Dual effects of caspase-1, interleukin-1β, tumour necrosis factor-α and nerve growth factor receptor in inflammatory myopathies

Z. Mackiewicz[3,4], M. Hukkanen[2,3], D. Povilenaite[4], A. Sukura[5], J.E Fonseca[6], I. Virtanen[3], Y.T. Konttinen[1,2,3]

[1]Department of Medicine/invärtes medicin, Helsinki University Central Hospital;
[2]ORTON Research Institute and the Orthopaedic Hospital of the Invalid Foundation;
[3]Institute of Biomedicine/Anatomy, Biomedicum Helsinki, University of Helsinki, Finland;
[4]Department of Cell Biology, University of Opole, Poland;
[5]Department of Veterinary Pathology, University of Helsinki, Finland;
[6]Unidade de Reumatologia and Institute for Molecular Medicine Santa Maria Hospital, University of Lisbon, Portugal.

Abstract

Objective

To analyse the expression of factors potentially involved in skeletal muscle degeneration and regeneration in dermatomyositis (DM), systemic sclerosis (SSc), polymyositis (PM), systemic lupus erythematosus (SLE) and non-inflammatory myopathies.

Methods

Immunohistochemical staining of skeletal muscle biopsies (10 DM, 10 SSc, 10 PM, 10 SLE, 10 non-inflammatory myopathies) for tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), activated caspase-1, pan-macrophage marker CD68, inducible nitric oxide synthase (NOS2) and nerve growth factor receptor (NGFR). TechMate™ staining robot and biotin-streptavidin protocol were used.

Results

Expression of TNF-α, IL-1β, caspase-1 and NOS2 was found in the cytoplasm and sarcolemma of dystrophic skeletal muscle fibres. TNF-α and IL-1β immunoreactive profiles were faint and few and close to satellite nuclei-containing regenerating muscle fibres both in inflammatory and non-inflammatory myopathies. NGFR expression was found in comparable areas. In non-inflammatory inherited myopathies more nuclei were caspase-1 immunoreactive whereas caspase-1 expression was rarely seen in inflammatory myopathies, implying regeneration of the affected muscle fibres.

Conclusion

Prominent expression of the proinflammatory factors TNF-α, IL-1β and NOS2 and caspase-1 is associated with muscle fibre damage, albeit when expressed to a low degree these factors may, like NGFR, contribute to muscle regeneration and healing.

Key words
Inflammatory myopathy, degeneration, regeneration, skeletal muscle, biopsy, immunohistochemistry.

Zygmunt Mackiewicz, PhD, DSc, Professor; Mika Hukkanen, PhD, Associate Professor; Danute Povilenaite, MD, Assistant Professor; Antti Sukura, DVM, PhD, Professor; Jõão E. Fonseca, MD, Ismo Virtanen, MD, PhD, Professor; Yrjö T. Konttinen, MD, PhD, Professor.

This study was supported by CIMO, the Sigrid Juselius Foundation, the Academy of Finland, the Ministry of Education, Finska Läkaresällskapet, HUS evo-grant, Finland, the and Ministry of Higher Education of Lithuania.

Please address correspondence to:
Prof. Yrjö T. Konttinen
Biomedicum Helsinki
PO Box 700 (Haartmaninkatu 8)
FIN-00029 HUS, Finland.
E-mail: yrjo.konttinen@helsinki.fi

Received on July 15, 2002; accepted on December 3, 2002.

Introduction

Contrary to earlier belief, the muscle cells are immunologically active and, accordingly, are involved in a number of disease conditions (1-3). The idiopathic inflammatory myopathies comprise a group of primary muscle diseases of unknown aetiology and obscure pathogenesis, characterized by a long-lasting and often treatment-refractory, symmetrical, proximal muscle weakness and pain. Polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM) are the chief members of this group of diseases (4-6). Inflammatory myopathies can occur in association with other systemic autoimmune diseases, e.g. systemic sclerosis (SSc), systemic lupus erythematosus (SLE), polymyalgia rheumatica and rheumatoid arthritis. Various other diseases such as cancer and congestive heart failure can also lead to muscle catabolism and loss of muscle function (7). Some myopathies arise as a consequence of intoxications, infection or innervation disturbances.

The pathogenesis of the myopathies leading to cachexia and weakness is largely unknown. Autoantibodies are formed against many muscle-specific (Jo-1, Mi-2, $tRNR^{His}$) and non-specific autoantigens (PM-Scl, U1RNP, native Ro, Ro60, Ro52, La) in myopathies (8, 9). In addition to autoantibodies, some proinflammatory cytokines, such as TNF-α and IL-1β, are involved. There is growing evidence suggesting that they may play a role not only in muscle damage, but possibly also in muscle regeneration (10). Therefore, the aim of the present study was to analyse some cytokine cascades and molecules involved in regeneration in relation to the primary idiopathic inflammatory myopathies and secondary inflammatory myopathies, and to assess their relation to muscle fibre damage and their possible regenerative effects. As additional comparative groups, a set of non-inflammatory myopathies associated with acquired disturbances in the innervation (osteoarthritis-caused radiculopathies) and genetically determined muscle/nerve diseases were included.

