Matrix metalloproteinases in inflammatory myopathies: enhanced immunoreactivity near atrophic myofibers


Objectives – To further examine the role of proteolytic enzyme expression of matrix metalloproteinases (MMP) and T-cell markers in inflammatory myopathies and controls. Material and methods – We studied the expression of MMP-2, MMP-7, and MMP-9 in 19 cases of inflammatory myopathies and controls using immunocytochemistry. Results – Inflammatory myopathies showed distinct patterns of up-regulation of MMP. MMP-9 was strongly expressed in atrophic myofibers in all inflammatory myopathies. MMP-2 immunoreactivity was similar in its distribution, however, to a weaker intensity. In dermatomyositis the perifascicular atrophy showed pronounced MMP-9 immunoreactivity, probably reflecting denervated patterns of myofibers. Moreover, MMP-7 strongly immunolabeled invaded myofibers in polymyositis cases only. Conclusion – These patterns confirm, that MMP-7 up-regulation is prominent in PM, while MMP-2 immunoreactivity is only slightly elevated in inflamed muscle. In general, MMP-9 up-regulation appears to be an important additional molecular event in the multistep process of all inflammatory myopathies.

Introduction

Chronic lymphocytic and macrophagic infiltration of muscle parenchyma is the hallmark of myositis. Although various studies demonstrated accumulation of CD-4, and CD-8 lymphocytes and up-regulation of their respective ligands, the major histocompatibility (MHC) antigen classes I and II, at sites of muscle inflammation (1–6) and further aspects of the molecular events of cell adhesion and components of the extracellular matrix (ECM) mediating myofiber damage have still to be characterized.

Matrix metalloproteinases (MMP) are proteolytic zinc-containing endoproteinases that are involved in the remodelling of the ECM. The MMP family consists of 23 members encompassing collagenase, stromelysin and membrane-type MMP subfamilies (7, 8). Typically, MMPs are secreted as inactive precursors that are subsequently activated by physiological stimuli, including plasmin, other members of the MMP are the family and endogenously and differently inducible tissue inhibitors of MMPs. Cytokines, such as tumor necrosis factor alpha, interleukin-1, and transforming growth factor beta, and eicosanoids like prostaglandine E2 can induce or suppress MMP expression directly. MMPs share important physiological functions and are involved in pathophysiological processes, e.g. in tumor invasion, inflammation, and atherosclerosis (9–13). There is ongoing evidence that MMPs are playing major roles in the pathogenesis of inflammatory demyelinating diseases of the central and peripheral nervous system, especially in multiple sclerosis and in Guillain–Barre syndrome (14–20).

The knowledge of possible roles of MMPs in muscle biology and pathology is limited as yet. MMP-2 is constitutively synthesized and secreted by human skeletal muscle satellite cells (SMSC).
Phorbol ester treatment of SMSC induced MMP-9 activity, and little amounts of MMP-1 and no MMP-3 activity (21). Increased MMP-7 activity was detectable in serum of Duchenne muscular dystrophy (22). Additionally, after experimentally performed denervation, MMP-2 and MMP-9 immunoreactivity in normal muscle was localized at the neuromuscular junctions (NMJ), in Schwann cells and the perineurium of the intramuscular nerves. In denervated muscle MMP-2 persisted at NMJ but decreased in the nerves, whereas MMP-9 also persisted at NMJ but was enhanced in degenerated intramuscular nerves (23, 24). MMP expression in human neurogenic atrophy revealed strong MMP-9 immunoreactivity in all studied biopsies (25). As compared with controls, MMP-2 expression appeared to be unaltered in these pathological contexts. Interestingly, in biopsies of acute denervation atrophy, none of the MMP immunomarkers used labeled structures other than the NMJ. Therefore, MMP expression might reflect later or chronic stages of denervation atrophy in human muscle.

Overexpression of MMP-2 and -9 in inflammatory myopathies has been reported recently (26). Additionally, quantitative polymerase chain reaction analysis revealed significantly elevated mRNA expression of MMP-1 and MMP-9 in polymyositis and dermatomyositis and to a lesser extent in inclusion body myositis, whereas the levels of expression of tissue inhibitors of metalloproteinases remained unchanged (27).

Because of the probable role of distinct MMPs in inflammatory myopathies, we tried to merge our knowledge of MMPs in neurogenic processes and recently growing understanding of specific MMPs in inflammatory myopathies, examining the expression of MMP-2, MMP-7, and MMP-9 in inflammatory myopathies of humans.

