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STUDIES IN SPORADIC INCLUSION BODY MYOSITIS

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Stockholm 2003

ISBN 91-7349-572-7
Printed by Karolinska University Press 2003

TO MY FAMILY

ABSTRACT

Aims: The general aims of the present study were to increase the understanding of the disease process in s-IBM by analysing the cytoskeletal structure in muscle fibres and their regeneration potential; and also to evaluate sensory function in patients with s-IBM to test the hypothesis of an associated neurogenic component or a concomitant neuropathy in s-IBM; and to evaluate therapeutic options by analysing the effect of IVIg treatment on the muscle inflammation; and to evaluate the effect of training on muscle function and muscle inflammation.

Patients: Twenty-six patients with the diagnosis of s-IBM participated in these studies. The diagnosis was based on clinical symptoms and signs as well as histopathological changes in muscle biopsy, using the diagnostic criteria for s-IBM according to Griggs et al. (1995). All patients had light microscopic muscle biopsy findings with inflammatory infiltrates including invasion of non-necrotic muscle fibres and rimmed vacuoles. The diagnosis was also verified by means of electron microscopy showing cytoplasmic tubulofilaments, 15-18 nm in diameter.

Methods: The cytoskeleton and regeneration activity in muscle from s-IBM patients were analysed by immunohistochemical methods, as was expression of T lymphocytes, macrophages, endothelial cells, cytokines, adhesion molecules and class I and II human leukocyte antigen (HLA). Repeated muscle biopsies were performed before and after IVIg treatment and before and after physical training. Safety and benefit of training was evaluated in a 12-week home training program. Quantitative sensibility tests and electrophysiology were used to analyse the sensory function in patients with s-IBM.

Results: The expression of the intermediate filament vimentin and upregulation of desmin as well as expression of the satellite cell antigen CD56, indicates an ongoing active muscle fibre regeneration in s-IBM. This is further supported by the presence of neonatal myosin. Sensibility dysfunction found in s-IBM patients indicates involvement of both large myelinated and unmyelinated nerve fibres. The effect of IVIg treatment on immunological markers, including HLA class I and II expression on muscle fibres, adhesion molecules and cytokine expression in capillaries and muscle inflammation is surprisingly small when muscle biopsies taken before and after IVIg treatment are compared. The home training program was well tolerated by patients with s-IBM and gave subjective positive effects on muscle function.

Conclusion: The muscle fibres in s-IBM do have the ability to regenerate and there is an ongoing regeneration during the disease process, which may be a powerful compensatory phenomenon. However, it is not enough effective to overcome the disease process. IVIg treatment, which in some reports has been suggested to have a beneficial effect in s-IBM, has little effect on the presence of inflammatory cells or on the expression of cytokines and adhesion molecules in muscle tissue. Thus these results do not support the contention that IVIg treatment can be effective in s-IBM. Moderate physical training is safe for patients with s-IBM and can possibly have positive effect on muscle strength and function. Finally, the sensory dysfunctions found in this study support the notion that there is a peripheral nerve involvement in s-IBM.

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ABBREVIATIONS

ALS	Amyotrophic lateral sclerosis
CK	Creatine kinase
CMT	Charcot-Marie-Tooth disease
CP	Cricopharyngeus
CT	Cold threshold
DM	Dermatomyositis
FI	Functional index in myositis
H&E	Haematoxylin-eosin
h-IBM	Hereditary IBM
HLA	Human leukocyte antigen
IBM	Inclusion Body Myositis
ICAM	Intracellular adhesion molecule
IFN	Interferon
IIM	Idiopathic inflammatory myositis
IL	Interleukin
IVIg	Intravenous immunoglobulin
MAA	Myositis associated autoantibodies
mATPase	Myosin adenosine triphosphatase
MCSA	Mean cross-sectional area
MHC	Myosin heavy chain
MMT	Manual muscle test
MRC	Medical Research Council
MSA	Myositis specific autoantibodies
NCV	Nerve conduction velocities
PEF	Peak Expiratory Flow
PM	Polymyositis
QST	Quantitative somatosensory threshold screening
s-IBM	Sporadic IBM
TA	Tibialis anterior
TGF	Transforming growth factor
TNF	Tumor necrosis factor
VCAM	Vascular cell adhesion molecule
VL	Vastus lateralis
VT	Vibratory thresholds
WDM	Welander distal myopathy
WT	Warm threshold

LIST OF PAPERS

This thesis is based on the following papers which will be referred to by their Roman numerals (I-IV):

I **Arnardottir S, Borg K, Ansved T.**
Sporadic Inclusion Body Myositis - Morphology, regeneration and cytoskeletal structure of muscle fibres.
Submitted for publication.

II **Arnardottir S, Alexanderson H, Borg K, Lundberg IE.**
Immunological markers in muscle tissue from patients with sporadic inclusion body myositis before and after treatment with IVIg.
Submitted for publication.

III **Arnardottir S, Alexanderson H, Lundberg IE, Borg K.**
Sporadic Inclusion Body Myositis: Pilot study on the effects of a home exercise program on muscle function, histopathology and inflammatory reaction.
J Rehab Med 2003; 35: 31-35

IV **Arnardottir S, Svanborg E, Borg K.**
Inclusion body myositis – sensory dysfunction revealed with quantitative determination of somatosensory thresholds.
Accepted for publication in Acta Neurologica Scand.

INTRODUCTION

IDIOPATHIC INFLAMMATORY MYOPATHIES (IIM)

The idiopathic inflammatory myopathies (IIMs) are chronic inflammatory muscle disorders classified into three major groups based on clinical and histopathological features: dermatomyositis (DM), polymyositis (PM) and inclusion body myositis (IBM). IIMs share some common clinical manifestations, such as muscle weakness in both upper and lower extremities. In PM and DM the muscle weakness has a symmetrical distribution in proximal muscles but in IBM the distribution is distal in the upper extremities and proximal in the lower extremities. The second feature these diseases share is muscle fibrosis and muscle fibre degeneration (Dalakas 1998). The third common feature is pathological changes with infiltration of mononuclear cells in the muscle tissue. The infiltrating inflammatory cells are mainly T cells and macrophages (Arahata and Engel 1984). In sporadic IBM (s-IBM) and PM the inflammatory infiltrates are endomysial and usually invade non-necrotic muscle fibres. The CD8+ T lymphocytes predominate over B lymphocytes, which might indicate a cell-mediated immune process (Arahata and Engel 1984, 1986 and 1988 A, B). Conversely, in DM the inflammatory infiltrates are perivascular and the B lymphocytes predominate over T lymphocytes, which might indicate a humorally mediated immune process (Arahata and Engel 1984, 1986 and 1988 A, B). The pathogenesis and aetiology of these diseases are still unclear, but at least PM and DM are considered to be autoimmune diseases.

The main topic of this thesis is s-IBM.

IMMUNOLOGICAL FEATURES IN IIM

A common finding in the IIMs is chronic inflammation. There are several characteristics in these disorders that suggest that autoimmune mechanisms are involved in the pathogenesis. These are: presence of inflammatory cell infiltrates which are composed of CD4+ and CD8+ T lymphocytes and to some extent also B lymphocytes; association to certain human leukocyte antigen (HLA) class II genotypes; presence of autoantibodies; and an association to other autoimmune diseases.

In s-IBM and PM, the immune response has been believed to be cell-mediated (Arahata and Engel 1984, 1986 and 1988 A, B). Characteristic in the endomysial inflammatory infiltrates are CD8+ cytotoxic T cells, which invade non-necrotic muscle fibres. The CD8+ T cells are

activated against an unknown antigen, which they probably recognise when it is presented by HLA class I molecules on the surface of the muscle fibre. In DM the inflammatory infiltrates are mainly perivascular and perimysial with a predominance of CD4+ T cells and B cells and therefore a humoral-mediated immune process has been believed to be involved (Arahata and Engel 1984, 1986 and 1988 A, B). However, the dividing line between the immunopathogenic mechanisms involved in these diseases may not be quite so clear-cut: may be all three diseases have a combination of both cell-mediated and humoral-mediated immune responses. The autoantibodies found in both PM and s-IBM support that a humoral-mediated immune response can be involved (Brouwer et al 2001). In DM the presence of cytotoxic T cells supports a possible involvement of a cell-mediated immune response (Hohlfeld and Engel 1991).

One important function of lymphocytes and macrophages is to produce pro- and anti-inflammatory molecules, of which cytokines have important roles in chronic inflammation, as they both promote and suppress inflammation. Cytokines could also have a role in tissue destruction. Another group of molecules, which are important in inflammation, are adhesion molecules, which are upregulated on endothelial cells in inflammation and promote homing of inflammatory cells to tissues. The most frequently reported cytokines in IIM are the proinflammatory cytokines IL-1α, IL-1β and TNF-α. The anti-inflammatory and profibrotic cytokine TGF-β is also a prominent molecule in the muscle tissue of patients with IIM (Lundberg 1997). These observations suggest that these cytokines are important in the disease mechanisms in IIM. There are also some reports showing increased expression of intracellular adhesion molecule type 1 (ICAM-1) and vascular cell adhesion molecule type 1 (VCAM-1) (De Bleecker and Engel 1994, Lundberg 2000).

IIM is strongly associated with certain HLA class II genotypes, which further suppports a role of T cell-mediated immune mechanism. The strongest association is to HLA DRB1*0301 and DQA1*0501 (Shamim et al 2000).

Various autoantibodies against nuclear and cytoplasmic antigens are found in IIM.

These are often subclassified as myositis specific autoantibodies (MSAs) and myositis associated autoantibodies (MAAs). In a large European study by Brouwer et al (2001), where serum samples from 417 patients with IIM were analysed, autoantibodies were found in 56%. In this study the most commonly detected MSA was anti-Jo-1 (18%) and the most commonly detected MAA was anti-Ro52 (25%). The presence of the MSA autoantibodies has been associated to different clinical symptoms, e.g., patients with anti-Jo-1 have more frequent interstitial lung disease (Hengstam et al 2002).

Further support for an autoimmune pathogenesis is the association to systemic connective tissue disease or other autoimmune disorders (Lotz et al 1989, Mantegazza et al 1997, Koffman et al 1998).

An immunological reaction in the blood vessels is suggested to be involved in the pathogenesis of IIM. In DM, microvascular changes are found at both early and advanced stages of the disease and capillaries are suggested to be an early and specific target of the disease process in the muscle (De Visser et al 1989, Emslie-Smith and Engel 1990). More recently, an involvement of the microvessels also in PM and s-IBM was suggested by the observation of signs of endothelial cell activation in capillaries in muscle tissue also in these conditions (Nyberg et al 2000, Lundberg et al 1997). These activation markers include the adhesion molecules ICAM-1 and VCAM-1 as well as the proinflammatory cytokine IL-1α, and they showed a pattern similar to that observed in patients with DM (De Bleecker and Engel 1994, Lundberg 2000). Interestingly the endothelial cells in the capillaries seemed thicker than normal, with an appearance similar to that of endothelial cells in high endothelium venules (HEV), which also indicates that they are activated. These observations suggest that the microvessels might have an important role in the inflammatory process not only in DM, as previously suggested, but also in PM and IBM, although few previous investigations have focused on the role of the microvessels in the pathogenesis of s-IBM.

INCLUSION BODY MYOSITIS

History

In 1971 Yunis and Samaha applied the term Inclusion Body Myositis (IBM) to a chronic progressive myopathy that presented with a light microscopical picture of polymyositis and characteristic nuclear and cytoplasmic inclusions (Yunis and Samaha 1971). A few similar cases had been reported earlier with a myopathy and cellular inclusions, the first by Adams et al. (1965), and two years later Chou (1967) described a myxovirus-like structure in a case of chronic polymyositis (PM). In 1970 Carpenter et al. (1970) described virus-like filaments and phospholipid accumulations in a patient with myopathy. At that time it was speculated that the disease was due to a viral infection. Sato et al. (1971) reported a case in which viral studies failed to show any evidence of viral infection.

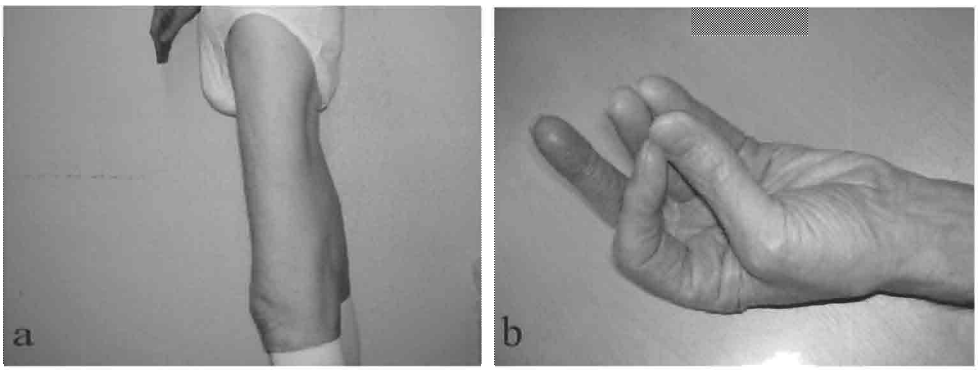
Since then, many papers have been published about IBM and the understanding of the disease process has increased, but still the aetiology, pathogenesis and pathophysiology of IBM are relatively unknown.

There are two different forms of IBM, a sporadic disease (s-IBM) and a hereditary form (h-IBM). H-IBM encompasses several different myopathies with different clinical manifestations and heredity. In this thesis, s-IBM is the subject.

Clinical features

S-IBM is an acquired slowly progressive inflammatory myopathy (Yunis 1971). The disease is at least three times more common among men than women (3 M:1 F) and most of the patients are over 50 years of age (Carpenter et al 1978, Lotz et al 1989). The typical clinical findings are muscle weakness and muscle atrophy most prominent in the quadriceps muscles in the lower extremities and wrist and finger flexors in the upper extremities (Griggs et al 1995) (Fig.1). There is a selective involvement of flexor digitorum profundus early in the disease (Sekul et al 1997).

