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AUTOANTIBODIES AND THE TYPE I INTERFERON SYSTEM IN IDIOPATHIC INFLAMMATORY MYOPATHIES

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AUTOANTIBODIES AND THE TYPE I INTERFERON SYSTEM IN IDIOPATHIC INFLAMMATORY MYOPATHIES

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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To Ebba, Emma, Alexander and Elsa ♥

ABSTRACT

Idiopathic inflammatory myopathies (IIM), also known as myositis, are rare autoimmune diseases, characterized by proximal muscle weakness and inflammatory cells in skeletal muscle tissue. The most common subgroups are polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM). New subgroups have been recognized, such as immune-mediated necrotizing myopathy (IMNM) and the antisynthetase syndrome (ASS). Autoantibodies are common and some of them specific for myositis. The most frequent is the anti-Jo-1 autoantibody, which is associated not only with myositis but also with interstitial lung disease (ILD) and arthritis. The etiology of IIM is still unknown but environmental and genetic factors are believed to contribute to disease susceptibility. Accumulating data indicate a role of the type I interferons (IFNs) in myositis. The treatment of IIM (glucocorticoids and immunomodulatory drugs) has limited effect. New treatments are needed, thus increased understanding of molecular disease mechanisms in IIM is required.

The overall aim of my thesis was to get an increased understanding of molecular mechanisms that are involved in IIM with a focus on the type I IFN system, autoantibodies and mechanisms that may induce immune reactivity, to be able to subclassify patients.

Several new observations were made. Firstly, we found that line blot is a suitable serological test in myositis and is a reliable alternative to more time-consuming assays such as immunoprecipitation (paper I). Secondly, we concluded that smoking is associated with IIM patients who are either anti-Jo-1 autoantibody and/or HLA-DRB1*03 positive (paper II). These associations point towards a gene-environment interaction in the pathogenesis for IIM. Thirdly, we found that a high IFN score was not only associated with the subset DM, as previously reported, and IBM, but also with autoantibody monospecificity against RNA-binding proteins or with autoantibody multispecificity (paper III). Furthermore, we identified IFN-α in sera as a trigger for activation of the type I IFN pathway in peripheral blood, which supports IFN-α as a possible target for therapy in these patients. Finally, we found that PM and DM are associated with dysregulation of endothelial progenitor cell (EPC) phenotype and function that may be attributed, at least in part, to aberrant IL-18 and type I IFN pathways (paper IV).

In conclusion, this thesis confirms a role of the type I IFN system in myositis, especially in subgroups of patients, based on their autoantibody status, and implicates a relationship between the type I IFN system and endothelial disruption. Furthermore, smoking may be a trigger in the pathogenesis of IIM in genetic susceptible persons. However, the implication of our findings to disease prognosis and treatment remain to be determined.

LIST OF SCIENTIFIC PAPERS

I. A.Ghirardello, M.Rampudda, **L.Ekholm,** N.Bassi, E.Tarricone, S.Zampieri, M.Zen, G.A.Vattemi, I.E.Lundberg, A.Doria. Diagnostic performance and validation of autoantibody testing in myositis by a commercial line blot assay. *Rheumatology (Oxford) 2010;49(12):2370-4.*

II. H.Chinoy, S.Adimulam, F.Marriage, P.New, M.Vincze, E.Zilahi, A.Kapitány, A.Gyetvai, **L.Ekholm**, P.Novota, M.Remakova, P.Charles, N.J.McHugh, L.Padyukov, L.Alfredsson, J.Vencovsky, I.E. Lundberg, K.Danko, W.E.Ollier, R.G.Cooper. Interaction of *HLA-DRB1*03* and smoking for the development of anti-Jo1 antibodies in adult idiopathic inflammatory myopathies: a European-wide case study. *Ann Rheum Dis, 2012;71(6):961-5.*

III. **L.Ekholm***, S.Vosslamber*, A.Tjärnlund, T.D. de Jong, Z. Betteridge, N.McHugh, L.Plestilova, M.Klein, L.Padyukov, A.E.Voskuyl, I.E.M. Bultink, D.M.Pegtel, C.P.Mavragani, M.K.Crow, J.Vencovsky, I.E.Lundberg, C.L.Verweij. Autoantibody specificities and type I Interferon pathway activation in Idiopathic Inflammatory Myopathies. *Scand J Immunol. 2016;84(2):100-9.*

IV. **L.Ekholm***, J.M. Kahlenberg*, S.Barbasso Helmers, A.Tjärnlund, S. Yalavarthi, W.Zhao, N.Seto, Z.Betteridge, I.E.Lundberg, M.J.Kaplan. Dysfunction of endothelial progenitor cells is associated with the type I Interferon pathway in patients with polymyositis and dermatomyositis. *Rheumatology (Oxford) 2016;doi:10.1093/rheumatology/kew288 (E-pub ahead of print).*

*The authors contributed equally to the work

CONTENTS

LIST OF ABBREVIATIONS

ACR	American College of Rheumatology
ANA	Antinuclear antibody
ALT	Alanine aminotransferase
APC	Antigen-presenting cell
ASS	Antisynthetase syndrome
ARS	Aminoacyl-tRNA synthetases
AST	Aspartate aminotransferase
BAFF	B cell activating factor
BlyS	B lymphocyte stimulator
CAC	Circulating angiogenic cell
CAM	Cancer-associated myositis
CADM	Clinically amyopathic dermatomyositis
CCP	Cyclic citrullinated peptide
CEC	Circulating endothelial cell
CD	Cluster of differentiation
CPK	Creatine phosphokinase
CTD	Connective tissue disease
CVD	Cardiovascular disease
DC	Dendritic cell
DM	Dermatomyositis
DNA	Deoxyribonucleic acid
DVT	Deep venous thrombosis
EC	Endothelial cell
ELISA	Enzyme-linked immunosorbent assay
EMG	Electromyography
EPC	Endothelial progenitor cell
ER	Endoplasmic reticulum
FC	Fold change
HC	Healthy control
HLA	Human leucocyte antigen
IBM	Inclusion body myositis
IC	Immune complex

ICAM-1	Intracellular adhesion molecule-1
IFN	Interferon
IFI44	Interferon-induced protein 44
IFIT1	Interferon-induced protein with tetratricopeptide repeats 1
IFNAR	Type I Interferon receptor
IIM	Idiopathic inflammatory myopathies
ILD	Interstitial lung disease
IL	Interleukin
IMACS	International Myositis Assessment and Clinical Studies Group
IMNM	Immune-mediated necrotizing myopathy
IPP	Immunoprecipitation
IRG	Interferon-regulated gene
JDM	Juvenile dermatomyositis
LDH	Lactate dehydrogenase
MAA	Myositis-associated antibodies
MCTD	Mixed connective tissue disease
MHC	Major histocompatibility complex
MMT	Manual muscle test
MSA	Myositis-specific antibodies
MX-1	Myxovirus resistance-1
NET	Neutrophil extracellular trap
PRKR	Double-stranded RNA-activated protein kinase
RA	Rheumatoid arthritis
PBMC	Peripheral blood mononuclear cell
pDC	Plasmacytoid dendritic cell
PCR	Polymerase chain reaction
PE	Pulmonary embolism
PM	Polymyositis
RA	Rheumatoid arthritis
RCT	Randomized controlled trial
RF	Rheumatoid factor
RNA	Ribonucleic acid

RTX	Rituximab
SD	Standard deviation
SE	Shared epitope
SR	Sarcoplasmic reticulum
SSc	Systemic sclerosis
SLE	Systemic lupus erythematosus
SNP	Single nucleotide polymorphism
SLEDAI	Systemic lupus erythematosus disease activity index
T_H cell	T helper cell
TLR	Toll-like receptor
TNF	Tumor necrosis factor
UK	United Kingdom
VAS	Visual Analogue Scale
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelium growth factor

1 INTRODUCTION

1.1 IDIOPATHIC INFLAMMATORY MYOPATHIES

Idiopathic inflammatory myopathies (IIM), also known as myositis, are rare chronic autoimmune diseases, characterized clinically by proximal muscle weakness and histologically by inflammation in skeletal muscle tissue. The most common subgroups in adults, classified on the basis of clinical and histopathological features are: polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM) [1] and in children, juvenile DM (JDM). However, new subgroups have been recognized, such as immune-mediated necrotizing myopathy (IMNM) [2] and the antisyntethase syndrome (ASS), due to the discovery of new myositis-specific autoantibodies (MSAs), associated with these distinct clinical phenotypes [3]. The etiology of IIM is still unknown but environmental and genetic factors are believed to contribute to disease susceptibility. The treatment of IIM, consisting of high-dose glucocorticoids and immunomodulatory drugs, has limited effect. New treatments are needed, thus increased understanding of molecular disease mechanisms in IIM is required.

The first clinical description of PM was published by E. Wagner in 1886. Clinical manifestations described were stiffness, pain and limited motion of muscles, especially in the arm muscles and symmetrical edema. Lung affection and skin involvement were also described. PM had already been established at the time when DM was reported in 1891 by H. Unverricht. IBM was initially described by Chou in 1967 [4], but the term IBM was coined by Yunis and Samaha in 1971 [5].

This thesis concerns mainly adult patients with PM and DM.

1.1.1 Classification and diagnostic criteria for idiopathic inflammatory myopathies

1.1.1.1 Polymyositis and dermatomyositis

The most commonly used criteria for diagnostic purposes and classification of PM and DM were proposed by Bohan and Peter in 1975 [6, 7]. The criteria consists of five variables (Table 1).

The criteria of Bohan and Peter do not distinguish between IBM and PM.

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), alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LD) and aldolase
3. Electromyographic (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 met. The diagnosis of PM is *probable* when three criteria (not including skin rash) are met 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 diagnosis of DM is *probable* when three criteria (including skin rash) are met and *possible* when two criteria (including skin rash) are fulfilled.

1.1.1.2 Inclusion body myositis

Several diagnostic criterions for IBM have been proposed, first by Calabrese et al in 1987 [8], and then by Dalakas in 1991[1]. More recent and commonly used diagnostic criteria were proposed by Griggs et al in 1995 [9] (Table 2).

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 of 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.

New myositis classification criteria are currently under review by the EULAR and ACR criteria subcommittees, and thereafter these criteria will be submitted for publication. These new criteria are data driven, and based on clinical and histopathological data from patients and comparators from centres worldwide. A webcalculator has been designed to calculate a probability for the classification of myositis, based on the 16 variables included. Furthermore, the webcalculator will provide a subgroup of myositis if sufficient information is available [10].

1.1.2 Clinical manifestations and laboratory findings

1.1.2.1 Skeletal muscle

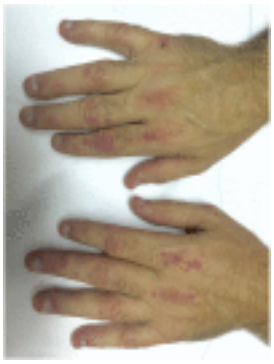
Most patients with PM or DM present with subacute or slowly progressive proximal muscle weakness of the shoulder and pelvic girdle, that is usually symmetric and is a major cause of disability in patients with myositis. Neck flexor muscle weakness can also occur. The patients experience difficulties in getting up from a chair, working with their arms above the head, walking stairs and difficulties in raising the head when supine. Besides muscle weakness, patients with PM or DM often suffer from decreased muscle endurance and muscle fatigue (exhaustion and tiredness). Muscle pain is less common than muscle weakness and fatigue. Typically the pain arises after a work load. Serum levels of muscle enzymes such as CPK, AST, ALT and LD are often increased.

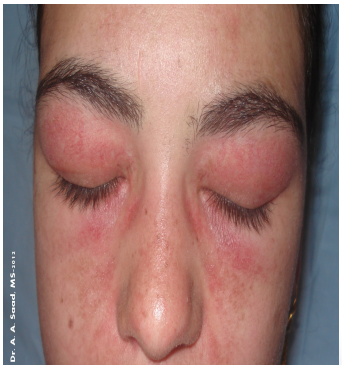
IBM patients differ clinically mainly through involvement not only of proximal thigh muscles but also of distal muscles. Involvement of the quadriceps muscle and of the deep finger flexors with weakness and muscle atrophy are often clues to diagnosis. In the beginning, it is not uncommon that IBM is misdiagnosed as treatment-resistant PM. Patients often present with falls because their knees collapse due to quadriceps muscle weakness, or they have difficulties in certain tasks, such as turning keys, owing to weakness of finger flexors. Neck flexors are often affected. Dysphagia may occur in 60 % of patients with IBM [11]. Disease progression is slow but steady and may resemble that of a muscle dystrophy. Most patients with IBM will require an assistive device, such as a walker or a wheelchair, within several years of onset. IBM patients rarely have muscle pain. Serum levels of CPK are usually only moderately elevated or may be normal.

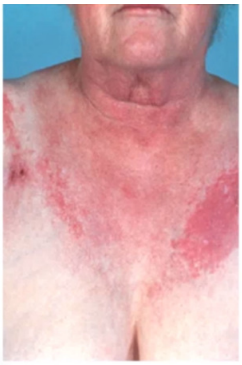
1.1.2.2 Extramuscular manifestations

Extramuscular manifestations are common in PM and DM, but less frequent in IBM. The most common extramuscular manifestations are from the skin, affecting patients with DM. In both PM and DM the lungs, heart, joints and gastrointestinal tract may be affected. This indicates that PM and DM are systemic inflammatory diseases. Lung involvement, such as interstitial lung disease (ILD) [12, 13], and cardiac involvement [14] is associated with worse prognosis of survival.

Cutaneous manifestations. In approximately 60 % of classical DM, the cutaneous and muscle changes appear at the same time, but in 30% of classical DM patients the skin symptoms precede the muscle symptoms by weeks or months. There are also approximately 10-20 % of the DM patients that do not develop muscle weakness, for 6 months of longer, referred to as clinically amyopathic DM (CADM) or "dermatomyositis sine myositis" [15]. Although the muscle symptoms may respond to treatment, the cutaneous lesions often persist despite treatment, which could indicate that the molecular pathways are different in muscles and skin [16]. Characteristic skin changes of DM are Gottron's papules (figure 1) and the heliotrope rash (figure 2) and they are considered pathognomonic manifestations of DM. Gottron's papules are scaly, erythematosus plaques located over the extensor side of joints e.g. metacarpophalangeal joints and proximal-and distal interphalangeal joints of the hands and found in 60-80% of DM patients. Gottron's sign is symmetrical, confluent, macular, violaceous erythema on the extensor side with a similar distribution as Gottron's papules. The heliotrope rash is a violaceous periorbital oedema and found in 50% of DM patients. Other less specific cutaneous findings are V-sign (figure 3), shawl-sign (figure 4), holster sign, calcinosis, nailfold capillary changes and mechanic's hands.







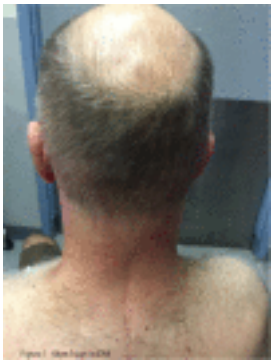


Figure 1. Gottron's papules**. Figure 2**. Heliotrope rash. **Figure 3.** V-sign. **Figure 4.** Shawl sign**.**

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Pulmonary manifestations. In PM and DM, pulmonary involvement is common and includes respiratory muscle weakness, aspiration pneumonia, interstitial ILD and fibrosis. ILD is recognized as a direct manifestation of PM and DM and occurs in 20-65% of all patients [17, 18] and has been reported to be a major cause of death in patients with PM and DM and contributes to morbidity [13, 19]. The clinical manifestations of ILD may vary from asymptomatic to rapidly progressive dyspnea and to fatal outcome. Cough and dyspnea are the most common symptoms of ILD [17, 20]

Cardiac involvement. Clinically manifest heart problems are relatively infrequent in patients with PM or DM. However, epidemiological studies on cardiovascular disease and IIM are rare. Subclinical cardiac involvement is not uncommon and the frequency varies depending on the methods used. Electrocardiography changes are the most common and include atrial and ventricular arrhythmias or alterations of the conducting system [21, 22]. Congestive heart failure and ischemic heart disease may also occur. In a recent study, traditional cardiovascular

risk factors and coronary artery calcification were commonly seen in patients with PM and DM. However, coronary artery calcification was not associated with PM and DM per se, but rather with age and smoking in these patients [23].

Gastrointestinal tract involvement. The most common gastrointestinal tract symptom is dysphagia, due to weakness of the tongue, pharynx or esophagus and disturbed esophageal motility. Esophageal dysfunction occurs in 15-50 % of patients.

Joint involvement. Arthritis is a common feature of myositis. It is often present at disease onset and may precede muscular symptoms of IIM [24]. The arthritis often present as symmetrical non-erosive polyarthritis, affecting mostly small joints of the hands, wrist and shoulders. Non-erosive polyarthritis is particularly common in the subset antisynthetase syndrome, a clinical phenotype characterized by the presence of myositis, antisynthetase autoantibodies, Raynaud's phenomenon, ILD and mechanic's hands and in overlap syndromes, where PM and DM occur in combination with one or several other rheumatic diseases such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA) and systemic sclerosis (SSc).

Malignancies. There is an association between malignancies and IIM, particularly for DM where up to 20% may have a malignancy around time of DM diagnosis. Therefore, recommendations are to let all newly diagnosed DM patients undergo age-appropriate malignancy screening. Many types of malignancies have been associated with IIMs, but they generally reflect those malignancies found in age-and sex-matched populations, e.g. breast cancer, lung-and colorectal cancer for Western country cohorts [25, 26]. However, different studies have shown significant differences in degree of cancer risk in patients with IIM and this can partly be attributed to the different definitions of cancer-associated myositis (CAM) between different studies. CAM is usually defined based on close relationship in time between onset of myositis and diagnosis of cancer [27]. The most often used definition of CAM is the onset of cancer within 3 years of the diagnosis of myositis, but this definition has not been uniformly adopted. In recent years, studies have shown that patients with certain MSAs (anti-TIF1-γ autoantibodies and anti-NXP-2 autoantibodies) are at increased risk of malignancy [28-30]. Some studies suggest that anti-HMGCR autoantibodies also have an increased risk of malignancy, but further studies are needed. For further information about these autoantibodies, see chapter 1.1.9.

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

1.1.3 Clinical outcome measures

1.1.3.1 IMACS disease activity core set measures

The International Myositis Assessment and Clinical Studies Group (IMACS), is an international consensus collaboration of health care providers and researchers with interest and experience in the IIMs. The goals of IMACS are to improve the lives of IIM patients through greater understanding of the pathogenesis of IIM and by discovering better therapies. IMACS has developed a three component core set outcome measure for adult and juvenile

myositis patients composed of myositis disease activity, myositis damage index and a health related quality of life assessment [31, 32]. In this thesis we used the first outcome measure, myositis disease activity. This core set consists of 6 domains:

- Physician's global disease activity assessment on a visual analogue scale (VAS) 0-100 mm
- Patient's global disease activity assessment on a VAS (0-100 mm)
- Manual muscle test (MMT) in 8 muscle groups on the dominant side (0-80)
- Health assessment questionnaire (HAQ) (0.00-3.00) [33]
- Assessment of serum levels of muscle associated enzymes (CPK, AST, ALT, LD)
- Extramuscular global assessment on a VAS (0-100 mm)

Improvement was defined according to IMACS, as 3 of any 6 measures improved by 20% or more, with no more than 2 worsened by 25% or more, which could not include MMT [32]. Worsening was defined by 30 % or more reduction in any 3 of 6 myositis disease activity core sets [34]. The changes at all time points were defined as changes compared to study start.

1.1.4 Treatment

The aims of the treatment are mainly to relieve the symptoms, slow disease progression, improve muscle function, reduce or prevent organ damage and make it possible for the patients to keep a good quality of life for as long as possible. Due to the lack of understanding of the disease mechanisms, there is no specific curing therapy for patients with IIM so far. Evidence based treatment for IIMs is extremely limited, as few trials have been conducted and due to the heterogeneity and rarity of these diseases, therefore the choice of treatment is often empirical.

1.1.4.1 Glucocorticoid therapy

Glucocorticoids are the first line of treatment in myositis patients and were introduced in the late 1940s, and had a major impact with reduced mortality and morbidity in myositis patients. There are only a few controlled trials of treatment in PM and DM patients and treatment recommendations are mainly based on clinical experience and open trials [35]. The exact mechanism of action of glucocorticoids are not fully understood, but involves mechanisms such as interference in binding or function of transcription factors involved in inflammatory pathways [36], thereby suppressing important cytokines such as tumor necrosis factor (TNFα) [37] and type I IFN [38, 39]. Glucocorticoids can also hinder the function of immune cells, such as proliferation, phagocytosis, antigen presentation and migration [40, 41]. In myositis, high doses of glucocorticoids, 0,75-1 mg/kg/day, are often given for several weeks, and then slowly tapered [35]. Many patients (70%) respond with improved muscle function, but few recover their former physical capacity [42] and side effects are common, such as hypertension, diabetes mellitus and osteoporosis [43, 44]. Less than 20% of the patients go into drug free remission. Most IBM patients do not respond to glucocorticoids or other immunosuppressive therapies [45].

1.1.4.2 Other therapies

Since glucocorticoids are not a sufficient treatment alone, many experts recommend combination therapies by including another immunosuppressive in order to improve the clinical response and reduce the need of glucocorticoids. The frequently used first line drugs are methotrexate at 15-20 mg/week or azathioprine at 2 mg/kg/day [46] . If these are without effect or not tolerated the recommended second line agents are: cyclosporine A, which has been found to be equally effective as methotrexate [47] or mycophenylate mofetil, as supported by case reports [48-50]. Patients (both PM and DM) refractory to first line, or above treatments, can be given intravenous immunoglobulins [35, 51, 52].

1.1.4.3 Biological therapies

The experience of treatment with biological agents in myositis is still limited.

Biological agents have been available for more than two decades and the first agents available were tumour necrosis factor (TNF)α-blockers. The mechanism of action is by TNFα-receptor blockage and by inhibitory binding to transmembrane TNFα, thereby reducing inflammation. The efficacy of TNFα-blockade in myositis has only been evaluated in case reports and case series with diverse outcomes. In an uncontrolled study including 13 patients with refractory myositis, 16 weeks of TNFα-blockade resulted in disease flares in some patients suggesting that TNFα is not a major molecular pathway in this phase of IIM disease [53].

A recent review of 48 studies, assessed 458 refractory myositis patients treated with Rituximab (RTX) until July 2015. RTX is a chimeric monoclonal antibody binding the CD20 antigen on the surface of B cells, resulting in depletion of CD20-positive B cells. In this review the rate of response to RTX was 78,3 % [54]. Furthermore, a study has shown that patients with autoantibodies (mainly anti-Jo-1 autoantibodies and anti-Mi-2 autoantibodies) were more likely to respond to RTX compared to autoantibody negative patients [54]. A very recent small (10 patients) randomized, double-blind, placebo-controlled clinical trial, showed that PM and DM patients who responded (defined by a consensus-driven definition of improvement) to RTX had higher levels of certain type I IFN gene expression (*STAT4, IFNAR2, IRF4, IFI44*) in muscle, and the responders had a greater decrease in the expression of these type I IFN genes than non-responders [55]. This suggests that the type I IFN signature is a biomarker of disease activity and also a predictor of therapeutic response.

Interleukin-1 receptor blockade, anakinra, has been studied in a mechanistic study with 15 patients during a 12 month follow up in patients with refractory PM/DM/IBM. Clinical improvement was noted in seven out of 15 patients, but the inflammation in muscle biopsies persisted [56]. One case reports has shown beneficial effect of anakinra in PM and DM [57]. Further studies are needed.

Interleukin-6 receptor blockade, Tocilizumab, has shown beneficial effect concerning disease activity in a few case reports [58, 59].

An agent blocking T-cell co-stimulation, abatacept, has shown beneficial effect on clinical symptoms and on CPK levels in three case reports [60-62]. A pilot study with randomized

treatment delayed-start with abatacept including 20 patients with PM and DM, showed improvement according to the IMACS definition of improvement. The improvement was also significant for MMT8 and health-related quality of life physical component in 50 % of the patients, but further investigations on the effects of abatacept in myositis are needed (Lundberg, I et al, Arthritis Rheum, ACR abstract no 2361, 2015).

IFN-blocking agents are on the market, and clinical trials with anti-IFNα monoclonal antibodies suggest beneficial effects in SLE [63, 64]. A randomised, double-blind, placebo controlled, dose-escalation, multicentre clinical trial was conducted to evaluate the effects of sifalimumab, an anti-IFN-α monoclonal antibody, in the blood and muscle of PM and DM patients by measuring neutralisation of a type I IFN gene signature following sifalimumab administration [65]. Both IFN-inducible transcripts (in muscle biopsies) and proteins (in peripheral blood) were suppressed following sifalimumab administration. Patients with 15% or greater improvement from baseline MMT scores showed greater neutralisation of the IFN gene signature than patients with less than 15% improvement in both blood and muscle. This trial included 51 patients, therefore these observations will require confirmation in a larger trial.

Sera from 48 of the PM/DM patients from this trial were also collected, to compare the effects of sifalimumab and placebo on protein markers, such as IL-18, IL-2 receptor antagonist (IL2RA) and B cell activating factor (BAFF). All of these proteins showed reduced levels after sifalimumab administration, but this was not seen in the placebo group. Furthermore, there was an overexpression of these proteins in those patients with an elevated type I IFN signature at baseline. Down-regulation of IL2RA correlated with improved MMT-8 in patients treated with sifalimumab [66].

In contrast to PM and DM, above-mentioned immunosuppressive drugs usually fail in IBM and currently there is no effective pharmacological treatment for IBM [67, 68]

1.1.4.4 Physical exercise

Earlier patients with PM and DM were advised against physical activity since it was believed to worsen the inflammation. The earliest two studies came in 1993, showing that exercise improved the muscle function, without clinically significant rises in serum levels of CPK [69, 70]. Further studies have shown that physical exercise improved strength, oxygen capacity, quality of life and that exercise is also safe to perform [71-73]. In a multicenter randomized controlled trial (RCT) from 2013, the effect of exercise in 23 established myositis patients was evaluated [74]. The trial revealed reduced disease activity in the exercise group compared to the control group. Another RCT, with recent onset PM and DM patients, supports the safety of exercise in these patients, i.e. no increased inflammation in muscle biopsies or increased CPK levels. However, no in between-group differences in effect of the exercise were shown [75]. In a pilot study, modest resistance exercise led to improved clinical performance and in muscle tissue there was a reduced expression of genes involved in inflammation and fibrosis [76]. Exercise has evolved as an important part of treatment of myositis patients. The amount of exercise and the intensity of the exercise program need to be adapted to each individual myositis patient. More studies to determine the optimal form of exercise in myositis patients are needed.

