

The immune system in Duchenne muscular dystrophy: Friend or foe

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Duchenne muscular dystrophy (DMD) is a genetic disease caused by mutations in the X-linked dystrophin gene, resulting in reduced or absent protein production, subsequently leading to the structural instability of the dystroglycan complex (DGC), muscle degeneration, and early death in males. Thus, current treatments have been targeting the genetic defect either by bypassing the mutation through exon skipping or replacing the defective gene through gene therapy and stem cell approaches. However, what has been an underappreciated mediator of muscle pathology and, ultimately, of muscle degeneration and fibrotic replacement, is the prominent inflammatory response. Of potentially critical importance, however, is the fact that the elements mediating the inflammatory response also play an essential role in tissue repair. In this opinion piece, we highlight the detrimental and supportive immune parameters that occur as a consequence of the genetic disorder and discuss how changes to immunity can potentially ameliorate the disease intensity and be employed in conjunction with efforts to correct the genetic disorder.

Role for Inflammation and Regulation in DMD

Indirect evidence supporting a role for the immune system in the pathogenesis of DMD comes from gene array, histological, and magnetic resonance imaging studies demonstrating skeletal muscle inflammation and the accumulation of immune cells in muscle of muscular dystrophy (MD) patients and animal models of the disease.^{1,2,3,4} Studies aimed at

characterizing the immune cells that invade dystrophic muscle have shown that CD4+ and CD8+ T cells, macrophages, eosinophils and natural killer T cells infiltrate both human and mouse dystrophic muscle.¹⁻⁶ Evidence supporting a functional role for the immune system in the exacerbation of muscular dystrophy comes from mouse studies in which specific depletion of either myeloid or lymphocyte populations greatly reduces myonecrosis.^{7,8,9} Moreover, the pharmacological inhibition^{10,11,12} or genetic ablation of inflammatory mediators largely expressed by immune cells, such as IFN γ ,¹³ also reduced the severity of muscle pathology in mdx mice, an animal model of DMD caused by a spontaneously-arising mutation in the dystrophin gene.¹⁴ Although the effect of immunodeficiency on acute pathology is not well described, multiple studies suggest that T and B lymphocytes contribute to the development of muscle fibrosis in aged (greater than 12 months) SCID-mdx¹⁵ and nu/nu-mdx mice.¹⁶ Specifically, the absence of B and T lymphocytes resulted in reduced fibrosis, accompanied by a reduction of TGF- β 1, suggesting the importance of the adaptive immune system in DMD.¹⁵

The empirical observation that immunosuppressive drugs, such as glucocorticoids, can moderate muscle weakness in a number of patients and in animal models of DMD further support a pathogenic role for the immune system in DMD. For instance, prednisone-treated DMD patients demonstrate improved muscle strength,¹⁷ accompanied by a decreased number of mononuclear inflammatory cells in the tissues.¹⁸ Similarly, glucocorticoid therapy reduced myofiber injury and immune cell infiltration of muscle in mdx mice.¹⁹ These reductions in

Keywords: autoimmunity, biologics, Duchenne muscular dystrophy, dystrophin-specific immunity, gene therapy, inflammation, interleukin-10, regulatory T cells

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Submitted: 11/21/2014

Revised: 01/07/2015

Accepted: 01/19/2015

<http://dx.doi.org/10.1080/21675511.2015.1010966>

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histopathological features of dystrophinopathy were attributed to reduced expression of cellular adhesion molecules that facilitate trafficking and extravasation of inflammatory cells into tissues, although the precise effects of this pleiotropic drug remain controversial. Importantly, the immune system appears to also contribute to the pathogenesis of other dystrophies such as facioscapulohumeral dystrophy and limb-girdle muscular dystrophy, although, in some settings, steroids were ineffective suggesting a functional difference in the inflammatory responses in these forms of MD.²⁰⁻²³

