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Immune-mediated Mechanisms and Immunotherapy of Duchenne Muscular Dystrophy

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Abstract

The immune response is a critical mechanism for dystrophic muscle pathology and muscle wasting in Duchenne Muscular Dystrophy (DMD). The inflammatory processes are supposed to associate with activation of the innate immune response like macrophages infiltration, T cell-mediated immune response, generation of cytokines and chemokines and the aberrant activation of NF-κB signaling pathway, which contribute to severe muscle necrosis and fibrosis. Immunotherapy has been developed to manage DMD, and glucocorticoids are used to treat DMD patients due to their anti-inflammatory functions. Furthermore, immune targeting therapies and Mesenchymal stem cells (MSCs) transplantation could be promising therapeutic approaches in the future.

Keywords

Duchenne muscular dystrophy, Inflammation, Macrophages, NF-κB signalling pathway, Immunotherapy

Introduction

Duchenne muscular dystrophy (DMD) is a lethal, X-linked genetic degenerative myopathy, affecting 1 in every 3500 live male births [1]. It is the most prevalent form of muscular dystrophies that is characterized by difficulty in rising from the floor, abnormal gait, calf hypertrophy and evaluation of serum creatine kinase (SCK). DMD patients usually present clinical signs between 2 and 5 years of age. The rapidly progression of muscle wasting and weakness can result in loss of ambulation and wheelchair dependence at a mean age of 12 years, and lead to death in the second decade due to the cardiac and respiratory complications [2]. The deficiency of functional dystrophin protein caused by DMD gene mutation is responsible for the muscle damage in DMD [3]. Dystrophin is a critical protein component of the dystrophin-glycoprotein complex (DGC), which locates at the cytoplasmic side of the sarcolemma and forms a link between the cytoskeleton and the extracellular matrix (ECM) [4]. The integrity of DGC is thought to impart structural stability to the membranes of myofibers and preserve important signaling roles. It is believed that the absence of dystrophin as well as the damage of DGC in myofibers contributes to contraction-induced membrane injury and necrosis of muscular cells. However, neither mechanical damage nor sarcolemmal defects could fully interpret the onset and progression of DMD [5]. More intriguingly, the disruption of DGC results in aberrant activation of multiple components of the innate immune system and a number of inflammatory signaling cascades during the early stages of disease, leading to aggravated myofiber degeneration and progressive muscle weakness [6]. This indicates that inflammatory and immune processes play a vital role in muscle pathology and disease progression of DMD. The review will focus on immune-mediated pathogenesis in DMD and highlight the potential therapeutic approaches related to the immune-regulation.

The Immune-Mediated Mechanisms in DMD

Acute injury in normal muscle is associated with transient inflammation, which is part of necessary process for tissue repair. Nevertheless, in dystrophic muscles, the inflammatory responses caused by myofiber membrane instability are persistent from the initial period to later stage of disease, which involve activated immune cell infiltrates, up-regulated inflammatory gene expression and aberrant activation of inflammatory signal pathways like Nuclear Factor-kappa B (NF-κB) pathway [7-9]. The chronic inflammatory state contributes to the successive courses of myofiber degeneration and regeneration.

The innate immune response

The early immune cell infiltration precedes the initial onset of disease and is considered to represent a significant aspect of dystrophic muscle pathology. The infiltration of macrophages is detected in 2-year-old DMD patients. Also, in DMD mouse model--mdx mice, the concentration of macrophages is increased in dystrophic muscles as early as 2 weeks of age and peak between 4 and 8 weeks of age [10]. A previous study has demonstrated that the elimination of macrophages by monoclonal antibody leads to dramatic reduction of muscle lesions in mdx mice, which is due to decreasing the production of nitric oxide from macrophages [11]. Macrophages have a variety of important immunoregulatory functions. Recently, there are two identified subpopulations of macrophages that may have influence on muscle degeneration and regeneration depending on the proportion of these cells present. M1 macrophages are proinflammatory and promote the myofiber lysis via producing nitric oxide and proinflammatory cytokines [12]. On the contrary, the M2 macrophages are characterized by anti-inflammatory and modulatory functions, which are able to secrete anti-inflammatory cytokines and enhance muscle regeneration by inducing satellite cell proliferation [13]. During acute muscle injury, predominant M1 macrophages infiltrate early to operate phagocytosis and remove necrotic debris, and then convert to M2 phenotype later to reduce the inflammation and sustain tissue healing and fiber growth [14,15]. In chronic