1.1.5 Epidemiology

PM and DM are rare disorders with an overall incidence of approximately 2 to 10 new cases per million persons and year [77]. The variations reflect ethnical, geographic and inclusion criteria used in the studies [78-80]. The peak of incidence in adults is said to be between 50 to 60 years of age, although other age groups may also be affected [81]. In a yet unpublished study from our research group, the estimated overall incidence of IIM was 11 per 1.000.000 and year and the prevalence 14 per 100.000 in Sweden (Svensson, J. et al, personal communication). Furthermore, the incidence increased with age and peaked at the 50-79 year age groups. However, in earlier studies the incidence pattern of IIM is said to be bimodal in PM and DM with an initial peak in childhood before the age of 20 and another adult peak between 45-69 years in adults [82, 83]. The incidence sex ratio is 2:1 (female:male) and the incidence race ratio is 3-4:1 (Afro-American:white) [79, 84].

In a systematic literature review from 2015 (46 articles published between 1966-2013 were reviewed), the prevalence of IIM ranged from 2-34 per 100.000 inhabitans [85]. The differences in prevalence may be explained by methodological issues or that there may be regional or ethnical differences. The ratio between PM and DM varies in different parts of the world, although PM and DM are present all over the world. A latitude gradient has been observed with DM being more common closer to the equator [86, 87].

There have been few population-based studies in IBM and the incidence ranges from 2.2-4.9 cases per million persons and year [88]. The mean age at diagnosis is more than 65 years and the incidence sex ratio is 1:2 (female:male) [89]. In a study from Norway from 2014, a prevalence of 35 per 1.000.000 was observed [90].

1.1.6 Etiology

The cause of IIM is yet unknown. However, there is most likely an interplay between genetic susceptibility and exposures to certain environmental factors. Multiple factors such as immunologic, genetic and environmental (e.g. infections, drugs, smoking, hormonal factors, ultraviolet (UV) radiation and stress) probably contribute to disease susceptibility.

1.1.6.1 Genetic association

The known genetic risk and protective factors in myositis are common alleles at polymorphic immune response loci and vary depending on clinical phenotype. Genetic risk factors include polymorphisms of many genes that regulate responses to environmental agents, particularly human leukocyte antigens (HLA), cytokines and immunoglobulin agents [91]. Certain polymorphic loci, such as *HLA DRB1*0301* and *Gm 2 23 5,13* are risk factors for all of the major clinical groups. *HLA DRB1*0301/*0501* and *DQA1*0501* on chromosome 6 are the strongest known risk factors for myositis in Caucasians especially in patients with ILD and/or anti-aminoacyl-tRNA synthetase autoantibodies [92, 93]. HLA genes are the most consistently identified genetic factors associated with autoimmune inflammatory diseases. HLA genes code for antigen-presenting molecules that play important regulatory roles in immune activation. Genetic associations are stronger with phenotypes defined by autoantibodies and by phenotypes defined by clinical features, than with myositis as a whole.

Risk genes for one phenotype are often protective for another, possibly explaining the mutual exclusivity of many myositis subgroups [94]. Genetic factors for myositis also vary by age of onset, ethnicity and environmental exposure group [95]. A genome-wide association study in DM has confirmed the importance of the major histocompatibility complex (MHC) complex but also suggested non-MHC genetic features overlapped with other autoimmune diseases [96]. In the largest genetic study to date in IIM (2566 myositis patient and 15 651 controls), it was confirmed that *PTPN22* and HLA, are the most strongly associated regions in IIM. Interestingly, *STAT4*, also reached a suggestive level of significance [97]. *STAT4* is a susceptibility locus for many autoimmune diseases, and has been associated with DM in the Japanese population and is an important transcription factor for many genes involved in T cell differentiation. A risk variant of *STAT4* has been shown to increase the sensitivity to IFNα in SLE-patients [98].

1.1.6.2 Infectious association

Viral infections that have been associated with IIMs are hepatitis C [99-101], parvovirus B19 [102], influenza viruses [103], and coxsackie virus [104, 105]. Retroviruses such as human T cell lymphotropic virus (HTLV-1) and human immunodeficiency virus (HIV) [106-108] have been associated with myositis, but muscle biopsies have failed to show viral nuclei acid [109]. It has also been reported that bacterial infections, such as staphylococcus aureus and streptococci [110], can invade muscle and cause an acute syndrome, pyomyositis. Parasites such as schistosoma and trichinella can be associated with myopathies. Elevated titers of antibodies of borrelia and toxoplasma, have been found in myositis patients, even though the antigens cannot be found histologically in the muscle tissue from myositis patients [111, 112]. Furthermore, mice infected with a parasite, trypanosoma cruzii, can develop a PM-like disease with similar histopatology [113]. In a recently accepted article by S. Barbasso Helmers et al, it was shown that subjects with preceding inflammatory lung disease, such as pneumonia, tuberculosis or sarcoidoisis tend to have an increased risk of developing myositis compared to those without. A history of gastrointestinal or respiratory symptoms, suggestive of infections, have been reported to precede the symptoms of muscle weakness and typical skin rash in JDM [114, 115].

1.1.6.3 Drug association

Many drugs, such as, statins, chlorochine, D-penicillamine and colchicines, can cause a myopathy which resembles myositis [116, 117]. However, the mechanism of drug-related myopathies is not understood, and whether they contribute to the development of IIM is unclear.

1.1.6.4 UV-light association

One environmental factor of interest in the pathogenesis of IIM is UV radiation. UV radiation has several immunomodulatory effects, such as triggering of cytokine production [118], regulation of surface expression of adhesion molecules [119], affecting cellular mitosis [120] and by inducing apoptotic cell death [121]. In DM, muscle and cutaneous manifestations have been shown to relapse more often in summer, whereas PM patients relapses was more evenly distributed during all four seasons, although lowest in summer [122]. DM is also more

frequent in countries around the equator than in countries on other latitudes [86, 87], supporting a role of UV radiation as an environmental factor that could contribute to disease onset. An association between UV radiation and anti-Mi-2 autoantibodies has also been shown [86]. Low serum levels of vitamin D have been reported in several autoimmune disorders, e.g. SLE [123], RA [124] and also in adult myositis [125], which suggest that vitamin D deficiency may be a risk factor for the development of autoimmune diseases including IIM.

1.1.6.5 Smoking

Tobacco smoking is a risk factor for several rheumatic diseases, such as SLE, RA and SSc. Smoking also affects both the outcome and course of rheumatic diseases. An association between smoking and dsDNA autoantibody production has been reported [126]. Furthermore, an association between smoking and an increased disease activity in SLE has been shown [127]. Smoking can predict the first cardiovascular event in SLE patients [128]. Tobacco smoking is a well established risk factor for the development of RA associated with rheumatoid factor (RF) positive but not with RF negative disease [129-131]. Tobacco smoking has also been associated with anti-cyclic citrullinated antibody (anti-CCP) positive but not anti-CCP negative RA [132]. Furthermore, a gene-environment interaction between smoking and *HLA-DR shared epitope (SE)* genes was present for the anti-CCP positive but not anti-CCP negative RA patients [132]. In affected subjects, exposure to tobacco smoking has also been associated with several measures of disease severity such as the presence of radiographic erosions, nodules, pulmonary disease and anti-RF-and anti-CCP autoantibodies [132-134]. Tobacco smoking increases the risk of digital ischemia and increases the severity of Raynaud's phenomenon in SSc [135], and also increases the risk of atherosclerosis [136]. The effects of smoking on the immune system are complex and not fully understood. Several plausible hypotheses have been presented. Genetic susceptibility certainly may have a role. Smoking can cause tissue damage and increase apoptosis, induce inflammation and have an immunosuppressive effect [137].

Limited data are available on smoking in patients with IIM. In an abstract to American college of Rheumatology (ACR) from Schiffenbauer A. et al in 2015, data suggest that smoking in PM and DM Caucasian patients is a risk factor for developing ILD, anti-Jo-1 autoantibodies and antisyntethase antibodies. In contrast, this was not seen in African/American PM/DM patients. This suggests that smoking can modulate serological and clinical status between different racial populations. The role of tobacco smoking for development of IIM has not been fully investigated, therefore this was something we wanted to study further (paper II).

1.1.7 Pathogenesis

Although IIMs were first described more than a century ago, many questions concerning the pathogenesis remain unclear. Muscle weakness, the most prominent symptom, is shared by all subsets of IIM, suggesting that some pathogenic mechanisms are shared. There are however some discrepancies, PM and DM often present with proximal muscle weakness and decreased endurance in proximal muscles, often without muscle atrophy, whereas in IBM the

weakness also involves distal muscle groups, with atrophy of thigh and finger flexor muscles. This suggests that some pathogenic mechanisms may differ between the subsets.

The presence of frequently detected autoantibodies and the presence of T cells in muscle tissue in a majority of patients with myositis, indicate that immune mechanisms are involved, and that both T and B cells may have a pathogenic role in these diseases.

Three major effector mechanisms have been proposed to be important for development of chronic muscle inflammation, muscle weakness and muscle fatigue.

- direct effect of infiltrating leukocytes, mainly T cells and macrophages on muscle cells (cytotoxicity) [138, 139], promoting the production of autoantibodies from B-cells.
- indirect effects of molecules from the immune system [cytokines, for instance interferons (IFNs) and others] on muscle funtion and metabolism.
- involvement of microvessels and a disturbed microcirculation, leading to metabolic disturbances and reduced muscle function.

T cells

Due to prominent findings of cluster of differentiation (CD)4+ T cells and CD8+ T cells in muscle tissue of myositis patients, a role of T cells in disease mechanisms of myositis has been suggested. T cells may have direct myotoxic effects on muscle fibers [140], leading to necrosis and regeneration of muscle fibers promoting the expression of specific muscle autoantigens in regenerating muscle cells which may induce production of myositis related autoantibodies. T cells may also have indirect effects through production of molecules, such as cytokines, which can affect the muscle fiber phenotype, including the characteristic MHC class I expression on muscle fibers in myositis patients. In DM, the inflammatory infiltrate involves primarily CD4+ T cells, macrophages and a small number of B cells and plasma cells [138]. It localizes mainly in the perivascular and perimysial level. A pivotal role may be played by complement membranolytic attack complex that may be involved in damage of endothelial cells (ECs) with vascular injury and subsequent muscle inflammation [139, 141]. PM and IBM, however, are characterized by an endomysial mononuclear inflammatory infiltrate complex including CD8+ T cells, CD4+ T cells, macrophages and myeloid dendritic cells. CD8+ T cells and CD4+ T cells, may surround and invade non-necrotic muscle fibers that aberrantly express MHC class I, and may cause a perforin-mediated cytotoxic injury [142, 143]. In another subset of myositis, called IMNM, muscle fiber necrosis is seen as the dominating histopathological feature, but with few or no inflammatory infiltrates. The mechanisms for the fiber necrosis has not be clarified. However, the immune system is suggested to be involved, due to recently detected autoantibodies in this disease subset [2, 144].

The $CD28^{null}$ T cells, a subset of T cells, are another interesting observation in the pathogenesis of IIM. These are cells that lack the co-stimulatory molecule CD28, but have expression of other receptors associated with natural killer cells. They further have proinflammatory and cytotoxic features and are apoptosis resistant. It has been shown that the muscle-infiltrating T cells are mostly CD8+ $CD28^{null}$ T cells and CD4+ $CD28^{null}$ T cells in

PM and DM [145, 146]. These findings suggest that the $CD28^{null}$ T cells are capable of inducing persistent inflammation and are capable of attacking muscle fibres.

The role of the inflammatory cell infiltrates in causing muscle weakness is still unclear. From clinical observations it has been suggested that there is no correlation between the degree of inflammatory infiltrates and degree of muscle weakness [147-149]. This suggests that other mechanisms than T cell mediated cytotoxicity may contribute to muscle weakness. This could be by effects from molecules in the inflammatory milieu in the muscle. For instance this has been shown for the cytokine, TNF, which may affect muscle fiber contractility inducing muscle weakness [150].

B cells

High serum levels of BAFF, also known as B lymphocyte stimulator (BLyS), in myositis patients [151, 152], presence of B cells and plasma cells in muscle tissue [153] and autoantibodies in peripheral blood, support a role of these cells in the pathogenesis of myositis. This role is also supported by the beneficial effect of B cell depleting therapy by RTX, at least in a subgroup of patients with PM or DM.

MHC class I

The muscle cells in myositis patients frequently express the MHC class I antigen, independent of inflammatory cell infiltrates. This has been seen in early and late chronic phases of the disease [154]. This is a well known myopathological feature and can be used as a support for IIM in the diagnostic work-up in patients with muscle weakness [155]. MHC class I antigen is normally not expressed on differentiated muscle fibers. A role of MHC class I antigens 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 and histological and immunological features similar to human myositis patients [156]. MHC expression turns the muscle fibres into active participants in the cellular immune reactions, as it allows them to present antigen and activate T cells. One of the strongest inducers of MHC class I expression are the type I IFNs [157]. However, if muscle cells with MHC class I upregulation, present antigens to naive T cells to initiate a response against muscle antigens is not known.

Dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells that are involved in both the adaptive and innate immune systems. Their activation in peripheral tissue and lymphoid tissue leads to activation and migration of CD4+ T cells and CD8+ T cells and of B cells. DCs are of two types: myeloid (the conventional DCs) and plasmacytoid DCs (pDCs). The myeloid DCs play a key role in humoral immunity with activation of B cells and autoantibody production. The plasmacytoid DCs (pDCs) produce type I interferon (IFN) and have a key role in the innate immunity. For further information about IFNs, see chapter 1.1.8.

HMGB1

High mobility group box 1 (HMGB1) is a ubiquitous non-histone protein present in the nucleus of cells. It binds to DNA and helps regulate transcription and chromatin structure. When cells die HMGB1 can translocate from the nucleus to the cytosol and be released out of the cells and mediate chemotaxis, cytokine induction, tissue damage and affect fiber contractility. Recent studies have reported cytoplasmic HMGB1 expression in inflammatory cells, endothelial cells and also in muscle fibers of patients with myositis, suggesting a possible role of HMGB1 in the pathogenesis of myositis [158, 159]. HMGB1 has been found to reversibly induce MHC class I expression on muscle fibres and irreversibly impair Ca^{2+} release from the sarcoplasmic reticulum (SR) during induction of fatigue, indicating a direct effect of HMGB1 on generation of muscle force [159].

Cytokines

Several cytokines, such as interleukin (IL)-1 and IL-15 have been studied, in the disease pathogenesis. In a study with 15 patients with refractory myositis, 7 responded to IL-1 receptor blocking, with improvement according the IMACS definition of improvement. However, no difference in IL-1 expression was seen in muscle tissue, before and after treatment with IL-1 receptor blocking, questioning the role of IL-1 in the pathogenesis of myositis [56]. However, larger studies are needed. In another study, myositis patients with a high number of IL-15 expressing cells in muscle tissue had less improvement in muscle performance after treatment with immunosuppressive agents for a median of 8 months and this persisted after 5 years [160]. This might suggest that IL-15 may have a role in chronic muscle inflammation and IL-15 may be a possible new target for treatment in subgroups of PM/DM. T helper (T_H) 17 cell, preferentially produces IL-17. The differentiation of T_H17 cells depend on IL-6, IL-23 and transforming growth factor (TGF)-β. All these cytokines are locally produced by macrophages, monocytes, endothelial cells and dendritic cells. Serum levels of IL-15 and IL-17 have been shown to be higher in IIM patients than in healthy controls (HCs), and a strong correlation between IL-15 and IL-17 in DM was shown, suggesting a role of these cytokines in the pathogenesis of IIM [161].

IL-18 is a T_H1 inflammatory cytokine, mainly produced by antigen presenting cells (APCs). It is over-expressed in endomysial areas in PM and in perivascular areas in DM [162]. Serum IL-18 levels have been shown to be elevated in patients with DM and was associated with ILD complication [163]. Furthermore, a study has shown high serum levels of IL-18 in both PM and DM [162]. In addition, IL-18 was overexpressed in muscle biopsies from patients with PM and DM. ECs, smooth muscle cells (i.e. cells of the arterial wall) and CD8+ T cells expressed a high content of IL-18 receptors. These findings suggest a dysregulation of the IL-18/IL-18 receptor pathway, which may be a pathogenic mechanism in IIMs. Also, in patients with SLE, IL-18 has been reported with elevated serum levels. The IL-18 levels correlated with disease severity and the presence of lupus nephritis [164, 165]. The inflammasome is a cytosolic multiprotein platform, whose assembly results in rapid activation of caspase-1, the enzyme responsible for generation of the active forms of IL-1β and IL-18. Type I IFN have been proposed to have a regulatory role in inflammasome activity in SLE patients, because the IFN-α responsive inflammasome scaffold absent in melanoma-2 (AIM2) (a cytoplasmic receptor for dsDNA) activates caspase-1. Caspase-1 also appears to be regulated by IFNs,

because the IFN-regulated transcription factor IRF-1 is essential for caspase-1 transcription and activation of IL-18 in response to IL-12 administration [166, 167]. IL-18 levels have been correlated with intima media thickness and increased vascular stiffness in men without evidence of coronary artery disease, suggesting a role of IL-18 in early stages of atherosclerosis development [168]. If IL-18 has a role in the pathogenesis of myositis is still unclear, and will need further investigations. A potential role of IL-18 and type I IFNs in myositis is discussed in paper IV.

Vasculopatic mechanisms

Another pathogenic mechanisms in IIM patients could possibly be explained by involvement of microvessels. A decreased number of capillaries in muscle biopsies has been reported in DM patients compared to healthy individuals, even in early cases without detectable inflammatory infiltrates [169]. A recent study from our research group has shown that a loss of capillaries is seen in early disease without inflammatory infiltrates in both PM and DM patients [170]. This finding correlated to an upregulated expression of the angiogenic factor, vascular endothelium growth factor (VEGF) in muscle fibres, suggesting that local muscle hypoxia could be a contributing factor to the impaired muscle function seen in myositis patients [170]. It has been demonstrated that ECs of the microvessels in muscle tissue from PM and DM patients are morphologically changed and express activation markers such as the proinflammatory cytokine IL-1α, the intercellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1. The phenotypic changes of the capillaries were related to the clinical signs of muscle weakness indicating a role of the microvasculature in the mechanisms causing the clinical symptoms [148].

Non-immune mechanisms

The degree of inflammation in skeletal muscle is not always correlated to disease severity. Some patients do not respond to anti-inflammatory treatment and the clinical disease can progress, even though inflammation declines [147-149, 171]. This suggests a potential role for non-immune mechanisms in the pathogenesis of IIM, such as hypoxia, endoplasmic reticulum (ER) stress and autophagy. Hypoxia can induce muscle weakness through reduced levels of phosphocreatine and ATP and production of adhesion molecules and proinflammatory cytokines and HMGB1 [172]. Hypoxia is also strictly connected with ER stress and/or impaired autophagy. ER stress is generated when accumulation of unfolded or misfolded proteins accumulate in the ER. Since several proteins that bind and/or regulate calcium homeostasis exist in the SR, a specialized form of ER, unfolded or misfolded proteins can cause muscle fatigue, by reducing Ca^{2+} release from SR [173]. A potential inducer of ER stress in IIM is the upregulation of MHC class I in muscle fibers. The ER stress can result in NF-κB activation that promotes the transcription of cytokine genes and thereby induces a self-sustaining inflammatory response that contributes to muscle damage [174].

Autophagy is a very important process for removing unnecessary cellular material by degradation to lysosomes. If this does not work properly, it can induce autophagic celldeath. This mechanisms may be particular important for IBM [175].

There are several ways to learn about the pathogenic mechanisms in human diseases, through studies on animal models, through *in vitro* studies of tissue or cell cultures and from studies of the disease course and the target organs during therapies targeting distinct mechanisms or molecules. Although investigators have previously developed several spontaneous (genetic) and induced animal models possessing some features of IIM [176], none of these models fully reproduces human disease. A very exciting antigen-induced model of IIM to emerge over the last several years is based on immunization with the murine form of histidyl-tRNA synthetase (Jo-1). Mice immunized with murine Jo-1 developed a combination of inflammation of muscle and lungs, that replicates features of the human anti-synthetase syndrome [177].

It has become increasingly important that the pathogenesis of IIMs is explored in detail so that more targeted and specific therapies, that are likely to be more effective and safe than the therapies that we have today, may be developed.

In the following parts the autoantibodies, the inflammatory molecules (type I IFNs), the autoantibodies and endothelial progenitor cells (EPCs)/ECs, that were investigated in this thesis, are introduced in more detail regarding their basic properties and potential role in the pathogenesis of myositis.

1.1.8 Interferons

In England, the Briton Alick Isaacs and the Swiss Jean Lindemann, first characterized IFN in 1957, to signify a protein, that was originally defined by its capacity to interfere with viral replication in cell cultures [178]. They noticed that cells already infected with a virus appeared to be resistant to infection by other viruses for a certain period of time. The first infection was said to "interfere" with (inhibit) the second. The protein isolated from these cell cultures was therefore given the name interferon (IFN).

The IFNs are a group of non-antibody proteins, the so called cytokines, that act as mediators between cells. TNFα and ILs are other examples of cytokines. Cytokines in general are typically not stored as preformed proteins, rather their synthesis is initiated by gene transcription and their mRNAs are short lived, when immune response is needed. Therefore, the cellular response to cytokines is slow (hours).

There are three types of IFNs, type I, type II and type III, based on their structural features, receptor usage and biological activities. The type I IFNs are the largest family that can be divided into 5 classes (IFNα, -β, -ω, -ε, and κ), of which IFN-alpha (IFNα) can be further divided into 12 subtypes encoded by 13 genes clustered on chromosome 9. The IFNα is a protein consisting of 165 amino acids without a glucose unit that is maintained in its three-dimensional loop structure by two disulfide bridges. The type I IFNs bind to the same heterodimeric type I IFN receptor (IFNAR), consisting of two membrane spanning polypeptide chains, IFNAR1 and IFNAR2. The type II IFN, which sole member is IFN-gamma (IFNγ), binds to the IFNγ receptor, IFNGR, a receptor complex, which consists of IFNGR1 and IFNGR2 chains. Type III IFNs (discovered in 2003) consists of IFN-lambda (IFN-λ1, λ2, λ3), also known as interleukin-28A/28B/29, and mediates their biological activities through the receptor, IFNλR1. Type I, II and type III IFNs activate the same

intracellular signaling pathway (of which the Janus kinase signal transducer and activator of transcription (STAT) pathway are the best characterized [179] and share many of the same biological activities. However, IFNARs are broadly expressed on most celltypes, whereas the IFNλ- receptors are largely restricted to cells of epithelial origin.

Most types of cells can produce small amounts of type I IFN, but the principal type I IFN producer is a phenotypically and functionally distinct subset of DCs, the pDCs. The pDCs represent 0.2-0.8% of peripheral blood mononuclear cells (PBMCs) in both humans and mice [180]. They selectively express toll-like receptors (TLRs), such as TLR7 and TLR9, and are specialized in rapidly secreting massive amounts of type I IFN following viral stimulation. pDCs can promote the function of natural killer cells, B cells, T cells, and myeloid DCs during an antiviral immune response. At a later stage of viral infection, pDCs differentiate into a unique type of mature dendritic cell, which directly regulates the function of T cells and thus links innate and adaptive immune responses. The type I IFN genes are strictly regulated and normally almost no IFNα production can be detected in healthy individuals.

The type I IFN is rapidly produced during viral invasion and constitutes our major defense system against viral infections. Furthermore, bacterial products [181] and autoantibody-nucleic acid complexes have been shown to trigger pDCs production of IFNα [182]. Type I IFNs combat viruses both directly by inhibiting virus replication in the cells and indirectly by stimulating the innate and adaptive immune responses. The direct antiviral activity of type I IFNs is exerted by a number of different mechanisms, e.g. blockage of viral entry into the cell, control of viral transcription, cleavage of RNA, and preventing translation. In addition to the direct effects, type I IFN induces general activation of immune cells. For instance, type I IFN induces DC maturation and activation, with increased expression of chemokines and chemokine receptors, as well as co-stimulatory molecules [183]. IFNα has been shown to have many effects on the endothelium. IFNα has been shown to mediate dysfunction in EPC/circulating angiogenic cell (CAC) differentiation in SLE [184]. From the cancer literature it has been shown that IFNα is a potent antiangiogenic factor [185], inhibits EC invasion and migration and downregulates proangiogenic molecules [186]. Furthermore, type I IFNs have an inhibitory effect on bone marrow precursors [187]. However, the exact pathways in which IFNα inhibits angiogenesis are not well known. Type I IFNs are known to induce MHC class I expression. Furthermore, the co-localization of muscle fibres with expression of MHC class I and infiltrating immune cells expressing IFNα/β/γ proteins that has been described [157], strengthens the view that type I IFNs indeed are the inducers of MHC class I expression on muscle fibres, and that possibly IFNγ acts as an additional stimulator. However, discordant results have been published in regards to IFNγ expression in IIM. In regards to IFNβ, less data is currently available. Another role of type I IFNs is to promote the development of helper T cells along the Th1 pathway, and cytotoxic T cells are stimulated by type I IFNs. Type I IFNs can also cause B cell activation, differentiation, antibody production, and Ig isotype class switching. They also influence cell communication and are involved in the breakdown of unstable cellular messenger RNA, which plays an important role in the retardation of the uncontrolled division of tumor cells. IFNα is therefore used against malignant diseases, such as malignant melanoma and lymphomas.

Another role of the type I IFNs that has been suggested in SLE, is the interaction with neutrophils and the forming of neutrophil extracellular traps (NETs). In SLE there is a relationship between IFNα and NETosis. NETosis is a form of death in neutrophils in which nuclear material is extruded from the cell forming NETs to kill extracellular bacteria, viruses and parasites [188]. NETs contain decondensed DNA chromatin, granular and cytoplasmic proteins and histones. A variety of stimuli can trigger NETosis such as immune complexes, pathogens, IFNα and endothelial cells [189, 190]. NETs can activate pDCs to produce IFNα [189], and IFNα in turn can stimulate additional NETosis. Some components of NETs can directly injure ECs [191]. However, the removal of NETs should be closely regulated and failure to do so can cause tissue damage and may provide a new source of autoantigens and may be involved in SLE pathogenesis, and perhaps also in IIM which needs to be addressed in future studies. One first report concerning NETs formation in myositis has been performed [192]. Compared with healthy controls (HCs), PM and DM patients exhibited a significantly enhanced capacity for inducing NETs and NETs could not be degraded completely because of decreased DNase I activity in PM and DM patients, especially in patients with ILD. These findings suggest that abnormal regulation of NETs may be involved in the pathogenesis of PM and DM. Glucocorticoids seem to improve DNase I activity. Although NETs formation is excessive *in vitro*, NETs formation *in vivo* remains unknown in PM and DM.

