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Characterization of a Rabbit Antihuman Mechano Growth Factor (MGF) Polyclonal Antibody against the Last 24 Amino Acids of the E Domain

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Abstract. *The human insulin-like growth factor-1 (IGF-1) gene gives rise to multiple, heterogeneous mRNA transcripts by alternative splicing, thus producing different IGF-1 isoforms. The mechano growth factor (MGF) is an IGF-1 isoform that was found to be markedly up-regulated in exercised or damaged muscle. The specific E domain of the MGF splice variant may act as an independent growth factor. The aim of the present study was to characterize a rabbit antihuman MGF polyclonal antibody. New-Zealand rabbits were immunized by injections of a purified synthetic peptide corresponding to the last 24 amino acids of the human C-terminal of the MGF E domain. Western blotting and immunohistochemical techniques were used to characterize the specificity of the polyclonal anti-MGF antiserum. The anti-MGF antiserum was found to recognize the MGF E-peptide and not the common part of the IGF-1 isoforms, i.e. the mature IGF-1 peptide. Furthermore, it specifically bound to the MGF protein in human skeletal and in rat cardiac muscle, apparently due to the considerable homology between the human and rat MGF E-peptide sequences. Immunostaining analysis showed that this polyclonal anti-MGF antibody was able to detect MGF in human muscle and in rat cardiomyocytes and vessels' smooth muscle cells. We conclude that this rabbit polyclonal anti-human/rat MGF antibody could become a valuable tool in the study of IGF-1 isoforms in human and rat tissues.*

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The human insulin-like growth factor-1 (IGF-1) gene spans a region of about 90 kb of genomic DNA and contains six exons (1), which give rise to multiple, heterogeneous mRNA transcripts by an elaborate alternative splicing whereby exons are spliced in different combinations from the primary RNA transcript (2, 3). Alternative splicing results in different IGF-1 isoforms that contain exon 5, generally classified as IGF-1 Eb, or exon 6 classified as IGF-1 Ea (4, 5), while a third isoform, the IGF-1 Ec, contains both exon 5 and 6 (6), (Figure 1).

The different IGF-1 mRNA transcripts encode several IGF-1 precursor proteins, which differ by the length of the amino-terminal (signal) peptide and the structure of the extension peptide (E-peptide) on the carboxy-terminal end (7, 8). The mature IGF-1 peptide, encoded by exons 3 and 4, is a 7.6-kDa, or 70-amino acid long single chain peptide resulting from post-translational cleavage of precursor polypeptide, by which the signal and the E-peptide are removed. The 5' end of exons 1, 2 and 3 encodes for the signal peptide of the prohormone (precursor IGF-1). The first 16 amino acids of the E-peptide (*i.e.* the amino-terminal portion of the extension peptide), which are common to all three different IGF-1 E-peptides, are coded by exon 4 while exons 5 and 6 encode, by alternative splicing, distinct portions of the E-peptide (called the E domain) with alternative carboxy-terminal sequences of the extension peptide (6). The IGF-1 Ec splice variant is an exon 4-5-6 variant, firstly identified in human liver (6) but also in skeletal muscle (9). It contains exon 4, only 49 bp from exon 5 and then exon 6, and produces an E-peptide, termed as Ec-peptide, with a predicted length of 40 amino acids, *i.e.* 16 amino acids from exon 4, 16 from exon 5 and 8 amino acids from exon 6 (6, 8). Hence, it results in a different carboxy (*C*)-terminal peptide sequence of the IGF-1 Ec isoform due to a reading frame shift, which leads to a different mode of action compared with the IGF-1 Ea or IGF-1 Eb isoforms (9-11). The predicted

