	32
Negative Cells	239
Positive Expression	11.86%
Total area	298959 px^2

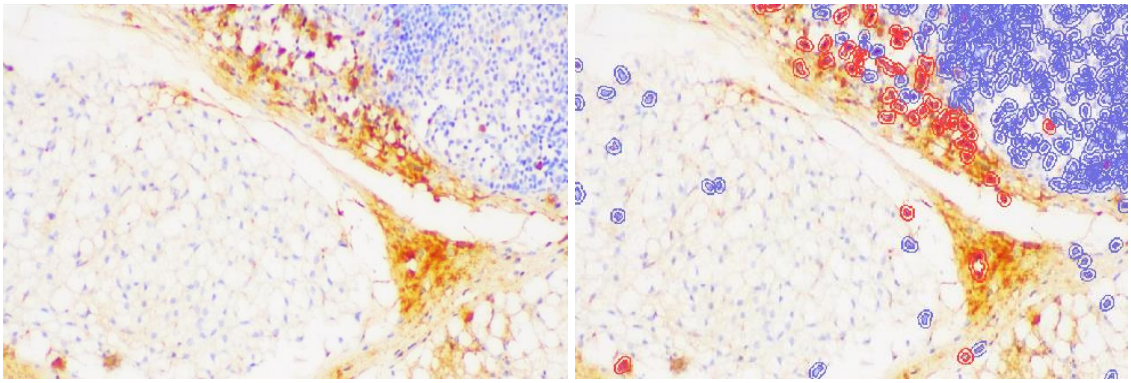

Figure 1. Representative immunohistochemical images of **CD20 expression in the thymus of 4-month-old outbred rats during experimental intestinal cicatricial processes**: original micrograph (left) and computer-assisted identification of CD20-positive and CD20-negative cells (right). Magnification ×200

The CD-20 immune marker is a B-cell membrane-expressed glycoprotein that allows for the determination of the distribution, functional activity, and involvement of B-lymphocytes in the mechanisms of the humoral immune response in lymphoid tissues.

During the study of tissue structures, it was established that in the thymus of the 7-month-old control rat, CD-20-positive cells were found in small numbers, primarily located in the medullary part, in areas close to blood vessels, and among lymphoid elements. Since the primary function of the thymus is related to the maturation of T-lymphocytes, this indicates a limited involvement of B-lymphocytes in this organ.

When assessing immunopositive reactions, CD-20 expression in the thymus of 7-month-old white outbred rats showed characteristics characteristic of a period of relatively stable immune system formation.

Under conditions of experimental intestinal scarring, signs of re-development were observed in the expression of the CD-20 marker in the thymus tissue compared to the control group. The long-term impact on the immune system, caused by chronic pathological processes in the intestine, formed functional changes in the lymphoid environment of the thymus, affecting the location and reactivity of B-lymphocyte cells.

According to microscopic analysis data, certain changes in the distribution of CD-20-labelled cells were identified in the experimental group. The accumulation of immunopositive cells in certain areas or their relative dispersion represents adaptive reactions formed by the body's immune system to chronic inflammatory processes. As a result of comparing the morphological state of the lymphoid microenvironment, it was observed that the localization of the B-lymphocyte component in the thymus of 7-month-old rats has different characteristics compared to the 4-month-old group. In young animals, CD-20-positive cells are expressed in connection with the developmental processes of the immune system, while at 7 months of age, their expression is determined by regulatory mechanisms aimed at ensuring the body's immune homeostasis. Comparative immunohistochemical assessments showed that the main difference between 4-month-old and 7-month-old rats was reflected in the characteristics of CD-20 expression. Changes in the young organism are associated with the processes of formation and improvement of the immune system, while changes in 7-month-old animals are characterized by the predominance of adaptation and compensation mechanisms under conditions of functional maturity (Fig. 2).

