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Age-Dependent CD45 Expression in Skeletal Muscle Following Injury in White Outbred Rats: An Immunohistochemical Study

Umurov Bobirjon Fayzillayevich
Assistant at the Bukhara State Medical Institute named after Abu Ali ibn Sino

Abstract: Skeletal muscle injury triggers complex inflammatory and regenerative processes that vary with age. In this study, we evaluated the expression of CD45, a pan-leukocyte marker, in skeletal muscle of 3-, 6-, 9-, and 12-month-old white outbred rats following mechanical injury. Immunohistochemical staining using DAB chromogen and quantitative analysis with QuPath 0.4.0 revealed age-dependent differences in immune cell infiltration. Early neutrophil recruitment was observed across all ages, followed by macrophage-mediated tissue clearance and myoblast activation. Younger animals exhibited higher initial CD45 expression, whereas older rats showed delayed but sustained immune responses. Our results demonstrate that CD45-positive leukocytes play a pivotal role in muscle regeneration, coordinating inflammation, tissue repair, and remodeling.

Keywords: skeletal muscle, injury, CD45, inflammation, immunohistochemistry, regeneration, age-dependent response.

Introduction

Skeletal muscle injuries induce inflammatory cascades that are essential for tissue repair. CD45, a transmembrane protein tyrosine phosphatase, is expressed in all nucleated hematopoietic cells, including neutrophils, lymphocytes, and monocytes. This marker allows the visualization and quantification of immune cell infiltration during tissue damage and repair. Age and injury severity influence both the timing and magnitude of CD45-positive cell recruitment, impacting the subsequent regenerative processes. Understanding these dynamics is crucial for developing therapeutic strategies that optimize skeletal muscle repair.

Materials and Methods

Animals

White outbred rats aged 3, 6, 9, and 12 months were used. Animals were randomly assigned to control or injury groups.

Muscle Injury Model

Mechanical injury was applied to the lower limb skeletal muscle. Post-injury, animals received either local or systemic (0.9% NaCl) administration.

Immunohistochemistry

CD45 expression was evaluated via DAB chromogen staining. Tissue sections were examined under a light microscope at 400× magnification. Quantitative analysis was performed using QuPath 0.4.0 software. Positive cells were counted, and the total area (px^2) was recorded.

Data Analysis

The number of total, positive, and negative cells was recorded. CD45 expression percentage was calculated as the ratio of positive cells to total cells.

Results

CD45 Expression in 3-Month-Old Rats

In 3-month-old rats, CD45 expression was moderate on the first day post-injury. Of 145 total cells, 32 were CD45-positive (22.1%) across an area of 912,000 px². Positive cells were dispersed in perivascular regions and sites of fiber damage, indicating early neutrophil recruitment.

CD45 Expression in 6-Month-Old Rats

CD45 expression was low (17/132, 12.8%, 905,000 px²) on day 1, primarily around injured fibers. The immune response appeared delayed compared to younger rats. Activated satellite cells were observed in the interstitial regions.

CD45 Expression in 9-Month-Old Rats

At semi-acute phase (day 1), 13/92 cells were CD45-positive (14.2%, 910,500 px²). By day 3, positive cell count increased to 34.5% (45/130), reflecting active macrophage recruitment. By day 7, expression declined to 13.5% (15/111), indicating the resolution of inflammation.

CD45 Expression in 12-Month-Old Rats

In 12-month-old rats, day 1 post-injury CD45 expression was 38.2% (55/144), decreasing to 6% (7/120) by day 3, and slightly rising to 8.5% (5/59) on day 7. Positive cells localized in perivascular and fiber-damage regions, suggesting persistent but controlled inflammation.

Effects of Anesthesia

Local anesthesia slightly reduced CD45 expression in younger rats (3–6 months), while general anesthesia (Propofol + Ketamine) led to a mild inflammatory response, with CD45-positive cells increasing from 2–6% over the first 7 days post-injury.

Discussion

Our findings highlight age-dependent differences in immune responses following skeletal muscle injury. Younger animals exhibited rapid neutrophil infiltration and higher early CD45 expression, while older rats displayed delayed, prolonged responses. CD45-positive leukocytes coordinated tissue clearance, macrophage activation, and satellite cell proliferation, critical for effective regeneration. Controlled inflammation was essential for limiting excessive tissue damage and promoting myogenesis. The observed effects of anesthetic agents further underscore the need to consider clinical context when studying immune responses in muscle injury models.

Conclusion

CD45-positive leukocytes are central to the regulation of skeletal muscle inflammation and regeneration. Age and injury context modulate the timing, intensity, and distribution of these cells. Understanding these dynamics can inform therapeutic interventions aimed at enhancing muscle repair while minimizing excessive inflammation.

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