Recently, the immune system has been shown to have opposing roles in diseases ranging from cancer to autoimmune diseases, as it can mediate tissue destruction as well as wound healing and tissue repair. Similarly, there has been an increasing appreciation that inflammation can play a dual role in DMD.² These opposing functions can be explained by subpopulations of immune cells that, rather than promoting pathogenicity, exhibit specialized functions that suppress immunity and promote muscle repair either directly or indirectly.²⁴ For instance, muscle macrophages display a broad continuum of activation *in vivo*. The so-called M1 macrophages are elicited by pro-inflammatory cytokines such as IFN γ and TNF α and acquire a cytotoxic function that contributes to muscle degeneration in MD through increased inducible nitric oxide synthase (iNOS) and production of nitric oxide.²⁵ In contrast, M2 macrophages are induced by Th2 cytokines such as IL-4 and IL-13,²⁶ inhibit damaging inflammation, and can suppress M1 macrophage-mediated cytotoxicity.²⁵ IL-6 can also prime the alternative activation of macrophages by inducing the expression of the IL-4 receptor.²⁷ However, the relationship between IL-6 and muscle regeneration is complex and extends beyond the regulation of macrophage function.²⁸ Studies have shown that IL-6 promotes hypertrophic muscle growth and enhances myogenesis through stimulation of satellite cell proliferation,^{29,30} but transgenic overexpression promotes atrophy and muscle wasting in mice.^{31,32} The pleiotropic nature of IL-6 during muscle injury and repair may be attributed to its

temporospatial regulation, acting either early or late during myogenesis, and whether chronic inflammation is a facet of the muscle injury and repair process.³³ For example, studies have shown exacerbation of muscle degeneration in mdx mice (i.e. chronic inflammation),³⁴ but improved regeneration when IL-6 signaling is blocked during acute injury.³⁵ Moreover, M2 macrophages are a rich source of growth factors such as insulin-like growth factor 1,³⁶ which was previously shown to promote muscle regeneration,³⁷ although this has not been confirmed in MD patients.

Less clear are the external immune regulatory factors that govern immune activation in patients with muscular dystrophy. Recently, we showed that regulatory T cells (Tregs) provide a layer of regulation controlling the activation status of muscle macrophages.³⁸ Depletion of Tregs enhanced M1 activation of muscle macrophages in mdx mice. Moreover, we and others found that muscle Tregs were a rich source of IL-10³⁹ and that increasing Treg content in mdx muscle caused a subsequent increase in the expression of IL-10 in the whole muscle.³⁸ A role for IL-10 in the suppression of muscle macrophages is supported by the observation that muscle macrophages in IL-10-deficient mdx mice have an amplified M1 activation status and these mice show increased cytolytic activity *in vitro*.⁴⁰ However, whether Treg-specific IL-10 is the critical cellular source of IL-10 responsible for the suppression of M1 macrophages in dystrophic muscle is not yet clear but is consistent with the “bystander” suppressive effects that Tregs are known to promote.⁴¹ Nonetheless, our investigations warrant further testing of IL-10-based therapeutics for the treatment of muscular dystrophy. Based on recent studies suggesting that Tregs modulate myogenesis in an amphiregulin-dependent manner during acute muscle injury,³⁹ further studies should also examine the capacity of muscle Tregs to directly enhance muscle regeneration in mdx mice and DMD patients and determine the factors produced in addition to amphiregulin that enhance satellite cell proliferation and or differentiation. In this regard, there are a number of presumed tissue repair molecules produced by tissue

resident Tregs that may play a key role in moderating the damage induced in this disease.³⁹

Does Muscular Dystrophy Also Involve an Autoimmune Component?

