Insulin-like Growth Factor I in Inclusion-Body Myositis and Human Muscle Cultures

ALDOBRANDO BROCCOLINI, MD*, ENZO RICCI, MD*, MARIO PESCATORI, PhD, MANUELA PAPACCI, BSc, CARLA GLIUBIZZI, MSc, ADELE D’AMICO, MD, SERENELLA SERVIDEI, MD, PIETRO TONALI, MD, and MASSIMILIANO MIRABELLA, MD, PhD

Abstract. Possible pathogenic mechanisms of sporadic inclusion-body myositis (sIBM) include abnormal production and accumulation of amyloid β (Aβ), muscle aging, and increased oxidative stress. Insulin-like growth factor I (IGF-I), an endocrine and autocrine/paracrine trophic factor, provides resistance against Aβ toxicity and oxidative stress in vitro and promotes cell survival. In this study we analyzed the IGF-I signaling pathway in sIBM muscle and found that 16.2% ± 2.5% of nonregenerating fibers showed increased expression of IGF-I, phosphatidylinositol 3′OH-kinase, and Akt. In the majority of sIBM abnormal muscle fibers, increased IGF-I mRNA and protein correlated with the presence of Aβ cytoplasmic inclusions. To investigate a possible relationship between Aβ toxicity and IGF-I upregulation, normal primary muscle cultures were stimulated for 24 hours with the Aβ(25-35) peptide corresponding to the biologically active domain of Aβ. This induced an increase of IGF-I mRNA and protein in myotubes at 6 hours, followed by a gradual reduction thereafter. The level of phosphorylated Akt showed similar changes. We suggest that in sIBM, IGF-I overexpression represents a reactive response to Aβ toxicity, possibly providing trophic support to vulnerable fibers. Understanding the signaling pathways activated by IGF-I in sIBM may lead to novel therapeutic strategies for the disease.

Key Words: β-amyloid; Inclusion-body myositis; Insulin-like growth factor; Muscle cultures.

INTRODUCTION

Sporadic inclusion-body myositis (sIBM) is the most frequent acquired neuromuscular disorder occurring in patients over age 50 (1, 2). Although numerous lines of evidence argue in favor of an immuno-mediated pathogenic mechanism (i.e. presence of endomysial inflammation with increased production of pro-inflammatory cytokines), sIBM patients respond poorly to immunosuppressive treatments and have a steadily worsening clinical course that leads to severe disability (1-4). In addition, sIBM muscle is characterized by a unique array of pathologic features, including i) muscle fibers with “rimmed” vacuoles, ii) the presence of 15- to 20-nm filaments (mainly composed of hyperphosphorylated tau) and amyloid β (Aβ)-containing inclusions within the muscle fibers, and iii) the abnormal accumulation of proteins commonly observed in Alzheimer disease (AD) brain (2, 3). The etiology of sIBM is largely unknown but it has been proposed that abnormal production and accumulation of Aβ, muscle aging and increased oxidative stress may have a key pathogenic role (2). The forced overexpression of Aβ precursor protein (AβPP) in both cultured myotubes and in muscle from animal models produces cellular abnormalities similar to those observed in sIBM (5-8). This suggests that an abnormal AβPP metabolism triggers a cascade of cellular events (i.e. increased oxidative stress, mitochondrial malfunction, and abnormal protein processing and aggregation) that possibly contributes to muscle fiber degeneration (2).

Insulin-like growth factor I (IGF-I) is a pleiotropic growth factor with both endocrine and autocrine/paracrine functions. It belongs to a family of structurally and functionally related polypeptides, including insulin and IGF-II, and has a broad spectrum of actions in both central and peripheral nervous systems (9, 10). In skeletal muscle, IGF-I plays a key role in the plastic adaptation following increased stretch and contraction and in the course of muscle fiber regeneration (11-13). In animal models, the muscle-targeted overexpression of IGF-I promotes hypertrophy and sustains the regeneration potential of aged muscle after injury (14).

