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STUDIES ON INTERLEUKIN-1 IN IDIOPATHIC INFLAMMATORY MYOPATHIES

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To my family

ABSTRACT

The idiopathic inflammatory myopathies (IIM): polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM) are chronic rheumatic muscle diseases of unknown origin that are characterized by muscle weakness and by inflammatory infiltrates in the skeletal muscle. Treatment with glucocorticoids and other immunosuppressive agents has improved outcome but not all patients respond to this treatment and most patients have only a partial response and side effects are common. Thus insights into the disease mechanisms of the IIMs are strongly needed and subsequently new therapies could emerge.

Aims: The main aim of this thesis has been to gain more insight into the pathogenesis of IIMs by studying inflammation, with particular focus on the proinflammatory cytokine, interleukin-1 (IL-1), in the muscle tissue and at the clinical level as IL-1 is one of the most consistently found cytokines in muscle tissue of IIMs. Muscle biopsies from patients with IIMs and healthy controls were studied using immunohistochemistry and immunofluorescence. Clinical outcome measures included disease activity and muscle performance using the functional index (FI) of myositis.

Results: As a prerequisite for studies on muscle tissue, we confirmed that muscle biopsy with the percutaneous conchotome technique is simple, safe and gives adequate yield to study inflammation and cytokine expression on the molecular level. The most important observation was that inflammation in muscle tissue in patients with relatively new onset PM or DM is general and not restricted to proximal muscles. We also found expression of the IL-1 receptors, IL-1RI and IL-1RII, on muscle fibers and a higher expression of the receptors and their ligand IL-1 in muscle tissue from PM and DM patients than in healthy individuals. Drug intervention with IL-blockade, anakinra, in 15 patients with refractory PM, DM and IBM revealed beneficial effects on clinical outcome including disease activity in 7 responders and muscle performance measured by FI in 4/7 responders. Notably, one of the responders had IBM, a disease which currently has no effective treatment. We could, however, not explain the clinical improvement by reduced inflammation or IL-1 expression in muscle tissue. Nevertheless, IL-1Ra expression was observed in more patients in post-treatment than in pre-treatment biopsies, all being responders.

Conclusions: The expression of IL-1 receptors on muscle fiber membrane and their colocalization with IL-1 support the hypothesis that IL-1 has an important role in the pathogenesis of PM and DM. This is further supported by the beneficial clinical effects of IL-1 blockade, at least in a subgroup of patients, but this needs to be confirmed in a larger, controlled trial. However, it is likely that different molecular pathways are predominating in different subsets of myositis patients and this needs further investigations. The mechanisms for improvement could not be explained in our study. In addition, the observation that muscle inflammation in IIMs is general and not restricted to muscles with clinical symptoms implies that other disease mechanisms than can be explained by signs of inflammation in muscle tissue should be searched for. Finally, muscle biopsy with the percutaneous conchotome technique is a method that gives sufficient yield to analyze muscle tissue for diagnostic evaluation and for research and based on the observations of the general muscle inflammation, the range of muscles available for biopsy is not dependent on clinical symptoms.

LIST OF PUBLICATIONS

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LIST OF ABBREVIATIONS

| | |
|---------------|--|
| ALAT | Alanine aminotransferase |
| ANA | Antinuclear antibody |
| ASAT | Aspartate aminotransferase |
| AZA | Azathioprine |
| CPK | Creatine phosphokinase |
| DC | Dendritic cell |
| DM | Dermatomyositis |
| DMARD | Disease-modifying antirheumatic drug |
| EMG | Electromyography |
| ER | Endoplasmic reticulum |
| FI | Functional index |
| GC | Glucocorticoid |
| HAQ | Health assessment questionnaire |
| HEV | High endothelial venule |
| HLA | Human leukocyte antigen |
| IBM | Inclusion body myositis |
| ICAM-1 | Intracellular adhesion molecule-1 |
| IFN | Interferon |
| IIM | Idiopathic inflammatory myopathy |
| IL-1 | Interleukin-1 |
| IL-1Ra | Interleukin-1 receptor antagonist |
| IL-1RAcP | Interleukin-1 receptor accessory protein |
| IL-1RI | Interleukin-1 receptor type I |
| IL-1RII | Interleukin-1 receptor type II |
| IL-1 α | Interleukin-1 alpha |
| IL-1 β | Interleukin-1 beta |
| ILD | Interstitial lung disease |
| IMACS | International myositis assessment and clinical studies |
| IvIg | Intravenous immunoglobulin |
| LD | Lactate dehydrogenase |
| MHC | Major histocompatibility complex |
| MMT-8 | Manual muscle test in 8 muscle groups |
| MRI | Magnetic resonance imaging |
| mRNA | Messenger ribonucleic acid |
| MSA | Myositis-specific autoantibody |
| MTX | Methotrexate |
| PM | Polymyositis |
| RA | Rheumatoid arthritis |
| SLE | Systemic lupus erythematosus |
| SS | Sjögren's syndrome |
| TNF | Tumor necrosis factor |
| tRNA | Transfer ribonucleic acid |
| VAS | Visual analogue scale |
| VCAM-1 | Vascular cell adhesion molecule-1 |

1 INTRODUCTION

The idiopathic inflammatory myopathies (IIMs) are a heterogeneous group of muscle disorders characterized clinically by proximal symmetrical muscle weakness, skin rash in dermatomyositis (DM) and histopathologically by inflammatory infiltrates in the skeletal muscle. Based on clinical and histopathological features, IIMs can be grouped into three main disorders: polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). These are chronic diseases of unknown aetiology that untreated may lead to severe impairment. Treatment with glucocorticoids (GCs) has improved outcome but high doses over long periods of time are often required. To reduce the risk of side effects and to potentiate the effects of glucocorticoids, combinations with other immunosuppressive agents such as azathioprine (AZA) or methotrexate (MTX) are often recommended. Not all patients respond to this treatment and most patients have only a partial response. Thus insights into the pathogenesis of the IIMs are strongly needed and subsequently new therapies could emerge.

1.1 CLASSIFICATION AND DIAGNOSIS

1.1.1 PM and DM

Bohan and Peter's diagnostic criteria proposed in 1975 [1, 2] is considered the gold standard for diagnosis and classification of PM and DM and is the most commonly used for both research and diagnostic purposes. The criteria consist of five parameters (Table 1).

Table 1. Bohan and Peter's diagnostic criteria for polymyositis and dermatomyositis

| |
|--|
| 1. Symmetric proximal muscle weakness of limb-girdle muscles and anterior neck flexors |
| 2. Elevation of serum skeletal muscle enzymes, creatine phosphokinase (CPK), serum alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LD) and aldolase |
| 3. Electromyography (EMG) indicating short, small, polyphasic motor unit potentials; fibrillations, positive sharp waves and insertional irritability; and high-frequency repetitive discharges |
| 4. Muscle biopsy pathology with inflammatory exudates, regeneration with basophilia, type I and II fiber phagocytosis, large vesicular sarcolemmal nuclei and prominent nucleoli, muscle fiber atrophy in a perifascicular distribution and variation in muscle fiber size |
| 5. Specific skin rash of dermatomyositis, including heliotrope rash and Gottron's papules which are pathognomonic |

The diagnosis of PM is considered *definite* when four criteria (not including skin rash) are satisfied. The PM diagnosis is *probable* when three criteria (not including skin rash) are satisfied and *possible* when two out of five criteria (not including skin rash) are fulfilled. The diagnosis of DM is considered *definite* when four criteria (including skin rash) are met. The DM diagnosis is *probable* when three criteria (including skin rash) are satisfied and *possible* when two criteria (including skin rash) are fulfilled.

As new models of disease pathogenesis emerge and new diagnostic investigations develop, a revision of Bohan and Peter's criteria have been proposed.[3] This classification includes the presence of myositis-specific autoantibodies (especially the anti-aminoacyl transfer ribonucleic acid (tRNA) synthetases – anti-Jo-1 and others, anti-Mi-2 and anti-signal recognition particle (anti-SRP)) and of oedema on magnetic resonance imaging (MRI).

1.1.2 IBM

Diagnostic criteria for IBM were first proposed in 1987 [4] and then by Dalakas et al 1991[5]. More recent and commonly used diagnostic criteria were proposed by Griggs et al in 1995 (Table 2).[6]

Table 2. Grigg's diagnostic criteria for inclusion body myositis

-
1. Duration > 6 months
 2. Disease onset at age > 30 years
 3. Muscle weakness affecting proximal and distal muscles of arms and legs with at least one of the following features:
 - a. Finger flexor weakness
 - b. Wrist flexor > wrist extensor weakness
 - c. Quadriceps muscle weakness
 4. Serum CPK < 12 times normal
 5. Muscle biopsy findings with:
 - a. Inflammatory myopathy characterized by mononuclear cell invasion of non-necrotic muscle fibers
 - b. Rimmed vacuoles in muscle fibers
 - c. Intracellular amyloid deposits or tubulofilaments by electron microscopy
 6. EMG consistent with features of an inflammatory myopathy
-

The diagnosis of IBM is *definite* if muscle biopsy demonstrates the findings above, irrespective of clinical features. The diagnosis of IBM is *possible* even if muscle biopsy findings are negative providing that clinical and laboratory findings are satisfied.

1.2 CLINICAL FEATURES

1.2.1 Skeletal muscle manifestations

PM and DM have clinical features characteristic of the diagnostic criteria. Muscle weakness is symmetric and affects proximal muscle of the shoulder and pelvic girdle and usually presents subacutely or slowly. Neck flexor muscle weakness can also occur. Mild muscle pain and tenderness are not as common as muscle weakness, but occur in about half of the patients. Another hallmark is that patients experience decreased muscle endurance and muscles are easily fatigued. Patients thus report difficulty with everyday activities which uses mainly proximal muscles. Patients describe, for example, difficulty in climbing steps, working with their arms over their head, carrying, and getting up from a chair. Patients also describe muscle pain after exertion. CPK levels are often increased at some time in the course of the disease and are in some patients a helpful indicator of disease activity. Up to 80 % of PM and DM

patients have non-specific antinuclear antibody (ANA).[7] About 50 % of PM and DM patients have defined autoantibodies, divided into the myositis-specific antibodies (MSA) and myositis-associated antibodies (MAA).[8] MSAs occur in about 38 % of patients. The MSAs target RNAs involved in protein synthesis, the most common being anti-aminoacyl-tRNA synthetases of which the most frequent is anti-histidyl-tRNA synthetase (anti-Jo-1) found in 16-30 % of PM and DM patients.[9] Other MSAs include anti-signal recognition particle (anti-SRP) autoantibodies and anti-Mi-2 autoantibodies.

IBM usually presents after the age of 50 years and can begin insidiously. Thus diagnosis is often delayed. In the beginning, it is not uncommon that IBM is misdiagnosed as treatment-resistant PM. Even though symptoms of IBM can be similar those of PM, the presentation is more typically characterized by painless focal, proximal and distal asymmetric muscle weakness. The distal muscles most commonly affected are finger flexors and foot extensors. CPK levels are normal to only moderately elevated, and myositis specific autoantibodies are absent except for the occasional non-specific ANA.

1.2.2 Extramuscular manifestations

Extramuscular manifestations are more common in PM and DM rather than in IBM patients.

1.2.2.1 Skin

The presence of a characteristic skin rash indicates the clinical subset of DM. Gottron's papules and the heliotrope rash are considered pathognomonic manifestations of DM. Gottron's papules are scaly, erythematous plaques located over the bony prominences, particularly the metacarpophalangeal and proximal and distal interphalangeal joints of the hands and found in 60-80 % of DM patients. The heliotrope rash is a purplish periorbital oedema and found in 50 % of DM patients. Other less specific cutaneous findings are photosensitivity, "V sign", "shawl sign", nailfold capillary changes, calcinosis and mechanic's hands.

