



The role of inflammatory factors in skeletal muscle injury

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Abstract: Injured skeletal muscle produces a series of inflammatory cytokines that activate the immune system. The pro-inflammatory factors in the lesions cause muscle loss most by activating the ubiquitin-proteasome degradation pathway and enhancing autophagy-lysosome degradation pathway. Also it can affect the repair and remodeling of muscle tissue by inhibiting the proliferation and differentiation of myogenic cells. However, the inflammation after injury is not all harmful. Destruction of tissue by phagocytic cells infiltrated by early inflammation will change the microenvironment. That is beneficial for muscle regeneration. The hallmark event of the muscle regeneration is that the macrophage transforms their phenotype. In that phase, immune cells produce cytokines with anti-inflammatory properties. These cytokines could promote muscle regeneration by down-regulating the expression of pro-inflammatory factors and affecting stem cell proliferation and differentiation.

Keywords: Skeletal muscle injury; muscle atrophy; muscle regeneration; inflammatory factor

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Introduction

After skeletal muscle injury, it undergoes successive stages of degeneration, inflammation, regeneration, and fibrosis. The mainstream view is that apoptosis and regeneration occur simultaneously in muscle tissue and form a dynamic equilibrium situation. When the tissues are stimulated by certain stimuli, such as drugs, physical injuries, autoimmune disorders, and so on, the balance will be broken, resulting in muscle atrophy or hypertrophy (1). Acute injury of skeletal muscle will cause fiber breakage and tearing of blood vessels. Inflammatory factors can enter the damaged tissue arbitrarily and trigger the early inflammatory response. Subsequently, the immune cells awaken and release a variety of inflammatory cytokines and chemokines. That will lead exacerbation of protein hydrolysis and apoptosis in the tissue. Cause to loss of myofibrillar protein, and decreased fiber cross-sectional area, finally, resulting in muscle

atrophy. In the later stage of inflammation, the reaction gradually inclines to the direction of anti-inflammatory, such as macrophage conversion phenotype, down-regulation of pro-inflammatory cytokine expression, etc. The microenvironment changes in the tissue to promote the proliferation and differentiation of the satellite cells, which is beneficial to the repair and remodeling of the muscles. Studies have shown that inflammatory factors have effects on protein degradation and synthesis rate in muscle tissue, autophagy pathway, proliferation and differentiation of stem cells. The purpose of this article is to describe the activities and effects of inflammatory factors in various stages of atrophy and regeneration after muscle injury.

Response of the immune system to muscle injury

Acute skeletal muscle injury can lead to fiber rupture,

oxidative stress and inflammation. Once the muscle injury is activated, myogenic cells secrete chemokines of circulating monocytes and recruit innate immune system cells (polymorphonuclear leukocytes and monocytes/macrophages). Neutrophils being the first infiltrate, and is considered by the superoxide dependent mechanism of direct cracking of muscle the cell membrane increased the initial damage (2,3). Within a few hours after injury, the infiltrating neutrophils released inflammatory cytokines, such as Tumor necrosis factor α (TNF- α), interleukin (IL)-1 β , interferon (IFN)- γ , transforming growth factor (TGF)- β 1, and IL-12, and reached the peak of concentration at 24 hours after injury, and could regulate the regeneration process of skeletal muscle (1).

The content of macrophages increased within 1–3 days after injury (2). Histological analysis showed that after muscle injury, resident macrophages secreted monocyte chemoattractant protein 1 (MCP-1), triggering further invasion of macrophages and circulating mononuclear cells adjacent tissues in skeletal muscle (4). Cytotoxicity tests showed that macrophages dissolve muscle cells through nitric oxide (NO) dependent and superoxide dependent mechanisms, and their neutrophil presence increases their cytolytic ability (3). Macrophages have different phenotypes at different stages of tissue damage, such as type I macrophages (M1) and type II macrophages (M2) (5,6). M1 induces the production of pro-inflammatory cytokines and reactive oxygen species, which is associated with muscle necrosis and reflects the main state of macrophage activation during tissue injury or immune conflict. M2 is an anti-inflammatory macrophage and is associated with the regeneration of muscle fibers (7). *In vitro* experiments have demonstrated that the phenotypic conversion occurs after the phagocytosis of the necrotic myogenic cell (4). Studies have shown that the injured muscles are also infiltrated by specific regulatory T cells (Treg), and Treg has an important effect on the phenotypic conversion of macrophages (8).

