SHORT REPORT

Sporadic inclusion body myositis: morphology, regeneration, and cytoskeletal structure of muscle fibres

S Arnardottir, K Borg, T Ansved

J Neurol Neurosurg Psychiatry 2004;**75**:917–920. doi: 10.1136/jnnp.2003.018994

Objective: To characterise morphological abnormalities in relation to muscle fibre type in sporadic inclusion body myositis (s-IBM).

Methods: 14 muscle biopsies from 11 patients with s-IBM were characterised for morphological abnormalities and fibre type composition as well as muscle fibre regeneration and cytoskeletal structure, using histochemical and immunohistochemical techniques.

Results: Morphological abnormalities included inflammatory infiltrates and ''rimmed vacuoles,'' and pronounced variation in fibre size. There were no significant differences in fibre type composition between s-IBM patients and controls based on the myofibrillar ATPase staining. A differential effect on muscle fibre sizes was noted, type II fibres being smaller in the s-IBM patients than in the controls. Conversely, the mean type I muscle fibre diameter in the s-IBM patients was larger than in the controls, though this difference was not significant. An ongoing intense regeneration process was present in s-IBM muscle, as indicated by the expression of neonatal myosin heavy chain, vimentin, and CD56 (Leu-19) in most of the muscle fibres. The cytoskeletal proteins dystrophin and desmin were normally expressed in s-IBM muscle fibres that were not undergoing degeneration or regeneration.

Conclusions: There are extensive morphological and morphometric alterations in s-IBM, affecting different muscle fibre types in different ways. The cytoskeletal structure of type I and II muscle fibres remains unaffected in different stages of the disease.

Sporadic inclusion body myositis (s-IBM) is an acquired slowly progressive inflammatory myopathy with unknown aetiology.[1] The typical clinical findings are muscle weakness and atrophy, most prominent in the quadriceps muscles and the wrist and finger flexors.[2]

Light microscopic abnormalities on muscle biopsy include inflammation with mononuclear inflammatory cells and ''rimmed vacuoles,'' which distinguishes s-IBM from other inflammatory myopathies.[1 3] The inflammatory infiltrates consist of large numbers of CD8+ T cells and macrophages, suggesting involvement of a T cell mediated cytotoxic mechanism directed against muscle fibres.[3 4]

Although the histopathology of s-IBM has been well described,[5] little is known about the fate of the cytoskeletal proteins, fibre type composition, and morphological changes during the disease process.

Our aim in this study was to characterise morphological abnormalities in relation to muscle fibre type composition, muscle fibre regeneration, and muscle fibre cytoskeleton using histochemical and immunohistochemical techniques in s-IBM patients.

METHODS

We studied 14 muscle biopsies from 11 patients who had been diagnosed as having s-IBM according to the criteria of Griggs *et al*[2] (table 1).

Data were compared with age and sex matched control muscle biopsies from 13 individuals without muscle disease and with biopsies from two patients with motor neurone disease and two with Charcot-Marie-Tooth disease type I (CMT I).

The study was approved by the local ethics committee of the Karolinska Hospital.

Muscle biopsy

Muscle biopsy was done in the tibialis anterior muscle (TA; n = 5) or the vastus lateralis (VA; n = 8) using the percutaneous conchotome method,[6] or in the cricopharyngeus muscle (CP; n = 1) during cricopharyngeotomy.

Cross sections were stained with haematoxylin and eosin (H&E) and modified trichrome,[7] and for myofibrillar ATPase (mATPase).[8 9] The classification of muscle fibre types was based on their mATPase staining characteristics, described by Brooke and Kaiser.[9]

The histopathological findings in the muscle biopsies were graded from 0 to IV and were defined according to Arnardottir *et al*.[10]

We measured the cross sectional area of 200 muscle fibres from each biopsy specimen stained with mATPase.