In recent years, genomic studies have shown marked overexpression of type I IFN inducible genes in the peripheral blood of patients with different autoimmune diseases such as SLE, DM, PM, multiple sclerosis, RA, SSc and Sjögren's syndrome [193-197]. In SLE and SSc, immune complex (IC)-containing DNA or RNA are taken up by pDCs through Fc-receptors, where triggering of certain intracellular TLRs initiate down-stream signaling, which eventually results in transcription of type I IFN genes [198]. ICs containing DNA and RNA have the capacity to activate pDCs and RNA-containing ICs that trigger TLR7 seem to be especially potent as IFNα inducers [199]. In SLE there is a correlation between serum IFNα activity and presence of autoantibodies against RNA-binding proteins [200]. For further associations between type I IFNs and SLE and endothelial cells, see chapter 1.1.10. An activated type I IFN pathway has also been proposed to be involved in the pathogenesis of IIM, particularly in the DM subgroup, but the mechanisms driving the type I IFN pathway has not been clarified. Early evidence of an overactived innate immune system in DM was seen in 2005, when a strong IFN-inducible gene expression was detected in muscle and skin tissues of patients with DM [194]. In the following years, several groups have studied the IFN signature in muscle tissue and in peripheral blood in patients with myositis and from later reports the IFN signature has been linked to both DM and PM [193, 194, 201]. Case reports on development of PM/DM during IFNα/β therapy have been published [202, 203]. In one study, serum levels of IFNβ, measured by enzyme-linked immunosorbent assay (ELISA), but not IFNα, where shown highly associated with DM [204]. Another study showed increased IFNβ transcripts in PM and DM muscle [205]. In JDM a correlation was shown between presence of autoantibodies against RNA-containing autoantigens and serum IFNα activity and the capacity to generate interferonogenic ICs [206]. Upregulation of IFN-inducible genes has also been observed in the skin of patients with DM [207, 208] and the presence of pDCs within the epidermis of DM skin suggests that the IFN-mediated processes may take place in the skin, as well as in the muscles of these patients by mechanisms other than the IC-

mediated mechanisms discussed above [209]. Furthermore, increased numbers of pDCs in muscle biopsies from patients with DM and JDM have been shown [210, 211]. However, there are contradictory results regarding correlation between the IFN signature in peripheral blood cells of IIM patients and disease activity [193, 212-214], which could be reflected by the fact that different IFN assays have been used in the different studies and that the definition of disease activity differed between the studies. The inducer of the type I IFN system in patients with IIM has not been clarified, but there is a suggestion of viral infections although this has not been confirmed. A subgroup of patients with PM and DM with anti-Jo-1 or anti-Ro52/60 autoantibodies sera together with RNA, induced IFNα production in peripheral blood cells [215], suggesting that these antibodies may serve as endogenous IFN inducers in pDCs similarly as has been reported for antibodies against RNA binding proteins in patients with SLE. These observations also suggest that there may be a role for the type I IFN system not only in DM, but also in other subtypes of myositis, where patients have antibodies against RNA or RNA-binding proteins and that autoantibodies may have a role in driving the type I IFN pathway in subsets of patients with IIM.

1.1.9 Autoantibodies

One of the most important characteristics of IIM and of other systemic autoimmune diseases, is the immune response to self-antigens, manifested by the production of autoantibodies that target either nuclear or cytoplasmic components of the cell, involved in gene transcription, protein translocation and antiviral responses. Whether autoantibodies are simply epiphenomena or directly linked to pathogenesis remains unclear. In many autoimmune diseases, autoantibodies may be present several years before disease onset [216, 217]. This has also been reported in occasional individuals with IIM, indicating a role of autoantibodies in the initiation of the disease [218]. However, the mechanism for this is yet unknown. In myositis, autoantibodies may contribute to the disease mechanism, by direct damage to the cell that carries the antigen, via antibody-dependent cellular cytotoxicitiy or complement activation. Autoantibodies can also form ICs with the autoantigen and contribute to inflammation via activation of Fc-receptor-expressing cells or via the complement system. Over the past 40 years several autoantibodies have been discovered that are associated with IIM. In recent years it has become even more apparent that autoantibodies have a role in distinguishing between subtypes of myositis patients and clinico-serological classifications have been proposed [219]. Myositis autoantibodies can be categorized into myositis-specific autoantibodies (MSAs) and myositis-associated antibodies (MAAs). MSAs are exclusively found in IIM and correlate with distinct clinical manifestations and genotypes [92, 219]. The most prevalent MSA is the anti-Jo-1 autoantibody (antihistidyl-tRNA synthetase). Eight anti-aminoacyl-tRNA syntethase autoantibodies (ARSs) are known to date. The other ARSs identified are anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, anti-KS, anti-Ha (YRS) and anti-Zo. Together with the other traditional MSAs, these antibodies can be identified in 40-50% of adult myositis patients. Other traditional MSAs are anti-SRP and anti-Mi-2. More recently, a number of novel MSAs have been identified, including anti-TIF1-γ (p155/140) anti-SAE, anti-MDA5 (CADM-140), anti-HMGCR and anti-NXP-2. When including these novel MSAs, it is now possible to identify a positive MSA or MAA in about 80 % with PM and DM [3]. In contrast, MAAs (i.e. anti-Ro52, anti-Ro60, anti-PM/Scl, anti-La, anti-U1RNP and

anti-Ku) are commonly found in patients who have features of other connective tissue disease (CTD), particular in overlap with systemic sclerosis. For target autoantigens and frequencies of MSAs/MAAs see table 3. For detection procedures see chapter 4.2.

Table 3 | Autoantibodies in myositis, their target antigens and frequencies.

Autoantibodies	Nature of target antigens	Frequency in IIM (%)
MYOSITIS-SPECIFIC AUTOANTIBODIES (MSAs)		
anti-ARS		
anti-Jo-1	Histidyl-tRNA synthetase	15-20
anti-PL-7	Threonyl-tRNA synthetase	5-10
anti-PL-12	Alanyl-tRNA synthetase	<5
anti-EJ	Glycyl-tRNA synthetase	5-10
anti-OJ	Isoleucyl-tRNA synthetase	<5
anti-KS	Asparaginyl-tRNA synthetase	<5
anti-Zo	Phenylalanyl-tRNA synthetase	<1
anti-YRS	Tyrosyl-tRNA synthetase	<1
anti-SRP	Signal recognition particle (SRP)	<4
anti-Mi-2	Mi-2α/Mi-2β helicase family proteins (Mi-2)	5-10
anti-MDA5	Melanoma differentiation-associated gene 5 (MDA5)	13-35
anti-TIF1-γ	Transcriptional intermediary factor 1γ (TIFI-1γ)	20
anti-NXP-2	Nuclear matrix protein (NXP-2)	17
anti-SAE	Small ubiquitin-like modifier activating enzyme (SAE)	8
anti-HMGCR	HMG-CoA reductase	5-7
MYOSITIS-ASSOCIATED AUTOANTIBODIES (MAAs)		
anti-U1RNP	U1 small nuclear RNP	10
anti-Ro52/Ro60	Ro-52/TRIM21 and Ro-60 proteins	≥35
anti-Ku	DNA-PK regulatory subunit	1-3
anti-PM/Scl	Nucleolar protein complex	4-12

ARS, aminoacyl-tRNA synthetases; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; DNA-PK, DNA-dependent protein kinase.

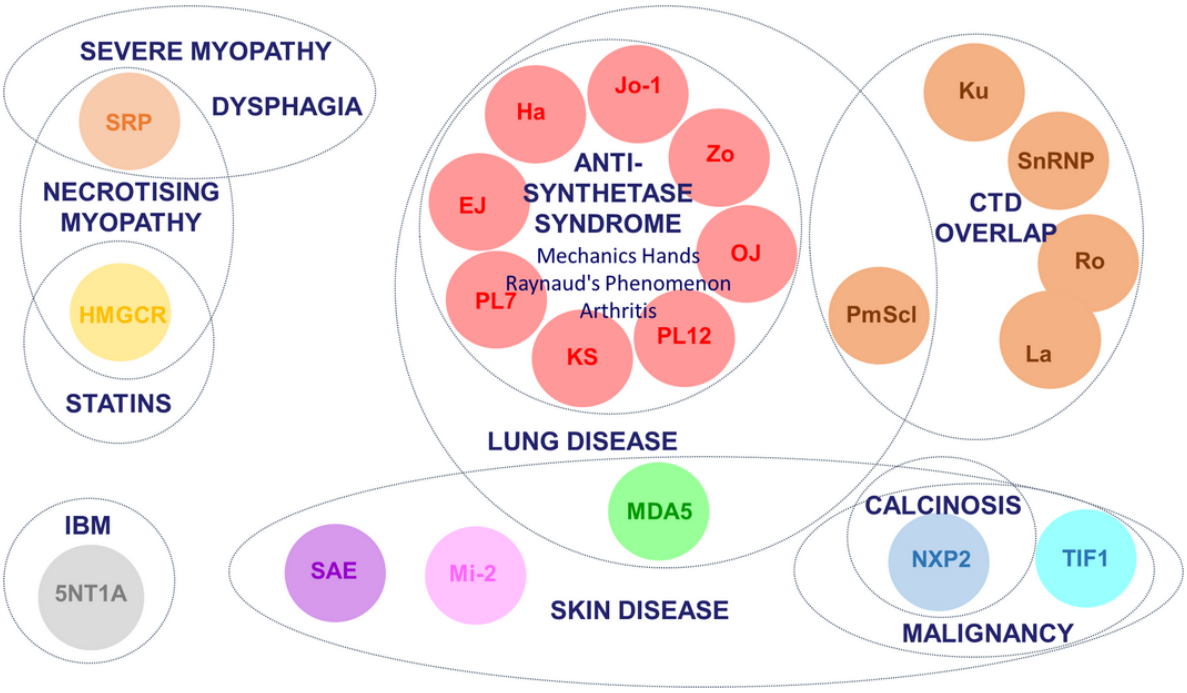

Figure 5. Myositis autoantibodies and their key clinical associations. IBM, inclusion body myositis; CTD, connective tissue disease; SRP, signal recognition particle; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; TIF1, transcription intermediary factor 1; NXP-2, nuclear matrix protein 2; MDA5, melanoma differentiation-associated gene 5; SAE, small ubiquitin-like modifier activating enzyme; 5NT1A, cytosolic 5'nucleotidase 1A; Mi-2, nucleosome-remodelling deacetylase complex; Jo-1, histidyl-tRNA synthetase; PL7, threonyl-tRNA synthetase; PL12, alanyl-tRNA synthetase; OJ, isoleucyl-tRNA synthetases; EJ, glycyl-tRNA synthetase; KS, asparaginyl-tRNA sythetase; Zo, phenylalanyl-tRNA synthetase, Ha; tyrosyl-tRNA synthetase; snRNP, small nuclear ribonucleic protein. *Reprinted with permission from John Wiley & Sons, Inc: Betteridge Z, McHugh N. Myositis-specific autoantibodies: an important tool to support diagnosis of myositis. J Intern Med 2016;280(1):8-23.*

1.1.9.1 Myositis-specific autoantibodies (MSAs)

MSAs are disease serological markers, nearly always mutually exclusive and closely associated with distinct disease subsets, making MSAs important diagnostic biomarkers (figure 5). Their detection in the early phase of the disease might be helpful in the prediction of clinical course and disease prognosis.

Anti-aminoacyl-tRNA syntethase autoantibodies (ARSs)

The ARSs target the aminoacyl-tRNA syntethases which catalyze the binding of amino acids to the corresponding tRNAs, so that the amino acid can be incorporated into a growing peptide chain. Each amino acid has a separate aminoacyl-tRNA synthethase and autoantibodies targeting 8 of the 20 aminoacyl-tRNA synthetases have been identified. The most common of these is the anti-Jo-1 antibody (anti-histidyl-tRNA syntetase), which is found in about 20% of adult IIM patients. The remaining antisynthetase autoantibodies: anti-PL-12 (alanyl), anti-PL-7 (threonyl), anti-EJ (glycyl), anti-OJ (isoleucyl), anti-KS (asparginyl), and the more recently identified anti-Ha (YRS) (tyrosyl) and anti-Zo (phenylalanyl) - have been collectively described in a further 20%- 30% of patients, and the

frequency of each individual autoantibody is between 1% and 5% [219]. With a few exceptions, each antisynthetase-positive patient develops autoantibodies to only a single aminoacyl-tRNA synthetase. Patients with these autoantibodies are classified as having the antisynthetase syndrome (ASS) when associated with clinical manifestations of myositis, ILD, non-erosive arthritis, mechanic's hands, Raynaud phenomenon, and fever [220, 221]. Some patients also have a DM rash. One of the most frequent symptoms, contributing to high morbidity and mortality, is ILD. It has been reported that between 67 and 100% of ASS patients are affected by ILD [222-224].

Anti-Jo-1 autoantibodies

Support for the role of anti-Jo-1 autoantibodies in the pathogenesis in IIM are: early observations that these autoantibodies are present at disease onset and can even precede the development of myositis symptoms [218], anti-Jo-1 positive sera may induce type I IFN production by pDCs [215] and anti-Jo-1 positive serum levels correlate with disease activity [225]. Additionally, in a conditional MHC class I mice model, mice developed myositis-like disease and autoantibodies to the Jo-1-autoantigen [156]. Furthermore, MHC class I as well as type I IFN-induced genes are highly expressed in muscle fibres from myositis patients in comparison to normal muscle [157]. Since type I IFN are strong MHC class I inducers [226], one mechanism by which anti-Jo-1 autoantibodies may mediate pathogenesis is via the IFNs, although whether anti-Jo-1 autoantibodies have a pathogenic role in disease mechanisms or are merely epiphenomena is still under debate.

Anti-SRP autoantibodies

Autoantibodies to the signal recognition particle (SRP) were first demonstrated in IIM by Reeves and colleagues in 1986 [227]. SRP is a cytoplasmic protein with a role in the recognition and translocation of newly synthesized proteins across the ER. Anti-SRP autoantibodies are present in approximately 1% of JDM patients [228] and 5% of adult myositis patients and have been associated with acute onset severe necrotizing myopathy and with systemic features (such as dysphagia [229]) that may be refractory to standard immunomodulatory treatments [230]. Anti-SRP autoantibody-positive patients have a decreased likelihood of cutaneous involvement [231]. Muscle biopsies from anti-SRP autoantibody positive patients classically demonstrate muscle fiber necrosis with minimal inflammatory cell infiltrate [144, 230].

Anti-Mi-2 autoantibodies

The autoantigen target, Mi-2, is a nuclear helicase protein, that forms part of the nucleosome-remodeling deacetylase complex, which plays a role in gene transcription [232]. Anti-Mi-2 autoantibodies are found in 11–59% of adult DM patients [233, 234], as well as 4–10% of JDM patients [235]. In work similar to studies on Jo-1 expression in target tissues, Mi-2 has been shown to be overexpressed in myositis muscle compared with normal muscle and in particular is upregulated in human DM myofibers expressing markers of regeneration [236, 237]. This autoantibody has been demonstrated to be a specific marker for DM [233] and have typical cutaneous DM lesions, including Gottron’s papules, heliotrope rash, cuticular overgrowth, and V-sign and shawl sign rashes. Patients who have the anti-Mi-2 autoantibodies often have a more favourable prognosis, with milder muscle involvement

and a decreased risk of ILD and malignancy and show good response to therapy compared to other myositis subgroups [220, 238].

Anti-TIF1-γ autoantibodies

These autoantibodies are found in 20–30% of adult DM as well as in JDM [239, 240]. They target nuclear transcription factors belonging to the human transcriptional intermediatory factor (TIF1) family, primarily TIF1-γ, but also TIF1-α and TIF1-β [241] .The TIF1 family is a subgroup of the tripartite motif (TRIM)-containing proteins. TIF1-γ is a nuclear protein involved in controlling DNA transcription. Anti-TIF1-γ autoantibody is significantly associated with aggressive skin lesions (diffuse photoerythema, verucca-like papules, scalp- and facial psorasiform lesions and back rashes), both in adult and juvenile DM [242], whereas the established association with paraneoplastic DM is largely confined to adult patients, especially to older individuals (>50 years). Patients with both anti-TIF1-α autoantibodies and anti-TIF1-γ autoantibodies more frequently had a malignancy, compared to patients with anti-TIF1-γ autoantibodies alone [241]. Furthermore, patients with anti-TIF1-γ autoantibodies have a reduced prevalence of Raynaud's phenomenon, arthritis, calcinosis and ILD than anti-TIF1-γ-negative patients [242]. However, pruritus was found to be positively associated. Interestingly, Ro52 (also known as TRIM21) belongs to the same TIF1 family, and is an E3 ligase and it ubiquitinates IRF8 [243].

Anti-SAE autoantibodies

Anti-SAE autoantibodies were originally described by Betteridge and colleagues in 11 DM patients (8%) in a European myositis cohort. Anti-SAE autoantibodies target small ubiquitin-like modifier activating enzyme (SAE) 1 and 2, which are involved in the post-translational modification of numerous targets, including protein kinases and transcription factors [244]. In all of the anti-SAE autoantibody studies completed, there is an association with DM and cutaneous involvement. Furthermore, the skin manifestations usually develop months before the onset of muscle weakness [244, 245].

Anti-MDA5 autoantibodies

Antibodies against MDA5 (originally termed “anti-CADM-140” antibodies) were first described in 2005 [246]. In studies performed, these DM-specific autoantibodies have been detected in 13–35% of adult and juvenile DM patients [246, 247]. MDA5 is a cytoplasmic RNA-specific helicase that recognizes single-stranded RNA viruses [248]. It belongs to a family of retinoic acid-inducible gene I-like receptors that function as cytoplasmic sensors of pathogen-associated molecular patterns within viral RNA. These proteins drive type I IFN production and anti-viral gene expression, thus mediating the intracellular immune response to control virus infection. It has also been shown that Ro52 autoantibodies (another IFN-inducing autoantigen) are found in 30% of anti-MDA5 autoantibody positive patients. The initial studies showed that patients with these autoantibodies typically have absent or mild muscle disease, so called amyopathic DM, but are at increased risk for developing rapidly progressive ILD with poor prognosis [249, 250]. Characteristic mucocutaneous features are also frequently found in DM patients with this specificity. These consist of skin ulceration

and/or tender palmar papules [251]. They also noted that patients with anti-MDA5 autoantibodies had a higher risk of experiencing oral pain/ulceration and arthritis/arthralgia.

Anti-NXP-2 autoantibodies

Anti-NXP-2 autoantibodies, originally called "anti-MJ" were first detected in approximately 25% of juvenile myositis patients [252]. The target autoantigen was subsequently identified as nuclear matrix protein 2 (NXP-2) and has been reported in 1-17% of adult cohorts [29, 30]. This autoantibody specificity is associated with severe muscle weakness, polyarthritis, joint- and muscle contractures, calcinosis and intestinal vasculitis in JDM [253]. Anti-NXP-2 autoantibodies have also been found to be associated with an increased risk of cancer in adult males [30].

Anti-HMGCR autoantibodies

A new autoantibody directed toward the 200-kDa/100-kDa complex, associated with necrotizing myopathy was described in 2010 [2]. Studies identified the autoantigen target as 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) (the pharmacologic target of statin medication). Patients with anti-HMGCR autoantibodies have been characterized by an increased risk of muscle weakness, elevated CPK levels and myopathic changes on EMG. This autoantibody has been associated with statin use, but studies have also demonstrated the presence of anti-HMGCR autoantibodies in statin-naïve patients, leading to the proposal that anti-HMGCR autoantibodies are a marker of IMNM as opposed to statin-induced myositis [254, 255]. Anti-HMGCR autoantibodies are found in 5-7 % of the myositis patients [2, 256].

Anti-FHL1 autoantibodies

Anti-FHL1 autoantibodies have been detected in 25 % of IIM patients, while patients with RA, Sjögren's syndrome, SLE, SSc, mixed connective tissue disease (MCTD) and muscular dystrophies were largely anti-FHL1 autoantibody negative [257]. Myositis patients with anti-FHL1 autoantibodies often present with severe skeletal muscle involvement. Furthermore, patients with anti-FHL1 autoantibodies develop dysphagia more often than anti-FHL1 autoantibody negative patients. The presence of these autoantibodies has been associated with distal muscle weakness and clinical muscle atrophy and vasculitis. The autoantibodies target a muscle-specific and novel autoantigen, FHL1, four-and-a-half LIM domain 1. Immunization of myositis-prone mice with the autoantigen, FHL1, worsened muscle weakness and increased mortality. The anti-FHL1 autoantibodies may be a potential prognostic biomarker for a subset of severe IIM.

1.1.9.2 Myositis-associated autoantibodies (MAAs)

MAAs are found in approximately 20% of adult myositis patients along with other connective tissue diseases. Although, they are less specific for myositis, they are still an important diagnostic marker and can correlate with clinical features.

Anti-Ro52 autoantibodies/Anti-Ro60 autoantibodies/Anti-La autoantibodies

Anti-Ro autoantibodies are found in some autoimmune diseases such as Sjögren's syndrome, SLE, SSc and IIM. Anti-Ro autoantibodies recognize two distinct antigens, with molecular weights of 52 kD (Ro52/TRIM21) and 60 kDa (Ro60), respectively. Autoantibodies to Ro60/Ro52 occur in 9–35% of adult myositis patients, approximately 6% of JDM patients and 14–25% of myositis overlap patients. Anti-La autoantibodies occur in 2–7% and 4–12% of myositis and myositis-overlap patients. Ro52, also denoted TRIM21, belongs to the TRIM family of proteins, and is an E3 ligase that functions in the process of ubiquitination that regulates cell death and signal transduction [258, 259]. Anti-Ro52 autoantibodies occur frequently together with anti-Jo-1 autoantibodies, with studies demonstrating the presence of anti-Ro52 autoantibodies in 56–72% of anti-Jo-1-positive patients. Patients with both anti-Jo-1 and anti-Ro52 autoantibodies have an increased risk of mechanic's hands and malignancy and a poorer outcome (more severe myositis and joint impairment) than patients with anti-Jo-1 autoantibodies alone. [260]. Furthermore, patients with anti-Jo-1 and anti-Ro-52 autoantibodies have a poorer prognosis concerning ILD than patients with anti-Jo-1 autoantibodies alone. Therefore it is important to test anti-Ro52 alongside the MSAs.

Anti-Ro52 autoantibodies have been demonstrated to have a pathogenic role in development of congenital heart block in mothers with these autoantibodies [261]. Overexpression of Ro52 can lead to ubiquitination of IFN regulatory factor (IRF)-8, which is a transcription factor for macrophages, DCs and B cells, as well as a regulator of type I IFNs which could become activated [243].

Anti-Ro60 autoantibodies are strongly connected to patients with Sjögren's syndrome and SLE and frequently co-occur with anti-Ro52 autoantibodies. The La-antigen also co-occurs with Ro52/Ro60 antigens. Anti-Ro60 autoantigens bind to cytoplasmic Y RNAs, which may have a role in mRNA stability and translation [262].

Anti-U1RNP autoantibodies

These autoantibodies are only found in 3-8% of adult and juvenile myositis, but are more common in patients with overlap conditions (25-40%). These patients rarely have myositis at initial presentation and have been reported to respond favourably to steroid treatment, suggesting that anti-U1-RNP autoantibodies are a marker of good prognosis in myositis [228, 263]. The U1-small nuclear ribonucleic proteins (snRNPs) are involved in pre-messenger RNA processing and collectively are composed of at least 11 polypeptides and five snRNP molecules.

Anti-PM/Scl autoantibodies

One of the most commonly occurring MAAs is the anti-PM/Scl autoantibody, which targets the 75-kDa or 100-kDa subunits of the nucleolar exosome complex. These autoantibodies are reported to occur in 4–12% of patients [263]. Anti-PM/Scl autoantibodies have been identified in various CTDs [264], but are most commonly associated with PM and scleroderma overlap, with an increased risk of ILD, inflammatory joint disease, Raynaud's phenomenon and mechanic's hands. Anti-PM/Scl autoantibodies were originally reported to be a marker of good prognostic outcome, however in a study of long-term outcome of anti-PM/Scl patients with myositis, only 10% of the patients achieved remission and 20% had worsened clinical status [265].

Anti-Ku autoantibodies

The target antigen of anti-Ku autoantibody is a heterodimer of 70 kDa and 80 kDa proteins that is thought to be an activation subunit of DNA-dependent protein kinase. The Ku antigen has key roles in various cellular processes, including DNA double-strand break repair. These antibodies can be seen in 1-3% of PM and DM patients and in 9–19% of myositis patients with overlap syndromes [263]. The inflammatory myopathy is usually mild in anti-Ku positive patients and responds to corticosteroid treatment, but the lung involvement (ILD) is severe and refractory to corticosteroids [266].

IBM

Only 17–43% of IBM patients are routinely found to be autoantibody positive, with the majority of these cases involving MAAs as opposed to MSAs [267]. However a novel autoantibody was found to target the autoantigen, cytosolic 5'nucleotidase 1A (5cN1A), which is a protein involved in the hydrolysis of adenosine monophosphate, leading to physiological control of energy balance, metabolic regulation and cell replication [268]. This autoantibody has also been found in 20 % of patients with SLE and in 36 % of patients with Sjögren's syndrome. The rarity of anti-5cN1A autoantibodies in PM and DM patients still makes this autoantibody a key marker for differentiating between myositis subtypes [269].