Fig 1.



(a) Atrophic thigh muscles in 75-year-old man, picture taken 4 years after the diagnosis of s-IBM. (b) Weakness in finger flexors in the same patient.

Heart involvement has not been described as a manifestation of s-IBM and respiratory muscles are usually spared. There is, however, one report by Cohen et al. (1993) describing progressive respiratory failure in a patient with s-IBM. At least 30% of the patients have dysphagia due to cricopharyngeal muscle dysfunction, which sometimes is the first symptom of the disease (Lotz et al 1989, Riminton et al 1993).

An association to other immune-mediated diseases is described, e.g., Sjögren syndrome, psoriasis, idiopathic trombocytopenic purpura and other autoimmune conditions (Lotz et al

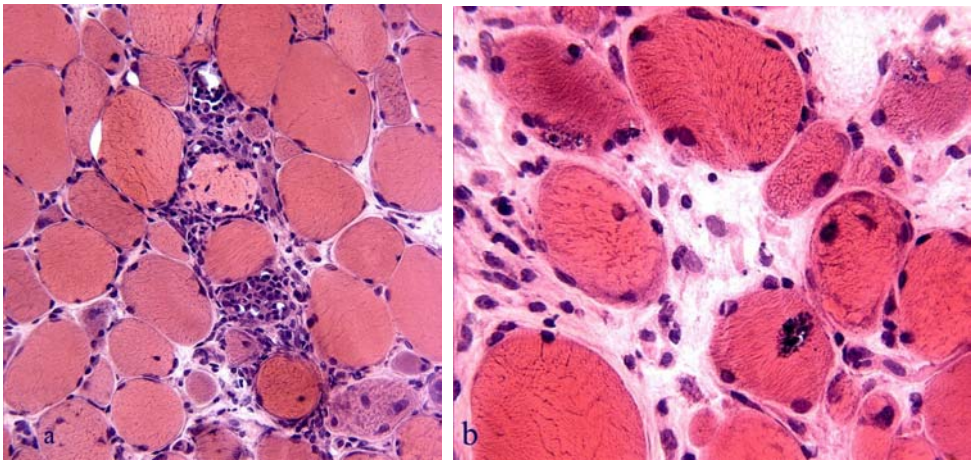
1989, Koffman et al 1998). Few cases of malignancy associated with s-IBM have been described in the literature. Arnardottir et al (2001) described a patient with s-IBM and CD8+ T cell chronic lymphocytic leukaemia (T-CLL). Furthermore, an association between s-IBM and malignant disease was verified in a population-based cohort study (Buchbinder 2001).

Laboratory features

Serum creatine kinase (CK) is normal or moderately elevated to two- to fivefold the normal value but never more than 12 times the normal value (Griggs et al 1995). Myositis specific autoantibodies (MSAs) and myositis associated autoantibodies (MAAs), which are more commonly found in the other inflammatory myopathies, PM and DM, have been described in a few patients with s-IBM (Brouwer et al 2001, Hengstam et al 2002).

Muscle biopsy abnormalities are diagnostic for s-IBM (Fig 2-3). Light microscopical abnormalities in the muscle biopsy include inflammation characterised by mononuclear inflammatory cells invading non-necrotic muscle fibres and "rimmed vacuoles", which distinguishes s-IBM from other inflammatory myopathies (Engel and Arahata 1984,Yunis and Samaha 1971). The inflammatory infiltrates have been shown to consist of large numbers of CD8+ T cells and macrophages, suggesting an involvement of a T cell-mediated cytotoxic mechanism directed against muscle fibres (Engel and Arahata 1984, Holhfeld and Engel 1994).

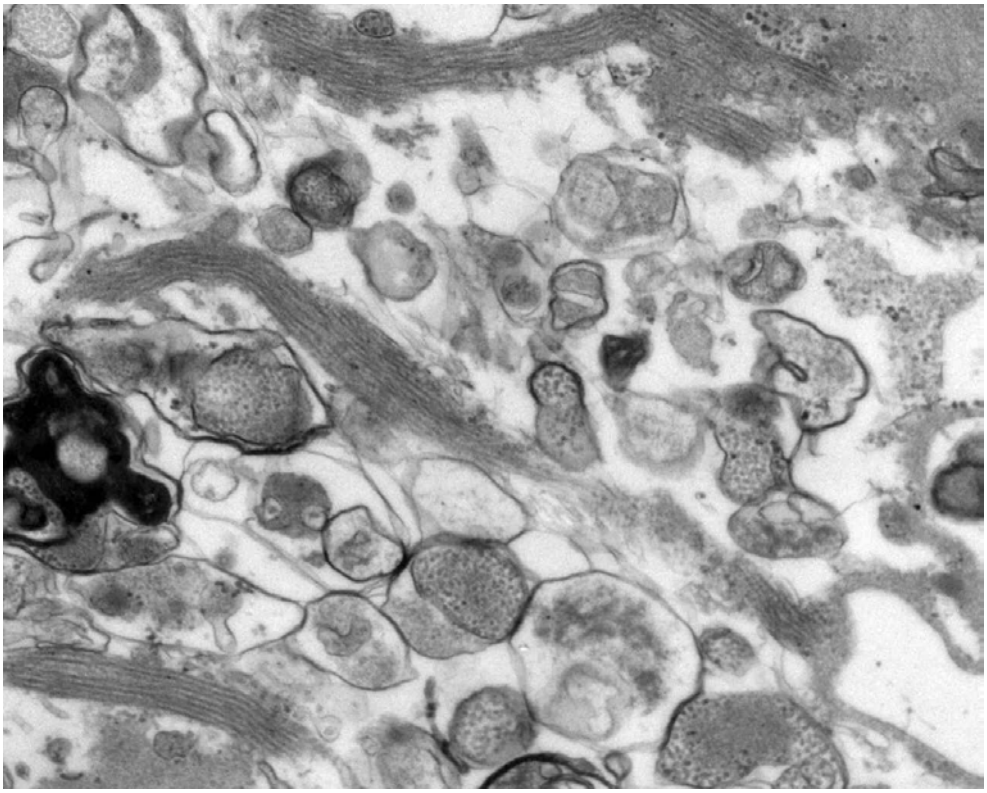
Fig 2.



Muscle biopsy from s-IBM patient stained with H&E. a. Note the inflammatory infiltrates. b. Note the rimmed vacuoles. (Orginal magnification x 450 in a and x 850 in b).

The muscle fibres with rimmed vacuoles have accumulation of proteins as amyloid-β, amyloid-β precursor protein, phosphorylated tau, apolipoprotein E, presenil-1, ubiquitin, α-synuclein, cellular prion protein and other proteins that are characteristic of Alzheimer disease (Askanas and Engel 2001).
Electron-microscopic examination demonstrates cytoplasmic and nuclear tubulofilaments, 15-18 nm in diameter, often in close association with the rimmed vacuoles (Yunis and Samaha 1971) (Fig.3).

Fig 3.



Clusters of cytoplasmic tubulofilaments within the vacuole of a muscle fibre. (Original magnification x 30.000)

According to the diagnostic criteria recommended by Griggs et al (1995), a definitive diagnosis of s-IBM requires that a histological examination of a muscle biopsy shows inflammatory infiltrates invading non-necrotic muscle fibres, rimmed vacuoles and intracellular amyloid deposit or tubulofilaments.

Pathogenesis

The cause of s-IBM is unknown and the pathogenetic mechanism still remains unclear. The most interesting question is whether s-IBM is an autoimmune disease in which the inflammation is a primary event, or if the disease is due to a degenerative process and the inflammation is secondary.

In s-IBM, inflammatory infiltrates are prominent at the early stages of the disease. At later stages, when the muscle atrophy and fibrosis are more prominent, the inflammation decreases. The presence of inflammatory infiltrates characterised by mononuclear cell infiltrates, mainly composed of CD8+ cytotoxic T-cells and macrophages, which surround and invade non-necrotic muscle fibres expressing HLA class I antigen, supports the theory that s-IBM has an autoimmune pathogenesis. An association to other immune-mediated diseases and presence of autoantibodies provide a further support for an immune basis for s-IBM. An association to HLA class II haplotype also gives support to a possible autoimmune aetiology; the strongest association is with HLA-DR3 (Garlepp et al 1994, Koffman et al 1998, Love et al 1991).

As mentioned above, a large number of different proteins accumulate in the muscle fibres with rimmed vacuoles and many of them also accumulate in the brain of Alzheimer patients. Moreover, the tubulofilaments in s-IBM morphologically resemble the neurofibrillary tangles in Alzheimer's disease (Askanas et al 1994). This has led to speculations on a pathogenic analogy between s-IBM and Alzheimer's disease (Askanas et al 1994, Askanas and Engel 2001). However, no direct association has been shown between these two diseases. In a post-mortem analysis of the brain of a patient with s-IBM there were only a few diffuse plaques in different cortical regions and some occasional neuritic plaques in the brain; no congophilic angiopathy was present. These changes are not compatible with Alzheimer's disease (Arnardottir et al 2001). Askanas et al (2001) postulated that the aged muscle fibres may possible have a decreased or diminshed cellular defense mechanism and may therefore be more susceptible to, e.g., a virus causing s-IBM by activate a putative master-gene which leads to overexpression of amyloid-β precursor protein which in turn induces various abnormalities including oxidative stress.

One might speculate that the pathogenesis involves a combination of an immune-mediated reaction and a degenerative process and that the autoimmune reaction starts the degenerative process in the muscle. Maybe there is some infection or other aetiologic agent that triggers the

autoimmune reaction in a muscle that is susceptible because of age and/or genetic factors and the chronic inflammation starts the degenerative process in the muscle tissue, which leads to accumulation of proteins and vacuolation of the muscle fibres and subsequent degeneration.

An associated neurogenic component or a concomitant neuropathy in s-IBM has been discussed. Abnormal nerve conduction velocity (NCV) and nerve and muscle biopsy abnormalities pointing to a neurogenic affection have been described in s-IBM (Arnardottir et al 2001, Carpenter et al 1978, Eisen et al 1983, Hermanns et al 2000, Joy et al 1990, Lindberg et al 1990, 1991 and 1994, Lotz et al 1989, Schroder et al 1997).

Therapeutic options

Despite the presence of inflammatory cell infiltrates in muscle tissue, treatment with corticosteroids and other immunosuppressive agents has had limited effect on recovery of muscle strength and function in patients with s-IBM (Lotz et al 1989, Griggs et al 1995). Lotz et al (1989) observed 25 patients for 2 or more years; all of them showed progression of weakness despite prednisone therapy. A few of the patients reported a temporary arrest of disease progression. A double-blind placebo-controlled study in which the effect of oral methotrexate in 44 patients with IBM was investigated did not show any retardation of the progression of muscle weakness (Badrising et al 2002).

Treatment with intravenous immunoglobulin (IVIg) in high doses has been a promising therapy. The first pilot study using high dose IVIg in s-IBM was published ten years ago and showed improvement of muscle strength (Soueidan and Dalakas 1993). Following this pilot study, a few small trials have been published with less convincing results but still indicating that IVIg might have a beneficial effect on muscle strength, or at least prevent disease progression (Amato et al 1994, Dalakas et al 1997, Dalakas et al 2001, Walter et al 2000). Moreover, a dramatically improved swallowing function after IVIg treatment was recently reported (Cherin et al 2002). There are few studies where the effect of IVIg on the muscle inflammation in muscle biopsies has been examined. A controlled study of IVIg in combination with prednisone showed that the endomysial inflammation in terms of CD2+ cells and necrotic fibres was significantly reduced, albeit without a clinical effect (Dalakas et al 2001). In another study the effect of IVIg on expression of TGF-β in muscle biopsies was examined, but there were no changes after IVIg treatment (Amemiya et al 2000). There are no studies published where the effect of IVIg treatment on IL-1 expression in muscle biopsies is

reported. Thus the beneficial effect of IVIg in s-IBM is still uncertain. Furthermore, the mode of action of IVIg is complex and not fully understood and the optimal dosage is not known. Recently a randomised pilot trial of β-interferon-1a in patients with s-IBM was published, which showed no significant difference concerning muscle strength and muscle mass at 6 months, but the authors concluded that β-interferon-1a was well tolerated in s-IBM patients and there was a need for further studies to establish its therapeutic usefulness (The muscle study group 2001). One could speculate that the next therapeutic approach will be the use of inflammatory cytokine blockers or protective cytokines.

Previously, training has not been recommended for patients with inflammatory myopathies, due to the notion that physical activity could increase the inflammatory process in the muscle (Jones et al 1986, Dalakas 1989). This cautious attitude was based, e.g., on the knowledge that strenuous exercise such as marathon running leads to muscle inflammation and skeletal muscle injury in healthy subjects (Warhol et al 1985). However, in recent years studies have shown that training of patients with polymyositis (PM) and dermatomyositis (DM) results in improved muscle strength without increase of the inflammatory reaction (Alexanderson et al 1999 and 2000, Wiesinger et al 1998 A, B). In a study by Spector et al (1997) the effects of a 12-week strength-training program in patients with IBM were analysed and it was concluded that training could lead to a gain in strength without causing muscle injury in this subgroup of myositis patients.

MUSCLE FIBRE TYPES AND REGENERATION

The classification of muscle fibre types has been based on their histochemical reaction to myosin adenosine triphosphatase (mATPase) and pH lability (Brooke and Kaiser 1970, Padykula and Herman 1955). Thus, fibres with high content of acid-stable mATPase and low content of alkali-stable mATPase are termed "type I" (slow-twitch) while fibres with the opposite staining pattern are termed "type II" (fast-twitch). Subtypes of type II, i.e., IIa, IIb and IIc, are also observed by using different pH levels for acid preincubation, 4.3 and 4.6 (Brooke and Kaiser 1970). Type I fibres are the most oxidative ones and type IIb fibres the least, but there is a wide range of enzyme activity levels within each fibre type (Essen et al 1975, Hintz et al 1984). Immunohistochemical analysis of myosin heavy chain (MHC) isoforms in muscle have shown that type I fibres contain only slow myosin, type IIa and IIb fibres contain only fast myosin and IIc fibres contain both fast and slow myosin in varying proportions (Billeter et al 1980).