Recently, several studies have focused on the possible involvement of IGF-I in the pathogenesis of neurodegenerative disorders such as AD, Huntington disease, and amyotrophic lateral sclerosis (ALS) (15-19), and a potential therapeutic use of IGF-I has been proposed (17, 20). In vitro experiments have shown that IGF-I has a protective role against Aβ toxicity (21), provides resistance in conditions of increased oxidative stress, and promotes neuronal cell survival (22). Similar to other growth factors, IGF-I mediates cell survival mainly through the activation of the phosphatidylinositol 3′OH-kinase (PI3K)/Akt pathway. The recruitment of PI3K, following the binding of IGF-I with its receptors, results in phosphorylation of inositol lipids. These operate as second messengers activating the serine-threonine kinase Akt.
Fig. 1. Peroxidase immunocytochemistry. A: sIBM: vacuolated (solid arrowheads) and nonvacuolated (empty arrowheads) abnormal muscle fibers show increased expression of IGF-I protein mainly in the form of a diffuse cytoplasmic staining, presence of cytoplasmic granular deposits, or both. Original magnification: ×100. B, C: sIBM vacuolated muscle fiber, characterized by the presence of SMI 31-immunoreactive cytoplasmic inclusions (B), shows increased diffuse cytoplasmic immunoreactivity for IGF-I, associated with IGF-I sub-sarcolemmal granular deposits (C). Original magnification: ×400. D–G: Peroxidase immunocytochemistry for developmental myosin (D, F) and IGF-I (E, G) on consecutive sections of sIBM muscle biopsies. Two nonregenerating muscle fibers, identified by lack of developmental myosin expression (solid arrowheads), show increased immunoreactivity for IGF-I; 1 small regenerating muscle fiber, immunopositive for developmental myosin (D, empty arrowhead), shows increased IGF-I expression (E, empty arrowhead). Original magnification: ×200. H–K: Peroxidase immunocytochemistry for Aβ (H, J) and IGF-I (I, K) on serial sections of sIBM muscle biopsies. Muscle fibers with Aβ immunopositive inclusions have increased IGF-I immunoreactivity. Original magnification: ×200. L–O: A vacuolated muscle fiber (L, hematoxylin and eosin staining) with increased IGF-I immunoreactivity (M, peroxidase immunocytochemistry) shows also increased levels of PI3K (N) and Akt (O) on serial sections. Original magnification: ×200.
with subsequent increased expression of pro-survival factors (23).

These observations provide the rationale to investigate a possible involvement of the IGF-I signaling system in the pathophysiology of sIBM muscle.

**MATERIALS AND METHODS**

**Patients**

Muscle biopsies from 7 patients with clinical features indicative of sIBM (i.e. age of onset over 50 years, muscle weakness in both proximal and distal compartments of 4 limbs, thinning of the forearm muscles, and prominent involvement of the quadriceps) were included in this study. All specimens showed typical sIBM morphological characteristics, including the presence of i) inflammatory cells often invading non-necrotic fibers, ii) muscle fibers with rimmed vacuoles, iii) Congo-red-positive deposits within the muscle fibers, iv) SMI 31-immunoreactive cytoplasmic inclusions, and v) 15- to 20-nm cytoplasmic tubulofilamentous inclusions by electron microscopy analysis (2, 3). Controls were polymyositis (n = 5), dermatomyositis (n = 5), peripheral neuropathies (n = 4), ALS (n = 4), spinal muscular atrophy (n = 4), Duchenne muscular dystrophy (n = 3), Becker muscular dystrophy (n = 3), and normal muscles (n = 4). All muscle samples were obtained for diagnostic purposes with informed consent.

**Immunocytochemistry on Muscle Biopsies**

Immunocytochemistry was performed on 8-μm-thick unfixed cryostat sections using standard protocols. The following primary antibodies were used: i) monoclonal anti-IGF-I (clone Sm1.2, Celltech, Italy), diluted 1/100; ii) polyclonal anti-PI3K subunit p110 (Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1/50; iii) polyclonal anti-Akt1/2 (Santa Cruz Biotechnology), diluted 1/50; iv) monoclonal anti-developmental myosin (YLEM, Newcastle upon Tyne, UK), diluted 1/100; v) monoclonal anti-SMI 31 (Sternberger Monoclonals, Lutherville, MD) diluted 1/1,000; and vi) monoclonal anti-Aβ (Biosource International, Camarillo, CA), recognizing residues 1–16 of the protein, diluted 1/100. The reaction with all primary antibodies was performed for 16 to 18 hours at 4°C in a humid chamber, followed by incubation with the appropriate secondary antisera.