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muscle damage, due to progressive disruption of muscle cells, both M1 and M2 macrophages invasion persist from onset to later stage, and the increasing number of M2 macrophages with age contribute to excessive connective tissue deposition and muscle fibrosis [16,17]. However, this finding has not been demonstrated in dystrophic muscles of DMD patient. Moreover, proinflammatory cytokines like Tumor necrosis factor-α (TNF-α) and Interferon-γ (IFN-γ) ultimately promote the activation of M1 macrophages and suppress M2 macrophages activation, resulting in persistent inflammatory responses and dystrophic muscle damage [18]. However, increased levels of anti-inflammatory cytokines like Interleukin-10 (IL-10) deactivate these proinflammatory M1 macrophages and promote transition from M1 to M2 phenotype, resulting in tissue repair [16,19]. Thus, increased, persistent presence of macrophages and the interaction between macrophages and the factors they release contribute to increased myofibers necrosis and the replacement of muscle with fibrotic and fat tissue [20].

The T cell-mediated immune response

Normal skeletal muscle cells have very little or no detectable MHC I proteins on their surface. However, the MHC I proteins and specific antigens are detectable on the surface of dystrophic myofibers and could be recognized by T cells [10]. In a previous study, increased concentrations of activated CD8+ and CD4+ T cells have been observed in dystrophic muscles of mdx mice, whereas no elevation of these T cells are present in axillary or inguinal lymph nodes, indicating that their T cells activation is occurring in muscle tissue, which may target to specific antigens on dystrophic muscle [21]. In mdx mice, CD8+ T cells are the first to invade dystrophic muscle. The mechanisms through which CD8+T cells cause muscle damage involve perforin-induced cytotoxitity and non-perforin-induced process. They can also recruit inflammatory cells such as eosinophils to promote muscle injury [22]. The prominence of CD8+ T cell–induced pathology in DMD is further supported by the effect of steroid treatment in DMD patients. The dramatic reduction of CD8+ T cells has been detected in DMD patients treated with steroids, correlating with the improvement of muscle strength [23]. On the other hand, CD4+ T cells could generally differentiate into effector T cells, mainly Th1 and Th2, both of which participate in immune responses. Th1 cells are known to support M1 macrophages by producing proinflammatory cytokines like IL-1, TNF-α and INF-γ, contributing to the process of innate inflammatory response [7]. On the contrary, Th2 cells could sustain M2 macrophages by producing anti-inflammatory cytokines like IL-4, IL-13, and IL-6 [7]. But the interaction between Th1 cells and M2 macrophages has not been discussed. Utilizing antibody to eliminate CD8+ T cells or CD4+ T cells in mdx mice respectively contributes to a 75% and 61% reduction in muscle histopathology, which suggesting that the depletions of T cells can alleviate inflammatory responses and muscle lesions [21]. In a most recent study, 75 DMD patients are studied at different stages of disease, and it is observed that the percentage of circulating CD49d (+) T cells are correlated with the severity and progression of DMD. CD49d (+) T cells infiltrations are also detected in dystrophic muscles. Therefore, CD49d antibodies can serve as a potential therapeutic approach to reduce inflammatory response and tissue damage in DMD [24].

The infiltrating CD4+ T cells can differentiate into Regulatory T cells (Tregs), which are critical regulatory cells during the immune response in dystrophic muscles. Tregs are known to highly express CD25 and Foxp3 transcription factor. In general, several factors like specific T cell receptor (TCR) signals and cytokines such as IL-2, IL-7 and IL-15 have contributed to the differentiation of Tregs. The TCR signals of increased strength and IL-2 facilitate the induction of Foxp3, therefore help the CD4+T cells differentiate into thymic and peripheral Tregs. In a recent study, the depletion of Tregs with anti-CD25 monoclonal antibody in mdx mice would aggravate histopathological damage and up-regulate genes encoding osteopontin and connective tissue growth factor, both of which are associated with fibrosis [25]. Tregs can also produce anti-inflammatory cytokines such as IL-10 [26]. The increased number of Tregs can alleviate inflammation and dystrophic muscle injury and cause a remarkable decrease in creatine kinase, which probably due to the elevated secretion of IL-10 by Tregs [27]. These findings indicate that Tregs likely inhibit the inflammatory response and fibrotic processes in dystrophic muscles. Rapamycin is known to induce the production of Tregs. This drug is proved to conserve Tregs in dystrophic muscles of mdx mice and decrease the number of CD4+ and CD8+ T cells [28]. In a recent study, it has been demonstrated that rapamycin had a positive influence on autophagy, which is defective in DMD muscles, and increase muscle strength in mdx mice [29]. Thus, Rapamycin could serve as candidate drug for DMD in the future.