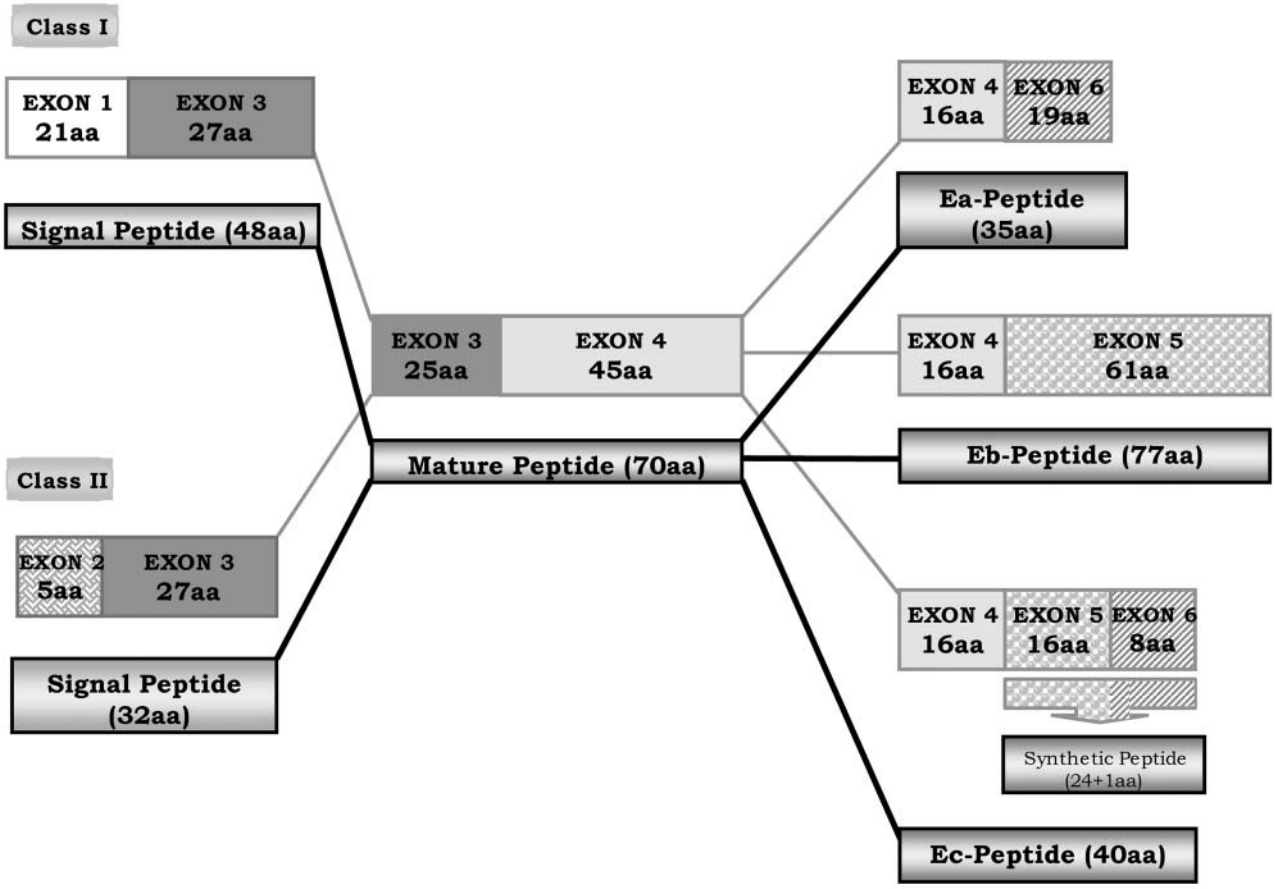

Figure 1. *Human IGF-1 alternative splicing and encoded precursor peptides in skeletal muscle (see text for details).*

prepro-IGF-1 Ec isoform (precursor) polypeptide, based on the amino acid sequence derived from its mRNA transcript (6, 12), is a molecule of 15.6 kDa. This isoform is named mechano growth factor (MGF) to reflect its mechanosensitive qualities, since it was found to be markedly up-regulated in exercised and damaged skeletal muscle (2, 13-16).

Currently, the existing studies provide information on MGF expression mainly at the mRNA level, and, as yet, there is only little information regarding the identification of the MGF peptide *in vivo* (17, 18), especially in muscle (19). The development of epitope-specific antibodies for distinguishing the different IGF-1 peptides is needed for the identification and differential quantitation of the IGF-1 isoforms at the protein level. The latter has prompted investigators to develop polyclonal (17, 19) or monoclonal (18) antibodies against human MGF. Herein, we present our work on the production and characterization of a polyclonal antibody against the human MGF which specifically recognizes the MGF E domain in human skeletal and in rat cardiac muscles.

Materials and Methods

MGF E-peptide synthesis. A part of the MGF E-peptide, predicted from the human cDNA sequence of the human IGF-I Ec isoform (6), was synthesized. It corresponded with the last 24 amino acids of the *C*-terminal of the E-peptide of MGF (exons 5 and 6) with the addition of a cysteine to its *C*-terminal in order to couple it with a keyhole limpet haemocyanin (KLH). The use of KLH was as a hapten carrier in order to elicit a better immune response from the conjugated synthetic peptide, as KLH is an extremely potent antigen (20). The MGF E-peptide was synthesized by Eastern Quebec Proteomic Core Facility (Ste-Foy, Quebec, Canada); it was purified to >90% by high performance liquid chromatography (HPLC) and its amino acid sequence was analysed by mass spectrometry and HPLC. The nucleotide and amino acid sequences of the synthetic peptide are shown in Figure 2 and it was predicted to be of 2,967 Da. The human MGF E-peptide (Ec peptide) shares 73% homology with the rat MGF E-peptide (termed Eb peptide in rodents), which is its potential counterpart (6). The corresponding part of the rat E-peptide, with respect to the human E-peptide that was synthesized, contains the last 25 amino acids of the *C*-terminal of rat E-peptide of MGF (also exons 5 and 6) and its nucleotide and amino acid

