IGF-I does not prevent myotube atrophy caused by proinflammatory cytokines despite activation of Akt/Foxo and GSK-3β pathways and inhibition of atrogin-1 mRNA

Mishaël Dehoux,1 Catherine Gobier,1 Pascale Lause,1 Luc Bertrand,2 Jean-Marie Ketelslegers,1 and Jean-Paul Thissen1
Divisions of 1Diabetology and Nutrition and 2Cardiology, Université catholique de Louvain, Brussels, Belgium

Submitted 21 February 2006; accepted in final form 14 August 2006

Dehoux M, Gobier C, Lause P, Bertrand L, Ketelslegers J-M, Thissen J-P. IGF-I does not prevent myotube atrophy caused by proinflammatory cytokines despite activation of Akt/Foxo and GSK-3β pathways and inhibition of atrogin-1 mRNA. Am J Physiol Endocrinol Metab 292:E145–E150, 2007. First published August 22, 2006; doi:10.1152/ajpendo.00085.2006.—Myofibrillar protein loss occurring in catabolic situations is considered to be mediated by the release of proinflammatory cytokines and associated with a decrease in circulating and muscle levels of insulin-like growth factor I (IGF-I). In this paper, we investigated whether the C2C12 myotube atrophy caused in vitro by TNF-α/IFN-γ cytokines might be reversed by exogenous IGF-I. Our results showed that, despite the presence of TNF-α/IFN-γ, IGF-I retained its full ability to induce the phosphorylation of Akt, Foxo3α, and GSK-3β (respectively, 16-fold, 9-fold, and 2-fold) together with a decrease in atrogin-1 mRNA (−39%, P < 0.001). Although this ubiquitin ligase has been reported to accelerate the degradation of MyoD, a myogenic transcription factor driving the transcription of myosin heavy chain (MHC), IGF-I failed to blunt the reduction of MyoD and MHC caused by TNF-α/IFN-γ. Moreover, IGF-I only very slightly attenuated the myotube atrophy induced by TNF-α/IFN-γ (TNF-α/IFN-γ 15.48 μm alone vs. TNF-α/IFN-γ/IGF-I 16.97 μm, P < 0.001). In conclusion, our data show that IGF-I does not reverse the myotube atrophy induced by TNF-α/IFN-γ despite the phosphorylation of Foxo and GSK-3β and the downregulation of atrogin-1 mRNA. Our study suggests therefore that factors other than IGF-I decrease are responsible for the muscle atrophy caused by proinflammatory cytokines.

growth factors; ubiquitin-proteasome system; tumor necrosis factor-α; transduction pathway; muscle atrophy; glycogen synthase kinase-3β

MUSCLE ATROPHY is a classical consequence of many inflammatory situations such as sepsis, cancer, or renal failure and contributes greatly to morbidity (23, 27). Rapid loss of muscle protein, especially the contractile components, is believed to result from the activation of the ubiquitin-proteasome proteolytic pathway (27). In this proteolytic pathway, the atrogin-1/MAFbx is an E3 ubiquitin ligase known to be specifically involved in the initiation of muscle atrophy (13, 36, 38).

There is clear evidence that the proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) play a critical role in muscle atrophy. First, circulating levels of TNF-α and IFN-γ are markedly increased in catabolic states complicated by muscle loss (2, 3, 12). Second, accelerated muscle loss has been observed after chronic administration of TNF-α (19, 40) and IFN-γ (26), in TNF-α transgenic animals (24), and in animals bearing TNF-α- and IFN-γ-producing tumors (7, 31). Third, the inhibition of TNF-α production by torbafylline attenuates skeletal muscle proteolysis in cancer-bearing and in septic rats (6). Also, in vitro experiments performed with C2C12 myotubes have shown that TNF-α induces an early activation of the atrogin-1 gene expression (29) and, together with IFN-γ, reduces the myosin heavy chain (MHC) content (20).