Cytokines and Chemokines

Numerous cytokines and chemokines have been reported to associate with DMD pathology and disease progression, as determined by studies both in DMD patients and mdx mice. The increasing expression of TNF-α is detected in the serum of DMD patients. Consistently, elevated TNF-α and IL-1β expression are observed in mdx mice age 16 and 60 days [10]. Even though there are several evidences for the important role of these factors in the dystrophic muscle pathology, the mechanisms are not definitely illustrated.

TNF-α is an important proinflammatory mediator in many immune processes [30]. In the serum of DMD patients, the average concentration of TNF-α is about 1000 times higher than that in healthy subjects by sensitive immuno-PCR assay [31]. A recent research indicated that the level of TNF-α upregulated in diaphragm of mdx mice at early stage (1 and 4 months of age), and a positive correlation between increased TNF-α level and histopathology damage was observed [32]. The primary source of TNF-α in DMD muscle is the inflammatory cells, particularly the macrophages in response to antigens. However, the dystrophic muscle cells in later stages could also produce this cytokines, making the muscle pathology much more severe in DMD [8]. Recently, studies in mdx mice reveal that TNF-α appears to have a dichotomous role in muscle physiology. On one hand, TNF-α is known to accelerate the muscle damage and wasting through inducing the production of nitric oxide which will activate the NF-κB inflammatory pathway [33]. On the other hand, TNF-α has a prominent role in promoting muscle maintenance and repair. A study has shown that the mRNA and protein level of TNF-α is much higher in regenerating muscle fibers, suggesting that TNF-α is firmly associated with muscle regeneration [34]. The mdx mice with genetic deletion of TNF-α show evidence of accelerated pathological progression in diaphragm muscles, whereas mdx mice treated with infliximab (a TNF-α inhibitor) show a delayed appearance and improvement of muscle damage. The probable reason for the different results between the two conditions exists that the treatment of infliximab does not eliminate total TNF-α expression, and the remaining low level of TNF-α could contribute to muscle regeneration process [35].

IL-1β is believed to enhance the inflammation, and consistently dystrophin-deficient muscle cells have been shown to produce a large amount of IL-1β [36]. IL-6 reveals possible anti-inflammatory function during immune response in DMD. It is demonstrated by evidence that the blockade of IL-6 with monoclonal antibody increase inflammation in mdx mice [37]. However, in a very recent study, Pelosi et al. provides evidence that increased levels of IL-6 exacerbate the dystrophic muscle phenotype in mdx mice. In a DMD dog model, the expressions of inflammatory cytokine genes (like IL-6, IL-8) are upregulated in dystrophic dog neonates, especially in the damaged dystrophic diaphragm [38]. Thus, the role of IL-6 in DMD progression has yet to be elucidated [39].

Transforming growth factor-β (TGF-β) plays important roles in inflammation, cell growth, and tissue repair. It is also a crucial cytokine that contribute to fibrotic process and accumulation of extracellular matrix [8]. The elevated expressions of TGF-β in DMD patients and mdx mice are consistent reported in many studies [40]. Interestingly,

the age-related increases of TGF-β are shown in DMD muscles and associate with increased fibrotic replacement of dystrophic tissue. Interestingly, TGF-β also acts as a significant suppressor of the immune response in dystrophic muscles, as determinate by evidence that Antibody-mediated depletions of TGF-β results in a dramatic increase in CD4+ T cells concentration in mdx diaphragm muscles [41]. Thus, the elevated expression of TGF-β may serve as an inhibitory attempting to suppress the inflammatory response in dystrophic muscle of DMD, but ultimately contribute to muscle fibrosis. TGF-β is produced as an inactive protein that requires additional processing to transfer it into active form. The recent studies have demonstrated that the identification of two genetic modifier loci, SPP1 and LTBP4, both known to alter TGF-β -mediated pathways and associate with DMD disease severity [42,43]. In fact, data about genetic modifiers indicate that TGF-β pathways may be more important than any other downstream pathway in exacerbating the progressive muscle damage, fibrosis and wasting observed in DMD.