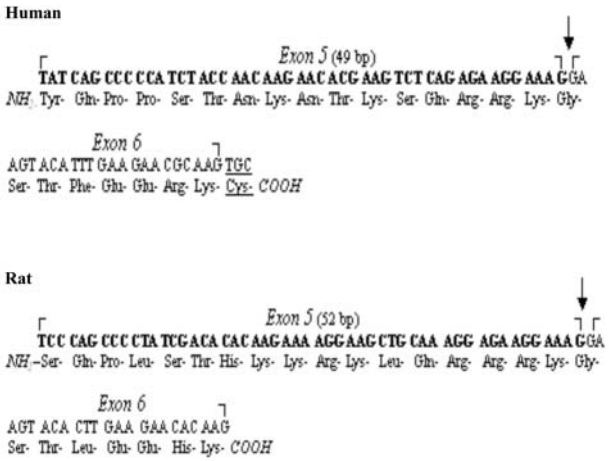

Figure 2. *Human amino acid sequence of the synthetic MGF E-peptide aligned with its nucleotide (cDNA) sequence, corresponding to exon 5 and 6. Note that because 49 is not a complete multiple of 3, the last nucleotide of exon 5 forms a triplet (codon) with the next two nucleotides of exon 6 (see arrow), resulting in a reading frame shift that finally introduces an early stop codon in exon 6 of the MGF E-peptide. Cysteine, added to the C-terminal of the synthetic MGF E-peptide, is underlined. In addition, we present the rat amino acid and nucleotide (cDNA) sequences, corresponding to the synthetic part of human MGF E-peptide used in the immunization protocol.*

sequences (6) are also shown in Figure 2. Different concentrations of the synthetic peptide were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) in a 20% acrylamide resolution gel and subsequent staining of the gel with Coomassie blue.

Immunization of rabbits against the synthetic MGF E-peptide. New-Zealand white rabbits (NZW) (n=3, female, aged 3 months, approximately 2.5 kg mass), were immunized by three injections of 1 mg of MGF E-peptide-KLH conjugate in 0.5 ml phosphate-buffered saline (PBS) to each animal, administered subcutaneously to multiple sites, at 28-days intervals. The first immunization (day 0) was performed with the addition of 0.5 ml of complete Freud's adjuvant. The second immunization, to boost the rabbits, was performed with incomplete Freud's adjuvant, as well as the third one, which was also performed with incomplete Freud's adjuvant. Blood samples from the rabbits were obtained immediately before (on day 0) and on days 42 and 70 after the first immunization and serum was subsequently collected. The animals were sacrificed on day 70 and blood was collected by heart puncture. The immunization process was carried out according to the guidelines for the use of laboratory animals and of the local Ethics Committee for animal research.

Characterization of the anti-MGF antiserum to MGF E-peptide. Immunodetection of the synthetic MGF E-peptide was performed using the anti-MGF E-peptide antiserum raised in rabbits. Western blotting experiments were used to determine antiserum specificity to the E-peptide of MGF.

Samples containing different concentrations (*i.e.* 0.1, 0.2, 0.4, 0.6, 0.8, 1 μg) of the synthetic MGF E-peptide non-coupled to KLH, as well as 0.2 μg of recombinant human IGF-1 used as negative control (a 7.6 kDa protein corresponding to mature IGF-1 peptide; Chemicon International Inc., Temecula, CA, USA), were subjected to SDS-PAGE (20% acrylamide resolution gel) and then electrophoretically transferred to a polyvinylidene fluoride (PVDF) membrane. The blot was incubated with a blocking solution containing 5% (v/v) nonfat milk powder in TBS-T (TBS/0.1% Tween 20) for 1 h at room temperature and, after three washes with TBS-T, it was incubated with the rabbit polyclonal anti-MGF antiserum (dilution 1:10,000) in TBS-T overnight at 4˚C with gentle shaking. Following three washes with TBS-T, the membrane was incubated with horseradish peroxidase-conjugated secondary anti-rabbit IgG (goat anti-rabbit, dilution 1:2,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) in TBS-T for 1 h at room temperature. Following three washes with TBS-T, bands were visualized by exposure of the membrane to X-ray film after incubation with an enhanced chemiluminescent (ECL) substrate (SuperSignal, Pierce Biotechnology, Rockford, IL, USA).

Controls for the specificity of the reactions obtained were performed by replacement of the primary anti-MGF antiserum by non-immune rabbit serum and by pre-absorption (neutralization) of the primary antiserum with different concentrations of the MGF synthetic peptide (*i.e.* 0.04, 0.4, 1.6, 4 and 20 μg/ml diluted anti-MGF antiserum) for 2 h at room temperature. In the pre-absorption control procedure, 0.2 μg of the MGF-synthetic peptide was loaded in each of the five different sample wells of a BioRad Mini Trans-blot device (BioRad, Hercules, CA, USA), subjected to SDS-PAGE (20% acrylamide resolution gel) and electrophoretically transferred to a PVDF membrane. The blot was incubated with a blocking solution, as described above, and then cut into five strips, each of which was subsequently incubated with one of the above five differentially neutralized anti-MGF antisera overnight at 4˚C. Thereafter, the incubation procedure with the horseradish peroxidase-conjugated secondary anti-rabbit IgG was followed as described above, and bands were again visualized by simultaneous exposure of the five membrane strips to x-ray film after their incubation with the ECL substrate.

Characterization of the anti-MGF antiserum in human skeletal and in rat cardiac muscles. Due to the considerable homology that human MGF E-peptide shares with that of the rat, the anti-MGF specificity of the obtained antiserum was further characterized not only in human skeletal muscle but also in rat myocardium, by using Western blotting and immunohistochemical analysis.