1.1.10 Endothelial progenitor cells

Generally, in the adult human organism, new blood vessel formation can occur in 2 ways: by endothelial sprouting from preexisting endothelial cells/angioblasts (angiogenesis) or by peripheral recruitment of endothelial progenitor cells (EPCs) (vasculogenesis) [270]. EPCs, primarily described by Asahara et al in 1997, [271] represent a population of bone marrow-derived, CD34 positive, VEGF receptor-2 (VEGFR-2 or kinase-insert domain receptor [KDR]) positive, CD133-positive cells [272] that have the ability to differentiate into ECs and thus, make a significant contribution to new blood vessel formation [273] and play an important role in endothelial repair [274]. Progenitor cells are defined by their clonogenic (ability to form a colony from a single cell) and proliferative potential. Total EPCs are typically 0.001% of total peripheral blood mononuclear cells (PBMCs). EPCs have been shown to be a surrogate marker of endothelial health, and a reduction in the number of these cells is associated with increased risk of cardiovascular disease [275-277]. Depletion of EPCs has been reported in some diseases with vascular complications, including diabetes and RA

[278, 279]. Moreover, depletion of EPCs has been shown to be caused by excessive serum levels of type I IFNs and this may be linked to endothelial dysfunction and increased cardiovascular risk in SLE [280]. EPCs have been extensively studied since 1997 and are considered as a potential marker for endothelial regeneration ability as mentioned above. On the other hand, circulating endothelial cells (CEC) were studied as biomarker for endothelial injury [281]. CECs are mature ECs sloughed off from the vessel walls [282]. Yet, in the literature, there is also a huge incoherency in regards to terminology and protocols used. This results in misleading conclusions on the role of so called EPCs, especially in the clinical field. The discrepancies are mainly due to strong phenotypic overlap between EPCs and circulating angiogenic cells (CACs), which are monocyte and macrophage progenitors. Likely both EPCs and CACs are important in generation of ECs. Furthermore, SLE patients have decreased amount of circulating EPCs/CACs and have a decreased capacity to differentiate into mature ECs, even during quiescent disease [184, 283]. Thus, patients with SLE have an impaired repair of the damaged endothelium, and one proposed mechanism could be through the effect of the type I IFNs. Type I IFN levels are increased in sera of SLE patients [284] and lupus cells appear to be more sensitive to the effects of the type I IFNs [98]. Also, an IFN signature in PBMCs of patients with severe SLE correlates with disease activity [285]. As the microvasculature is known to be affected in patients with myositis, particularly in patients with DM, with loss of capillaries in muscle tissue as a well recognized phenomenon, and with the more recent observations of activation of the type I IFN system in patients with myositis, we wanted to further investigate if the phenotype and function of EPCs is altered also in myositis and if so, if there is an association to the type I IFNs. Therefore, we initiated a study which resulted in paper IV.

2 AIMS

The overall aim of my thesis was to get an increased understanding of molecular mechanisms that are involved in IIM with a focus on the type I IFN system, autoantibodies and mechanisms that may induce immune reactivity, to be able to subclassify patients.

The specific aims were:

- To assess the diagnostic accuracy of a novel and commercially available line blot assay with myositis-related autoantibodies and to validate the test results against those obtained using established autoantibody detection techniques **(Paper I)**.
- To investigate if smoking is a risk factor for development of subgroups of PM/DM, in particular, a subset with anti-Jo-1 autoantibodies and a certain HLA-type **(Paper II)**.
- To investigate whether autoantibody profiles in myositis are associated with activation of the type I IFN system and whether there is a correlation with disease activity, clinical manifestations, or HLA haplotype **(Paper III)**.
- To investigate whether type I IFN and IL-18 is linked to EPC depletion, activation and dysfunction in IIM patients **(Paper IV)**.

3 PATIENTS

Patients included in the thesis were recruited from the Rheumatology Clinic, Karolinska University Hospital, Stockholm, Sweden, the University of Padova, Padova, Italy, the VU University medical center, Amsterdam, the Netherlands, the Institute of Rheumatology, Prague, Czech Republic, the outpatient Rheumatology Clinic, University of Michigan, Ann Arbor, Michigan, USA, University of Manchester, Salford, United Kingdom (UK) and from the University of Debrecen, Debrecen, Hungary. Patients were diagnosed with definite or probable PM/DM according to Bohan and Peter's criteria [6, 7] and patients with IBM were diagnosed according to Grigg's criteria [9]. All patients and controls gave their written informed consent to participate in the studies.

Several different groups of patients were included.

3.1 PAPER I

208 patients [PM (n=100), DM (n=63), JDM (n=2), overlap (n=27), cancer-associated myositis (CAM)(n=9), ASS (n=7)] were included in this study. 137 patients from the Division of Rheumatology, University of Padova, Padova, Italy, between January 1999 and May 2008, and 71 patients from the Rheumatology Unit of Karolinska University Hospital, Stockholm, Sweden, within the above mentioned period, were enrolled. Overlap myositis was defined when the patient satisfied one or more classification criteria of at least one CTD in addition to the myositis diagnosis. 50 age-and sex-matched HCs or 180 patients with various diseases including 11 non-autoimmune myopathies, 23 sporadic or genetic muscular dystrophies, 11 CTDs, 68 SLE, 36 SSc, 22 primary Sjögren's syndrome and 9 arthropathies, where tested as healthy or disease controls. The patients' clinical characteristics are presented in table 4.

3.2 PAPER II

A cohort of 557 patients with PM (n=276), DM (n=213) or overlap (n=68) from the rheumatology units in Hungary, UK, Sweden and the Czech Republic were included in this study. The inclusion criteria were PM, DM or myositis-overlap with concurrent information about *HLA-DRB1* genotype, smoking history and anti-Jo-1 autoantibody status. Patients with myositis-CTD overlap either had their primary disease with probable or definite myositis according to Bohan and Peter's criteria [6, 7] or had possible myositis but additionally had a confirmed MSA or MAA. The patients' clinical characteristics are presented in table 4.

3.3 PAPER III

A cohort of one hundred and eight patients with PM (n=40), DM (n=46) and IBM (n=6) who fulfilled the criteria for definite or probable PM/DM [6, 7] or possible IBM [9] were included in this study. The patients were enrolled between 2006-2009 from the Rheumatology Unit, Karolinska University Hospital, Stockholm, Sweden and from the Institute of Rheumatology, Prague, Czech Republic. Twenty-nine patients of these were Czech and 79 patients were Swedish. Exclusion criteria were treatment with a biological agent, presence of overlap syndrome or no available antinuclear antibody (ANA) status. Twelve of the Swedish patients

were excluded according to the exclusion criteria and four patients due to missing IFN data due to technical issues. The remaining 92 were included. Ten of the Swedish patients and 2 of the Czech patients were newly diagnosed and had at time of blood sampling no medication. Forty-seven patients with SLE were recruited at the Rheumatology department, VU University medical center, Amsterdam, The Netherlands, as a comparator group. Forty-one healthy controls (HCs) (23 female and 18 male; mean age 35 years) were recruited at the VU University Medical Center, Amsterdam, the Netherlands. The patients' clinical characteristics are presented in table 4.

3.4 PAPER IV

Two cohorts (cohort 1 and 2) were included in this study. Thirty-six patients with PM (n=24) or DM (n=12) from the well-characterized myositis cohort at Karolinska University Hospital, Stockholm, Sweden were included in cohort 1. Twenty-five patients with PM (n=17) or DM (n=8) were recruited between 2010-2012 at the outpatient Rheumatology Clinic, University of Michigan, Ann Arbor, USA (cohort 2). Exclusion criteria were IBM, current or recent infections (within 1 week), pregnancy or cancer. Matched HCs were recruited by advertisement at the University of Michigan. The patients' baseline characteristics are presented in table 4.

Table 4. **Patient characteristics at baseline**

Characteristics*	Paper I	Paper II	Paper III	Paper IV	
				Cohort 1	Cohort 2
Diagnosis, n (%)	208	557	92	36	25
PM	100 (48)	276 (50)	40 (43)	24 (67)	17 (68)
DM	65° (31)	213 (38)	46 (50)	12 (33)	8 (32)
IBM	0	0	6 (7)	0	0
MCTD	0	0	0	0	0
Overlap/CAM/ ASS	27 (13)/ 9 (5)/7 (3)	68 (12)/0/0	0	0	0
Sex, n (%)					
Men/women	49 (24)/ 159 (76)	139 (25)/ 418 (75)	30 (33)/ 62 (67)	10 (28)/ 26 (72)	11 (44)/ 14 (56)
Age, years	n/a	48 (37- 58)	58 (49-68)	60 (48-69)	57 (33-68)
Disease duration,years	1 (0-31) (mean+SD)	n/a	3.0 (0.3-9.6)	2.2 (0.1-6.7)	n/a
Physician's global disease activity assessment,mm	n/a	n/a	7 (0-23)	13 (3-40)	n/a
Patient's global disease activity assessement,mm	n/a	n/a	36 (16-64)	46 (24-63)	n/a
MMT8, (0-80)	n/a	n/a	91 (72-99)	75 (68-79)	n/a
HAQ (0.00-3.00)	n/a	n/a	0.50 (0.00-1.25)	0.94 (0.40-1.50)	n/a
CPK, µkat/liter	n/a	n/a	2.00 (1.05-8.23)	3.00 (1.10-12.38)	n/a
AST, µkat/liter	n/a	n/a	0.50 (0.39-0.73)	0.53 (0.35-0.97)	n/a
ALT, µkat/liter	n/a	n/a	0.53 (0.35-1.05)	0.41 (0.27-0.90)	n/a
LDH, µkat/liter	n/a	n/a	3.60 (3.08-4.42)	5.60 (3.42-7.58)	n/a
Extramuscular global assessment, mm	n/a	n/a	5 (0-14)	12 (0-29)	n/a
Immunomodulatory drugs, n (%)	n/a	n/a	55 (60)	18 (50)	21 (84)
Daily dose of Prednisolone, mg	n/a	n/a	10 (4-18)	4 (0-10)	8 (5-50)
Antibody profile†, n					
Jo-1	s	115 (21)	23	14	8
PL7	e	n/a	1	1	2
EJ	e	n/a	0	0	1
Ku		n/a	1	1	0
Ro52/Ro60	r	n/a	22/9	6/0	3/0
Mi-2	e	n/a	0	3	1
PM/Scl 75/100	s	n/a	7	0	0
SRP	u	n/a	2	1	0
U1RNP	l	n/a	8	3	3
La	t	n/a	4	1	1
TIF1 gamma	s	n/a	0	2	0

*All data are from time of blood sampling and presented as median (IQR; interquartile range) if not stated otherwise. PM, Polymyositis; DM, Dermatomyositis; MCTD, Mixed Connective Tissue Disorder; Disease duration, years from diagnosis till sampling date; Physician's global disease activity assessment (Visual Analogue Scale (VAS), from 0-100 mm,) Patient's global disease activity assessment (VAS from 0-100 mm); Extramuscular global assessment (VAS from 0-100 mm); MMT8, Manual muscle test (0-80); HAQ, Health Assessment Questionnaire (potential score 0.00-3.00); CPK, creatine phosphokinase (normal levels: 0.6-3.5 μkat/liter); AST, Aspartate aminotransferase (normal levels <0.61 μkat/liter); ALT, Alanine aminotransferase (normal levels <0.76 μkat/liter); LDH, lactate dehydrogenase (normal levels <3.5 μkat/liter); Immunomodulatory drugs includes cyclophosphamide, methotrexate, azathioprine, cyclosporin A, and intravenous immunoglobulin; (n/a), not assessed. CAM, Cancer-associated myositis; ASS, Antisyntethase syndrome with subclinical myositis. One patient could have several autoantibody specificities. ° two of these patients have the diagnosis juvenile DM

4 METHODS

4.1 GENOTYPING

HLA-typing was performed for all the myositis patients in paper II, in order to examine whether smoking was associated with development of anti-Jo-1 autoantibodies in *HLA-DRB1*03* positive IIM. HLA-typing was also done for all patients in paper III, in order to investigate a possible link between HLA type and IFN activity, by sequence-specific primers PCR [286] in CMM Research Laboratory, Stockholm, Sweden (Swedish patients) and in the Institute of Rheumatology, Prague, Czech Republic (Czech patients).

In the paper II and III, primers were designed for DR "low –resolution" typing by sequence-specific primers PCR i.e. identifying polymorphisms corresponding to the serologically defined series DR1-DRw18.

Sequence-specific primers PCR is an accurate typing technique with high sensitivity, specificity and reproducibility. The method is rapid (two hours) and inexpensive, and is suited for analyzing small numbers of samples simultaneously. The method contains DNA preparation, PCR amplification, post-amplification processing, gel detection, documentation and interpretation.

4.2 AUTOANTIBODY ANALYSES

Autoantibodies are important for the evaluation of patients with systemic autoimmune diseases. This is because of their inclusion in diagnostic and classification criteria, their correlation to certain clinical manifestations and their association to disease activity. Assays to detect autoantibodies should therefore be reproducible, reliable, easy to perform and available in everyday clinical practice.

In paper I line blot assay was validated to standard immunoprecipitation (IPP) and immunoblot. In paper II, only line blot assay was used and in paper III both line blot assay and IPP were used to detect autoantibodies. In paper IV, line blot assay and IPP (cohort 1) and a multiplex assay (cohort 2) were used. In papers III and IV, patients were defined as positive for an autoantibody if the autoantibody status was positive in one of the two used methods. Multispecific autoantibody status was defined as presence of two or more autoantibodies, antinuclear antibody (ANA) not included.

4.2.1 Line blot assay

This test is based on the principal of line immunoassay in which recombinant antigens, synthetic peptides or natural antigens are coated as discrete, parallel lines on a membrane. These strips are incubated sequentially with diluted patient sample, horseradish peroxidase labeled secondary antibody and a substrate. Readings are made by comparing the intensity of the reaction with control lines. The strip is coated with multi-antigens which allows for simultaneous detection of different autoantibodies using small serum volumes and make it possible to discriminate between unspecific background signal and real reaction with the present antigen. It is also a semi-quantitative method, since each autoantibody can be quantified and divided into a low or high titer. A similar method is the immunoblot (Western blot), where the separation of the proteins are made with gelelectrophoresis by molecular weight. Thereafter the proteins are transferred (blotted) to a membrane (nitrocellulose or nylon) and identification of the protein is made by incubation of the patient sample and a labeled secondary antibody and substrate as mentioned above.

4.2.2 Immunoprecipitation

IPP is a technique of precipitating a protein antigen out of a solution using an antibody that specifically binds to that particular protein. This process can be used to isolate and concentrate a non-abundant protein from a sample containing many thousands of different proteins, such as a whole cell extract. IPP requires that the antibody is coupled to a solid substrate at some point in the procedure, with assays generally immobilizing antibodies using superparamagnetic microbeads or microscopic agarose (non-magnetic) beads. For radiolabeled IPP, a S^{35} methionine labelled protein mixture is added to the bead-autoantibody complex and proteins targeted by the autoantibodies are immunoprecipitated and captured onto the beads. After heating, proteins are fractionated by gel electrophoresis, enhanced, fixed and dried. Labeled proteins are analyzed by autoradiography (X-ray film). IPP has been regarded as the gold standard testing method for autoantibody serology due to its high sensitivity and ability to detect a wide repertoire of known and unknown autoantigen targets [287]. However, the test requires a specialist centre and is both time-consuming and labour-intensive. IPP also has the disadvantage of not being able to distinguish between autoantibodies targeting proteins of the same molecular weight and it is a non-quantitative assay [288].

4.2.3 Multiplex assay (Bioplex 2200)

In paper IV, cohort 2, serum samples were screened for autoantibodies with the Bioplex 2200 assay. This method is an immunoassay, which uses very small magnetic beads, each coated with a different autoantigen. The bead sets are then incubated with the patient sera, containing the autoantibodies. A secondary antihuman-IgG antibody conjugated to a fluorophore is added to detect the autoantibodies bound to the antigen-coated beads. A dual-laser detects the individual bead results for each assay. The multiplex assay is a quantitative, fully automated assay, requiring no user input after the initial loading of primary tubes. The system can automatically process up to 100 samples per hour. First results are available in 40-60 minutes. This assay still needs to be fully validated [289].

4.2.4 Immunofluorescent assay

This assay is one of the most commonly used methods for testing autoantibodies. In papers III and IV, ANA was screened by an immunofluorescent assay, which is the gold standard method for ANA screening. This is a method in which a patient's blood sample is mixed with Hep-2 cells that are affixed to a slide, enabling autoantibodies that exist in the blood to react with the cells. The slide is treated with a fluorescent tagged secondary antibody reagent and examined under a fluorescent microscope. The presence or absence and pattern of fluorescence is noted. This method is labor intensive, but an excellent screening test in expert hands, who know how to process and read the slides. Furthermore, in the case of myositis autoantibodies, the results are often negative or nonspecific, as many of the MSAs are cytoplasmic. In some laboratories this method has been replaced by multiplex methods (see chapter 4.2.3).

4.3 IFN ASSAYS

In this thesis we have had collaboration with colleagues in the USA and in Europe, and the IFN assays used have been decided depending on what IFN assay is mostly used in the different laboratories that we collaborated with.

There are four main types of assays used to measure IFNα/β production. ELISAs and bioassays can be used to measure secreted IFNα and IFNβ. Polymerase chain reaction (PCR) is commonly used to detect IFNα and IFNβ mRNA expression by pure cell populations. Intracellular cytokine staining protocols detect IFNα/β production by flow cytometry. Measuring circulating IFN-α in serum is less sensitive compared to measuring type I IFN-inducible genes, as has been reported in many studies [200, 290-292]. The bioassay is the most sensitive assay, followed by ELISA and then the intracellular staining protocol [293]. These assays have different advantages and disadvantages. Detection of mRNA levels by PCR will not identify the IFN-producing cell unless a pure cell population is used and the results may not reflect protein production. However, several subtypes of IFNα production can be assayed simultaneously. ELISAs allow unambiguous detection of secreted protein. The IFN bioassays are more complex experiments, take longer time (5-6 days), but use less expensive reagents and equipment and detect production of functional IFN proteins using a relevant bioassay system. Intracellular staining protocol is a quick method (3-4 hours) for detecting the production of specific types of IFN protein by identified cell types within mixed cell population.

4.3.1 IFN signature in whole blood

This method was used in paper III. For whole blood RNA isolation, 2.5 ml blood was drawn in PAXgene tubes and RNA isolation, quantification and purification was performed as previously described [294]. RNA was reversely transcribed into cDNA by using a cDNA synthesis kit. Real-time PCR analysis was performed. Relative quantities were calculated using the ddCT method. *GAPDH* was used as a housekeeping gene and all arrays contained two samples for calibration.

The average expression of eight IFN-regulated genes (IRG), *IFI3, IFIT2, MxA, IFI44L, HERC5, IFIT1, RSAD2 and OAS3,* (all corrected versus *GAPDH*, $\log^2$ according to earlier studies) was used as the IFN score [193, 214]). Since the IRGs were highly correlative, we calculated an IFN score by averaging the expression levels of all IRGs per sample [295-297]. The mean + 2 standard deviation (SD) of the IFN score in HC (n=41) was used as a cut off to define if an IFN signature was present (IFN-score > 4.84=IFN high) or absent (IFN-score <4.84=IFN low).

4.3.2 IRG induction assay and neutralization

The IRG induction assay was used in paper III, to test if patient sera had the property to activate the type I IFN pathway. Briefly, healthy PBMCs were incubated with 20% patient serum from 20 randomly selected patients with IIM for 4 hours or 8 hours at 37°C and 5 % CO_2. Next, to identify the specificity of type I IFN, 25 randomly selected samples from patients with IIM were co-cultured with neutralizing anti-IFNα antibody or neutralizing anti-IFNα-receptor (IFNAR2) antibody. After incubation, cells were harvested, washed and lysed in both experiments. RNA isolation from cells and reverse transcription of cDNA and real time quantitative (q)PCR was performed and assessed (see chapter 4.3.1) with the exception that the expression levels of target genes were calculated relative to housekeeping gene *18S ribosomal RNA* (*18SrRNA*). To correct for any variations between experiments, all expression values are relative to healthy controls. The overall IRG induction was determined by calculating the average expression of three known IRGs; *RSAD2, IFI44L* and *MX1* [295, 296]. The average *ex-vivo* whole blood IRG induction for all samples (1.96) was used to subdivide patients into IFN high and IFN low.

4.3.3 Type I IFN serum activity

In paper IV, sera from 36 IIM patients recruited from Karolinska University Hospital were analyzed for their IFN-inducing ability using a validated bioassay [200] employing cell cultures [283]. This bioassay measures the functional effects of serum on the gene expression of cultured target cells, an epithelial cell line (HeLa cells). The induction of three type I IFN-inducible genes *IFI44, IFIT1* and *PRKR* was quantified by real-time qPCR. Samples were normalized to media alone after normalization to house-keeping gene *HPRT-1* and fold change (FC) was calculated as compared to samples exposed to serum from healthy controls. The IFN score was calculated as previously reported [290]: [myositis patients_{FC}-mean_{FC} of healthy controls (HC)]/standard deviation HC) and was calculated for each gene followed by summation of all three gene scores to generate the composite index.

4.4 ELISA

ELISA is commonly used for detection of proteins and autoantibodies in fluid, such as sera or plasma. ELISA is a quick method and produces quantitative data, but the drawback is the dependence on a standard curve.

In paper IV we used a human quantitative ELISA for detection of IL-18. The ELISA plates are precoated with an anti-human IL-18 antibody onto microwells. Human IL-18 present in the sample binds to the IL-18 antibodies in the microwells. An enzyme-labelled (e.g. biotin)

secondary antibody binds to human IL-18 captured by the first antibody. This is followed by the substrate-chromogen which forms a colored reaction product that is visible, in proportion to the amount soluble human IL-18 present in the sample. A standard curve is prepared from 7 human IL-18 standard dilutions and human IL-18 sample concentration determined.

4.5 ASSAYS FOR EPC DIFFERENTIATION AND QUANTIFICATION

Currently, it is accepted standard to measure the circulating numbers of EPCs by flow cytometry using either antibodies against CD34 and KDR or CD133, whereas the functional, clonogenic capacity should be evaluated using colony-forming unit assays [276] or monolayer formation. ECs initially form colonies (around day 7) from which mature ECs then migrate and form a monolayer (between day 14-21). Ideally, cells are stained with markers to prove they are mature ECs, but morphology (monolayer formation) is another way to judge. Monolayer formation is one way to measure endothelial cell differentiation. In paper IV we wanted to study the differentiation into ECs, and not just early EPC function, therefore we studied the monolayer formation.

4.5.1 Flow cytometry

Flow cytometry provides rapid characterstics of single cells. The information obtained is both quantitative (cell counting) and qualitative (cell size, internal complexity, that is cell sorting).

The basic principle of flow cytometry is the passage of cells in single file in front of a laser, in a flow cytometer, so they can be detected, counted and sorted. Cell components are fluorescently labeled and then excited by the laser to emit light at varying wavelengths. The fluorescence can then be measured to determine the amount and type of cells present in a sample.

In paper IV circulating EPCs were characterized and quantified by flowcytometry in patients of cohort 2 and in matched controls.

4.5.2 *In vitro* differentiation into mature endothelial cells

PBMC differentiation into EC-like cells is an accepted method to assess the functionality of EPCs [279, 283, 298], that we used in paper IV. Briefly, PBMCs were cultured in EC-specific enrichment medium on fibronectin-coated wells. Media was changed 120 hours after plating, then every 2 days. Typically in 2-3 weeks, these cells differentiate into ECs by forming a monolayer. Wells that had monolayer formation (detected by light microscopy) were scored as EC-monolayer forming or not-EC-monolayer forming. This method (monolayer formation) was used in cohort 2 in paper IV. This is a quick way of evaluating if there has been a differentiation from EPCs to ECs. Staining with acetylated-LDL (ac-LDL) and UEA-1, followed by fluorescent microscopy, is a confirmation of this assay as it detects specific EC markers (see 4.5.3). This is labor intensive, but more specific than monolayer formation. If cells are stained with ac-LDL and UEA-1, then we can be sure that a monolayer has been formed.

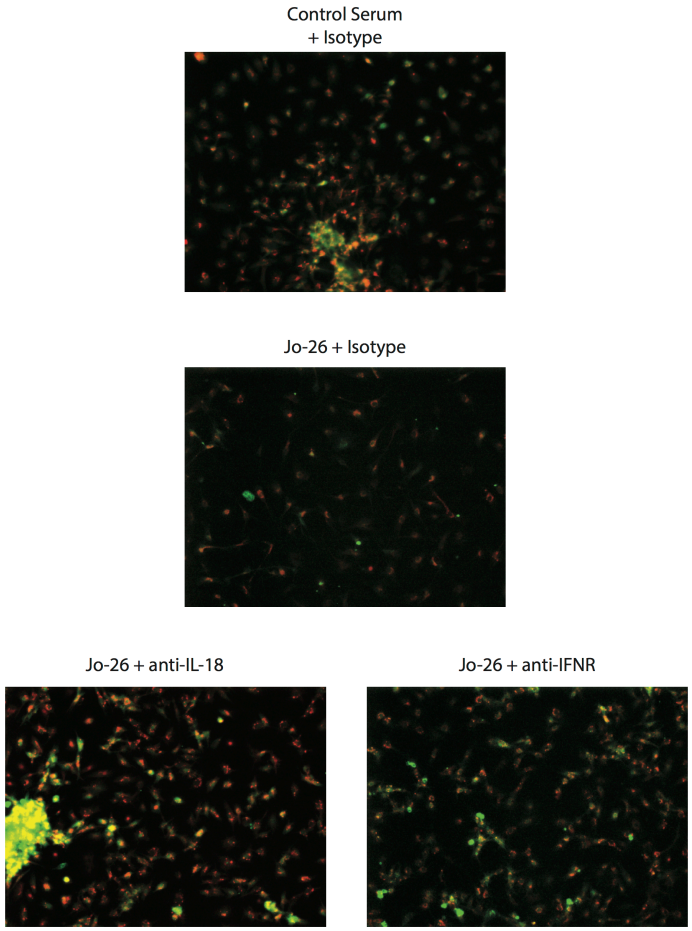

Figure 6. Myositis sera inhibits EPC differentiation in vitro. Representative fluorescent photomicrograph examining staining of ac-LDL (red) and UEA-1 (green) in EC cultures at day 14 with and without type I IFN receptor blockade (IFNR) or IL-18 neutralization. The overlap yellow staining represents cells that are double positive and most likely a mature EC. Jo-26 represents sera from a representative myositis patient. The photomicrograph is published courtesy of Dr Michelle Kahlenberg.