Patients and methods

Patients and samples

After their informed consent was obtained, 50 patients (Table I) were clinically examined prior to biopsies. All biopsies were taken from deltoid or quadriceps muscles and were primarily for diagnostic purpose.

The insidious, painless onset of proximal muscle weakness was the dominant clinical feature of all patients with inflammatory myopathies. They met the diagnostic criteria established by the American College of Rheumatology for the diagnosis of SSc and SLE, and the criteria for PM and DM according to the Bohan and Peter classification (11,12). Biopsy samples were fixed in formalin and ethanol and embedded in paraffin.

Table I. Clinical characteristics of the patient groups studied.

Condition	No. of pts.	Mean age, years (range)	Sex M/F	Disease duration (months)
Dermatomyositis	10	36.8 (19-62)	2/8	5.3
Systemic sclerosis	10	43.7 (18-62)	2/8	17.4
Polymyositis	10	45.9 (19-70)	2/8	5.1[1]
Systemic lupus erythematosus	10 (25-72)	48.7	1/9	26.7
Non-inflammatory myopathies[2]	10	44.5 (29-69)	4/6	Inborn in 5 cases, 5 mos. in other cases

[1]One patient with juvenile PM complicated by Teutschländer syndrome who had a 23-year long disease history has not been included. [2]Five cases of genetically determined myopathies: Leyden-Mobius disease, myastenia gravis,Duchenne disease, Charcot-Marie-Tooth disease and spinal amyotrophy; and 5 cases of acquired myopathies secondary to osteoarthritis-caused radiculopathies.

Primary antibodies

The primary antibodies used were: affinity purified rabbit anti-human caspase-1 IgG produced against a peptide mapping at the amino terminus of the caspase-1, active form (A-19): sc-622, 1:100, Santa Cruz Biotechnology, Inc., CA, USA); rabbit anti-human TNF-α (Code PS030, 1:400, Monosan, Uden, The Netherlands); monoclonal mouse anti-human low affinity nerve growth factor receptor IgG1/κ (NGFR, Code M3507, 1:25, DAKO Corporation, CA, USA); monoclonal mouse anti-human CD68 IgG1/κ (recognizes KP1 epitope, 1:100, DAKO A/S, Glostrup, Denmark) as a marker for macrophages; affinity-purified polyclonal rabbit anti-human IL-β, IgG (1:200, Genzyme Diagnostics, Cambridge, USA); and affinity-purified polyclonal rabbit anti-human inducible nitric oxide synthase (NOS2) IgG raised against a peptide mapping at the carboxy terminus (C-19): sc-649, 1:100, Santa Cruz Biotechnology, Inc., CA, USA).

Immunohistochemistry

5-μm paraffin sections were mounted on DAKO capillary slides (TechMate™, DAKO, Glostrup, Denmark), deparaffinized in xylene and rehydrated in graded ethanol series to 10 mM phosphate-buffered 0.9 M saline, pH 7.4 (PBS). For antigen retrieval, the slides were placed into Antigen Retrieval Buffer for the use with TechMate™ Instruments (DAKO A/S, Denmark) and microwaved for 10 minutes at 600W, then cooled at room temperature for 30 minutes, washed in PBS and immunostained automatically using the following protocol: 1) the primary antibody, diluted with DAKO ChemMate™ antibody diluent, for 1 hour; 2) secondary antibody containing both biotinylated goat anti-rabbit IgG and biotinylated goat anti-mouse IgG antibodies for 30 minutes; 3) peroxidase block for 30 minutes; 4) peroxidase-conjugated streptavidin 3 times for 3 minutes; 5) HRP Substrate Buffer and 6) incubated with substrate solution containing 3,3'-diaminobenzidine tetrachloride (ChemMate™ Detection Kit) for 5 minutes. Between each step, the sections were washed with DAKO ChemMate™ washing buffers three times and dried with absorbent pads. Replacement of primary antibodies with normal rabbit or mouse IgG with irrelevant specificity diluted to the same concentration as the primary antibodies were used as negative staining controls. After immunostaining the sections were counterstained with haematoxylin or left without counterstaining, washed, dehydrated in ethanol series, cleared in xylene and mounted in synthetic mounting medium (Diatex, Beckers Industrifäg AB, Märsta, Sweden).

Semi-quantitative microscopic assessment

Semi-quantitative assessment was done using digital MTV-3 camera on an Olympus BH2-RFCA microscope. The whole section area under magnification 400x (high power field) was analysed and scored to four grades: 0 meant no immunoreactivity present in the field; ± one or few positive immunoreactive profiles; + many profiles; or ++ abundant profiles. Two histopathologists independently scored all sections.

