Integrins, muscle agrin and sarcoglycans during muscular inactivity conditions: an immunohistochemical study

G. Anastasi,[1] G. Cutroneo,[1] G. Santoro,[1] A. Arco,[1] G. Rizzo,[1] C. Trommino,[1] P. Bramanti,[2] L. Soscia,[3] A. Favaloro[1]

[1]Department of Biomorphology and Biotechnologies, Policlinico Universitario G. Martino, University of Messina, Messina; [2]Study Centre for Neurological Patients, University of Messina, Messina; [3]Department of Biomorpholocial and Functional Science, University of Naples Federico II, Napoli, Italy

Sarcoglycans are transmembrane proteins that seem to be functionally and pathologically as important as dystrophin. Sarcoglycans cluster together to form a complex, which is localized in the cell membrane of skeletal, cardiac, and smooth muscle. It has been proposed that the dystrophin-glycoprotein complex (DGC) links the actin cytoskeleton with the extracellular matrix and the proper maintenance of this connection is thought to be crucial to the mechanical stability of the sarcolemma. The integrins are a family of heterodimeric cell surface receptors which play a crucial role in cell adhesion including cell-matrix and intracellular interactions and therefore are involved in various biological phenomena, including cell migration, and differentiation tissue repair. Sarcoglycans and integrins play a mechanical and signaling role stabilizing the systems during cycles of contraction and relaxation. Several studies suggested the possibility that integrins might play a role in muscle agrin signalling. On these basis, we performed an immunohistochemical analyzing sarcoglycans, integrins and agrin, on human skeletal muscle affected by sensitive-motor polyneuropathy, in order to better define the correlation between these proteins and neurogenic atrophy due to peripheral neuropathy. Our results showed the existence of a cascade mechanism which provoke a loss of regulatory effects of muscle activity on costameres, due to loss of muscle and neural agrin. This cascade mechanism could determine a quantitative modification of transmembrane receptors and loss of α7B could be replaced and reinforced by enhanced expression of the α7A integrin to restore muscle fiber viability. Second, it is possible that the reduced cycles of contraction and relaxation of muscle fibers, during muscular atrophy, provoke a loss of mechanical stresses transmitted over cell surface receptors that physically couple the cytoskeleton to extracellular matrix. Consequently, these mechanical changes could determine modifications of chemical signals through variations of pathway structural integrins, and α7A could replace α7B.

Key words: sarcoglycan, neural agrin, muscle agrin, integrin, skeletal muscle.

Correspondence: Angelo Favaloro,
Università degli Studi di Messina, Dipartimento di Biomorfologia e Biotecnologie Policlinico Universitario, Torre Biologica, Via C. Valeria 98125, Messina, Italy
Tel: +39.090/2213361.
Fax: +39.090/692449.
E-mail: angelofavaloro@tiscali.it

Paper accepted on October 6, 2006
European Journal of Histochemistry
2006; vol. 50 issue 4 (October-December):327-336

Sarcoglycans are transmembrane proteins of the dystrophin-glycoprotein complex (DGC) and they seem to be functionally and pathologically as important as dystrophin. Sarcoglycans cluster together to form a complex, sarcoglycan complex, which is localized in the cell membrane of skeletal, cardiac, and smooth muscle (Ervasti *et al.,* 1990; Yoshida *et al.,* 1990). Five sarcoglycans have been isolated and characterized to date (α, 50kD; β, 43kD; γ; 35kD; δ, 35kD; and ε, 50kD). Four of them (α, β, γ, and δ) are known to be part of the DGC in the sarcolemma of skeletal muscle (Roberds *et al.,* 1993; Bönnemann *et al.,* 1995; Lim *et al.,* 1995). ε-sarcoglycan, homologous to α-sarcoglycan, is widely expressed in most tissues and so is not included in the DGC complex (Ettinger *et al.,* 1997; Chan *et al.,* 1998). The sarcoglycan complex requires coordinated translation and assembly of its subunits for maintenance in the muscle membrane (Holt & Campbell, 1998; Hack *et al.,* 2000a; Hack *et al.,* 2000b). In fact, it has been proposed that the DGC links the actin cytoskeleton with the extracellular matrix (Ohlendieck, 1996) and the proper maintenance of this connection is thought to be crucial to the mechanical stability of the sarcolemma (Ervasti & Campbell, 1991). About vinculin-talin-integrin system, the integrins are a family of heterodimeric cell surface receptors; each integrin subunit has a large extracellular domain, a single membrane spanning region, and usually a short cytoplasmic domain (McDonald, 1989; Hemler, 1990; Dustin & Springer, 1991; Ruoslahti, 1991). Integrins play a crucial role in cell adhesion including cell-matrix and intracellular interactions and therefore are involved in various biological phenomena, including cell migration, and differentiation tissue repair. More than 20 different integrin heterodimeric receptors have been identified, whereas 8 integrin β subunits and 15 α subunits have been described so far. Among different β subunits, the β1-integrin subunit is usually the most abundant integrin expressed by adhesion-dependent

cell types (Hynes, 1992). Currently, there are two broad views on the mechanism of integrin-mediated signalling; these may turn out to be complementary rather than mutually exclusive. One concept suggests that integrins transmit signals by organizing the cytoskeleton, thus regulating cell shape and internal cellular architecture (Horwitz *et al.,* 1986; Otey *et al.,* 1990). Recently, however, an alternative paradigm for integrin-mediate signal transduction has emerged. In this view, integrins are deemed to be true receptors capable of giving rise to biochemical signals within the cell. Confirming the hypothesis of a bidirectional signalling in rat L6 skeletal muscle cell line (Yoshida *et al.,* 1998), in our recent investigation on normal human skeletal muscle (Anastasi *et al.,* 2004a), we presented evidence that the integrin system involved in the bidirectional interaction with the sarcoglycan complex. This hypothesis has been confirmed, also, by our results on human skeletal muscle affected by sarcoglycanopathies which showed a reduced α7B- and β1D-integrin staining together with absence of β-, γ-, and δ-sarcoglycans (Anastasi *et al.,* 2004b). Because integrins serve as cellular receptors for many basal lamina components, several lines of evidence suggested the possibility that integrins might play a role in muscle agrin signalling. Agrin, a basal lamina-associated proteoglycan, is a crucial nerve-derived organizer of postsynaptic differentiation at the skeletal neuromuscular junction. Integrins mediate most well-characterized developmental effects of laminin, a basal lamina protein that shares several regions of homology with agrin (Rupp *et al.,* 1991; Ruegg *et al.,* 1992). Besides, the neural isoform of agrin, which released by motor nerve terminals into the synaptic cleft (McMahan, 1990), is essential for the formation of neuromuscular junction (Gautam *et al.,* 1996) since is most effective in clustering acetylcholine receptors (AChRs) *in vitro* (Gesemann *et al.,* 1995). Although numerous studies have been carried out on muscular diseases (Jung *et al.,* 1996; Hack *et al.,* 2000b; Bönnemann *et al.,* 2002), there are insufficient data on correlation between costameric proteins and muscle agrin during muscular inactivity condition. Here, we have studied sarcoglycans, integrins, and agrin, with a immunohistochemical analysis, in order to value and to better define the behaviour of these proteins in human skeletal muscle affected by sensitive-motor polyneuropathy.