4.5.3 Blocking experiments

In cohort 1 in paper IV, we wanted to assess the effect of PM and DM serum on EPC differentiation into mature ECs. We therefore proceeded to isolate healthy control EPC-containing PBMCs and cultured them in proangiogenic conditions as above (see chapter 4.5.2), in the presence or absence of 30 % healthy control or PM/DM serum from cohort 1, for the first three days of culture. EC differentiation was assessed as above (see chapter 4.5.2). On day 14 of culture, by incubation with markers of mature ECs (ac-LDL and fluorescein labeled UEA-1), followed by live cell fluorescent microscopy, images were acquired at room temperature at 100X total magnification (figure 6). Three random fields of view were acquired per every triplicate well. The data was presented as one combined mean. Cells that co-expressed ac-LDL and UEA-1 were quantified as ECs. In some experiments, neutralizing antibodies to IL-18 or the type I IFN receptor or isotype control were added 30

min prior to addition of human serum. We could then study if neutralizing antibodies to IL-18 or antibodies to the type I IFN receptor affected the EPC differentiation into mature ECs.

4.6 TAQMAN PCR

TaqMan reverse transcription qPCR (TaqMan qPCR) is one of two types of quantitative PCR methods. Unlike the other type of quantitative PCR which uses a fluorescent dye that can bind any double-stranded DNA, Taqman qPCR uses a fluorogenic probe, which is a single stranded oligonucleotide of 20-26 nucleotides and is designed to bind only the DNA sequence between the two PCR primers. So only specific PCR products can generate fluourescent signals in TaqMan qPCR. This method allows a sensitive, specific and reproducible sequence detection down to single nucleotide resolution. Expression of IFN-regulated genes involved in IFN production in paper III and IV, was assessed using TaqMan qPCR following RNA extraction and cDNA synthesis.

4.7 STATISTICAL ANALYSES

In **paper I** differences in frequencies of dichotomous variables were analyzed by chi-squared test or Fisher's exact test, when appropriate. P-values below 0.05 were considered statistically significant. Concordance between methods was evaluated by contingency tables. Diagnostic accuracy for IIM was measured by sensitivity, specificity, positive and negative predictive values for any antibody specificity investigated. Sensitivity (%) for IIM = (number of MSA/MAA positive patients with IIM/total patients with IIM) x 100. Specificity for IIM (with respect to healthy subjects) = (number of MSA/MAA negative healthy subjects/total healthy subjects) x 100. Specificity for IIM (with respect to disease controls) = (number of MSA/MAA negative disease controls/ total disease controls) x 100. Data were statistically analysed using SPSS 15.0I statistical package (Windows version)

In **paper II**, individual associations were derived from 2x2 contingency tables using the chi-squared test, or two-tailed Fisher's exact test when individual cells valued five or less. Data were expressed as odds ratio (OR) with exact 95 % confidence interval (CI). Pointwise p-values were not corrected as the association with *HLA-DRB1*03* and IIM is well established in the literature [299, 300]. The described analyses were also repeated after stratification by smoking and anti-Jo1-status using multinominal logistic regression. Data were analysed by the statistical package Stata (release 9.2).

In **paper III,** the significance of differences between groups was calculated by Mann-Whitney U-test or Student's t-test when appropriate for continuous variables, or by Pearson's chi square tests or Fischer's exact test for categorical variables. Differences in IRG induction over time were tested using a paired t-test. Correlation analyses were done using Pearson r or Spearman r tests. P-values <0.05 were considered statistically significant. Data were analyzed using GraphPad Prism 4 or 5 Software**.**

In **paper IV,** the significance of differences between groups was calculated by Mann-Whitney U-test. Correlation analyses were done using linear regression analysis. Comparison of improvement with IL-18 or type I IFN receptor blockade was performed using paired, 2-sided Student's t-test. Difference between the means in the EPC quantification and

monolayer assay was analyzed using an unpaired Student's t-test. P-values <0.05 were considered statistically significant. All analyses were done using GraphPad Prism 6 Software.

4.8 ETHICS

All studies included in this thesis were approved by the Local Regional Ethical Review Boards.

Approval numbers: Paper I (dnr 2005/792-31/4, 2011/1374-32 Karolinska Institutet, prot.n. 2542P, Padova, Italy). Paper II (dnr: 2005/792-31/4, 2007/1121-32, 2011/1374-32 Karolinska Institutet and MREC ref.nr: 98/8/86 Manchester, United Kingdom). Paper III (dnr: 2005/792-31/4, 2011/1374-32 Karolinska Institutet, ref.nr 2007/125 Amsterdam, Netherlands, ref.nr 3233/2007 Prague, Czech Republic). Paper IV (dnr: 2005/792-31/4, 2011/1374-32 Karolinska Institutet and ID: HUM 00066116, HUM 00044257 Ann Arbor, Michigan, USA).

None of the studies in this thesis involved any medical risks to the patients, as they were observational. The collection of data and publication of results have been carried out so as to guarantee the anonymity and integrity of the patients.

5 RESULTS AND DISCUSSION

In the following sections the main results of paper 1-IV are summarized and together with unpublished data, discussed in the context of recent findings and literature.

5.1 PAPER I

Serological testing for MSAs and MAAs is useful for the diagnosis, prognosis and treatment response of IIMs. However, available assays need to be standardized and validated. The objective of this paper was to evaluate the accuracy of a novel commercial line blot assay compared to established autoantibody detection methods (IPP and immunoblot).

We found that with the line blot assay, any MSA or MAA was detected in 98/208 (47%) of the myositis patients, in 2/50 (4%) of the healthy subjects and in 70/180 (39%) of disease controls. Regarding IPP/immunoblot, any MSA or MAA was detected in 106/208 (51%) of the myositis patients, in 3/50 (6%) of the healthy subjects and 47/180 (26%) of the disease controls.

The diagnostic specificity of line blot with respect to healthy/disease controls was 100% for anti-Jo-1, anti-PL-7 or PL-12, anti-PM/Scl; 96% for anti-Ku, 98% for anti-Mi-2 and 76% for anti-Ro52. Notably, anti-Ku and anti-Mi-2 false positive cases were mainly found in SLE patients.

The diagnostic specificity of IPP/immunoblot with respect to healthy/disease controls was 100% for anti-Jo-1, anti-Mi-2, anti-PM/Scl, 97% for anti-ARS non Jo-1 (94% towards healthy subjects and non-immune myopathies, and 98% towards CTD patients), 98% for anti-Ku (100% vs. healthy subjects and non-immune myopathies, and 99% vs. CTD patients),

82% for anti-Ro52 (100% vs healthy subjects, 94% vs non-immune myopathies and 73% vs CTD patients).

In summary, IPP/immunoblot confirmed the line blot assay results as regard to anti-Jo-1, anti-PM/Scl and anti-Ku positivities, but IPP/immunoblot was somewhat more sensitive than line blot in detecting anti-Mi-2 and anti-ARS non-Jo-1 autoantibodies in patients with IIM.

The sensitivity of the anti-Mi-2 autoantibody was lower when it was detected by line blot assay than by immunoblot. This could depend on the nature of the antigen, since the Mi-2 autoantigen is composed of at least two isoforms, Mi-2α and Mi-2β. In this version of the line blot assay only Mi-2β was detected, in contrast to in immunoblot. Improvement of the sensitivity has been made in the new version of line blot strips, where both the Mi-2α and Mi-2β epitopes can be detected. Furthermore, the sensitivity of anti-ARS non-Jo-1 autoantibodies was lower when these autoantibodies were detected by line blot than by IPP. This can partly be due to the limited number of anti-ARS specificities detectable at that time in the line blot assay (i.e. PL-7, PL-12), contrary to IPP where any anti-ARS autoantibody can be detected.

Another limitation in this paper was that the healthy and disease controls were from Italy, but the patients were from both Italy and Sweden. However, there was no difference in clinical characteristic or antibody profile between the Italian and the Swedish cohort, apart from a lower relative prevalence of the anti-Mi-2 autoantibody and DM in the Swedish patients. This is likely due to differences in environmental factors or HLA profiles between the two populations. One known environmental factor that is different in Sweden compared to Italy is the degree of UV-light radiation. As it has been reported previously, that UV-light exposure increases risk to develop both Mi-2 autoantibodies and clinical features of DM this is a plausible explanation for the observed difference in our two-center study, but influence of other environmental or genetic factors cannot be excluded.

We could confirm that the anti-Ro52 autoantibody is not specific for IIM by means of either line blot or IPP/immunoblot and this reduces the accuracy of both tests to be used as diagnostic tests. By excluding the anti-Ro52 autoantibody detection both methods achieved a specificity higher than 90% but without losing sensitivity (40%). However, anti-Ro52 autoantibodies are often present in combination with one MSA such as anti-Jo-1 autoantibodies, and it has an additional value to detect anti-Ro52 autoantibodies as they may confer a worse prognosis when present together with an MSA [260].

In our study, the line blot assay available at that time, detected seven myositis antigens, but nowadays 16 myositis antigens (Mi-2α, Mi-2β, TIF1-γ, MDA5, NXP-2, SAE-1, Ku, PM/Scl100, PM/Scl75, Jo-1, SRP, PL-7, PL-12, EJ, OJ, Ro52) can be detected by this assay. We nowadays have the possibility to test sera from myositis patients in the department of Clinical Immunology, Uppsala Akademiska Hospital, Sweden, where the novel line blot assay is used and analyses are performed several times per week.

The line blot assay is not only a qualitative method, it can also be used as a semi-quantitative method, since each autoantibody can be quantified and divided into low or high titer.

Furthermore, the line blot assay is fast and reproducible, and is easy to perform and can therefore be used in routine clinical laboratories.

In conclusion, we found that the line blot test is an accurate serological test. Positive MSAs, more than MAAs, support the diagnosis of myositis and with a high likelihood rule out other systemic rheumatic diseases or myopathies, thus representing a reliable alternative to the more time-consuming and complex IPP and immunoblot and feasible to use in clinical routine testing. For research projects where we want to test many sera or if we want to search for new autoantibody specificities we suggest an algorithm to first screen with the line blot assay and if patients are "seronegative" by the line blot assay, they can then proceed to the "gold standard" IPP and/or immunoblot.

5.2 PAPER II

The aim of this study was to examine whether smoking was associated with anti-Jo-1 autoantibodies in *HLA-DRB1*03*-positive IIM.

Interestingly, the frequency of smoking was higher in anti-Jo-1 autoantibody positive myositis patients across all four cohorts, compared to anti-Jo-1 autoantibody negative patients. In a cohort of 557 patients from four European centers, we found that the frequency of anti-Jo-1 autoantibodies was 21%. The frequency of anti-Jo-1 autoantibodies when compared between countries was broadly similar, although the highest frequency was noted in the Czech cohort, and the lowest in the Swedish cohort. We defined a patient as "ever smoker" if a patient stated that they had ever smoked as much as one cigarette a day for as long as a year. The overall frequency of "ever smoking" was 39% and a difference was noted between the countries. The lowest frequency was noted in the Hungarian cohort, the highest in the UK cohort. The overall frequency of "ever smoking" 39%, is a high value. Furthermore, in the Swedish cohort 54 % were ever smokers, and the current smoking prevalence in the overall population in Sweden is about 21% (men 23%, women 20%, 16-84 years old) (WHO, www.who.int.). There is no universal definition of a smoker, and therefore the smoking prevalence varies from study to study and within countries and between countries. One explanation for the variations in frequency of smoking between the countries could possibly be due to variations in methods on how the information on smoking habits was retrieved. Patients in our study cohort, from the other three countries received a questionnaire where the smoking question could be answered. However, for the Swedish cohort the patients were interviewed over the phone. It could be so, that it is easier to capture smoking habits during a telephone call than by paper, explaining the higher smoking frequency in the Swedish cohort. Hungary had the lowest frequency 22%, which is in line with reported smoking prevalence in the overall Hungarian population (WHO, www.who.int.). Perhaps differences in tendency to admit smoking habits also contributes to the reported different smoking frequencies between the countries.

As has been previously described, we found a strong association between *HLA-DRB1*03* and anti-Jo-1 status across all four cohorts combined [92, 93]. Furthermore, the frequency of *HLA-DRB1*03* was higher in smokers than in non-smokers, and this association again reached statistical significance in the Hungarian cohort, like the association between smoking

and anti-Jo-1 autoantibody positivity also reached statistical significance in the Hungarian cohort. Our results suggest that there is an additional risk to develop anti-Jo-1 autoantibodies in *HLA-DRB1*03*-positive ever smokers.

In conclusion, this study has shown that smoking is associated with IIM patients who are either anti-Jo-1 autoantibody and/or *HLA-DRB1*03* positive. The association of the presence of anti-Jo-1 antibodies is further increased in IIM patients who are both smokers and possess one or more copies of *HLA-DRB1*03*. It would be interesting to further subgroup the smokers with the help of an environmental questionnaire, in regards to, how many cigarettes were smoked per day, the duration of smoking in years, the cumulative dose of cigarette smoking (in some studies referred to as pack-years) and when cessation of smoking was in relationship to clinical disease onset. Furthermore, it would be interesting to assess potential confounding factors such as residential area, alcoholic drinking habits and occupation. We did not address this in paper II, and this is a limitation. Another limitation was that we did not have any matched healthy controls in this study, so we were therefore not able to establish that smoking was a risk factor for myositis per se.

Several studies have demonstrated that smoking is a risk factor for SLE, RA and multiple sclerosis and contributes to an increased disease activity and disease severity (see chapter 1.1.6.5). Furthermore, smoking can trigger immune responses (anti-CCP autoantibodies in RA and dsDNA in SLE) and the genetics may contribute to which immune responses that may be triggered by smoking [131]. Since 70 % of the newly diagnosed myositis patients have ILD [17], and anti-Jo-1 autoantibody positive myositis patients have an incidence of ILD that approaches 90% [222], it is tempting to speculate that the immune response in the pathogenesis of IIM could be triggered in the lungs by cigarette smoke. In this sense efficient anti-smoking information should be given to our rheumatology patients and their families, on a regular basis.

5.3 PAPER III

The hypothesis to test in this study was that the autoantibody profile in myositis patients is associated with a type I IFN signature, as has been reported in patients with SLE.

We found that the IFN activity in whole blood was significantly higher in patients with myositis compared to HCs, and equal to patients with SLE. We categorized patients into two groups, IFN high (n=41, 45%) and IFN low (n=51, 55%), based on the cut off value for the average gene expression in HC, and found that patients with DM and IBM had higher IFN scores compared to patients with PM. Significantly more patients with DM, regardless of autoantibody status were observed in the IFN high compared to the IFN low group. Furthermore, we found that almost all patients with multispecific autoantibodies have an IFN signature and the IFN score was significantly higher, compared to patients with one or no autoantibodies.

Patients with monospecific autoantibody status were selected (n=30), to determine whether the IFN signature was associated with distinct autoantibody specificities. The IFN signature was clearly present in patients with monospecificity for autoantibodies against RNA-binding proteins (anti-Jo-1 autoantibodies, anti-Ro60 autoantibodies and anti-U1RNP autoantibodies) and was absent in most patients with mono-specificity for autoantibodies against other targets

than RNA-binding proteins (anti-Ro52 autoantibodies and anti-PM/Scl autoantibodies). Furthermore, we found that the IFN score was significantly higher for patients with autoantibodies against RNA-binding proteins compared to the patients without these autoantibodies. When looking at the whole cohort of 92 patients, only one of the IBM patients had detectable autoantibodies targeting RNA binding proteins, and approximately half of the patients with DM diagnosis. Therefore, in these subsets other explanations for the type I IFN signature must be searched for.

Next we investigated if sera from IIM patients (n=20) could activate the type I IFN pathway, i.e. upregulate the IRG expression in healthy PBMCs. Patients were subdivided into IFN high or IFN low, based on the average ex-vivo whole blood IRG induction for all samples. Sera from IFN high patients induced IRG expression in healthy PBMCs after 4 h of incubation, whereas sera from IFN low patients induced significantly lower IRG induction. None of the patients' sera induced IRG expression at 8 h after serum addition, suggesting that the IRG induction is a rapid process. By adding neutralizing IFNAR or anti-IFNα antibodies to sera in another subset of IIM patients (n=25), we wanted to determine whether type I IFNs were responsible for the observed activation of the type I IFN pathway. The type I IFN bioactivity was determined after 4 hours. We found that the IFN bioactivity in IRG high samples, but not IRG low, was significantly inhibited by both anti-IFNα antibodies and IFNAR antibodies, indicating that IFNα in serum was responsible for most of the observed IRG induction in healthy PBMCs.

To further increase the understanding of the role of type I IFN in pathogenesis of IIM, we wanted to explore whether the IFN score correlated to clinical manifestations or laboratory variables. The only correlation we found, was a low degree of correlation between the extent of the IFN score and disease activity (physician's global disease activity assessment) for patients with DM, but not for the whole group with IIM. There was no correlation seen between disease activity measures or laboratory variables and IFN score for PM and IBM. This observation may suggest that the IFN activity could possibly be genetically determined.

One of the limitations in this study could be that the selection of controls and the SLE patients were recruited from the Netherlands, while the IIM patients were from Sweden and the Czech republic. Importantly all the analyzes were done in the same laboratory. Another limitation was that when we divided our 92 patients into subgroups, based on either diagnosis or autoantibody mono-and multispecificity the number of patients in each group is small. A strength on the other hand, was that the subgroups of myositis were well defined. If we have had unlimited blood sample volumes for all 92 patients, it would have been interesting to do the IRG induction assay in all 92 samples, to get a more complete view of the IRG induction process. A technical limitation was the absence of isotype controls in the experiments with anti-IFNα-and anti-IFNAR antibodies. However, we did not have these isotype controls available at that time. Moreover, we did not have a specific anti-IFNβ antibody that would have enabled us to distinguish between IFNα-and IFNβ-mediated responses. We found that patients with autoantibodies against RNA-binding proteins had a significantly higher IFN score compared to other patients. However, a causal relationship was not demonstrated and was not in the scope of this paper and would need additional studies. ICs containing RNA binding proteins could contribute to the IFN signature and other factors in sera e.g.

interleukins could also contribute to the IFN activity as well as gene variants in the type I IFN signaling pathway.

In one of our previous studies (abstract and poster ACR, November 2011, Ekholm et al), we studied a cohort of 132 myositis patient to assess if the type I IFN activity in sera (using an *in vitro* system with a cell line) correlated with disease activity and/or autoantibody status and genetic variables. Patients were categorized into two groups, IFN high (n=13) and IFN low (n=119), based on an IFN score. We found a trend for higher self-perceived pain among the IFN high patients compared to the IFN low patients (figure 7a). Furthermore, the IFN low patients had significantly higher doses of prednisolone compared to the IFN high patients (p=0.002) (figure 7b) and this can possibly be explained by the fact that glucocorticoid treatment may suppress the type I IFN activity. Significantly more IFN high patients were positive for ANA compared to IFN low patients (p=0.001)(figure 7c). This is in line with reports demonstrating nucleic acid-containing ICs as endogenous inducers of type I IFN. In this cohort there were too few patients in the IFN high group to subgroup the patients into MSA specificities.

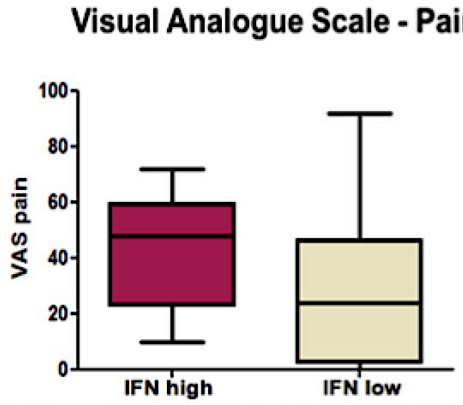

Figure 7a. VAS pain in IFN high and IFN low patients.

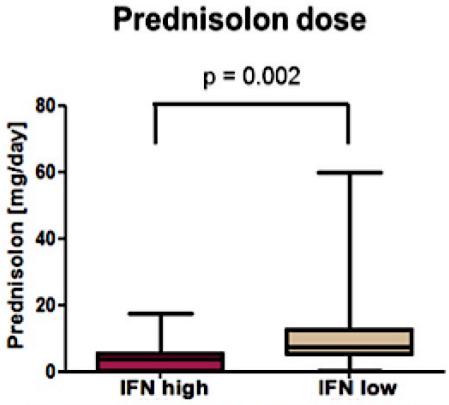

Figure 7b. Prednisolone dose in IFN high and IFN low patients.

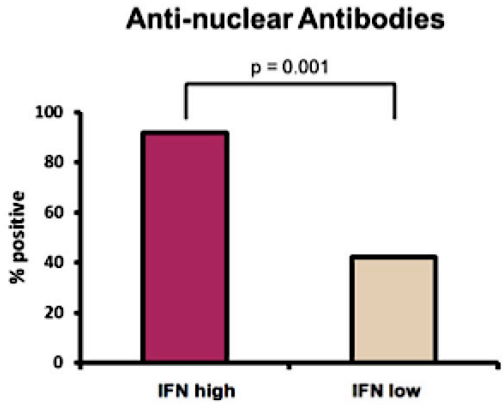

Figure 7c. ANA positivity in IFN high and IFN low patients.

In conclusion our study underscores that different molecular pathways, such as the type I IFN pathway, may predominate in different subsets of myositis, emphasizing the need for careful molecular phenotyping of patients to gain better understanding of molecular pathogenesis and to improve and individualize treatment. Patients with a multispecific autoantibody profile,

patients with autoantibodies against RNA-binding proteins and patients with DM/IBM regardless of autoantibody profile, have an activated type I IFN pathway. This indicates that the mechanism behind the IFN induction may differ between DM/IBM patients and patients with antibodies to RNA-binding proteins, where the latter group has a potential endogenous factor that can activate pDCs to produce type I IFN, whereas the mechanisms for IFN induction in patients with DM without these antibodies remain to be defined. Gene variants in the type I IFN signaling pathway and other factors in sera, such as ILs and NETs could play a role. IFNα-blocking drugs are on the market, and clinical trials with anti-IFNα monoclonal antibodies suggest beneficial effects in SLE [63], as well as in myositis [65]. Further studies concerning the role of the IFN signature in carefully phenotyped myositis patients are needed.

5.4 PAPER IV

In this study our hypothesis was that patients with PM and DM have dysregulation of EPCs driven by type I IFN and IL-18 similar to other autoimmune diseases, such as SLE.

We found a significantly lower number of circulating EPCs in patients with PM and DM compared to controls. Furthermore, there was a significant reduction in circulating EPCs when comparing PM alone vs controls, but not when comparing DM alone vs controls. We also demonstrated that EPCs from both PM and DM patients had a significantly lower capacity to differentiate into mature ECs, i.e. they had defect monolayer formation, when compared to healthy controls. This significance persisted when DM was compared to controls ($p=0.0350$), but not when PM was compared to controls.

Next, we investigated if sera from patients with PM or DM inhibited EPCs differentiation into mature ECs. Patients were categorized into two groups: a) serum that inhibited EPC differentiation or b) serum that left unchanged EPC differentiation (based on a cut-off of 90% of healthy controls). Significant inhibition of EPC differentiation into ECs compared to controls was detected in 25 patients (70%), whereas no significant inhibition was detected in 11 patients (30%). Of the 25 patients in which sera had an inhibitory effect on EPC differentiation compared to healthy control serum, 60% (15 patients) showed improvement of abnormal EPC phenotype with IL-18 neutralization and 52% (13 patients) showed improvement with type I IFN receptor neutralization. In 2 of these patients (8%) the effect was type I IFN receptor specific and in 4 patients (16%), the effect was IL-18 blocking specific. However, in 11 patients (44%), the effect was reversed by both strategies and in 8 patients (32%), the inhibition of EPC differentiation could not be abrogated by any of the blocking strategies.

Furthermore, we found that patients with PM and DM had significantly higher serum levels of IL-18, 73 (5-285) pg/ml versus undetectable in controls ($p=<0.0001$). However, IFN score or IL-18 concentration did not correlate with *in vitro* improvement of the EPC differentiation with respective cytokine blockade. Interestingly, sera displaying EPC inhibitory activity had significantly higher type I IFN serum activity (i.e. IFN score) than sera that did not display inhibition of EPC function. In contrast, significantly higher LDH levels and CRP levels were seen in those patients whose sera had a lower type I IFN serum activity i.e. did not inhibit

EPC differentiation. This would be in line with results seen in SLE, where type I IFNs inhibit CRP upregulation [301].

Clinical signs of capillary loss in muscle tissue and microvasculature disturbances in nailfold vessels are well known clinical features of both adult and juvenile DM, but the underlying mechanisms behind this disturbance of microvessels have not been clarified. Likewise, endothelial dysfunction and activation has been described previously in JDM [302]. It was shown that VCAM-1 expression was increased in both muscle biopsies and in blood, in children with untreated JDM with short disease duration (≤ 2 months) and this supports a disturbance in the ECs early in the disease. Activated ECs (but not quiescent ECs) can release VCAM-1 resulting in soluble vascular cell adhesion molecule-1 (sVCAM-1) (Lebranchu,Y et al,1997), promoting inflammation and muscle tissue damage in JDM [303]. In DM, VCAM-1 has been inconsistently found in muscle and blood [304-307]. To determine if the EPC numbers reflect ongoing EC activation, we decided to evaluate the levels of sVCAM-1 in our patient cohort (unpublished data), but we did not see any correlation between sVCAM-1 and EPC numbers. This might be because EC damage is not necessarily correlated with low numbers, rather it is related to a defect bone marrow synthesis. Furthermore, we did not see a correlation with sVCAM-1 and monolayer formation nor did we see a correlation between IL-18 and sVCAM-1. The correlation for sVCAM-1 and serum induced EPC inhibition was inverse, but not significant. There was no significant statistical difference between serum levels of sVCAM-1 in controls and myositis patients (figure 8), but there was a trend for an increase in the myositis group. Our control numbers are very low, likely keeping the difference from possibly being significant. The median disease duration in our cohort was 2.2 years and most patients were treated with immunosuppressive treatment, so that might be an explanation why we did not see a correlation to sVCAM-1.

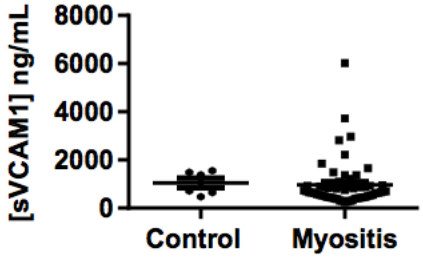

Figure 8. Soluble VCAM-1 levels in control and myositis patients. Serum or plasma levels were measured in control (n=6) and myositis (n=53) patients. p=0.21 between control and myositis patients via Mann-Whitney U-test.