More specifically, a skeletal muscle biopsy was obtained from the middle portion of the vastus lateralis muscle of one male volunteer (age: 22 years, height: 183 cm, mass: 75 kg) under local anesthesia by using the percutaneous needle biopsy technique (21) with applied suction. The volunteer gave his informed consent and participated in the study that had been approved by the Ethics Committee of National and Kapodistrian University of Athens. For the cardiac muscle biopsy, a male Wistar rat (age: 3 months, mass: 300 g) was sacrificed under total anaesthesia and following death a small part of its heart was rapidly excised. The University's Animal Ethics Committee also approved the animal manipulations. In both cases, the obtained muscle samples were quickly dipped on a gauze swab, to remove superficial blood, and divided into two pieces. The first piece was quick-frozen in liquid nitrogen for use in Western blotting analysis. The second piece was orientated longitudinally and fixed in formaldehyde (10% final concentration) for subsequent immunohistochemical analysis.

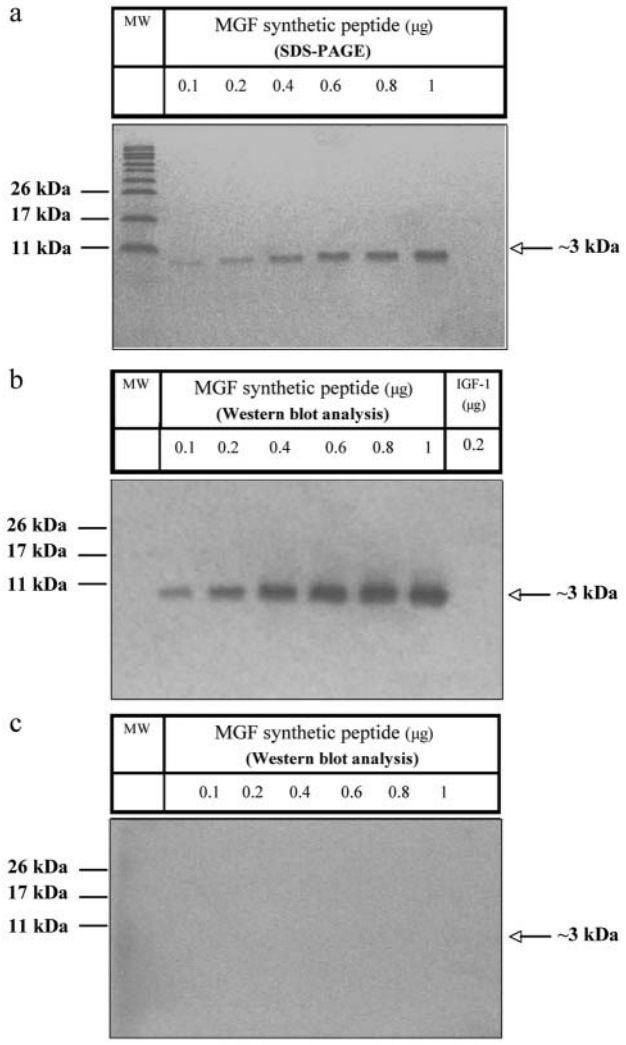

Figure 3. *(a) Coomassie blue staining of electrophoresed increasing concentrations of the synthetic MGF E-peptide analyzed by SDS-PAGE. A single band was labelled at the peptide's predicted molecular weight (i.e. ~3 kDa; arrow). (b) SDS-PAGE and immunodetection of increasing concentrations of the synthetic peptide by the anti-MGF antiserum gave only one band at the appropriate molecular weight in Western blot analysis, reproducing the results from Coomassie blue staining, while no binding was detected using the recombinant human IGF-1 (i.e. the mature peptide of IGF-1). (c) No MGF E-peptide immunoreactivity was detected when the primary anti-MGF antiserum was replaced by non-immune pre-immunization rabbit serum.*

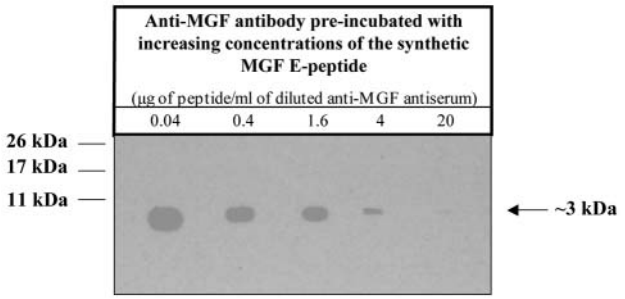

Figure 4. *SDS-PAGE and Western blot analysis using 0.2 μg of the synthetic peptide with the anti-MGF polyclonal antibody (1:10,000) pre-incubated with increasing concentrations of the MGF synthetic peptide. Note that the immunoreactions obtained were reduced by pre-absorption (neutralization) of the anti-MGF antibody with increasing concentrations of the MGF synthetic peptide.*