1.2.2.2 Joints

Arthralgia can be present although arthritis is not as common. Non-erosive polyarthritis is frequent with overlap syndromes, where PM or DM occurs in combination with one or several other rheumatic diseases such as systemic sclerosis (SSc), Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD) or rheumatoid arthritis (RA) and the antisynthetase syndrome, a clinical phenotype characterized by the presence of antisynthetase autoantibodies such as anti-Jo-1, myositis, Raynaud's phenomenon, interstitial lung disease (ILD), arthritis and mechanic's hands.

1.2.2.3 Lungs

Lung involvement is common in PM and DM. Dyspnoea may result from ventilatory muscle weakness, alveolitis or lung fibrosis. Parenchymal changes in pulmonary tissue are more often seen in association with anti-aminoacyl-tRNA synthetase autoantibodies

such as anti-Jo-1, as part of the antisynthetase syndrome. Aspiration pneumonia, ventilatory insufficiency and ILD are considered three distinct types of lung involvement associated with PM and DM.[10] The reported prevalence of pulmonary involvement in PM or DM varies between 5 and 70 %.[11-18] These pulmonary complications are a major cause of morbidity and mortality in PM and DM patients.[11, 12, 19, 20]

1.2.2.4 Heart

The most common findings are arrhythmias due to inflammatory or fibrotic alteration of the conducting system. Congestive heart failure and ischemic heart disease may occur, but is uncommon. Cardiovascular manifestations are, nonetheless, a major cause of death.[21]

1.2.2.5 Gastrointestinal tract

Dysphagia is the most common manifestation due to weakness of the tongue, involvement of striated pharyngeal muscle, cricopharyngeal muscle dysfunction and/or esophageal dysmotility.

1.2.2.6 Other

Systemic symptoms such as fatigue, morning stiffness, weight loss, Raynaud's phenomenon and fever may occur.

1.3 EPIDEMIOLOGY

PM and DM are rare disorders where the reported annual incidence varies between 2 to 10 new cases per million persons and year.[22] The variations reflect geographic, ethnical and inclusion criteria used in the studies.[23-27] There is a trend towards increasing incidence, probably due to a higher awareness from physicians rather than a true increase in disease occurrence. The mean age at diagnosis of PM and DM onset is 40-45 years, the incidence sex ratio is 2:1 (female:male) and the incidence race ratio is 3-4:1 (Afro-American:white).

IBM is an even rarer disease with incidence rates ranging from 2.2-4.9 cases per million persons and year.[28, 29] The mean age at diagnosis is older, >65 years, and the incidence sex ration is 1:2 (female:male).

1.4 INVESTIGATIONS – MUSCLE BIOPSY

Muscle biopsy is an important tool for the diagnosis of IIMs, to exclude other myopathies or vasculitides, and to assess the effect of treatment on the tissue level. Results of histopathological muscle evaluation are included in Bohan and Peter's and Grigg's criteria, and muscle biopsy is considered the gold standard of all investigations and a prerequisite for diagnosis of IIM.[22] Muscle biopsy is also an important tool in research in order to study muscle tissue on the molecular level and thus gain insight into the etiology and pathogenesis of IIMs. Thus, a muscle biopsy technique that provides a tissue sample large enough with sufficient quality to allow for a high

diagnostic yield is essential. Moreover, the technique has to be safe and easily performed.

The focal or patchy nature of the inflammatory infiltrates in myositis is a problem in muscle biopsy sampling.[30-32] Thus, some patients with active myositis may have normal muscle biopsies. To overcome this problem, multiple biopsies can be taken from the same site at the same occasion. Also, one could use the presence of oedema in muscles on MRI, to direct the biopsy.[33, 34]

There are three muscle biopsy methods available – the percutaneous conchotome or semi-open muscle biopsy technique, the percutaneous needle biopsy technique and the open muscle biopsy technique. However, direct comparisons between the three techniques have not been carried out.

1.4.1 The percutaneous conchotome muscle biopsy technique

The percutaneous conchotome or semi-open biopsy technique has become widely used in Sweden and other Scandinavian countries, including in our Rheumatology Clinic, during the past 30 years, but has received little attention outside of Scandinavia.[35-39] For details on the muscle biopsy procedure, see Paper I, Materials and Methods. The percutaneous muscle biopsy technique has been reported to have the following advantages: it can be performed in local anesthesia in an outpatient clinic, the procedure involves only slight discomfort and inconvenience for the patient, and it only leaves a small scar. The method has also been reported to widen the range of muscles available for biopsy, compared to the open and needle biopsy techniques. It is acceptable for repeat biopsies and is considered less painful than the percutaneous needle technique. However, it has been questioned whether this biopsy technique gives adequate yield for histological and immunohistochemical investigations.

1.4.2 The percutaneous needle muscle biopsy technique

The percutaneous needle biopsy technique is more commonly used in other parts of the world.[31, 40-44] The use of the needle technique was introduced by Duchenne [45] in 1865. The needle biopsy method did not receive any more attention until the 1940s [46] and then when Bergström published work on muscle chemistry of needle biopsy samples in 1962.[47] There have been numerous reports on different instruments used for the needle muscle biopsy.[44] On the whole, the procedure is as follows. The skin and subcutaneous tissue is infiltrated with local anaesthesia. An about 5 mm skin incision is made. The needle is then inserted into the muscle at a depth of 2-5 cm below the level of the skin and the operator applies firm pressure to the muscle in the region of the needle tip. The needle consists of an outer cylinder with tapered end and a window at the distal segment and an inner rod with a cutting end. The sample is then guillotined, with several cuts being made in a quick succession. The needle is then withdrawn from the muscle and firm pressure is applied to the biopsy site. The procedure has been shown to have several advantages similar to the percutaneous conchotome technique. It is rapid, safe, can easily be performed under local anaesthesia in an outpatient ward and leaves only a small scar. It also provides adequate amounts of muscle tissue for diagnostic purposes and is more acceptable to repeat biopsies than the open biopsy technique. The procedure has also been reported to increase the tissue

amount obtained by applying suction to the cutting cylinder.[48, 49] Samples can be taken from several sites and depths at the same occasion. Nonetheless, the procedure has also been criticized for providing muscle specimens too small or traumatized for histopathological and electron microscopy investigations and can be painful.

1.4.3 The open muscle biopsy technique

The third technique, the open surgical muscle biopsy method, is well established. It usually results in larger biopsy samples than the two methods described above, and thus one obtains plenty of material for investigations, but it is invasive and produces scarring.[50-52] Also, in most studies, roughly 25-30 % of patients with myositis have negative muscle biopsies, even with the open biopsy technique.[36, 53-55] In addition, the range of muscles available for biopsy is limited and the method is time-consuming. Open biopsies are nonetheless useful to locate vessels in vasculitis and could be preferable for use children with atrophic muscles, even though general anesthesia may be necessary.

1.5 TREATMENT

The main aim of treatment of myositis is to improve muscle function and reduce muscle inflammation. The recommended treatment for PM and DM is initially, high-dose GCs which nonetheless often are required over long periods of time.[56-61] GC related side-effects have been shown to contribute to increased disease disability over time.[62] There are no randomized controlled trials with GC treatment in myositis patients. To reduce the risk of side-effects and to potentiate the effects of GCs, empirical evidence and uncontrolled studies suggest that combinations with other immunosuppressive agents such as MTX or AZA could be effective and have steroid sparing effect.[60, 63-65] However, there are only a few controlled studies of PM and DM management with MTX and AZA and all of them have been performed on small groups of patients.[66-68]

Other disease modifying antirheumatic drugs (DMARDs) are also used. Plasmapheresis has been claimed to be beneficial. But a controlled trial with plasmapheresis and leukapheresis has failed to show efficacy.[69] Administration of high intravenous immunoglobulin (IvIg) doses was beneficial in only DM patients in one placebo-controlled trial but this could not be confirmed in a later open study where repeat muscle biopsies showed persisting inflammation.[70-72] IvIg has been effective in juvenile DM [73] and in patients with severe esophageal involvement.[74] Other DMARDS such as cyclophosphamide, cyclosporine and antimalarials (reported to be beneficial in treating skin rash in DM patients) are also used in patients who fail therapy with MTX and/or AZA, but experience is limited.[75-78] Not all patients respond to treatment and most patients have only partial response.[79, 80] Thus new therapies are needed.[81]

Response to the above treatment among IBM patients has been considered so unsuccessful that some have recommended that these patients should not be treated with drugs.[82, 83] Others have reported good results in some IBM patients, but improvements are often not maintained.[84, 85]

Generally accepted old guidelines have been that patients with active IIMs should refrain from physical exercise due to fear of causing flare-ups. However, recent studies have shown that exercise not only is not damaging, but also beneficial for muscle function. Present recommendations are active rehabilitation early in the disease course to improve the possibility of a faster and more complete recovery.[86-92]

Taken together, there is no specific effective drug for use in IIMs. The currently used immunosuppressive treatment has limited effects on muscle strength and performance in PM and DM and hardly any effect in IBM. Thus there is a strong need for new and more targeted therapies. A prerequisite for drug development is to have good information on molecular disease mechanisms.

1.6 PATHOGENETIC MECHANISMS

1.6.1 The immune system – general remarks

The immune system has been developed to defend the host against infection. The cells in the immune system are mainly produced by the bone marrow. The immune system is divided into the non-specific or innate and specific or adaptive immune system with overlapping functions.[93]

The innate system is the first line of defense against infection. This system includes physical barriers such as the skin and mucosa as well as phagocytic cells that recognize common components on pathogens. Phagocytic cells engulf the invader and induce secretion of cytokines that can lead to local inflammatory reactions.

The adaptive system is antigen-specific and is divided into the humoral immunity mediated by antibody-producing B cells and cell-mediated immunity mediated by T cells. Each B and T cell expresses a single type of receptor with unique specificity. When the cell receptor interacts with a specific antigen, the cell is activated into effector cells. This process normally takes place in lymphoid organs. In IIMs, the gain of knowledge in pathogenesis is concentrated on immunological studies. This is due to that histopathology found in muscle tissue of patients with IIMs suggests that the adaptive immune system is involved. Also, the presence of autoantibodies, especially MSAs, suggests that the immune system is involved. More recently, the presence of dendritic cells (DCs), interferon- α (IFN- α), and high mobility group box chromosomal protein-1 (HMGB-1) in muscle tissue of patients with IIMs suggests that the innate system could also be involved.[94-96]

1.6.2 Pathogenetic mechanisms and histopathology in skeletal muscle tissue in IIMs

The main histopathologic findings in skeletal muscle tissue of patients with IIM are focal inflammation and injury and death of muscle cells (myocytes). Histopathologic findings are best visualized in the hematoxylin and eosin stains. Signs of injury and cell death are degenerating and necrotic muscle cells, muscle cell atrophy and replacement of muscle tissue by fibrosis and fat but also regeneration and hypertrophy of muscle cells. The type of inflammatory cells which dominate in PM, DM and IBM are T cells, macrophages and DCs. The inflammatory cells can be found between muscle cells (in

the endomysium) and between fascicles (in the perimysium) and surrounding vessels in the interstitial nearby tissue (perivascular). Inflammatory cells may also be found inside normal-looking muscle cells. Irregular red-rimmed vacuoles and inclusions inside muscle cells on the trichrome stain are characteristic of IBM. Studies have shown that the inclusions contain β -amyloid, ubiquitin, phosphorylated τ -protein, apolipoprotein E and prion protein.[6] The role of these proteins in the pathogenesis is unclear. In IBM, as the muscle weakness becomes more severe, it is accompanied by muscle fiber atrophy and fatty replacement of the muscle tissue.