Immunohistochemistry

The muscle biopsy cross sections were stained for different isoforms of myosin using monoclonal antibodies directed against the following:

- fast, slow, and neonatal myosin (NCL-MHCf clone WB-MHCf; NCL-MHCs clone WB-MHCs; NCL-MHCn clone WB-MHCn; Novocastra Laboratories, Järfälla, Sweden);
- HLA class I (HLA-ABC, clone W6/32; Dako, Glostrup, Denmark);
- cytoskeletal protein dystrophin (NCL-DYS 2 clone Dy8/6C5 and NCL-DYS 3 clone Dy10/12B2; Novocastra Laboratories);
- intermediate filaments vimentin (NCL-VIM-V9 clone V9, Novocastra Laboratories) and desmin (NCL-DES-DER11 clone DE-R-11; Novocastra Laboratories)[11]
- CD56 (Leu-19, clone MY31; Becton Dickinson, Cowley, Oxfordshire, UK), a marker for regeneration.[12]

Sections were examined in a light microscope. The expression of the different myosin isoforms in 100 fibres in

Abbreviations: CMT, Charcot-Marie-Tooth disease; CP, cricopharyngeus muscle; mATPase, myofibrillar ATPase; MCSA, mean cross sectional area; MHCf, fast myosin; MHCn, neonatal myosin; MHCs, slow myosin; s-IBM, sporadic inclusion body myositis; TA, tibialis anterior muscle; VA, vastus lateralis muscle

consecutive sections was analysed. Sections stained for dystrophin, desmin, vimentin, and CD56 were graded and defined as: +, present but only to a minor degree, less than 10% of the fibres stained; ++, present, 10–50% of the fibres stained; +++, present and very prominent, more than 50% of the fibres stained; A, present in all fibres.

Statistical analyses

Muscle fibre cross sectional areas are presented as mean (SD). Data were compared between patients and controls using the Wilcoxon matched pairs test. A probability (p) value of <0.05 was considered statistically significant.

RESULTS

Histopathology

All biopsies from s-IBM patients contained rimmed vacuoles and showed inflammatory infiltrates partially invading non-necrotic muscle fibres. There was a marked variation in fibre size. Centralised nuclei were seen in a few fibres in each biopsy. Fibre necrosis and regenerating fibres were encountered in all biopsy specimens, together with increased amounts of fat and connective tissue. Increased staining for HLA class I was seen in all biopsies.

Morphometry

Mean cross sectional areas (MCSA) of muscle fibres are presented in table 1 and fig 1. MCSA of type I fibres was 6205 and 5125 μm^2 in patients and controls, respectively, but this difference was not statistically significant ($p = 0.16$). Type II fibres were generally smaller in the s-IBM patients. Type IIa had a smaller MCSA than the controls ($p = 0.03$) but the difference for type IIb MCSA was not significant ($p = 0.3$).

Fibre type composition

The number of type I fibres tended to be greater in both TA and VL of s-IBM patients than in the controls, but this difference did not reach statistical significance ($p = 0.2$ in VL; $p = 0.09$ in TA). As judged from the mATPase histochemical data, in the s-IBM patients 83% of the muscle fibres in TA were of type I ($n = 6$) and 71% in VL ($n = 7$); in the controls 71% of the muscle fibres in TA were of type I and 50% in VL.

A higher percentage ($p = 0.002$) of type IIc fibres was found in the s-IBM biopsies than in the controls. Type IIc fibres were found in all biopsies from the s-IBM patients, comprising 2–32% of muscle fibres counted, but they were

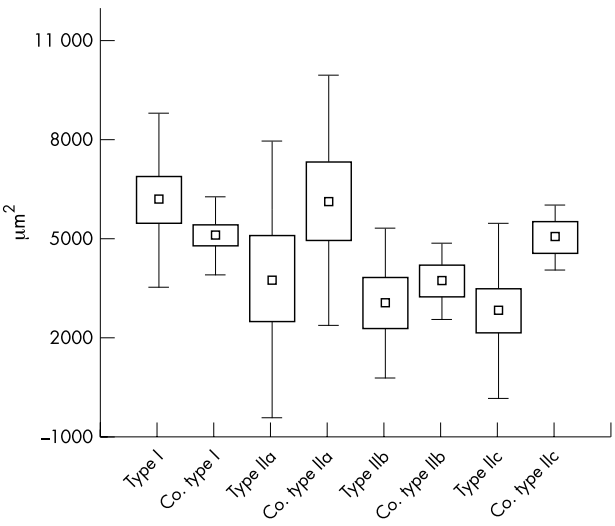

Figure 1 Box and whisker plot of mean cross sectional areas of the different fibre types (I/IIa/IIb/IIc); Co., control.