Table 1. Staining intensity in the different fibre types at various pH values using mATPase immunohistochemistry, MHC content is also shown.

Fibre type	pH 4.3	pH 4.6	MHC
I	●	●	Slow
IIa	○	○	Fast
IIb	○	◍	Fast
IIc	◍	◍	Fast and slow

Two MHC isoforms predominate during development, embryonic and neonatal MHCs. They disappear during maturation but can reappear under certain pathological circumstances and then reflect muscle regeneration (Fitzsimons and Hoh 1981, Thornell et al 1984).

Repair and regeneration of muscle fibres occurs from myogenic precursor cells, satellite cells that lie between the sarcolemma and basement membrane (Mauro 1961, Muir et al 1965). When activated in response to stimuli such as myotrauma or muscle degeneration, the satellite cells proliferate rapidly to produce myoblasts that fuse with pre-existing fibres and with one another to replace part or all of any damaged fibres (Schultz and McCormick 1994).
The intermediate filament protein desmin and vimentin are components of the cytoskeleton of immature muscle fibres. In regenerating muscle fibres the expression and intracellular expression of desmin and vimentin resembles that in foetal muscle. After 2-4 weeks vimentin disappears whereas desmin persists (Bornemann and Schmalbruch 1992). The desmin expression is stronger in regenerating fibres than in mature fibres (Bornemann and Schmalbruch 1992). Therefore anti-vimentin and anti-desmin antibodies are useful indicators for muscle fibre regeneration. However, a disadvantage of anti-desmin is that all muscle fibres contain desmin and the difference between mature and regenerating fibres is not always obvious.

QUANTITATIVE SENSORY TESTING

Quantitative determination of somatosensory thresholds (QST) is a technique mostly employed for evaluation of peripheral nerve disorders. Testing of thermal and vibration modalities enables assessment of the different types of sensory fibres. With the Marstock

method described by Fruhstorfer et al (1976) four perception thresholds can quickly be estimated: those for warmth, cold, cold pain and heat pain. Thermal senses are mediated via small diameter myelinated and unmyelinated nerve fibres; warm sensation is mediated by unmyelinated C-fibres, cold sensation by small diameter myelinated fibres, heat pain by small diameter myelinated and unmyelinated nociceptors and cold pain by unmyelinated nociceptors as well as cold fibres.
Vibration thresholds are determined by means of a handheld vibrator (Goldberg and Lindblom 1979). Vibration sensation is mediated via large diameter myelinated nerve fibres.

Electrophysiological measurements reflect only the function of large myelinated nerve fibres. With QST, sensory function in small diameter nerve fibres can be tested, and significant abnormalities can be found in patients in whom conventional electrophysiology is within the normal range (Jamal et al 1987). By using the QST technique a peripheral sensory neuropathy has been found in "pure" myopathies such as myotonic dystrophy (Jamal et al 1986) and Welander distal myopathy (WDM) (Borg et al 1987).

AIMS

The general aims of the present study were to increase the understanding of the disease process in s-IBM and to evaluate therapeutic options.

Specific aims were:

Paper I: To characterise morphological abnormalities and muscle fibre type composition as well as muscle fibre regeneration and the muscle fibre cytoskeleton by means of histochemical and immunohistochemical analyses in patients with s-IBM.

Paper II: To evaluate the effect of IVIg treatment on muscle inflammation and particularly the cytokine expression in muscle biopsies taken from s-IBM patients before and after treatment with IVIg. The muscle biopsies were analysed by immunohistochemical methods to evaluate the expression of T-cells, macrophages and pro-inflammatory cytokines. Possible changes in expression of HLA class I and II on muscle fibres were evaluated, as well.

Paper III: To evaluate the safety and effects of training on disease activity and muscle strength in patients with s-IBM using a home exercise program.

Paper IV: To evaluate sensory function in patients with s-IBM by means of performing sensibility screening and quantitative determination of somatosensory thresholds. These data were correlated to results from electrophysiological examination and biopsy of the vastus lateralis muscle.

METHODS

PATIENTS (paper I-IV)

Twenty-six patients with the diagnosis of s-IBM participated in these studies, 17 men and 9 women. Eleven of the patients participated in more than one of the studies, 2 patients participated in three of the studies, I II and III, and 9 patients participated in two of the studies, 7 in study I and IV and 2 in study II and IV. The diagnosis was based on clinical symptoms and signs as well as histopathological changes in muscle biopsy, using the diagnostic criteria for s-IBM according to Griggs et al. (1995). All patients had light microscopic muscle biopsy findings with inflammatory infiltrates including invasion of non-necrotic muscle fibres and rimmed vacuoles. The diagnosis was also verified by means of electron microscopy showing cytoplasmic tubulofilaments, 15-18 nm in diameter.

Table 2. Data on the patients in papers I-IV.

Paper	No of patients	Mean age at examination or muscle biopsy	Sex Male /Female (M/F)	Age and sex matched controls without inflammatory muscle disease
I	11	68	7 M / 4 F	13 *
II	12	63	9 M / 3 F	12
III	7	60	7 M	0
IV	9	68	4 M / 5 F	9

* Data were also compared with data from muscle biopsies from 2 patients with ALS and 2 patients with CMT I.

MUSCLE BIOPSY (paper I-IV)

Muscle biopsy procedure (paper I-IV)

Muscle biopsies were performed in m. vastus lateralis (VL) or m. tibialis anterior (TA) using a "semi-open" muscle biopsy technique, the percutaneous conchotome method (Henriksson 1979). In paper III a biopsy from m. cricopharyngeus (CP) was included, which was obtained during a cricopharyngeotomy. The muscle biopsy material obtained was immediately frozen

in Freon 22, which was kept at its melting point (-190°C) with liquid nitrogen, and then placed in a freezer at -75°C until further processed. Sections of 10-15 µm were cut in a cryostat operating at -25°C.

Histochemistry (paper I-IV)

Muscle biopsy cross-sections were stained with haematoxylin-eosin (H&E) for routine histopathology and evaluated by light microscope. Two or three independent observers evaluated each cross-section at the same occasion. The histopathological findings in the muscle biopsies were graded and defined as; 0 = normal muscle, + (I) = mild changes, increased variability in muscle fibre sizes, internal nuclei, slight degenerative and regenerative changes, ++ (II) = moderate changes, prominent degenerative and regenerative changes, +++ (III) = moderate to severe changes, slight fibrosis and fatty infiltration, ++++ (IV) = severe changes, no fibres of normal size, fibrosis and fatty infiltration, * = end stage muscle, mostly fibrosis and fat, few scattered atrophic muscle fibres.

In paper I-III, the cross-sections were stained for myofibrillar ATPase (mATPase) and classified as type I and type II A, II B and II C (Brooke and Kaiser 1970, Padykula and Herman 1955). The classification of muscle fibre types was based on their mATPase-staining characteristics, as described by Brooke and Kaiser (1970). Thus, fibres with high content of acid-stable mATPase and low content of alkali-stable mATPase were termed ”type I” (slow-twitch) while fibres with the opposite staining pattern were termed ”type II” (fast-twitch). Subtypes of type II were also observed by using different pH levels (4.3 and 4.6) for acid preincubation (Brooke and Kaiser 1970).

Morphometric techniques (paper I and III)

The cross-sectional area of the muscle fibres stained with mATPase was measured directly from the microscope via a CCD camera (Hamamatsu C3077, Hamamatsu Photonics KK Japan) connected to an image-analysis processor (VIDAS, Kontron Bildanalyse, Gmbh, Munich, Germany). Measurements were made on about 200 fibres from each biopsy specimen. In biopsies where the number of measurable fibres was less than 200 all fibres were measured.

In paper I the same technique was used to measure cross-sectional area of capillaries stained with EN-4 an antibody to human endothelium.

Immunohistochemistry (paper I, II and III)

In paper II and III T lymphocytes, macrophages, endothelial cells, cytokines, adhesion molecules and class I and II human leukocyte antigen (HLA), were analysed using an immunohistochemical technique.

T lymphocytes, macrophages (CD68) and adhesion molecules (ICAM-1 or CD54 and VCAM-1 or CD106) as well as HLA class I (HLA ABC) and class II (HLA DR) were analysed using an immunohistochemical method for detection of cell types described by Frostegård et al (1999). Cytokine staining of the cross-sections was performed using anti-IL-1α, anti-IL-1β, anti-INF-γ, TNF-α and IL-8 according to a modified immunohistochemistry protocol for intracellular staining using paraformaldehyde for fixation and saponin as a detergent as described in detail by Lundberg et al (1997). The endothelium antibody (EN4) was used as an endothelial cell marker. The reaction was developed with diaminobenzidine exept in stainings for IL-1α and EN-4 where 3-amino-9-ethylcarbazole was used.

In paper I the muscle biopsy cross-sections were stained for different isoforms of myosin using monoclonal antibodies directed against fast, slow and neonatal myosin heavy chain (MHC) (NCL-MHCf, NCL-MHCs and NCL-MHCn). The muscle biopsy cross-sections were also labelled with monoclonal antibodies directed against HLA class I (HLA-ABC) and against the cytoskeletal protein dystrophin (NCL-DYS 2 and NCL-DYS 3), and the intermediate filaments vimentin (NCL-VIM-V9) and desmin (NCL-DES-DER11) (Virtanen et al 1986) and Leu 19 (CD56) a marker for muscle fibre regeneration (Schubert et al. 1989). The secondary antibody was a biotinylated sheep anti-mouse antibody, which was detected using an avidin-biotin complex kit (Vector Laboratories) and visualised with 3-amino-9-ethylcarbazole (Sigma).

Immunohistochemical assessment was done by conventional light microscopic evaluation and computerised image analysis.

In paper II and III a light microscopic evaluation was used to count the number of positively stained cells for different cytokines. In paper II the sections were coded and analysed by two independent observers on two different occasions. In paper III the sections were coded and analysed twice by the same person on two occasions. The mean values of the two assessments were used. The total section area of the patients` muscle biopsies varied; therefore a mean value was calculated, based on measurements by computerised image analysis. The number of positively stained cells per total section area was counted and a ratio was calculated on

positively stained cells per mean mm² area of the cross-section and evaluated according to the following scoring system: 0 = 0-10 positively stained inflammatory cells, + = 11-25 cells, ++ = 26-50 cells, +++ = 51-100 cells, ++++ = > 100 cells per 5.5 mm².

In paper I the expression of different myosin isoforms in 100 fibres in consecutive sections was analysed. Serial photos were taken directly from the microscope via a CCD camera connected to an image-analysis processor (see morphometric techniques) and printed on paper. The same fibres were identified in consecutive sections and numbered from 1-100.

The light microscopic evaluation for paper I was done by three independent observers on the same occasion. 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.

For evaluation of HLA class I and II expression in paper II, positively stained muscle fibres per 100 muscle fibres in the cross-section area were counted. The sections were coded and analysed by two independent observers on two different occasions. The mean values of the two assessments were used. The values from these two independent occasions were most often very similar and it is unlikely that the observers have looked at the same area in the cross-sections, which speaks for the reliability of these assessments.

In paper II and III a computerised image analysis system was used to quantitate percentage of the total area of the tissue tissue section that was positively stained for T cells and macrophages, adhesion molecules expressed both in endothelial cells and inflammatory cells as well as the endothelial cell marker and IL-1α, a cytokine expressed both in endothelial cells and inflammatory cells. The reason for choosing this method was that the inflammatory cells often were found in clusters and therefore it was difficult to count the cells. Capillaries in patients with s-IBM have larger diameter than in healthy controls. Thus, calculating the total positively labelled area is probably a better way to describe the capillary content, than simply counting them. The muscle biopsies were assessed in a Quantiment 600 (Q600) image analyser (Leica, Cambridge, UK), directed by a PC computer and connected to a microscope equipped with a 3CCD video camera (Sony Corp, Tokyo, Japan) that digitised the microscope images. A special software program, written in high-level language, QUIPS, was used for this application.

Table 3. Antibody used in the study.

Antibody against	Clone and source	Paper
CD3+ Tcell	SK7 [1]	II and III
CD4+ Tcell	Leu-3a [1]	II and III
CD68 (macrophages)	PGM1 and Ber-MAC3 [2]	II and III
CD8+ Tcell	Leu-2a [1]	II and III
EN-4 (endothelial cells)	EN-4 [3]	II and III
HLA ABC	W6/32 [2]	II and III
HLA DR	L243 [1]	II
ICAM-1 (CD54)	84H10 [4]	II and III
Il-1ra	AF-280 [5]	III
IL-1α	1277-89-7 [6]	II and III
IL-1β	2D8 [6]	II and III
IL-8	Nap-1[7]	II
INF-γ	7B6 and DIK-1[8]	II
Leu-19 (CD56)	MY31 [1]	I
NCL-DES-DER-11 (Desmin)	DER-11[9]	I
NCL-DYS 2 (Dystrophin 2)	Dy8/6C5 [9]	I
NCL-DYS 3 (Dystrophin 3)	Dy10/12B2 [9]	I
NCL-MHCf	WB- MHCf [9]	I
NCL-MHCn	WB- MHCn [9]	I
NCL-MHCs	WB- MHCs [9]	I
NCL-VIM-V9 (Vimentin)	V9 [9]	I
TNF- α	MAB I and MAB II [10]	II
VCAM-1 (CD106)	51-10C9 [10]	II and III

Sources: [1] Becton Dickinson, San Jose, CA, USA,[2] DAKO, Denmark, [3] Sanbio, Uden, Netherland, [4] Serotec, Oxford, UK, [5] R&D Systems LTD, UK, [6] Towbin Heusser, Basel, Switzerland, [7] Pharmacia, Sweden, [8] Mabtec, Sweden, [9] Novocastra Laboratories LTD, Newcastle, UK, [10] Pharmingen, San Diego, CA, USA.