Several lines of evidence indicate that the decrease of local insulin-like growth factor I (IGF-I) also could contribute to muscle wasting. First, circulating and muscle concentrations of IGF-I are decreased in many catabolic states (10, 32). Second, IGF-I inhibits muscle atrophy induced by thermal injury or glucocorticoids (16, 35, 37). Third, in several catabolic states, such as fasting and diabetes, IGF-I strongly attenuates the activation of the atrogin-1 gene expression, a factor believed to be crucial in the regulation of skeletal muscle protein degradation (10). It is therefore possible that IGF-I blocks muscle atrophy by downregulating atrogin-1 expression. The use of the in vitro C2C12 myotube model allowed uncovering of the signaling pathway by which IGF-I inhibits the atrogin-1 gene expression. Dissection of this pathway has revealed that IGF-I stimulates Akt by phosphorylation, which causes in turn the phosphorylation of the forkhead transcription factors (Foxo) and glycogen synthase kinase-3β (GSK-3β) (14, 28) and, hence, their inactivation (36, 38). These two factors, in their dephosphorylated state, indeed play a cardinal role in the control of the E3 ubiquitin ligase atrogin-1 expression (13, 36, 38) by activating its transcription. Even if the targets of atrogin-1 have not yet been clearly defined, recent studies indicate that atrogin-1 could interact with and degrades MyoD (39), a myogenic transcription factor driving the transcription of MHC (1). Taken together, these observations suggest that IGF-I might inhibit muscle atrophy by preventing the atrogin-1-dependent decline of MyoD and consequently the decrease of MHC-inducing muscle atrophy.

In vitro observations indicate that the muscle cell atrophy caused by TNF-α alone (30) or in combination with IFN-γ (1) is associated with a local decrease of the muscle growth factor IGF-I (18). We therefore investigated the capacity of IGF-I to prevent, through atrogin-1 inhibition, the muscle cell atrophy caused by TNF-α/IFN-γ in cultured C2C12 myotubes.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
EFFECT OF IGF-I ON TNF-α/IFN-γ-TREATED MYOTUBES

MATERIALS AND METHODS

Chemicals and reagents. Recombinant murine TNF-α (rmTNF-α) and interferon-γ (rmIFN-γ) were purchased through R&D Systems (Abingdon, UK). Recombinant human IGF-I (rhIGF-I) was a kind gift from Pharmacia (Upsala, Sweden). NUNCLOn plastic dishes were purchased from NUNC (Roskilde, Denmark), and Dulbecco’s modified Eagle’s medium (DMEM), fetal calf serum, horse serum, nonessential amino acids, and 1-glutamine were purchased from Life Technologies (Paisley, UK).

C2C12 cell culture. Myoblasts from the muscle-derived C2C12 cell line were obtained from American Type Culture Collection (ATCC, Manassas, VA). The seeding density used throughout the experiments was 0.4 × 10^5 cells/plate of 10-cm diameter. Undifferentiated cells were grown in DMEM supplemented with 10% heat-inactivated fetal calf serum, 1% penicillin-streptomycin (100 U·100 μg·1 ml−1), 1% nonessential amino acids, and 1-glutamine at 37°C in the presence of 5% CO2. When cells reached 90–100% confluence (after 3 days), 10% fetal calf serum was replaced by 2% heat-inactivated horse serum to induce myogenic differentiation. Muscle cells were examined for evidence of myotube formation and growth by using an inverted Olympus IMT microscope (Olympus Optical, Hamburg, Germany). The medium was changed every 48 h, and differentiation was allowed to continue for 96 h (4 days) before the experimentation period. To preserve the characteristics of the C2C12 cell line, the splitting of cells was done up to a maximum of 7 times.