The role of inflammatory factors in muscle atrophy

Muscle atrophy is usually caused by the selective degradation of the regulatory proteins and structural proteins in the tissues, and the obstruction of the proliferation and differentiation of the stem cells. After skeletal muscle injury, cells release various pro-inflammatory factors, trigger inflammatory reactions, break the balance of muscle homeostasis, and increase the activity of proteolytic

system, resulting in decreased muscle mass.

TNF- α also known as cachectin, is the prototype of TNF ligand superfamily, produced by a variety of immune cells, plays a central role in inflammation, apoptosis and immune system development, including leukocyte activation and chemotaxis, adhesion molecule expression and regulation of other pro-inflammatory cytokine secretion (9). Studies have shown that the expression of TNF- α activity is associated with insufficient muscle production, mainly by acting on NF- κ B and MyoD to cause muscle loss. TNF- α activates the transcription factor NF- κ B (nuclear factor kappa-light-chain enhancer of activated B cells) leading to the degradation of specific muscle proteins (3,9,10), NF- κ B activates the E3 ubiquitin ligase, which degrade most of the protein in the skeletal muscle. NF- κ B can also regulate the expression of many pro-inflammatory factors, such as TNF- α , IL-1, IL-6 and so on, and these inflammatory factors stimulate the activity of NF- κ B and establish a positive feedback loop (11), causing continuous atrophy of muscle tissue. By affecting the differentiation of the stem cells in the muscles, the regeneration of the muscle fibers is blocked after the injury and atrophy. *In vitro* cell culture experiments, C2C12 myotubes that differentiated for 3 days treatment with TNF- α in 24 hours, exhibited obvious atrophy. In skeletal muscle, TNF- α can accelerate the transformation from satellite cell G1 phase to S phase and influence its proliferation (12). TNF-p38 signal transduction can promote the proliferation of satellite cells but inhibit its differentiation, thus affecting the growth of muscle tissue (13).

The pro-inflammatory cytokine, TWEAK induces muscle atrophy mainly by stimulating proteasome-dependent proteolysis. Cell culture studies show that TWEAK treatment increases the expression of muscle specific E3 ubiquitin ligase MuRF1 and MAFbx (12,14), and stimulates the binding of ubiquitin to MyHC in C2C12 myotubes, that through the activation of UPS (ubiquitin-proteasome system) led to the degradation of MyHC (15,16). TWEAK can also induce the expression of autophagy lysosomal system components, and the activation of caspase-3, especially in cultured myotubes, which may also promote myofibrillar protein hydrolysis. TWEAK could inhibit the PI3K/Akt pathway that may be the reason of its strong effect on the decomposition of myotubes (15). PI3K/Akt pathway can promote protein synthesis to increase muscle mass. In addition to activating proteolysis, TWEAK also up-regulated the expression of matrix metalloproteinases (MMPs), especially MMP-9, and

MMP-9 may be one of the reasons for the degradation and fibrosis of extracellular matrix in atrophic skeletal muscle (15).

IL-1 is a peptide substance, including two active proteins, IL-1 α and IL-1 β , which are secreted by monocytes, participate in mobilization of neutrophils, trace metal elements redistribution and increase proteolysis of skeletal muscle at the early stage of inflammation (17). Cell culture experiments showed that IL-1 β induced apoptosis by increasing MMP-13 expression, which reduced cell density, but had no significant effect on cell proliferation and fusion. It is indicated that IL-1 system is involved in the regulation of cell density during normal muscle tissue growth.

IL-8 produced by monocytes, macrophages, endothelial cells, fibroblasts and skeletal muscle cells, which belongs to the CXC chemokine family, has a strong chemotactic effect, can promote cell proliferation and induce infiltration of inflammatory cells. The biological effects are chemotaxis and activation of neutrophils, promote the lysosomal enzyme activity of neutrophils and phagocytosis (18-20). In the study of cancer cells, IL-8 has the role of promoting mitosis and inducing the migration of endothelial cells. Lipopolysaccharide (LPS) also called endotoxin, can enhanced atrogin-1/MAFbx gene promoter activity, and up-regulate the expression of MCP-1 and IL-6 in myotubes by induce JNK phosphorylation (21), which Increases the chemotactic effect of macrophages and promotes inflammatory response. The addition of LPS resulted in a decrease in the diameter of myotubes cultured mouse myotubes in C2C12 (22).