MUSCLE STRENGTH/FUNCTION MEASUREMENTS (paper I and IV)

Manual muscle test (papers II and III)

Clinical examination of muscle strength, manual muscle test (MMT), was done by using the Medical Research Council (MRC) scale, 5 degrees (Guarantors of Brain 1986). In paper III the maximum possible total score was 140. Muscle strength was assessed by MMT in neck flexors and extensors and in the following muscles bilaterally: shoulder abductors, elbow flexors and extensors, wrist flexors and extensors, finger flexors and extensors, hip flexors, knee flexors and extensors, foot plantar flexors and dorsal flexors and extensor hallucis longus. In paper II muscle strength was also tested in shoulder adductors, hip extensors, abductors and adductors as well as finger abductors and thumb opposed little finger grip. The maximum possible total score was 200.

Functional index in myositis (papers II and III)

Muscle function was assessed and scored with the "functional index in myositis" (FI) (Josefson et al 1996). Total score of the FI is 64 per right and left sides respectively, which indicates normal muscle function. The FI sets out to measure function in the upper and lower limbs as well as a person`s ability to transfer from side to side when lying down, to rise up to a sitting position and finally Peak Expiratory Flow (PEF). The FI was modified as the Grippit instrument was used instead of the sphygmomanometer originally used for measuring of grip strength (Nordenskiöld et al 1993).

Isokinetic strength measurement (paper III)

Maximal voluntary knee extension and flexion were measured in isokinetic, concentric, movements carried out with isokinetic dynamometer (KIN-COM 500, Chattex Corp., Chattanooga, TN) at angular velocity 120°/s, and peak torque values were determined. The best of three trials was recorded.

TRAINING PROGRAM (paper III)

Each patient was individually instructed to perform a standardised 15 minute home exercise program and to take a 15-minute self paced walk five days a week for 12 weeks (Alexanderson et al 1999). There were two versions of the exercise program: one moderate and one easy program. Patients with FI score > 38 were given the moderate program and the resistance was individually adjusted with weight cuffs from 0.25-2 kg. The moderate program included exercises for shoulder mobility with a pulley apparatus, resistive exercises for

shoulder muscles, hip muscles and quadriceps as well as neck and trunk muscles. The easy program did not include resistive exercise for shoulder muscles and the resistive exercise for hip muscles was easier and did not employ any weight cuffs. At the end of both programs careful stretching of neck muscles, quadriceps, hamstrings, and trunk and shoulder muscles was performed. The programs included 10 repeats of each exercise.
Along with the exercise program the patients were given tape-recorded instructions with music and they were also instructed to write an exercise diary. The same physiotherapist instructed all patients, and also contacted the patients by telephone once a week during the 12-week exercise period.

SENSIBILITY TESTING (paper IV)

Sensibility screening

Sensibility screening was carried out on upper and lower extremities according to Hansson et al (1991) including the use of cotton wool for touch, pin-prick with a disposable pin for mechanically evoked pain, warm and cold metallic rollers (40°C and 20°C, respectively) for temperature. In addition, tactile discrimination was tested by figure writing with a blunt pencil (graphesthesia). The results were graded as - = normal and + = abnormal finding.

Quantitative somatosensory threshold screening (QST)

QST included determination of vibratory, thermal and thermal pain thresholds.
Vibratory thresholds (VT) were determined according to Goldberg and Lindblom (1979), with a handheld electromagnetic vibrator (SomedicAB, Stockholm, Sweden) giving a 100 Hz sine wave. The vibrator was applied over the carpal, tibial and tarsal bones and VT determined with the methods of limits, i.e. as the average of appearance and disapperance thresholds when the stimulus was successively increased and decreased. An average from at least three measurements at each location was calculated. Values were compared with data from a reference material of healthy subjects and graded as - = normal and + = abnormal finding.

Thermal and thermal pain thresholds were determined with a thermostimulator according to the Marstock method as described by Fruhstorfer et al (1976) in the thenar region of the hand and on the lateral aspect of the foot. The warm and cold threshold (WT and CT) was defined as the average temperature of at least three measurements within two minutes of recording and the warm-cold difference limen (W-C D) as the difference between these two values. The heat and cold pain thresholds were defined as when heat or cold was perceived as painful. The

threshold for heat pain was defined as the average of the last two out of three recordings. The cold pain threshold was based on one recording. The stimulus range was 5-50 ˚C. Values of the patients were considered abnormal (+) when outside mean ± 2 SD of the values of the controls. To compensate for skin temperature the determined temperature threshold was corrected as follows: WT or CT = measured WT or CT + (30˚C - skin temperature) x 0.4 (Habermann-Horstmeier 1986).

ELECTROPHYSIOLOGY (paper II)

Electrophysiological examination was carried out with standardised methods, electroneurography (ENeG) and electromyography (EMG). ENeG included determinations of motor and sensory nerve conduction velocities (NCV). Sensory nerve conduction velocity was determined in the median, peroneal and sural nerves and motor nerve conduction velocities in the median and peroneal nerves (Daube 1986). EMG was performed with Keypoint Dantec equipmentwith concentric needle electrodes for computerised analysis of interference pattern and motor unit potentials (Aminoff 1986, Stalberg et al 1983). On EMG examination, small polyphasic motor unit potentials were considered as myopathic findings and reduced interference pattern and giant motor unit potentials as neuropathic findings.

RESULTS AND DISCUSSION

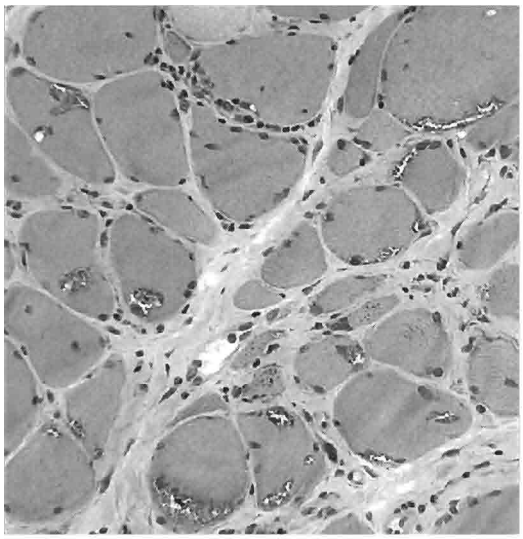
ANALYSIS OF MUSCLE BIOPSIES (paper I-IV)

Histopathology (paper I-IV)

On light microscopic examination of VL and TA muscle biopsies taken from patients with s-IBM, inflammatory infiltrates were found to a varying degree, including mononuclear cells invading non-necrotic muscle fibres. Rimmed vacuoles were found in all biopsies in the H&E stained sections. There was a marked variation in fibre sizes with an abundance of atrophic fibres, mostly rounded, but some of them were also angulated (Fig. 7). Centralized nuclei were seen in a few fibres in each biopsy. Fibre necrosis and regenerating fibres were encountered in all biopsy specimens and an increased amount of fat and connective tissue was found. An increased staining for HLA class I was seen in all biopsies, thus confirming the presence of an inflammatory process. Inflammatory infiltrates were also noted in muscle biopsy from m. cricopharyngeus (CP) and the rimmed vacuoles were larger than in other biopsies (Fig 4.).

Electron microscopy showed 15-18 nm tubulofilament.

Fig 4.

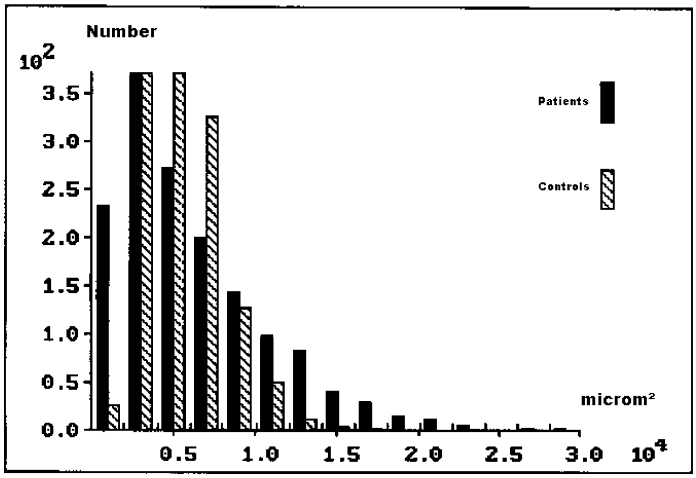


Cross-section from m. cricopharyngeus, H&E staining. (Original magnification x 450).

Muscle fibre size and fibre types (paper I and III)

In paper I the mean cross-sectional area (MCSA) of the muscle fibres in biopsies from 13 patients with s-IBM patients was measured and compared with data from biopsies from 13 sex- and age-matched control individuals without muscle disease. MCSA of type I fibres was 6205 μm² and 5125 μm² in patients and controls, respectively. However, the difference was not statistically significant (p=0.16). This may be explained by the great variation of the size of the type I fibres: the patients had both atrophic and hypertrophic muscle fibres, as seen in Fig. 5. Type II fibres were smaller in the s-IBM patients than in the controls. Type IIa muscle fibres had a significantly smaller MCSA (p=0.03): 3725 μm² in s-IBM patients versus 6159 μm² in the controls. The difference in type IIb MCSA was not statistically significant (p=0.3): 2564 μm² and 4071 μm² in patients and controls, respectively. In the training study (paper III) the muscle fibres in the biopsies taken before training were both atrophic and hypertrophic and the MCSA of type II fibres were significantly smaller than that of type I fibres (p=0.03): 2840 μm^2 in type I fibres versus 4064 μm^2 in type I fibres. There was a trend to an increase in the MCSA of type I fibres after the training. The lack of statistically significant changes may be due to that there were only 4 muscle biopsies that could be analysed before and after training.

Fig 5. Cross-sectional area of type I muscle fibres from s-IBM patients and controls (paper I).



Both atrophy and hypertrophy of muscle fibres in s-IBM have been described earlier but the difference between the different fibre types is a new finding. Carpenter et al (1978) described muscle biopsies from 6 patients: atrophic fibres were present in all biopsies and in 4 of the patients the atrophic muscle fibres comprised up to 80 % of the total fibre population. Verma et al (1992) studied muscle fibre hypertrophy in IIM and concluded that the proportion of hypertrophic fibres was higher in IBM than PM and DM and could be used to help to differentiate IBM from other clinically similar IIM.

No significant difference was found between s-IBM patients and controls in fibre type composition as judged from histochemical staining for type I and II muscle fibres (paper I). However, there was a trend that the number of type I fibres was higher in both TA muscle and VL muscle of s-IBM patients than in controls. However, this difference did not reach statistical significance ($p= 0.2$ in VL and $p= 0.09$ in TA, respectively). In TA, 83% of the muscle fibres were of type I and 71% in VL, whereas the controls had 71% type I in TA and 50% in VL. There was a significantly ($p=0.002$) higher percentage of type IIc fibres in biopsies from the s-IBM patients than in those from controls. Type IIc fibres were found in all biopsies from the s-IBM patients and constituted 2-32 % of muscle fibres counted, but were only found in 4 control biopsies (1-7% of muscle fibres counted).
Thus, a tendency to a higher type I fibre percentage was found, together with a statistically significant increase of type IIc fibres. Type IIc muscle fibres coexpress adult MHCf and MHCs and this coexpression may represent a transitional process from fast to slow or slow to fast myosin (Billeter et al 1980, Staron and Hikida 1992). The findings of a higher percentage of type I fibres and an increase of type IIc fibres in muscles from patients with s-IBM may therefore indicate a transitional process from fast to slow muscle fibres. In other words, the type IIc fibres may be in transformation from type II to type I. A most probable cause of the fibre type conversion is an increased use of remaining motor units due to progressive loss of neighbouring muscle fibres, as has been reported in other neuromuscular disorders (Borg et al 1993, Borg and Edström 1993). However, one cannot exclude other causes such as regeneration or an increased unspecific coexpression of different MHC due to the disease process itself.

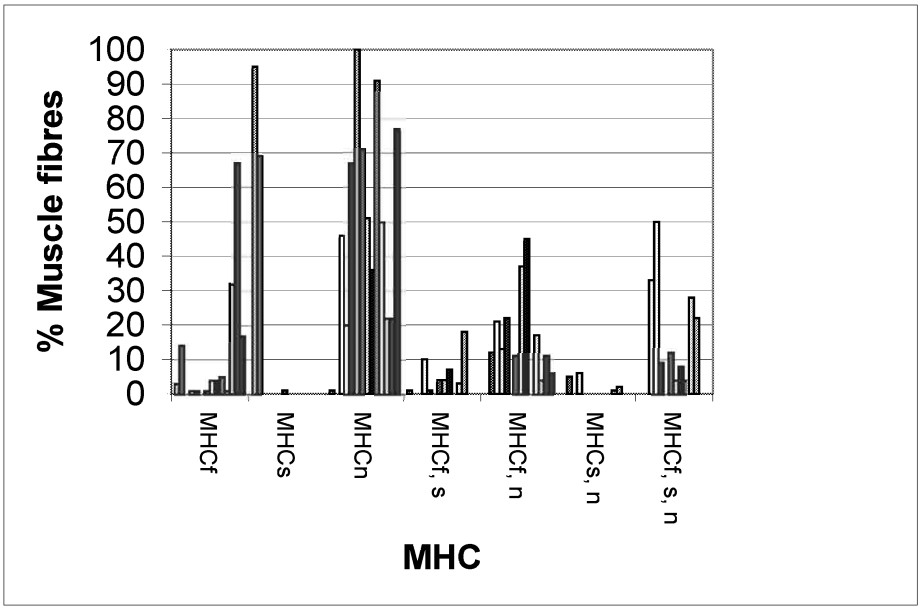
One might speculate that type II muscle fibres are more sensitive and vulnerable to the disease process, e.g., the inflammation, than type I fibres. It is thus suggested that type I fibres initially compensate the muscle weakness that results from the atrophy of type II fibres, by

increasing their amount of contractile tissue, thus becoming hypertrophic. However, as the disease progresses they will eventually also become atrophic. Another possibility is that the type II muscle fibre atrophy is due to decreased activity as a results of the disease or to the combination of the muscle inflammation and inactivity.

