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Novel activity of an anti-inflammatory cytokine: IL-10 prevents TNFα-induced resistance to IGF-I in myoblasts ☆

Klemen Strle [a], Robert H. McCusker [a], Lynn Tran [a], Alexandra King [a], Rodney W. Johnson [b], Gregory G. Freund [c], Robert Dantzer [a,c], Keith W. Kelley [a,c,*]

[a] *Laboratories of Integrative Immunophysiology, College of Medicine, University of Illinois, Urbana, IL 61801, United States*

[b] *Integrative Biology, Integrative Immunology and Behavior Program, Department of Animal Sciences, College of Medicine, University of Illinois, Urbana, IL 61801, United States*

[c] *Department of Pathology, College of Medicine, University of Illinois, Urbana, IL 61801, United States*

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Abstract

IL-10 is an anti-inflammatory cytokine that suppresses synthesis of proinflammatory cytokines and their receptors. Here we tested the possibility that TNFα-induced hormone resistance in myoblasts might be overcome by IL-10. We found that IL-10 restores myogenesis by suppressing the ability of exogenous TNFα to inhibit IGF-I-induced myogenin. This protection occurs without decreasing global activity of TNF receptors since IL-10 does not impair TNFα-induced IL-6 synthesis or ERK1/2 phosphorylation. Instead, IL-10 acts to prevent TNFα-induced phosphorylation of JNK. These findings demonstrate that IL-10 serves a previously unrecognized protective role in muscle progenitors by overcoming TNFα-induced resistance to IGF-I.

Keywords: Inflammation; Cytokines; Hormone resistance; Muscle; JNK; IGF-I

1. Introduction

It is now clear that cells of the immune system interact with muscle cells to regulate muscle development, including responses to injury and repair and a variety of dystrophies (Tidball, 2005). The potential role of inflammation in muscle development was most recently shown in young dystrophic mice by depletion of neutrophils or by injections of Etanercept, a soluble TNF receptor antagonist (Hodgetts et al., 2006): both treatments significantly reduced muscle necrosis. At the other end of the spectrum, prolonged elevations of proinflammatory cytokines are closely associated with muscle wasting that occurs during the sarcopenia of aging and in cachectic AIDS and cancer patients. These clinical disorders occur along with a decline in IGF-I anabolic activity, which is consistent with *in vitro* findings in muscle progenitor cells. Very low concentrations of TNFα (0.01–1 ng/ml) inhibit IGF-I-induced protein synthesis (Broussard et al., 2003; Strle et al., 2004) and expression of the critical muscle differentiation factors, MyoD (Strle et al., 2004) and myogenin (Broussard et al., 2003; Strle et al., 2004). This ability of TNFα to induce hormone resistance is mediated by c-Jun N-terminal kinase (JNK) (Strle et al., 2006). JNK is a stress-activated protein kinase (SAPK) that is implicated in the pathophysiology of major metabolic and inflammatory disorders mainly because it serves as a key signaling intermediate downstream of two major proinflammatory cytokine receptors, TNFα receptor (TNFR)1 and IL-1β receptor (Manning and Davis, 2003; Wajant and Scheurich, 2004). Potential treatments that might overcome TNFα-induced hormone resistance in myoblasts are unknown.

Abbreviations: SAPK, stress-activated protein kinase; JNK, c-jun N-terminal kinase; IL-10, interleukin 10; IGF-I, insulin like growth factor I; TNFα, tumor necrosis factor α; IL-1β, interleukin-1 β.

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* Corresponding author. University of Illinois, Laboratory of Immunophysiology, Integrative Immunology and Behavior Program, Department of Animal Sciences, 227 Edward R. Madigan Laboratory, 1201 West Gregory Drive, Urbana, IL 61801, United States. Tel.: +1 217 333 5141; fax: +1 217 244-5617.

E-mail address: kwkelley@uiuc.edu (K.W. Kelley).

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IL-10 is the protypical anti-inflammatory cytokine that suppresses the innate immune system and subsequently blunts Th1 activation (Moore et al., 2001; Strle et al., 2001). IL-10 acts in a multifaceted manner by inhibiting antigen presentation, decreasing cell-surface expression of cytokine receptors and inducing expression of endogenous cytokine antagonists (Moore et al., 2001). However, the hallmark of IL-10 anti-inflammatory activity is generally considered to reside in its ability to suppress the synthesis of chemokines and pro-inflammatory cytokines, particularly TNFα and IL-1β, because they are early-acting proteins that promote the synthesis of other cytokines (Fiorentino et al., 1991; Moore et al., 2001; Strle et al., 2001). Since IL-10 inhibits the synthesis of TNFα and IL-1β, it could play a protective role in muscle by abrogating the activation of key intermediary inhibitor proteins such as JNK, a possibility that has not yet been explored. This is surprising because IL-10 is expressed *in vivo* in skeletal muscle of young and elderly individuals (Nieman et al., 2003; Przybyla et al., 2006) and *in vitro* by myotubes derived from immortalized murine myoblasts (Alvarez et al., 2002). These results establish that muscle cells are capable of synthesizing IL-10 independently of myeloid cells such as macrophages that are known to reside in muscle. In addition, evidence is slowly accumulating that muscle cells not only produce IL-10 but also respond to it. For instance, IL-10 ameliorates insulin resistance in skeletal muscles of diabetic mice, as assessed by kinase activity of protein kinase C (PKC), phosphatidylinositol (PI) 3-kinase and AKT (Kim et al., 2004). Similarly, IL-10-encoding plasmids expressed in skeletal muscle notably improve whole-body insulin responses in diabetic animals (Zhang et al., 2003) and alleviate clinical severity of collagen-induced arthritis (Saidenberg-Kermanac'h et al., 2003). It has recently been shown that transfection of myoblasts with IL-10-encoding plasmids prevents weakness of the diaphragm and reduces mortality following infection with *Pseudomonas aeruginosa* (Divangahi et al., 2006). Collectively, these data establish that skeletal muscle is an IL-10 sensitive tissue, but the potential interaction of IL-10 with receptors for proinflammatory cytokines in development of muscle tissue has yet to be determined.