Materials and Methods

Ten adult patients affected by sensitive-motor polyneuropathy were identified and analyzed using immunohistochemistry, techniques. Muscle biopsies (gastrocnemius) were obtained during orthopedic surgery. All patients gave informed consent. The biopsies were fixed in 3% paraformaldehyde in a 0.2 M phosphate buffer at pH 7.4. After numerous rinses in 0.2 M phosphate buffers and saline-phosphate buffers (PBS), the biopsies were infiltrated with 12% and 18% saccharose, then frozen in liquid nitrogen and sliced on a cryostat, following the protocol used to carry out indirect immunofluorescence. Sections 20 μm thick were cut on a cryostat, collected on glass coated with 0.5% gelatine and 0.005% chromium potassium sulphate. To block non-specific sites and to render the membranes permeable, the sections were preincubated with 1% BSA and 0.3% Triton X-100 in PBS at room temperature for 15 min. Finally, the sections were incubated with primary antibodies. The following primary antibodies were used: anti-α-sarcoglycan diluted at 1:100, anti-β-sarcoglycan diluted at 1:200, anti-γ-sarcoglycan diluted at 1:100, anti-δ-sarcoglycan diluted at 1:50, anti-α7B-integrin diluted at 1:50, anti-β1D-integrin diluted at 1:50, anti-α7A-integrin diluted at 1:100 (synthetic peptides from the COOH terminal region, kindly provided by laboratories of Professor Tarone, University of Torino) and anti-agrin diluted at 1:100 (Santa Cruz Biotechnology Inc.), to evidence this protein in extrajunctional regions. Primary antibodies were detected with Texas Red conjugated secondary IgG (Jackson Immuno-Research Laboratories, Inc.). Slides were finally washed in PBS and sealed with mounting medium. Sections were observed and photographed using a Zeiss LSM 510 confocal laser scanning microscope, equipped with Argon laser (458 nm, 488 nm) and two HeNe lasers (543 and 633 nm). All images were digitized at resolution of 8 bits into an array of 2048x2048 pixels. Optical sections of fluorescence specimens were obtained using HeNe laser (543 nm) and Argon laser (458 nm) at a 1 min 2 s scanning speed with up to 8 average. 1.50 μm thick sections were obtained using a pinhole of 250. For each reaction, at least 100 fibers were observed. Contrast and brightness were established by examining the most brightly labelled pixels and choosing setting that allowed clear visualization of structural details while keeping the highest pixel intensities near 200.

The same settings were used for all the images obtained from the other samples that had been processed in parallel. The function, called *display profile*, allowed us to show the intensity profile across the image along a freely selectable line. The intensity curves are shown in a graph below the scanned image. Digital images were cropped and figure montages prepared using Adobe Photoshop 7.0.

Results

It is well known that sarcoglycans and integrins also are expressed under the sarcolemma of skeletal, cardiac and smooth muscle. To better define immunostaining patterns of these proteins, in skeletal muscle fibers of patients affected by sensitive-motor polyneuropathy, were performed immunofluorescence reactions. The sections were analyzed using a stack of 16 sections (0.8 μm of scan step) on 20 μm-thick cryosections of skeletal muscle.

The control reactions, carried out with single localization reaction, on normal adult human skeletal muscle, showed that all tested sarcoglycans had a costameric distribution and a normal staining patterns (Figure 1). The control reactions, on normal adult human skeletal muscle, carried out with antibody against α7A- (Figure 2a), α7B- (Figure 2b), β1D-integrin (Figure 2c) and muscle agrin (Figure 2d), showed the same normal staining pattern of sarcoglycans.

Our immunofluorescence analysis of longitudinal sections of tested samples, carried out with single localization reaction, on muscle fibers affected by sensitive-motor polyneuropathy, showed that immunostaining patterns of all tested sarcoglycans were detectables, to variable degrees, along sarcolemma of skeletal muscle (Figure 3a, b, d). In some case, any sarcoglycan immunostaining was slightly reduced (Figure 3c).

Regarding other tested proteins, our data showed that α7A-integrin immunostaining pattern appeared normal, and, any time, increased (Figure 4a), with regard to reactions on normal samples. However, immunofluorescence patterns for α7B-integrin (Figure 4b), and β1D-integrin (Figure 4c) were severely reduced. Finally, the study of the muscle agrin immunostaining show a nearly absence of his proteins (Figure 4d).

The α7B-, β1D-integrin, and agrin staining patterns (Figure 4b, c, d) was usually seen as continuous lines along each muscle fiber. However, when we looked carefully at those fluorescent lines, they were not uniformly stained, showing intermittent or periodic staining patterns along the sarcolemma.

In addition, to substantiate our results and to investigate the real presence of tested protein stainings, we applied the software function of *display profile* to some reaction. This further analysis, showing the intensity profile across the image along a freely selectable line, confirmed that the α7A-integrin fluorescence intensity was increased (Figure 5a), showing intensity values included between 50 and 250, while α7B- (Figure 5b), β1D-integrin (Figure 5c) and muscle agrin (Figure 5d) fluorescence peaks revealed severely reduced values, as demonstrated by graphs which not overstep limits of 50 intensity values.

Finally, in any our observations, we showed a longitudinal stripes of fluorescence with, in the same fiber, trasversal stripes of fluorescence (Figure 6a). It possible to see the stripes also in light transmitted image (Figure 6b). This behaviour could be caused by gathering of fluorochrome in sarcolemmal folds, due at reduction of myofibrillar compartment.

Discussion

The presence of motor innervation controls many of the physiological and biochemical properties of skeletal muscle, including the expression of particular isoforms of contractile proteins and other proteins characteristic of muscle fiber types (Guth, 1968; Pette & Vrbova, 1985; McLennan, 1994). The sarcoglycan complex, together dystrophin and other DGC proteins, are believed to stabilize the membrane. These proteins may also be involved in creating distinctive domains at the sarcolemma that link the contractile apparatus to the extracellular matrix, allowing the lateral transmission of contractile force (Ervasti & Campbell, 1993; Patel & Lieber, 1997).

