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Research paper

# Hierarchical signaling transduction of the immune and muscle cell crosstalk in muscle regeneration

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ABSTRACT

The muscle regeneration is a complicated bioprocess that involved in many cell types, including necrotic muscle cells, satellite cells, mesenchymal cells, pericytes, immune cells, and other cell types present at the injury site. Immune cells involved in both innate and adaptive immune responses regulate the progress of muscle regeneration. In this review, we discussed the roles of different immune cells in muscle regeneration. The immune cells regulate muscle regeneration through cytokine production, cell-cell contacts, and general immune environment regulation. We also describe the current known mechanism of how immune cells regulating muscle regeneration.

## 1. Skeletal muscle and satellite cells

As the biggest organ in human body, skeletal muscle accounts for about half of the body weight, and is essential for posture maintenance, breathing, locomotion and glucose and lipid metabolism. The loss of muscle tissue in aging or other muscle degenerative diseases causes the mobility loss and mortality [1]. The functional unit of the skeletal muscle is the long multi-nucleated cylindrical muscle fibre. Each myofiber contains hundreds of myonuclei by the fusion of many myoblasts [2]. The muscle fibers are post-mitotic cells unable to divide due to the functions of retinoblastoma protein (RB) [3,4]. They are very slow to turn over with no more than 2% of myonuclei replaced per week under the physiological condition [5].

In everyday life of human beings, the skeletal muscles undergo a variety of injuries including small tears, minor lesions, and even trauma. Skeletal muscles retain the ability to regenerate and remodel upon injury [6,7]. Multiple cell types have been shown to have skeletal muscle differentiation potentials [8]. However, satellite cells (muscle stem cells) are the major player for the regeneration of skeletal muscles. They were first discovered by Alexander Mauro in 1961 [9]. They are mononucleated cells localized between the basement membrane and the sarcolemma of muscle fibers [10–15]. These cells usually remain in quiescent stage and are activated upon injury to regenerate the damaged muscles [13,16–22].

The self-renewal and differentiation are tightly regulated by a group of transcription factors. Pax7 is the key transcription factor, which is required for satellite cell specification and maintenance [23]. It is a paired-homeobox transcription factor, and considered as the hall mark of satellite cells [23]. Pax7 plays a dominant role in transcriptional regulation in satellite cells. The Pax7−/− mice are viable but lack functional satellite cells [23]. Pax3 is another member of paired-homeobox transcription factor. It also plays a critical role in satellite cell specification and maintenance [24,25]. In most cases, Pax3 and Pax7 bind identical DNA motifs and activate a large number of genes involved in muscle stem cell functions [26], though they also have distinct targets [27].

Under normal condition, muscle satellite cells are quiescent and located in protected membrane enclosed niche between the sarcolemma (plasma membrane) and the basal lamina surrounding the myofiber. In response to exercise or trauma, satellite cells are activated and undergo several rounds of divisions. It is called the activation of satellite cells which is a tightly regulated process [26]. After satellite cell activation, a group of transcription factors named myogenic regulatory factors (MRFs), including Myogenic factor 5 (Myf5), muscle-specific regulatory factor 4 (Mrf4), myoblast determination protein (MyoD), and myogenin [28,29], regulates the progressions of satellite cells differentiation. Myf5 and MyoD are the downstream targets of Pax7 [30,31]. Myogenin is one of the target genes of MyoD, which cooperates with Mrf4 to regulate fusion of myotubes and myofiber maturation [32]. Although many great works have been performed to elucidate the mechanism of satellite cell mediated muscle regeneration, many aspects, such as the transition between each stage, the mechanism of satellite cell activation, the orchestration of multiple cell type to achieve timely muscle regeneration, require more investigations. Despite the many regulatory

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facets of myogenesis, we will focus on the crosstalk between immune cells and muscle cells and its functions in orchestrating efficient muscle regeneration in this review.

## 2. Immune cells in muscle regeneration

Muscle regeneration process can largely divided into two stages: myolysis and reconstruction [33]. These two stages can be further characterized by the following major events: myofiber necrosis, local inflammation at the injury site, satellite cell activation, migration, and differentiation, new muscle fiber maturation, and new muscle fiber remodeling. Similar to most mammalian tissues, skeletal muscle contains a population of resident immune cells, mainly macrophages and dendritic cells. More types of immune cells, like monocytes, neutrophils, and lymphocytes, infiltrate the injury site or under pathological conditions. The immune system and immune cells play critical regulatory roles during muscle regeneration process.

### 2.1. Innate immune cells

#### 2.1.1. Complement system, mast cells, and neutrophils

The activation of the complement system leads to the production of complement fragments that can play important roles in the inflammatory response. The components C5b-9 of complement system formed the membrane attack complex that can induce a loss in membrane integrity and necrosis of the targeted cells [34]. Complement-mediated cytolysis has been reported to play a significant role in muscle diseases, including dermatomyositis [35] and myasthenia gravis [36]. Meanwhile, in the serum of the prolonged exercise group, cleavage products of C3a and C4a were upregulated [37], revealing the involvement of the complement-mediated inflammation in muscles. The initial phase of muscle repair is characterized by necrosis of damaged fibers and activation of immune responses. Complement system serves as the first sensor of the muscle injury. It is rapidly activated in injured muscle, causing infiltration of immune cells to the lesion site [38]. Whether the complement system directly interacts with muscle cells during muscle regeneration remains to be explored.