Myosin heavy chain (MHC) expression (paper III)

In normal controls, there were almost no fibres that stained positively for neonatal myosin (MHCn). The fibres that were positively stained for slow myosin (MHCs) were identified as type I fibres and the fibres that were positively stained for fast myosin (MHCf) were identified as type II fibres by mATPase staining.

Fig 6. MHC expression in individual s-IBM patients. (Each bar represents one patient).



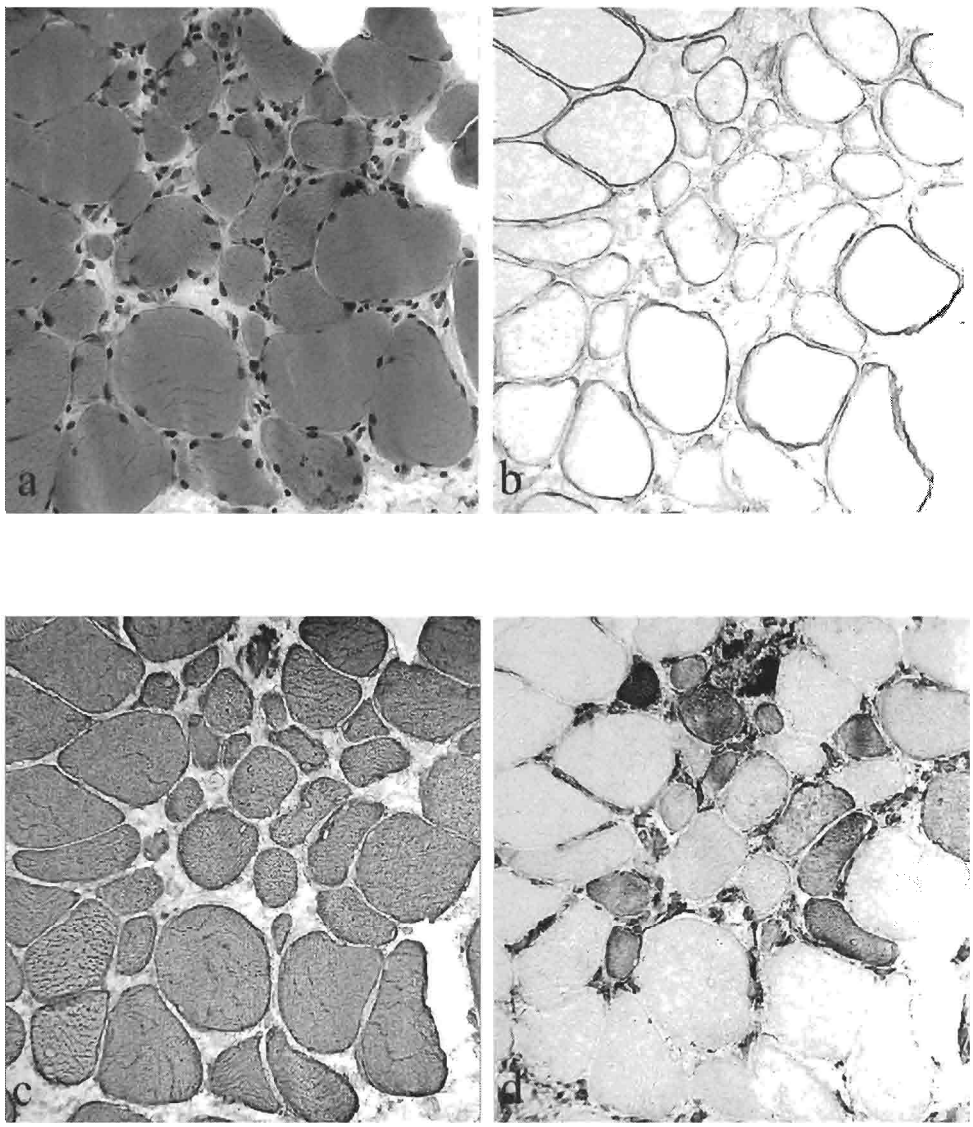
In contrast to the controls, all biopsies from patients with s-IBM contained MHCn positive fibres (1-100 % of the fibres). Most of the fibres that stained positive for MHCn were of type I according to mATPase histochemistry. Furthermore, all biopsies contained fibres that were positive for more than one isoform of myosin (1-80 % of the fibres), i.e., hybrid fibres as

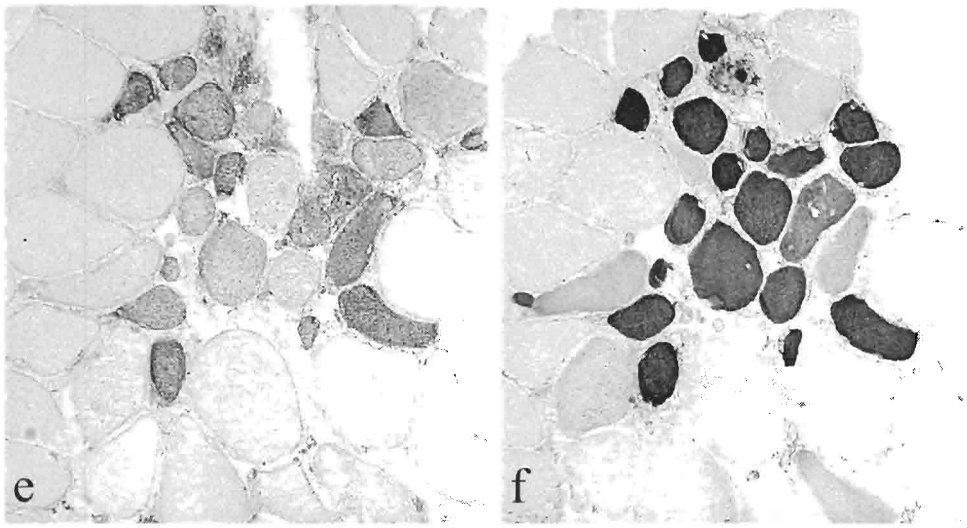
earlier described by Hamalainen and Pette (1995) (Fig 7.). Hybrid fibres containing MHCf and MHCs constituted 0-18% of all muscle fibres. Other hybrid fibres coexpressed MHCn and MHCs/ MHCf or all three isoforms. MHCn is a myosin isoform that is found in developing and regenerating muscle (Fitzsimons and Hoh 1981, Thornell et al 1984). The hybrid fibres are most likely regenerating ones, since they contain MHCn. Fibres coexpressing both MHCf and MHCn were of type I as shown by mATPase histochemistry after preincubation at pH 4.3 and 4.6, respectively. This can be explained by the fact that fibres containing MHCn exhibit dual activity regarding stability for mATPase (Staron and Hikida 1992, Fitzsimons and Hoh 1981, Thornell et al 1984, Biral et al 1989). Therefore care must be taken in drawing conclusions regarding fibre types on the basis of mATPase histochemistry alone in the present patients and also in other patients with muscle diseases, especially when there is an ongoing regeneration. Further evaluation of muscle fibre contractility and isomyosin content will elucidate the nature of these muscle fibres.

Muscle fibre regeneration (paper I)

In contrast to the controls, a majority of the s-IBM patients had muscle fibres that stained positively for vimentin and CD56. Vimentin is mainly expressed during myogenesis and reappears transiently after necrosis in muscle tissue as an indicator of muscle fibre regeneration (Bornemann and Schmalbruch 1993). In inflammatory myopathies a marker for vimentin has been shown to detect a broader spectrum of regenerating fibres than MHCn (Winter and Bornemann 1999). CD56, which is a satellite-cell antigen, is also considered to be a marker for muscle fibre regeneration (Schubert et al 1989, Illa et al 1992). However, one has to be cautious when analysing immunostained biopsies from patients with degenerative muscle disorders since several proteins may be upregulated as a part of a pathological process, irrespective of its origin. One of the concerns about using the CD56 antibody is that it is an antibody to a neural cell adhesion molecule (NCAM), which is also expressed in denervated muscle fibres; thus CD56 might be positive both in regenerating muscle fibres and in denervated ones (Covault and Sanes 1985, Illa et al 1992). Vimentin and CD56 stainings seem to be more sensitive as markers of regeneration than conventional histological stainings with e.g., H&E, since there were many fibres that appeared normal in the routine histological preparations, but expressed vimentin and CD56. Expression of these molecules persists longer than the histopathological abnormalities in the muscle tissue (Bornemann and Schmalbruch 1993, Schubert et al 1989).

Fig 7. Serial cross-sections from m. vastus lateralis from patient with s-IBM: (a) H&E staining; (b) Dystrophin expression; (c) Desmin expression; (d) Vimentin expression; (e) CD56 epression; (f) Neonatal MHC expression; (g) Fast MHC expression; (h) Slow MHC expression. (Original magnification x 450).

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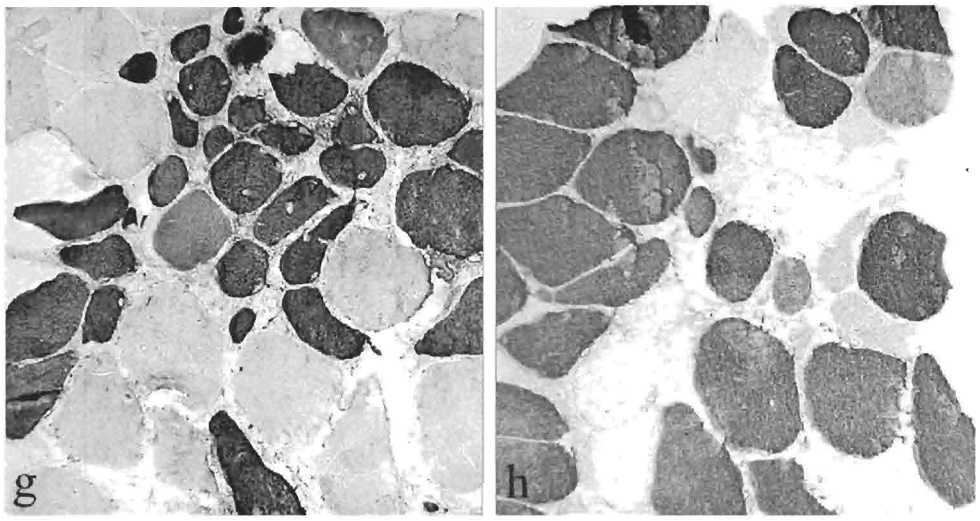
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Thus, the positive vimentin staining together with positive staining for MHCn in the muscle and CD56 in the muscle biopsies from s-IBM patients suggest that these fibres have activated a regenerative process. One might speculate that an active ongoing regeneration mechanism in s-IBM is favourable as it compensates the loss of contractile tissue. If one could slow down the pathological process it would lead to a gain of muscle tissue and muscle function.

Cytoskeletal structure (paper I)

Dystrophin and desmin were normally expressed in hypertrophic and normal sized muscle fibres, but in some atrophic muscle fibres and fibres undergoing de- or regeneration the staining for dystrophin was decreased and staining for desmin increased. More intensive staining for desmin was noted in fibres that also stained positive for vimentin, which supports that these fibres are regenerating.

Inflammatory cells and HLA expression (paper II and III)

An inflammatory reaction was found in the muscle biopsies of all s-IBM patients. In all muscle biopsies in paper II, positive staining for the HLA class I antigen on muscle fibres was detected; in most patients all muscle fibres stained positive. The staining was localised both to the sarcolemma and diffusely in the sarcoplasm. The HLA class I staining was more distinct in atrophic muscle fibres. Positive staining for the HLA class II antigen on muscle fibres was observed in 6 out of 13 biopsies and detected in approximately one third of the calculated fibres and was expressed both in the sarcolemma and sarcoplasm.

There was a statistically significant increase in staining for macrophages (CD68), CD3+ and CD8+ T cells in the muscle biopsies from the s-IBM patients when compared with the controls ($P<0.05$). However, there was no significant difference in CD4+ T cell staining as compared to the controls. It is interesting to note that there was no difference between the expression of inflammatory T cells and macrophages in the muscle biopsies from the s-IBM patients, in paper II, who had previously been treated with immunsuppressives or were still taking corticosteroids and those who were untreated. This is in contrast with an earlier report in which decreased inflammation in muscle biopsies from eight s-IBM patients was observed after corticosteroid treatment (Barohn et al 1995). On the other hand, Lindberg et al (1995) reported no clear change in inflammation in four IBM patients after prednisolon treatment, and Pruitt et al (1996) reported no changes in frequency of invaded fibres after prednisone treatment. It is, thus, obvious that the inflammation in s-IBM does not respond to immunosuppressive treatment. This raises the question of the difference between the

inflammation in s-IBM and the steroid sensitive inflammation in the other inflammatory myopathies. Today no one has the answer to this question and it is debated whether s-IBM is a primary inflammatory myopathy or a degenerative myopathy with a secondary inflammation (Askanas and Engel 1998, Oldfors and Fyhr 2001, Sivakumar and Dalakas 1997).