The causes of clinical muscle symptoms in myositis are not known. The clinical presentation of the three subsets give clues to that there might be different pathogenetic mechanisms involved in the different subsets. In PM and DM there is suggestive evidence that the diseases are secondary to an autoimmune process that could lead to muscle cell injury. Both humoral and cellular mechanisms of muscle fiber and capillary injury have been proposed.[97-99] Autoantibodies, as described earlier, are more common in PM and DM than in IBM patients and could contribute to pathogenesis.

In PM and IBM patients there is a dominance of activated CD8+ T cells and macrophages located in the endomysium. These infiltrates are often surrounding non-necrotic fibers and can appear to invade the muscle cells. In DM patients there is a dominance of CD4+ T cells, macrophages and sometimes B cells located perivascularly and in the perimysium.[100, 101]

Capillaries are important components of the environment in muscles and among other roles, transports oxygen to muscle cells. There are about 3-8 capillaries per muscle cell. A role of microvessels in the pathogenesis of DM has been suggested due to the loss of capillaries in muscle tissue.[102] Morphological abnormalities of capillaries, resembling high endothelial venules (HEV) suggesting that the endothelial cells are activated, have also been reported in DM and PM lending further support to a role of the microvessels in the disease mechanisms of both these subsets of myositis.[98]

The major histocompatibility complex (MHC) class I antigen, which is normally not expressed on differentiated muscle fibers, is frequently expressed on muscle cells in IIMs independent of inflammatory cell infiltrates. This has been observed both in early and late chronic phases of the disease.[103-109] MHC class I has been found strongly expressed on the surface of muscle cells that histologically appear unaffected by inflammation. As this has been seen in early phase of disease it suggests that MHC class I has a role early in the pathogenesis of myositis. A role of MHC class I in causing muscle weakness is supported by the observation that transgenic mice with specific up-regulation of MHC class I antigens on muscle fibers develop muscle weakness before infiltration of inflammatory cells.[110] The finding of these molecules in muscle tissue of IIMs suggests an interaction with T cells. But whether MHC-expressing muscle cells present antigens to naive T cells to initiate a response against muscle antigens is not known. Another hypothesis is that the MHC class I expression may lead to endoplasmic reticulum (ER) stress that can have a negative effect on muscle contractility and thereby lead to muscle weakness and fatigue.[111]

The function of invading T cells and macrophages in muscle tissue is not clear. Serologic and molecular studies have shown restricted T cell receptor usage by muscle-infiltrating cells. This suggests that subpopulations of T cells are selected and expanded in response to yet undefined antigens and may explain the presence of T cells in muscle tissue.[112] A main function of T cells is to produce cytokines which are inter- and intracellular signalling molecules. They are potent mediators of cell functions and are important in regulating inflammatory responses in a paracrine or autocrine fashion. Cytokines found most consistently in IIMs are presented in chapter 1.8. The role of these cytokines in IIMs needs yet to be defined. Also, the presence of T cells requires the existence of antigen presenting cells (APC). DCs are such cells and have been found in PM, DM and IBM patients.[95, 113]

Still, there is not always such a distinct localization of inflammatory infiltrates. Some cases have very few inflammatory cells, may be diffusely distributed throughout the muscle tissue or may not have the typical localization as described above for each of the subsets of myositis. This observation has led to the hypothesis that other mechanisms than immune mediated cell death may lead to muscle weakness. One such is the ER stress hypothesis mentioned above. Another hypothesis is that cytokines could have direct effects on muscle fiber contractility, as has been demonstrated for tumor necrosis factor (TNF) by an effect on Ca^{2+} transportation.[114] Another theory is based on that the clinical symptoms of muscle fatigue could be caused by the loss of, as well as, the phenotypically changed capillaries that may lead to hypoxia and thereby to an acquired metabolic myopathy. This hypothesis is further supported by the beneficial effects of exercise.

Based on these findings, PM, DM and IBM may have different pathological mechanisms and different mechanisms may predominate in different phases of disease.

1.7 ETIOLOGY

The etiology of IIMs is unknown. To date there is some support which suggests that environmental factors such as infectious agents, drugs, ultraviolet (UV)-light and toxins together with genetic factors could contribute to disease onset.

1.7.1 Infectious association

There is only indirect proof that points towards infectious causes of DM, PM and IBM. Bacteria such as *Staphylococcus aureus* can invade muscle and cause an acute syndrome, pyomyositis. However, there is no evidence that this kind of infection precedes PM, DM or IBM. Parasites such as *Trichinella* and *Schistosoma* can transiently resemble myositis, but clinical, laboratory and histopathologic features do not resemble PM, DM and IBM. Exceptions are *Toxoplasma* and *Borrelia* [115, 116] where elevated titers of antibodies against these organisms have been found in myositis patients, even though they cannot be cultured from muscle and are not found histologically in muscle tissue from myositis patients. Viral myositis is known for at least three viruses – *influenza adenovirus*, *coxsackievirus* and *echovirus*. The *influenza adenovirus* has been cultured from a patient with inclusion body myositis.[117] There is a serological association between the *coxsackievirus* and adult and juvenile

myositis.[118, 119] There is also evidence that several *picornaviruses* can cause myositis.[120, 121] *Retroviruses* including human immunodeficiency virus (HIV) and human T-cell leukemia/lymphoma virus (HTLV-1) [122-124] have been associated with myositis, but muscle biopsies have failed to show viral nuclei acid.[125]

1.7.2 Drug association

Many drugs can cause a disease which resembles myositis, these drugs include statins, corticosteroids, D-penicillamine and colchicines.[126, 127] But the mechanisms of drug-related myopathies is not understood, so whether they could contribute to idiopathic myositis is unclear.

1.7.3 UV-light association

DM is more frequent around the equator than in countries on other latitudes, which supports the role of UV-light as an environmental factor which could contribute to disease onset.[23, 128] Furthermore, muscle and skin manifestations of DM have been shown to relapse more often in the summer whereas in PM patients relapse was more evenly distributed during all four seasons.[129] UV radiation is known to increase clinical symptoms of diseases such as SLE and DM, where photosensitive rashes are common.[130] The effects of UV radiation could be mediated through triggering of cytokine production.[131] UV radiation may also regulate surface expression of adhesion molecules,[132] affect cellular mitosis [133] and induce apoptotic cell death.[134]

1.7.4 Genetic association

The occurrence of familial myositis and the association of specific human leukocyte antigen (HLA) and non-HLA genes with groups of myositis patients is evidence of the role of genetic factors in myositis onset. Particularly HLA DRB1*0301 and DQA1*0501 on chromosome 6 are the strongest known risk factors for myositis in Caucasians especially in patients with ILD and/or aminoacyltransfer synthetase autoantibodies.[135-139] HLA genes code for antigen-presenting molecules that play important regulatory roles in immune activation. HLA genes are, on the whole, the most consistently identified genetic factors associated with autoimmune inflammatory diseases.[140]

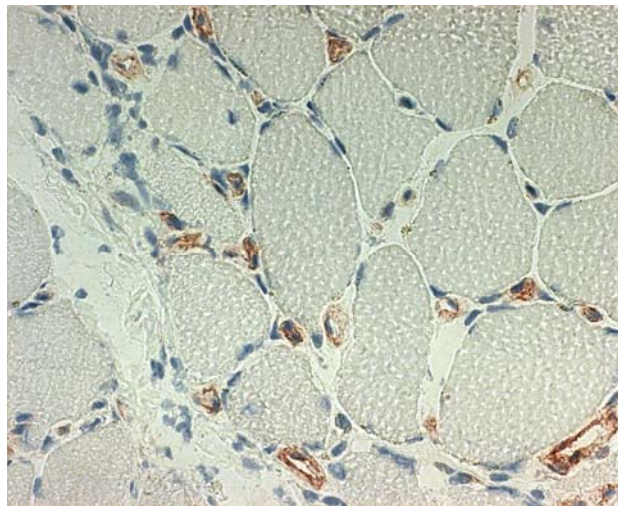
Since several proinflammatory cytokines are found consistently in myositis patients, researchers have tried to find if there is a genetic predisposition for these genes. The interleukin-1 receptor antagonist (IL-1Ra) A1 allele in Caucasians and the A3 allele in African-Americans has been found to be a risk factor for juvenile myositis versus normal race-matched controls.[141] Associations of TNF-308A polymorphism have been found in small studies of juvenile and adult onset myositis.[142-145]

1.8 INTERLEUKIN-1 AND OTHER CYTOKINES

Cytokines are inter- and intracellular signaling molecules. They are potent mediators of cell functions and are important in regulating inflammatory responses in a paracrine or autocrine fashion. They can be produced by many different types of cells and have both anti- and proinflammatory effects.

Despite the differences in inflammatory cellular phenotypes and localization between subsets of myositis the cytokine pattern in muscle tissue is strikingly consistent. Two of the most consistently expressed cytokines in muscle tissue of PM, DM and IBM patients are interleukin-1 α (IL-1 α) and interleukin-1 β (IL-1 β).[108, 109, 146-153] These cytokines have been found in muscle tissue of patients with impaired muscle performance both in the early phase before treatment and in a chronic phase of disease with persisting muscle weakness, making IL-1 a potential target of therapy in myositis.[108, 109, 151](Figure 1)

Figure 1. Immunohistochemical localization of IL-1 α in endothelial cells, visualized as red staining, in muscle tissue from a patient with PM.



1.8.1 The interleukin-1 family

There are three structurally related polypeptides in the IL-1 family: IL-1 α , IL-1 β and the endogenous IL-1Ra.[154, 155] IL-1 α and IL-1 β are agonist molecules that act through the same cell-surface receptors and share similar biologic activities.[154-156] IL-1Ra, on the other hand, is an endogenous specific receptor antagonist that at least partly regulates IL-1 α and IL-1 β activity and thus provides some protection against the disease-provoking effects of IL-1.[157]

IL-1 is a proinflammatory cytokine mainly produced by activated monocytes and macrophages and by endothelial cells [108, 109, 151, 155, 156] and may increase the expression of certain proinflammatory genes. IL-1 induces activation of T cells; promotes chemotaxis of polymorphonuclear leukocytes, lymphocytes and monocytes; stimulates protease release by tissue macrophages; and enhances infiltration of these molecules into inflamed tissues.[156] One relevant role is its ability to increase expression of adhesion molecules such as intracellular adhesion molecule-1 (ICAM-1) on mesenchymal cells and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, both which have been found in muscle tissue in patients with myositis.[158-161] This leads to infiltration of inflammatory cells into the extravascular space. IL-1 production is induced by other cytokines such as TNF, IFN and IL-1 itself.[162] IL-1 may also exert several effects on skeletal muscle. It can be toxic to human muscle cells

in *in vitro*. [163] It can disturb muscle cell metabolism *in vitro* via inhibition of glucose transport and lactate production by blocking the effect of the insulin-like growth factor. [164] IL-1 α can suppress myoblast proliferation and myoblast fusion, leading to poor muscle cell regeneration. [165]

IL-1 α and IL-1 β are synthesized as precursor molecules without a leader peptide sequence characteristic of secreted proteins. The IL-1 α precursor (proIL-1 α) is synthesized in association with cytoskeletal structures, unlike most proteins which are translated in the ER. ProIL-1 α is fully active as a precursor and remains active intracellularly. Even under conditions of cell stimulation, do human blood monocytes not process or secrete mature IL-1 α . Because of the lack of leader peptide, proIL-1 α remains in the cytosol after translation, and there is no accumulation of IL-1 in any specific organelles. In studies of experimental inflammatory bowel disease, there is a better correlation between disease severity with colonic tissue levels of IL-1 α than of IL-1 β , probably due to the nature of IL-1 α . [166] Also as a consequence, IL-1 α is not found in the circulation or in body fluids except during severe disease where the cytokine is released from dying cells. IL-1 β is often found in the circulation and levels often, but not always, correlate with disease activity in RA. [167, 168] IL-1 α is also constitutively expressed in epithelial cells and in the skin of healthy controls, suggesting that in these tissues, it may function as an intracellular messenger. [168]

The IL-1 β precursor (proIL-1 β), on the other hand, is not fully active and a considerable amount is secreted as mature IL-1 β following cleavage by a specific protease, IL-1 β converting enzyme (ICE) or caspase-1. Thus IL-1 β is the predominant secreted form. [169] IL-1 β , in contrast to IL-1 α , is frequently found in the circulation and levels may correlate to disease severity. [167] IL-1 β is produced by monocytes, macrophages, DCs, B cells and natural killer (NK) cells. Similar to IL-1 α , hypoxia induces synthesis of large amounts of IL-1 β messenger ribonucleic acid (mRNA) in monocytic cells. [170] The biological role of IL-1 β is not clear and a null mutation in the IL-1 β gene results in a phenotypically normal mouse.