**Materials and methods**

**Muscle tissue specimens**

We studied limb muscle biopsies (biceps brachii, vastus lateralis, tibial anterior muscle) from 19 patients (aged 33–76 years) with an inflammatory myopathy confirmed by clinical examination and routine histological preparations (Table 1). Muscle biopsy specimens from five patients without clinical and histological evidence of a neuromuscular disorder (aged 35–72 years) served as normal controls. All patients gave written informed consent to the muscle biopsy.

The biopsy specimens were frozen immediately after excision in liquid nitrogen and stored at −80°C. Serial transverse sections were cut on freezing microtome at 5-μm thickness, dried at room temperature, and were stored at −20°C until staining.

**Immunohistological procedures**

The cryosections were pretreated with 2% H2O2 in phosphate-buffered saline (PBS) for 10 min to eliminate endogenous peroxidase activity. The primary monoclonal antibodies were applied (diluted 1:100) and incubated for 1 h at room temperature (all MMPs from Oncogene, UK; anti-CD4, anti-CD8, anti-MHC-1 from Dakopatts, Germany). After several rinses in PBS after each step, for secondary staining, the standard peroxidase–antiperoxidase technique (diluted 1:100; incubation time 30 min LSAB 2-Kit, K676, DAKO, Germany) or fluorescein isothiocyanate (FITC)-labeled secondary antibodies (Sigma-Aldrich Chemie, Steinheim, Germany) were used. The cryosections were counterstained with Harris’s hematoxylin and mounted in glycerol–gelatine medium for light microscopy. Negative controls were obtained by omission of the primary antibodies and substitution with human IgG (25).

**Immunohistochemical analysis and inflammation score**

To determine the grade of inflammation we defined a representative area with cellular infiltration including an average of 500 myofibers in a routine hematoxylin and eosin stained section. Accumulation of mononuclear cells in such an area were scored as follows: grade 0: no cells; grade 1: 1–20 cells; grade 2: 20–100 cells; grade 3: 100–150 cells; grade 4: more than 150 cells (28).

**Results**

**Normal controls**

In histopathological non-diseased human muscles MMP-2 immunoreactivity (−IR), MMP-7-IR, and...
MMP-9-IR was predominantly found at NMJ, on endomysial capillaries and on perimysial venules and arterioles, and on the perineurium of terminal nerve branches (Table 2).

Inflamed muscle

Routine histological preparations revealed inflammatory myopathy infiltrates with myofiber necrosis, myophagocytosis as well as degenerating and regenerating myofibers. In chronic cases a replacement of myofibers by connective tissue stages was seen. Subclassification of myositis was based on the clinical picture as well as on the distribution of the inflammatory infiltrates and the distribution and portion of the cellular subtypes (1, 3, 4, 28). The respective grades of inflammation for the different subtypes of myositis are shown in Table 1.

Dermatomyositis (DM)

In DM cases a strong sarcolemmal expression of MMP-9 at atrophic myofibers and some of CD-4

<table>
<thead>
<tr>
<th>Biopsy</th>
<th>n</th>
<th>Tissue</th>
<th>MMP-2</th>
<th>MMP-7</th>
<th>MMP-9</th>
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<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>Nerve/vessel/NMJ muscle</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Dermatomyositis</td>
<td>8</td>
<td>Nerve/vessel/NMJ muscle</td>
<td>+</td>
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<tr>
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<td>Nerve/vessel/NMJ muscle</td>
<td>+</td>
<td>+</td>
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</tr>
<tr>
<td>IBM</td>
<td>4</td>
<td>Nerve/vessel/NMJ muscle</td>
<td>+</td>
<td>+</td>
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Grade of staining: (+++) strong; (++) moderate; (+) weak; (−) negative. IBM: Inclusion body myositis.

MMPs in inflamed muscle

**Table 2** Expression patterns of MMPs in human inflammatory myopathies

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Dermatomyositis (DM)

In DM cases a strong sarcolemmal expression of MMP-9 at atrophic myofibers and some of CD-4

Figure 1. Serial sections: A: MMP-9 immunoreactivity at perifascicular atrophy in dermatomyositis (arrow heads). B: CD-4 immunoreactivity interstitially and at vessels (arrow head, A, B: bar 100 μm). C–E: Serial sections of polymyositis (asterix marks identical myofibers in C–E, bar: 75 μm). C: Sarcolemmal MMP-9 expression at atrophic myofibers and lymphocytes invading myofibers (arrow head). D: Strong sarcolemmal MMP-7 immunoreactivity at atrophic myofibers (arrows) and lymphocyte clusters (arrow head). E: Weaker MMP-2 immunoreactivity at atrophic myofibers and lymphocyte clusters (arrow head). F: MHC-I expression in inclusion body myositis (arrow, bar 50 μm). G: MMP-9 immunoreactivity at phagocytotic myofibers and clusters of lymphocytes in inclusion body myositis (arrow). H: Enhanced MMP-9 immunoreactivity in inclusion body myositis around vacuoles (upper section: MMP-9 staining; lower section: HE staining).
positive lymphocytes in areas of perifascicular atrophy was noticed (Figs 1A, B). MMP-7 and MMP-2 expression was much weaker at some atrophic myofibers only. Peri- or endomysial vessels did not reveal additional strong up-regulation of any MMPs (Figs 1A, B; Table 2).