Although the infiltration of immune cells in dystrophic muscle is viewed as a generalized inflammatory response orchestrated by cytokines and chemokines induced early in the pathogenesis of muscular dystrophy,^{42,43} critical studies suggest that the immune response to injured muscle may also involve an antigen-specific response to ill-defined muscle antigens.^{2,44} Vetrone and colleagues demonstrated that the frequency of V β 8.1/8.2⁺ T cells isolated from mdx muscle was increased relative to other V β populations, suggesting the oligo-expansion of antigen-specific T cells.⁶ Similarly, an over-representation of V β 2 transcripts and a conserved amino acid motif, RVSG, in the third complementary determining region (CDR3) of the T cell receptor (TCR), was observed in muscle of DMD patients, indicating recognition of an undefined antigen in a subset of DMD patients.⁴⁵ Moreover, recent studies examining the TCR repertoire of Tregs in mdx muscle using deep sequencing methods revealed an enrichment of several TCR rearrangements,³⁹ suggesting, together with the biased reactivity to self-antigens,⁴⁶ that Tregs react to multiple self-antigens in dystrophic muscle. Although the identity of these antigens remains elusive, it is interesting to speculate that the instability of the dystroglycan complex (DGC) and the chronic inflammatory environment contribute to a bona fide autoimmune response to native or altered proteins resulting from unique post-translational modifications in DGC components or other muscle proteins. This can lead to altered conformational epitopes, antigen presentation, and the breaking of tolerance. It is important to note that recent work by Davis and colleagues suggest that thymic deletion of potentially autoreactive T cells can be incomplete, and inflammatory responses, or even certain infections, can trigger these self-reactive cells and promote autoimmunity.^{47,48}

The observation that dystrophin transcripts are expressed in the thymus⁴⁹ of healthy animals supports the view that the prevalence of dystrophin-reactive T cells may be attributed to a defect in thymic deletion, because dystrophin is unlikely expressed to any extent in the thymus of DMD patients.⁵⁰ Thus, patients likely retain a pool of dystrophin-reactive T cells that escape into the periphery, and may be further activated by expression of dystrophin on revertant fibers or dystrophin introduced exogenously by gene therapy. Indeed, a recent study showed that a substantial number of DMD patients had a pre-existing pool of circulating dystrophin-reactive T cells,⁵¹ raising the suspicion that dystrophin gene therapy will prime a dystrophin-specific immune response that may limit efficacy. Although the evidence is circumstantial and anecdotal, the findings of a recent dystrophin gene therapy trial corroborate the concern that pre-existing dystrophin-reactive T cells may limit the clinical efficacy of gene therapy.⁵² Moreover, recent studies showing that Treg identity and function may become destabilized in inflammatory settings,⁵³ further suggest that peripheral tolerance mediated by Tregs in dystrophic muscle may be compromised due to the pro-inflammatory cytokines generated by the muscle damage locally in the tissue, further compromising the efficacy of dystrophin gene therapy.

The Use of Biologics and Cell Therapy to Modulate Muscle Inflammation

In the last decade, the FDA has approved a number of novel biologics that block tumor necrosis factor α (TNF α), interleukin-6 (IL-6) or IL-17 signaling for the treatment of inflammatory disorders such as psoriasis, rheumatoid arthritis, multiple sclerosis, and Crohn's disease.^{54,55,56} In addition, drugs that target T cell co-stimulation, such as CTLA4Ig, and cell trafficking such as anti-VLA4 and fingolimod (FTY720) are showing increasing efficacy in a variety of autoimmune settings.^{57,58,59} The potential promise of using biologics to treat muscular dystrophy is exemplified by studies showing that TNF α blockade

reduced muscle necrosis, contractile dysfunction, fibrosis, and led to improved muscle function in mdx mice.^{11,60} Studies showing that TNF α can also stimulate myogenesis raise safety concerns regarding the targeting of TNF α as a therapy for muscular dystrophy.^{61,62} This concern is highlighted by the recent study from Spencer and colleagues, which showed that although TNF α blockade reduced cardiac fibrosis, deficits in cardiac function were also observed.⁶³ Thus, it will be essential to modify the unwanted immune responses without compromising the reparative aspects of inflammation in mdx mice.