NF-κB signaling pathway

The transcription factor NF-κB is involved in regulating numerous processes including the expression of many inflammatory genes, acute stress response, cellular proliferation and differentiation [44]. The NF-κB family contains five members: RelA (also known as p65), RelB, c-Rel, p105/p50, and p100/p52, among which p50 and p52 are precursors to the mature proteins. The mature proteins RelA, c-Rel, and RelB have nuclear localization signals, whereas p50 and p52 do not. They have to dimerize with either RelA, c-Rel, or RelB to gain the function of signal transcription [45]. The upstream activation of inhibitors of κB (IκB) kinase-β (IKKβ) caused by extracellular stimulus and subsequent phosphorylation and degradation of IκB protein would trigger the nuclear localization signal to NF-κB. NF-κB is then free to enter the nucleus and induce gene expression for inflammatory factors [44].

Previous studies have elucidated several important functions of NF-κB in the regulation of immune response in dystrophic muscle and progression of DMD. Evidently, there are three potential mechanisms by which induced activation of NF-κB leads to muscle pathology: Firstly, NF-κB can augment the expression of several proteins relate to ubiquitin-proteasome system that are involved in degradation of specific muscle proteins. Moreover, NF-κB may enhance the expression of proinflammatory cytokine, chemokines and cell adhesion molecules, which promote the muscle loss. Finally, NF-κB can also interfere with the process of myogenic differentiation in dystrophic muscles [46,47].

The earlier reports have demonstrated that the activation of NF-κB pathway caused by mechanical stretch is observed in the diaphragm muscle of dystrophin-deficient adult mdx mice (170 ± 12days), as well as the elevated expression of proinflammatory cytokines like IL-1β and TNF-α. Mechanical stretch also augments the PI3K/Akt signaling pathway in the skeletal muscles of 12-week-old mdx mice, which subsequently contributes to the activation of NF-kB signaling pathway. The findings suggest that the activation of NF-κB signaling pathway may contribute to the persistent concentration of proinflammatory cytokines in dystrophic muscle, which leading to skeletal muscle wasting [48,49]. Furthermore, persistently elevated NF-κB signaling has also been detected in dystrophic muscle of DMD patients and mdx mice [50]. The role of NF-κB in muscle pathogenesis and pathology of DMD is recently investigated by genetic approaches. Acharyya et al. demonstrated that the activation of NF-κB is associated with the severity of muscle damage in 3 weeks and 5 weeks old mdx mice [50]. The deletion of NF-κB subunit p65 of in the mdx mice is enough to decrease the infiltration of macrophages and fiber necrosis, and augment regeneration of myofibers [50]. Moreover, depletion of IKKβ in macrophage dramatically reduces the expression of proinflammatory cytokines and augments myofiber regeneration, which suggests that macrophages play a critical role in DMD pathology probably via increased activation of NF-κB signaling pathway [50]. The recent study has also illustrated that inhibition of NF-κB expression improves the proliferation, differentiation, and transplantation of muscle-derived stem cells from mdx mice [51]. Also, shRNA-mediated silence of RelA with adeno-associated virus (AAV) also ameliorates pathology and enhances regeneration in muscles of 1-month-old mdx mice [52].

Immune Therapeutic Options in DMD

No effective treatment is currently available for DMD, with glucocorticoids being the unique drugs in clinical use to ameliorate symptoms partially and delay disease progression due to their anti-inflammatory functions, in spite of their remarkable side effects [53]. Efforts have been devoted to exploring safer and more promising immune therapeutic approaches including immune targeting treatment and stem cell therapy.

Glucocorticoids and immune suppressants

Glucocorticoids like prednisolone are main drugs in treatment of DMD. The anti-inflammatory functions of glucocoticoids firmly associate with suppressing CD8+ T cell responses. A recent study has demonstrated that prednisone and deflazacort can benefit subjects with DMD probably through dystrophin-specific T cell immunity. DMD patients who receive either prednisone or deflazacort show a reduction in dystrophic muscle-specific T cells, compare with no treatment for patients or healthy controls. Furthermore, the treatment effect of Deflazacort appears to be much more dramatic than that of prednisone in this research [54]. However, dramatic side effects from prednisone or deflazacort greatly limit their therapeutic application on patients particularly children patients. VBP15 is a new steroid-like oral drug and has novel membrane-stabilizing potential, anti-inflammatory effects and potent NF-kB inhibition [55] In vivo, VBP15 improves dystrophic phenotypes in mdx mice with both preventive and post-onset treatment. Research indicates that the capability to block the NF-κB signaling pathway is essential for steroid efficacy of glucocorticoids treatment. In addition, VBP15 reveals its membrane stabilization properties, which may counteract the membrane mechanical damage caused by dystrophin deficiency in dystrophic muscles. Importantly, VBP15 avoids the severe side effects caused by glucocorticoid [56]. Therefore, VBP15 could provide strong efficacy and better safety, and has potential to replace glucocorticoids in the treatment of DMD as well as other chronic inflammatory diseases.