There are some limitations in this study, such as not having flow cytometry data, monolayer data and the data from sera's effects on EPC differentiation from the same patients. Furthermore, some of the serum samples were from 2004 and these older serum samples might compromise the IFN assays, since the IFN molecules are unstable and in general difficult to measure directly in humans and freezing and thawing of samples will degrade the

IFN molecules. Yet another limitation is that we were not able to make clinical implications related to the muscle biopsy findings. This was because we had very few biopsies available with staining for CD-31 (endothelial cell marker), and of the muscle biopsies we had, we could only detect perifascicular atrophy (as a sign of loss of capillaries) in 3 out of the 36 patients. We further did correlations between Functional Index 2 (FI-2) [308] (i.e., how well patients perform in repetitive muscle endurance) and EPC differentiation, and we did not see any significant difference in FI-2 between those patients with impaired EPC differentiation and those without impaired EPC differentiation. However, most of our patients were at time of blood sampling under treatment with immunosuppressive agents and had a median disease duration of 2.2 years, which might explain that no correlation was seen. Somewhat surprisingly we found the aberrant EPC differentiation in patients diagnosed with DM as well as with PM. This supports a role of the vascular tree in both these subsets and is supported by a previous report from our research group where both patients with DM and PM had elevated expression of VEGF in muscle biopsies as an indication of disturbed microcirculation in muscle tissue [170].

The EPC dysfunction seen in both PM and DM patients could possibly contribute to an increased risk of thromboembolic events such as deep venous thrombosis (DVT), pulmonary embolism (PE), acute myocardial infarction (AMI) and atherosclerosis. An increased risk of cardiovascular disease (CVD) has been shown in other autoimmune diseases, including SLE [309], RA [310] and SSc [311]. Data on CVD in myositis patients are rare, and have mainly focused on atherosclerotic disease [312, 313]. In a case-control study with 798 myositis patients an increased risk of DVT and PE was seen in the myositis patients compared to controls, especially within the initial years of diagnosis [314]. Another case-control study has shown that DM patients are at an increased risk of AMI and ischemic stroke [312]. However, further research is needed in this field and the need for thromboprofylaxis may be considered.

In conclusion, EPCs isolated from patients with PM and DM were decreased in numbers and in function. These findings are likely triggered by the type I IFN/IL-18 axis. Further studies are needed to evaluate whether progression of vascular damage, muscle-and organ specific outcomes in PM and DM are associated to EPC dysregulation in association with the type I IFN pathway. IFNα blocking treatment is currently being explored in PM and DM. It will be important to incorporate diagnostic markers of vascular repair and vasculopathy as part of the evaluation of therapy and its efficacy.

6 SUMMARY AND HYPOTHESIS

In this thesis we found that MSAs are important tools for the diagnosis and subclassification of IIM and that the line blot assay, which is a rapid and simple method, is a reliable alternative to more time consuming procedures such as IPP. Furthermore, in paper II our results led us to hypothesize that a gene-environmental (smoking) interaction may prime the development of the subphenotype with anti-Jo-1 autoantibodies in myositis patients. In paper III we found that the IFN score was significantly higher in myositis patients compared to HCs and equal to that seen in SLE patients. Also, the IFN score in patients with autoantibodies to

RNA-binding proteins (compared to patients without these autoantibodies) and in patients with a multispecific autoantibody status (compared to those patients with one or no autoantibodies), and in DM and IBM patients, was increased. A low degree of correlation was seen between the IFN score and physician's global disease activity assessment in DM patients. Interestingly, in paper IV we found that type I IFNs and IL-18 play a role in inducing abnormal function of EPCs/ECs in both PM and DM in a majority of patients, and that the type I IFN serum activity is associated to disruptions in EPC function.

Apart from our results above, type I IFNs are known to have many immunomodulatory functions such as inhibitory effects on the bone marrow precursors, such as EPCs, which may lead to defect vascular repair. IFNα can mediate EPC/CAC dysfunction via inflammasome upregulation and scewing toward IL-18 production in SLE, and has been shown to be antiangiogenic. We also know that the type I IFN can cause T cell/B cell activation and autoantibody production. In response to viral or bacterial invasion or RNA-containing ICs, pDCs rapidly produce IFNα (figure 9, B).

Since SLE and IIM are both systemic autoimmune diseases, autoantibodies are common features, and accumulating data suggest a role of type I IFN system in both SLE and IIM, it is possible to hypothesize that there might be similarities in their disease pathogenesis (figure 9). Smoking has been related to autoantibodies (dsDNA) in SLE and we have also shown an association between smoking and autoantibodies (anti-Jo-1) in genetic susceptible myositis patients (figure 9, A). New evidence points toward a plausible role for NETosis, in the pathogenesis of SLE. One earlier study suggest a role of NETs in myositis, especially in patients with ILD [192]. NETs contain DNA/antimicrobial peptide complexes and are released in response to ICs (containing anti-RNP autoantibodies) or bacterial/viral infection (figure 9, C). Furthermore, EC activation can elicit NETosis, and NETs in turn can induce vascular damage [315] (figure 9, D). NETs may result in exposure to antigenic material, toward which autoantibodies can be directed (anti-NET-autoantibody) [315]. This can be triggered by IFNα. NETs might also contribute to B-cell activation and autoantibody production (figure 9, E). NETs have been shown to enhance IFNα production in pDCs [189, 316] and IFNα can in turn prime neutrophils for enhanced NETosis (figure 9, F). Furthermore, NETs can activate the inflammasome in macrophages to induce the synthesis of IL-1β and IL-18, both of which can stimulate NETosis [317] (figure 9, G). We hypothesize that NETosis might play a role in the pathogenic mechanism, not only in SLE, but also in myositis, at least in some subgroups, in addition to similarities in the type I IFN system, presence of autoantibodies, IL-18 and EPC/EC dysfunction. Furthermore, positive feedback loops between type I IFN, NETs and inflammasome can perpetuate the inflammation.

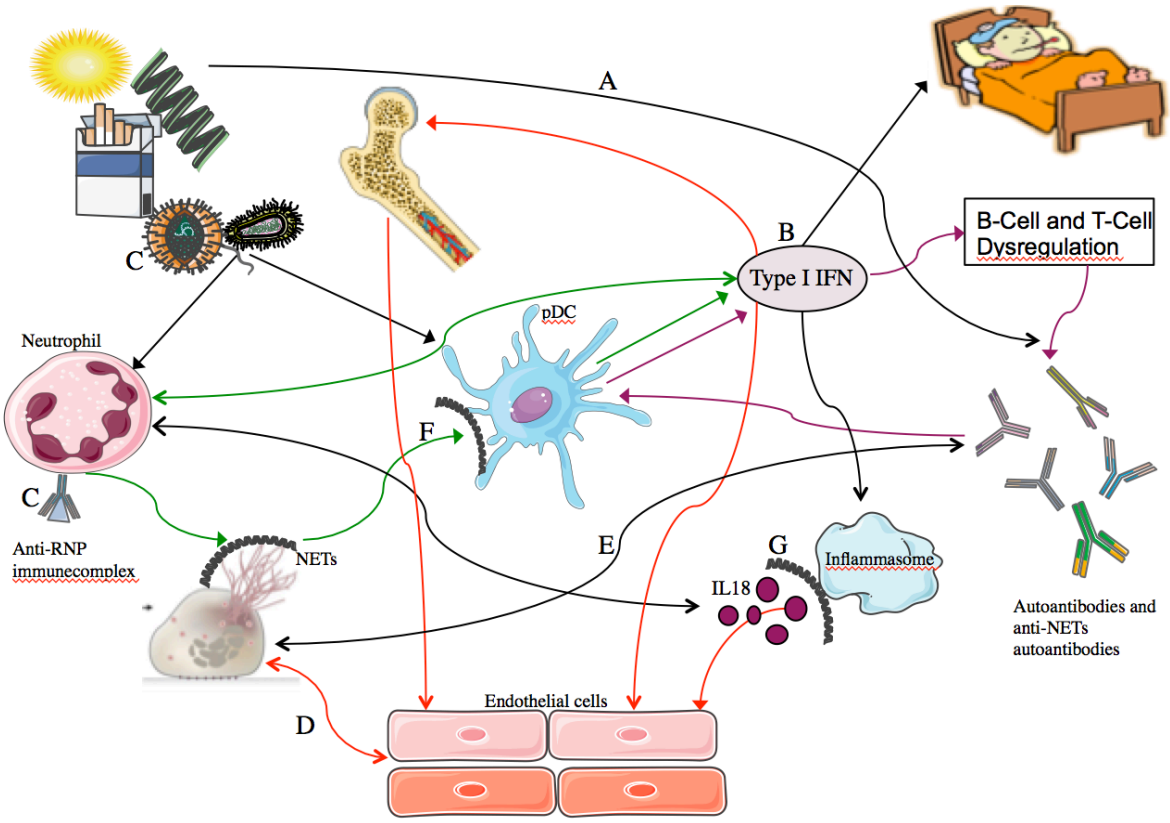

Figure 9. Simplified schematic illustration of the major findings in this thesis and a potential hypothesis associated to these findings. Some symbols are provided by courtesy of Servier. Red arrows indicate inhibition and black arrows stimulation, green arrows indicate a loop of NETs activating pDCs to produce IFNα which in turn stimulates NETosis. Purple arrows indicate a loop where type I IFN activates B-cells to autoantibody production which in turn stimulates pDCs to produce type I IFN.

7 CONCLUSIONS AND FUTURE PERSPECTIVES

In this thesis I aimed to explore and learn more about the pathogenesis in IIM and I conclude the following:

- MSAs more than MAAs are useful tools for the diagnosis and classification of IIM
- Line blot assay is a reliable alternative method to IPP.
- Smoking appears to be associated with an increased risk of possession of anti-Jo-1 autoantibodies in *HLA-DRB1*03*-positive IIM patients, and we hypothesis that an interaction between *HLA-DRB1*03* and smoking may prime the development of anti-Jo-1 autoantibodies.
- The type I IFN pathway is activated in IIM patients with (i) autoantibodies against RNA-binding proteins and in patients with (ii) DM/IBM, regardless of autoantibody status and in patients (iii) with autoantibody multispecificity.
- A low degree of correlation between the extent of the IFN score and disease activity was found in patients with DM.
- IFNα was mainly responsible for the type I IFN activity in myositis patients.
- PM and DM is associated with dysregulation of EPC phenotype and function that may be attributed, at least in part, to aberrant IL-18 and type I IFN pathways.

Myositis is a heterogeneous group of autoimmune diseases and this thesis has shown that different pathogenic mechanisms are likely to contribute to the different subsets of disease. It is therefore of utter importance to carefully identify the different clinical subgroups, taken into account such factors as the autoantibody profile, the IFN signature and vascular endothelial markers. IFNα blocking treatment is already on the market, and could potentially be used in patients where an increased IFN-signature is associated with disease activity, and/or endothelial dysfunction.

In paper I, we validated the line blot assay containing 7 antigens. The new line blot strips have 9 additional antigens. These 9 new antigens also need to be validated compared to IPP/immunoblot and there is an ongoing validation study within Myonet (www.myonet.eu, a global multicenter, interdisciplinary research project on IIM, involving clinicians and scientists) in collaboration with Professor Johan Rönnelid from Uppsala Akademiska Hospital, Uppsala, Sweden.

In paper II we could not show a correlation between smoking and the risk of myositis per se, due to the lack of healthy controls. Further studies are required to address this question. There is an international (Europe and USA) case study ongoing, with an epidemiological questionnaire concerning smoking and other environmental triggers, to be able to identify risk factors for myositis. Also a case-control study in Sweden is ongoing using this epidemiological questionnaire.

All studies in this thesis are cross-sectional. Therefore, to take the results from paper III and IV further, it would be interesting to make a study with newly diagnosed IIM patients (with autoantibody profiles) and evaluate their IFN-signature, serum IL-18 levels and disease activity and treat those patients who have an IFN signature, with IFNα blocking treatment and study the effect of this treatment regarding disease activity and effects on the type I IFN

signature and IFNα and IFNβ levels in longitudinally followed patients. Furthermore, it would be interesting to enroll myositis patients and study the phenotype and function of EPCs and also study the effect of myositis sera in vitro from a longitudinal study, before and after treatment (IFNα blocking), with regards to the IFN signature and IL-18 levels, and also make clinical correlations to disease activity and muscle endurance. In such a study it would be very interesting to study muscle biopsies, before and after treatment, to detect possible vascular rarefication and correlate this to the IFN signature, IL-18 levels and disease activity. Similar to studies in JDM, it would be interesting to address the role of the adhesion molecules, such as VCAM-1, in newly diagnosed IIM patients as compared to those with longer disease duration. The different subgroups in such a study would need to be carefully defined according to disease duration, autoantibody status, disease activity, smoking status and HLA-typing.

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9 REFERENCES

1 Dalakas MC. Polymyositis, dermatomyositis and inclusion-body myositis. N Engl J Med 1991;325(21):1487-98.

2 Christopher-Stine L, Casciola-Rosen LA, Hong G, Chung T, Corse AM, Mammen AL. A novel autoantibody recognizing 200-kd and 100-kd proteins is associated with an immune-mediated necrotizing myopathy. Arthritis Rheum 2010;62(9):2757-66.

3 Betteridge ZE, Gunawardena H, McHugh NJ. Novel autoantibodies and clinical phenotypes in adult and juvenile myositis. Arthritis Res Ther 2011;13(2):209.

4 Chou SM. Myxovirus-like structures in a case of human chronic polymyositis. Science 1967;158(3807):1453-5.

5 Yunis EJ, Samaha FJ. Inclusion body myositis. Lab Invest 1971;25(3):240-8.

6 Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). N Engl J Med 1975;292(7):344-7.

7 Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). N Engl J Med 1975;292(8):403-7.

8 Calabrese LH, Mitsumoto H, Chou SM. Inclusion body myositis presenting as treatment-resistant polymyositis. Arthritis Rheum 1987;30(4):397-403.

9 Griggs RC, Askanas V, DiMauro S, et al. Inclusion body myositis and myopathies. Ann Neurol 1995;38(5):705-13.

10 Lundberg IE, Miller FW, Tjarnlund A, Bottai M. Diagnosis and classification of idiopathic inflammatory myopathies. J Intern Med 2016;280(1):39-51.

11 Badrising UA, Maat-Schieman ML, van Houwelingen JC, et al. Inclusion body myositis. Clinical features and clinical course of the disease in 64 patients. J Neurol 2005;252(12):1448-54.

12 Arsura EL, Greenberg AS. Adverse impact of interstitial pulmonary fibrosis on prognosis in polymyositis and dermatomyositis. Semin Arthritis Rheum 1988;18(1):29-37.

13 Takizawa H, Shiga J, Moroi Y, Miyachi S, Nishiwaki M, Miyamoto T. Interstitial lung disease in dermatomyositis: clinicopathological study. J Rheumatol 1987;14(1):102-7.

14 Danko K, Ponyi A, Constantin T, Borgulya G, Szegedi G. Long-term survival of patients with idiopathic inflammatory myopathies according to clinical features: a longitudinal study of 162 cases. Medicine (Baltimore) 2004;83(1):35-42.

15 Sontheimer RD. Skin manifestations of systemic autoimmune connective tissue disease: diagnostics and therapeutics. Best Pract Res Clin Rheumatol 2004;18(3):429-62.

16 Callen JP, Wortmann RL. Dermatomyositis. Clin Dermatol 2006;24(5):363-73.

17 Fathi M, Dastmalchi M, Rasmussen E, Lundberg IE, Tornling G. Interstitial lung disease, a common manifestation of newly diagnosed polymyositis and dermatomyositis. Ann Rheum Dis 2004;63(3):297-301.

18 Marie I, Hachulla E, Cherin P, et al. Interstitial lung disease in polymyositis and dermatomyositis. Arthritis Rheum 2002;47(6):614-22.

19 Lakhanpal S, Lie JT, Conn DL, Martin WJ, 2nd. Pulmonary disease in polymyositis/dermatomyositis: a clinicopathological analysis of 65 autopsy cases. Ann Rheum Dis 1987;46(1):23-9.

20 Chen YJ, Wu CY, Shen JL. Predicting factors of interstitial lung disease in dermatomyositis and polymyositis. Acta Derm Venereol 2007;87(1):33-8.

21 Taylor AJ, Wortham DC, Burge JR, Rogan KM. The heart in polymyositis: a prospective evaluation of 26 patients. Clin Cardiol 1993;16(11):802-8.

22 Sharratt GP, Danta G, Carson PH. Cardiac abnormality in polymyositis. Ann Rheum Dis 1977;36(6):575-8.

23 Diederichsen LP, Simonsen JA, Diederichsen AC, et al. Cardiac abnormalities assessed by non-invasive techniques in patients with newly diagnosed idiopathic inflammatory myopathies. Clin Exp Rheumatol 2015;33(5):706-14.

24 Klein M, Mann H, Plestilova L, et al. Arthritis in idiopathic inflammatory myopathy: clinical features and autoantibody associations. J Rheumatol 2014;41(6):1133-9.

25 Hill CL, Zhang Y, Sigurgeirsson B, et al. Frequency of specific cancer types in dermatomyositis and polymyositis: a population-based study. Lancet 2001;357(9250):96-100.

26 Stockton D, Doherty VR, Brewster DH. Risk of cancer in patients with dermatomyositis or polymyositis, and follow-up implications: a Scottish population-based cohort study. Br J Cancer 2001;85(1):41-5.

27 Chinoy H, Fertig N, Oddis CV, Ollier WE, Cooper RG. The diagnostic utility of myositis autoantibody testing for predicting the risk of cancer-associated myositis. Ann Rheum Dis 2007;66(10):1345-9.

28 Trallero-Araguas E, Rodrigo-Pendas JA, Selva-O'Callaghan A, et al. Usefulness of anti-p155 autoantibody for diagnosing cancer-associated dermatomyositis: a systematic review and meta-analysis. Arthritis Rheum 2012;64(2):523-32.

29 Ichimura Y, Matsushita T, Hamaguchi Y, et al. Anti-NXP2 autoantibodies in adult patients with idiopathic inflammatory myopathies: possible association with malignancy. Ann Rheum Dis 2012;71(5):710-3.

30 Fiorentino DF, Chung LS, Christopher-Stine L, et al. Most patients with cancer-associated dermatomyositis have antibodies to nuclear matrix protein NXP-2 or transcription intermediary factor 1gamma. Arthritis Rheum 2013;65(11):2954-62.

31 Miller FW, Rider LG, Chung YL, et al. Proposed preliminary core set measures for disease outcome assessment in adult and juvenile idiopathic inflammatory myopathies. Rheumatology (Oxford) 2001;40(11):1262-73.

32 Rider LG, Giannini EH, Brunner HI, et al. International consensus on preliminary definitions of improvement in adult and juvenile myositis. Arthritis Rheum 2004;50(7):2281-90.

33 Fries JF, Spitz P, Kraines RG, Holman HR. Measurement of patient outcome in arthritis. Arthritis Rheum 1980;23(2):137-45.

34 Rider LG, Werth VP, Huber AM, et al. Measures of adult and juvenile dermatomyositis, polymyositis, and inclusion body myositis: Physician and Patient/Parent Global Activity, Manual Muscle Testing (MMT), Health Assessment Questionnaire (HAQ)/Childhood Health Assessment Questionnaire (C-HAQ), Childhood Myositis Assessment Scale (CMAS), Myositis Disease Activity Assessment Tool (MDAAT), Disease

Activity Score (DAS), Short Form 36 (SF-36), Child Health Questionnaire (CHQ), physician global damage, Myositis Damage Index (MDI), Quantitative Muscle Testing (QMT), Myositis Functional Index-2 (FI-2), Myositis Activities Profile (MAP), Inclusion Body Myositis Functional Rating Scale (IBMFRS), Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI), Cutaneous Assessment Tool (CAT), Dermatomyositis Skin Severity Index (DSSI), Skindex, and Dermatology Life Quality Index (DLQI). Arthritis Care Res (Hoboken) 2011;63 Suppl 11:S118-57.

35 Dalakas MC. Immunotherapy of myositis: issues, concerns and future prospects. Nat Rev Rheumatol 2010;6(3):129-37.

36 Guiducci C, Gong M, Xu Z, et al. TLR recognition of self nucleic acids hampers glucocorticoid activity in lupus. Nature 2010;465(7300):937-41.

37 Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991;34(9):1125-32.

38 Crow MK, Wohlgemuth J. Microarray analysis of gene expression in lupus. Arthritis Res Ther 2003;5(6):279-87.

39 Ronnblom L, Alm GV. A pivotal role for the natural interferon alpha-producing cells (plasmacytoid dendritic cells) in the pathogenesis of lupus. J Exp Med 2001;194(12):F59-63.

40 Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 1984;5(1):25-44.

41 Rook GA, Hernandez-Pando R, Lightman SL. Hormones, peripherally activated prohormones and regulation of the Th1/Th2 balance. Immunol Today 1994;15(7):301-3.

42 Henriksson KG, Sandstedt P. Polymyositis--treatment and prognosis. A study of 107 patients. Acta Neurol Scand 1982;65(4):280-300.

43 Souverein PC, Berard A, Van Staa TP, et al. Use of oral glucocorticoids and risk of cardiovascular and cerebrovascular disease in a population based case-control study. Heart 2004;90(8):859-65.

44 de Vries F, Pouwels S, Lammers JW, et al. Use of inhaled and oral glucocorticoids, severity of inflammatory disease and risk of hip/femur fracture: a population-based case-control study. J Intern Med 2007;261(2):170-7.

45 Barohn RJ, Amato AA, Sahenk Z, Kissel JT, Mendell JR. Inclusion body myositis: explanation for poor response to immunosuppressive therapy. Neurology 1995;45(7):1302-4.

46 Zong M, Lundberg IE. Pathogenesis, classification and treatment of inflammatory myopathies. Nat Rev Rheumatol 2011;7(5):297-306.

47 Vencovsky J, Jarosova K, Machacek S, et al. Cyclosporine A versus methotrexate in the treatment of polymyositis and dermatomyositis. Scand J Rheumatol 2000;29(2):95-102.

48 Caramaschi P, Volpe A, Carletto A, Bambara LM, Biasi D. Long-standing refractory polymyositis responding to mycophenolate mofetil: a case report and review of the literature. Clin Rheumatol 2007;26(10):1795-6.

49 Gelber AC, Nousari HC, Wigley FM. Mycophenolate mofetil in the treatment of severe skin manifestations of dermatomyositis: a series of 4 cases. J Rheumatol 2000;27(6):1542-5.

50 Lopez de la Osa A, Sanchez Tapia C, Arias Diaz M, Terrancle de Juan I. [Antisynthetase syndrome with good response to mycophenolate mofetil]. Rev Clin Esp 2007;207(5):269-70.

51 Dalakas MC, Illa I, Dambrosia JM, et al. A controlled trial of high-dose intravenous immune globulin infusions as treatment for dermatomyositis. N Engl J Med 1993;329(27):1993-2000.

52 Mastaglia FL, Phillips BA, Zilko PJ. Immunoglobulin therapy in inflammatory myopathies. J Neurol Neurosurg Psychiatry 1998;65(1):107-10.

53 Dastmalchi M, Grundtman C, Alexanderson H, et al. A high incidence of disease flares in an open pilot study of infliximab in patients with refractory inflammatory myopathies. Ann Rheum Dis 2008;67(12):1670-7.

54 Fasano S, Gordon P, Hajji R, Loyo E, Isenberg DA. Rituximab in the treatment of inflammatory myopathies: a review. Rheumatology (Oxford) 2016.

55 Nagaraju K, Ghimbovschi S, Rayavarapu S, et al. Muscle myeloid type I interferon gene expression may predict therapeutic responses to rituximab in myositis patients. Rheumatology (Oxford) 2016.

56 Zong M, Dorph C, Dastmalchi M, et al. Anakinra treatment in patients with refractory inflammatory myopathies and possible predictive response biomarkers: a mechanistic study with 12 months follow-up. Ann Rheum Dis 2014;73(5):913-20.

57 Furlan A, Botsios C, Ruffatti A, Todesco S, Punzi L. Antisynthetase syndrome with refractory polyarthritis and fever successfully treated with the IL-1 receptor antagonist, anakinra: A case report. Joint Bone Spine 2008;75(3):366-7.

58 Narazaki M, Hagihara K, Shima Y, Ogata A, Kishimoto T, Tanaka T. Therapeutic effect of tocilizumab on two patients with polymyositis. Rheumatology (Oxford) 2011;50(7):1344-6.

59 Kondo M, Murakawa Y, Matsumura T, et al. A case of overlap syndrome successfully treated with tocilizumab: a hopeful treatment strategy for refractory dermatomyositis? Rheumatology (Oxford) 2014;53(10):1907-8.

60 Musuruana JL, Cavallasca JA. Abatacept for treatment of refractory polymyositis. Joint Bone Spine 2011;78(4):431-2.

61 Arabshahi B, Silverman RA, Jones OY, Rider LG. Abatacept and sodium thiosulfate for treatment of recalcitrant juvenile dermatomyositis complicated by ulceration and calcinosis. J Pediatr 2012;160(3):520-2.

62 Kerola AM, Kauppi MJ. Abatacept as a successful therapy for myositis-a case-based review. Clin Rheumatol 2015;34(3):609-12.

63 Merrill JT, Wallace DJ, Petri M, et al. Safety profile and clinical activity of sifalimumab, a fully human anti-interferon alpha monoclonal antibody, in systemic lupus erythematosus: a phase I, multicentre, double-blind randomised study. Ann Rheum Dis 2011;70(11):1905-13.

64 McBride JM, Jiang J, Abbas AR, et al. Safety and pharmacodynamics of rontalizumab in patients with systemic lupus erythematosus: results of a phase I, placebo-controlled, double-blind, dose-escalation study. Arthritis Rheum 2012;64(11):3666-76.

65 Higgs BW, Zhu W, Morehouse C, et al. A phase 1b clinical trial evaluating sifalimumab, an anti-IFN-alpha monoclonal antibody, shows target neutralisation of a type I IFN signature in blood of dermatomyositis and polymyositis patients. Ann Rheum Dis 2014;73(1):256-62.

66 Guo X, Higgs BW, Rebelatto M, et al. Suppression of soluble T cell-associated proteins by an anti-interferon-alpha monoclonal antibody in adult patients with dermatomyositis or polymyositis. Rheumatology (Oxford) 2014;53(4):686-95.