The role of inflammatory factors in the process of regeneration

In animal models, cellular inflammatory reactions seem to have a beneficial regenerative effect. For example, infiltration of neutrophils and macrophages into muscle tissue at early stage of injury leads to membrane lysis, and later macrophages transform phenotype and promote myosin membrane repair. These closely related reactions mean that the extent of the tissue repair may depend on the destructive stage of the inflammation (2).

Immune cells infiltrate into muscle injury sites, release a series of cytokines and growth factors, and regulate muscle repair and regeneration through direct interaction with satellite cells. Within a few hours after injury, infiltrating neutrophils and macrophages cleaned up the necrotic cell debris and released inflammatory factors, such as TNF- α , to prevent premature differentiation of myogenic cells.

The second stage of muscle repair is characterized by the transformation of macrophage M1 into an anti-inflammatory phenotype M2 (4,5,7,8), which Inhibits of environmental inflammatory signals and the release of growth promoting factors such as IGF-1 to directly support myogenesis and muscle fiber growth. IGF-1 prolongs the regenerative potential of skeletal muscle by acting as a survival signal of the myogenic precursor cells (7), and also reduce proteolysis and apoptosis (9). IGF-1 also inhibits the expression and activity of macrophage migration inhibitory factor (MIF), high mobility group box 1 (HMGB1) and transcription factor NF- κ B, and directly regulates the process of inflammation. In vitro experiments of human myogenic cells and macrophages confirmed the effect of macrophages on the behavior of myocytes. The results showed that macrophages stimulated the growth of myogenic cells in a dose-dependent manner. The two cells are more effective when they are allowed to contact each other. This stimulatory effect is caused by the following reasons: (I) release of mitogenic factors of myogenic cells. These cells may be known as growth factors secreted by macrophages and effective response of myocytes; (II) to establish cell-cell contact to protect myogenic cells from apoptosis. In the rat injury model, anti-inflammatory drugs were applied to inhibit macrophages and neutrophil activity, showing muscle regeneration slowly, tendon weakness, and tissue removal slowly (affecting repair) (4).

IL-4 is produced most by eosinophils, and can also be produced by NK cells, activated T cells, mast cells and basophils. The expression of MHC II molecules can be induced and the expression of pro-inflammatory cytokines TNF- α and IL-1 can be downregulated. In myocytes, IL-4 induced by NFATc2 may promote myoblast fusion by increasing the cell adhesion. It is also possible to promote muscle regeneration by controlling the function of FAPs (fibroblast and adipocyte progenitors). IL-4 stimulation guides FAPs as fibroblast proliferation and supports myogenesis by scavenging necrotic debris (23). FAPs are a pluripotent stem cell that can selectively differentiate into fibroblasts and adipocytes. IL-4 can inhibit the expression of many genes during adipogenesis, inhibit its differentiation into adipocytes, and differentiate into fibroblasts as much as possible, which is conducive to muscle tissue regeneration. In the absence of IL-4 or its receptor, FAPs differentiation into adipocytes, leading to skeletal muscle fatty degeneration, and also to muscle cell formation of damaged myotubes (1,12).

IL-10 is famous for its function in inhibiting pro-

inflammatory cytokines, such as IFN- γ , TNF- α and IL-6. Its inhibitory effect is considered to be protective, as IL-10 saves the blockade of myocyte by IGF-1 induced by TNF- α . In the injured muscles, IL-10 is considered to be the key to guiding the switching between M1 and M2 macrophages (12).

IL-15 is secreted by many types of cells, including macrophages, neutrophils and skeletal muscle cells (24). It is an important anabolic factor. It can also increase the production of other pro-inflammatory cytokines such as TNE, IFN and IL-17 in T cells (9). In the studies of human skeletal muscle derived cultures, addition the recombinant IL-15 induces a hypertrophic phenotype in myotubes (immature muscle fiber). In this study, IL-15 does not stimulate the proliferation or differentiation of muscle precursor cells (myoblasts), but as hypertrophic factor in cultured myotubes, its main function is to stimulate muscle cell activity (25). Another mechanism of the anabolic effect of IL-15 on skeletal muscles is to reduce the rate of proteolysis. Both in cell culture and animal experiments show that it has the effect of promoting protein synthesis (9,25,26). Up-regulated the expression of IL-15 in transgenic mice, in the absence of effects on myoblasts, the results shown protein synthesis rate increased and the degradation rate decreased in myotubes (25).