A structural model of the sarcoglycan complex was proposed, in which the sarcoglycans are separated into two subunits: one consisting of α-sarcoglycan and other consisting of β-, γ-, and δ-sarcoglycan. Since the sarcoglycans often exists as oligomers and serve as receptors, it is possible that β-γ-δ-sarcoglycan subunit also functions as receptor for a yet unidentified ligand, while α-sarcoglycan, as separate subunit, could be the downstream effector (Chan *et al.,* 1998).

In fact, the DGC, and then sarcoglycan complex, has been linked with other focal adhesion assembly proteins such as integrins (Labonishok *et al.,* 1992;

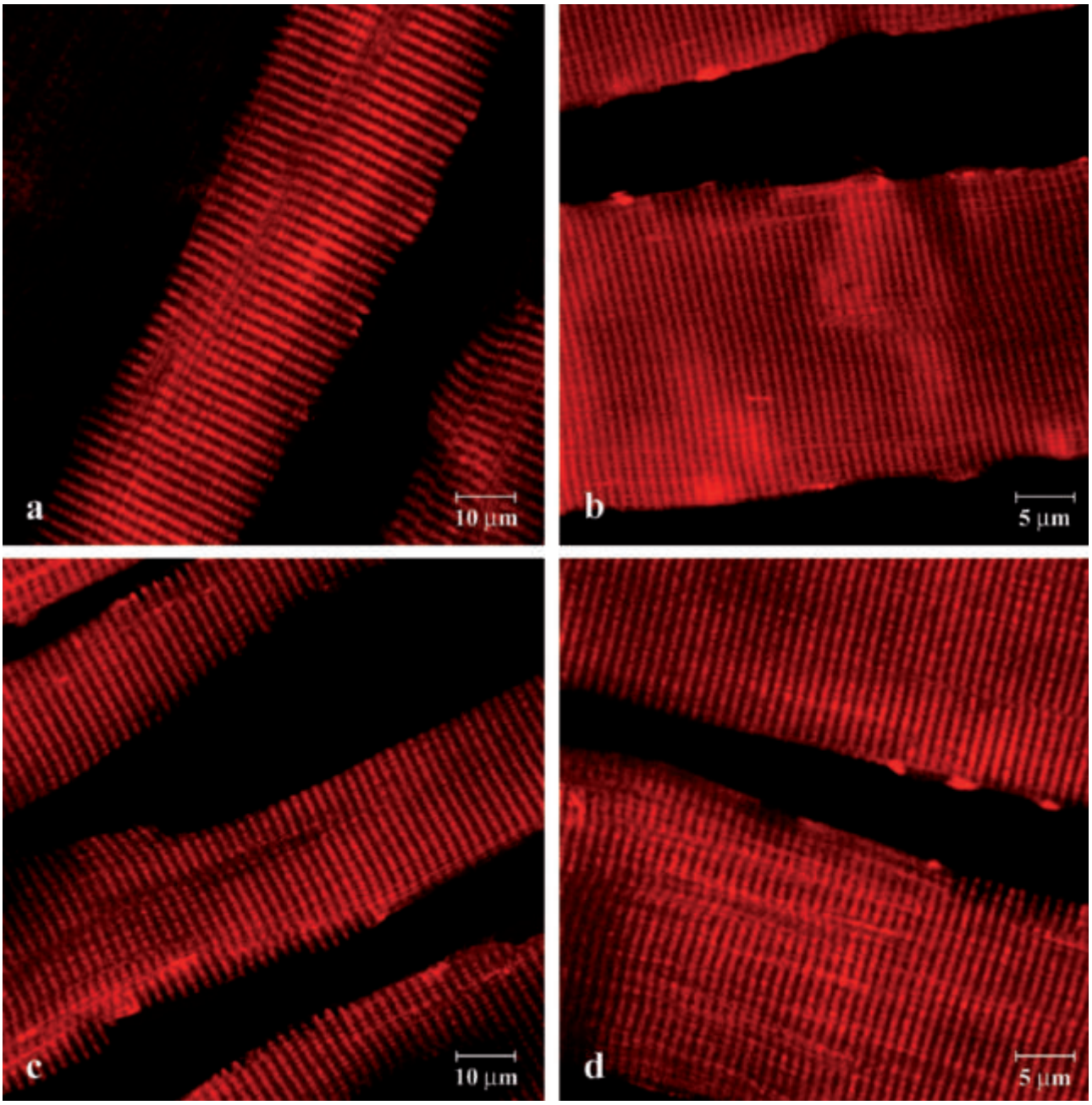

Figure 1. Longitudinal sections of control human skeletal muscle immunolabeled with α- (a), β- (b), γ- (c), and δ-sarcoglycan (d) antibodies. All proteins of sarcoglycan subcomplex showed a costameric distribution and a normal pattern of immunostaining.

Yoshida *et al.,* 1998; Thompson *et al.,* 2000) it would work analogously to the integrins as a mechanochemical transducer in skeletal muscle (Street, 1983). In support of this hypothesis, it was showed the existence of a bidirectional signalling between sarcoglycan and integrins adhesion system (Yoshida *et al.,* 1998; Anastasi *et al.,* 2004a; Anastasi *et al.,* 2004b). The integrin system seems to be mainly engaged in the cell-matrix adhesive reaction, because adhesion induced physical association of the sarcoglycan complex with focal adhesion-associate proteins is required for the tyrosine phosphorilation of α- and γ-sarcoglycan (Yoshida *et al.,* 1998). Consequently, it was demonstrated that α7-integrin upregulation might to compensate for the loss of the transmembrane sarcoglycan linkage in sarcoglycan-deficient muscle (Allikian et al. 2004). Therefore, these proteins work together, in the context of costameres, to protect the sarcolemma against contraction-induced damage and to