Endogenous IL-1Ra acts as a natural antagonist, at least partially responsible for the regulation of IL-1 α and IL-1 β by competitively binding with high avidity to IL-1 receptor type I (IL-1RI). [157] Several studies have suggested that 10-100 fold excess amounts of IL-1Ra are required to inhibit 50 % of the IL-1 response in cells that express IL-1RI. [157, 171-173] IL-1Ra protein can both have and not have a leader peptide. Thus, some IL-1Ra remains in the cell and some is secreted out of the cell. Soluble IL-1Ra is produced mainly by monocytes and macrophages, but may be synthesized by almost any cell producing IL-1. Both forms inhibit the activity of IL-1 by binding to IL-1RI. [174] The amino acid sequence for IL-1Ra is more homologous to IL-1 β than between IL-1 α and IL-1 β , yet IL-1Ra has no agonist activity. [175-177] IL-1Ra has two binding sites to IL-1RI. The back-side binding site of IL-1Ra is more homologous to that of IL-1 β than the second binding site. Thus, it is speculated that the back-side of IL-1Ra binds so tightly to IL-1RI and occupies the receptor. Since this binding site thus is not available, IL-1Ra does not recruit the IL-1 receptor accessory protein (IL-1RAcP) required to trigger a signal. IL-1Ra-deficient mice spontaneously develop a destructive inflammatory arthropathy [178] and arteritis, [179] suggesting that endogenous IL-1Ra is important to suppress inflammation in mice living in a normal

environment. Mice overexpressing the IL-1Ra gene had significant reduction in disease incidence and severity.[180]

IL-1 has two receptors that are expressed on endothelial cells [181]: IL-1RI and IL-1 receptor type II (IL-1RII). The agonist function of IL-1 α and IL-1 β results from the interaction between these and IL-1RI, causing signal transduction and cell activation.[156] When IL-1 binds to IL-1RI, conformational changes in the receptor occurs which permits the required second cell membrane molecule, IL-1RAcP, to interact with the receptor-ligand complex. The intracellular domains of both receptor chains contain Toll domains which are important for signal transduction. The complex now recruits the IL-1 receptor activating kinase (IRAK) close to the Toll domains, leading to signal transduction in the cell and activation of nuclear genes. (see Figure 2, p. 23)

IL-1 binding to IL-1RI is very high in affinity thus receptor number is low. IL-1RII has no intracellular signalling domain and is a decoy receptor.[182-184] Also, proteolytic cleavage of IL-1RII releases its extracellular domain. This domain is more likely to bind to free IL-1 β than IL-1 α , which in practice can result in a diminished response to IL-1 β since it cannot bind to the cellular IL-1RI.[154, 185] Soluble receptors of IL-1RI and IL-1RII also circulate in health and disease, functioning as natural buffers binding IL-1 α , IL-1 β and IL-1Ra.

There are different binding affinities for IL-1 α , IL-1 β and IL-1Ra which has clinical importance, especially when using soluble receptors such as therapeutic anti-IL-1.[186-189] IL-1Ra binds with the highest affinity to surface and soluble IL-1RI and is thus a very effective blocker of IL-1 activity. IL-1 α binds to surface and soluble IL-1RI with the same affinity, but to a lesser degree than IL-1Ra. IL-1 β binds with the lowest affinity of the three to IL-1RI. The most dramatic difference is the high affinity in which IL-1 β binds to the soluble form of IL-1RII. This bond is nearly irreversible due to the long dissociation rate (about 2 hours).

1.8.2 Interleukin-1 in RA

The significant role of cytokines in chronic rheumatic inflammatory diseases has been demonstrated by successful targeted therapies such as TNF blockade in RA.[190, 191] Pathogenesis of RA, though unknown, is thus believed to include proinflammatory cytokines such as TNF.[22, 192, 193] IL-1 is also a key mediator and has been implied in the pathogenesis of RA.[154, 194]

Patients with RA have increased plasma and synovial fluid levels of IL-1 compared with healthy controls, and these levels and histomorphologic patterns of synovitis also correlate with disease activity.[195-197] In a study with symmetrical and asymmetrical knee joint inflammation, nearly equal levels of IL-1 β were detected in knees of patients with symmetrical involvement whereas levels were considerably higher in inflamed knees of patients with asymmetrical involvement.[198]. IL-1 induces proliferation of fibroblasts leading to pannus formation, stimulates the production of prostaglandin E₂, and contributes to destruction of cartilage, bone and periarticular tissues in RA.[169]

In vitro studies have showed the chemotactic activity of synovial fluid specimens from patients with RA, verifying the presence of IL-1 in the RA synovial membrane.[199, 200] IL-1 can also alter the production of collagen by chondrocytes, decreasing the production of collagen type II, the main constituent of cartilage.[169]

Some experimental animal models of chronic arthritis have confirmed a role of IL-1 in joint inflammation. IL-1 β directly induces arthritis when injected into joints of animals, [201] stimulates cartilage and bone resorption [202] and inhibits the synthesis of articular collagen and proteoglycan.[203]

This evidence of an important role of IL-1 in the pathophysiology of RA has led to the effective approach to treat RA patients with recombinant human IL-1Ra (anakinra, Kineret[®]).[204-210]

1.8.3 Interleukin-1 and other cytokines in IIMs

Despite the differences in inflammatory cellular phenotypes and localization between subsets of myositis the cytokine pattern in muscle tissue is strikingly consistent. Two of the most consistently expressed cytokines in muscle tissue of PM, DM and IBM patients are IL-1 α and IL-1 β . [108, 109, 146-153] These cytokines have been found in muscle tissue of patients with impaired muscle performance both in the early phase before treatment and in a chronic phase of disease with persisting muscle weakness, making IL-1 a potential target of therapy in myositis.[108, 109, 151] IL-1 α is a potent up-regulator of MHC class I expression on various cells, including cultured muscle cells.[211, 212] In IIMs, IL-1 α is expressed in infiltrating inflammatory cells, endothelial cells of capillaries and large vessels.[108, 109, 150, 151] IL-1 β has a more sparse expression pattern than IL-1 α in IIMs muscle tissue. If seen, it is found in infiltrating mononuclear cells. IL-1 β could not be detected in serum of patients with IIMs and healthy controls.[213] IL-1 β expression in muscle tissue has also been suggested to be more associated with IBM.[146]

Further support of a role of IL-1 in myositis are the elevated serum levels of IL-1Ra mRNA and protein in patients with active PM/DM compared with inactive PM/DM and normal controls.[214] Serum IL-1Ra levels have also been shown to be significantly higher in DM/PM compared to patients with spondylarthropathies and normal controls. IL-1Ra levels were particularly elevated in patients with active myositis and decreased in response to therapy.[213] A study has shown significantly increased concentrations of IL-1Ra in immunoassays of homogenized IIM muscle compared to healthy controls.[148] Moreover, the IL-1Ra A1 allele in Caucasians and the A3 allele in African-Americans, which are associated with low IL-1Ra production, were found to be a risk factor for juvenile myositis versus normal race-matched controls.[141]

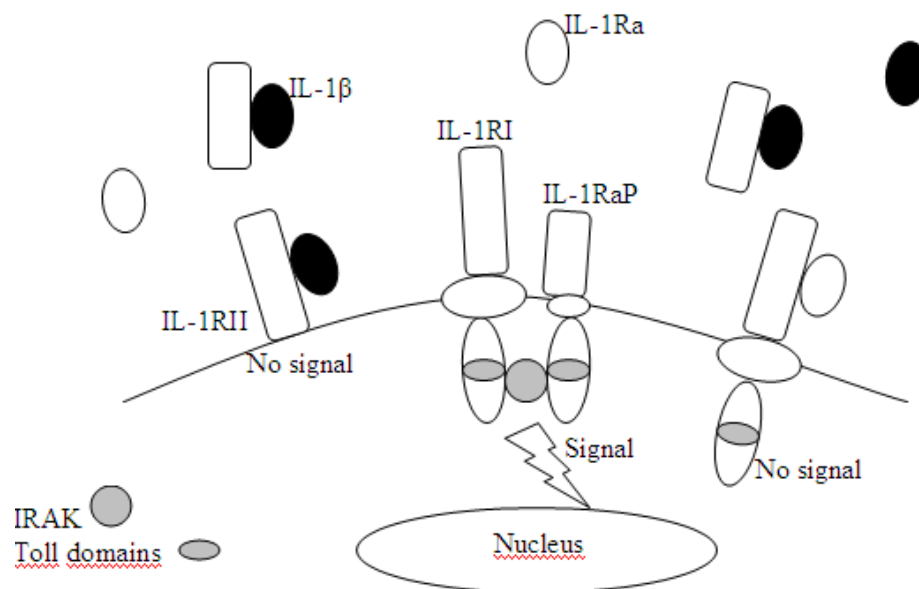
Other proinflammatory cytokines such IFN- α , TNF and HMGB-1 have also been demonstrated in muscle tissue in patients with inflammatory infiltrates in muscle tissue in patients with muscle weakness and muscle fatigue.[96, 108, 109, 143, 151, 215-218] These findings suggest that cytokines could be involved in the pathogenesis of myositis and that blocking cytokines could be possible therapy targets. However, TNF blockade

has had varying effects on clinical symptoms [219-222]. In one study, some patients who were treated with infliximab worsened in their muscle symptoms and inflammatory cell infiltrates were still present in muscle tissue after 14 weeks of infliximab treatment suggesting that TNF does not have a key role in the pathogenesis of myositis.[219] This suggests that other key molecules need to be searched for in myositis. Based on the findings in muscle tissue described above, IL-1 is an interesting target for therapies in myositis.

1.9 ANAKINRA

Anakinra is a human recombinant form of IL-1Ra, approved for treatment of RA in Sweden since 2002. In RA, IL-1Ra production by synovial macrophages is deficient relative to the total production of IL-1.[156, 157, 223, 224] Hence, an imbalance exists favoring the proinflammatory effects of IL-1.[225] Thus the objective of IL-1Ra based therapy is to reverse this deficiency and occupy enough IL-1 receptors with IL-1Ra to block IL-1 signaling. Anakinra binds to soluble and membrane-bound receptors on T cells, synovial cells and chondrocytes with an affinity equal to that of IL-1.[157] Recombinant human IL-1Ra differs only slightly from the endogenous IL-1Ra by the addition of an N-terminal methionine.[168] Naturally occurring and recombinant IL-1Ra has comparable ability to inhibit IL-1.[175-177, 226] It has been shown that >95 % receptor occupancy by IL-1Ra is necessary to block IL-1 signaling.[227] Anakinra, when bound to IL-1R, prevents IL-1AcP from interacting with the receptor and thus no response occurs.[225] See Figure 2 which demonstrates the mechanism of action of both endogenous and recombinant IL-1Ra, which operate in similar manners. Anakinra's mechanism of action was also described in chapter 1.8.