For the Western blotting, total proteins were extracted from the muscle samples using Trizol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's recommendations for protein isolation, and the protein concentration of samples was assessed by the Bradford method (22). Thereafter, increasing concentrations of muscle total protein extracts (*i.e.* 0.25, 0.5, 1, 2, 4 and 8 μg from the human skeletal muscle sample and 10, 30, 50 and 70 μg from the rat cardiac muscle), as well as 0.1 μg of the synthetic MGF E-peptide used as a positive control, were subjected to SDS-PAGE (16% acrylamide resolution gel) and then electrophoretically transferred to two different PVDF membranes. Thereafter, the same procedure, as described above, was followed and bands were again visualized by exposure of the membranes to x-ray film after their incubation with the ECL substrate. The specificity of the reactions obtained in Western blot analyses were determined by labeling a single and specific band by the anti-MGF antiserum with increasing concentrations of muscle total protein extracts subjected to SDS-PAGE and by replacement of the primary anti-MGF antiserum by non-immune rabbit serum.

For the immunohistochemical analysis, formaldehyde-fixed skeletal and cardiac muscle samples were paraffin wax embedded and processed for paraffin sections. Microtome sections of 3 μm were prepared from the paraffin-fixed samples, allowed to adhere to glass slides, dried at 37˚C overnight, dewaxed in xylene and rehydrated in serial dilutions of ethanol. Endogenous peroxidase activity was quenched with 1% hydrogen peroxide in distilled water for 15 minutes. After two serial washings in distilled water and PBS buffer, the sections were then incubated with the polyclonal anti-MGF antiserum at a dilution of 1:1,000 in PBS overnight at 4˚C. After repeated PBS buffer washing, secondary biotinylated goat anti-rabbit igG (Dako Real EnVision) was added for 25 minutes at room temperature, followed again by repeated PBS buffer washes. Visualization of the immunocomplex was obtained by incubating the sections in a solution of 3,3-diaminobenzidine (DAB, Dako Real EnVision Glostrup, Denmark) in PBS for 10 minutes. Sections were stained in hematoxylin for 5 minutes, washed in distilled water, dehydrated in serial dilutions of ethanol and xylene and finally mounted in dibutyl phthalate xylene. Tissue sections were visualized under light microscopy. Controls for the specificity of the reactions obtained in immunohistochemical analysis were performed by substituting the primary anti-MGF antiserum with the antibody diluent (PBS) minus the primary anti-MGF antiserum.