The mast cell degranulation is one of the earliest innate immune system responses that leads to the consequent inflammatory events [39]. Thus, the mast cells represent another early-phase immune cell population involved in muscle damage and repair. Upon the stimulation of the muscle injury, the mast cells are activated to rapidly degranulate and release pro-inflammatory cytokines [40]. Resident mast cells in skeletal muscle are the immediate source of pro-inflammatory cytokines, such as TNFα, IL-1, and histamine, allowing the recruitment of immune cells at the injury site [41]. The immune cells, including more mast cells, neutrophils and other leukocytes were further recruited and infiltrated into the damaged muscle tissues within 30 min and produce more pro-inflammatory cytokines, especially TNF-α [42].

Neutrophils can phagocyte necrotic debris and release variety of factors such as cytokines, enzymes, and oxidative factors [43,44]. Similar to the mast cells, neutrophils are also among the first wave of immune cells to infiltrate the muscle lesions, and are involved in the initiation of pro-inflammatory response following muscle injury. In healthy muscle, neutrophils were recruited by mast cells and show a very quick response after injury to clean up cellular debris [45]. The neutrophils at the muscle lesion then release interleukin 1 (IL-1) and interleukin 8 (IL-8) to induce the macrophage infiltration to the injury site [46].

#### 2.1.2. Macrophage

Macrophages are mainly derived from blood monocytes and can be recruited to peripheral sites and further induced to differentiate by pro-inflammatory stimuli [47]. The macrophages constitute very diverse cell populations and have very distinct phenotypes in different tissues [48]. The tissue-resident macrophages play important roles to maintain homeostasis of the tissues in which they reside. For example, peritoneal cavity macrophages control the production of gut immunoglobulin [49]. The brain-resident macrophages named microglia prune synapses during development [50].

The resident macrophages in muscle originate from bone marrow monocytes [51]. The most popular macrophage classification is M1 and M2. M1 macrophages represent classically activated macrophages, while M2 macrophages refer to alternatively activated macrophages [52]. M1 macrophages are pro-inflammatory, which are induced by IFNγ, TNF-α, and GM-CSF in response to environmental stimuli. M1 macrophages secrete more pro-inflammatory cytokines and chemokines attracting other types of immune cells. M2 macrophages are anti-inflammatory. They are induced by IL-10 and TGF-β. M2 macrophages are mainly involved in tissue repair and remodeling [53].

The monocyte/macrophages have been implicated as key players in skeletal muscle regeneration [51]. Depletion of macrophages leads to impaired muscle regeneration [54,55]. After the first wave of the immune cell infiltration that contains mast cells and neutrophils, the monocytes were subsequently recruited from the peripheral blood to the lesion site. The monocytes were then differentiated into macrophages at the lesion and replace neutrophils to become the major population of immune cells at the injury site. M1 macrophages play several roles during muscle regeneration. They initially function in the removal of the muscle debris at the injury site. Meanwhile, they can attract muscle stem cells to the injured site and stimulate the proliferation of the muscle stem cells [56]. M1 macrophages produce large amount of cytokines such as TNF-α and IL-1β. In addition, they also express inducible nitric oxide synthase (iNOS), which is required for normal muscle repair after injury [57].

Alternatively activated M2 macrophages down-regulate the inflammatory reactions by releasing large amount of anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 to avoid excess tissue damage [55,57,58]. The anti-inflammatory cytokines such as IL-4, IL-10, and IL-13 promote the dynamic transition from M1 macrophages to M2 macrophages [59]. Mice carrying specific M2 macrophage inhibition display muscle regeneration defects [60], suggesting that M2 macrophages are required for timely muscle regeneration. Depletion of M1 macrophages and other circulating pro-inflammatory monocytes at the beginning of the muscle injury completely blocks muscle regeneration [54], suggest that macrophages are indispensible for muscle regeneration. While depletion of M2 macrophages at the late stage of muscle regeneration leads to smaller fiber size after muscle regeneration [54], suggesting the function of M2 macrophage in the differentiation of myoblasts and maturation of nascent myofibers.

### 2.2. Adaptive immune cells

#### 2.2.1. T cells

Besides the innate immune cells, the adaptive cells including T cells are also involved in the process of muscle regeneration. T cells play a central role in the cell-mediated immunity. T cells populating the peripheral bold or secondary lymphoid organs were distinguished with the exclusive marker CD4 (CD4+ T cell, T helper) or CD8 (CD8+ T cell, cytotoxic T). During muscle damage, CD8+ T cells were recruited by the macrophages and infiltrated into the injury site [61]. The sustained T-cell presence throughout the regenerative process suggests the involvement of T-cells in skeletal muscle repair [62].

Muscle regeneration was impaired in T cell deficient mice [63]. Transplantation of T cells to the T cell deficient mice can fully rescue the muscle regeneration defects [63]. Further analysis indicates that transplantation of either CD4+ or CD8+ T cells can improve the muscle regeneration in T cell deficient mice [63]. Consistent with these results, CD8+ T cell deficient mice also display defects in muscle regeneration [61]. Transplantation of CD8+ T cell improves the muscle regeneration and reduce the matrix deposit in CD8+ deficient mice [61]. Meanwhile, the infiltration of CD8+ T cells was essential for the