67 Zschuntzsch J, Voss J, Creus K, et al. Provision of an explanation for the inefficacy of immunotherapy in sporadic inclusion body myositis: quantitative assessment of inflammation and beta-amyloid in the muscle. Arthritis Rheum 2012;64(12):4094-103.

68 Dalakas M. IVIg in other autoimmune neurological disorders: current status and future prospects. J Neurol 2008;255 Suppl 3:12-6.

69 Escalante A, Miller L, Beardmore TD. Resistive exercise in the rehabilitation of polymyositis/dermatomyositis. J Rheumatol 1993;20(8):1340-4.

70 Hicks JE, Miller F, Plotz P, Chen TH, Gerber L. Isometric exercise increases strength and does not produce sustained creatinine phosphokinase increases in a patient with polymyositis. J Rheumatol 1993;20(8):1399-401.

71 Dastmalchi M, Alexanderson H, Loell I, et al. Effect of physical training on the proportion of slow-twitch type I muscle fibers, a novel nonimmune-mediated mechanism for muscle impairment in polymyositis or dermatomyositis. Arthritis Rheum 2007;57(7):1303-10.

72 Alexanderson H, Stenstrom CH, Jenner G, Lundberg I. The safety of a resistive home exercise program in patients with recent onset active polymyositis or dermatomyositis. Scand J Rheumatol 2000;29(5):295-301.

73 Alexanderson H, Dastmalchi M, Esbjornsson-Liljedahl M, Opava CH, Lundberg IE. Benefits of intensive resistance training in patients with chronic polymyositis or dermatomyositis. Arthritis Rheum 2007;57(5):768-77.

74 Alemo Munters L, Dastmalchi M, Andgren V, et al. Improvement in health and possible reduction in disease activity using endurance exercise in patients with established polymyositis and dermatomyositis: a multicenter randomized controlled trial with a 1-year open extension followup. Arthritis Care Res (Hoboken) 2013;65(12):1959-68.

75 Alexanderson H, Munters LA, Dastmalchi M, et al. Resistive home exercise in patients with recent-onset polymyositis and dermatomyositis -- a randomized controlled single-blinded study with a 2-year followup. J Rheumatol 2014;41(6):1124-32.

76 Nader GA, Dastmalchi M, Alexanderson H, et al. A longitudinal, integrated, clinical, histological and mRNA profiling study of resistance exercise in myositis. Mol Med 2010;16(11-12):455-64.

77 Hochberg MC. Epidemiology of polymyositis/dermatomyositis. Mt Sinai J Med 1988;55(6):447-52.

78 Medsger TA, Jr., Robinson H, Masi AT. Factors affecting survivorship in polymyositis. A life-table study of 124 patients. Arthritis Rheum 1971;14(2):249-58.

79 Oddis CV, Conte CG, Steen VD, Medsger TA, Jr. Incidence of polymyositis-dermatomyositis: a 20-year study of hospital diagnosed cases in Allegheny County, PA 1963-1982. J Rheumatol 1990;17(10):1329-34.

80 Weitoft T. Occurrence of polymyositis in the county of Gavleborg, Sweden. Scand J Rheumatol 1997;26(2):104-6.

81 Tan JA, Roberts-Thomson PJ, Blumbergs P, Hakendorf P, Cox SR, Limaye V. Incidence and prevalence of idiopathic inflammatory myopathies in South Australia: a 30-year epidemiologic study of histology-proven cases. Int J Rheum Dis 2013;16(3):331-8.

82 Benbassat J, Geffel D, Zlotnick A. Epidemiology of polymyositis-dermatomyositis in Israel, 1960-76. Isr J Med Sci 1980;16(3):197-200.

83 Peloro TM, Miller OF, 3rd, Hahn TF, Newman ED. Juvenile dermatomyositis: a retrospective review of a 30-year experience. J Am Acad Dermatol 2001;45(1):28-34.

84 Medsger TA, Jr., Dawson WN, Jr., Masi AT. The epidemiology of polymyositis. Am J Med 1970;48(6):715-23.

85 Meyer A, Meyer N, Schaeffer M, Gottenberg JE, Geny B, Sibilia J. Incidence and prevalence of inflammatory myopathies: a systematic review. Rheumatology (Oxford) 2015;54(1):50-63.

86 Okada S, Weatherhead E, Targoff IN, Wesley R, Miller FW, International Myositis Collaborative Study G. Global surface ultraviolet radiation intensity may modulate the clinical and immunologic expression of autoimmune muscle disease. Arthritis Rheum 2003;48(8):2285-93.

87 Hengstman GJ, van Venrooij WJ, Vencovsky J, Moutsopoulos HM, van Engelen BG. The relative prevalence of dermatomyositis and polymyositis in Europe exhibits a latitudinal gradient. Ann Rheum Dis 2000;59(2):141-2.

88 Lindberg C, Persson LI, Bjorkander J, Oldfors A. Inclusion body myositis: clinical, morphological, physiological and laboratory findings in 18 cases. Acta Neurol Scand 1994;89(2):123-31.

89 Badrising UA, Maat-Schieman M, van Duinen SG, et al. Epidemiology of inclusion body myositis in the Netherlands: a nationwide study. Neurology 2000;55(9):1385-7.

90 Dobloug GC, Antal EA, Sveberg L, et al. High prevalence of inclusion body myositis in Norway; a population-based clinical epidemiology study. Eur J Neurol 2015;22(4):672-e41.

91 O'Hanlon TP, Rider LG, Schiffenbauer A, et al. Immunoglobulin gene polymorphisms are susceptibility factors in clinical and autoantibody subgroups of the idiopathic inflammatory myopathies. Arthritis Rheum 2008;58(10):3239-46.

92 Chinoy H, Salway F, Fertig N, et al. In adult onset myositis, the presence of interstitial lung disease and myositis specific/associated antibodies are governed by HLA class II haplotype, rather than by myositis subtype. Arthritis Res Ther 2006;8(1):R13.

93 O'Hanlon TP, Carrick DM, Targoff IN, et al. Immunogenetic risk and protective factors for the idiopathic inflammatory myopathies: distinct HLA-A, -B, -Cw, -

DRB1, and -DQA1 allelic profiles distinguish European American patients with different myositis autoantibodies. Medicine (Baltimore) 2006;85(2):111-27.

94 O'Hanlon TP, Miller FW. Genetic risk and protective factors for the idiopathic inflammatory myopathies. Curr Rheumatol Rep 2009;11(4):287-94.

95 Shamim EA, Rider LG, Pandey JP, et al. Differences in idiopathic inflammatory myopathy phenotypes and genotypes between Mesoamerican Mestizos and North American Caucasians: ethnogeographic influences in the genetics and clinical expression of myositis. Arthritis Rheum 2002;46(7):1885-93.

96 Miller FW, Cooper RG, Vencovsky J, et al. Genome-wide association study of dermatomyositis reveals genetic overlap with other autoimmune disorders. Arthritis Rheum 2013;65(12):3239-47.

97 Rothwell S, Cooper RG, Lundberg IE, et al. Dense genotyping of immune-related loci in idiopathic inflammatory myopathies confirms HLA alleles as the strongest genetic risk factor and suggests different genetic background for major clinical subgroups. Ann Rheum Dis 2016;75(8):1558-66.

98 Kariuki SN, Kirou KA, MacDermott EJ, Barillas-Arias L, Crow MK, Niewold TB. Cutting edge: autoimmune disease risk variant of STAT4 confers increased sensitivity to IFN-alpha in lupus patients in vivo. J Immunol 2009;182(1):34-8.

99 Kase S, Shiota G, Fujii Y, et al. Inclusion body myositis associated with hepatitis C virus infection. Liver 2001;21(5):357-60.

100 Ueno Y, Kondo K, Kidokoro N, Kobashi R, Kanaji K, Matsumura T. Hepatitis C infection and polymyositis. Lancet 1995;346(8970):319-20.

101 Nishikai M, Miyairi M, Kosaka S. Dermatomyositis following infection with hepatitis C virus. J Rheumatol 1994;21(8):1584-5.

102 Chevrel G, Calvet A, Belin V, Miossec P. Dermatomyositis associated with the presence of parvovirus B19 DNA in muscle. Rheumatology (Oxford) 2000;39(9):1037-9.

103 Yoshino M, Suzuki S, Adachi K, Fukayama M, Inamatsu T. High incidence of acute myositis with type A influenza virus infection in the elderly. Intern Med 2000;39(5):431-2.

104 Travers RL, Hughes GR, Cambridge G, Sewell JR. Coxsackie B neutralisation titres in polymyositis/dermatomyositis. Lancet 1977;1(8024):1268.

105 Christensen ML, Pachman LM, Schneiderman R, Patel DC, Friedman JM. Prevalence of Coxsackie B virus antibodies in patients with juvenile dermatomyositis. Arthritis Rheum 1986;29(11):1365-70.

106 Dalakas MC, Pezeshkpour GH, Gravell M, Sever JL. Polymyositis associated with AIDS retrovirus. JAMA 1986;256(17):2381-3.

107 Morgan OS, Rodgers-Johnson P, Mora C, Char G. HTLV-1 and polymyositis in Jamaica. Lancet 1989;2(8673):1184-7.

108 Wrzolek MA, Sher JH, Kozlowski PB, Rao C. Skeletal muscle pathology in AIDS: an autopsy study. Muscle Nerve 1990;13(6):508-15.

109 Leff RL, Love LA, Miller FW, et al. Viruses in idiopathic inflammatory myopathies: absence of candidate viral genomes in muscle. Lancet 1992;339(8803):1192-5.

110 Collazos J, Mayo J, Martinez E, Blanco MS. Comparison of the clinical and laboratory features of muscle infections caused by Salmonella and those caused by other pathogens. J Infect Chemother 2001;7(3):169-74.

111 Reimers CD, Pongratz DE, Neubert U, et al. Myositis caused by Borrelia burgdorferi: report of four cases. J Neurol Sci 1989;91(1-2):215-26.

112 Magid SK, Kagen LJ. Serologic evidence for acute toxoplasmosis in polymyositis-dermatomyositis. Increased frequency of specific anti-toxoplasma IgM antibodies. Am J Med 1983;75(2):313-20.

113 Andersson J, Englund P, Sunnemark D, et al. CBA/J mice infected with Trypanosoma cruzi: an experimental model for inflammatory myopathies. Muscle Nerve 2003;27(4):442-8.

114 Pachman LM, Lipton R, Ramsey-Goldman R, et al. History of infection before the onset of juvenile dermatomyositis: results from the National Institute of Arthritis and Musculoskeletal and Skin Diseases Research Registry. Arthritis Rheum 2005;53(2):166-72.

115 Rider LG, Wu L, Mamyrova G, Targoff IN, Miller FW, Childhood Myositis Heterogeneity Collaborative Study G. Environmental factors preceding illness onset differ in phenotypes of the juvenile idiopathic inflammatory myopathies. Rheumatology (Oxford) 2010;49(12):2381-90.

116 Le Quintrec JS, Le Quintrec JL. Drug-induced myopathies. Baillieres Clin Rheumatol 1991;5(1):21-38.

117 Love LA, Miller FW. Noninfectious environmental agents associated with myopathies. Curr Opin Rheumatol 1993;5(6):712-8.

118 Takashima A, Bergstresser PR. Impact of UVB radiation on the epidermal cytokine network. Photochem Photobiol 1996;63(4):397-400.

119 Krutmann J. The interaction of UVA and UVB wavebands with particular emphasis on signalling. Prog Biophys Mol Biol 2006;92(1):105-7.

120 Bielenberg DR, Bucana CD, Sanchez R, Donawho CK, Kripke ML, Fidler IJ. Molecular regulation of UVB-induced cutaneous angiogenesis. J Invest Dermatol 1998;111(5):864-72.

121 Clydesdale GJ, Dandie GW, Muller HK. Ultraviolet light induced injury: immunological and inflammatory effects. Immunol Cell Biol 2001;79(6):547-68.

122 Phillips BA, Zilko PJ, Garlepp MJ, Mastaglia FL. Seasonal occurrence of relapses in inflammatory myopathies: a preliminary study. J Neurol 2002;249(4):441-4.

123 Kamen DL, Cooper GS, Bouali H, Shaftman SR, Hollis BW, Gilkeson GS. Vitamin D deficiency in systemic lupus erythematosus. Autoimmun Rev 2006;5(2):114-7.

124 Cutolo M, Plebani M, Shoenfeld Y, Adorini L, Tincani A. Vitamin D endocrine system and the immune response in rheumatic diseases. Vitam Horm 2011;86:327-51.

125 Azali P, Barbasso Helmers S, Kockum I, et al. Low serum levels of vitamin D in idiopathic inflammatory myopathies. Ann Rheum Dis 2013;72(4):512-6.

126 Freemer MM, King TE, Jr., Criswell LA. Association of smoking with dsDNA autoantibody production in systemic lupus erythematosus. Ann Rheum Dis 2006;65(5):581-4.

127 Ghaussy NO, Sibbitt W, Jr., Bankhurst AD, Qualls CR. Cigarette smoking and disease activity in systemic lupus erythematosus. J Rheumatol 2003;30(6):1215-21.

128 Gustafsson J, Gunnarsson I, Borjesson O, et al. Predictors of the first cardiovascular event in patients with systemic lupus erythematosus - a prospective cohort study. Arthritis Res Ther 2009;11(6):R186.

129 Heliovaara M, Aho K, Aromaa A, Knekt P, Reunanen A. Smoking and risk of rheumatoid arthritis. J Rheumatol 1993;20(11):1830-5.

130 Stolt P, Bengtsson C, Nordmark B, et al. Quantification of the influence of cigarette smoking on rheumatoid arthritis: results from a population based case-control study, using incident cases. Ann Rheum Dis 2003;62(9):835-41.

131 Padyukov L, Silva C, Stolt P, Alfredsson L, Klareskog L. A gene-environment interaction between smoking and shared epitope genes in HLA-DR provides a high risk of seropositive rheumatoid arthritis. Arthritis Rheum 2004;50(10):3085-92.

132 Klareskog L, Stolt P, Lundberg K, et al. A new model for an etiology of rheumatoid arthritis: smoking may trigger HLA-DR (shared epitope)-restricted immune reactions to autoantigens modified by citrullination. Arthritis Rheum 2006;54(1):38-46.

133 Mattey DL, Dawes PT, Fisher J, et al. Nodular disease in rheumatoid arthritis: association with cigarette smoking and HLA-DRB1/TNF gene interaction. J Rheumatol 2002;29(11):2313-8.

134 Saag KG, Cerhan JR, Kolluri S, Ohashi K, Hunninghake GW, Schwartz DA. Cigarette smoking and rheumatoid arthritis severity. Ann Rheum Dis 1997;56(8):463-9.

135 Harrison BJ, Silman AJ, Hider SL, Herrick AL. Cigarette smoking as a significant risk factor for digital vascular disease in patients with systemic sclerosis. Arthritis Rheum 2002;46(12):3312-6.

136 Hudson M, Lo E, Lu Y, et al. Cigarette smoking in patients with systemic sclerosis. Arthritis Rheum 2011;63(1):230-8.

137 Harel-Meir M, Sherer Y, Shoenfeld Y. Tobacco smoking and autoimmune rheumatic diseases. Nat Clin Pract Rheumatol 2007;3(12):707-15.

138 Reed AM, Ernste F. The inflammatory milieu in idiopathic inflammatory myositis. Curr Rheumatol Rep 2009;11(4):295-301.

139 Szodoray P, Alex P, Knowlton N, et al. Idiopathic inflammatory myopathies, signified by distinctive peripheral cytokines, chemokines and the TNF family members B-cell activating factor and a proliferation inducing ligand. Rheumatology (Oxford) 2010;49(10):1867-77.

140 Goebels N, Michaelis D, Engelhardt M, et al. Differential expression of perforin in muscle-infiltrating T cells in polymyositis and dermatomyositis. J Clin Invest 1996;97(12):2905-10.

141 de Padilla CM, Reed AM. Dendritic cells and the immunopathogenesis of idiopathic inflammatory myopathies. Curr Opin Rheumatol 2008;20(6):669-74.

142 Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. I: Quantitation of subsets according to diagnosis and sites of accumulation and

demonstration and counts of muscle fibers invaded by T cells. Ann Neurol 1984;16(2):193-208.

143 Emslie-Smith AM, Arahata K, Engel AG. Major histocompatibility complex class I antigen expression, immunolocalization of interferon subtypes, and T cell-mediated cytotoxicity in myopathies. Hum Pathol 1989;20(3):224-31.

144 Hengstman GJ, ter Laak HJ, Vree Egberts WT, et al. Anti-signal recognition particle autoantibodies: marker of a necrotising myopathy. Ann Rheum Dis 2006;65(12):1635-8.

145 Fasth AE, Dastmalchi M, Rahbar A, et al. T cell infiltrates in the muscles of patients with dermatomyositis and polymyositis are dominated by CD28null T cells. J Immunol 2009;183(7):4792-9.

146 Pandya JM, Venalis P, Al-Khalili L, et al. CD4+ and CD8+ CD28(null) T Cells Are Cytotoxic to Autologous Muscle Cells in Patients With Polymyositis. Arthritis Rheumatol 2016;68(8):2016-26.

147 Plotz PH, Dalakas M, Leff RL, Love LA, Miller FW, Cronin ME. Current concepts in the idiopathic inflammatory myopathies: polymyositis, dermatomyositis, and related disorders. Ann Intern Med 1989;111(2):143-57.

148 Nyberg P, Wikman AL, Nennesmo I, Lundberg I. Increased expression of interleukin 1alpha and MHC class I in muscle tissue of patients with chronic, inactive polymyositis and dermatomyositis. J Rheumatol 2000;27(4):940-8.

149 Lundberg I, Brengman JM, Engel AG. Analysis of cytokine expression in muscle in inflammatory myopathies, Duchenne dystrophy, and non-weak controls. J Neuroimmunol 1995;63(1):9-16.

150 Reid MB, Lannergren J, Westerblad H. Respiratory and limb muscle weakness induced by tumor necrosis factor-alpha: involvement of muscle myofilaments. Am J Respir Crit Care Med 2002;166(4):479-84.

151 Krystufkova O, Vallerskog T, Helmers SB, et al. Increased serum levels of B cell activating factor (BAFF) in subsets of patients with idiopathic inflammatory myopathies. Ann Rheum Dis 2009;68(6):836-43.

152 Salajegheh M, Pinkus JL, Amato AA, et al. Permissive environment for B-cell maturation in myositis muscle in the absence of B-cell follicles. Muscle Nerve 2010;42(4):576-83.

153 Greenberg SA, Bradshaw EM, Pinkus JL, et al. Plasma cells in muscle in inclusion body myositis and polymyositis. Neurology 2005;65(11):1782-7.

154 Inukai A, Kuru S, Liang Y, et al. Expression of HLA-DR and its enhancing molecules in muscle fibers in polymyositis. Muscle Nerve 2000;23(3):385-92.

155 Karpati G, Pouliot Y, Carpenter S. Expression of immunoreactive major histocompatibility complex products in human skeletal muscles. Ann Neurol 1988;23(1):64-72.

156 Nagaraju K, Raben N, Loeffler L, et al. Conditional up-regulation of MHC class I in skeletal muscle leads to self-sustaining autoimmune myositis and myositis-specific autoantibodies. Proc Natl Acad Sci U S A 2000;97(16):9209-14.

157 Isenberg DA, Rowe D, Shearer M, Novick D, Beverley PC. Localization of interferons and interleukin 2 in polymyositis and muscular dystrophy. Clin Exp Immunol 1986;63(2):450-8.

158 Ulfgren AK, Grundtman C, Borg K, et al. Down-regulation of the aberrant expression of the inflammation mediator high mobility group box chromosomal protein 1 in muscle tissue of patients with polymyositis and dermatomyositis treated with corticosteroids. Arthritis Rheum 2004;50(5):1586-94.

159 Grundtman C, Bruton J, Yamada T, et al. Effects of HMGB1 on in vitro responses of isolated muscle fibers and functional aspects in skeletal muscles of idiopathic inflammatory myopathies. FASEB J 2010;24(2):570-8.

160 Zong M, Loell I, Lindroos E, et al. Effects of immunosuppressive treatment on interleukin-15 and interleukin-15 receptor alpha expression in muscle tissue of patients with polymyositis or dermatomyositis. Ann Rheum Dis 2012;71(6):1055-63.

161 Notarnicola A, Lapadula G, Natuzzi D, Lundberg IE, Iannone F. Correlation between serum levels of IL-15 and IL-17 in patients with idiopathic inflammatory myopathies. Scand J Rheumatol 2015;44(3):224-8.

162 Tucci M, Quatraro C, Dammacco F, Silvestris F. Interleukin-18 overexpression as a hallmark of the activity of autoimmune inflammatory myopathies. Clin Exp Immunol 2006;146(1):21-31.

163 Gono T, Kawaguchi Y, Sugiura T, et al. Interleukin-18 is a key mediator in dermatomyositis: potential contribution to development of interstitial lung disease. Rheumatology (Oxford) 2010;49(10):1878-81.

164 Tucci M, Quatraro C, Lombardi L, Pellegrino C, Dammacco F, Silvestris F. Glomerular accumulation of plasmacytoid dendritic cells in active lupus nephritis: role of interleukin-18. Arthritis Rheum 2008;58(1):251-62.

165 Calvani N, Richards HB, Tucci M, Pannarale G, Silvestris F. Up-regulation of IL-18 and predominance of a Th1 immune response is a hallmark of lupus nephritis. Clin Exp Immunol 2004;138(1):171-8.

166 Horiuchi M, Yamada H, Akishita M, Ito M, Tamura K, Dzau VJ. Interferon regulatory factors regulate interleukin-1beta-converting enzyme expression and apoptosis in vascular smooth muscle cells. Hypertension 1999;33(1):162-6.

167 Fantuzzi G, Reed D, Qi M, Scully S, Dinarello CA, Senaldi G. Role of interferon regulatory factor-1 in the regulation of IL-18 production and activity. Eur J Immunol 2001;31(2):369-75.

168 Vlachopoulos C, Ioakeimidis N, Aznaouridis K, et al. Association of interleukin-18 levels with global arterial function and early structural changes in men without cardiovascular disease. Am J Hypertens 2010;23(4):351-7.

169 Emslie-Smith AM, Engel AG. Microvascular changes in early and advanced dermatomyositis: a quantitative study. Ann Neurol 1990;27(4):343-56.

170 Grundtman C, Tham E, Ulfgren AK, Lundberg IE. Vascular endothelial growth factor is highly expressed in muscle tissue of patients with polymyositis and patients with dermatomyositis. Arthritis Rheum 2008;58(10):3224-38.

171 Nawata Y, Kurasawa K, Takabayashi K, et al. Corticosteroid resistant interstitial pneumonitis in dermatomyositis/polymyositis: prediction and treatment with cyclosporine. J Rheumatol 1999;26(7):1527-33.

172 Park JH, Olsen NJ, King L, Jr., et al. Use of magnetic resonance imaging and P-31 magnetic resonance spectroscopy to detect and quantify muscle dysfunction in the amyopathic and myopathic variants of dermatomyositis. Arthritis Rheum 1995;38(1):68-77.

173 Kim I, Xu W, Reed JC. Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 2008;7(12):1013-30.

174 Nagaraju K, Casciola-Rosen L, Lundberg I, et al. Activation of the endoplasmic reticulum stress response in autoimmune myositis: potential role in muscle fiber damage and dysfunction. Arthritis Rheum 2005;52(6):1824-35.

175 Shtifman A, Ward CW, Laver DR, et al. Amyloid-beta protein impairs Ca2+ release and contractility in skeletal muscle. Neurobiol Aging 2010;31(12):2080-90.

176 Nagaraju K, Plotz PH. Animal models of myositis. Rheum Dis Clin North Am 2002;28(4):917-33.

177 Katsumata Y, Ridgway WM, Oriss T, et al. Species-specific immune responses generated by histidyl-tRNA synthetase immunization are associated with muscle and lung inflammation. J Autoimmun 2007;29(2-3):174-86.

178 Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci 1957;147(927):258-67.

179 O'Shea JJ, Plenge R. JAK and STAT signaling molecules in immunoregulation and immune-mediated disease. Immunity 2012;36(4):542-50.

180 Liu YJ. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. Annu Rev Immunol 2005;23:275-306.

181 Mancuso G, Gambuzza M, Midiri A, et al. Bacterial recognition by TLR7 in the lysosomes of conventional dendritic cells. Nat Immunol 2009;10(6):587-94.

182 Bene MC, Feuillard J, Jacob MC, Groupe d'Etude Immunologique des L. Plasmacytoid dendritic cells: from the plasmacytoid T-cell to type 2 dendritic cells CD4+CD56+ malignancies. Semin Hematol 2003;40(3):257-66.

183 Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 2007;13(5):543-51.

184 Westerweel PE, Luijten RK, Hoefer IE, Koomans HA, Derksen RH, Verhaar MC. Haematopoietic and endothelial progenitor cells are deficient in quiescent systemic lupus erythematosus. Ann Rheum Dis 2007;66(7):865-70.

185 Albini A, Marchisone C, Del Grosso F, et al. Inhibition of angiogenesis and vascular tumor growth by interferon-producing cells: A gene therapy approach. Am J Pathol 2000;156(4):1381-93.

186 Lara PN, Jr., Quinn DI, Margolin K, et al. SU5416 plus interferon alpha in advanced renal cell carcinoma: a phase II California Cancer Consortium Study with biological and imaging correlates of angiogenesis inhibition. Clin Cancer Res 2003;9(13):4772-81.

187 Broxmeyer HE, Lu L, Platzer E, Feit C, Juliano L, Rubin BY. Comparative analysis of the influences of human gamma, alpha and beta interferons on human multipotential (CFU-GEMM), erythroid (BFU-E) and granulocyte-macrophage (CFU-GM) progenitor cells. J Immunol 1983;131(3):1300-5.

188 Brinkmann V, Reichard U, Goosmann C, et al. Neutrophil extracellular traps kill bacteria. Science 2004;303(5663):1532-5.

189 Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. Sci Transl Med 2011;3(73):73ra19.

190 Gupta AK, Joshi MB, Philippova M, et al. Activated endothelial cells induce neutrophil extracellular traps and are susceptible to NETosis-mediated cell death. FEBS Lett 2010;584(14):3193-7.

191 Saffarzadeh M, Juenemann C, Queisser MA, et al. Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones. PLoS One 2012;7(2):e32366.

192 Zhang S, Shu X, Tian X, Chen F, Lu X, Wang G. Enhanced formation and impaired degradation of neutrophil extracellular traps in dermatomyositis and polymyositis: a potential contributor to interstitial lung disease complications. Clin Exp Immunol 2014;177(1):134-41.