Results

Apart from the topological intra-sample differences with respect to the degree of expression of the molecules studied in degenerating and regenerating muscle fibres, the general semi-quantitative pattern of immunoreactivity is shown in Table II. The total score of immunoreactivity in patient groups with DM and SSc (the diseases characterised *inter alia* by progressive blood vessel damage and their further atresia, especially in SSc) dominated slightly over the groups of PM and SLE (in which vascular damage often can be reversible and not as serious as in SSc). In SSc patients, the indices studied were high, and signs of vascular and muscle fibre regeneration were generally absent. The total value of the indices in the DM patient group was comparable to that of PM. The regeneration in the muscle fibres and especially in the vasculature seemed more evident in PM than in DM. Satellite cells formed one of the principal signs of muscle fibre regeneration (13). In the myopathy secondary to radiculopathy, only IL-1β was expressed at levels comparable to the other inflammatory myopathies. Histopathological signs of muscle degeneration and regeneration were weak in PM. In genetically determined myopathies the highest indices were related to TNF-α, NOS2 and NGFR. In this group also the markers of regeneration were most evident. There were numerous satellite cells, with intensive expression of all the epitopes studied.

TNF-α was expressed in some skeletal muscle fibres (Fig. 1A). TNF-α immunoreactivity was strong only in the atrophic and dystrophic fibres. Often different degrees of TNF-α expression were seen in the same fibre (Fig. 1B). TNF-α was also found in some mononuclear inflammatory cells infiltrating the endo- and perimysium (Fig. 1C). Generally, there was no TNF-α expression in the blood vessel walls, or the expression was very faint. A more intense TNF-α expression was found in the muscle spindles, the stretch receptors of muscles. Scarcely distributed cells with low TNF-α expression were

Table II. Semi-quantitative scores[1] for immunoreactive profiles in different myopathies.

Disease	TNF-α	IL-1β	Caspase-1	CD68	NOS2	NGFR
Dermatomyositis	+	0/±	0/±	+	+	0/±
Systemic sclerosis	+	+	±	+	+	0/±
Polymyositis	+	+	0/±	0/±	+	0/±
Systemic lupus erythematosus	+	+	0/±	±	+	0/±
Myopathy secondary to radiculopathy	0/±	+	0/±	0/±	0/±	0/±
Genetically determined myopathy	+	0/±	±/+	0/±	+	+

[1]Results were scored as follows:0:no immunoreactivity in the field; ±: one or few positive profiles; +: numerous profiles; ++: abundant profiles.

