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ScienceDirect
Neuromuscular Disorders 22 (2012) 26–33
www.elsevier.com/locate/nmd

Heat shock protein families 70 and 90 in Duchenne muscular dystrophy and inflammatory myopathy: Balancing muscle protection and destruction ☆

Boel De Paepe [a,*], Kim K. Creus [a], Joachim Weis [b], Jan L. De Bleecker [a]

[a] Department of Neurology and Neuromuscular Reference Center, Ghent University Hospital, Belgium
[b] Institute for Neuropathology, Medical Faculty, RWTH Aachen University, Germany

Received 23 May 2011; received in revised form 1 July 2011; accepted 8 July 2011

Abstract

Heat shock proteins are important factors in skeletal muscle physiology and stress response. We examined the effects of chronic inflammation on the distribution of heat shock protein families 70 and 90 using immunofluorescence and Western blotting, in muscle biopsies from 33 idiopathic inflammatory myopathy patients [aged 26–66 (dermatomyositis), 17–78 (polymyositis) and 57–80 (sporadic inclusion body myositis) years], and seven Duchenne muscular dystrophy patients (aged 3–19 years). Our results reveal the multifaceted role played by chaperones in inflammatory muscle tissue. On the one hand, regenerating, atrophic and vacuolated muscle fibers displayed upregulation of both protein families. Higher levels of chaperones in challenged fibers point to the myocyte's attempt to restore and regenerate. On the other hand, heat shock proteins of the 90 family were strongly upregulated in macrophages and cytotoxic T-cells actively invading nonnecrotic muscle fibers of sporadic inclusion body myositis and polymyositis, probably conferring enhanced myocytotoxic capacity. Our data provide positive arguments for exploring heat shock protein 90-based therapy in inflammatory muscle disease.

Keywords: Duchenne muscular dystrophy; Heat shock proteins; Inflammatory myopathy

1. Introduction

Chaperoning heat shock proteins (HSP) [1] are important factors in skeletal muscle physiology and adaptation to exercise and stress [2]. In addition, HSP are immune-active compounds that induce the maturation and activation of inflammatory cells [3–7], and regulate the expression of pro-inflammatory factors [8]. These characteristics combined make them putative mediators of inflammatory muscle disease [9]. The HSP70 and HSP90 families are of special interest as the glucocorticoid (GC)-receptor is among their client proteins [10]. HSP70 act early in the translational process, preventing incorrect folding of the nascent polypeptide chain [11,12]. The two cytosolic HSP90, termed HSP90α (HSP86), and HSP90β (HSP84) [13], bind their client proteins near their active conformation state. HSP90 target many protein kinases and transcription factors and are therefore important cellular regulators [14].

Infiltration of skeletal muscle tissue by inflammatory cells is observed in myopathies of different etiology. In Duchenne muscular dystrophy (DMD), a severe deficiency of the sarcolemmal dystrophin-complex results in sarcolemmal damage, disturbed calcium homeostasis, and proteolysis [15]. These processes trigger a secondary inflammatory response mediated by macrophages and T-cells [16]. The idiopathic inflammatory myopathies (IIM) are sporadic autoimmune diseases caused by different immune effector

☆ This study was presented, in part, at the 14th International Congress of the World Muscle Society.

* Corresponding author. Address: Department of Neurology, Ghent University Hospital, De Pintelaan 185, B9000 Ghent, Belgium. Tel.: +32 9 3324391; fax: +32 9 3324971.
E-mail address: boel.depaepe@ugent.be (B. De Paepe).

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doi:10.1016/j.nmd.2011.07.007

mechanisms. Dermatomyositis (DM) is a complement-mediated endotheliopathy characterized by perimysial accumulation of helper T-cells, dendritic cells (DCs) and B-cells. In sporadic inclusion body myositis (IBM) and polymyositis (PM), myocytotoxicity mediated by cytotoxic T-cells and macrophages is directed against muscle fibers, which results in the typical nonnecrotic muscle fibers invaded by an autoaggressive infiltrate. The antigen(s) at which the cytotoxic immune reaction is directed remain(s) elusive. IBM muscle fibers display additional degenerative phenomena [17], and IBM patients are notably resistant to GC-therapy.