Figure 2. Mechanism of action of IL-1Ra and IL-1 β



The antagonist effects of anakinra on IL-1 have been showed in *in vitro* studies and *in vivo* animal models of arthritis with improvements in joint inflammation.[172, 173, 228-237] Several randomized clinical studies have shown clinical efficacy of anakinra in RA patients. A 24-week, double-blind, placebo-controlled multicenter trial included 472 European patients with early RA who had been treatment-resistant for other DMARDs or who were DMARD naive.[204] Three different doses of anakinra (30, 75, 150 mg/day subcutaneously) was compared to placebo. There was a 24-week extension of this study where the patients who had received active treatment continued this and the placebo patients were re-randomized to therapy in three doses.[238, 239] Outcome was ACR20 (American College of Rheumatology 20) response at 24 weeks. In the 150 mg/day group, significant improvements versus placebo were observed for all ACR clinical parameters. Rates of changes of clinical improvement were greatest during the first 6 weeks of the study. Radiologic evaluation also showed a statistically significant slowing in rate of progressive joint damage after treatment with anakinra compared with placebo. The extension study showed that clinical efficacy was maintained up to 48 weeks and was well tolerated and safe. Radiographic joint damage also continued to significantly slow down. Anakinra in combination with MTX was evaluated in a 24-week, multicenter, double-blind, placebo-controlled study of MTX alone versus MTX/anakinra and in an extension study which showed similar results.[205, 240] Thus clinical studies have showed that anakinra in RA is more effective than placebo. However, clinical trials and practice have shown that the effects of anakinra on disease activity in RA are much weaker than those of TNF-blockers.

Small uncontrolled studies and case reports have, on the other hand, indicated that anakinra is very effective in autoimmune systemic disorders such as adult-onset Still's disease and the equivalent disease in children, systemic-onset juvenile idiopathic arthritis. Both are characterized by fever, rashes and arthritis. Treatment with anakinra has quickly resolved systemic symptoms and laboratory markers of inflammation. Arthritis improved and significant steroid-sparing effect was observed. There are also rare disorders related to genetic disruptions of the regulated processing of IL-1 β called Muckle-Wells syndrome, familial Mediterranean fever, familial cold autoinflammatory syndrome, neonatal onset multisystem inflammatory disease and the cryopyrin-associated periodic syndrome. Rapid and favorable responses to anakinra have also been reported in these rare disorders.[241]

2 AIMS OF THE STUDY

The main aim of this thesis has been to gain more insight into the pathogenesis of IIMs by studying inflammation, with particular focus on IL-1, on the muscle tissue level. Ultimately new therapies could emerge. Specific aims were:

To study the safety and value of muscle biopsy with the percutaneous conchotome technique for diagnostic and research purposes in a rheumatology setting

To study whether clinical muscle symptoms can be correlated to inflammation and cytokine expression, especially IL-1, in muscle tissue of patients with PM, DM and healthy controls

To study whether muscle fibers in patients with PM, DM and healthy controls express IL-1 receptors and if so is there a colocalization with reciprocal ligands

To study the clinical outcome and effects of inflammation and cytokine expression, especially IL-1, in muscle tissue when using IL-1 blockade, anakinra, in patients with refractory IIMs

3 MATERIALS AND METHODS

3.1 STUDY POPULATION (PAPERS I-IV)

Patients included in the thesis were recruited from the Rheumatology Clinic at Karolinska University Hospital, Stockholm, Sweden. This is a nonselective referral center for rheumatic diseases for Stockholm and the nearby counties. Patients with PM or DM were diagnosed according to Bohan and Peter's criteria and patients with IBM were diagnosed according to Grigg's criteria.

Several groups of patients are included:

In **paper I**, the group included all patients (n = 122) who underwent muscle biopsy (n = 149) at the Rheumatology Unit, Karolinska University Hospital, Stockholm during 1996 and 1997. Thus, both patients already diagnosed with a rheumatic disease and patients where a rheumatic diagnosis was suspected were included.

In **papers II and III**, the group included consecutive patients (8 PM in papers II and III; 3 DM in paper II and 2 DM in paper III) who agreed to have muscle biopsies performed from two different muscle groups (symptomatic muscle where the patients experienced subjective muscle weakness, tenderness or pain; and non-symptomatic muscle where the patients did not experience subjective muscle weakness, tenderness or pain), at the same occasion, for diagnostic purposes. Thus the patients had active disease and were newly diagnosed with PM or DM. The reason why there was one less patient in paper III is due to that we did not have enough muscle tissue to allow for analysis for one patient.

In **paper IV**, the group included patients with treatment-resistant PM (n = 6), DM (n = 4) or IBM (n = 5) followed at the Rheumatology Clinic, Karolinska University Hospital. All patients had previously been treated with high-dose prednisolone and at least one of AZA or MTX with no or limited effect.

Healthy controls without clinical or histopathological signs of muscle disease were included in **papers II** (n=5) and **III** (n=7). They were age and sex matched and provided through collaboration with professor Kristian Borg, Rehabilitation Medicine, Danderyds Hospital, Stockholm, Sweden.

3.2 MUSCLE BIOPSY METHOD (PAPERS I-IV)

In all papers, muscle biopsy with the percutaneous conchotome biopsy technique or semi-open muscle biopsy technique was used. **Paper I**: n = 149. **Papers II and III**: n = 22 resp 20 for patients and n = 5 resp 7 for healthy controls. **Paper IV**: n = 17. In paper IV, muscle biopsies were also obtained by the open surgical method (n = 11) due to pronounced muscle atrophy and/or muscle tissue consisting of much fat and connective tissue.

At least two biopsies were taken from the same site and at the same occasion to minimize the risk of missing focal histopathological changes.

3.3 CLINICAL OUTCOME MEASURES

3.3.1 IMACS disease activity core set parameters (Paper IV)

An international consensus collaboration, the International Myositis Assessment and Clinical Studies (IMACS) group, has reached agreement on three component outcome measures in adult and pediatric PM and DM patients: myositis disease activity, myositis damage index and health-related quality of life.[242-245] For this thesis we have only used the first outcome measure, myositis disease activity. In this tool, the core set consists of 6 domains:

1. Patient's assessment of disease on a visual analogue scale (VAS) scale
2. Physician's assessment of global disease activity on a VAS scale
3. Manual muscle test in 8 muscle groups on the dominant side (MMT-8)
4. Assessment of serum levels of muscle associated enzymes (CPK, LD, ASAT, ALAT)
5. Health assessment questionnaire (HAQ)
6. Extramuscular disease activity score on a VAS scale (divided into constitutional, mucocutaneous, articular, gastrointestinal, pulmonary, cardiac and muscular symptoms)

Improvement was defined according to the suggestion by IMACS as $\geq 20\%$ improvement in three or more of the six core set parameters and no more than two worsened $\geq 25\%$ which could not include MMT-8. Worsening was defined by $\geq 30\%$ reduction in any three of the core set parameters. The changes, at all time points, were defined as changes compared to study start.

3.3.2 Muscle performance

3.3.2.1 Functional index (FI) (Paper IV)

The functional index (FI) was the first functional impairment outcome measure developed specifically for patients with PM and DM.[246] The FI assesses the number of correctly performed repetitions in 10 muscle groups with a total score of 64, representing good muscle performance. It is validated regarding its ability to discriminate patients from healthy controls. The FI is useful in assessing patients with moderate to severe muscle impairment.[86, 87] However, it is time-consuming, some tasks may be inadequate and ceiling effects have been observed in patients with only mild impairment. Improvement of FI was defined as $\geq 20\%$ increase in FI whereas worsening was defined as $\geq 20\%$ reduction in FI. The changes, at all time points, were defined as changes compared to study start.

3.3.2.2 Manual muscle test in eight muscle groups (MMT-8) (Paper IV)

MMT-8 is included in the disease activity core set proposed by IMACS. MMT-8 is based on the medical research council scale and is the most frequently used measure of muscle strength in clinical trials.[82, 247-249] It has been partly validated and inter-rater reliability was good in six out of eight tested muscles in myositis patients.[242]

3.4 MUSCLE BIOPSY ASSESSMENT

3.4.1 Immunohistochemistry and immunofluorescence techniques (Papers II-IV)

Since skeletal muscle is easily available for biopsy, subsequently immunohistochemistry and immunofluorescence are good techniques to analyze molecules on the protein level in muscle tissue. It gives information on the structural and intracellular localization of molecules examined by using antibodies, preferably monoclonal, for detection. Vital to the success of these methods are tissue preparation and fixation. It is important to freeze the biopsy samples as quickly as possible to maintain morphology. For intracellular stainings, we have used fixation with formaldehyde. A high degree of cellular morphology and antigenicity is preserved after formaldehyde fixation. For cell surface markers, we have used fixation with acetone.

Permeabilization of cell surface membranes is also important to allow for penetration of antibodies, essential to enable intracellular staining. We have used saponin as a permeabilizing agent. Saponin intercalates in the membrane and reversibly replaces cholesterol. Also critical to the success of these methods are that antibodies are carefully tested and titrated. This is a procedure which occurs continually in the laboratory. Primary antibodies must be well-validated to show that the staining corresponds with the protein of interest i.e. be specific. Antibodies have been tested on peripheral blood monocytes, tonsil tissue, synovial tissue and muscle tissue. Negative controls of irrelevant isotype-matched control antibodies have also been included.

Advantages of immunohistochemistry are that one can use an ordinary light microscope, tissue structures can be seen and the staining does not fade as fast as immunofluorescence. Immunofluorescence, on the other hand, enables colocalization of cells and is simpler and requires fewer numbers of steps. However, immunofluorescence does not allow for visualization of structures in tissue and the color fades fast.

3.4.2 Confocal microscopy technique (Paper III)

The immunohistochemistry technique alone cannot distinguish staining to specific cellular structures. But confocal microscopy improves the quality of images compared to conventional microscopy. Confocal microscopy permits to visualize fluorescence in a single plane of focus, creating a greatly sharper image. Immunofluorescence in combination with confocal microscopy and double staining can localize protein expression to specific cellular structures such as the sarcolemma and nuclei. Disadvantages to confocal microscopy are that the intense illumination from the laser can fade fluorescent samples and samples have to be with a strong fluorescent to be detected.

3.4.3 Manual assessment (Papers II-IV)

A manual evaluation was used to assess certain stainings. Manual evaluation was performed according to an arbitrarily chosen scale or by counting, for example, the number of positive cells per tissue section and then presenting the results as the number of positive cells per square millimeter (no of positive cells/mm²) in each tissue section.

3.4.4 Assessment by computerized image analysis (Papers II-IV)

Computerized image analysis is a method to reduce the subjectivity when performing evaluations of immunohistochemistry. Because of the adaptive nature of the human eye, the method allows for quantitative measurements and is more sensitive to variations in staining compared to manual visual assessment of immunohistochemical stainings. Disadvantages could be setting the color threshold for positivity and its time-consuming nature. To overcome this, the color threshold is set manually, and not by the computer, to account for differences in staining intensity among tissue sections and between different image frames.