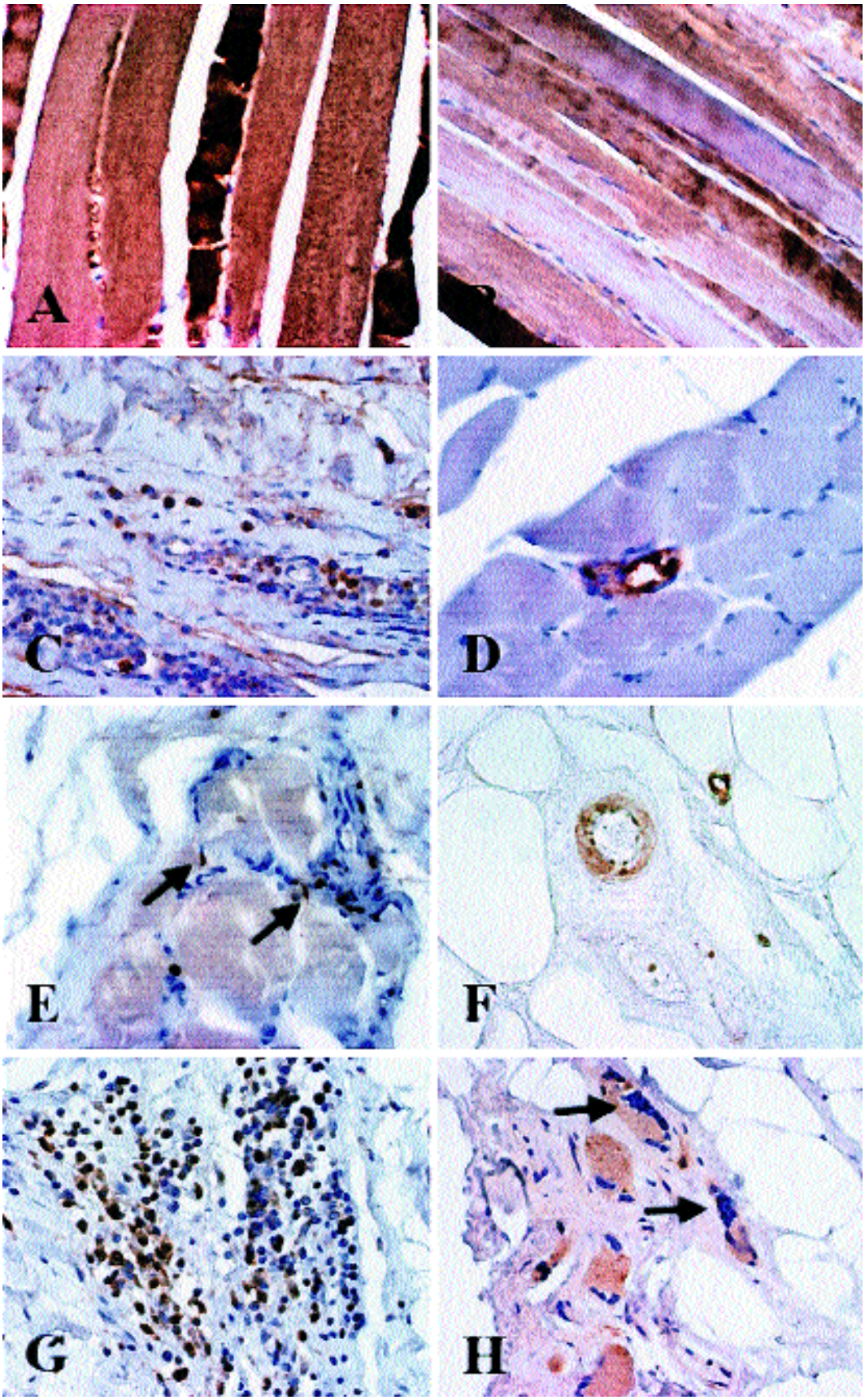

Fig 1. Expression of TNF-α, IL-1β and caspase-1 in myopathies. Immunostaining for TNF-α in polymyositis (panel **A**), systemic lupus erythematosus (panel **B**) and systemic sclerosis (panel **C**). Immunostaining for IL-1β in skeletal muscle affected by SLE (panel **D**) and SSc (panel **E**). Expression of caspase-1 in vascular wall (panel **F**) and in perimysium (panel **G**) in a patient with SSc, and in satellite cells (panel **H**) in a patient with myopathy related to progressive demyelinating neuropathy (Charcot-Marie-Tooth disease). Original magnification x400.