In the past, chaperones have received some attention in muscle disease. Enhanced expression of αB-crystallin [18], HSP65 [19], HSP70 [20], and HSP90 [21], has been described in IIM; for HSP65 and HSP90, increased expression has also been reported in DMD [22]. Recently, we showed that HSP90:HSP70 ratios are positively related to severity of inflammation in IIM patients [23] pointing to a differential upregulation of these two chaperone families.

In this study we describe in detail the expression of HSP70 and HSP90 chaperone families in human skeletal muscle from patients with DMD and IIM, offering a comparison between presumed secondary and primary muscle inflammation.

2. Materials and methods

2.1. Patients

Patient material was obtained from the University Hospitals of Ghent, Antwerp and Aachen. Frozen diagnostic limb muscle biopsies were collected from patients diagnosed with DMD ($n = 7$), and IIM ($n = 33$) (Supplementary data, Table S1). The diagnosis of DM, PM and IBM was based upon conventional criteria [24]. PM was diagnosed only when nonnecrotic invaded muscle fibers (NNIF) were present in the diagnostic biopsy and when patients had subsequently reacted to immune therapy. None of the DMD and IIM patients received immunosuppressive therapy prior to the biopsy and all patients had progressive disease at the time of biopsy. Muscle specimens from subjects with no clinical, electromyographic or histological evidence of myopathy served as healthy controls ($n = 20$). Disease controls included patients with polyneuropathy or motor neuron disease ($n = 9$). Ages in years were: healthy controls (21–74, mean 48), disease controls (40–70, mean 52), DMD (3–19, mean 8), DM (26–66, mean 44), PM (17–78, mean 51), and IBM (57–80, mean 66) at time of biopsy. The study was approved by the Medical Ethics Committee and the Ghent University Hospital, and written informed consent was obtained from all patients or patient's guardians.

2.2. Immunofluorescence

Six micron cryostat sections were cut from frozen muscle biopsies. Sections were treated with blocking solution containing 5% donkey serum, 10% heat-inactivated human serum and 2% bovine serum albumin in phosphate buffered saline (PBS). Incubations with primary antibodies were carried out in the same solution (for dilutions see Supplementary data, Table S2). Secondary antibodies were used, labeled with CY3 (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and AlexaFluor488 (Invitrogen, Carlsbad, CA, USA). Appropriate negative control IgGs were tested and displayed no staining. Immunoreactivity was interpreted with a Zeiss fluorescence microscope (Zeiss, Gottingen, Germany) and CellF software (Olympus, Hamburg, Germany). Inflammatory cells were identified with antibodies specifically staining immune cell phenotypes. In different microscopic fields of a selection of PM and IBM patients, CD4+, CD8+ and CD68+ cells were identified, counted using the touch count function of the CellF software, and quantitatively evaluated by two independent observers.

2.3. Western blotting

Total protein extracts were prepared by homogenizing frozen muscle samples in two volumes of extraction buffer (50 mM TrisHCl, 2 mM EDTA pH 7.4) supplemented with protease inhibitor (TM mini protease inhibitor cocktail; Roche, Indianapolis, IN, USA). To pellet debris, samples were centrifuged at 2000*g* for 10 min. Samples were prepared for electrophoresis by adding lithium dodecyl sulfate sample buffer and reducing agent (Invitrogen, Carlsbad, CA, USA). Samples were boiled for 3 min and loaded onto tris–acetate gels. Proteins were transferred to nitrocellulose membranes by electroblotting. The upper part of the blots was incubated with anti-HSP antibodies, the lower part with anti-β-actin. All incubations were carried out for 4 h at room temperature, and immunoreaction was visualized using the chromogenic Western Breeze kit (Invitrogen). Densities of protein bands were calculated with GeneTools software (Syngene, Cambridge, UK). For statistical analysis, the Student t-test was performed, *p*-values below 0.05 were considered statistically significant.

3. Results

Results of immunolocalization studies are summarized in Table 1 and representative images are shown in Figs. 1 and 2. In a selection of DMD and IIM sections CD4+, CD8+ and CD68+ cells were counted and the intensity of HSP90α and HSP90β staining was scored bright, low or negative (Supplementary data, Table S3).

3.1. Normal and disease controls

3.1.1. Muscle fibers

In healthy controls, weak sarcoplasmic HSP70, HSP90α and HSP90β staining was observed. Satellite cells, identified morphologically and as neural cell adhesion molecule (NCAM)+, were negative. In patients with neurogenic