mRNA analysis. Total RNA was isolated from the C2C12 muscle cells using TRIzol reagent as described by the manufacturer. Total RNA recovery was ~100 μg/pls of cells. Reverse transcription, using 1 μg total RNA, was performed as described previously (11). Specific primers were selected using Primer Express software (Applied Biosystems, Foster City, CA). Forward and reverse oligonucleotides used were as follows: atrogin-1/MAFbx, 5′-TGT-T-3′/H11032 and 5′-CCA-TCA-GGA-GAA-GTG-GAT-CTA-3′/H9253; TGT-T-3′/H11032 and 5′-CCA-TCA-GGA-GAA-GTG-GAT-CTA-3′/H9253; glyceraldehyde3-phosphate dehydrogenase (GAPDH), 5′-TGC-ACC-ACC-ACC-TGC-TTA-3′ and 5′-GGA-TGC-AGG-GAT-GAT-CTT-C-3′. Primers were tested to avoid primer dimers, self-priming formation, or unspecific amplification. Real-time quantitative PCR (RTQ-PCR) was carried out using the following cycle parameters: 10 min at 95°C, followed by 40 cycles of 1 min at 60°C and 15 s at 95°C. For each gene, RTQ-PCR was conducted in duplicate with a 25-μl reaction volume of 1 ng of cDNA. To ensure the quality of the measurements, each plate included for each gene a negative control and a positive control. For each sample, a value or threshold cycle (CT) was calculated based on the time (measured by the no. of PCR cycles) at which the reporter fluorescent emission increased beyond a threshold level (based on the background fluorescence of the system). The samples were diluted in such a manner that the CT value was observed between 15 and 30 cycles. Results were expressed using the comparative CT method as described in User Bulletin 2 (Applied Biosystems). Briefly, the ΔCT values were calculated in every sample for each gene of interest as follows: CT gene of interest – CT housekeeper gene, with GAPDH as the reporter gene. Calculation of relative changes in the expression level of one specific gene (ΔΔCT) was performed by subtraction of ΔCT from the Ct pl group to the corresponding ΔCT from the treated groups (11, 41). The values and ranges given in different figures were determined as follows: 2−ΔΔCT with ΔΔCT = SE and ΔCT = SE, where SE is the standard error of the mean of the ΔCT value (User Bulletin 2, Applied Biosystems).

Western blots and antibodies. For immunobLOTS of MyoD, MHC fast, and ubiquitinated proteins, cells were washed twice in cold PBS and incubated 30 min on ice with lysis buffer A (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 2% NP-40, 0.1% SDS) containing protease and phosphatase inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 1 mM sodium vanadate) followed by centrifugation at 14,000 rpm for 15 min. For Akt, Foxo3a, and GSK-3β immunobLOTS, cells were washed twice in cold PBS and incubated 30 min on ice with lysis buffer B (50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM MgCl2·6H2O, 1% NP-40) containing protease and phosphatase inhibitors (1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 20 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium vanadate) followed by centrifugation at 14,000 rpm for 15 min. Protein concentrations were determined using the Bradford method (Bio-Rad Protein Assay) as described by the manufacturer. Equal amounts of total cellular proteins were resolved by a SDS-polyacrylamide gel 10% electrophoresis (except for MHC 7%) and transferred to polyvinylidene difluoride membrane (Immobilon P/PVDF, Millipore). Membranes were probed with anti-MyoD antibody (1:300, Santa Cruz Biotechnology), anti-MHC (1:10,000, Novoceastra), anti-phospho-Akt (1:1,000, Cell Signaling), anti-Akt (1:1,000, Upstate), anti-phospho-Foxo3a (1:1,000, Upstate), anti-Foxo3a, (1:1,000, Upstate), anti-phospho-GSK-3β (1:2,000, Cell Signaling), and anti-GSK-3β (1:5,000, Cell Signaling) followed by horseradish peroxidase-coupled secondary antibody, anti-mouse or anti-rabbit (1:40,000, Amersham Biosciences), and developed with a chemiluminescence-based detection system (ECL Plus, Amersham Biosciences). Developed film was scanned (Image Scanner, Amersham Pharmacia Bio-tech) and analyzed using ImageMaster TotalLab software v2.0 (Amersham Pharmacia Bio-tech).

Myotube morphological analysis. Myotubes were photographed directly in culture plate without fixation using an Olympus digital camera coupled to an Olympus IX71 microscope with the use of AnalySIS 3.2 software (Soft Imaging System). Briefly, the cell diameter was measured on 100 myotubes in each condition (from 2 independent experiments). For each myotube, three random measurements were performed along the length of the myotube (n = 3 measurements/myotube) using the computer program Scion Image Beta 4.02 (Scion), and the average of these three measurements was considered as one single value.