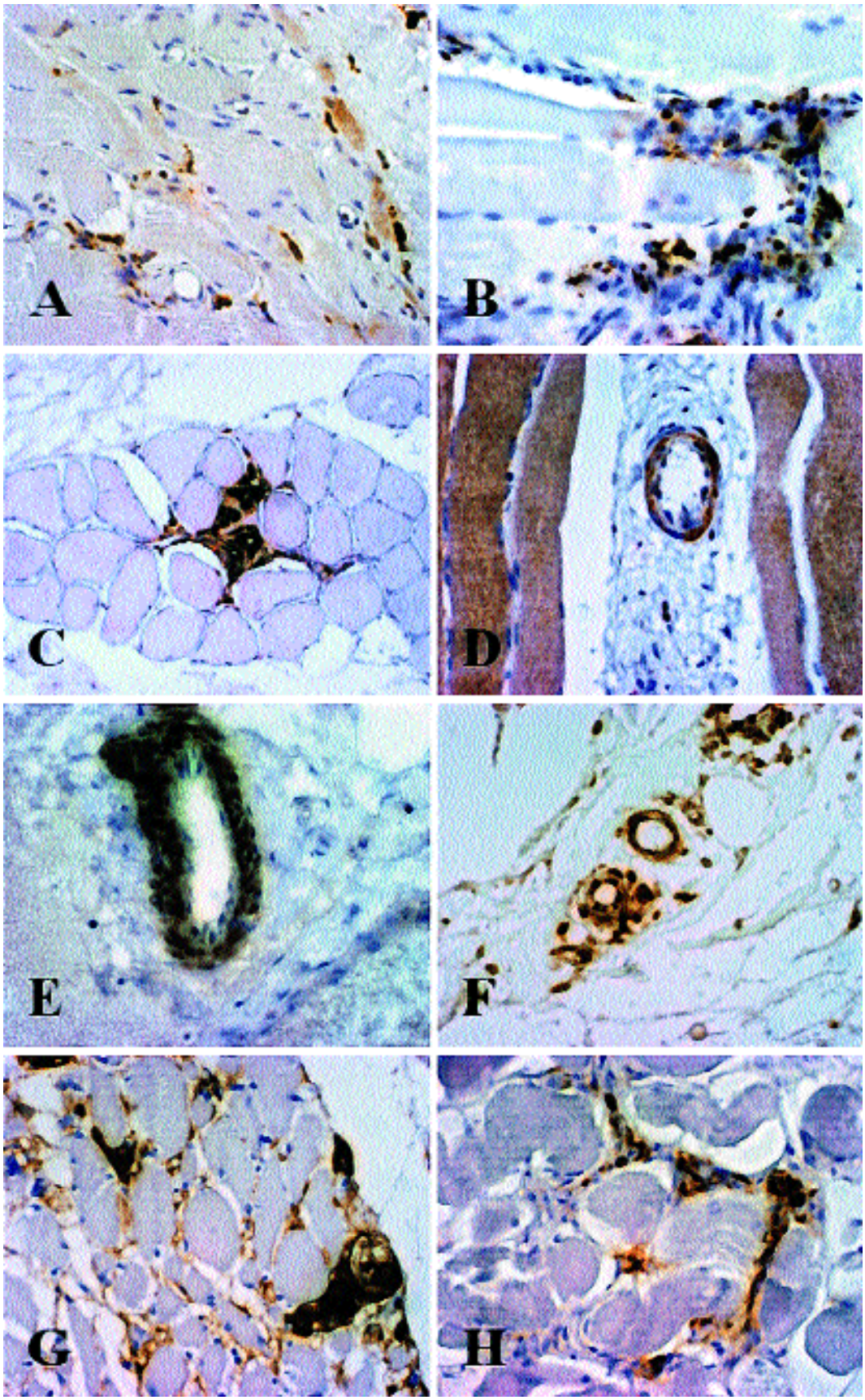

Fig 2. Expression of macrophage (CD68) antigens,NOS2 and NGFR in myopathy-affected muscles. Immunostaining for CD68 in DM (panels **A** and **B**), and in PM (panel **C**). Immunostaining for inducible NOS2 in PM (panel **D**), SSc (panel **E**), and SLE (panel **F**). Immunostaining for NGFR in Charcot-Marie-Tooth-related non-inflammatory myopathy (panel **G**) and in DM (panel **H**). Original magnification x400.

detected close to the regenerating satellite nuclei-containing skeletal muscle fibres in the inflammatory and inborn myopathies. No histopathological signs of muscular destruction were seen in these areas.

IL-1β and active caspase-1 (also known as IL-1 converting enzyme, ICE) showed similar patterns of immunoreactivity, with IL-1β reactivity being somewhat more intense. IL-1β was found in the vascular walls (Fig. 1D) and in some inflammatory cells infiltrating the endo- and perimysium (Fig. 1E). Caspase-1 was similarly distributed and also found in some of the blood vessels (Fig. 1F) and inflammatory cells infiltrating the endo- and perimysium (Fig. 1G). Very occasional caspase-1 immunoreactivity was also found in the nuclei of muscle fibres, except for some non-inflammatory myopathies, which were characterized by small areas containing caspase-1 immunoreactive muscle fibres and endothelial cell nuclei. Caspase-1 localized also to satellite cells of regenerating muscle fibres (Fig. 1H).

Some of the cells infiltrating the endo- and perimysium were CD68-positive macrophages (Fig. 2A and B). Occasionally these cells were phagocytosing parts of the muscle fibres (Fig. 2C). Amongst the CD68-negative mononuclear infiltrating cells there were also scarce lymphocytes.

NOS2 immunoreactivity was found in some blood vessel walls, mostly in the media and the adventitia (Fig. 2D and E), infiltrating mononuclear inflammatory cells (Fig. 2F) and diffusely in muscle fibres.

The highest level of NGFR expression was detected in the genetically determined myopathies (Fig. 2G). In addition, NGFR immunoreactivity was found in endomysium in the inflammatory myopathies (Fig. 2H) and in blood vessel walls and perivascularly.

Discussion

Inflammatory myopathies are characterized by antibody- and cell-mediated responses against mostly unknown muscle tissue antigens. IL-1β and TNF-α are expressed in human myoblasts after stimulation with pro-inflammatory cytokines. This indicates that muscle cells behave as immunologically active cells during inflammation (14,15). Muscle cells may be actively involved in the process of mononuclear cell recruitment from the blood stream to the areas of inflammation.

TNF-α is a key factor in the initiation and perpetuation of inflammation, and for *in situ* cytokine expression by leukocytes, endothelial cells and mesenchymal cells (16). It has been established that TNF-α diminish muscle strength via both contractile dysfunction and accelerated protein loss (2, 17). It has been shown that many endothelial cells in DM express TNF-α (10). TNF-α can induce its own expression (15) and perpetuate and amplify inflammatory process within muscle tissue (7). TNF-α upregulates intercellular adhesion molecules on endothelial cells (18,19). TNF-α in inflammatory myopathies increases cell trafficking through adhesion molecule upregulation, activation of T and B cells and macrophages, induction of apoptosis and induction of nitric oxide production.

In general, TNF-α has been considered a central mediator of immune and inflammatory responses in myopathies. TNF-α was also detected in invading macrophages, in myonuclei of regenerating muscle fibres and freely dispersed in endomysial and perimysial connective tissue (17). However, this role of TNF-α is directed towards proliferation rather than tissue degradation (20). TNF-α induces inflammatory angiogenesis (21) and increases capillary permeability (22). Despite its potential importance in myopathies, the direct effects of TNF-α on skeletal muscle remain unelucidated (23, 24). TNF-α inhibits myoblast differentiation and limits the regenerative response of satellite cells (25-27). In our study TNF-α, when strongly expressed by many cells, especially in clusters, was undoubtedly related to muscle fibre damage. On the other hand, in the biopsy areas where the expression of TNF-α was low, positively stained profiles were few and histological signs of proliferation were evident with no signs of any deterioration. It was obvious that TNF-α in these cases participated in muscle proliferation and healing, or at least in attempts at reparation. This was also confirmed by the expression of neurotrophin binding receptors in such areas.

TNF-α does not usually function as a solitary stimulus (23). IL-1β has been suggested to be the next most important proinflammatory factor in the inflammatory myopathies (10,17, 28,29),and is often associated with TNF-α. In our study, expression of IL-1β was found in all patient groups and in all biopsies, usually within vascular walls and in some of the infiltrating mononuclear inflammatory cells. Thus, the localization of TNF-α and IL-1β were to an extent apparently overlapping but not identical, especially in the blood vessel walls, where the expression of TNF-α was not found at all or was very faint. In contrast, immunolocalization of IL-1β was usually absent or very faint in the muscle fibres and different from that of TNF-α.