Table 1 Degree of histopathology and results from immune staining and muscle fibre size measurements

Patient/muscle	Age (years)/sex	Degree of histopathological changes*	Dystrophin†	Desmin†	Vimentin†	CD56†	MCSA type I fibre μm^2 (SD)	MCSA type IIa fibre μm^2 (SD)	MCSA type IIb fibre μm^2 (SD)	MCSA type IIc fibre μm^2 (SD)
1/TA	80/F	II	A	A	+	+	5243 (3117)	2306 (649)	2651 (197)	2129 (1876)
2/VL	76/M	III	A	A	+++	+	5872 (2153)	5097 (1639)	1857 (1145)	1920 (1523)
3/CP	76/M	III	A	A	+	++	3993 (2751)	756 (365)	1421 (819)	1756 (2563)
3/VL	75/M	III	A	A	++	+++	2140 (1581)	602 (277)	ND	1153 (683)
3/TA	75/M	II	A	A	+	+	7890 (4647)	0	0	2459 (490)
4/VL	75/M	IV	A	A	+	+	6663 (4455)	0	0	1024 (567)
5/TA	74/F	II	A	A	+	+	4591 (2665)	1621 (352)	1193 (297)	2275 (1940)
6/VL	69/M	III	A	A	+	+	10716 (3450)	5029 (3117)	3119 (1712)	3691 (3232)
6/VL	69/M	III	A	A	+	++	5560 (2950)	1506 (936)	1263 (991)	1245 (1168)
7/VL	67/M	III	A	A	+	+	10233 (7174)	0	0	1513 (910)
8/TA	60/M	III	A	A	+	+	5616 (4491)	1142 (831)	1025 (274)	1086 (528)
9/VL	57/F	III	A	A	+	+	2683 (1685)	2604 (2018)	3724 (1582)	2102 (1270)
10/VL	57/F	II	A	A	+	+	4490 (2678)	2434 (1416)	2636 (858)	7043 (1269)
11/TA	45M	I	A	A	+	+	8861 (5018)	14912 (4546)	8169 (2144)	10363 (2542)

*0, normal muscle; I, mild changes; II, moderate changes; III, moderate to severe changes; IV, severe changes.
†Expression of dystrophin decreased and that of desmin increased in some degenerating or regenerating fibres. A, all fibres stained; +, <10% stained; ++, 10–50% stained; +++, >50% stained.
CP, cricopharyngeus muscle; F, female; M, male; TA, tibialis anterior muscle; VL, vastus lateralis muscle.

only found in four control biopsies (1–7% of muscle fibres counted).

Immunohistochemistry

In normal controls, less than 1% of fibres stained positively for neonatal myosin (MHCn). The fibres that were positively stained for slow myosin (MHCs) were identified as type I fibres and those that were positively stained for fast myosin (MHCf) were identified as type II fibres by mATPase staining.

In contrast to the controls, MHCn positive fibres were found in all the biopsies from s-IBM patients (1–100% of the fibres). Furthermore, all patient biopsies contained fibres that were positive for more than one isoform of myosin (1–80% of the fibres)—that is, hybrid fibres.[13] Most of the fibres that stained positively for MHCn were of type I according to mATPase histochemistry. In the biopsies from patients with CMT and motor neurone disease fewer than 1% of the fibres stained positively for neonatal myosin, and some of these fibres were hybrid; other fibres stained positively either for MHCs or MHCf.

Normal subsarcolemmal staining for dystrophin was seen in all normal sized and hypertrophic muscle fibres from the patients with s-IBM (table 1). The staining for desmin showed normal localisation to the sarcoplasm and the subsarcolemmal region, forming an intramyofibrillar network. There was more intensive staining for desmin in those muscle fibres that also stained positive for vimentin. For further details about staining for desmin in normal controls see Jakobsson *et al*.[14] In contrast to the controls, positive vimentin staining and CD56 staining was found to varying degree (table 1) in muscle fibres from all patients with s-IBM.