Immunocytochemistry. Cells were fixed in 4% paraformaldehyde for 20 min at room temperature and permeabilized with 0.2% Triton-PBS for 15 min at room temperature before blocking in BSA (0.5%)–PBS normal goat serum (2%) for 30 min at room temperature. Primary antibodies diluted in 0.5% BSA-PBS were added for 1 h at room temperature (anti-MHC, 1:10 dilution). After washing steps, cells were incubated for 1 h at room temperature in goat anti-mouse FITC (green) diluted to 1:100 in 0.5% BSA-PBS. Images were captured with a Olympus numeric device coupled to an Olympus IX71 fluorescent microscope by use of AnalySIS 3.2 software (Soft Imaging System). For fusion index determination, the same procedure was used, except that MHC antibody was replaced by anti-desmin (1:100 dilution), and nuclei were revealed by 4′,6-diamidino-2-phenylindole staining. The numbers of myotubes (cells with at least 2 nuclei) and myoblasts (cells with 1 nucleus) were counted on three different fields. Fusion index was determined as the ratio of number of myotubes divided by total number of myotubes (myoblasts and myotubes).

DNA content and creatine phosphate kinase release. DNA content was quantitated by fluorometry (Jobin Yvon Spectroflow JY3D). Creatine phosphate kinase release was assessed biochemically on cytoplasmic extract and medium via determination of muscle creatine kinase (CK) activity with a Synchro L2X20 systems reagent kit (Beckman Coulter), following the manufacturer’s instructions.

Statistical analysis. Results are presented as means ± SE. Treatment effect was assessed by use of one-way ANOVA. When the treatment effect was significant (P < 0.05), multiple comparisons among groups were performed using Student-Newman-Keuls test.

RESULTS

IGF-I stimulates the Akt/Foxo and GSK-3β pathways even in the presence of TNF-α/IFN-γ. To determine whether IGF-I signaling is still inducible in the presence of proinflammatory cytokines, we first studied Akt phosphorylation induced by IGF-I treatment for 15 min in differentiated myotubes previ
ously exposed for 24 h to TNF-α/IFN-γ. IGF-I induced a marked phosphorylation of Akt (13.4-fold, \( P < 0.001 \)), and this response to IGF-I was not affected by TNF-α/IFN-γ (16.2-fold, \( P < 0.001 \)). Stimulation of Akt phosphorylation occurred without modification of Akt protein content (Fig. 1). This Akt activation was associated with enhanced phosphorylation of Foxo3a, a downstream target of Akt (Fig. 2). Total amount of Foxo3a increased markedly in cells exposed to TNF-α/IFN-γ (2.3-fold, \( P < 0.05 \)) (Fig. 2). Nevertheless, when the ratio of phosphorylated Foxo3a to total Foxo3a was calculated in individual samples, an increase of phosphorylation in IGF-I-treated cells was still evident, after IGF-I alone (2.5-fold, \( P < 0.05 \)) or combined with TNF-α/IFN-γ (3.3-fold, \( P < 0.01 \)). We also investigated the phosphorylation of GSK-3β. The GSK-3β phosphorylation was slightly decreased by TNF-α/IFN-γ (−27%, \( P < 0.05 \)), and this effect was reversed by IGF-I addition (2.13-fold, \( P < 0.001 \)) (Fig. 3). No effect on GSK-3β protein content was observed (Fig. 3).

IFG-I inhibits the induction of atrogin-1 induced by TNF-α/IFN-γ. The activation of the Akt pathway by IGF-I is known to decrease the expression of atrogin-1 E3 ligase. We therefore investigated the relationship between IGF-I and the proinflammatory cytokines in the regulation of the atrogin-1 mRNA levels (Fig. 4). Exposure of myotubes to TNF-α/IFN-γ increased two-fold the atrogin-1 gene expression (\( P < 0.001 \)). We then evaluated whether the effect of proinflammatory cytokines could be blunted by IGF-I. IGF-I alone was able to suppress the basal expression of atrogin-1 mRNA (−53%, \( P < 0.001 \)). More importantly, this consistent activation of atrogin-1 mRNA by TNF-α/IFN-γ was completely suppressed by IGF-I (Fig. 4).