**TABLE**

<table>
<thead>
<tr>
<th>Nonregenerating IGF-I-positive muscle fibers (mean ± SE)</th>
<th>p value sIBM vs control myopathies</th>
</tr>
</thead>
<tbody>
<tr>
<td>sIBM (n = 7)</td>
<td>16.2 ± 2.5%</td>
</tr>
<tr>
<td>Dermatomyositis (n = 5)</td>
<td>3.2 ± 2.3%</td>
</tr>
<tr>
<td>Polymyositis (n = 5)</td>
<td>0%</td>
</tr>
<tr>
<td>Muscular dystrophies (n = 6)</td>
<td>0%</td>
</tr>
</tbody>
</table>
Fig. 3. In situ hybridization studies. A, B: sIBM: An abnormal muscle fiber bearing Aβ cytoplasmic inclusions (A, peroxidase immunocytochemistry) shows increased signal for IGF-I mRNA by in situ hybridization (B). Original magnification: ×100. C, D: Polymyositis: Small regenerating muscle fiber with increased IGF-I mRNA (C, in situ hybridization) and protein (D, Texas red immunofluorescence) expression. Original magnification: ×400.

For quantitative analysis, the relative amount of immunopositive fibers was evaluated in at least 500 muscle fibers from 4 representative microscopic fields of each experimental section.

Human IGF-I cDNA and 35S-Labeled cRNA Synthesis

The human IGF-I cDNA was generated by PCR from total human cDNA using the following primers: forward 5'-TGC TCT CAA CAT CTC CCA TCT CTC-3'; reverse 5'-CAG CCC GAG TTG TGT AGA AAG AAG-3' (Genbank accession number M27544). The 999 base pair PCR product was cloned into the pPCR-script plasmid (Stratagene, La Jolla, CA). Sense and antisense 35S-labeled cRNA probes were then generated using the Riboprobe In Vitro Transcription System (Promega, Madison, WI), according to the manufacturer’s instructions.

In Situ Hybridization

In situ hybridization was performed in 8-μm-thick cryostat sections according to previously published protocols (24) using sense or antisense 35S-labeled IGF-I cRNA probes at the final activity of 800,000 cpm/μl. After being coated with NBT-2 photographic emulsion (Eastman Kodak, Rochester, NY), the slides were kept at 4°C in the dark and developed after 4 to 7 weeks.

Primary Muscle Cultures

Human primary muscle cultures were obtained from the biopsies of 5 healthy individuals using the explantation re-explantation method (25). The fusion of confluent mononucleated myoblasts into multinucleated myotubes was obtained using a culturing medium with 5% fetal bovine serum (differentiation medium) for 5 days. Fusion index was expressed as number of myonuclei/number of total nuclei, visualized by Hoechst 33258 staining (Molecular Probes Inc., Eugene, OR), and varied between 0.8 and 0.9 for all the culture sets used in this study. Each set of cultured myotubes was treated for 24 hours with the Aβ(25–35) peptide (Bachem AG, Bubendorf, Switzerland) corresponding to the biologically active domain of Aβ, at the concentration of 25 μM and after being aged in differentiation medium at 37°C for 5 days as previously described (26). Sister cultures treated for the same amount of time with the corresponding scrambled and biologically inactive peptide Aβ(35–25) (Bachem AG) were used as controls.

mRNA Expression Studies on Muscle Cultures

Total RNA was extracted from experimental cultures before treatment (time 0) and at 6, 12, 18, and 24 hours during stimulation with Aβ(25–35) or Aβ(35–25) using Trizol reagent (Invitrogen, Carlsbad, CA). One μg of total RNA was used for reverse transcription using the oligo-dT_{12–18} primer and the SuperScript® II Reverse Transcriptase (Invitrogen), according to the manufacturer’s instructions. Total cDNAs were used for semiquantitative PCR analysis of IGF-I transcript with the pair of primers described above. The housekeeping gene Glyceraldehyde-3-phosphate dehydrogenase (GAPD) was also amplified and used as internal control. For quantitative real-time PCR
Western Blot Analysis on Muscle Cultures