At least fourteen members of the caspase family have been described in mammalian cells (30). Caspase-1 and caspase-11 are primarily involved in the processing of proinflammatory cytokines, and are not crucial in apoptosis. The active form of caspase-1, the IL-1β converting enzyme (ICE), had in inflammatory muscle diseases a distribution similar to that of IL-1β, suggesting that it was involved in the activation of proIL-1β to its active form. Caspase-1 is also involved in cell proliferation (31, 32). There is a relationship between caspase-1, interleukins and NOS (33). In our analysis the expression of caspase-1 was mostly related to the sites of tissue regeneration.

Skeletal muscle constitutively expresses NOS (34). Inflammatory mediators can further stimulate NO production by muscle cells (35, 36). Transcription factor NF-κB plays a key role in the transcriptional regulation of several genes involved in immune and inflammatory responses including TNF-α, IL-1β, and iNOS (37). In a mouse model of TNF-α-induced cachexia, muscle wasting was partially inhibited by systemic administration of NOS inhibitor (38). TNF-α, via an effect on NOS synthesis, may cause focal muscle fibre

atrophy in inflammatory myopathies (39). NO is also known to promote angiogenesis (40,41). NO released locally by vascular endothelium and muscle fibres during exercise contributes to blood flow response and regulates mitochondrial respiration (42). In our study NOS2 expression was not always found in the same tissue structures as TNF-α but the degree of expression in the same patient groups were comparable though somewhat lower. That implies that like TNF-α, NOS2 also probably takes part both in muscle fibre degeneration and regeneration.

During the differentiation muscle cells express neurotrophin-binding receptors (43). Neurotrophins have broad physiological actions and are by no means limited to nerve tissues only. Their role in skeletal muscle tissue has not been clarified as yet (44-46), although it is known that NGFs and their receptors take part in muscle regeneration (43), especially in denervated areas (47). Our findings support and extend these findings by showing that NGFR is expressed in areas with histological signs of regeneration.

Skeletal muscle is a stable post-mitotic tissue with a contractile mechanism organised in long syncytial myofibres containing many myonuclei. Growth and regeneration does occur through myogenic precursor cells, mainly the satellite cells that lie between the plasmalemma and basement membrane of each fibre, and are involved in permanent minor on demand repairs of muscle. Also bone marrow-derived cells can contribute to muscle regeneration (48-50).

Generally, our study contributes to the suggestion that in inflammatory myopathies two processes – damage and regeneration – exist in parallel. TNF-α and other inflammation-related factors seem to play a role in both tissue destruction and reparation. TNF-α expression occurs during the early stages of myoblast differentiation, and evokes a myogenic response (24). The myogenic response to TNF-α is short and reversible, whereas the catabolic response is long lasting. Activation of NFκB by overexpression of IκB kinase is sufficient to block myogenesis, but this inhibition is reversible (51). Our results and the sparse pre-existing data provide the basis for suggesting that TNF-α exerts biphasic effects on skeletal muscle, promoting early myogenesis in undifferentiated myocytes and stimulating catabolism in more mature muscle fibres.

Yet, in spite of naturally occurring reparative attempts, degenerative processes dominate in myopathies and severe symptoms develop and usually persist. A better understanding of the destructive and reparative processes in muscle tissue would provide clues to appropriate sites of intervention, not only in the inflammatory tissue destructive cascades but also in the regenerative muscle tissue response. The naturally occurring basic processes of muscle tissue repair in the inflammatory and non-inflammatory myopathies seem to have much in common. Our data show the coexistence of both destructive and reparative signs related partially to the same cytokines studied. This raises the hypothesis that short lasting suppression of TNF and interdependent cytokines alternating with short lasting expression could provide an attractive model for the management of refractory inflammatory myopathies.

Acknowledgements

We are grateful to bioanalyst Eija Kaila for her professional help and to Ms Aili Takkinen for skilful technical assistance.