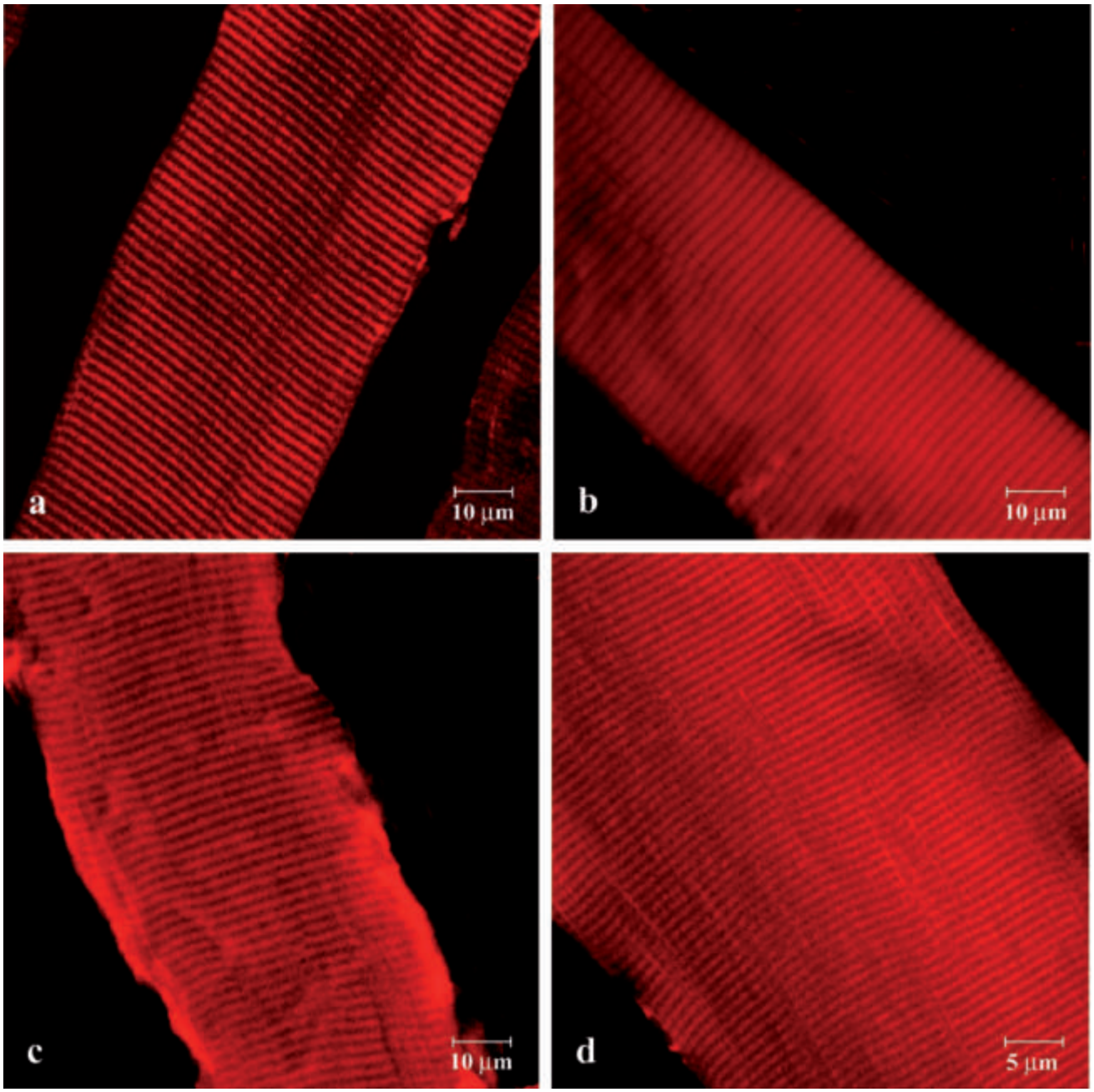

Figure 2. Longitudinal sections of control human skeletal muscle immunolabeled with α7A- (a), α7B- (b), β1D-integrin (c), and muscle agrin (d) antibodies. All protein showed a costameric distribution and a normal pattern of immunostaining.

transmit the forces of contraction laterally, to the extracellular matrix (Ervasti *et al.,* 1990; Yoshida & Ozawa, 1990; Ervasti & Campbell, 1995). These forces are generated in sarcomeres and transmitted longitudinally to myotendinous junctions and laterally to extracellular matrix (Street, 1983; Monti *et al.,* 1999).

In a previous study (Bezakova & Lomo, 2001), it was demonstrated that costameric proteins, in denervated muscles, changed their orientation in the context of muscle fiber axis, appearing as longitudinal stripes, and that electrical stimulation causes these stripes to change back to their normal transverse orientation.

Furthermore, in muscle injected with neural agrin, the induced AChR aggregates appear as transverse or longitudinal stripes in electrically and inactive muscle, respectively. Thus, the organization of costameric proteins is plastic, depends on muscle activity, and is important for the organization of

