From the DEPARTMENT OF MEDICINE
Karolinska Institutet, Stockholm, Sweden

POLYMYOSITIS AND DERMATOMYOSITIS – INFLAMMATION, MUSCLE STRUCTURE & IMMUNOSUPPRESSIVE TREATMENT

Ingela Loell

Karolinska Institutet

Stockholm 2012

"If it is to be, it is up to me."
~Wordsworth

ABSTRACT

Polymyositis and dermatomyositis are chronic, inflammatory disorders characterized by muscle weakness, low muscle endurance and by inflammation in skeletal muscle tissues. The pathogenesis is not completely understood and several mechanisms are believed to be involved. Conventional immunosuppressive treatment for patients is primarily with glucocorticoids in combination with another immune-modulating drug. Although most patients respond to the treatment to some degree, persisting muscle weakness as well as continued presence of muscle tissue inflammation is often evident.

The main aim was to investigate different pathways that may play a role in the disease pathogenesis and contribute to the muscle weakness in patients with polymyositis and dermatomyositis. We wanted to determine whether these mechanisms were associated with clinical outcome and if immunosuppressive treatment could alter the processes. Studies were thus performed by characterizing muscle tissue samples from patients before and after immunosuppressive treatment, and through comparison with samples from healthy individuals. We also collected *in vivo* samples from quadriceps muscles by microdialysis before and after exercise in both patients and healthy individuals.

Some novel observations were made. Firstly, we determined that the pro-inflammatory cytokine interleukin (IL) 15 and the IL-15 receptor alpha (IL-15Rα) were over-expressed in muscle tissues from the patients compared to in healthy individuals. Immunosuppressive treatment had no effect on IL-15 expression in 1/3 of the patients along with a poorer functional outcome. Secondly, we investigated the prostaglandin (PG) E_2 and leukotriene (LT) B_4 pathways. Here we found that PGE_2 pathway is up-regulated in muscle tissue from patients with polymyositis or dermatomyositis compared to healthy individuals. A higher expression of cyclooxygenase (COX) 1, COX-2 and microsomal PGE synthase (mPGES)-1 in the patients was apparent and while COX-2 was down-regulated by treatment, COX-1 and mPGES-1 levels remained high. LTB_4 pathway was also found to be active in patients and the expression of both 5-lipoxygenase (5-LO) and the LTB_4 receptor 1 (BLT1) were higher compared to in healthy individuals. 5-LO activating protein (FLAP), was highly expressed in a subgroup of patients who did not respond to immunosuppressive treatment. These results may suggest an involvement of PGE_2 and LTB_4 in vascular permeability and chemotaxis for infiltrating immune cells, but perhaps also to serve a purpose in skeletal muscle regeneration in patients with polymyositis and dermatomyositis. Lastly, we could report that patients with newly diagnosed polymyositis or dermatomyositis did not display a low proportion of oxidative type I muscle fibers typically seen in muscle tissue of patients in a chronic state of disease. This finding implies that the proportion of oxidative fibers does not contribute to the low muscle endurance, at least not in early polymyositis and dermatomyositis.

The conclusions drawn from this thesis are that several mechanisms may contribute to the muscle weakness in different subgroups of polymyositis and dermatomyositis patients. New pathways discovered in this thesis include IL-15 and the eicosanoids PGE_2 and LTB_4, but the fiber type composition in the muscle tissue might play a lesser role, at least in early disease. The mechanisms identified could provide a basis for further studies and possibly new therapies.