DISCUSSION

In this study we found that there are extensive morphological and morphometric alterations in s-IBM, affecting different muscle fibre types in different ways. A tendency to a greater percentage of type I fibres was found, together with a significant increase in type IIc fibres. Type IIc fibres co-express adult MHCf and MHCs and may represent a transitional process from fast to slow or slow to fast myosin.[15 16] Our findings of a higher percentage of type I fibres and an increase in type IIc fibres in s-IBM muscles may therefore indicate a transitional process from fast to slow fibres.

Mean MCSA for type I fibres was larger in nine of 13 s-IBM biopsies than in the matched controls. Type II fibre MCSA was significantly reduced in patients with s-IBM. In a recent training study, it was noticed that type I fibres were larger than type II fibres in s-IBM patients and that after training there was slight hypertrophy of type I fibres, whereas type II fibres remained unchanged or were atrophic.[10] Taken together, these observations indicate that type II muscle fibres are more sensitive and vulnerable to the disease process—that is, the inflammation—than type I fibres. It is suggested that type I fibres initially compensate the muscle weakness by increasing their amount of contractile tissue. However, as the disease progresses they will eventually also become atrophic. Another possibility is that the type II muscle fibre atrophy is caused by decreased activity accompanying the disease, or by the combination of muscle inflammation and inactivity.

Muscle fibres staining positively for MHCn were found in all biopsies from the s-IBM patients. MHCn is found in developing and regenerating muscle.[17 18] Hybrid fibres co-expressing two or three isoforms of myosin were found in all muscle biopsies from s-IBM patients. Hybrid fibres containing MHCf and MHCs constituted 0–18% of all muscle fibres. Other hybrid fibres co-expressed MHCn and either MHCs or MHCf, or all three isoforms. These hybrid fibres are most probably regenerating ones as they express MHCn. Fibres co-expressing both MHCf and MHCn were of type I according to mATPase histochemistry. This can be explained by the fact that fibres containing MHCn show dual activity with respect to stability for mATPase.[16–19] Thus care must be taken when drawing conclusions about fibre types using mATPase histochemistry alone in patients with muscle diseases, especially when there is ongoing regeneration.

All s-IBM patients had muscle fibres that stained positively for vimentin and CD56. Vimentin is mainly expressed during myogenesis and is an indicator of muscle fibre regeneration.[20] CD56, which is a satellite cell antigen, is also considered to be a marker for muscle fibre regeneration.[12 21] These staining patterns seem to be more sensitive as markers of regeneration than conventional histological staining with, for example, H&E, as there were many fibres that appeared normal on routine histological preparations but expressed vimentin and CD56. Expression of these molecules persists longer than the histopathological abnormalities in the muscle tissue.[12 20] Furthermore, almost all biopsies from patients with s-IBM in the present study contained muscle fibres positive for MHCn. Thus the positive vimentin staining, together with positive staining for MHCn and CD56, in the muscle biopsies from patients with s-IBM suggest that these fibres have activated a regenerative process.

Dystrophin and desmin were normally expressed in hypertrophic and normal sized muscle fibres in all patient biopsies, which suggests that the cytoskeleton is well preserved in different disease stages of s-IBM.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (3875), the Swedish Association of Neurologically Disabled (NHR), the Swedish Sports Medicine Research Council, and the Karolinska Institute. We are indebted to Ylva Friberg, Birgitta Hedberg, and Margareta Samuelsson-Liljedahl for their excellent technical assistance.