In addition to the well-known approved and investigational biological therapeutics that have surfaced as potential therapies for DMD, either directly or in conjunction with gene therapies, there are a number of new immune targets on the horizon that could be approached based on preclinical studies. For instance, it is clear from our studies that targeting the regulatory T cell compartment would be effective in this setting. One could attempt to enhance the regulatory T cells that are already resident and increased in the muscle³⁹ using the Treg growth factor, IL-2, which has been shown to be efficacious in the mdx animal model of DMD³⁸ and has shown promise in humans with a variety of autoimmune diseases and graft-versus-host disease (GvHD).^{64,65} In addition, there is increasing interest in using IL-10 and other Treg-derived factors such as IL-35.⁶⁶ However, it should be emphasized that the effects of these therapies may be double-edged as the generalized immune suppression may lead to reduced tissue repair, which can moderate the clinical efficacy of the therapy. Thus, it is our belief that ultimate success may be achieved through the use of Treg therapy. Tregs are natural components of the anti-inflammatory response, and thus provide a salutary effect on inflammation but also produce key molecules involved in tissue repair and regeneration. Importantly, the cells can act as Trojan horses able to deliver a variety of therapeutic gene products that promote repair or induce tolerance to avoid the generation of autoimmune or anti-foreign protein responses. Recent studies suggest that Treg therapy can be used in a variety of immune-mediated diseases such as Type 1 Diabetes, GvHD, and uveitis but

can also be adapted to non-immune based diseases such as atherosclerosis and, most recently, hemophilia.⁶⁷⁻⁷²

In further support of mechanisms to increase the number and enhance the function of muscle-resident Tregs is the contention that the therapeutic approach to DMD must be viewed not solely in terms of short-term benefits, which include delaying the loss of muscle mass, the requirement for ventilator dependency, and death by a period of several years, but rather in terms of longer-lasting definitive treatments that are now at our doorstep. Taming the inflammatory response, while beneficial in the short-term, does not address the fundamental pathology of the disease, i.e., the lack of dystrophin expression, whose loss perpetuates the cycle of inflammation, muscle loss, and fibrosis. As such, the advent of potentially curative, or certainly more effective therapies for DMD heightens the need to fully control the inflammatory response in DMD muscle such that dystrophin-expressing therapies may be enabled.

Conclusion

The strong inflammatory response in DMD muscle may abrogate the efficacy of dystrophin replacement therapies by rendering them into dystrophin vaccines that induce or boost extant dystrophin-specific T cells. The potential of priming dystrophin-specific immunity would likely worsen the severity of disease; thus, therapeutic approaches that thoroughly subdue this response must be used in conjunction with gene therapies to ensure efficacy. Given the panoply of possible immunomodulatory therapeutic interventions, most of which appear to have dichotomous effects (favorable suppression of inflammation and immunity, while unfavorable effects on muscle healing and growth), as well as requiring continuous treatment, as mentioned above, investigation of Tregs in DMD rises to prominence in offering the best chance of suppressing dystrophin-directed immunity and inhibiting inflammation, and thus facilitating dystrophin expression and function. From a scientific perspective,

therapies that would generate Tregs in vivo, such as low dose/mAb complexed IL-2 or by ex-vivo generation and subsequent administration, may provide benefit for an unmet medical need in a serious condition. From a regulatory perspective, therapies that address unmet medical needs in the treatment of a serious or life-threatening condition may be considered for an accelerated development and approval track (Guidance for Industry: Expedited Programs for Serious Conditions-Drugs and Biologics-<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM358301.pdf>). Moreover, if promising in early studies, such therapeutics may qualify as “breakthrough” and gain additional regulatory considerations. Given the recent advances in dissecting the pathophysiology of DMD, the improvements in our ability to generate regulatory T cells for clinical use, the advent of gene therapy and exon skipping therapeutics, and the devastating nature of this disease with its pressing need for more definitive therapeutics, we should move forward, not with the aim to decrease morbidity and delay mortality for a short interval, but rather with the aim of a normal lifespan with an acceptable quality of life for these patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Funding

These studies were supported by a Ruth L. Kirschstein National Research Service Award training grant (1F32AR063575–01A1 to SAV); grants R01 AI50834, R01 AI46643, P01 AI35297, P30DK63720 from the US National Institutes of Health to J.B; and by the NIH/NIAMS in partial support for the Center for Duchenne Muscular P30AR057230–0 and by the Muscular Dystrophy Association to MJS.

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