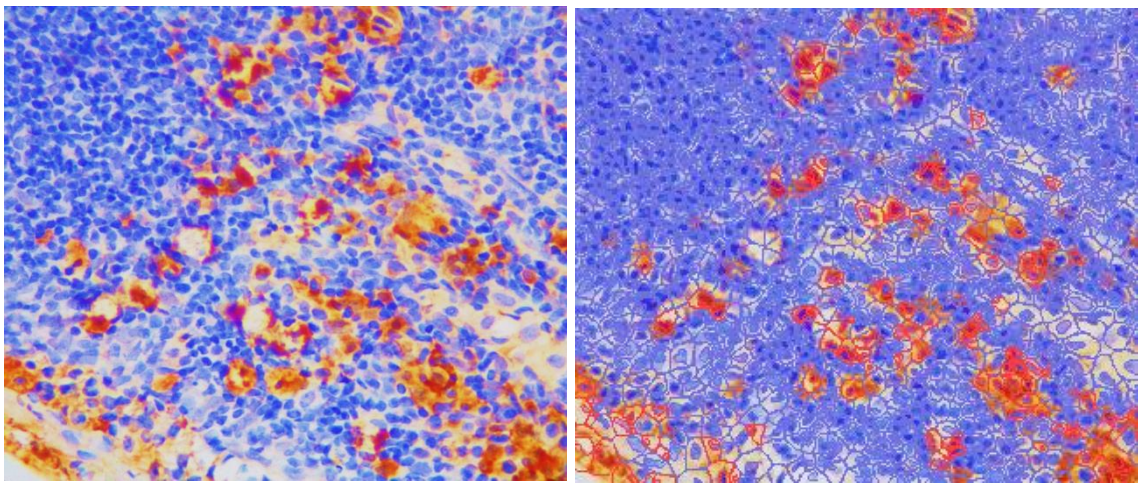

Figure 2. Representative immunohistochemical images of **CD20 expression in the thymus of 7-month-old outbred rats during experimental intestinal cicatricial processes**: original micrograph (left) and computer-assisted identification of CD20-positive and CD20-negative cells (right). Magnification ×200

Table 2. Quantitative analysis of CD20-positive and CD20-negative cells and CD20 expression in the thymus of 7-month-old outbred rats during experimental intestinal cicatricial processes

Total detected number of cells	1177
Positive cells	175
Negative Cells	1002
Positive Expression	14.92%
Total area	999843 px^2

Based on the results of immunohistochemical studies, the features of CD-20 marker expression in the thymus of 10-month-old white outbred rats under the conditions of cicatricial processes in the experimental intestine were analyzed in detail. CD-20 is a membrane antigen characteristic of differentiated forms of B-lymphocytes and is an important immunohistochemical criterion for assessing the functional state of humoral immunity and the degree of distribution of the B-cell population in lymphoid tissues. Although the density of lymphoid components decreases to a certain extent due to the onset of physiological involution processes in the thymus at this age stage, it was observed that CD-20-positive cells persist as a component of the immune control system. Analysis of the experimental model revealed certain rearrangements in the expression of the CD-20 marker in animals with intestinal cicatricial processes compared to the control group. Long-term inflammatory and fibrous processes increased the antigenic load in the body and influenced immunological processes occurring in the lymphoid environment of the thymus involving B-lymphocytes. During the general assessment of immunohomostatic changes, it was established that in 10-month-old rats, CD-20 expression manifests in combination with age-related involuntary processes, reflecting immune rearrangements formed under the influence of chronic intestinal scarring. This indicates a gradual limitation of the functional capabilities of the central immune system and a specific nature of the response to external pathological stimuli.

Based on the generalized results, it can be concluded that the immunohistochemical expression of the CD-20 marker in the thymus of 10-month-old white outbred rats during cicatricial processes of the experimental intestine reliably reflects the morphofunctional changes occurring in the state of humoral immunity. The identified immunohistochemical changes are formed as a result of the interaction of chronic pathological processes and age-related physiological restructuring, further demonstrating the participation of the thymus in the immune regulation system.