Polymyositis (PM)
In PM cases MMP-9 expression was strongly up-regulated at the sarcolemma of atrophic myofibers. Additionally, a few CD-8 and MHC-I positive lymphocytes were stained positively (Fig. 1C). Strong sarcolemmal MMP-7 expression was found at some normal sized, invaded and atrophic myofibers in all cases (Fig. 1D). Weaker MMP-2 expression was found at some atrophic myofibers and CD-8 and MHC-1 positive lymphocytes (Fig. 1E; Table 2).

Inclusion body myositis (IBM)
MMP-2 and -7 showed weak expression in a few single atrophic myofibers only. MMP-9 immunoreactivity was found at MHC-1 positive lymphocytes and myofibers with rimmed vacuoles (Figs 1F, G, H; Table 2).

Discussion
In this study of MMPs expression in inflammatory myopathies, we have been able to document additional distinct patterns of sarcolemmal immunoreactivity of MMPs in inflammatory myopathies.

In PM without evidence of microangiopathy and muscle ischemia, but of an antigen-directed cytotoxicity mediated by cytotoxic T cells with the presence of CD8+ cells, which initially surround healthy myofibers and later invade and destroy them, and linked to areas with strong expression of (MHC-I) (3, 4, 6, 29). We could demonstrate intensive MMP-7 and MMP-9 expression in areas with strong expression of major-histocompatibility-complex class I (MHC-I) at atrophic and invaded myofibers as well as at CD-8 positive lymphocytes and macrophages. Furthermore, MMP-2 immunoreactivity was slightly seen at atrophic myofibers and invading lymphocytes. Therefore, MMP expression seems to contribute to invading mononuclear cells and myophagocytosis and myofiber lysis. Our results confirm Choi and Dalakas findings of MMP-2 and MMP-9 expression in PM (26). In addition, induced MMP-7 expression has been discussed as one possible mechanism in restoring the integrity of muscle membrane, as proposed by studies of experimental autoimmune neuritis (16, 30, 31) or human neurogenic process (25).

Immunopathogenesis of endomysial infiltrates in DM encompasses a higher ratio of CD4+ helper T-cells to CD8+ suppressor-cytotoxic T-cells, and a complement C5b-9 membranolytic attack complex mediated mechanism directed against the intramuscular vessels leading to perifascicular myofiber atrophy, which is diagnostic in DM even in the absence of inflammation (1, 3, 4, 6). Moreover, expressions of interleukin-1 in myofibers undergoing ischemic damage and associated with myofibrillar protein breakdown were described (32). In our DM cases affected myofibers strongly expressed MMP-9 in areas of typical perifascicular atrophy. Findings of perifascicular up-regulation of MMP-9 in DM and expression patterns in neurogenic processes (25) combined with data on interleukin-1, tumor necrosis factor alpha, and transforming growth factor beta as inductor or suppresser of MMP expression (33, 34) proposed that MMP expression in DM may contribute at later stages of the disease progression for destabilization and degeneration of the ECM and myofiber under inflammation as, for example postulated in other diseases (7, 35). Our results support recent results of Kieseier et al. (27) which were able to demonstrate MMP-9 expression in DM at both mRNA and protein level without significant MMP-2 overexpression. But, these results are in contrast to the study of Choi & Dalakas (26) which failed to detect any expression of MMPs in DM.

In our cases of IBM only MMP-9 revealed a strong immunoreactivity at MCH 1 positive myofibers invaded by CD8+ T cells. This finding is comparable with recent reports (26), and suggests that MMP-9 may play a promoting factor of adhesion of autoinvasive T cells to the muscle, as proposed for other autoimmune disease.

Nevertheless, future studies of inflammatory myopathies may include data on MMPs expression of serial biopsies to confirm these distinct expression patterns.

In conclusion, our samples of untreated cases of inflammatory myopathies confirm, that MMP-7 up-regulation is prominent in PM, while MMP-2 immunoreactivity is only slightly elevated in inflamed muscle. In general, MMP-9 up-regulation appears to be an important additional molecular event in the multistep process of all inflammatory myopathies.
Acknowledgements

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References