Proteins were extracted from experimental cultures at the same time points described above during treatment with Aβ(25–35) or Aβ(35–25) using RIPA buffer supplemented with protease inhibitors. Fifty μg of proteins were loaded in each well of a 12% polyacrylamide gel, separated by electrophoresis, and blotted onto a nitrocellulose membrane (Schleicher & Schuell, Relliehausen, Germany) using standard protocols. Blots were incubated overnight using one of the following antibodies: i) monoclonal anti-IGF-I (Biosource International) diluted 1/200; ii) polyclonal anti-Akt1/2 (Santa Cruz Biotechnology) diluted 1/200; and iii) polyclonal anti-phospho-Akt (Cell Signaling Technology Inc., Beverly, MA), recognizing the active form of Akt that is phosphorylated at Thr-308 and Serine 473 (23), diluted 1/500. After being incubated with the appropriate peroxidase-conjugated secondary antibody, blots were developed using the ECL Western Blotting Analysis System (Amersham Biosciences, Piscataway, NJ). For quantitative study, each blot was then probed with a monoclonal anti-muscle specific Actin antibody (YLEM) diluted 1/2,000. Densitometry on autoradiographic films was carried out using the TotalLab 2.01 software (Nonlinear Dynamics Ltd., Newcastle upon Tyne, UK).

Immunocytochemistry on Muscle Cultures

Muscle cultures were grown on glass coverslips placed on the bottom of Petri dishes. At each time point during treatment with Aβ peptides, coverslips were collected from the culture dish. This allowed evaluating changes in the expression of IGF-I protein in the same culture at different moments during the experimental procedure. Cultures were incubated with the monoclonal anti-IGF-I antibody (Clone sm1.2, Celltech) diluted 1/20 overnight at 4°C. Detection of immunocomplexes was performed using a fluorescein isothiocyanate (FITC)-conjugated anti-mouse antibody (Amersham Biosciences). Cultures were photographed using the same exposure time to allow a correct comparison of staining intensities.

Statistical Analysis

All data were expressed as mean ± SE. Analysis of repeated measures was performed by ANOVA and comparisons between groups were assessed by Student t-test. A p value ≤ 0.05 was considered significant.

RESULTS

IGF-I Expression in sIBM and Other Muscle Disorders

In the 7 sIBM muscle biopsies, IGF-I expression was increased in 16.2% ± 2.5% of nonregenerating muscle fibers (identified by the lack of immunoreactivity for developmental myosin), mainly in the form of a diffuse cytoplasmic staining, presence of cytoplasmic granular deposits, or both (Fig. 1A, C, E, G, I, K, M). These included both vacuolated (48.4% ± 5.1% of vacuolated muscle fibers had increased immunoreactivity for IGF-I) and nonvacuolated muscle fibers. On serial sections, 60% to 70% of nonregenerating muscle fibers with increased IGF-I expression showed the presence of typical Aβ-immunoreactive cytoplasmic inclusions (Fig. 1H–K). On serial sections, all IGF-I-immunopositive muscle fibers also showed increased expression of PI3K and Akt (Fig. 1L–O).

In sIBM and other myopathies, regenerating muscle fibers (identified by a strong and diffuse expression of developmental myosin) had increased cytoplasmic immunoreactivity for IGF-I and the downstream factors PI3K and Akt (Fig. 2A–D).

Among non-sIBM inflammatory myopathies, only in dermatomyositis muscle biopsies a minority of nonregenerating fibers showed IGF-I-immunopositive granular cytoplasmic deposits (3.2% ± 2.3%, p < 0.05 when confronted with sIBM; Table), whereas in polymyositis and in muscular dystrophies included in this study, increased IGF-I expression was exclusively localized in regenerating muscle fibers (Table). No evidence of increased expression of IGF-I was found in acute and chronic neurogenic disorders and in normal muscle biopsies (Fig. 2E–G).