Here we show for the first time that IL-10 plays a protective role in skeletal muscle myoblasts by directly inhibiting TNFα-induced IGF-I resistance. This protective action of IL-10 is not caused by inhibition of pro-inflammatory cytokine synthesis or regulation of ERK 1/2 activation. Instead, IL-10 completely suppresses TNFα-induced phosphorylation of JNK. These data establish a novel protective activity for IL-10 in skeletal muscle progenitor cells which is to prevent TNFα-induced IGF-I resistance by a mechanism that is not caused by a reduction in cytokine or cytokine receptor expression.

2. Materials and methods

2.1. Reagents

Recombinant murine TNFα and human IGF-I were purchased from Intergen (Purchase, NY) and recombinant murine IL-10 was from Pharmingen, (San Hose, CA). Enzyme-linked immunosorbent assay (ELISA) kits for measuring IL-6 and IL-1β concentrations were obtained from Pierce Biotechnology (Rockford, IL). Dulbecco's Modified Eagle's Medium (DMEM with 4.5 g/L glucose, 0.584 g/L glutamine), penicillin/streptomycin, sodium pyruvate and fetal bovine serum (FBS;<0.25 EU/ml of endotoxin) were purchased from HyClone (Logan, UT). Mouse monoclonal antibodies were obtained from the following vendors: the IgG_1 antibody to myogenin (F5D), the IgG_{2b} antibody to TNF-receptor 1 (TNFR1; H-5) and the IgG_{2a} antibody to phosphorylated ERK1/2 (E-4) were from Santa Cruz Biotechnology (Santa Cruz, CA); the IgG_1 antibody to α-tubulin (B-5-1-2) was from Sigma Aldrich. Rabbit polyclonal antibodies to C-terminus (M-20, C-20) of the IL-10 receptor (IL-10R) and an antibody to ERK1 (K-23) were purchased from Santa Cruz Biotechnology. Horseradish peroxidase (HRP)-linked secondary antibodies were purchased from GE Healthcare (United Kingdom), Antibodies specific for JNK (9252) and phosphorylated JNK (P-JNK; 9251) were from Cell Signaling Biotechnology (Danvers, MA). All other reagents were obtained from Sigma Aldrich (St. Louis, MO).

2.2. Cell culture

Skeletal murine C_2C_{12} myoblasts were obtained from the American Type Culture Collection (Manassas, VA) and cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 4 mM L-glutamine, 4.5 g/L glucose and supplemented with 10% heat inactivated FBS, 1 mM sodium pyruvate, 100 U/ml of penicillin and 100 μg/ml of streptomycin at 37 °C, 7% CO_2 and 95% humidity. Myoblasts were grown to 70% confluence, washed three times in DMEM devoid of FBS to remove growth factors and deprived of serum for 4 h prior to initiation of experiments.

In all experiments using IL-10, myoblasts were pretreated with IL-10 (10, 25 or 50 ng/ml) for 1 h prior to addition of an optimal concentration of TNFα (1 ng/ml). For experiments measuring myogenin expression, myoblasts were treated with TNFα for 1 h before adding IGF-I (50 ng/ml) for an additional 24 h. In experiments measuring JNK and ERK 1/2 phosphorylation, myoblasts were pretreated with IL-10 (10 ng/ml) for 1 h prior to adding TNFα for another 10, 15 or 30 min. ELISA time-course experiments were conducted by pretreating myoblasts with IL-10 (10 ng/ml) for 1 h before adding TNFα for additional 24, 16, 8, 4 and 2 h. Myoblasts in control wells were left untreated for the duration of experiments. In ELISA experiments that utilized IL-10 dose-responses, myoblasts were pretreated with 0, 10, 25 and 50 ng/ml IL-10 for 1 h prior to treatment with TNFα for an additional 24 h. The highest concentration of IL-10 (50 ng/ml) served as a control in ELISA dose-response experiments. Whole cell lysates from untreated myoblasts were used to detect IL-10R1 and TNFα receptor 1 TNFR1 expression.

2.3. Western blotting for myogenin, JNK and ERK

At the termination of experiments, myoblasts were homogenized in 200 μl of homogenization buffer containing 50 mM