Adhesion molecules and cytokine expression (paper II and III)

The capillaries stained with EN-4 in the muscle biopsies from s-IBM patients had a thicker endothelium than the capillaries in the control biopsies, which indicate that the endothelial cells in the capillaries of the patients are activated. This was further supported by the increased expression of the adhesion molecules ICAM-1 and VCAM-1 in the microvessels. Adhesion molecules, ICAM-1 and VCAM-1 were detected in endothelial cells of capillaries, arterioles and venules as well as inflammatory cells. A statistically significant increase in staining for ICAM-1 and VCAM-1 was noted in the s-IBM patients as compared to the control muscle biopsies (P=0.04) (paper II).

IL-1α was detected in the endothelial cells in capillaries and in perifascicular arterioles and venules as well as in endomysial inflammatory cells. IL-1α was not detected in the muscle fibres. There was a statistically significant increase in the expression of IL-1α in the muscle biopsies from s-IBM patients compared to the controls. In the controls, IL-1α was only expressed in perifascicular vessels. Inflammatory cells expressing IL-1β were found in most muscle biopsies in the s-IBM patients. No significant number of cells expressing TNF-α, INF-γ or IL-8 was detected in muscle biopsies from the s-IBM patients or in the control muscle biopsies.

The increased expression of IL-1α and the adhesion molecules, together with the morphological changes in the blood vessels, support the notion that the blood vessels possibly play a role in the pathogenesis of s-IBM, but the functional importance of this observation is not clear. One possibility is that activated microvessels contribute to the sustained muscle tissue inflammation, which could induce damage to the muscle fibres. In paper I, different muscle fibre sizes were noted with type II fibres being significantly smaller in the s-IBM patients. A possible explanation for this observation could be that the changes in the blood vessels could affect the microcirculation in muscle tissue and the type II fibres to a greater extent since type II muscle fibres have more capillaries per fibre than type I fibres (Jakobsson et al 1990). Whether these changes of the microvessels are primary phenomena or secondary changes to the muscle inflammation is not known. However, if the changes in the

microvessels play a role in the pathogenesis, they may be of importance as targets for future treatments.

EFFECTS OF IVIG TREATMENT (paper IV)

On muscle inflammation (paper IV)

When a muscle biopsy taken 24 hour after an IVIg treatment was compared to a muscle biopsy taken before treatment, no changes of histopathology or in HLA expression were noted. The staining for macrophages (CD68) increased significantly as did the staining for CD4+ and CD8+ T cells in the muscle biopsies from s-IBM patients after the IVIg treatment compared to baseline muscle biopsies. Staining for CD3+ T-cells was not significantly changed after the treatment. The CD4+/CD8+ ratio was unchanged. No significant changes were noted in the percentage of areas positively stained for ICAM-1 or VCAM-1, in the muscle biopsy from s-IBM patients taken 24 hours after IVIg treatment compared to the baseline biopsy. The cytokine (IL-1α, IL-1β, TNF-α, INF-γ and IL-8) expression was also unchanged after the treatment. Furthermore, when a third muscle biopsy (n=4) was taken 6 – 30 months after the first IVIg treatment in four s-IBM patients, no changes in histopathology or in area positively stained for inflammatory cell markers or cytokine expression were seen in 3 of the muscle biopsies. In the one biopsy taken 30 months after the first biopsy an histopathology grade was increased, including increased amount of fat infiltration and fibrosis. In that biopsy, the inflammation was decreased compared to the baseline biopsy from the same s-IBM patient.

The reason for the increased staining for macrophages (CD68), CD3+ and CD8+ T cells is unknown. Aukrust et al. (1997) have described an increase in circulating CD8+ T cells in blood after IVIg infusion in patients with hypogammaglobulinaemia. They also describe a decrease in CD4+/CD8+ ratio and an activation of monocytes/macrophages. In a placebo-controlled tria,l high-dose IVIg was beneficial in patients with dermatomyositis (DM) not only in terms of clinical symptoms but also against inflammatory changes in muscle tissue (Dalakas 1993).

For patients with s-IBM the effect of IVIg on clinical symptoms has been more controversial and there are no previous studies in which the effect of IVIg on immunological markers such as IL-1 or adhesion molecules in muscle has been investigated. It cannot be ruled out that the dosage used in the present study, 30 g/day for 3 days, was too low to give a significant effect on the immunological markers in the muscle tissue and that the results might have been

different if a higher dose of IVIg had been used. Secondly, the time for the second biopsy, within 24 hours after the first infusion, may have been too early to catch a significant change of the immunological markers. This time was chosen based on the rapid effect of IVIg on cytokine production both in vitro and in vivo, particularly induction of the anti-inflammatory cytokine IL-1 ra, which could block both IL-1α and IL-1β (Andersson et al 1994, Aukrust et al 1999). No effect was on the expression of these two latter pro-inflammatory molecules in muscle tissue was seen after the first IVIg infusions, although the pre-treatment expression of both IL-1α and IL-1β was significantly higher than in healthy individuals. Unfortunately no antibody to detect IL-1ra expression in muscle tissue was available at this time, and an effect of IVIg treatment on IL-1ra cannot be excluded.

A decrease in mean diameter of capillaries after IVIg treatment has been reported in dermatomyositis (Dalakas 1993). In the present study there was no effect on the total endothelial cell size assessed as the percentage of the whole tissue area that was labelled with the endothelial cell marker, EN-4, after the IVIg treatment. This would have been expected if the total capillary area was decreased. For determination of the endothelial cell area, computerised image analysis was used that measures the percentage of positive expression of certain markers in the muscle tissue. This method does not exclude the possibility that the area of each capillary decreased and that the number of capillaries increased, making the percentage of positive endothelial cell area of the total tissue area unchanged.

On muscle strength (paper II)

In 6 of the 12 s-IBM patients the effect of IVIg treatment on muscle strength and muscle function was evaluated. No improvement in muscle function was noted. Both MRC value and FI were lower after 6 months, but these changes were not statistically significant. This might be explained by a stabilisation of the disease and slowing of the disease process, or by an insensitivity in the tests that were used to measure muscle function and strength. On the other hand, one may conclude that an improvement in muscle strength was not detected after the IVIg treatment. However, a possible positive effect on muscle strength cannot be excluded.

A lack of improved strength and function is not surprising as no effect was detected after the IVIg treatment on the inflammation or on the immunological markers that are upregulated in the inflamed tissue and are likely to influence the function of the muscle fibres.

EVALUATION OF TRAINING (paper III)

Muscle strength and function: After 12 weeks training with the home training program no significant improvement in muscle strength or muscle function was found. However, there was no deterioration of muscle strength or function either. Six of seven patients reported a subjective positive effect on muscle function, when they were asked about various activities in their daily lives and none of the patients experienced deterioration of muscle function.

Table 4. Median and mean values of MRC and FI before and after training.

	MRC Baseline	MRC Pretraining	MRC Posttraining	FI Baseline	FI Pretraining	FI Posttraining
Median value (25^{th} to 75^{th} percentiles)	116 (98-122)	108 (104-118)	110 (101-118)	34.5 (13.5-58.0)	37.5 (14.5-60.0)	34.5 (17.0-62.5)
Mean value	112	109	111.5	36	35	36.5

The changes in MRC and FI values between baseline and pretraining and between pretraining and posttraining were not significant.

In earlier studies a decline in muscle strength has been measured over a period of few months (Lindberg et al 1994, Rose et al 2001). Therefore a decrease in muscle strength was expected in the three-month period in between measurement of the baseline value and pretraining values. Rose et al. (2001) reported a 4% mean decline in muscle strength from baseline in a 6-month period, as assessed by myometry determining a maximum voluntary isometric contraction. Lindberg et al. (1994) reported a 1.4% decline in strength from baseline per month as assessed by hand-held myometer. In the present study, there was a trend towards a decreased strength when looking at mean and median value for MRC as well as mean value for FI, and after the training there was a slight increase in these values (table 4). The lack of significant changes might be due to the small number of patients included in the study or lack of sensitivity in the tests which were used to measure muscle function and strength. The MMT using MRC 5 graded scale is insensitive and small changes in strength are not detected. One must also take into consideration that the home training program is a form of endurance training with relatively low intensity and the training period was relatively short. In the study

by Spector et al. (1997) a strength-training program was analysed and it was concluded that the training could lead to a gain in muscle strength. The data from present study conclude that the home exercise program was safe for the patients and did not lead to any deterioration in muscle function or strength and can possibly prevent loss of muscle strength. However, there is a need for further studies in this field with evaluation of the different types of training and their effect on muscles in patients with s-IBM.

Histopathological changes: No statistically significant changes were found concerning histopathological degree after training. When the histopathological changes in general were assessed, the degree of histopathology was unchanged or decreased in the s-IBM patients except in two of the patients. In those patients an increased degree of histopathological abnormalities with end-stage changes was found after the training. These two patients had had the disease longest and were the oldest in the group. One possibility to consider is that the increase in pathology grade was due to a muscle biopsy bias, since no clinical deterioration was observed and creatine kinase (CK) values were unchanged during the training. Furthermore the muscle fibres abnormalities have a patchy distribution in IBM, as has been shown in muscle specimens from an autopsy study (Arnardottir et al. 2001). However, since two muscle biopsies from the same patient were often similar, the problem of muscle biopsy bias because of a small sample and/or patchy distribution of pathology seems not to be a major one, at least when the "semi-open" biopsy technique is used.

Muscle fibre size and fibre type could not be determined in two of the biopsies after the training because of end-stage changes. In the other four biopsies, the mean cross-sectional area of type I fibres increased whereas the mean cross-sectional area of type II fibres decreased in two, was unchanged in one and increased in one after the training. The trend to an increase in the cross-sectional area of type I muscle fibres after the training may be related to the type of exercise used in the training program. The training program was more an endurance training rather than strength training and may therefore lead to hypertrophy of type I fibres rather than type II.
Increase in the percentage of type IIc fibres was seen in all biopsies and there was a decrease in type IIb fibres in three of four biopsies after the training. The increase in the percentage of type IIc training was not statistically significant but one could speculate that this could be due to a muscle fibre transformation.

No significant changes were seen in the mean diameter of the capillaries after the training. The mean diameter of the capillaries in the biopsies before training was 90 μm^2 but 40 μm^2 in the two control biopsies. However, the endothelial marker EN-4 was significantly decreased ($p<0.05$) after the training, which indicates a decreased endothelial cell area. This could be explained by the trend to an increase in fibre size after the training leading to a lower capillary density, as no significant changes were observed in capillary diameter.

Muscle inflammation: All patients had an inflammatory reaction in the muscle biopsies before the training. After the training there were no significant changes of inflammatory cell infiltrates in muscle biopsies assessed as expression of macrophages and CD3+, CD4+ and CD8+ T-cells by immunohistochemistry and image analyses. In all but one of the patients a decrease of macrophage (CD68) staining was found after the training. There were no significant changes in the number of ICAM-1 positive endothelial cells, IL-1α positive area or the number of IL-1β positive cells. However, there was a decrease in the number of IL-1Ra positive cells in five of the six patients after the training, which was statistically significant ($p< 0.05$). This may suggest a decrease in inflammatory activity after training.

In conclusion, this training program was safe in terms of muscle inflammation and histopathology. All patients experienced a subjective positive effect, muscle strength was not decreased and there may have been a decrease in inflammatory activity, indicating that physical activities may be recommended for patients with s-IBM.

ANALYSIS OF SENSORY FUNCTION (paper II)

In Table 5 results from sensibility screening, QST, neurophysiology and muscle biopsy are presented. Sensibility impairment was found in eight of nine patients with s-IBM on screening. The abnormalities were more pronounced in the lower extremities.

Temperature was the modality most often abnormal on sensibility screening and was more often abnormal than on thermal thresholds. This has earlier been described by Borg et al. (1987) in patients with Welander distal myopathy (WDM), and has been suggested to due to a hypoesthesia confined to the suprathreshold range as described by Lindblom and Ochoa (1986).

Table 5. Number of patients with abnormalities on sensibility screening and QST, and neurogenic changes on neurophysiological examination and on muscle biopsy, of the nine patients tested.

	Hands	Feet
Sensibility screening		
Touch	1	2
Pain	3	4
Graphaesthesia	2	4
Temperature	6	7
QST		
Vibration	4	5
Thermal thresholds	1	4
Thermal pain thresholds	4	4
Neurophysiology		
NCV	3	3
EMG	6	6
Muscle biopsy		8

Heat pain thresholds were significantly higher in patients with s-IBM than in controls, while thermal thresholds did not differ between the groups. Vibration thresholds were significantly higher in the s-IBM patients as compared to the controls and were more often abnormal than thermal thresholds. Abnormal vibration thresholds indicate that myelinated nerve fibres are affected. This point to that unmyelinated nerve fibres (C-fibres) as well as large diameter myelinated nerve fibres are affected. In order to exclude variations of sensory thresholds because of age and gender, data were compared to data from an age- and sex-matched control group of patients with prior poliomyelitis, and compared with published reference values (Fruhstorfer et al 1976, Goldberg and Lindblom 1979). Furthermore, the controls were selected with the same degree of paresis as the s-IBM patients in order to rule out the possibility that the increased thresholds might be secondary to the paresis.