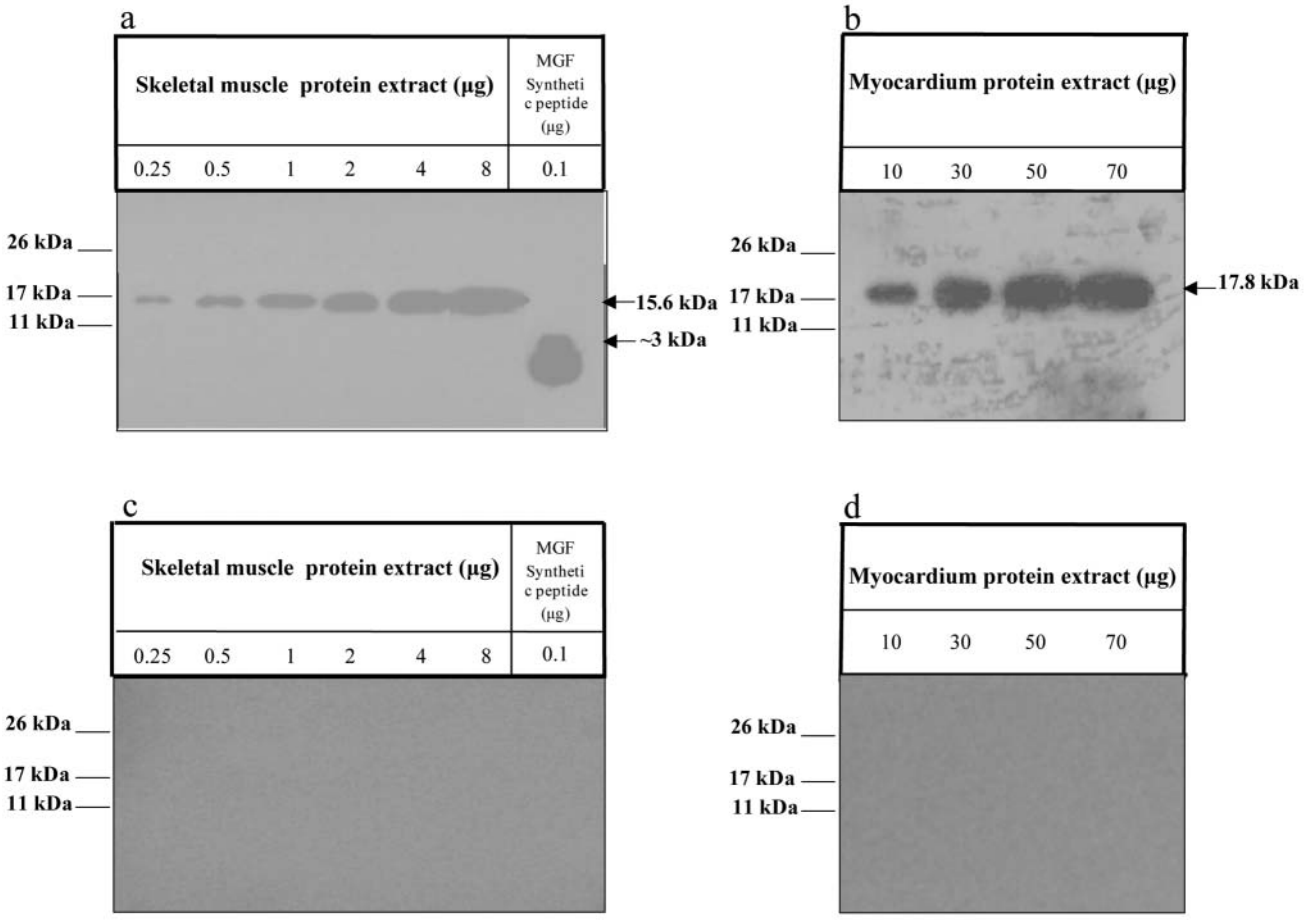

Figure 5. *(a) Western blotting immunodetection of MGF protein following SDS-PAGE using increasing concentrations of proteins extracted from human skeletal muscle. A single band at the appropriate molecular weight (15.6 kDa), with no cross-reacting bands, was detected. The synthetic MGF E-peptide is also shown as a positive control. (b) A single band was detected at the predicted molecular weight of the MGF protein in rat myocardium (17.8 kDa) using the anti-MGF antiserum in Western blot analysis. No MGF immunoreactivity was detected by non-immune rabbit serum using skeletal (c) and cardiac (d) muscle protein extracts.*

Results

MGF E-peptide verification. A single band was labelled at the appropriate (predicted) molecular weight by gel staining with Coomassie blue after SDS-polyacrylamide gel electrophoresis of increasing concentrations of the synthetic MGF E-peptide (Figure 3 a).

*Characterization of the anti-MGF antiserum to MGF E-peptide.*The antiserum raised in rabbits against the synthetic part of the E-peptide of MGF was demonstrated to be specific by Western blot analysis, since against increasing concentrations of the synthetic peptide it only gave one band at the appropriate molecular weight in accordance with the results from Coomassie blue staining, while no binding with the recombinant human IGF-1 (*i.e.* the mature peptide of IGF-1) was demonstrated (Figure 3 b). No MGF E-peptide immunoreactivity was detected when the primary anti-MGF antiserum was replaced by non-immune rabbit serum (Figure 3c). Moreover, the immunoreactions obtained were inversely reduced by pre-absorption of the primary anti-MGF antiserum with increasing concentrations of the MGF synthetic peptide (Figure 4).

Characterization of the anti-MGF antiserum in human skeletal and rat cardiac muscles. The anti-MGF antiserum was also demonstrated to be specific to precursor MGF protein in human skeletal muscle as well as in rat myocardium. A single band at the appropriate (expected) molecular weight (*i.e.* 15.6 and 17.8 kDa for human and rat samples respectively) (6), with no cross-reacting bands, was labeled by the anti-MGF antiserum against increasing concentrations of total protein extracts from the human skeletal and rat cardiac muscle subjected to SDS-PAGE (Figure 5 a, b). Furthermore, no

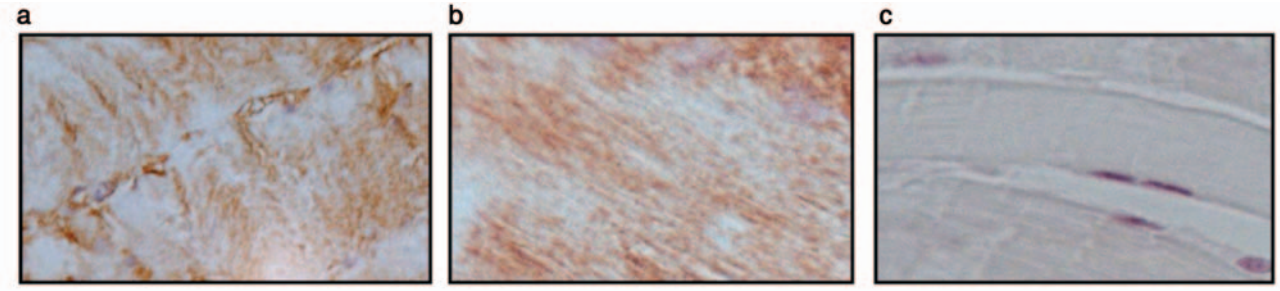

Figure 6. *Immunohistochemical analysis of human skeletal muscle using the anti-MGF antiserum (a, b; magnification x40). Note the positive (brown) cytoplasmic staining. Specificity of the immunohistochemical detection of MGF was confirmed by the absence of immunoreactivity in the control staining procedure using PBS buffer (c; magnification x40).*

MGF immunoreactivity was demonstrated when the primary anti-MGF antiserum was replaced by non-immune rabbit serum in Western blot analyses in either the skeletal or cardiac muscle samples (Figure 5 c, d).