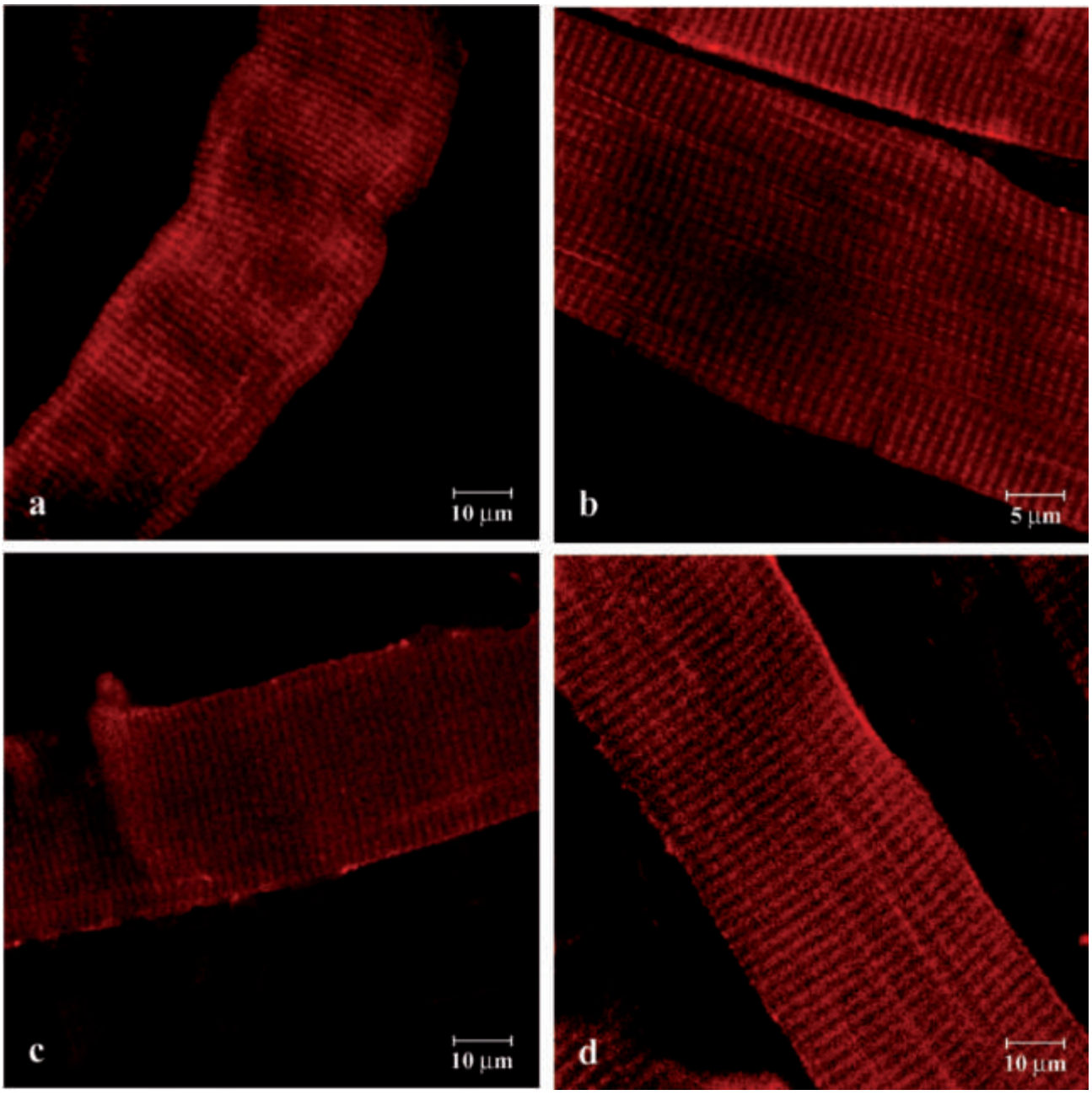

Figure 3. Longitudinal sections of human skeletal muscle affected by sensitive-motor polyneuropathy immunolabeled with α- (a), β- (b), γ- (c), and δ-sarcoglycan (d) antibodies. Immunostaining patterns of all tested sarcoglycans were detectables, to variable degrees, along sarcolemma of skeletal muscle (a, b, d). In some case, any sarcoglycan immunostaining was slightly reduced (c).

neural agrin-induced AChR aggregates.

On these basis, we performed a study of immunohistochemistry to define the correlation between sarcoglycans (α-, β-, γ-, and δ-), integrins (α7A, α7B, and β1D), and muscle agrin in human skeletal muscle affected by sensitive-motor polyneuropathy, a neurogenic atrophy due to peripheral neuropathy.

Our results showed a slightly reduced staining for all sarcoglycans, a severely reduced or almost absence for α7B-, β1D-integrin and muscle agrin staining and an increased α7A-integrin staining pattern. Additionally, in any our examinations we observed longitudinal stripes of fluorescence of tested proteins but, in our images, this behaviour, could be caused by gathering of fluorochrome in sarcolemmal folds. Our results which showed a decreased agrin staining pattern, in human samples, seem to be in contrast with results of previous reports which showed an increased of same protein in gastrocnemius samples of normal and dystrophic

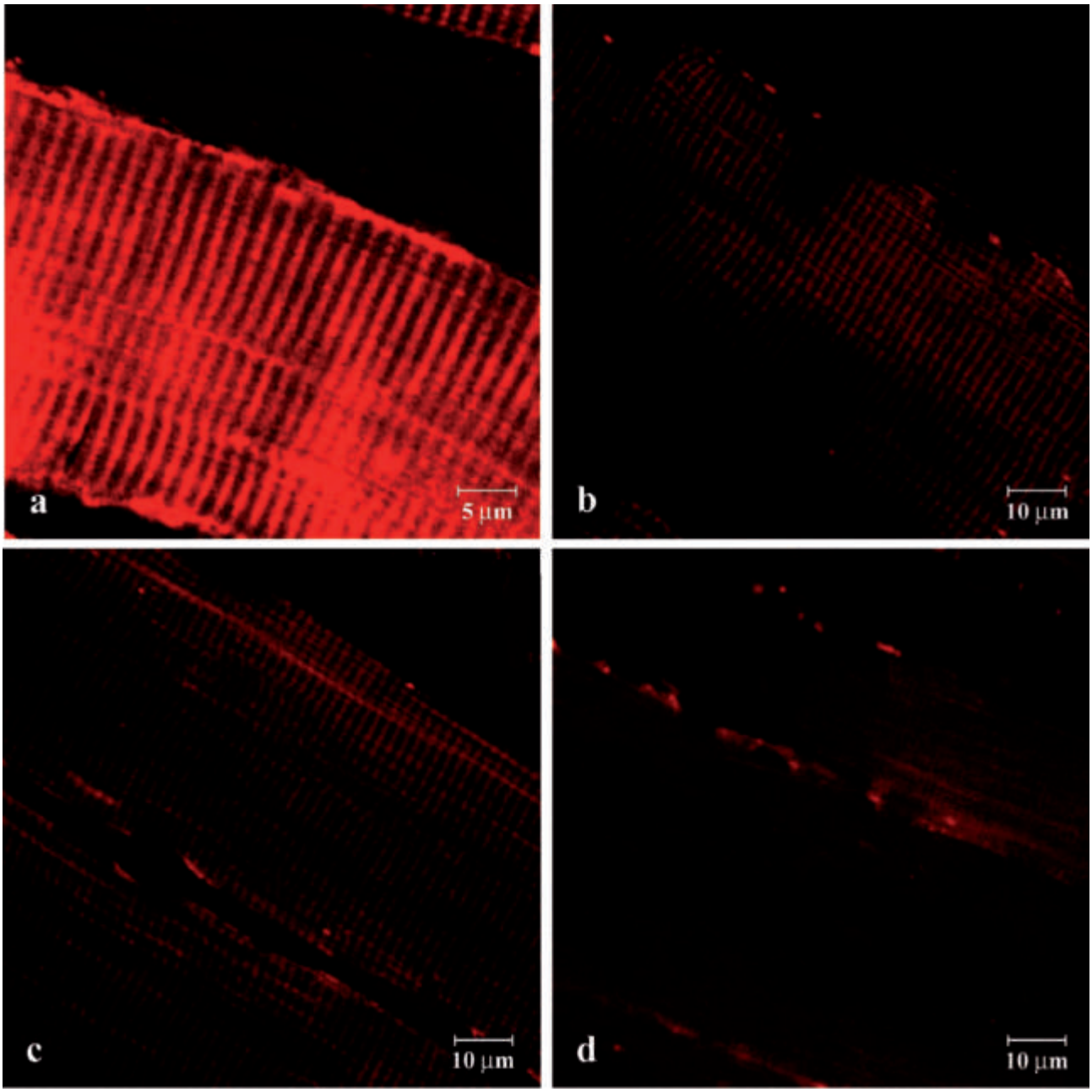

Figure 4. Longitudinal sections of human skeletal muscle affected by sensitive-motor polyneuropathy immunolabeled with α7A- (a), α7B- (b), β1D-integrin (c), and muscle agrin (d) antibodies. α7A-integrin immunostaining pattern appeared increased; immunofluorescence patterns for α7B-integrin (b), and β1D-integrin (c) were severely reduced. The muscle agrin immunostaining show a nearly absence of these proteins (d).