193 Walsh RJ, Kong SW, Yao Y, et al. Type I interferon-inducible gene expression in blood is present and reflects disease activity in dermatomyositis and polymyositis. Arthritis Rheum 2007;56(11):3784-92.

194 Greenberg SA, Pinkus JL, Pinkus GS, et al. Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. Ann Neurol 2005;57(5):664-78.

195 van der Pouw Kraan TC, Wijbrandts CA, van Baarsen LG, et al. Rheumatoid arthritis subtypes identified by genomic profiling of peripheral blood cells: assignment of a type I interferon signature in a subpopulation of patients. Ann Rheum Dis 2007;66(8):1008-14.

196 Bave U, Nordmark G, Lovgren T, et al. Activation of the type I interferon system in primary Sjogren's syndrome: a possible etiopathogenic mechanism. Arthritis Rheum 2005;52(4):1185-95.

197 Assassi S, Mayes MD, Arnett FC, et al. Systemic sclerosis and lupus: points in an interferon-mediated continuum. Arthritis Rheum 2010;62(2):589-98.

198 Ronnblom L. The type I interferon system in the etiopathogenesis of autoimmune diseases. Ups J Med Sci 2011;116(4):227-37.

199 Lovgren T, Eloranta ML, Kastner B, Wahren-Herlenius M, Alm GV, Ronnblom L. Induction of interferon-alpha by immune complexes or liposomes containing systemic lupus erythematosus autoantigen- and Sjogren's syndrome autoantigen-associated RNA. Arthritis Rheum 2006;54(6):1917-27.

200 Hua J, Kirou K, Lee C, Crow MK. Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti-RNA binding protein autoantibodies. Arthritis Rheum 2006;54(6):1906-16.

201 Greenberg SA. Type 1 interferons and myositis. Arthritis Res Ther 2010;12 Suppl 1:S4.

202 Shiba H, Takeuchi T, Isoda K, et al. Dermatomyositis as a complication of interferon-alpha therapy: a case report and review of the literature. Rheumatol Int 2014.

203 Kalkner KM, Ronnblom L, Karlsson Parra AK, Bengtsson M, Olsson Y, Oberg K. Antibodies against double-stranded DNA and development of polymyositis during treatment with interferon. QJM 1998;91(6):393-9.

204 Liao AP, Salajegheh M, Nazareno R, Kagan JC, Jubin RG, Greenberg SA. Interferon beta is associated with type 1 interferon-inducible gene expression in dermatomyositis. Ann Rheum Dis 2011;70(5):831-6.

205 Cappelletti C, Baggi F, Zolezzi F, et al. Type I interferon and Toll-like receptor expression characterizes inflammatory myopathies. Neurology 2011;76(24):2079-88.

206 Balboni I, Niewold TB, Morgan G, et al. Interferon-alpha induction and detection of anti-ro, anti-la, anti-sm, and anti-rnp autoantibodies by autoantigen microarray analysis in juvenile dermatomyositis. Arthritis Rheum 2013;65(9):2424-9.

207 Wong D, Kea B, Pesich R, et al. Interferon and biologic signatures in dermatomyositis skin: specificity and heterogeneity across diseases. PLoS One 2012;7(1):e29161.

208 Wenzel J, Schmidt R, Proelss J, Zahn S, Bieber T, Tuting T. Type I interferon-associated skin recruitment of CXCR3+ lymphocytes in dermatomyositis. Clin Exp Dermatol 2006;31(4):576-82.

209 McNiff JM, Kaplan DH. Plasmacytoid dendritic cells are present in cutaneous dermatomyositis lesions in a pattern distinct from lupus erythematosus. J Cutan Pathol 2008;35(5):452-6.

210 Page G, Chevrel G, Miossec P. Anatomic localization of immature and mature dendritic cell subsets in dermatomyositis and polymyositis: Interaction with chemokines and Th1 cytokine-producing cells. Arthritis Rheum 2004;50(1):199-208.

211 Lopez de Padilla CM, Vallejo AN, McNallan KT, et al. Plasmacytoid dendritic cells in inflamed muscle of patients with juvenile dermatomyositis. Arthritis Rheum 2007;56(5):1658-68.

212 Baechler EC, Bauer JW, Slattery CA, et al. An interferon signature in the peripheral blood of dermatomyositis patients is associated with disease activity. Mol Med 2007;13(1-2):59-68.

213 Krol P, Krystufkova O, Polanska M, et al. Serum levels of interferon alpha do not correlate with disease activity in patients with dermatomyositis/polymyositis. Ann Rheum Dis 2011;70(5):879-80.

214 Bilgic H, Ytterberg SR, Amin S, et al. Interleukin-6 and type I interferon-regulated genes and chemokines mark disease activity in dermatomyositis. Arthritis Rheum 2009;60(11):3436-46.

215 Eloranta ML, Barbasso Helmers S, Ulfgren AK, Ronnblom L, Alm GV, Lundberg IE. A possible mechanism for endogenous activation of the type I interferon system in myositis patients with anti-Jo-1 or anti-Ro 52/anti-Ro 60 autoantibodies. Arthritis Rheum 2007;56(9):3112-24.

216 Rantapaa-Dahlqvist S, de Jong BA, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum 2003;48(10):2741-9.

217 Arbuckle MR, McClain MT, Rubertone MV, et al. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med 2003;349(16):1526-33.

218 Miller FW, Waite KA, Biswas T, Plotz PH. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. Proc Natl Acad Sci U S A 1990;87(24):9933-7.

219 Gunawardena H, Betteridge ZE, McHugh NJ. Myositis-specific autoantibodies: their clinical and pathogenic significance in disease expression. Rheumatology (Oxford) 2009;48(6):607-12.

220 Love LA, Leff RL, Fraser DD, et al. A new approach to the classification of idiopathic inflammatory myopathy: myositis-specific autoantibodies define useful homogeneous patient groups. Medicine (Baltimore) 1991;70(6):360-74.

221 Marguerie C, Bunn CC, Beynon HL, et al. Polymyositis, pulmonary fibrosis and autoantibodies to aminoacyl-tRNA synthetase enzymes. Q J Med 1990;77(282):1019-38.

222 Richards TJ, Eggebeen A, Gibson K, et al. Characterization and peripheral blood biomarker assessment of anti-Jo-1 antibody-positive interstitial lung disease. Arthritis Rheum 2009;60(7):2183-92.

223 Hallowell RW, Ascherman DP, Danoff SK. Pulmonary manifestations of polymyositis/dermatomyositis. Semin Respir Crit Care Med 2014;35(2):239-48.

224 Bernstein RM, Morgan SH, Chapman J, et al. Anti-Jo-1 antibody: a marker for myositis with interstitial lung disease. Br Med J (Clin Res Ed) 1984;289(6438):151-2.

225 Stone KB, Oddis CV, Fertig N, et al. Anti-Jo-1 antibody levels correlate with disease activity in idiopathic inflammatory myopathy. Arthritis Rheum 2007;56(9):3125-31.

226 Halloran PF, Urmson J, Van der Meide PH, Autenried P. Regulation of MHC expression in vivo. II. IFN-alpha/beta inducers and recombinant IFN-alpha modulate MHC antigen expression in mouse tissues. J Immunol 1989;142(12):4241-7.

227 Reeves WH, Nigam SK, Blobel G. Human autoantibodies reactive with the signal-recognition particle. Proc Natl Acad Sci U S A 1986;83(24):9507-11.

228 Rider LG, Shah M, Mamyrova G, et al. The myositis autoantibody phenotypes of the juvenile idiopathic inflammatory myopathies. Medicine (Baltimore) 2013;92(4):223-43.

229 Kao AH, Lacomis D, Lucas M, Fertig N, Oddis CV. Anti-signal recognition particle autoantibody in patients with and patients without idiopathic inflammatory myopathy. Arthritis Rheum 2004;50(1):209-15.

230 Miller T, Al-Lozi MT, Lopate G, Pestronk A. Myopathy with antibodies to the signal recognition particle: clinical and pathological features. J Neurol Neurosurg Psychiatry 2002;73(4):420-8.

231 Targoff IN, Johnson AE, Miller FW. Antibody to signal recognition particle in polymyositis. Arthritis Rheum 1990;33(9):1361-70.

232 Wang HB, Zhang Y. Mi2, an auto-antigen for dermatomyositis, is an ATP-dependent nucleosome remodeling factor. Nucleic Acids Res 2001;29(12):2517-21.

233 Targoff IN, Reichlin M. The association between Mi-2 antibodies and dermatomyositis. Arthritis Rheum 1985;28(7):796-803.

234 Kang EH, Nakashima R, Mimori T, et al. Myositis autoantibodies in Korean patients with inflammatory myositis: anti-140-kDa polypeptide antibody is primarily associated with rapidly progressive interstitial lung disease independent of clinically amyopathic dermatomyositis. BMC Musculoskelet Disord 2010;11:223.

235 Rider LG, Miller FW, Targoff IN, et al. A broadened spectrum of juvenile myositis. Myositis-specific autoantibodies in children. Arthritis Rheum 1994;37(10):1534-8.

236 Casciola-Rosen L, Nagaraju K, Plotz P, et al. Enhanced autoantigen expression in regenerating muscle cells in idiopathic inflammatory myopathy. J Exp Med 2005;201(4):591-601.

237 Mammen AL, Casciola-Rosen LA, Hall JC, Christopher-Stine L, Corse AM, Rosen A. Expression of the dermatomyositis autoantigen Mi-2 in regenerating muscle. Arthritis Rheum 2009;60(12):3784-93.

238 Hengstman GJ, Vree Egberts WT, Seelig HP, et al. Clinical characteristics of patients with myositis and autoantibodies to different fragments of the Mi-2 beta antigen. Ann Rheum Dis 2006;65(2):242-5.

239 Targoff IN, Mamyrova G, Trieu EP, et al. A novel autoantibody to a 155-kd protein is associated with dermatomyositis. Arthritis Rheum 2006;54(11):3682-9.

240 Gunawardena H, Wedderburn LR, North J, et al. Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis. Rheumatology (Oxford) 2008;47(3):324-8.

241 Fujimoto M, Hamaguchi Y, Kaji K, et al. Myositis-specific anti-155/140 autoantibodies target transcription intermediary factor 1 family proteins. Arthritis Rheum 2012;64(2):513-22.

242 Fiorentino DF, Kuo K, Chung L, Zaba L, Li S, Casciola-Rosen L. Distinctive cutaneous and systemic features associated with antitranscriptional intermediary factor-1gamma antibodies in adults with dermatomyositis. J Am Acad Dermatol 2015;72(3):449-55.

243 Kong HJ, Anderson DE, Lee CH, et al. Cutting edge: autoantigen Ro52 is an interferon inducible E3 ligase that ubiquitinates IRF-8 and enhances cytokine expression in macrophages. J Immunol 2007;179(1):26-30.

244 Betteridge ZE, Gunawardena H, Chinoy H, et al. Clinical and human leucocyte antigen class II haplotype associations of autoantibodies to small ubiquitin-like modifier enzyme, a dermatomyositis-specific autoantigen target, in UK Caucasian adult-onset myositis. Ann Rheum Dis 2009;68(10):1621-5.

245 Fujimoto M, Matsushita T, Hamaguchi Y, et al. Autoantibodies to small ubiquitin-like modifier activating enzymes in Japanese patients with dermatomyositis: comparison with a UK Caucasian cohort. Ann Rheum Dis 2013;72(1):151-3.

246 Sato S, Hirakata M, Kuwana M, et al. Autoantibodies to a 140-kd polypeptide, CADM-140, in Japanese patients with clinically amyopathic dermatomyositis. Arthritis Rheum 2005;52(5):1571-6.

247 Chen Z, Cao M, Plana MN, et al. Utility of anti-melanoma differentiation-associated gene 5 antibody measurement in identifying patients with dermatomyositis and a high risk for developing rapidly progressive interstitial lung disease: a review of the literature and a meta-analysis. Arthritis Care Res (Hoboken) 2013;65(8):1316-24.

248 Barral PM, Sarkar D, Su ZZ, et al. Functions of the cytoplasmic RNA sensors RIG-I and MDA-5: key regulators of innate immunity. Pharmacol Ther 2009;124(2):219-34.

249 Sato S, Hoshino K, Satoh T, et al. RNA helicase encoded by melanoma differentiation-associated gene 5 is a major autoantigen in patients with clinically amyopathic dermatomyositis: Association with rapidly progressive interstitial lung disease. Arthritis Rheum 2009;60(7):2193-200.

250 Nakashima R, Imura Y, Kobayashi S, et al. The RIG-I-like receptor IFIH1/MDA5 is a dermatomyositis-specific autoantigen identified by the anti-CADM-140 antibody. Rheumatology (Oxford) 2010;49(3):433-40.

251 Fiorentino D, Chung L, Zwerner J, Rosen A, Casciola-Rosen L. The mucocutaneous and systemic phenotype of dermatomyositis patients with antibodies to MDA5 (CADM-140): a retrospective study. J Am Acad Dermatol 2011;65(1):25-34.

252 Espada G, Maldonado Cocco JA, Fertig N, Oddis CV. Clinical and serologic characterization of an Argentine pediatric myositis cohort: identification of a novel autoantibody (anti-MJ) to a 142-kDa protein. J Rheumatol 2009;36(11):2547-51.

253 Ceribelli A, Fredi M, Taraborelli M, et al. Anti-MJ/NXP-2 autoantibody specificity in a cohort of adult Italian patients with polymyositis/dermatomyositis. Arthritis Res Ther 2012;14(2):R97.

254 Watanabe Y, Suzuki S, Nishimura H, et al. Statins and myotoxic effects associated with anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase autoantibodies: an observational study in Japan. Medicine (Baltimore) 2015;94(4):e416.

255 Allenbach Y, Drouot L, Rigolet A, et al. Anti-HMGCR autoantibodies in European patients with autoimmune necrotizing myopathies: inconstant exposure to statin. Medicine (Baltimore) 2014;93(3):150-7.

256 Mammen AL, Gaudet D, Brisson D, et al. Increased frequency of DRB1*11:01 in anti-hydroxymethylglutaryl-coenzyme A reductase-associated autoimmune myopathy. Arthritis Care Res (Hoboken) 2012;64(8):1233-7.

257 Albrecht I, Wick C, Hallgren A, et al. Development of autoantibodies against muscle-specific FHL1 in severe inflammatory myopathies. J Clin Invest 2015;125(12):4612-24.

258 Horn EJ, Albor A, Liu Y, et al. RING protein Trim32 associated with skin carcinogenesis has anti-apoptotic and E3-ubiquitin ligase properties. Carcinogenesis 2004;25(2):157-67.

259 Dho SH, Kwon KS. The Ret finger protein induces apoptosis via its RING finger-B box-coiled-coil motif. The Journal of biological chemistry 2003;278(34):31902-8.

260 Marie I, Hatron PY, Dominique S, et al. Short-term and long-term outcome of anti-Jo1-positive patients with anti-Ro52 antibody. Semin Arthritis Rheum 2012;41(6):890-9.

261 Salomonsson S, Dorner T, Theander E, Bremme K, Larsson P, Wahren-Herlenius M. A serologic marker for fetal risk of congenital heart block. Arthritis Rheum 2002;46(5):1233-41.

262 Farris AD, O'Brien CA, Harley JB. Y3 is the most conserved small RNA component of Ro ribonucleoprotein complexes in vertebrate species. Gene 1995;154(2):193-8.

263 Lega JC, Fabien N, Reynaud Q, et al. The clinical phenotype associated with myositis-specific and associated autoantibodies: a meta-analysis revisiting the so-called antisynthetase syndrome. Autoimmun Rev 2014;13(9):883-91.

264 Muro Y, Hosono Y, Sugiura K, Ogawa Y, Mimori T, Akiyama M. Anti-PM/Scl antibodies are found in Japanese patients with various systemic autoimmune conditions besides myositis and scleroderma. Arthritis Res Ther 2015;17:57.

265 Marie I, Lahaxe L, Benveniste O, et al. Long-term outcome of patients with polymyositis/ dermatomyositis and anti-PM-Scl antibody. Br J Dermatol 2010;162(2):337-44.

266 Rigolet A, Musset L, Dubourg O, et al. Inflammatory myopathies with anti-Ku antibodies: a prognosis dependent on associated lung disease. Medicine (Baltimore) 2012;91(2):95-102.

267 Rojana-Udomsart A, Bundell C, James I, et al. Frequency of autoantibodies and correlation with HLA-DRB1 genotype in sporadic inclusion body myositis (s-IBM): a population control study. J Neuroimmunol 2012;249(1-2):66-70.

268 Salajegheh M, Lam T, Greenberg SA. Autoantibodies against a 43 KDa muscle protein in inclusion body myositis. PLoS One 2011;6(5):e20266.

269 Herbert MK, Stammen-Vogelzangs J, Verbeek MM, et al. Disease specificity of autoantibodies to cytosolic 5'-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. Ann Rheum Dis 2016;75(4):696-701.

270 Tepper OM, Galiano RD, Kalka C, Gurtner GC. Endothelial progenitor cells: the promise of vascular stem cells for plastic surgery. Plast Reconstr Surg 2003;111(2):846-54.

271 Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275(5302):964-7.

272 Peichev M, Naiyer AJ, Pereira D, et al. Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. Blood 2000;95(3):952-8.

273 Murayama T, Tepper OM, Silver M, et al. Determination of bone marrow-derived endothelial progenitor cell significance in angiogenic growth factor-induced neovascularization in vivo. Exp Hematol 2002;30(8):967-72.

274 Crosby JR, Kaminski WE, Schatteman G, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. Circ Res 2000;87(9):728-30.

275 Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001;89(1):E1-7.

276 Hill JM, Zalos G, Halcox JP, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003;348(7):593-600.

277 Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005;353(10):999-1007.

278 Tepper OM, Galiano RD, Capla JM, et al. Human endothelial progenitor cells from type II diabetics exhibit impaired proliferation, adhesion, and incorporation into vascular structures. Circulation 2002;106(22):2781-6.

279 Grisar J, Aletaha D, Steiner CW, et al. Depletion of endothelial progenitor cells in the peripheral blood of patients with rheumatoid arthritis. Circulation 2005;111(2):204-11.

280 Lee PY, Li Y, Richards HB, et al. Type I interferon as a novel risk factor for endothelial progenitor cell depletion and endothelial dysfunction in systemic lupus erythematosus. Arthritis Rheum 2007;56(11):3759-69.

281 Prater DN, Case J, Ingram DA, Yoder MC. Working hypothesis to redefine endothelial progenitor cells. Leukemia 2007;21(6):1141-9.

282 Goon PK, Lip GY, Boos CJ, Stonelake PS, Blann AD. Circulating endothelial cells, endothelial progenitor cells, and endothelial microparticles in cancer. Neoplasia 2006;8(2):79-88.

283 Denny MF, Thacker S, Mehta H, et al. Interferon-alpha promotes abnormal vasculogenesis in lupus: a potential pathway for premature atherosclerosis. Blood 2007;110(8):2907-15.

284 Kim T, Kanayama Y, Negoro N, Okamura M, Takeda T, Inoue T. Serum levels of interferons in patients with systemic lupus erythematosus. Clin Exp Immunol 1987;70(3):562-9.

285 Baechler EC, Batliwalla FM, Karypis G, et al. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci U S A 2003;100(5):2610-5.

286 Bunce M, Passey B. HLA typing by sequence-specific primers. Methods Mol Biol 2013;1034:147-59.

287 Satoh M, Vazquez-Del Mercado M, Chan EK. Clinical interpretation of antinuclear antibody tests in systemic rheumatic diseases. Mod Rheumatol 2009;19(3):219-28.

288 Phan TG, Wong RC, Adelstein S. Autoantibodies to extractable nuclear antigens: making detection and interpretation more meaningful. Clin Diagn Lab Immunol 2002;9(1):1-7.

289 Shovman O, Gilburd B, Barzilai O, et al. Evaluation of the BioPlex 2200 ANA screen: analysis of 510 healthy subjects: incidence of natural/predictive autoantibodies. Ann N Y Acad Sci 2005;1050:380-8.

290 Kirou KA, Lee C, George S, Louca K, Peterson MG, Crow MK. Activation of the interferon-alpha pathway identifies a subgroup of systemic lupus erythematosus patients with distinct serologic features and active disease. Arthritis Rheum 2005;52(5):1491-503.

291 Yao Y, Richman L, Higgs BW, et al. Neutralization of interferon-alpha/beta-inducible genes and downstream effect in a phase I trial of an anti-interferon-alpha monoclonal antibody in systemic lupus erythematosus. Arthritis Rheum 2009;60(6):1785-96.

292 Bengtsson AA, Sturfelt G, Truedsson L, et al. Activation of type I interferon system in systemic lupus erythematosus correlates with disease activity but not with antiretroviral antibodies. Lupus 2000;9(9):664-71.

293 Seeds RE, Gordon S, Miller JL. Characterisation of myeloid receptor expression and interferon alpha/beta production in murine plasmacytoid dendritic cells by flow cytomtery. J Immunol Methods 2009;350(1-2):106-17.

294 van Baarsen LG, Wijbrandts CA, Rustenburg F, et al. Regulation of IFN response gene activity during infliximab treatment in rheumatoid arthritis is associated with clinical response to treatment. Arthritis Res Ther 2010;12(1):R11.

295 Raterman HG, Vosslamber S, de Ridder S, et al. The interferon type I signature towards prediction of non-response to rituximab in rheumatoid arthritis patients. Arthritis Res Ther 2012;14(2):R95.

296 Vosslamber S, Raterman HG, van der Pouw Kraan TC, et al. Pharmacological induction of interferon type I activity following treatment with rituximab determines clinical response in rheumatoid arthritis. Ann Rheum Dis 2011;70(6):1153-9.

297 de Jong TD, Vosslamber S, Mantel E, et al. Physiological evidence for diversification of IFNalpha- and IFNbeta-mediated response programs in different autoimmune diseases. Arthritis Res Ther 2016;18(1):49.

298 Shao Z, Makinde TO, McGee HS, Wang X, Agrawal DK. Fms-like tyrosine kinase 3 ligand regulates migratory pattern and antigen uptake of lung dendritic cell subsets in a murine model of allergic airway inflammation. J Immunol 2009;183(11):7531-8.

299 Chinoy H, Ollier WE, Cooper RG. Have recent immunogenetic investigations increased our understanding of disease mechanisms in the idiopathic inflammatory myopathies? Curr Opin Rheumatol 2004;16(6):707-13.

300 Chinoy H, Lamb JA, Ollier WE, Cooper RG. An update on the immunogenetics of idiopathic inflammatory myopathies: major histocompatibility complex and beyond. Curr Opin Rheumatol 2009;21(6):588-93.

301 Enocsson H, Sjowall C, Skogh T, Eloranta ML, Ronnblom L, Wettero J. Interferon-alpha mediates suppression of C-reactive protein: explanation for muted C-reactive protein response in lupus flares? Arthritis Rheum 2009;60(12):3755-60.

302 Kim E, Cook-Mills J, Morgan G, Sredni ST, Pachman LM. Increased expression of vascular cell adhesion molecule 1 in muscle biopsy samples from juvenile dermatomyositis patients with short duration of untreated disease is regulated by miR-126. Arthritis Rheum 2012;64(11):3809-17.

303 Iademarco MF, McQuillan JJ, Dean DC. Vascular cell adhesion molecule 1: contrasting transcriptional control mechanisms in muscle and endothelium. Proc Natl Acad Sci U S A 1993;90(9):3943-7.

304 Figarella-Branger D, Civatte M, Bartoli C, Pellissier JF. Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies. Muscle Nerve 2003;28(6):659-82.

305 Sallum AM, Marie SK, Wakamatsu A, et al. Immunohistochemical analysis of adhesion molecule expression on muscle biopsy specimens from patients with juvenile dermatomyositis. J Rheumatol 2004;31(4):801-7.

306 Jain A, Sharma MC, Sarkar C, Bhatia R, Singh S, Handa R. Increased expression of cell adhesion molecules in inflammatory myopathies: diagnostic utility and pathogenetic insights. Folia Neuropathol 2009;47(1):33-42.

307 Tews DS, Goebel HH. Expression of cell adhesion molecules in inflammatory myopathies. J Neuroimmunol 1995;59(1-2):185-94.

308 Alexanderson H, Broman L, Tollback A, Josefson A, Lundberg IE, Stenstrom CH. Functional index-2: Validity and reliability of a disease-specific measure of impairment in patients with polymyositis and dermatomyositis. Arthritis Rheum 2006;55(1):114-22.

309 Yurkovich M, Vostretsova K, Chen W, Avina-Zubieta JA. Overall and cause-specific mortality in patients with systemic lupus erythematosus: a meta-analysis of observational studies. Arthritis Care Res (Hoboken) 2014;66(4):608-16.

310 Avina-Zubieta JA, Choi HK, Sadatsafavi M, Etminan M, Esdaile JM, Lacaille D. Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. Arthritis Rheum 2008;59(12):1690-7.

311 Man A, Zhu Y, Zhang Y, et al. The risk of cardiovascular disease in systemic sclerosis: a population-based cohort study. Ann Rheum Dis 2013;72(7):1188-93.

312 Lai YT, Dai YS, Yen MF, et al. Dermatomyositis is associated with an increased risk of cardiovascular and cerebrovascular events: a Taiwanese population-based longitudinal follow-up study. Br J Dermatol 2013;168(5):1054-9.

313 Tisseverasinghe A, Bernatsky S, Pineau CA. Arterial events in persons with dermatomyositis and polymyositis. J Rheumatol 2009;36(9):1943-6.

314 Carruthers EC, Choi HK, Sayre EC, Avina-Zubieta JA. Risk of deep venous thrombosis and pulmonary embolism in individuals with polymyositis and dermatomyositis: a general population-based study. Ann Rheum Dis 2016;75(1):110-6.

315 Villanueva E, Yalavarthi S, Berthier CC, et al. Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus. J Immunol 2011;187(1):538-52.

316 Garcia-Romo GS, Caielli S, Vega B, et al. Netting neutrophils are major inducers of type I IFN production in pediatric systemic lupus erythematosus. Sci Transl Med 2011;3(73):73ra20.

317 Kahlenberg JM, Carmona-Rivera C, Smith CK, Kaplan MJ. Neutrophil extracellular trap-associated protein activation of the NLRP3 inflammasome is enhanced in lupus macrophages. J Immunol 2013;190(3):1217-26.