Authors' affiliations

S Arnardottir, T Ansved, Department of Clinical Neuroscience, Division of Neurology, Karolinska Hospital, Stockholm, Sweden
K Borg, Department of Rehabilitation Medicine, Huddinge University Hospital, Karolinska Institutet, Stockholm

Competing interests: none declared

Correspondence to: Dr Snjolaug Arnardottir, Department of Neurology, Karolinska Hospital, S-171 76 Stockholm, Sweden; snjolaug.arnardottir@ks.se

Received 17 May 2003
In revised form 16 October 2003
Accepted 1 November 2003

REFERENCES

1 **Yunis EJ**, Samaha FJ. Inclusion body myositis. *Lab Invest* 1971;**25**:240–8.
2 **Griggs RC**, Askanas V, DiMauro S, *et al*. Inclusion body myositis and myopathies. *Ann Neurol* 1995;**38**:705–13.
3 **Engel AG**, Arahata K. Monoclonal antibody analysis of mononuclear cells in myopathies. II: Phenotype of autoinvasive cells in polymyositis and inclusion body myositis. *Ann Neurol* 1984;**16**:209–15.
4 **Hohlfeld R**, Engel AG. The immunobiology of muscle. *Immunol Today* 1994;**15**:269–74.
5 **Carpenter S**, Karpati G, Heller I, *et al*. Inclusion body myositis: a distinct variety of idiopathic inflammatory myopathy. *Neurology* 1978;**28**:8–17.
6 **Henriksson KG**. ''Semi-open'' muscle biopsy technique. A simple outpatient procedure. *Acta Neurol Scand* 1979;**59**:317–23.
7 **Engel WK**, Cunningham GC. Rapid examination of muscle tissue. An improved trichrome method for fresh-frozen sections. *Neurology* 1963;**13**:919–23.

8 **Padykula HA**, Herman E. The specificity of the histochemical method of adenosine triphosphatase. *J Histochem Cytochem* 1955;**3**:170–95.
9 **Brooke MH**, Kaiser KK. Muscle fiber types: how many and what kind? *Arch Neurol* 1970;**23**:369–79.
10 **Arnardottir S**, Alexanderson H, Lundberg I, *et al.* Sporadic inclusion body myositis: pilot study on the effects of a home exercise program on muscle function, histopathology and inflammatory reaction. *J Rehabil Med* 2003;**35**:31–5.
11 **Virtanen J**, Kalljock M, Nävänen O, *et al.* Peritubular myoid cells of human and rat testis are smooth muscle cells that contain desmin type intermediate filaments. *Anat Rec* 1986;**215**:10–20.
12 **Schubert W**, Zimmerman K, Cramer M, *et al.* Lymphocyte antigen Leu-19 as a molecular marker of regeneration in human skeletal muscle. *Proc Natl Acad Sci USA* 1989;**86**:307–11.
13 **Hämäläinen N**, Pette D. Patterns of myosin isoforms in mammalian skeletal muscle fibres. *Microsc Res Tech* 1995;**30**:381–9.
14 **Jakobsson F**, Borg K, Edström L. Fibre-type composition, structure and cytoskeletal protein location of fibres in anterior tibial muscle. Comparison between young adults and physically active aged humans. *Acta Neuropathol* 1990;**80**:459–68.
15 **Billeter R**, Weber H, Lutz H, *et al.* Myosin types in human skeletal muscle fibers. *Histochemistry* 1980;**65**:249–59.
16 **Staron RS**, Hikida RS. Histochemical, biochemical, and ultrastructural analyses of single human muscle fibers, with special reference to the c-fiber population. *J Histochem Cytochem* 1992;**40**:563–8.
17 **Fitzsimons RB**, Hoh JFY. Embryonic and foetal myosin in human skeletal muscle. *J Neurol Sci* 1981;**52**:367–84.
18 **Thornell LE**, Billeter R, Butler-Browne GS, *et al.* Development of fiber types in human fetal muscle. *J Neurol Sci* 1984;**66**:107–15.
19 **Biral D**, Scarpini E, Angelini C, *et al.* Myosin heavy chain composition of muscle fibers in spinal muscular atrophy. *Muscle Nerve* 1989;**12**:43–51.
20 **Borneman A**, Schmalbruch H. Anti-vimentin staining in muscle pathology. *Neuropathol Appl Neurobiol* 1993;**19**:414–19.
21 **Illa I**, Leon-Monzon M, Dalakas MC. Regenerating and denervated human muscle fibers and satellite cells express neural cell adhesion molecule recognized by monoclonal antibodies to natural killer cells. *Ann Neurol* 1992;**31**:46–52.