In situ hybridization studies showed that in sIBM, the majority of IGF-I-immunopositive nonregenerating muscle fibers also had increased signal for IGF-I mRNA. In 70% of abnormal muscle fibers, increased IGF-I mRNA correlated on serial sections with the presence of Aβ immunoreactive cytoplasmic inclusions (Fig. 3A, B). IGF-I mRNA also resulted in increased regenerating fibers in all myopathies (Fig. 3C, D). Hybridization with the sense-strand IGF-I cRNA probe produced only a faint and uniform background signal.
IGF-I mRNA and Protein in Primary Muscle Cultures

The evidence of colocalization of Aβ immunoreactive inclusions and increased IGF-I mRNA and protein in sIBM abnormal muscle fibers prompted us to further investigate in vitro a possible relationship between Aβ toxicity and IGF-I expression. Semiquantitative RT-PCR analysis in primary muscle cultures treated with 25 μM Aβ(25–35) showed that IGF-I mRNA expression was sharply increased at 6 hours. By 12 hours, IGF-I expression began to decline and by 24 hours returned to a level comparable to that found at time 0 (Fig. 4A). Quantitative real-time RT-PCR analysis of IGF-I transcript in Aβ(25–35)-treated muscle cultures confirmed a peak of expression at 6 hours, with a 7.1 ± 2-fold increase compared to the level at time 0 (p = 0.018; n = 5), and a progressive reduction thereafter (3.8 ± 1.3, 2.2 ± 0.6, and 2 ± 0.3-fold increase over time 0 level at 12, 18, and 24 hours respectively; Fig. 4B).

To determine whether changes in IGF-I mRNA levels were reflected by parallel changes in IGF-I protein, Western Blot analysis was performed on total proteins extracted from Aβ(25–35)- and Aβ(35–25)-treated muscle cultures. After 6 hours of treatment with Aβ(25–35), IGF-I protein expression in cultured myotubes was 2.1 ± 0.1 times higher than the baseline level (p = 0.002; n = 5) and progressively declined thereafter: 1.5 ± 0.3, 1.4 ± 0.1, and 1.2 ± 0.3-fold increase over time 0 level at 12, 18, and 24 hours, respectively (Fig. 5A, B). Treatment with the inactive Aβ(35–25) peptide did not result in significant changes of expression of both IGF-I mRNA and protein throughout the 24 hours (Figs. 4A, B, 5A, B). In agreement with the results of Western Blot analysis, treatment with Aβ(25–35) induced an increased IGF-I cytoplasmic immunoreactivity in myotubes collected between 6 and 12 hours compared to myotubes isolated at time 0. Accordingly, the intensity of IGF-I immunosignal in myotubes studied at 18 at 24 hours declined to a level...
Fig. 5.  A: Western Blot analysis on experimental muscle cultures. After 6 hours of treatment with Aβ(25–35), IGF-I protein level is increased compared to time 0. Similar to what is observed for IGF-I mRNA expression profile throughout the 24 hours of treatment, IGF-I protein level progressively declines starting at 12 hours and reaches a level comparable to the baseline between 18 and 24 hours. No change of IGF-I protein expression is observed after treatment with Aβ(35–25). The muscle-specific Actin bands confirm an equal loading of all samples. B: Graph representation of the averaged values of IGF-I protein expression in 5 different experimental sets of cultures during the 24 hours treatment with Aβ(25–35) and Aβ(35–25). C: Fluorescein isothiocyanate immunocytochemistry on Aβ(25–35)-treated cultures shows increased IGF-I expression in myotubes collected between 6 and 12 hours. The intensity of IGF-I immunosignal in myotubes studied at 18 at 24 hours declines to a level comparable to that seen at time point 0. Original magnification: ×200. D: Western Blot analysis of phosphorylated and total Akt on experimental muscle cultures. In Aβ(25–35)-treated cultures at each time point, the level of phosphorylated Akt parallels that of IGF-I (A, upper panel), whereas the level of total Akt is unmodified throughout the 24 hours of treatment.
comparable to that seen at time point 0 (Fig. 5C). Only a faint staining could be observed in fibroblasts, thus confirming that muscle cells primarily produced the protein detected on Western blots.

