Abstract

Muscular dystrophy and idiopathic inflammatory myopathy include a group of disorders characterized by muscle degeneration and chronic inflammation. Therapy for these disorders largely relies on glucocorticoids that broadly suppress inflammation, but provide only a short delay in disease progression with many adverse side effects. Moreover, the generalized suppression of inflammation by glucocorticoids may silence protective immune cell populations that are anti-inflammatory and critical orchestrators of muscle regeneration. Thus, it is imperative to delineate the mechanisms that regulate muscle degenerative versus regenerative inflammation, in order to design novel therapies that specifically inhibit pro-injurious inflammatory responses. Recent studies using mouse models of acute muscle injury and Duchenne muscular dystrophy (DMD) have shown that Tregs promote regeneration and inhibit a type 1, pro-inflammatory response in muscle. However, the mechanisms by which Tregs modulate muscle inflammation and promote regeneration are not well defined. This gap in knowledge hampers the clinical translation of animal studies, showing that Tregs promote regeneration, and reduce the severity of muscular dystrophy. Furthermore, the lack of a detailed characterization of Tregs in patients with DMD or other muscle diseases makes it difficult to ascertain the therapeutic potential of targeting Tregs in these patients.

Our long-term objective is to leverage the strength of our expertise in preclinical models of DMD and immunology with the clinical expertise of Dr. Tahseen Mozaffar, Director of the ALS and Neuromuscular Center, to perform an on-going assessment of the role and therapeutic potential of Tregs in patients with muscle disease. We will characterize muscle Treg responses in patients with DMD, FSHD, LMGD, and IBM to assess whether the Treg response to muscle degeneration is disease specific or a generalized response to muscle injury. We will also explore the potential biomarker application of Tregs by enumerating Tregs in whole blood and drawing correlative relationships between circulating and muscle Tregs responses. Moreover, we will generate an adoptive transfer system to functionally test the role of human Tregs in a humanized mouse model of DMD. In preliminary work using the mdx mouse, a null mutant of the dystrophin gene and model of DMD, we show that muscle Tregs are a large source of interleukin-10 (IL-10) and relaxin-3 (rln-3), which we hypothesize are critical in reducing muscle inflammation, and promoting regeneration, respectively. Considering the known anti-inflammatory function of IL-10 and the high conservation among relaxin family members across specifies, we predict that these molecules are also expressed by human Tregs and participate in the regulation of muscle inflammation and regeneration in human. Collectively, our studies will provide an understanding of Treg responses in a wide range of muscular disorders and establish a humanized system to study the functional role of human Tregs in muscular dystrophy. Importantly, this work will lay the conceptual and technical foundation for future studies aimed at characterizing the role of Tregs in muscle disorders by addressing the following specific aims:

Aim 1: Determine the regulation of human Treg responses in patients with muscle degenerative disorders. We show that muscle Tregs are elevated in human dystrophic patients. However, the clinical implications of these findings are difficult to assess, considering the difficulty in readily accessing muscle tissue for clinical testing and the lack of a quantitative and molecular analysis on blood-circulating Tregs in patients. Thus, in this aim we will compare the number of muscle and circulating Tregs in DMD, FSHD, LMGD and IBM patients, in effort to ascertain the utility of Treg number and/or frequency as a potential biomarker for the immunoregulatory status of muscle in these patients. Additionally, determining whether Treg responses are disease specific or a generalized response to muscle degeneration will provide important insights on the applicability of Treg promoting therapies over a broader class of muscle degenerative disorders.

Aim 2: Develop an adoptive transfer system to test the functional role of human Tregs in a humanized model of DMD. To examine the functional role of human Tregs in muscle disease, we will use a well-established humanized model of DMD. This system will allow us to adoptively transfer *ex vivo*-expanded human Tregs and study their ability to regulate muscle inflammation, injury, fibrosis, and/or regeneration. Based on our observation that IL-2c therapy ameliorates the severity of muscular dystrophy in mdx mice, and clinical trials have demonstrated that low-dose IL-2 therapy specifically activates human Tregs and reduces inflammation, we will adoptively transfer human Tregs into the humanized DMD mouse in the presence or absence of low-dose IL-2. These studies will allow us to assess the potential clinical translation of using IL-2 therapy in muscle degenerative disorders.