Assessment of the functional relationships between lymphoid cells showed that in 10-month-old animals, the involvement of the B-lymphocyte population is directed toward the overall regulation of the immune system. In the 7-month group, due to the relatively stable state of these processes, signs of functional coordination prevailed rather than sharp changes in CD-20 expression.

In 4-month-old rats, due to the high intensity of cellular metabolism, the immune response was dynamic in nature, whereas at 10 months of age, changes in cellular composition occurred relatively slowly, primarily due to functional readaptation.

When assessing the adaptive capabilities of the immune system, the changes identified in 10-month-old animals differed from both the high plasticity observed in the 4-month-old group and the relatively stable immune balance in 7-month-old animals. During this period, it was observed that the central links of humoral immunity are more likely to respond to external pathological stimuli through the functional re-development of existing immune resources rather than through the formation of new cells (Fig. 3).

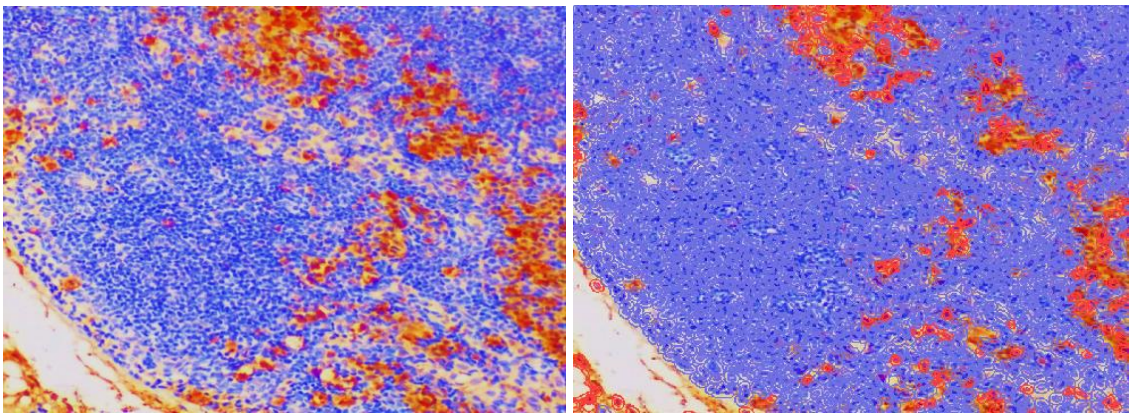

Figure 3. Representative immunohistochemical images of **CD20 expression in the thymus of 10-month-old outbred rats during experimental intestinal cicatricial processes**: original micrograph (left) and computer-assisted identification of CD20-positive and CD20-negative cells (right). Magnification ×200

Table 3. Quantitative analysis of CD20-positive and CD20-negative cells and CD20 expression in the thymus of 10-month-old outbred rats during experimental intestinal cicatricial processes

Total detected number of cells	1622
Positive cells	147
Negative Cells	1475
Positive Expression	9.06%
Total area	361046 px^2

A comparison of the total results of the experimental model showed that CD-20 expression does not change according to the same pattern with age. Conversely, the involvement of the B-lymphocytic component performs a different morphological function at each age stage.

Summarizing the obtained comparative results, it was established that the CD-20 expression observed in the thymus of 10-month-old outbred white rats under conditions of cicatricial intestinal processes is determined not only by increasing age but also by the refinement of the functional capabilities of the immune system.

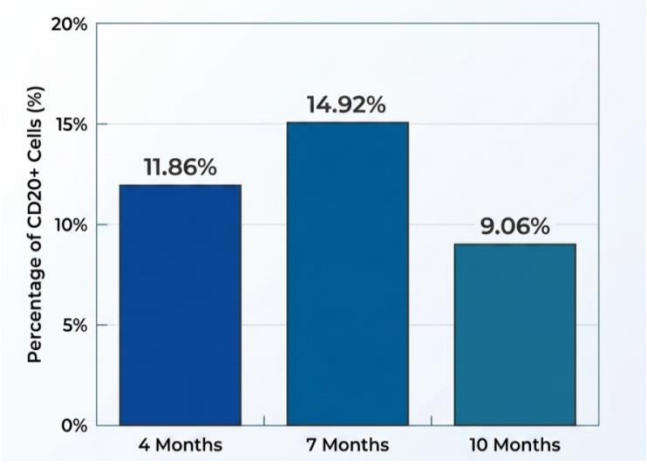

Figure 4. Age-related dynamics of CD20 expression in the thymus Level of CD20-positive cells in the rat thymus with experimental intestinal scarring