IFG-I does not prevent the decrease of MyoD or the decrease of MHC caused by TNF-α/IFN-γ. There is some evidence that MyoD is one of the atrogin-1 targets (39). We therefore investigated whether inhibition of atrogin-1 by the Akt/Foxo or GSK-3β pathway might prevent MyoD loss in cells exposed to TNF-α/IFN-γ. In agreement with previous observations (1), exposure of muscle cells to TNF-α/IFN-γ caused a dramatic decrease in MyoD (−91%, \( P < 0.001 \)) and in MHC (−50%, \( P < 0.001 \)) (Fig. 5). Surprisingly, despite marked inhibition of atrogin-1 expression (Fig. 4), IGF-I failed to prevent the decrease of MyoD and MHC caused by TNF-α/IFN-γ (Fig. 5). Furthermore, this profound reduction of the MyoD protein was accompanied by a severe downregulation of the MyoD mRNA (data not shown).

To demonstrate that decreased MHC content was not caused by decreased cell density, the effect of TNF-α/IFN-γ and IGF-I on DNA content was assessed after 72 h of incubation. TNF-α/IFN-γ decreased the DNA content (\( \mu \)g/plate) by 28% \( (P < 0.001) \), and this decrease was not prevented by coinubation with IGF-I. When MHC content was corrected for DNA content, a 50% decrease of MHC was still observed after TNF-α/IFN-γ treatment, demonstrating that the myotube atrophy was associated with MHC loss (data not shown, \( P < 0.001 \)). Moreover, neither cytokines nor IGF-I modified fusion index (94% of differentiated myotubes in all conditions) and cell necrosis, as assessed by the release of CK enzyme into the medium. Taken together, these data indicate that decline in

---

**Fig. 1.** Akt phosphorylation induced by IGF-I is not blocked by TNF-α/IFN-γ. Cells were treated with TNF-α/IFN-γ (5 ng/ml each) for 24 h and exposed to IGF-I (50 nM) for 15 min. IGF-I alone strongly activated Akt by phosphorylation. The action of IGF-I on Akt was not impaired by TNF-α/IFN-γ. Phospho-Akt (top) and total Akt (bottom) were quantified by Western immunoblot as described in MATERIALS AND METHODS using 80 µg of proteins. CTRL, control. Results are means ± SE of 3 independent experiments. ***\( P < 0.001 \) vs. control. **\( P < 0.001 \) vs. TNF-α/IFN-γ alone.

**Fig. 2.** Foxo3a phosphorylation induced by IGF-I is not blocked by TNF-α/IFN-γ. Cells were treated with TNF-α/IFN-γ (5 ng/ml each) for 24 h and exposed to IGF-I (50 nM) for 15 min. IGF-I alone phosphorylated Foxo3a, and this was not impaired by TNF-α/IFN-γ. Phospho-Foxo3a (top) and total-Foxo3a (bottom) were quantified by Western immunoblot as described in MATERIALS AND METHODS using 80 µg of proteins. Results are means ± SE of 3 independent experiments. *\( P < 0.05 \), **\( P < 0.01 \) vs. control. ***\( P < 0.001 \) vs. TNF-α/IFN-γ alone.

**Fig. 3.** GSK-3β phosphorylation induced by IGF-I is not blocked by TNF-α/IFN-γ. Cells were treated with TNF-α/IFN-γ (5 ng/ml each) for 24 h and exposed to IGF-I (50 nM) for 15 min. IGF-I phosphorylated GSK-3β, and this was not impaired by TNF-α/IFN-γ. Phospho-GSK-3β (top) and total-GSK-3β (bottom) were quantified by Western immunoblot as described in MATERIALS AND METHODS using 10 µg of proteins. Results are means ± SE of 3 independent experiments. *\( P < 0.05 \), **\( P < 0.01 \) vs. control. ***\( P < 0.001 \) vs. TNF-α/IFN-γ alone.
MHC content in response to TNF-α/IFN-γ is not caused by decreased cell number or decreased myotube fusion. IGF-I only slightly prevents the myotube atrophy caused by TNF-α/IFN-γ. Because the IGF-I effect on Akt/Foxo and GSK-3β pathways persisted despite the presence of proinflammatory cytokines, we investigated whether this effect was associated with changes in myotube size. In agreement with previous observations (34), IGF-I alone induced myotube hypertrophy (Fig. 6 and Table 1; P < 0.001). Despite its clear hypertrophic action, IGF-I inhibited only modestly the decrease in myotube size caused by TNF-α/IFN-γ (P < 0.001).