References

1. NAGARAJU K: Immunological capabilities of skeletal muscle cells. *Acta Physiol Scand* 2001; 171: 215-23.
2. LUNDBERG IE: The physiology of inflammatory myopathies: An overview. *Acta Physiol Scand* 2001; 171: 207-13.
3. ENGLUND P, NENNESMO I, KLAGESKOG L, LUNDBERG IE: Interleukin-α expression in capillaries and major histocompatibility complex class I expression in type II muscle fibbers from polymyositis and dermatomyositis patients: Important pathogenetic features independent of inflammatory cell clusters in muscle tissue. *Arthritis Rheum* 2002; 46: 1044-55.
4. DALAKAS MC: Polymyositis, dermatomyositis, and inclusion-body myositis. *N Engl J Med* 1991; 325: 1487-98.
5. DALAKAS MC: Inflammatory myopathies. Recent advances in pathogenesis and therapy. *Adv Neurol* 2002; 88: 253-71.
6. PLOTZ PH, RIDER LG, TARGOFF IN *et al*.: Myositis: immunologic contributions to understanding, cause, pathogenesis and therapy. *Ann Int Med* 1995; 122: 715-24.
7. REID MB, LI Y-P: Cytokines and oxidative signalling in skeletal muscle. *Acta Physiol Scand* 2001; 171: 225-32.
8. ROZMAN B, BOZIC B, KOS-GOLJA M, PLESIVCNIK-NOVLJAN, KVEDER T: Immunoserological aspects of idiopathic muscle disease. *Wien Klin Wochenschr* 2000; 112/15-16: 722-27.
9. HENGSTMAN GJ, BROUWER R,EGBERTS WT *et al*.:Clinical and serological characteristics of 125 Dutch myosistis patients. Myositis specific autoantibodies aid in the differential diagnosis of the idiopathic inflammatory myopathies. *J Neurol* 2002; 249: 69-75.
10. DE BLEECKER JL, MEIRE VI, DECLERCQ W, VAN AKEN EH: Immunolocalization of tumor necrosis factor-alpha and its receptors in inflammatory myopathies. *Neuromusc Dis - ord* 1999; 9: 239-46.
11. BOHAN A, PETER JB: Polymyositis and dermatomyositis. I. *N Engl J Med* 1975; 292: 344-7.
12. BOHAN A, PETER JB: Polymyositis and dermatomyositis. II. *N Engl J Med* 1975; 292: 403-7.
13. PARTRIDGE TA: Cells that participate in regeneration of skeletal muscle. *Gene Ther* 2002; 9: 752-3.
14. SHINDOH C, HIDA W, OHKAWARA K *et al*.: TNF-α mRNA expression in diaphragm muscle after endotoxin administration. *Am J Respir Crit Care Med* 1995; 152: 1690-6.
15. DE ROSSI M, BERNASCONI P, BAGGI F, DE WAAL MALEFYT, MANTEGAZZA R: Cytokines and chemokines are both expressed by human myoblasts: Possible relevance for the immune pathogenesis of muscle inflammation. *Internatl Immunol* 2000; 12: 1329-35.
16. PAUL WE,SEDER RA: Lymphocyte responses and cytokines. *Cell* 1994; 76: 241-51.
17. LUNDBERG I, ULFGREN A-K, NYBERG P, ANDERSSON U, KLARESKOG L: Cytokine production in muscle tissue of patients with idiopathic inflammatory myopathies. *Arthri - tis Rheum* 1997; 40: 865-74.
18. DHAWAN S, SINGH S, AGGARWAL BB: Induction of endothelial cell surface adhesion molecules by tumor necrosis factor is blocked by protein tyrosine phosphatase inhibitors: Role of the nuclear transcription factor NF-kappa B. *Eur J Immunol* 1997; 27: 2172-9.
19. MACKAY F, LOETSCHER H, STUEBER D, GEHR G, LESSLAUER W: Tumor necrosis factor alpha (TNF-α)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *J Exp Med* 1993; 177: 1277-86.
20. COLLINS RA,GROUNDS MD: The role of tumor necrosis factor-alpha (TNF-α) in skeletal muscle regeneration. Studies in TNF-alpha (-/-) and TNF-alpha (-/-) LT-alpha (-/-) mice. *J Histochem Cytochem* 2001; 49: 989-1001.
21. KIM I, KIM JH, RYU YS, LIU M, KOH GY: TNF-α upregulates angiopoetin-2 in human umbilical vein endothelial cells. *Biochem Biophys Res Commun* 2000; 269: 361-5.
22. JAHR J, GRANDE PO: *In vitro* effects of tu-