5. Discussion

The present study demonstrated age-related differences in the distribution and immunohistochemical expression of CD20-positive cells in the thymus of white outbred rats during experimental intestinal cicatrization. The findings indicate that chronic cicatricial and sclerotic changes in the intestine are associated not only with local structural alterations but also with remodeling of the immune microenvironment of the thymus.

The relatively higher representation of CD20-positive cells in 7-month-old rats may reflect a period of comparatively stable functional activity of the immune system, during which adaptive mechanisms are actively maintained in response to chronic pathological stimulation. The observed distribution of CD20-positive cells suggests that the B-cell component of the thymic microenvironment is not constant and may undergo compensatory and adaptive changes depending on both the duration of pathological exposure and the age-related functional state of the organism.

In 4-month-old animals, the immune response was characterized by greater cellular plasticity. The observed CD20-positive cell distribution may be associated with the developmental and functional maturation of the immune system. In contrast, the changes observed in 7-month-old rats appeared to reflect a more coordinated adaptive response to chronic intestinal injury. This may explain the relatively higher proportion of CD20-positive cells in this age group.

The decrease in CD20 expression observed in 10-month-old animals may indicate a reduction in the adaptive potential of the thymic immune microenvironment. Age-related changes in lymphoid tissues are known to be accompanied by gradual remodeling of cellular composition and reduced functional plasticity. In the context of chronic intestinal cicatrization, these age-dependent changes may limit the ability of the immune system to generate an adequate cellular response and may result in greater reliance on compensatory mechanisms involving previously established immune cell populations.

The morphological distribution of CD20-positive cells also demonstrated that their localization within the thymic tissue was heterogeneous. Areas of accumulation and relative dispersion of immunopositive cells may represent different functional states of the lymphoid microenvironment. Such redistribution can be interpreted as an adaptive response to persistent inflammatory and fibrotic stimulation originating from the intestine.

The findings obtained support the concept that chronic intestinal cicatrization has systemic immunomorphological consequences. Although the primary pathological process is localized in the intestinal tissue, prolonged inflammatory and sclerotic changes may influence central immune organs, including the thymus. The age-dependent differences identified in CD20 expression indicate that the magnitude and character of this response depend on the functional maturity of the immune system.

The results also provide a morphological basis for considering the thymus as an important component of the systemic response to chronic intestinal pathology. Assessment of CD20 expression complements conventional morphological examination and makes it possible to identify changes in the B-cell component of the thymic microenvironment that may not be evident from routine histological evaluation alone.

Overall, the results demonstrate that experimental intestinal cicatrization is accompanied by dynamic remodeling of CD20-positive cells in the thymus, with distinct age-related patterns. The observed changes can be regarded as manifestations of adaptive and compensatory restructuring of the immune system in response to chronic pathological stimulation.

6. Conclusions

Experimental intestinal cicatrization is accompanied by significant age-dependent changes in the immunohistochemical expression and distribution of CD20-positive cells in the thymus of white outbred rats.

The identified redistribution of CD20-positive cells indicates remodeling of the B-cell component of the thymus under conditions of chronic intestinal cicatrization. These changes appear to represent adaptive and compensatory reactions of the central immune system to persistent pathological stimulation.

Thus, immunohistochemical assessment of CD20 expression can be considered an informative additional method for characterizing age-related immunomorphological changes in the thymus during experimental chronic intestinal pathology. The obtained findings emphasize the systemic nature of the immune response to intestinal cicatricial processes and demonstrate the importance of considering the age of the organism when evaluating thymic immunomorphological changes.

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