Computerized image analysis measures the total positively stained area in percent for each muscle tissue section. It is possible to manually discard area which is determined by the eye to be negative, artifacts and non-muscle tissue.

3.4.5 Statistical analyses (Papers II-IV)

Due to the non-normality of data and limited number of patient, non-parametric methods were used to test for significance. A P-value < 0.05 was considered statistically significant. However, care should be taken when interpreting significance due to that non-parametric tests are less statistically powerful and due to the low number of patients included in the papers.

4 SUMMARY OF RESULTS

4.1 PAPER I

Muscle biopsy with the percutaneous conchotome technique is safe, simple and gives an adequate yield for diagnostic and research purposes in IIMs.

4.2 PAPERS II AND III

Signs of inflammation, IL-1 α and MHC class I and II are found in both symptomatic and asymptomatic skeletal muscle tissue in patients with PM and DM.

4.3 PAPER III

Increased expression of IL-1R on muscle fibers and the colocalization with its reciprocal ligands in patients with PM and DM, but not in healthy controls, support the hypothesis of a crucial role of IL-1 in the pathogenesis of PM and DM.

4.4 PAPER IV

Treatment with IL-1 blockade, anakinra, had beneficial effects on clinical outcome measures including disease activity, especially extramuscular disease activity and muscle performance, in a pilot study of patients with treatment-resistant PM, DM and IBM. This could not be confirmed by significantly reduced inflammation in muscle tissue. Nonetheless, the findings of beneficial clinical effects and of IL-1Ra expression in more responders than non-responders in post-treatment biopsies, should be confirmed in a larger placebo-controlled trial.

5 RESULTS AND DISCUSSION

5.1 PAPER I

It is important to study the target organ in diseases, to gain more knowledge about pathogenesis and to subsequently improve therapy options. Skeletal muscle is in this thesis the target organ in IIMs. Thus a safe, easy technique for muscle biopsy which allows for a good diagnostic yield and repeated biopsies is of paramount importance. Such a technique is central for both diagnostic and research purposes in a rheumatology setting. Therefore, we chose to evaluate the currently used technique for muscle biopsies at the Rheumatology Clinic, Karolinska University Hospital, Stockholm, Sweden, the percutaneous conchotome muscle biopsy technique.

This was a retrospective cohort study of medical records of all patients who were subject to muscle biopsies during 1996 and 1997. Due to that Sweden has strong laws concerning medical documentation and that there is a register of all muscle pathology reports, we are quite confident that all muscle biopsies performed 1996 and 1997 are included. A disadvantage could be that the study in fact relies on existing records, and sometimes information may be missing or unavailable. Nevertheless, a study of this kind has the advantages that it is least costly than a prospective study and produces results earlier.

All patients were subject to muscle biopsy with the percutaneous conchotome technique. 149 muscle biopsies were performed on 122 patients. 145 biopsies were sufficient for histopathological evaluation whereas 4 biopsies consisted of endstage muscle consisting of fat, connective tissue or subcutaneous fat. M. tibialis anterior, m. vastus lateralis and m. deltoideus were all readily accessible sights for muscle biopsy.

106/149 biopsies were carried out for primary diagnostic purposes. Biopsies were divided into four groups based on purpose for investigation. The sensitivity and specificity of muscle biopsy with the percutaneous conchotome technique for each group is also presented below.

1. suspicion of myositis and no previous other rheumatological diagnosis (n = 24)
83 % sensitivity, 100 % specificity
2. suspicion of overlap myositis and previous rheumatological diagnosis (n = 43)
89 % sensitivity, 100 % specificity
3. suspicion of systemic vasculitis (n = 19)
11 % sensitivity, 90 % specificity
4. myalgia as primary symptom and no proximal muscle weakness (n = 20)
No biopsy had positive histopathology for myositis or vasulitis, and no patient received myositis or vasculitis as diagnosis.

Among the 106 biopsies carried out for primary diagnostic purpose, the distribution by myositis diagnosis (probable or definite PM or DM according to Bohan and Peter's criteria) and by outcome of muscle biopsy histopathology is presented in Table 3 below.

Table 3. Distribution of patients suspected of having myositis by diagnosis and results of muscle biopsy

| <i>Muscle biopsy</i> | <i>Myositis</i> | <i>No myositis</i> | <i>Total</i> |
|----------------------|-----------------|--------------------|--------------|
| + | 18 | 0 | 18 |
| - | 3 | 85 | 88 |
| Total | 21 | 85 | 106 |

Thus, we see that the sensitivity or proportion of patients with PM or DM who also has positive muscle biopsy, is high:

Sensitivity of muscle biopsy results for myositis diagnosis = $18/21 = 86\%$

We can also observe that the specificity or proportion of patients who do not have PM or DM and who also have negative muscle biopsy, is high:

Specificity of muscle biopsy results for myositis diagnosis = $85/85 = 100\%$

The most useful tests for diagnosing a disease are those with both high sensitivity and specificity, which was found in this group of patients when myositis was suspected. The probability that a sick individual was classified as having myositis was high. Also, the probability that a healthy individual was classified as healthy was high. Thus the diagnostic value of muscle biopsy with the percutaneous conchotome technique was high when myositis was suspected. The high sensitivity of muscle biopsy with the percutaneous conchotome technique when suspecting myositis is comparable with other studies (see Paper I for references).

Among the 106 biopsies carried out for primary diagnostic purpose, the distribution by vasculitis diagnosis (according to the 1990 classification criteria of the American College of Rheumatology) [250] and by outcome of muscle biopsy histopathology is presented in Table 4 below.

Table 4. Distribution of patients suspected of having vasculitis by diagnosis and results of muscle biopsy

| <i>Muscle biopsy</i> | <i>Vasculitis</i> | <i>No vasculitis</i> | <i>Total</i> |
|----------------------|-------------------|----------------------|--------------|
| + | 3 | 2 | 5 |
| - | 10 | 91 | 101 |
| Total | 13 | 93 | 106 |

Hence, we can see that the sensitivity is low:

Sensitivity of muscle biopsy results for vasculitis diagnosis = $3/13 = 23\%$

and that the specificity is high:

Specificity of muscle biopsy results for vasculitis diagnosis = $91/93 = 98\%$

The probability that a sick individual was classified as having vasculitis was low. But the probability that a healthy individual is classified as healthy was high. Thus, the diagnostic value of muscle biopsy with the percutaneous conchotome technique was low when vasculitis was suspected. These results could reflect the nature of vasculitis

with skip lesions, and that vasculitis may primarily affect larger vessels than those captured by the conchotome-performed biopsy.

43/149 biopsies were rebiopsies, carried out to evaluate the effect of treatment or investigate an eventual flare-up. One third of these biopsies had persisting signs of inflammation and more intense immunotherapy was given. In general for all biopsies, therapy was initiated, terminated, increased or reduced after about half of all muscle biopsies. Normal as well as pathological biopsies were important in this aspect. Normal biopsies were also important to exclude myositis and other myopathies. Repeated biopsies are important to assess the effect of treatment and to gain insight into changes in histopathology over time and in different muscle groups. This has been considered controversial due to the trauma it may cause. Only a few studies report repeated biopsies.[5, 251, 252] Our study confirms that repeated biopsies are safe, simple to perform and gives diagnostic yield leading to active clinical decisions, such as treatment changes. Findings in Papers II and III also support that multiple biopsies from the same occasion are safe, straightforward and key to gain understanding of molecular expression in muscle tissue from myositis patients in different muscle groups. See results and discussion of Papers II and III.

The procedure is safe for the patient, with only 4/149 (3 %) biopsies reported to be followed by complications. Two complications were minor, transient muscle pain at the biopsy site. One patient had persisting hyperesthesia distal to the biopsy site in m. tibialis anterior after one year. One patient had a serious complication, a subfascial hematoma followed by deep vein thrombosis in the thigh. This patient, however, had risk factors predisposing to such a complication since he had advanced atherosclerosis and was treated with low dose acetylsalicylic acid. To prevent complications it is important to be well acquainted with the anatomy to prevent nerve damage and caution should be taken with patients undergoing anticoagulant treatment. We have since then also applied a pressure bandage after biopsy.

Despite the retrospective nature of the study which prevents comparison with other biopsy methods, muscle biopsy with the percutaneous conchotome technique is a sufficiently sensitive and specific tool to make new diagnostic evaluations of IIMs and assess the effect of a given therapy in patients already diagnosed with IIMs (repeated biopsies). The method is also simple, safe and comfortable for patients. Thus, the technique serves patients in a rheumatology clinic well since IIM diagnosis can be confirmed or disproved, since results of muscle biopsy findings lead to changes in clinical practice, since results can gain insight into pathogenetic mechanisms on the molecular level in muscle tissue and thus ultimately lead to improved therapies. Findings from this paper also support already known data that muscle biopsy is a “gold standard” to define IIMs.[22, 253]

5.2 PAPERS II AND III

Based on the background outlined in Chapter 1.8, IL-1 might affect muscle fiber function and contribute to muscle injury in IIMs. IL-1 has been, as described in chapter 1.8, one of the most consistently expressed cytokines in muscle tissue of patients with IIMs (in endothelial cells of capillaries and in inflammatory cells), both in an active and

chronic phase of the disease and during and after immunosuppressive treatment. Inflammation in it self could induce muscle weakness, for example, infiltrating inflammatory cells can induce muscle cell necrosis. Despite this, there is a lack of correlation between clinical symptoms and the degree of inflammatory infiltrates in patients with myositis.[67, 109, 251, 254] Other phenotypic changes that have tried to explain the lack of correlation of clinical symptoms and degree of inflammation in muscle tissue is the phenotypic expression of MHC class I and II on muscle fibers (normally not expressed on muscle fibers) independent of inflammatory cell infiltrates in myositis patients.[103-109, 150] A role of MHC class I antigen has also been proposed in that transgenic mice with specific upregulation of MHC class I antigens on muscle fibers develop muscle weakness before infiltration of inflammatory cells.[110]

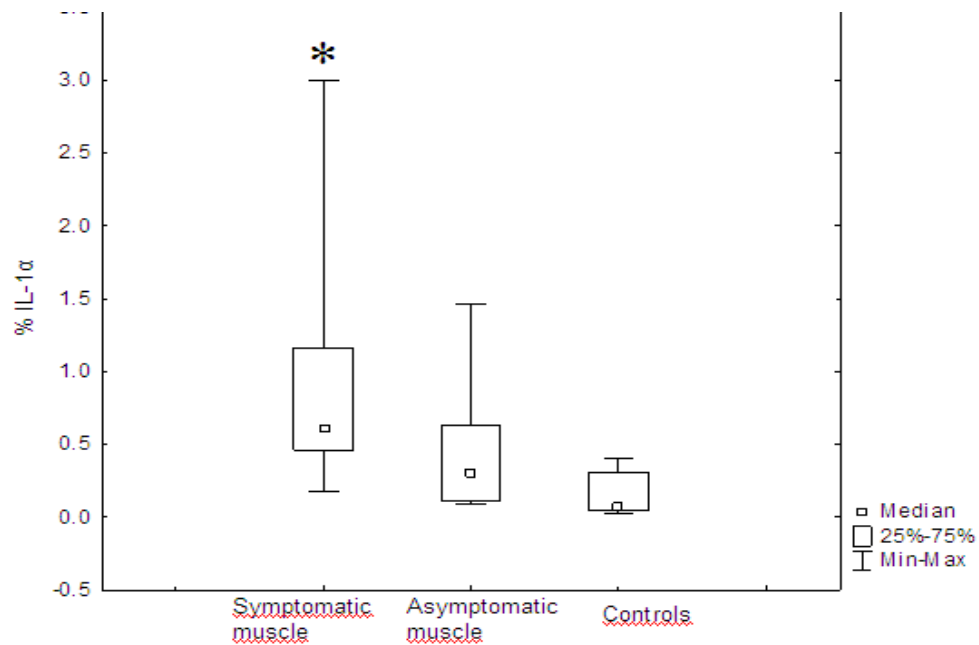
In Paper II, we wanted to further investigate the role of inflammation (T cells and macrophages), IL-1 and MHC class I and II in the cause of muscle symptoms, by comparing the expression of these molecules in muscle tissue from the same patient, one from a clinically symptomatic (all proximal muscles) and one from a clinically asymptomatic (all distal muscles, with the exception of two biopsies) muscle. Two biopsy samples were taken from each site, one for diagnostic and one for research purpose. We also wanted to compare the expression of these molecules between patients and healthy controls. The same patients were included in Paper II as in Paper III (see chapter 3.1). So stainings for IL-1 α were performed by two independent executors on two separate occasions. Also, analysis of IL-1 α in muscle biopsies was performed by two independent observers for computerized image analysis and by four independent observers for conventional microscopic assessment. This repetitive, independent practice in handling muscle biopsy stainings, strengthens our results, especially since the number of patients (n = 10 and 11) and healthy controls (n = 6 and 7) included were limited. Muscle biopsies were investigated by immunohistochemistry using antibodies against IL-1 α , CD3 (T cell marker), CD163 (macrophage marker), MHC class I and MHC class II. Quantification was performed by computerized image analysis. Conventional microscopic assessment was also performed by the observers and an experienced neuropathologist.