Immunohistochemical analysis of muscle samples showed positive staining with the anti-MGF antiserum, which was localized mainly in the cytoplasm (Figure 6 a, b and 7 a, b). Interestingly, noticeable immunoreactions were also observed in smooth muscle cells of myocardium vessels (Figure 7 e, f). The specificity of the immunohistochemical detection of MGF was confirmed by the absence of immunoreactivity, *i.e.* no background staining was given, in the control staining procedures where the primary anti-MGF antiserum was substituted with PBS buffer (Figure 6 c and 7 c, d).

Discussion

The results of the present study documented that the polyclonal antibody against a synthetic part of the human MGF E domain specifically recognized MGF E-peptide in human skeletal muscle and in rat myocardium, apparently due to the high homology that human MGF E-peptide shares with that of the rat (6).

This specificity was expected since immunization was performed using a unique part of the E-peptide of MGF encoded by a distinct splicing sequence of exons 5 and 6 (Figure 2) that is specific to this IGF-1 isoform (6). Immunoreactivity was inversely reduced by pre-absorption of the anti-MGF antibody with increasing concentrations of the synthetic MGF E-peptide. Indeed, the affinity and specificity of this polyclonal antibody to MGF was confirmed by its failure to recognize the common part of the IGF-1 isoforms, *i.e.* the mature peptide of IGF-1. Moreover, the detection of a single band at the appropriate molecular weight(s) without any cross-reacting bands in skeletal and cardiac muscles, using Western blot analysis, strongly indicates that this anti-MGF antibody specifically recognizes the MGF protein in human skeletal muscle and in rat myocardium. Furthermore, immunohistochemical analysis,

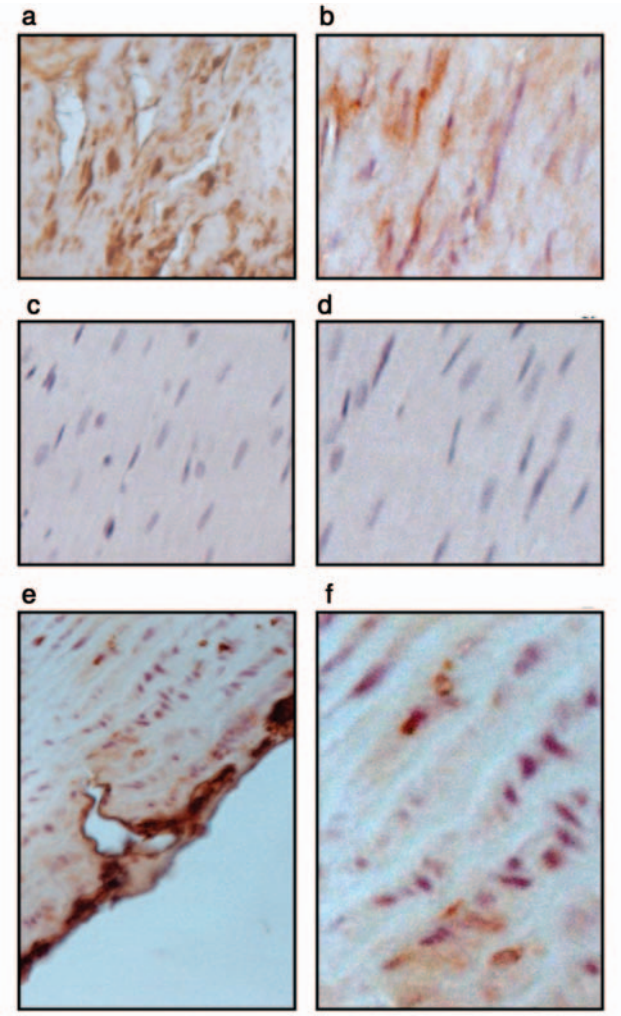

Figure 7. *Immunohistochemical analysis of rat cardiac muscle using the anti-MGF antiserum (a, b; magnification x20 and 40, respectively). Note the positive cytoplasmic staining. Interestingly, MGF immunostaining was also detected in smooth muscle cells of myocardium vessels (e, f; magnification x10 and 40, respectively). Specificity of the immunohistochemical detection of MGF in cardiac muscle sections was also confirmed by the absence of immunoreactivity in the control staining procedure using PBS buffer (c, d; magnification x20 and 40, respectively).*

using the polyclonal anti-MGF antibody, showed positive staining in human skeletal and in rat cardiac muscle cells. This MGF immunoreactivity was abolished when the primary anti-MGF polyclonal antibody was replaced by non-immune (*i.e.* pre-immunization) rabbit serum or by PBS in Western blot analysis and in immunohistochemistry.

The development of IGF-1 isoform-specific antibodies, *i.e.* raised against specific domains of the IGF-1 precursor polypeptides, constitutes an especially promising perspective, since one of the most attractive aspects recently developed is the potential differential role of these IGF-1 isoforms (2, 10, 16, 23-26) or, furthermore, of the isoform-specific peptides (27-30) in muscle physiology. Althought the biological significance of IGF-1 splice variants (isoforms) is currently under investigation and the molecular and physiological mechanisms that regulate their expression are unclear, it is conceivable that they probably mediate aspects of the complex IGF-1 actions in several tissues (31).