To verify whether the increased expression of IGF-I was indeed associated with activation of the metabolic pathway leading to the phosphorylation of the downstream factor Akt, both total and phosphorylated forms of this protein were studied on total protein extracts. Two distinct antibodies were used: one recognizing both phosphorylated and nonphosphorylated forms of Akt and one directed against p-Akt
\textsuperscript{Thr-308}. Western blot analysis showed an increase of p-Akt
\textsuperscript{Thr-308} at 6 hours after treatment with Aβ(25–35) and a gradual reduction thereafter, whereas no change of total Akt level was observed (Fig. 5D).

**DISCUSSION**

In recent years, a growing body of evidence has pointed out a potential role of IGF-I signaling in the pathogenic scenario of various neurodegenerative disorders (15–19). For example, hippocampal neurons in AD show increased expression of IGF-I (15), and it has been demonstrated in a mouse model of the disease that this growth factor has a direct role in reducing the burden of Aβ from the brain (16). A possible derangement in IGF-I signaling has also been found in the spinal cord of patients affected by ALS, a disorder characterized by the progressive degeneration of ventral horn motor neurons (19). Despite the fact that IGF-I plays a key role in many aspects of muscle physiology (11–14), little information is available about its possible involvement in the pathogenic mechanisms of human primary muscle disorders.

In the present study, using immunocytochemistry and in situ hybridization, we showed increased IGF-I expression in regenerating muscle fibers in all myopathies. This is in agreement with previous reports showing a role of IGF-I throughout the entire process of muscle repair and regeneration, from the recruitment of quiescent satellite cells within the basal lamina to the maturation of new multinucleated myofibers (12, 13).

We found IGF-I protein overexpression in a considerable amount (16.2% ± 2.5%) of nonregenerating fibers in muscle biopsies from 7 sIBM patients. The majority of these fibers also showed upregulation of IGF-I mRNA, suggesting that IGF-I overexpression was mainly due to increased transcription, mRNA stabilization, or both. In addition, the same fibers also displayed increased expression of the other members of the IGF-I signaling system (PI3K and Akt), suggesting that within sIBM abnormal muscle fibers, IGF-I acts mainly through an autocrine mechanism. It appears that the activation of the IGF-I signaling system in sIBM is not simply consequent to nonspecific myopathic abnormalities or to inflammation as in all muscular dystrophies (which showed the highest degree of myopathic changes), and in polymyositis (which has in common with sIBM the presence of CD8+ invading T-cells and an increased expression of various pro-inflammatory cytokines [4]) IGF-I upregulation was restricted to regenerating muscle fibers. The possibility exists that in sIBM nonregenerating abnormal muscle fibers, IGF-I upregulation may be secondary to the increased production and accumulation of Aβ, as suggested by the evidence that muscle fibers bearing Aβ-immunoreactive cytoplasmic inclusions also presented increased IGF-I mRNA and protein. The experimental results of our in vitro model appear to support this hypothesis. In fact, in cultured myotubes, treatment with the Aβ(25–35) peptide resulted in upregulation of both IGF-I mRNA and protein at 6 hours, with a gradual reduction thereafter. The simultaneous increased phosphorylation of Akt in our model was probably triggered by IGF-I through the activation of an autocrine signaling pathway. It does not appear that this profile of IGF-I expression is specific to our in vitro cell system, as similar results have been reported in vascular smooth muscle cells using different stressors (28). The mechanisms leading to increased IGF-I in sIBM and in myotubes treated with the Aβ peptide are not known, but oxidative stress may play an important role. The presence of numerous markers of increased oxidative stress has been demonstrated in sIBM abnormal muscle fibers (29–31). In vitro, treatment of cultured myotubes with the Aβ peptide results in increased expression of inducible nitric oxide synthase and production of the free radical nitric oxide, a short-lived gas known to promote the formation of highly reactive oxidizing molecules (32, 33). Moreover, reactive oxygen species are able to stimulate the synthesis of IGF-I mRNA and protein in vascular smooth muscle cells (28).