Results in Paper II and Paper III show that there was no statistically significant difference in histopathological changes (inflammation and regenerating and degenerating muscle fibers) characteristic of PM and DM between the symptomatic and asymptomatic muscle biopsies or between the two biopsy samples taken from each biopsy site (for research and diagnostic purposes). In addition, comparison of histopathologic changes in the first and last sections of each biopsy sample showed no differences. These findings were also confirmed when performing immunohistochemistry on symptomatic and asymptomatic muscle biopsies. We found no statistically significant difference in the number of T cells or macrophages between symptomatic and asymptomatic muscle tissue. Healthy controls showed no or very few scattered inflammatory cells. There was a statistically significant higher number of T cells and macrophages in patients (both symptomatic and asymptomatic muscle tissue) versus healthy controls.

Further validating that signs of inflammation are found both in symptomatic and asymptomatic muscle, is that papers II and III show that total IL-1 α expression, as

assessed by computerized image analysis, was significantly increased in patients (hence in both symptomatic and asymptomatic muscle) as compared with healthy controls ($P < 0.05$). Figure 2B in Paper II is incorrectly printed. The correct figure is presented down below (Figure 3). Also the number of IL-1 α positive capillaries per square millimeter was significantly increased in patients versus healthy controls. IL-1 α expression was localized to endothelial cells of capillaries, mononuclear inflammatory cells and larger vessels.

Figure 3. Total IL-1 α expression in symptomatic, asymptomatic and normal control muscle assessed by computerized image analysis, expressed as the percentage positive staining of the whole tissue section. $P < 0.05$ vs controls.



Paper II further shows that MHC class I and II expression on muscle fibers, as assessed by conventional microscopic assessment, was significantly higher in patients' symptomatic and asymptomatic muscle compared to healthy controls.

This is to our knowledge, the one of the first studies to investigate two different muscles from the same patient with PM or DM, one from a clinically symptomatic (all proximal) and one from a clinically asymptomatic (mostly distal) muscle. Since inflammatory changes within affected muscles in PM and DM patients is often focally distributed, one may miss sites of inflammation during the biopsy procedure. Nonetheless, biopsy samples from the two different muscles in patients demonstrated similar histopathological and immunohistochemical findings.

The choice of biopsy location was based on where the patients experienced subjective clinical symptoms such as weakness, tenderness and pain. All biopsies from symptomatic muscle were in fact taken from proximal muscles and those from asymptomatic muscle were mostly taken from distal muscle. This corresponds well to the classical distribution of muscle weakness in PM and DM according to textbooks.[22, 255] Muscle is recommended to be sampled from weak muscle. One of

the explanations for patients with active myositis to have normal muscle biopsies, besides the patchy nature of muscle inflammation, is that the wrong muscle is chosen for biopsy. We would like to challenge this established notion. Muscle biopsies could be taken from both proximal and distal muscle to demonstrate the characteristic muscle pathology of PM and DM, depending on what site the patient feels is more comfortable for biopsy and depending on the physician's judgement of biopsy feasibility. So, as previously mentioned, the degree of inflammation does not correspond to patient's subjective muscle symptoms and cannot explain why muscle symptoms occur.

There may be many different mechanisms underlying the clinical muscle symptoms in IIMs. This is supported by that in an early phase of myositis, muscle weakness can be severe without accompanying muscle atrophy, suggesting that factors other than loss of muscle mass contributes to muscle weakness.

What then causes muscle weakness, if it may not be signs of inflammation in muscle tissue? A possibility could be that clinical muscle symptoms in PM and DM are caused by different physical demands on proximal and distal muscles or by different characteristics of muscle fibers in diverse muscle groups. Similar mechanisms could account for the distribution of muscle weakness in other myopathies such as muscular dystrophies.[256] The physical demands of strength and endurance and of oxygen supply are higher on postural muscles like thigh muscles than on lower leg muscles. Local hypoxia, a consequence of inflammation, could thus make thigh muscles more disposed to clinical symptoms than distal muscles.[257-259] IL-1 α , ICAM-1 and VCAM-1, all found in muscle tissue of patients with IIMs, have been reported to be upregulated by hypoxia.[108, 151, 158, 260-262] In addition, previously reported reduced levels of adenosine triphosphate in muscle of patients with myositis support the hypothesis of local muscle tissue hypoxia.[258] A small pilot study has also showed that increased physical demand in a muscle tissue with hypoxia could lead to a further reduction in oxygen tension, which could explain the reduced functional capacity that the patients experience.[263] It has also been reported that patients with DM have a decreased number of capillaries, even when there are no inflammatory infiltrates.[102, 264-267] Another observation of disturbed microcirculation is the presence of morphologically changed endothelial cells resembling HEVs, indicating activation of endothelial cells.[151, 268] Reported benefits of exercise in patients with IIM also support that hypoxia may cause muscle symptoms.[269] In another paper, a low total number of capillaries in muscle tissue was found both in PM and DM patients compared to healthy controls, indicating capillary loss. The reduced number of capillaries corresponded to an increased expression of vascular endothelium growth factor (VEGF) which is induced by hypoxia and could function as an angiogenic growth factor *in vivo*. [270-272]

A drawback to our study is that the biopsy site was chosen based on where the patients experienced subjective muscle weakness. A more objective tool such as MRI was not used for validation. EMG and FI results are insufficient to use since the tests do not correspond exactly to the specific muscles investigated. In a previous study, increased amounts of inflammatory infiltrates were found in muscle samples taken from MRI-detected affected muscle tissue in comparison with those biopsied from MRI-detected

non-affected sites, yet inflammatory cells could also be found in non-oedematous appearing muscle.[273]

In conclusion, inflammatory cells, IL-1 α on endothelial cells in capillaries and MHC class I and II expression on muscle fibers are present in both proximal and distal muscle in patients with PM and DM. Thus signs of inflammation constitute a general phenotype in skeletal muscle tissue of patients with PM and DM. This suggests that other factors than inflammation in muscle tissue, hypothesized above, are more important in causing muscle symptoms. One can also propose that muscle biopsy from proximal **or** distal muscle are adequate to locate histopathological findings characteristic of PM and DM.

5.3 PAPER III

Based on the observations outlined in Chapter 1.8 and 5.2, IL-1 might still affect muscle fiber function and contribute to muscle injury in IIMs since it was not strictly found in clinically symptomatic muscle. For IL-1 to have direct molecular effect on muscle fibers most likely it would require the expression of IL-1 receptors on the muscle fiber membrane. Paper III is the first paper, to our knowledge, to investigate if muscle fibers in patients with PM and DM and healthy controls express IL-1 receptors. Thus, the aim of paper III was to further investigate the role of IL-1 in the pathogenesis of PM and DM by exploring if muscle fibers in patients express IL-1RI and/or IL-1RII, and if so, is there a quantitative and qualitative difference in their expression between muscle from patients and healthy controls. We also wanted to examine whether there is a colocalization between IL-1 receptor expression and its reciprocal ligands, IL-1 α , IL-1 β and IL-1Ra, in muscle from patients and healthy controls.

Symptomatic and asymptomatic muscle biopsies from 10 patients with PM or DM (see chapter 3.1) and 7 healthy controls were investigated with immunohistochemistry using antibodies against IL-1RI, IL-1RII, IL-1 α , IL-1 β , and IL-1Ra. Quantification was performed by computerized image analysis and localization of expression was verified by double staining using immunofluorescence and confocal microscopy.

Results from Paper III showed that both IL-1RI and IL-1RII were expressed in muscle fibers, in inflammatory cells and in endothelial cells. The muscle fiber expression was localized to the sarcolemma and to the nuclei. IL-1 α results are presented in Chapter 5.2. IL-1 β and IL-1Ra was expressed in inflammatory cells. The expression of IL-1Rs and their three ligands was significantly higher in patients (both symptomatic and asymptomatic muscle) compared to controls. The IL-1R expression on muscle fibers was most evident nearby IL-1 α and IL-1 β expressing cells.

Both receptors were expressed on the membrane of muscle cells, and a surface expression of IL-1Rs is likely to be found on most IL-1 responsive cells. Colocalization of expression of the receptors and ligands in muscle nuclei leads one to think of the possibility that IL-1 might act as a transcription factor. The mechanism for how this might work has however not been studied in this paper. It is known that proIL-1 α can act as an intracrine proinflammatory activator of transcription.[274] There is also evidence to support that intracellular IL-1 α can decrease the threshold of the

transcription factors nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) and activator protein-1 (AP-1) dependent gene expression.[274] NF- κ B is a major transcription factor modulating the cellular immune response and has been shown to be activated in IIMs.[111, 275-278] That cytokines can activate transcription factors intracellularly and independent of receptors on membranes is a role not previously characterized.

In other studies, IL-1 has been shown to be expressed by muscle fibers with a sarcoplasmic appearance in IIMs.[146, 260] We did not, however, find sarcoplasmic expression of IL-1. We confirmed already known data, that endothelial cells express IL-1 α and mononuclear inflammatory cells express IL-1 α , IL-1 β and IL-1Ra.[108, 146, 151] Elevation of IL-1Ra mRNA and protein has been found in sera and in culture supernatants of unstimulated monocytes from patients with active PM and DM.[214] This could reflect an increased production of IL-1Ra and that IL-1Ra may regulate inflammatory events in diseased muscle of PM and DM patients.[279] Also, IL-1 α and IL-1 β protein expression in muscle tissue and serum levels of IL-1Ra in PM and DM patients have been shown to be decreased in response to steroid treatment.[108, 213] In this paper, we did, however, not find differences in protein levels of IL-1 when comparing steroid-treated and untreated patients.

In conclusion, the increased expression of IL-1Rs and the colocalization with reciprocal ligands in both symptomatic and asymptomatic muscle from PM and DM patients but not in healthy controls support the hypothesis that IL-1 may have an important role in the pathogenesis of PM and DM. IL-1 could, as described in Chapter 1.8, have direct effects on muscle fibers and might affect muscle fiber metabolism and function. Whether IL-1 also could act as a transcription factor in muscle fibers still needs to be clarified.