NCV was decreased in three of nine patients. All s-IBM patients had abnormal EMG findings with both myogenic and neurogenic changes. Abnormal NCV and nerve biopsy and muscle biopsy abnormalities pointing to a neurogenic affection have been described before in patients with s-IBM (Arnardottir et al 2001, Carpenter et al 1978, Eisen et al 1983, Hermanns et al 2000, Joy et al 1990, Lindberg et al 1990, 1991 and 1994, Lotz et al 1989, Schroder et al 1997). The results from the present study show sensibility impairment in s-IBM both in myelinated nerve fibres - especially the larger ones - and in small unmyelinated nerve fibres. This gives further support to the notion that there is a peripheral nerve involvement in s-IBM. However, from the present study it is not possible to determine if the effects on nerve has the same pathogenesis and as the effects on muscle, or is secondary to the myopathy, or if the effects on nerve and muscle simply coincide. It is interesting to note that in the study by Hermanns et al (2000) where sural nerve biopsies from patients with s-IBM and h-IBM were analysed, neuropathy was detected both in s-IBM and h-IBM and mononuclear cell infiltrates were not detected in the nerve. H-IBM differs from s-IBM in that there is almost no muscle inflammation in h-IBM. Furthermore, a sensory dysfunction has been described as an early pathological feature of WDM, a hereditary distal myopathy with rimmed vacuoles (Borg et al 1991). One might speculate that the peripheral neuropathy may have another background than the inflammatory muscle disease, or that the inflammation in the muscle is secondary to a pathological process that leads both to the myopathic changes in the muscle and to the neuropathy. In the immunohistochemical studies (I and III) it was noted that capillaries from patients with s-IBM have larger diameter and thicker endothelium than control muscle biopsies from individuals with no known muscle disease. Thus, a decrease in the microcirculation could be a possible mechanism behind the neuropathy seen in patients with s-IBM.

SUMMARY

The etiology of s-IBM is unknown and the pathogenetic mechanism is unclear. Despite the presence of inflammatory cell infiltrates in muscle tissue, treatment with corticosteroids and other immunosuppressive agents has had limited effect in patients with s-IBM. However, there have been anecdotal reports and studies suggesting beneficial effects of treatments, especially IVIg.

The general aims of the present study were to increase the understanding of the disease process in s-IBM by analysing the cytoskeletal structure in muscle fibres and their regeneration potential, and also to analyse the sensory function, in order to evaluate the hypothesis of an associated neurogenic component or a concomitant neuropathy in s-IBM as well as to evaluate therapeutic options by analysing the effect of IVIg treatment on the muscle inflammation and evaluate the effect of training on muscle function and muscle inflammation.

The cytoskeleton is well preserved in s-IBM, since dystrophin and desmin were normally expressed in hypertrophic and normal sized muscle fibres. However, atrophic muscle fibres and fibres undergoing de- or regeneration may exhibit alterations of cytoskeletal proteins as suggested by decreased staining for dystrophin and increased expression of desmin. There seems to be active regeneration in muscle from s-IBM patients. All of the s-IBM patients had muscle fibres that stained positively for vimentin, an intermediate filament mainly expressed during myogenesis and is an indicator of muscle fibre regeneration, and CD56, which is a satellite-cell antigen, also considered to be a marker for muscle fibre regeneration. A further support for ongoing regeneration is that the intermediate filament desmin was upregulated in muscle fibres that often also expressed vimentin, and almost all biopsies from the s-IBM patients showed muscle fibres positive to MHCn, suggesting that these fibres have activated a regenerative response.

Thermal sensibility was more often abnormal on sensibility screening in the patients with s-IBM than on quantitative determination of thermal thresholds. This has earlier been described in patients with Welander distal myopathy (WDM). A sensibility dysfunction with abnormal vibratory thresholds and thermal pain thresholds found in patients with s-IBM indicates involvement of both large myelinated nerve fibres as well as unmyelinated nerve fibres. This corroborates earlier findings with abnormal NCV and abnormal changes in nerve biopsies.

When the effect of IVIg treatment on immunological markers including HLA class I and II expression on muscle fibres, adhesion molecules and cytokine expression in capillaries and

muscle inflammation was investigated in muscle biopsies from s-IBM patients, surprisingly little effect was recorded on the presence of inflammatory cells or on the expression of cytokines and adhesion molecules in muscle tissue. Furthermore, when muscle strength and function after IVIg treatment was tested, no improvement was noted, which was not surprising, as no effect was detected on the inflammation or on the immunological markers that are upregulated in the inflamed tissue and are likely to influence the function of the muscle fibres.

It is interesting to note that in cross-sections of muscle biopsies from s-IBM patients, the capillaries seem thicker and larger, and when capillaries stained with EN-4 in the muscle biopsies from s-IBM patients were compared with those from controls, the former had significantly increased staining, which indicates that these endothelial cells are activated. This was further supported by the increased expression of the adhesion molecules ICAM-1 and VCAM-1 as well as the proinflammatory cytokine IL-1α in the microvessels. These findings support the notion that the blood vessels may play a role in the pathogenesis of s-IBM. However, the functional importance of this observation is not clear.

The home exercise program was well tolerated by patients with s-IBM. The patients percieved a subjective positive effect on muscle function. However, there was no statistically significant increase in muscle strength or muscle function. The subjective positive effect may be due to an improved fitness. The lack of statistically significant improvement in functions that could be measured objectively can be due to the low number of patients included, the relatively short training period or to an insensitivity in the tests which was used to measure muscle function and strength.

It is concluded that the muscle fibres of patients with s-IBM do have an ability to regenerate and there is an ongoing regeneration during the disease process. The IVIg treatment, which in some reports been suggested to have a possible beneficial effect in s-IBM, has little effect on the presence of inflammatory cells or on expression of cytokines and adhesion molecules in muscle tissue. The data from the present study do not support the notion that IVIg can be effective against s-IBM. Moderate physical training is safe for patients with s-IBM and may have positive effect on musscle strength and function. The sensory dysfunctions found in this study give support for the notion that there is a peripheral nerve involvement in s-IBM. Finally it is speculated that an affection of microvessels may be a part of the pathogenetic process leading to myopathic and neuropathic changes.

ACKNOWLEDGEMENTS

I whish to express my sincere gratitude to all who have supported me, and especially to:

Associate professor, **Kristian Borg**, my tutor, for introducing me to the field of neuromuscular disorders and generously sharing his knowledge of scientific work together with excellent competent guidance.

Associate professor, **Tor Ansved**, my co-tutor, for encouragement and positive guidance and not least for being a good friend.

Associate professor, **Ingrid Lundberg**, my co-tutor, for introducing me to the immunological aspects of myositis, positive guidance and all the interesting and valuable scientific discussions.

Professor, **Lars Edström**, for generously sharing his knowledge and introducing me to the world of electron microscopy.

Heléne Alexanderson, for introducing me to the field of physiotherapy and functional index in myositis and for all interesting scientific discussions.

Pernilla Englund, for teaching me the immunohistochemistry skills.

Margareta Samuelsson-Liljedahl for professional asistance with muscle biopsy procedure.

Ylva Friberg, Birgitta Hedberg and Lillibil Stuart for excellent histopathological assistance and for all invaluable help and company in the " muscle lab".

Gullevi Bramert for professional skill with QST.

Eva Lindroos, for excellent technical assistance and invaluable help in the lab at CMM.

Members of the **"Myositis Group"**, for all interesting scientific discussions, journal club and meetings outside work.

All the patients, who generously participated.

The staff at ward R18 at the Neurology Clinic at Karolinska Hospital.

Berit Joélius, for being a good friend and unfailing help.

My friends and colleagues **Haukur Hjaltason, Finnbogi Jakobsson, UllaBritt Gripenstedt and Lou Brundin** for encouragement and showing interest in my works.

Last, but not least, my husband **Kolbeinn** for endless love and belief in me and for computer support during the writing process and our children **Örn and Hallgerður**.

REFERENCES

Adams RD, Kakulas BA, Samaha FA. A myopathy with cellular inclusions. Trans Am Neurol Assoc 1965;90:213-216.

Alexanderson H, Stenstrom CH, Jenner G, Lundberg I. The safety of a resistive home exercise program in patients with recent onset active polymyositis or dermatomyositis. Scand J Rheumatol 2000;29:295-301.

Alexanderson H, Stenstrom CH, Lundberg I. Safety of a home exercise programme in patients with polymyositis and dermatomyositis: a pilot study. Rheumatology 1999;38:608-611.

Amato AA, Barohn RJ, Jackson CE, Pappert EJ, Sahenk Z, Kissel JT. Inclusion body myositis: treatment with intravenous immunoglobulin. Neurology 1994;44(8):1516-1518.

Amemiya K, Semino-Mora C, Granger RP, Dalakas MC. Downregulation of TBF-β1 mRNA and protein in muscles of patients with inflammatory myopathies after treatment with high – dose intravenous immunoglobulin. Clin Immunol 2000;94(2):99-104.

Aminoff M. Clinical electromyography. In: Aminoff M ed. Clinical electromyography. Churchill Livingstone, New York 1986.

Andersson U, Björk L, Skansen-Saphir U, Andersson J. Pooled human IgG modulates cytokine production in lymphocytes and monocytes. Immunol Rev 1994;139:21-42.

Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and demonstration and counts of muscle fibers invaded by T-cells. Ann Neurol 1984;16(2):193-208.

Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. III: Immunoelectron microscopy aspects of cell-mediated muscle fiber injury. Ann Neurol 1986;19:112-125.

Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. IV: Cell mediated cytotoxity and muscle fiber necrosis. Ann Neurol 1988A;23:168-173.

Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies V: Identification and quantitation of T8+ cytotoxic and T8+ suppresor cells. Ann Neurol 1988B;23:493-499.

Arnardottir S, Ansved T, Nennesmo I, Borg K. Report of a patient with inclusion body myositis and CD8+ chronic lymphocytic leukemia – post mortem analysis of muscle and brain. Acta Neurol Scand 2001;103:131-135.

Askanas V, Engel WK, Bilak M, Alvarez RB, Selkoe DJ. Twisted tubulofilaments of inclusion body myositis muscle resemble paired helical filaments of Alzheimer brain and contain hyperphosphorylated tau. Am J Pathol 1994;144(1):177-187.

Askanas V, Engel WK. Inclusion-body myositis: newest concept of pathogenesis and relation to aging and Alzheimer disease. J Neuropathol Exp Neurol 2001;60(1):1-14.

Askanas V, Engel WK. Sporadic inclusion-body myositis and hereditary inclusion-body myopathies. Diseases of oxitative stress and aging? Arch Neurol 1998;55:915-920.

Aukrust P, Muller F, Nordoy I, Haug CJ, Froland SS. Modulation of lymphocyte and monocyte activity after intravenous immunoglobulin administration in vivo. Clin Exp Immunol 1997;107:50-56.

Aukrust P, Muller F, Svenson M, Nordoy I, Bendtzen K, Froland SS. Administration of intravenous immunoglobulin (IVIg) in vivo – down-regulatory effects on the IL-1 system. Clin Exp Immunol 1999;115:136-143.

Badrising UA, Maat-Schieman MLC, Ferrari MD et al. Comparison of weakness progression in inclusion body myositis during treatment with methotrexate or placebo. Ann Neurol 2002;51:369-372.

Barohn RJ, Amato AA, Sahenk Z, Kissel JT, Mendell JR. Inclusion body myositis: explanation for poor response to immunosuppressive therapy. Neurology 1995;45(7):1302-1304.

Billeter R, Weber H, Lutz H, Howald H, Eppenberger HM, Jenny E. Myosin types in human skeletal muscle fibers. Histochem 1980;65:249-259.

Biral D, Scarpini E, Angelini C, Salviati G, Margreth A. Myosin heavy chain composition of muscle fibers in spinal muscular atrophy. Muscle Nerve 1989;12(1):43-51.

Borg K, Borg J, Lindblom U. Sensory involvement in distal myopathy (Welander). J Neurol Sci 1987;80(2-3):323-332.

Borg K, Edström L. Prior poliomyelitis: an immunohistochemical study of cytoskeletal proteins and a marker for muscle fibre regeneration in relation to usage of remaining motor units. Acta Neurol Scand 1993; 87:128-132.

Borg K, Åhlberg G, Borg J, Edström L. Welander`s distal myopathy: clinical, neurophysiological and muscle biopsy observations in young and middle aged adults with early symptoms. J Neurol Neurosurg Psychiatry 1991;54(6):494-498.

Borg K, Åhlberg G, Hedberg B, Edström L. Muscle fibre degeneration in distal myopathy (Welander) - ultrastructure related to immunohistochemical observations on cytoskeletal proteins and Leu-19 antigen. Neuromusc Disorder 1993; 2:149-155.

Bornemann A, Schmalbruch H. Anti-vimentin staining in muscle pathology. Neuropathol Appl Neurobiol 1993;19(5):414-419.

Brooke MH, Kaiser KK. Muscle fiber types: How many and what kind? Arch Neurol 1970;23:369-379.

Brouwer R, Hengstam GJD, Vree Egberts W et al. Autoantibody profiles in the sera of European patients with myositis. Ann Rheum Dis 2001;60:116-123.

Buchbinder R, Forbes A, Hall S, Dennett X, Giles G. Incidence of malignant disease in biopsy-proven inflammmatory myopathy. A population-based cohort study. Ann Intern Med 2001;134(12):1087-1095.

Carpenter S, Karpati G, Heller I, Eisen A. Inclusion body myositis: A distinct variety of idiopathic inflammatory myopathy. Neurology 1978;28:8-17.

Carpenter S, Karpati G, Wolfe L.Virus-like filaments and phospholipid accumulation in skeletal muscle. Study of a histochemically distinct chronic myopathy. Neurology 1970;20(9):889-903.

Cherin P, Pelletier S, Teixeira A, Laforet P, Simon A, Herson S, Eymard B. Intravenous immunoglobulin for dysphagia of inclusion body myositis. Neurology 2002;58:326-327.

Chou SM. Myxovirus-like structures in a case of human chronic polymyositis. Science 1967;158(807)1453-1455.

Cohen R, Lipper S, Dantzker DR. Inclusion body myositis as a cause of respiratory failure. Chest 1993;104:975-977.

Covault J, Sanes JR. Neural cell adhesion molecule (N-CAM) accumulates in denervated and paralyzed skeletal muscles. Proc Natl Acad Sci U S A 1985;82(13):4544-4548.

Dalakas MC, Illa I, Dambrosia JM, Soueidan SA, Stein DP, Otero C, Dinsmore ST, McCrosky S. A controlled trial of high-dose intravenous immune globulin infusions as treatment for dermatomyositis. N Engl J Med 1993;329(27):1993-2000.