Double effects of inflammatory factors

Many inflammatory cytokines have double effects on muscle tissue, which are usually related to their concentration, changes in microenvironmental conditions and the way of drug delivery.

IL-6 can reduce the level of growth hormone and IGF-1 in muscle tissue, and has a negative effect on muscle growth. Its overexpression is accompanied by an increase in the expression of ubiquitin and cathepsin, resulting in skeletal muscle atrophy in mice (9). In animal experiments, muscle protein hydrolysis increased in rats and mice exposed to IL-6 at high doses or for a long time. Reduced muscle growth was also observed in several transgenic mice that overexpressed human IL-6. On the other hand, IL-6 has a strong anti-inflammatory effect by stimulating the production of anti-inflammatory cytokines such as IL-1 and IL-10, and inhibiting the formation of TNF- α in the human body (25). IL-6 in skeletal muscle promotes the proliferation of mouse satellite cells through regulating cell cycle related genes cyclin D1 and c-myc, and also plays a key role in the proliferation, differentiation and migration of myoblasts. There are experiments shown that

the elimination of IL-6 expression by specific siRNA can reduce the degree of differentiation and fusion of myoblast cells. In cell culture experiments, the myoblasts from IL-6 deficient mouse showed decreased differentiation and fusion capacity (27).

The expression of IFN- γ is up-regulated at the early stage of muscle regeneration, and its expression level is down-regulated as the myoblasts turn from proliferation to differentiation. When IFN- γ is combined with its receptor, it activates the JAK1-STAT1 pathway and plays an important role in myogenesis. *In vitro* experiments confirmed that high doses of IFN- γ could inhibit muscle differentiation. The mechanism of IFN- γ induces the expression of CIITA that is a trans-activating factor of MHC II, which acts as a function of CIITA by directly binding and inhibiting the myo-cytokine. Myo-cytokinin cannot function as a result of muscle specific gene expression and the reduction of transcription factors that drive terminal differentiation (12).

The TGF- β superfamily is essential for regulating normal physiological functions. The expression of TGF- β will be upregulated in skeletal muscle after injury or exercise, which regulates the proliferation of satellite cells, and inhibits cell apoptosis by increasing adhesion to extracellular matrix, thereby enhances the viability of myofibroblast (12,28). Further studies have shown that TGF- β blocks the transcriptional activity of myogenin and inhibits muscle differentiation. High dose of TGF- β stimulates collagen deposition, leading to the formation of fibrotic scar tissue (28).

IL-17 can stimulate pro-inflammatory cytokines TNF- α , IL-6, IL-1 β chemokine CXC motif ligand 1 (CXCL1), CC-motif ligand 2 (CCL2), CCL7, CCL20, MMP3 and MMP9 generation, important to protect the host from invasive pathogens. But IL-17 disorders that produce excessive cytokine production and chronic inflammation, resulting in tissue damage and inflammatory myopathy, such as Duchenne muscular dystrophy (12).

Prostaglandins and leukotrienes play an important role in skeletal myositis, including recruitment of inflammatory cells, necrosis of muscle fibers, and so on. On the other hand, some prostaglandins and leukotrienes are essential for promoting muscle growth and differentiation, and thus play an active role in muscle repair (29).

Conclusions

The regeneration process of skeletal muscle after injury

depends on the balance between pro-inflammatory and anti-inflammatory factors. The regeneration process can be divided into two stages according to the phenotype of macrophages. Each stage is related to different types of inflammatory cells, involving many autocrine and paracrine processes. The atrophy stage is mainly dominated by pro-inflammatory factors, and the inflammatory response is aggravated. Subsequently, the change of microenvironment in the tissue promotes macrophages to transform phenotype into regeneration stage. At this time, the balance gradually inclines to the anti-inflammatory side and restores the muscle tissue. If the tissue is severely damaged (such as the central nervous system is broken), the anti-inflammatory factors are not enough to compete with the pro-inflammatory factor, which leads to muscle necrosis. Inflammatory response at early stage of injury, such as macrophage and neutrophil phagocytosis, has a function of clearing debris, which is beneficial for subsequent repair of muscle tissue. Blocking early inflammatory signals may hinder muscle regeneration. Many inflammatory molecules play a completely opposite role in skeletal muscle under different dose or modes, which may provide some new ideas for the treatment of muscular atrophy.

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Footnote

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