mice (Eusebio *et al.,* 2003). These Authors hypothesized that the increased of agrin staining in gastrocnemius muscle and the decreased in soleus muscle might be correlate with muscle fiber type. Human soleus muscle contains a high number of slow twitch muscle fibers (89.0%), while the gastrocnemius muscle contains a mean of 50.3% slow twitch fibers (Johnson *et al.,* 1973). In our opinion, the hypothesis of Eusebio et al. is correct on soleus muscle, but is it not possible to advance this hypothesis on gastrocnemius muscle fibers.

Our data demonstrated that the muscular inactivity condition can alter and modify the protein organization in skeletal muscle. In our opinion, this proteic derangement could be caused in two ways.

First, the muscular atrophy and inactivity, determined by sensitive-motor polyneuropathy, could cause, besides that a natural ACh reduction, also a

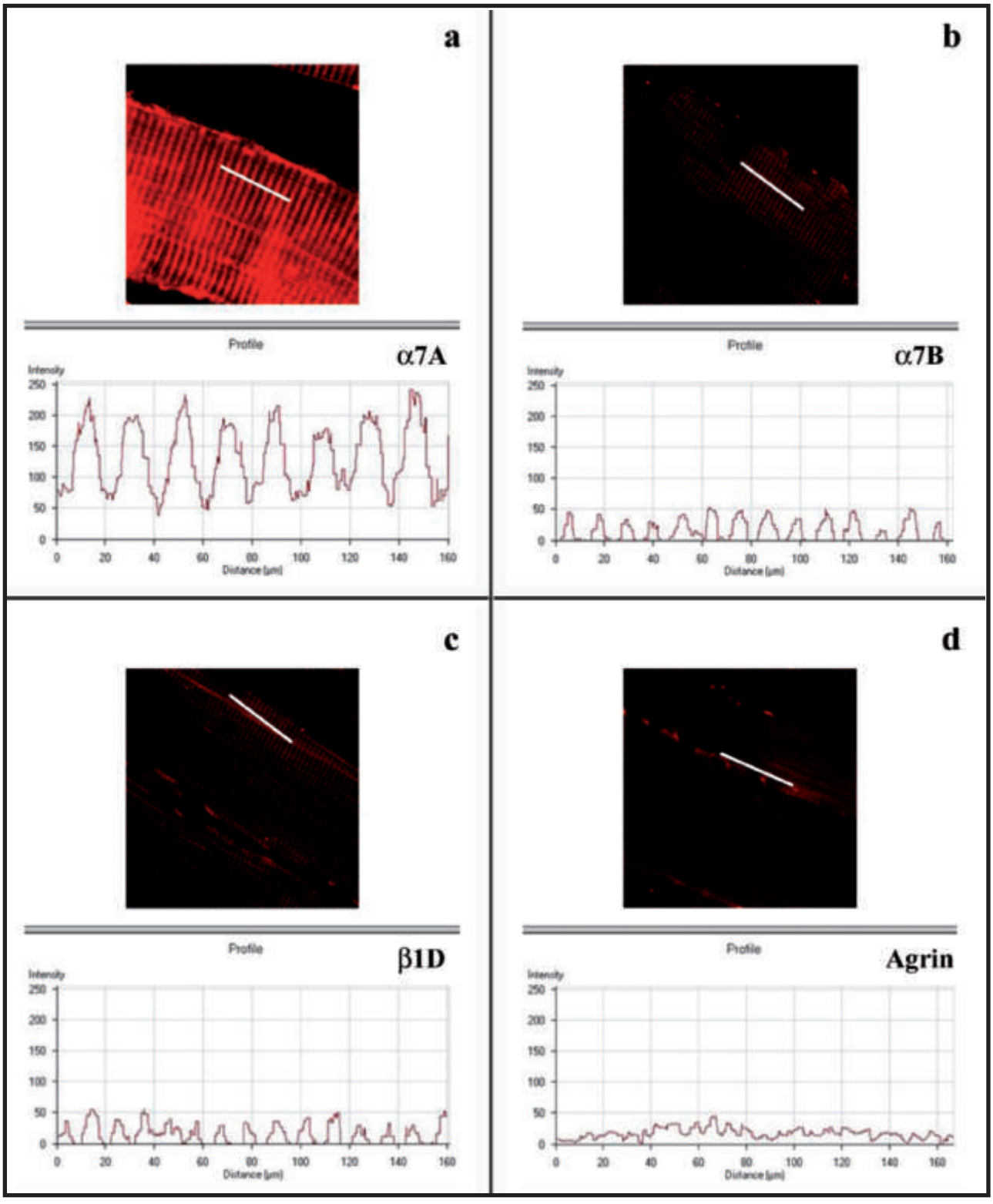

Figure 5. Display-profile, which shows the intensity profile across the image along a freely selectable line, of longitudinal sections of human skeletal muscle affected by sensitive-motor polyneuropathy. The α7A-integrin fluorescence intensity showed values included between intensity 50 and 250 (a), while α7B-integrin (b), β1D-integrin (c) and muscle agrin (d) fluorescence peaks not overstep limits of 50 intensity value, showing nearly absence of these proteins.