Some kinds of immune suppressants are delivered in pre-clinical drug tests. In a previous nonrandomized placebo controlled study, the treatment of cyclosporine A (CysA) has contributed to the improvements in muscle strength in DMD patients with a larger dose (5 mg/kg versus 3.5 to 4 mg/kg) [57]. Nevertheless, A recently randomized double-blind study of has shown that the agent fails to enhance muscle strength [58]. By contrast, another immunomodulatory drug----rapamycin, may serve as a better therapeutic option for DMD patients. Studies in mdx mice have suggested that rapamycin could reduce muscle pathology and ameliorate the dystrophic phenotype, to some extent through inhibiting the concentration of CD4+ or CD8+ T cells and preserving Tregs responses [29]. Furthermore, previous studies performed in mdx mouse models have indicated that histone deacetylases (HDACs) are deregulated in dystrophic muscles due to dystrophin deficiency, which would prevent muscle regeneration in DMD. HDACs inhibitors have been proved to promote muscle regeneration and prevent the fibroadipogenic degeneration of dystrophic mice, and are of great potential therapeutic interest for the treatment of DMD [59,60]. In a preclinical study in mdx mice, the long-term (3.5 months) treatment of HDACs inhibitor- givinostat at doses of 5 and 10 mg/kg/d shows an efficacy of recovering functional and histological muscle damage, promoting muscle regeneration and reducing fibrotic scars and fatty infiltration. Intriguingly, the decrease of inflammatory infiltrate has also been observed in dystrophic muscles in mdx mice [61].

Immune targeting therapies

As described above, the aberrant activation of transcription factor NF-κB may be responsible for muscle damage and wasting [44]. In

the regard, It seems that the inhibition of NF-κB serve as a potential therapeutic option for DMD. The activation of NF-κB signaling pathway is regulated by the interaction of IKK and NEMO protein [46]. The N-terminal region of NEMO protein combines with a sequence within the C-terminus of IKK, forming the NEMO-binding domain (NBD). In studies of mdx mice, a short cell-permeable peptide (named NBD peptide) is known to disrupt combination between NEMO and IKK, result in deactivation of NF-κB signaling pathway, and alleviate inflammatory responses in dystrophic muscle [50]. Moreover, NBD peptide is observed to decrease inflammation, improve dystrophic muscle histopathology and enhance motor functions in both mdx and mdx/utrophin double knockout mice [62,63]. The green tea extract possesses antioxidant capacity and reducing formation of free radicals, which could regulate the activation of NF-κB via blocking IKK activity [64]. Researches in mdx mice with green tea extract have illustrated that green tea extract improve contractile properties in dystrophic muscles and decreased muscle pathology [65,66]. The isoflavone, inhibitor of NF-κB pathway, MAPK pathway and cytokine TNF-α, could have promising treatment for DMD patients. In a study, mdx mice treated with the isoflavone show increased muscle strength, reduced levels of SCK, decrease in myofibers necrosis and enhanced muscle regeneration. The therapeutic effect of the isoflavone is comparable to the efficacy of glucocorticoids [67].

The DMD damaged muscles would release endogenous ligands for Toll-like receptor 2 (TLR2) and may be of great importance in activating the innate immune system especially macrophage behaviour following muscle injury. A recent study have suggested that the depletion of TLR2 in dystrophic muscles of mdx mice could not only reduce numbers of macrophage but additionally facilitate to shift their phenotype from inflammatory to more anti-inflammatory features [68]. Previous studies indicate that dystrophin mutations in DMD result in P2RX7 purinoceptor upregulation which is responsible for the muscles necrosis in the mdx mice and human DMD lymphoblasts. Sinadinoset et al. demonstrates that genetic depletion of P2RX7 successfully improves muscle structure and muscle strength of mdx mice. Furthermore, this treatment apparently reduces the SCK levels, cognitive impairment, as well as inflammation and fibrosis process [69].