We are aware of the fact that our in vitro model raises the question of a possible pathogenic role of extracellular Aβ. In sIBM the majority of Aβ deposits are within the muscle fibers. However, it has been shown that in highly degenerated sIBM fibers, Aβ deposits may reach the extracellular space possibly through breaks in the plasma membrane. Moreover, by immuno-electronmicroscopy, Aβ-immunoreactive material has been detected also outside the muscle fibers in association with collagen fibrils (34). Similar to AβPP-transfected myotubes that release an increased amount of Aβ molecules in the culture medium (35), in vivo muscle fibers overexpressing AβPP could also generate an extracellular pool of Aβ. Therefore, the possibility that in sIBM an additional toxic effect derives from extracellular Aβ cannot be excluded.

We also found increased IGF-I expression in a minority of nonregenerating muscle fibers (3.2% ± 2.3%) in dermatomyositis biopsies. In dermatomyositis muscle, the deposition within the microvascular bed of the C5b–9 membrane attack complex of complement suggests the presence of a complement-mediated pathogenic
mechanism (4, 36). The C5b–9 membrane attack complex is able to induce increased expression of IGF-I and upregulates IGF-I binding sites in vascular smooth muscle cells (37), and therefore it is possible that such mechanisms is responsible for the overexpression of IGF-I in a subpopulation of nonregenerating muscle fibers in dermatomyositis. However, additional studies are needed to verify whether this and/or other dermatomyositis-specific pathogenic mechanisms may contribute to IGF-I overexpression.

In sIBM it is possible that IGF-I induces a cascade of molecular events in the attempt to restore cellular homeostasis in vulnerable fibers. This effect may be mediated by different mechanisms, including i) activation of signaling pathways known to promote cell survival, ii) reduction of tau protein hyperphosphorylation, and iii) modulation of the immune response.

We found that IGF-I upregulation was accompanied by an increased expression of PI3K and Akt, indicating the activation of a signaling pathway known to promote cell survival (23, 38). This may happen in part through the activation of the nuclear factor-κB (NF-κB) and members of the mitogen activated protein kinases (MAPKs) (22, 39–41). Indeed, both NF-κB ad members of the MAPKs family have been found upregulated in sIBM muscle (42–44).

In cultured neurons it has been demonstrated that the IGF-I-induced activation of the PI3K/Akt pathway regulates phosphorylation of the microtubule-associated protein tau through the inhibition of glycogen-synthase kinase 3 (45). In sIBM muscle the characteristic SMI-31-immunoreactive cytoplasmic inclusions are mainly composed of hyperphosphorylated tau protein abnormally aggregated into paired helical filaments (PHFs) (46). Therefore, it is possible that increased IGF-I represents an attempt of the muscle fibers to counteract the formation of PHFs by reducing the abnormal phosphorylation of tau.

Finally, IGF-I overexpression may also be relevant in other aspects of sIBM pathology. In fact, IGF-I is able to mediate both T lymphocyte growth and chemotaxis (as these cells bear functional IGF-I receptors [47]) and to promote the production of superoxide anion and pro-inflammatory cytokines by macrophages (48). IGF-I could therefore also contribute to the modulation of the immune response in sIBM muscle by improving host defense against a yet unknown putative antigen (29).

We can only speculate on the reasons why in sIBM muscle fiber degeneration steadily progresses in spite of the activation of IGF-I-mediated cellular defense mechanisms. It is possible that such response is unable to counterbalance the pathogenic mechanisms taking place in sIBM muscle for 2 reasons: i) the impairment of other intracellular signaling systems within sIBM abnormal muscle fibers, as previously proposed (44), may render a putative protective function of IGF-I less effective, or ii) a parallel increased expression of IGF-I binding proteins, probably promoted by the action of pro-inflammatory cytokines such as tumor necrosis factor-α and interleukin-1β (48), results in reduced levels of free and metabolically active IGF-I. However, additional studies are necessary to further clarify this issue. Nevertheless, understanding the molecular signaling pathways activated by IGF-I in sIBM muscle may open the way to possible novel therapeutic strategies for this disease.

ACKNOWLEDGMENTS

The authors thank Ms. Sabrina de Simone for technical support.

REFERENCES


Received December 29, 2003
Revision received March 8, 2004
Accepted March 11, 2004