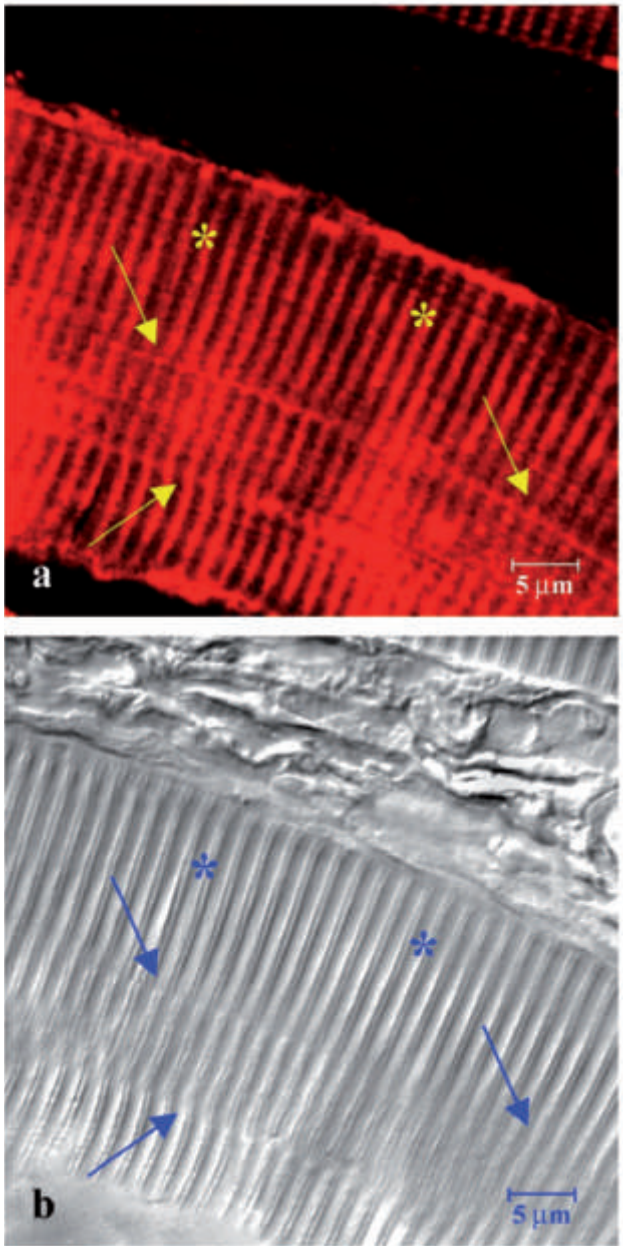

Figure 6. Longitudinal stripes of fluorescence (→) with, in the same fiber, trasversal stripes of fluorescence (*) in longitudinal sections of human skeletal muscle affected by sensitive-motor polyneuropathy immunolabeled with α7A-integrin (a) and relative light transmitted image (b).

lack of neural agrin and then a lack of muscle agrin, as demonstrated in our results. The neural agrin, besides that controls genic expression of muscle agrin, could show, directly and indirectly, a regulatory effect on genic expression of sarcoglycans and integrins. This situation could provoke a lower expression of muscle-specific integrins, α7B- and β1D-integrin. This condition could determine, thus, a quantitative modification of transmembrane receptors and the loss of α7B-integrin could be replaced and reinforced by enhanced expression of the α7A-integrin to restore the muscle fiber viability. Second, the reduced cycles of contraction and relaxation of muscle fibers, during muscular atrophy, provoke a loss of mechanical stresses transmitted over cell surface receptors that physically couple the cytoskeleton to extracellular matrix (e. g. integrins) or to other cells. Mechanical signals, therefore, could be integrated with other environmental signals and transduced into a biochemical response through force-dependent changes in scaffold geometry or molecular mechanism. In fact, physical forces of gravity, hemodynamic stresses, and movement play a critical role in tissues, since the cells use tensegrity architecture for their structural organization (Ingber, 1997).

Tensegrity is a neologism used to indicate the balance among tension and compression that causes some objects to maintain their structural integrity under tension (Connelly & Back, 1998). A structure characterized by tensegrity can be thought of as made up of rigid elements held together by elastic members (e. g. elastic tendons). More generally, the structural shape of a tensegret is guaranteed by interaction between a set of members in tension and a set of a members in compression. Tensegrity can be observed at different levels, from the macroscopic world to the atomic scale.

Other studies confirm that physical forces, including gravity, tension, and compression, influence growth and remodelling in all living tissue and show that these effects are exerted at the cell level (Joshi *et al.,* 1985; Stamenovic *et al.,* 1996). This model can be applied also in skeletal muscle fibers, in which tensional forces are borne by cytoskeletal microfilaments and intermediate filaments, and these forces are balanced by interconnected structural elements that resist compression, most notably, internal microtubule struts and extracellular matrix adhesion.

Consequently, these mechanical changes, caused by muscular inactivity, could determine modifications of chemical signals through variations of pathway structural integrins, and α7A- could replace α7B-integrin.

These data open a new line of research in understaining the real protein organization and the signaling structure in the context of the costameres during muscular inactivity. It is intringuing, besides, to integrate these studies with molecular biology techniques, in order to confirm the exact expression

of costameric proteins, even if the definition of patterns in the sarcoglycan immunohistochemical profile as a function of the primary mutation would be importan to guide the genetic analysis directly to the responsible gene and abbreviate molecular genetic investigation (Bönnemann *et al.,* 2002).

References

Allikian MJ, Hack AA, Mewborn S, Mayer U, McNally EM. Genetic compensation for sarcoglycan loss by integrin alpha7beta1 in muscle. J Cell Sci 2004; 117: 3821-30.

Anastasi G, Cutroneo G, Rizzo G, Arco A, Santoro G, Bramanti P et al. Sarcoglycan and integrin localization in normal human skeletal muscle: a CLSM (Confocal Laser Scanning Microscope) study. Eur J Histochem 2004a; 48: 245-52.

Anastasi G, Cutroneo G, Trimarchi F, Santoro G, Bruschetta D, Bramanti P et al. Evaluation of sarcoglycans, vinculin-talin-integrin system and filamin-2 in α- and γ-sarcoglycanopathy: an immunohistochemical study. Int J Mol Med 2004b; 14: 989-99.

Bezakova G, Lomo T. Muscle activity and muscle agrin regulate the organization of cytoskeletal proteins and attached acetylcholine receptor (AchR) aggregates in skeletal muscle fibers. J Cell Biol 2001; 153: 1453-63.

Bönnemann CG, Modi R, Noguchi S, Mizuno Y, Yoshida M, Gussoni E et al. β-sarcoglycan (A3b) mutations cause autosomal recessive muscular dystrophy with loss of the sarcoglycan complex. Nat Genet 1995; 11: 266-72.

Bönnemann CG, Wong J, Jones KJ, Lidov HGW, Feener CA Shapiro F et al. Primary γ-sarcoglycanopathy (LGMD 2C): broadening of the mutational spectrum guided by the immunohistochemical profile. Neuromuscul Disord 2002; 12: 273-80.

Brown SC, Lucy JA. Dystrophin as a mechanochemical transducer in skeletal muscle. Bioessays 1993; 15: 413-9.

Chan Y, Bönnemann CG, Lidov HGW, Kunkel LM. Molecular organization of sarcoglycan complex in mouse myotubes in culture. J Cell Biol 1998; 143: 2033-44.

Connelly R, Back A. Mathematics and tensegrity. Am Scientist 1998; 86: 142-51.