The inflammatory cytokines also represent critical drug targets. As mentioned previously, pharmacological blockade of TNF-α activity with specific antibody infliximab (Remicade) is proved to delay the disease progression and decrease muscle necrosis in young mdx mice [35]. Etanercept (Enbrel), an inhibitor of TNF-α soluble receptor, shows its efficacy in improving muscle strength and reducing SCK in adult mdx mice [70]. TGF-β is believed to strongly associate with fibrosis [40]. Thus, reducing the expression of TGF-β appears to be an effective curing approach in later stages of DMD, especially in controlling fibrosis process and increasing the population of Tregs in dystrophic muscle [71]. The treatment with losartan, an angiotensin II type 1 receptor antagonist, contributes to attenuate TGF-β-mediated signaling in mdx mouse models, resulting in alleviating fibrosis and improving muscle performance [72].

Mesenchymal stem cells transplantation therapy

Mesenchymal stem cells (MSCs) are originally derived from the bone marrow, as well as numerous tissues including adipose, cord blood, heart and peripheral blood [73]. MSCs are defined as a group of cells expressing non-hematopoietic markers such as CD90, CD105 and CD73, and being negative in hematopoietic markers [73]. Besides the ability to differentiate into various types of tissues, MSCs also have been shown to suppress inflammatory response and modulate immunity through several aspects, including suppressing macrophage activation, inhibiting NK and T cytotoxic cell function, inducing generation of Tregs and releasing anti-inflammatory cytokines like IL-10 and TGF-β [73,74]. Thus, the ability of MSCs to differentiate into different injured tissues and produce numerous immune-regulatory factors makes them an attractive therapeutic candidate for several autoimmune and chronic inflammatory diseases. MSCs have been utilized in treating Amyotrophic Lateral Sclerosis (ALS) [75], metachromatic leukodystrophy [76] and multiple sclerosis [77]. Though lacking large scale clinical trials, several studies have been conducted to investigate the therapeutic effects and safety of MSCs in mdx mice. The double-knockout (dko) mice refer to mice with both dystrophin and the dystrophin-related protein utrophin deficiency. Several researches have demonstrated that the dko mice show much more typical clinical signs of DMD than mdx mice, and they have onset of dystrophy in their diaphragm muscles at an earlier age. Therefore, the dko mice would be utilized as a more useful model for screening potential therapeutic approaches and exploring pathogenesis in DMD [78]. A study from Zhang et al. has demonstrated that the dko mouse models treated with bone marrow MSCs transplantation show increased expression of dystrophin and utrophin in sarcolemma of muscle tissues [79]. In another study, dko mice treated with bone marrow MSCs by injection from tail vein have gained dramatic improvements in motor functions, compare with untreated control mice [80]. A recent study on mdx mice indicates that the transplantation of bone marrow MSCs could induce expression of TSG-6 which is an anti-inflammatory protein. Furthermore, TSG-6 is proved to ameliorate muscle damage through decreasing inflammation and enhancing muscle regeneration [81]. MSCs transplantation possesses greatly promising therapeutic value for DMD but requires more clinical trials.

Conclusion

The membrane instability and mechanical stretch injury due to the dystrophin deficiency play significant roles in aberrant activation of immune responses during onset and progression of DMD. The immune changes contain increased immune cells infiltration, production of cytokines and chemokines and upregulation of transcription factor NF-κB signaling pathway, which contribute to the pathological damage and muscle wasting in dystrophic muscles. More importantly, the infiltrating immune cells like macrophages and T cells would produce proinflammatory cytokines that persistently stimulate immune cells and NF-κB pathway activation, creating a more proinflammatory environment in dystrophic muscles. In this case, many preclinical and clinical evidences have demonstrated that anti-inflammatory drugs such as steroid and immune suppressants have successfully improve the muscle pathology and conserve muscle strength in both DMD human patients and mouse models. The immune targeting and stem cell treatments also provide beneficial therapeutic interests for DMD. Nowadays, some kinds of new therapies such as exon skipping and genetic modification may provide long-term benefits on curing DMD. The immune therapies that could reduce inflammatory response and induce immune tolerant microenvironment may create conditions are good for the success of these new therapeutic approaches.

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