Table 1. Effect of TNF-α/IFN-γ and IGF-I on myotube diameter

<table>
<thead>
<tr>
<th></th>
<th>Diameter ± SE, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.17±0.13</td>
</tr>
<tr>
<td>TNF-α/IFN-γ</td>
<td>15.48±0.16*</td>
</tr>
<tr>
<td>IGF-I</td>
<td>23.04±0.23*</td>
</tr>
<tr>
<td>TNF-α/IFN-γ/IGF-I</td>
<td>16.97±0.16†</td>
</tr>
</tbody>
</table>

Cells were treated with TNF-α/IFN-γ (5 ng/ml each) and exposed to IGF-I (50 nM) for 72 h. Diameters were measured as described in MATERIALS AND METHODS. Results are means ± SE of 2 independent experiments (n = 50 diameters/experiment). *P < 0.001 vs. control. †P < 0.001 vs. TNF-α/IFN-γ alone.

DISCUSSION

Our study shows that IGF-I fails to reverse the muscle cell atrophy caused by TNF-α/IFN-γ despite stimulation of Akt/Foxo and GSK-3β pathways together with inhibition of the atrophy-related ubiquitin ligase atrogin-1. Moreover, our data showing that the inhibition of atrogin-1 by IGF-I was not sufficient to blunt the myotube atrophy caused by cytokines support the concept that the catabolic role of atrogin-1 in this model is limited.

The inefficiency of IGF-I to antagonize TNF-α/IFN-γ-dependent myotube atrophy contrasts with previous observations showing that IGF-I prevents myotube atrophy caused by glucocorticoids treatment (36, 38). Thus overexpression of IGF-I in tibialis anterior muscle by electroporation prevents muscle atrophy induced by glucocorticoid injection (37). In vitro studies using C2C12 myotubes have uncovered that the anti-atrophic action of IGF-I on glucocorticoid-treated myotubes is mediated by activation of the Akt/Foxo pathway (36, 38). In the same model, atrogin-1 activation by dexamethasone is in parallel suppressed by IGF-I addition (10, 35).

Data of the present study reveal that proinflammatory cytokines may promote wasting by mechanisms other than Akt/Foxo or GSK-3β pathways. Some evidence suggests that the
activation of NF-κB, a transcription factor induced in many inflammatory conditions, could be one of them. Indeed, in the C5C12 model, TNF-α and IFN-γ activate NF-κB (20). Moreover, overexpression of IkB, the natural NF-κB inhibitor, significantly prevents the strong reduction of MHC in myotubes exposed to TNF-α/IFN-γ (20). Finally, a recent report by Cai et al. (5) demonstrates that NF-κB activation by overexpression of IKKβ induces a very important muscle atrophy. However, in this model, muscle atrophy occurs without any atrogin-1 stimulation and seems to rely mainly on Muscle RING finger 1 (MuRF1) activation (5). This contrasts with our observations obtained in myotubes where NF-κB activation by cytokines fails to induce MuRF1 gene expression (data not shown). Therefore, even if a correlation between increased atrogin-1 and proteolysis has been described (35), the exact role of atrogin-1 in myofibrillar protein degradation still remains to be established. Nevertheless, our data and other studies (5, 17) suggest that muscle atrophy can occur independently of atrogin-1 activation.

Our observation that IGF-I cannot prevent muscle cell atrophy caused by proinflammatory cytokines is consistent with in vivo data showing that IGF-I fails to prevent muscle proteolysis in septic animals (15). In such models (LPS injection or cecal ligature followed by puncture), muscle atrophy occurs with atrogin-1 induction (11, 42), decreased IGF-I production (11), and NF-κB activation (33), all features observed in our in vitro model. Therefore, it could be suggested that delivery of IGF-I, and probably activation of Akt, may not be a viable option to counteract muscle atrophy caused by proinflammatory cytokines. IGF-I overexpression also failed to prevent muscle atrophy caused by immobilization (9). It is interesting to note that, in this model (21) as in sepsis, an NF-κB-dependent mechanism is thought to play a major role in the muscle atrophy process. It should be noted, therefore, that the beneficial effects of IGF-I may not be universal to all catabolic conditions.