5.4 PAPER IV

To further test the hypothesis that IL-1 may have a key role in the disease mechanisms in IIMs we targeted IL-1 in 6 PM, 4 DM and 5 IBM patients (total n = 15). For treatment with IL-1 blockade we used anakinra, which is a receptor antagonist that neutralizes IL-1 α and IL-1 β by competitive binding to IL-1RI and is approved for treatment of RA in Sweden since 2002.[204, 205, 210] This was a 12 month open label trial. Anakinra was self-administered subcutaneously, 100 mg per day. Clinical assessment of efficacy and adverse events, and laboratory tests were performed at study start and thereafter at months 1, 2, 3, 4, 5, 6, 9 and 12 by a physician. Disease activity was assessed as proposed by IMACS (see Chapter 3.3.1). A physical therapist unaware of the physician's scoring of disease activity assessed muscle performance by FI months 0, 3, 6, 9 and 12 (see Chapter 3.3.2). Fourteen patients consented to repeated muscle biopsy (months 0 and 6). Muscle biopsies were investigated with immunohistochemistry using antibodies against IL-1 α , IL-1 β , IL-1Ra, T cells (CD3), macrophages (CD68 and CD163) and MHC class I. Quantification was performed by conventional microscopic assessment and computerized image analysis.

5.4.1 Clinical results

Results from Paper IV shows that 9 patients completed 12 months, 11 completed 6 months and 13 concluded 3 months. 7/15 patients fulfilled improvement criteria according to IMACS definition and were classified as responders, and 4 of the 7 responders improved $\geq 20\%$ in FI. Patients and time points of improvement according to IMACS definition and according to FI are presented in Table 5.

Table 5. Responders according to the IMACS definition of improvement of disease activity

| <i>Patient</i> | <i>Month(s) of IMACS improvement</i> | <i>Month(s) of FI improvement</i> |
|----------------|--------------------------------------|-----------------------------------|
| PM | 3 | 3, 6, 12 |
| PM | 3 | |
| DM | 3 | 3 |
| DM | 3 | |
| IBM | 3, 6 | 3, 6, 12 |
| PM | 6, 12 | 12 |
| DM | 12 | |

The main core set parameters which improved $\geq 20\%$ in responders were physician's VAS and extramuscular global score. 3/4 patients with DM improved in the extramuscular mucocutaneous score. Arthralgia and fatigue were otherwise overall the clinical features that improved in the extramuscular score. MMT-8 improved in the 2 responders (PM and IBM) who also improved in FI months 3, 6 and 12. MMT-8 did not worsen by $\geq 25\%$ in any patient.

There were 8/15 patients which were defined as non-responders. 5 of these patients had unchanged disease activity according to IMACS definition and unchanged FI scores. 3 of these non-responders worsened according to IMACS definition, and 1 of these 3 patients worsened $\geq 20\%$ in FI. Patients and time points of worsening according to IMACS definition and according to FI are presented in Table 6.

Table 6. Patients with worsening according to the IMACS definition of disease activity

| <i>Patient</i> | <i>Month of IMACS worsening</i> | <i>Month of FI worsening</i> |
|---|---------------------------------|------------------------------|
| PM | 12 | 12 |
| DM (withdrew at month 3 due to headache) | 3* | |
| IBM (withdrew at month 6 due to lack of efficacy) | 3* | |

*drop out

Six patients withdrew from the study prematurely. Two were among the non-responders described above. One PM and one DM patient withdrew at month 1 due to local skin rash at the injection site. One PM patient withdrew at month 3 due to increased muscle symptoms, but fulfilled IMACS improvement criteria. One IBM

patient withdrew at month 6 due to increased muscle symptoms but did not fulfill criteria for worsening.

Other transient adverse events were antibiotic-treated infections of which one was serious, pneumonia. Six patients had temporary local skin reactions at the injection site.

5.4.2 Muscle biopsy findings

Inflammatory infiltrates were present in the first and last sections and in the both biopsy samples taken before the study. IL-1 α was expressed in inflammatory cells, endothelial cells of capillaries and in large vessels. IL-1 β and IL-1Ra were expressed in mononuclear inflammatory cells. Macrophages and T cells were detected in almost all pre-treatment and post-treatment biopsies. There was no statistically significant change in expression of these markers between the pre-treatment and post-treatment biopsies that could explain the improvement in responders and improvement in FI. Nonetheless, IL-1Ra was detected in post-treatment biopsies of all responders but in only 3 of 8 non-responders. The source of IL-1Ra in the post-treatment biopsies could not be determined, but one possibility could be that it reflects uptake from the anakinra treatment.

5.4.3 Discussion

The number of clinical responders is clearly higher than observed in our previously reported studies where we investigated the effect of TNF-blockade (infliximab) or high dose IvIg.[70, 219] To our knowledge there are only two previously published case reports using IL-1Ra treatment in myositis. In the first report, three patients with active SLE were treated and one of them had myositis. Two of these patients had transient effect on muscle pain and/or polyarthritis but there was no effect on lupus myositis in the third patient.[280] In the second case report, there was a rapid reduction of fever, polyarthritis and acute phase reactants following administration of anakinra in a patient with antisynthetase syndrome.[281]

How can we explain the lack of correlation between clinical improvement and muscle biopsy findings? One explanation is that the outcome measures defined by IMACS are yet untried in larger studies. Hence, the sensitivity of the definition of improvement according to IMACS is still unclear. More controlled studies using this as an outcome measure are needed. Considering this was a non-blinded study, physician's expectations could have an influence on outcome, for example in scoring the global assessment of disease. Nevertheless, improvement of 4 of the 7 responders was confirmed by improvement in FI measured by a physical therapist unaware of the physician's scoring. FI is a more validated outcome measure of muscle performance. Another explanation could be that anakinra could have positive effects on more systemic symptoms such as fatigue, arthralgia and pain, as was also seen in our study by a reduction of the extramuscular score. Improvement of systemic symptoms may lead patients and physicians to score lower values on VAS and HAQ scales. It may even improve patient's performance in the MMT-8 test. This systemic effectiveness of anakinra on patients with other chronic systemic inflammatory disorders, is supported by data previously described in chapter 1.9.

Following subcutaneous injection of anakinra, peak plasma levels are reached within 5 hours and return to normal within 24 hours due to rapid renal elimination.[282] This could be an explanation for the systemic rapid effects and the reason why most patients improved already by month 3. Anakinra has a short half-life (about 5 hours) which may lead to that the drug does not penetrate into the muscle. Also, because of the need to block more than 90 % of receptors, it could be an inefficient method of suppressing IL-1. The lack of effect on muscle tissue could thus be explained by that the concentration of IL-1Ra was too low to compete with IL-1 α and IL-1 β .

Three out of four patients with DM reported improvement in skin rash and the clinical improvement in one IBM patient is of large interest since IBM is a disease which currently has no effective treatment. Another interesting finding was that IL-1Ra was detected in all post-treatment biopsies of responders but only in three of eight non-responders, possibly reflecting uptake from anakinra treatment.

Nonetheless, the role of inflammation in muscle tissue in relation to muscle symptoms is still unclear. This is supported by the findings in Paper II, where there is a lack of correlation between the degree of clinically reported muscle symptoms and degree of inflammatory infiltrates, MHC class I expression in muscle fibers or IL-1 expression, where the muscle biopsy changes were equally present in symptomatic as well as asymptomatic muscle. Muscle inflammation may not consequently lead to muscle weakness according to mechanisms also described in chapter 5.2. Another explanation could be that IL-1 may not be an important target of treatment, nonetheless IL-1 is the most consistently found cytokine in muscle tissue from patients with IIMs. Could other molecules involved in the inflammation cascade be a more effective target? At present, other mechanisms of IL-1 inhibition are being explored, including the development of monoclonal antibodies against IL-1, soluble IL-1 receptors and inhibitors of caspase-1.[282-284] These therapies are being evaluated in randomized clinical trials in patients with systemic autoimmune disorders and RA.[285, 286] Maybe the mechanisms of these inhibitors could be more effective than anakinra and could resolve muscle tissue specific inflammation and expression of IL-1.

In this study, the number of patients is too low to allow for any definite conclusions, but we find that data from this open-label study are interesting enough and the safety of anakinra is well-established to give reason for a larger scale placebo-controlled trial with anakinra in patients with PM and DM.

6 CONCLUSIONS

The work in this thesis contributes to understanding the role of inflammation, especially IL-1, in muscle tissue from patients with PM and DM and to some extent patients with IBM.

We found that muscle biopsy with the percutaneous conchotome technique is simple, safe and gives adequate yield to study inflammation and cytokine expression on the molecular level. This finding confirms that one can be assured of the quality of muscle biopsies analysed in the following papers.

The thesis has revealed one important point, which has not been studied before: that inflammation in muscle tissue in patients with relatively newly onset PM or DM is general and not restricted to proximal muscle, as presently proposed in rheumatology textbooks. This finding leads to two main remarks. Muscle biopsy with adequate diagnostic yield can be performed on both proximal and distal muscle. Thus the range of muscles available for biopsy is widened, benefiting the patient. The second comment is that the causes of clinical muscle symptoms in PM and DM cannot solely be explained by signs of inflammation in muscle tissue. This is also supported by the fact that muscle weakness often persists despite the lack of inflammation in muscle tissue in patients with chronic myositis.

The finding of an increased expression of the cytokine IL-1 and its reciprocal receptors and their colocalization in muscle tissue from patients with PM and DM but not in healthy controls, nonetheless, support the hypothesis that IL-1 has a crucial role in the pathogenesis of PM and DM. The observation of IL-1R in the muscle fiber nuclei is challenging and indicates a possible role of IL-1 as a transcription factor, but this still needs to be investigated. Drug intervention with IL-1 blockade, anakinra, in patients with refractory PM, DM and IBM revealed beneficial effects on clinical outcome including disease activity in 7 responders and muscle performance measured by FI in 4 out of 7 responders. This supports a role of IL-1 in the disease mechanisms in a subset of myositis patients. Thus IL-1 could be a potential target of therapy in these patients, but this must be investigated in a randomized placebo-controlled trial. Notably, one of these patients had IBM, a disease which currently has no effective treatment, suggesting that the IL-1 molecular pathway could be important in some IBM patients. Nonetheless, this result is anecdotal and needs further investigations.

We could, however, not explain the clinical improvement by reduced inflammation or cytokine expression in muscle tissue. This also confirms that the role of inflammation in muscle tissue in relation to muscle symptoms is still unclear. On the other hand, concentrations of anakinra in muscle tissue could also be too low to compete with the proinflammatory effects of IL-1. Nevertheless, we saw IL-1Ra expression in more patients in post-treatment biopsies than in pre-treatment biopsies, all being responders, a possible reflection of anakinra uptake in muscle tissue. Other mechanisms of blocking IL-1 are currently being explored and may also be valuable as treatment and in elucidating the role of IL-1 in the pathogenesis of IIMs. A possible explanation for the clinical amelioration in remarkably half of the patients could be the beneficial systemic

effects ascribed to anakinra. Anakinra may be a therapeutic option in some patients with IIMs.

Further studies are needed to address the role of inflammation and cytokine expression in muscle tissue in relation to muscle symptoms in PM, DM and IBM patients. The number of patients included in this thesis reflects the rarity of the IIMs, even though we have about 90 of these patients followed regularly at the Rheumatology Clinic, Karolinska University Hospital. Larger controlled studies should preferably be performed in a multi-center study design. Ultimately more insight into the pathogenesis of IIMs and new therapies could emerge by careful longitudinal follow-up including outcome measures of disease activity, disease damage, muscle performance and molecular expression in repeated muscle tissue.

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