More specifically, alternative splicing results in different IGF-1 isoforms (4, 5), while the different transcription initiation sites of the IGF-1 gene also lead to two different classes of IGF-I isoforms: class 1 transcripts have their initiation site(s) on exon 1 (promoter 1), whereas class 2 use exon 2 as leader exon (promoter 2), (Figure 1). In human skeletal muscle, and also in other tissues or cell lines, alternative splicing of the IGF-1 gene also generates the IGF-1 Ec (MGF) isoform which corresponds to IGF-1 Eb in rodents (6). It results from a splice acceptor site in the intron preceding exon 6 and structurally its cDNA differs from the main systemic IGF-1 Ea isoform produced in liver by the presence of the first 49 base pairs from exon 5 (52 bp in the rat) (6). A differential mRNA expression of IGF-1 Ea and MGF isoforms was found following skeletal muscle damage in rats (2, 32) and humans (16), and it was postulated that IGF-1 isoforms act as different growth factors with different mRNA expression kinetics. MGF was found to be rapidly activated and subsequently depressed in damaged or overloaded muscles (27); therefore, it was suggested, both from *in vivo* and *in vitro* studies, that it is responsible for satellite cell activation (2, 23), for the prolongation of myogenic cell proliferation and for depression of their terminal differentiation into myotubes (16, 27). In contrast, IGF- Ea appeared to have a more delayed expression profile, increased the mitotic index, enhanced terminal differentiation and promoted fusion of the myogenic cells (2, 27). Although both, MGF and IGF-I Ea isoforms, appear to up-regulate protein synthesis in processes such as skeletal muscle regeneration and hypertrophy (2, 24), because they both share the same mature peptide which promotes protein synthesis, this role, however, is predominately mediated by IGF-I Ea, since most of the mature IGF-I peptide is derived from IGF-I Ea, whereas the E domain of the MGF peptide is thought to be involved in the activation of satellite cells (2, 16).

Furthermore, it has been proposed that the E-peptides of the IGF-1 precursors, which are proteolytically cleaved after post-translational processing by members of the subtilisin-related proprotein convertase family (33), may act as independent growth factors (34, 35). Indeed, a predicted synthetic MGF peptide, generated from a unique region within the E domain of MGF sequence (*i.e.* a part of Ec-peptide) was used *in vitro* and *in vivo* experiments with the C2C12 mouse myoblast cell line (27), and with human myogenic precursor cells (28, 29). These data documented that this distinct E domain of MGF enhanced proliferation and migration while it delayed or inhibited terminal differentiation of these muscle cell lines. However, the mature IGF-1 peptide was found to induce myoblast differentiation (27-29).

Interestingly, the MGF E domain acted *via* a different receptor, since blocking of the IGF-1 receptor with a specific antibody did not inhibit its action (17, 27-29). *C*-terminal MGF peptide lacks the domain present in the full-length IGF-1 precursor peptide which is responsible for the binding of the IGF-1 receptor (17). Moreover, using a proteomics approach it was reported that the specific E domain of MGF results in binding to a different binding protein and so points to a different mode of action compared to the IGF-1 Ea isoform (11, 36). Taking all the above findings into account, it was argued that MGF, and more specifically its E domain, acts as an independent autocrine factor in skeletal muscle (11, 17, 28, 29, 36).

Nevertheless, there is very little information regarding the endogenous MGF protein expression in skeletal or cardiac muscle and its regulation as a result of a local adaptation response to various stimuli, as has been found for MGF expression at the mRNA level (2, 10-15, 16, 24, 37-39). A positive immunostaining of levator ani muscle cells was found after the first vaginal delivery in women using an MGF specific antibody, indicating a significant up-regulation of MGF in this muscle probably as a result of vaginal delivery-induced muscle damage (19). There was also a report for MGF protein up-regulation in rabbit cardiac muscle following a pressure/volume overloading of myocardium and it was suggested that this reflects an adaptation response of myocardium to increased load (40). Our findings demonstrate the detection of the endogenous MGF protein expression both in human skeletal and in rat cardiac muscle by the rabbit antihuman MGF polyclonal antibody, analysed by both Western blotting and immunostaining.

The precise biological and functional characterization of IGF-1 isoforms in muscle would be particularly important, in terms of elucidating their specific signaling pathways that promote both the competing processes of cellular proliferation and differentiation and also cell survival, in skeletal or cardiac muscle regeneration and hypertrophy

(28-30). A potential alternative activation of these pathways *via* different receptor(s) or with different downstream effectors remains to be demonstrated. Thus, the development of IGF-1 isoform-specific antibodies would be essential for distinguishing the different IGF-I peptides and the quantification of their production and distribution in skeletal and cardiac muscles.

In conclusion, an anti-MGF polyclonal antibody was developed against a synthetic peptide within the E domain of the human MGF and its specificity was characterized in human skeletal and in rat cardiac muscles. The potential for detecting the endogenous MGF expression protein in these muscles could widen the research approaches to the study of this promising IGF-1 isoform, since it remains a challenge to elucidate and identify not only the specific stimuli by which MGF synthesis is regulated, but also the potentially differential signaling mechanisms that it modulates in muscle cellular processes.

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