Dalakas MC, Koffman B, Fujii M, Spector S, Sivakumar K, Cupler E. A controlled study of intravenous immunoglobulin combined with prednisone in the treatment of IBM. Neurology 2001;56:323-327.

Dalakas MC, Sonies B, Dambrosia J, Sekul E, Cupler E, Sivakumar K. Treatment of inclusion-body myositis with IVIg: a double blind, placebo-controlled study. Neurology 1997;48(3):712-716.

Dalakas MC. Molecular immunology and genetics on inflammatory muscle disease. Arch Neurol 1998;55:1509-1512.

Dalakas MC. Treatment of polymyositis and dermatomyositis. Curr Opin Rheumatol 1989;1:443-449.

Daube J. Nerve conduction studies. In: Aminoff M ed. Nerve conduction studies. Churchill Livingstone, New York 1986.

De Bleecker JL, Engel AG. Expression of cell adhesion molecules in inflammatory myopathies and Duchenne dystrophy. J Neuropathol Exp Neurol 1994;53:369-376.

De Visser M, Emslie-Smith AM, Engel AG. Early ultrastructural alterations in adult dermatomyositis. Capillary abnormalities precede other structural changes in muscle. J Neurol Sci 1989;94(1-3):181-192.

Eisen A, Berry K, Gibson G. Inclusion body myositis (IBM): Myopathy or neuropathy? Neurology 1983;33:1109-1114.

Emslie-Smith AM, Engel AG. Microvascular changes in early and advanced dermatomyositis: a quantitative study. Ann Neurol 1990;27(4):343-356.

Engel AG, Arahata K. Monoclonal antibody analysis of mononuclear cells in myopathies. II: Phenotypes of autoinvasive cells in polymyositis and inclusion body myositis. Ann Neurol 1984;16:209-215.

Essen B, Jansson E, Henriksson J, Taylor AW, Saltin B. Metabolic characteristics of fibre types in human skeletal muscle. Acta Physiol Scand 1975;95(2):153-165.

Fitzsimons RB, Hoh JFY. Embryonic and foetal myosin in human skeletal muscle. J Neurol Sci 1981;52:367-384.

Frostegard J, Ulfgren A-K, Nyberg P, Hedin U, Swedenborg J, Anderson U, Hanson GK. Cytokine expression in advanced human atherosclerotic plaques: dominance of pro-inflammatory (Th1) and macrophage-stimulating cytokines. Atherosclerosis 1999;145:33-43.

Fruhstorfer H, Lindblom U, Schmidt W.G. Method for quantitative estimation of thermal thresholds in patients. J Neurol Neurosurg Psych 1976;39:1071-1075.

Garlepp MJ, Laing B, Zilko PJ, Ollier W, Mastalgia FL. HLA associations with inclusion body myositis. Clin Exp Immunol 1994;98(1):40-45.

Goldberg J M, Lindblom U. Standardized method of determining vibratory perception thresholds for diagnosis and screening in neurological investigation. J Neurol Neurosurg Psych 1979;42:793-803.

Griggs RC, Askanas V, DiMauro S, Engel A, Karpati G, Mendell JR, Rowland LP. Inclusion body myositis and myopathies. Ann Neurol 1995;38:705-713.

Guarantors of Brain. Aids to the examination of the peripheral nervous system. London: Bailliére Tindall; 1986.

Habermann-Horstmeier L. Alterbedingte Schwellenänderungen der cutanen Vibrations-, Temperatur und Schmerzempfindung des Menschens. Thesis. (Institut für normale und pathologische Physiologie der Philips-Universität Marburg), 1-89, 1986.

Hamalainen N, Pette D. Patterns of myosin isoforms in mammalian skeletal muscle fibres. Microsc Rec Tech 1995;30(5):381-389.

Hansson P, Lindblom U, Lindstrom P. Graded assessment and classification of impaired temperature sensibility in patients with diabetic polyneuropathy. J Neurol Neurosurg Psych 1991;54:527-530.

Hengstam GJD, Brouwer R, Vree Egberts WTM, Seelig HP, Jongen PJH, van Venrooij WJ, van Engelen BGM. Clinical and serological characteristics of 125 Dutch myositis patients. J Neurol 2002;249:69-75.

Henriksson KG. ”Semi-open”muscle biopsy technique. A simple outpatient procedure. Acta Neurol Scand 1979;59:317-323.

Hermanns B, Molnar M, Schroder JM. Peripheral neuropathy associated with hereditary and sporadic inclusion body myositis: confirmation by electron microscopy and morphometry. J Neurol Sci 2000;179:92-102.

Hintz CS, Coyle EF, Kaiser KK, Chi MM, Lowry OH. Comparison of muscle fiber typing by quantitative enzyme assays and myosin ATPase staining. J Histochem Cytochem 1984;32(6):655-660.

Hohlfeld R, Engel AG. Coculture with autologous myotubes of cytotoxic T cells isolated from muscle in inflammatory myopathies. Ann Neurol 1991;29:498-507.

Hohlfeld R, Engel AG. The immunobiology of muscle. Immunol Today 1994;15(6):269-274.

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.

Jakobsson F, Borg K, Edström L. Fibre-type composition, structure and cytoskeletal protein location of fibres in anterior tibial muscle. Acta Neuropathol 1990;80:459-468.

Jamal GA, Hansen S, Weir AI, Ballantyne JP. The neurophysiologic investigation of small fiber neuropathies. Muscle Nerve 1987;10(6):537-545.

Jamal GA, Weir AI, Hansen S, Ballantyne JP. Myotonic dystrophy. A reassessment by conventional and more recently introduced neurophysiological techniques. Brain 1986;109(6):1279-1296.

Jones DA, Newham DJ, Round JM, Tolfree SEJ. Experimental human muscle damage: Morphological changes in relation to other indices of damage. J Physiol 1986;375:435-448.

Josefson A, Romanus E, Carlsson J. A functional index in myositis. J Rheumatol 1996;23:1380-1384.

Joy JL, Oh SJ, Baysal AI. Electrophysiological spectrum of inclusion body myositis. Muscle Nerve 1990;13(10):949-951.

Koffman BM, Rugiero M, Dalakas MC. Immune-mediated condition and antibodies associated with sporadic inclusion body myositis. Muscle Nerve 1998;21:115-117.

Koffman BM, Sivakumar K, Simonis T, Stroncek D, Dalakas MC. HLA allele distribution distinguishes sporadic inclusion body myositis from hereditary inclusion body myopathies. J Neuroimmunol 1998;84:139-142.

Lindberg C, Borg K, Edström L, Hedström A, Oldfors A. Inclusion body myositis and Welander distal myopathy: a clinical, neurophysiological and morphological comparsion. J Neurol Sci 1991;103:76-81.

Lindberg C, Oldfors A, Hedström A. Inclusion body myositis: peripheral nerve involvement. Combined morphological and electrophysiological studies on peripheral nerves. J Neurol Sci 1990;99:327-338.

Lindberg C, Oldfors A, Tarkowski A. Local T-cell proliferation and differentiation in inflammatory myopathies. Scand J Immunol 1995;41:421-426.

Lindberg C, Persson LI, Bjorkander J, et al. Inclusion body myositis: clinical, morphological, physiological and laboratory findings in 18 cases. Acta Neurol Scand 1994; 89: 123–131.

Lindblom U, Ochoa J. Somatosensory function and dysfunction. In: Ashbury AK, McKhann G, Mc Donald WI eds. Diseases of the nervous system. Clinical Neurobiology. Philadelphia, PA:WB Saunders Comp, 1986:283-298.

Lotz BP, Engel AG, Nishino H, Stevens JC, Litchy WJ. Inclusion body myositis. Observations in 40 patients. Brain 1989;112:727-747.

Love LA, Leff RL, Fraser DD, Targoff IN, Dalakas M, Plotz PH, Miller FW. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine (Baltimore) 1991;70(6):360-374.

Lundberg I, Ulfgren A-K, Nyberg P, Andersson U, Klareskog L. Cytokine production in muscle tissue of patients with idiopathic inflammatory myopathies. Arthritis Rheum 1997;40:865-874.

Lundberg IE. The role of cytokines, chemokines, and adhesion molecules in the pathogenesis of idiopathic inflammatory myopathies. Curr Rheumatol Rep 2000;2(3):216-224.

Mantegazza R, Bernasconi P, Confalonieri P, Cornelio F. Inflammatory myopathies and systemic disorders: a review of immunopathogenic mechanisms and clinical features. J Neurol 1997;244:277-287.

Mauro A. Satellite cell of skeletal muscle fibers. J Biophys Biochem Cytol 1961;9:493-498.
Muir AR, Kanji AH, Allbrook D. The structure of the satellite cells in skeletal muscle. J Anat 1965;99:435-444.

Nordenskiold UM, Grimby G. Grip force in patients with rheumatoid arthritis and fibromyalgia and in healthy subjects. A study with the Grippit instrument. Scand J Rheumatol 1993;22:14-19.

Nyberg P, Wikman AL, Nennesmo I, Lundberg I. Increased expression of interleukin 1α and MHC class I in muscle tissue of patients with chronic, inactive polymyositis and dermatomyositis. J Rheumatol 2000;27:940-948.

Oldfors A, Fyhr IM. Inclusion body myositis: genetic factors, aberrant protein expression, and autoimmunity. Curr Opin Rheumatol 2001;13:469-475.

Padykula HA, Herman E. The specificity of the histochemical method of adenosine triphosphatase. J Histochem Cytochem 1955;3:170-195.

Pruitt II JN, Showalter CJ, Engel AG. Sporadic inclusion body myositis: counts of different types of abnormal fibers. Ann Neurol 1996;39:139-143.

Riminton DS, Chambers ST, Parkin PJ, Pollock M, Donaldson IM. Inclusion body myositis presenting solely as dysphagia. Neurology 1993;43(6):1241-1243.

Rose MR, McDermott MP, Thornton CA, Palenski C, Martens WB, Griggs RC. A prospective natural history study of inclusion body myositis: Implications for clinical trials. Neurology 2001;57:548-550.

Sato T, Walker DL, Peters HA, Reese HH, Chou SM. Chronic polymyositis and myxovirus-like inclusions. Arch Neurol 1971;24:409-418.

Schroder JM, Molnar M. Mitochondrial abnormalities and peripheral neuropathy in inflammatory myopathy, especially inclusion body myositis. Mol Cell Biochem 1997;174(1-2):277-281.

Schubert W, Zimmerman K, Cramer M, Starzinski-Powitz A. Lymphocyte antigen Leu-19 as a molecular marker of regeneration in human skeletal muscle. Proc Natl Acad Sci 1989; 86:307-311.

Schultz E, McCormick KM. Skeletal muscle satellite cells. Rev Physiol Biochem Pharmacol 1994;123:213-257.

Sekul EA, Chow C, Dalakas MC. Magnetic resonance imaging of the forearm as a diagnostic aid in patients with sporadic inclusion body myositis. Neurology 1997;48(4):863-866.

Shamim EA, Rider LG, Miller FW. Update on the genetics of the idiopathic inflammatory myopathies. Curr Opin Rheumatol 2000;12:482-491.

Sivakumar K, Dalakas MC. Inclusion body myositis and myopathies. Curr Opin Neurol 1997;10:413-420.

Soueidan SA, Dalakas MC. Treatment of inclusion-body myositis with high-dose intravenous immunoglobulin. Neurology 1993;43:876-879.

Spector SA, Lemmer JT, Koffman BM, Fleisher TA, Feuerstein IM, Hurley BF, Dalakas MC. Safety and efficacy of strength training in patients with sporadic inclusion body myositis. Muscle Nerve 1997;20:1242-1248.

Stalberg E, Chu J, Bril V, Nandedkar S, Stalberg S, Ericsson M. Automatic analysis of the EMG interference pattern. Electroencephalogr Clin Neurophysiol 1983;56(6):672-681.

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;40:563-568.

The Muscle Study Group. Randomized pilot trial of βINF1a (Avonex) in patients with inclusion body myositis. Neurology 2001;57:1566-1570.

Thornell LE, Billeter R, Butler-Browne GS, Eriksson PO, Ringqvist M, Whalen RG. Development of fiber types in human fetal muscle. J Neurol Sci 1984;66:107-115.

Walter MC, Lochmuller H, Toepfer M, Schlotter B, Reilich P, Scroder M, Muller-Felber W, Pongratz D. High-dose immunoglobulin therapy in sporadic inclusion body myositis: a double blind, placebo-controlled study. J Neurol 2000;247:22-28.

Warhol MJ, Siegel AJ, Evans WJ, Silverman LM. Skeletal muscle injury and repair in marathon runners after competition. Am J Pathol 1985;118:331-339.

Verma A, Bradley WG, Soule NW, Pendlebury WW, Kelly J, Adelman LS, Chou SM, Karpati G, Brenner JF. Quantitative morphometric study of muscle in inclusion body myositis. J Neurol Sci 1992;112(1-2):192-198.

Wiesinger GF, Quittan M, Aringer M, Seeber A, Volc-Platzer B, Smolen J, Graninger W. Improvement of physical fitness and muscle strength in polymyositis/dermatomyositis patients by a training programme. Br J Rheumatol 1998 A;37:196-200.

Wiesinger GF, Quittan M, Graninger M, Seeber A, Ebenbichler G, Sturm B et al. Benefit of 6 months long-term physical training in polymyositis dermatomyositis patients. Br J Rheumatol 1998 B;37:1338-1342.

Winter A, Bornemann A. NCAM, vimentin and neontal myosin heavy chain expression in human muscle diseases. Neuropathol Appl Neurobiol 1999;25:417-424.

Virtanen J, Kalljock M, Navanen 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.

Yunis EJ, Samaha FJ. Inclusion Body Myositis. Lab Invest 1971;25(3):240-248.