Although proinflammatory cytokines have been reported to downregulate IGF-I receptor expression and impair its downstream activation signals (4), the fact that IGF-I failed to reverse the muscle cell atrophy caused by proinflammatory cytokines does not seem to result from a defect in the IGF-I receptor. Indeed, after IGF-I stimulation, Akt and Foxo were phosphorylated, leading to the inhibition of atrogin-1 mRNA expression. Although physiological IGF-I concentrations (5 and 10 nM) were still able to induce Akt phosphorylation and to inhibit atrogin-1 gene expression after proinflammatory cytokine exposure (data not shown). Our observation, therefore, does not support the existence of an IGF-I resistance state caused by proinflammatory cytokines in muscle cells.

The muscle cell atrophy caused by TNF-α/IFN-γ is mainly due to the dramatic reduction of MHC. Our data indicate that this decline is not caused by decreased cell number or decreased myotube fusion but by the parallel fall in the myogenic transcriptional factor MyoD. Indeed, the overexpression of MyoD after TNF-α/IFN-γ treatment completely blunts the loss of MHC (1). The reduction of MyoD by proinflammatory cytokines has been reported to result both from a decrease of its synthesis through an NF-κB-dependent posttranscriptional mechanism (20) and also from an increase of its degradation by the ubiquitin-proteasome system (25). In our study, the down-regulation by IGF-I of atrogin-1 mRNA, the ubiquitin ligase-targeting MyoD (39), did not prevent the decline of the MyoD protein. Furthermore, the persistent, dramatic downregulation of MyoD mRNA caused by TNF-α/IFN-γ, despite IGF-I stimulation, suggests a major role of the decreased synthesis in the reduction of MyoD in this model.

Although MyoD mRNA has been reported to increase in response to denervation (22), it has been shown to be decreased in a cancer-driven muscle atrophy model (8). Furthermore, downregulation of MyoD mRNA has been reported after injection of TNF-α/IFN-γ in gastrocnemius muscle (20). These last observations are in line with our in vitro results showing decreased MyoD mRNA in response to TNF-α/IFN-γ. Taken together, these observations suggest that the in vivo regulation of MyoD might be dependent on the cause of the atrophic stimulus. The observation that MyoD is decreased in inflammatory conditions is compatible with the hypothesis that a reduced expression of molecules involved in the regenerative response may contribute to muscle atrophy.

Because MyoD synthesis inhibition is not reversed by Akt activation in vitro, it is tempting to speculate that proinflammatory cytokines do not contribute to muscle wasting via downregulation of the Akt/Foxo or GSK-3β pathways but rather through activation of an NF-κB-dependent pathway (20).

In conclusion, our data show that, in contrast to the atrophy induced by glucocorticoids, the myotube atrophy induced by TNF-α/IFN-γ is not prevented by exogenous IGF-I despite the phosphorylation of Akt, Foxo, and GSK-3β together with the downregulation of atrogin-1 mRNA. In addition, the TNF-α/IFN-γ-induced MyoD loss is not prevented by the Akt pathway activation. This study suggests, therefore, that other intracellular pathways, probably NF-κB, play a major role in MyoD downregulation and in the muscle atrophy induced by proinflammatory cytokines. Investigations uncovering pathways that could dominantly suppress the activation of NF-κB and help in suppressing muscle atrophy caused by proinflammatory cytokines are therefore warranted.

GRANTS

This work was supported by grants from the Fund for Scientific Medical Research (Belgium) and the Fonds Spéciaux de Recherche (Université catholique de Louvain, Belgium). M. Dehoux is the recipient of a research fellowship from Fonds pour la formation à la Recherche dans l’Industrie et l’Agriculture from the Communauté Française (Belgium). L. Bertrand is a Research Associate of the Fonds National de la Recherche Scientifique, Belgium.

REFERENCES