mor necrosis factor-alpha on capillary permeability and vascular tone in a skeletal muscle. *Acta Anaesthesiol Scand* 1996; 40: 256-61.
23. REID MB, LI Y-P: Tumor necrosis factor-α and muscle wasting: A cellular perspective. *Respir Res* 2001; 2: 269-72.
24. LI Y-P, REID MB: Effect of tumor necrosis factor-α on skeletal muscle metabolism. *Curr Opin Rheumatol* 2001; 13: 483-7.
25. GUTTRIDGE DC, MAYO MW, MADRID LV, WANG CY, BALDWIN AS: NF-κB-induced loss of MyoD messenger RNR: Possible role in muscle decay and cachexia. *Science* 2000; 289: 2363-6.
26. LAYNE MD, FARMER SR: Tumor necrosis factor-alpha and basic fibroblast growth factor differentially inhibit the insulin-like growth factor-I induced expression of myogenin in C2C12 myoblasts. *Exp Cell Res* 1999; 249: 177-87.
27. THALOOR D, MILLER KJ, GEPHARY J, MITCHELL PO, PAVLATH GK: Systemic administration of NF-κB inhibitor curcumin stimulates muscle regeneration after traumatic injury. *Am J Physiol* 1999; 277: C320-9.
28. TEWS DS, GOEBEL HH: Cytokine expression profile in idiopathic inflammatory myopathies. *J Neuropathol Exp Neurol* 1996; 55:342-7.
29. SMITH MD, PARKER A, WEEDON H, COLEMAN M, ROBERTS-THOMPSON P, AHERN M: Cytokines and cell adhesion molecules in musculoskeletal disorders. *Drugs of Today* 1999; 35: 127-37.
30. WANG J, LENARDO MJ: Roles of caspases in apoptosis, development, and cytokine maturation revealed by homozygous gene deficiencies. *J Cell Sci* 2000; 113: 753-7.
31. RAMADANI M, YANG Y, GANSAUGE F, GANSAUGE S, BEGER HG: Overexpression of caspase-1 (IL-1β converting enzyme) in chronic pancreatitis and its participation in apoptosis and proliferation. *Pancreas* 2001; 22: 383-7.
32. FIORUCCI S, SANTUCCI L, CIRINO G *et al.*: IL-1β converting enzyme is a target for NO-releasing aspirin: New insights in the anti-inflammatory mechanism of NO-releasing nonsteroidal antiinflammatory drugs. *J Immunol* 2000; 165: 5245-54.
33. ZHAO M-L, KIM M-O, MORGELLO S, LEE SC: Expression of inducible nitric oxide syntase, IL-1 and caspase-1 in HIV-1 encephalitis. *J Neuroimmunol* 2001; 115: 182-91.
34. REID MB, KOBZIK L, BREDT DS, STAMLER JS: Nitric oxide modulates excitation-contraction coupling in the diaphragm. *Comp Biochem Physiol* 1998; 119: 211-8.
35. WILLIAMS G, BROWN T, BECKER M, RAGER M, GIROIR BP: Cytokine-induced expression of nitric oxide synthase in C2C12 skeletal myocytes. *Am J Physiol* 1994; 267: R1020-25.
36. TENG X, ZHANG H, NEAD C, CATRAVAS JD: Molecular mechanisms of iNOS induction by IL-beta and IFN-gamma in rat aortic smooth muscle cells. *Am J Physiol Cell Physiol* 2002; 282: C144-52.
37. BARNES PJ, KARIN M: Nuclear factor-κB: A pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med* 1997; 336: 1066-71.
38. BUCK M, CHOJKER M: Muscle wasting and dedifferentiation induced by oxidative stress in a murine model of cachexia is prevented by inhibition of nitric oxide synthesis and antioxidants. *EMBO J* 1996; 15: 1753-65.
39. BEUTLER B, CERAMI A: Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature* 1986; 320: 584-8.
40. MUROHARA T, WITZENBICHLER B, SPYRODOPOULOS *et al.*: Role of endothelial nitric oxide synthase in endothelial cell migration. *Arterioscler Thromb Vasc Biol* 1999; 19: 1156-61.
41. LEE PC, KIBBE MR, SCHUCHURT MJ *et al.*: Nitric oxide induces angiogenesis and upregulates alpha(v)beta(3) integrin expression on endothelial cells. *Microvasc Res* 2000; 60: 269-80.
42. GAVIN TP, SPECTOR DA, WAGNER H, BREEN EC, WAGNER PD: Nitric oxide synthase inhibition attenuates the skeletal muscle VEGF mRNA response to exercise. *J Appl Physiol* 2000; 88: 1192-8.
43. RENDE M, BRIZI E, CONNER J *et al.*: Nerve growth factor (NGF) influences differentiation and proliferation of myogenic cells *in vitro* via TrKA. *Int J Devl Neuroscience* 2000; 18: 869-85.
44. AMANO T, YAMAKUNI T, OKABE N, SAKIMURA K, TAKAHASHI Y: Production of nerve growth factor in rat skeletal muscle. *Neurosci Lett* 1991; 132: 5-7.
45. GRIESBECK O, PARSADANIAN AS, SENDTNER M, THOEN H: Expression of neurotrophins in skeletal muscle: Quantitative comparison and significance for motoneuron survival and maintenance of function. *J Neurosci Res* 1995; 42: 21-33.
46. STUERENBURG HJ, KUNZE K: Tissue concentrations of nerve growth factor in aging rat heart and muscle. *Muscle Nerve* 1998; 21: 404-6.
47. BARON P, SCARPINI E, MEOLA G *et al.*: Expression of low-affinity NGF receptor during human development, regeneration, and in tissue culture. *Muscle Nerve* 1994; 17: 276-84.
48. FERRARI G, CUSELLA-DE ANGELIS G, COLETTA M *et al.*: Muscle regeneration by bone marrow-derived myogenic progenitors. *Science* 1998; 279: 1528-30.
49. GUSSONI E, SONEOKA Y, STRICKLAND CD *et al.*: Dystrophyn expression in the mdx mouse restored by stem cell transplantation. *Nature* 1999; 401: 390-4.
50. SEALE P, ASAKURA A, RUDNICKI MA: The potential of muscle stem cells. *Dev Cell* 2001; 1: 133-42.
51. LANGEN RC, SCHOLS AM, KELDERS MC, WOUTERS EF, JANSSEN-HEININGER YM: Inflammatory cytokines inhibit myogenic differentiation through activation of nuclear factor-κB. *FASEB* 2001; 15: 1169-80.