Dustin ML, Springer TA. Role of lymphocyte adhesion receptors in transient cell interactions and cell locomotion. Annu Rev Immunol 1991; 9: 27-66.

Ervasti JM, Campbell KP. Membrane organization of the dystrophin-glycoprotein complex. Cell 1991; 66: 1121-31.

Ervasti JM, Campbell KP. A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. J Cell Biol 1993; 122: 809-23.

Ervasti JM, Ohlendieck K, Kahl SD, Gaver MG, Campbell KP. Deficiency of a glycoprotein component of the dystrophin complex in dystrophic muscle. Nature 1990; 345: 315-9.

Ettinger AJ, Feng G, Sanes JR. ε-sarcoglycan, a broadly expressed homologue of the gene mutated in limb-girdle muscular dystrophy 2D. J Biol Chem 1997; 272: 32534-8.

Eusebio A, Oliveri F, Barzaghi P, Ruegg MA. Expression of mouse agrin in normal, denervated and dystrophic muscle. Neuromuscul Disord 2003;13: 408-15.

Gautam M, Noakes PG, Moscoso L, Rupp F, Scheller RH, Merlie JP et al. Defective neuromuscular synaptogenesis in agrin-deficient mutant mice. Cell 1996; 85: 525-35.

Gesemann M, Denzer AJ, Ruegg MA. Acetylcholine receptor-aggregating activity of agrin isoforms and mapping of the active site. J Cell Biol 1995; 128: 625-36.

Guth L. Trophic influence of nerve on muscle. Physiol Rev 1968; 48: 645-87.

Hack AA, Groh ME, McNally EM. Sarcoglycans in muscular dystrophy. Microsc Res Tech 2000a; 48: 167-80.

Hack AA, Lam MY, Cordier L, Shoturma DI, Ly CT, Hadhazy MA et al. Differential requirement for individual sarcoglycans and dystrophin in the assembly and function of the dystrophin-glycoprotein complex. J Cell Sci 2000b; 113: 2535-44.

Hemler ME. VLA proteins in the integrin family; structures, functions and their role in leukocytes. Annu Rev Immunol 1990; 8: 365-400.

Holt KH, Campbell KP. Assembly of the sarcoglycan complex. Insights for muscular dystrophy. J Biol Chem 1998; 273: 34667-70.

Horwitz A, Duggan E, Buck C, Beckerle MC, Burridge K. Interaction of plasma membrane fibronectin receptor with talin-a transmembrane linkage. Nature (Lond.) 1986; 320: 531-3.

Hynes RO. Integrins: versatility, modulation and signalling in cell adhesion. Cell 1992, 69: 11-25.

Ingber DE. Tensegrity: the architectural basis of cellular mechanotransduction. Annu Rev Physiol 1997; 59: 575-99.

Joshi HC, Chu D, Buxbaum RE, Heidemann SR. Tension and compression in the cytoskeleton of PC 12 neurites. J Cell Biol 1985; 101: 697-705.

Jung D, Leturcq F, Sunada Y, Duclos F, Tome FMS, Moomaw C et al. Absence of γ-sarcoglycan (35 DAG) in autosomal recessive muscular dystrophy linked to chromosome 13q12. FEBS Lett 1996; 381: 15-20.

Labonishok M, Mushler J, Horwitz AF. The α5β1 integrin associates with a dystrophin-containing lattice during muscle development. Dev Biol 1992; 152: 209-20.

Lim LE, Duclos F, Broux O, Bourg N, Sunada Y, Allamand J, et al. β-sarcoglycan: characterization and role in limb-girdle muscular dystrophy linked to 4q12. Nat Genet 1995; 11: 257-65.

McDonald JA. Receptors for extracelluylar matrix components. Am J Physiol 1989; 257: L331-L7.

McLennan I. Neurogenic nand myogenic regulation of skeletal muscle formation: a critical re-evaluation: Prog Neurobiol 1994; 44: 119-40.

McMahan U.J. The agrin hypothesis. Cold Spring Harb Symp Quant Biol 1990; LV:407–18.

Monti RJ, Roy RR, Hodgson JA, Edgerton VR. Transmission of forces within mammalian skeletal muscle. J Biochem 1999; 32: 371-80.

Ohlendieck K. Towards an understanding of the dystrophin-glycoprotein complex: linkage between the extracellular matrix and the membrane cytoskeleton in muscle fibers. Eur J Cell Biol 1996; 69: 1-10.

Otey CA, Pavalko FM, Burridge K. An interaction between α-actinin and the β1 integrin subunit *in vitro*. J Cell Biol 1990; 111: 721-9.

Patel TJ, Lieber RL. Force transmission in skeletal muscle: from actomyosin to external tendons. Exerc Sport Sci Rev 1997; 25: 321:363.

Pette TJ, Vrbova G. Neural control of phenotypic expression in mammalian muscle fibers. Muscle Nerve 1985; 8: 676-89.

Roberds SL, Anderson RD, Ibraghimov-Beskrovnaya O, Campbell KP. Primary structure and muscle-specific expression of the 50-kDa dystrophin-associated glycoprotein (adhalin). J Biol Chem 1993; 268: 23739-42.

Ruegg MA, Tsim KWK, Horton SE, Kroger S, Escher G, Gensch EM et al. The agrin gene codes for a family of basal lamina proteins that differ in function and distribution. Neuron 1992; 8: 691-9.

Ruoslahti E. Integrins. J Clin Invest 1991; 87: 1-5.Rupp F, Payan DG, Magill-Solc C, Cowan DM, Scheller RH. Structure and expression of a rat agrin. Neuron 1991; 6: 811-23.

Stamenovic D, Fredberg J, Wang N, Butler J, Ingber DE. A microstructural approach to cytoskeletal mechanism based on tensegrity. J Theor Biol 1996; 181: 125-36.

Street SF. Lateral transmission of tension in frog myofibers: a myofibrillar network and transverse cytoskeletal connections are possible transmitters. J. Cell. Physiol 1983; 114: 346-64.

Thompson TG, Chan YM, Hack AA, Brosius M, Rajala M, Lidov HGW et al. Filamin 2 (FLN2): a muscle-specific sarcoglycan interacting protein. J Cell Biol 2000; 1: 115-26.

Yoshida M, Ozawa E. Glycoprotein complex anchoring dystrophin to sarcolemma. J Biochem 1990;108: 748-52.

Yoshida T, Pan Y, Hanada H, Iwata Y, Shigekawa M. Bidirectional signaling between sarcoglycans and the integrin adhesion system in cultured L6 myocytes. J Biol Chem 1998; 273: 1583-90.