LIST OF PUBLICATIONS

I. Mei Zong, <u>Ingela Loell</u>, Eva Lindroos, Gustavo A Nader, Helene Alexanderson, Christina Stål-Hallengren, Kristian Borg, Snjolaug Arnardottir, Ian B McInnes, Ingrid E Lundberg. **Effects of immunosuppressive treatment on interleukin-15 and interleukin-15 receptor α expression in muscle tissue of patients with polymyositis or dermatomyositis.** *Ann Rheum Dis 2012 Epub ahead of print*

II. Marina Korotkova, <u>Ingela Loell</u>*, Sevim Barbasso Helmers*, Helene Alexanderson, Cecilia Grundtman, Christina Dorph, Ingrid E Lundberg, Per-Johan Jakobsson
Effects of immunosuppressive treatment on micfosomal prostaglandin E synthase 1 and cyclooxyenases expression in muscle tissue of patients with polymyositis or dermatomyositis. *Ann Rheum Dis 2008, 67(11):1596-602*

III. <u>Ingela Loell</u>, Li Alemo Munters, Jayesh Pandya, Mei Zong, Helene Alexanderson, Andreas E Fasth, Christina Stål-Hallengren, Olof Rådermatomyositisark, Ingrid E Lundberg, Per-Johan Jakobsson, Marina Korotkova. **Activated LTB4 pathway in muscle tissue of patients with polymyositis or dermatomyositis.** *Manuscript*

IV. <u>Ingela Loell</u>, Sevim Barbasso Helmers, Maryam Dastmalchi, Helene Alexanderson, Li Alemo Munters, Inger Nennesmo, Eva Lindroos, Kristian Borg,Ingrid E Lundberg, Mona Esbjörnsson. **Higher proportion of fast-twitch (type II) muscle fibers in idiopathic inflammatory myopathies - evident in chronic but not in untreated newly diagnosed patients.** *Clin Phys Funct Imaging (2011) 31, 18-25*

*both authors contributed equally

RELATED PUBLICATIONS

I. <u>Ingela Loell</u>, Ingrid E Lundberg. **Can muscle regeneration fail in chronic inflammation: a weakness in inflammatory myopathies?** *J Intern Med. 2011 Mar;269(3):243-57*

II. Maryam Dastmalchi, Helene Alexanderson, <u>Ingela Loell</u>, Magnus Ståhlberg, Kristian Borg, Ingrid E Lundberg, Mona Esbjörnsson. **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 Oct 15;57(7):1303-10.*

1 TABLE OF CONTENTS

2 LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
CD	Cluster of differentiation
CK	Creatine Kinase
COX	Cyclooxygenase
CSA	Cross-sectional area
DC	Dendritic cell
DMARD	Disease modifying anti-rheumatic drug
ER	Endoplasmatic reticulum
FI	Functional Index
FLAP	Five lipoxygenase activating protein
GR	Glucocorticoid receptor
GRE	Glucocorticoid responsive element
HMGB1	High mobility group box 1
ICAM	Intracellular adhesion molecule
IFN	Interferon
IIM	Idiopathic inflammatory myopathies
IL	Interleukin
LO	Lipoxygenase
LT	Leukotriene
MHC	Major histocompatibility complex
MMT	Manual muscle test
NFκB	Nuclear factor kappa B
PG	Prostaglandin
PGES	Prostaglandin E synthase
TCR	T cell receptor
TNF	Tumor necrosis factor
Treg	Regulatory T cell
VCAM	Vascular cell adhesion molecule

3 POLYMYOSITIS & DERMATOMYOSITIS

Polymyositis and dermatomyositis, here also defined as myositis, belong to the idiopathic inflammatory myopathies (IIM). Their pathogenesis is not fully understood but both genetic susceptibility as well as environmental factors have been implicated to play a role in disease development [1, 2]. Polymyositis and dermatomyositis are rare diseases, characterized by muscle inflammation and proximal muscle weakness with a reported incidence of 1/100,000 cases per year, being more common in women than in men [3].

Polymyositis and dermatomyositis are heterogeneous disorders and the classification and diagnostic criteria proposed by Boham and Peters 1975 is used in routine practice [4, 5]. The use of the additional information derived from the autoantibody profile in myositis patients can provide indications of clinical subtypes in disease progress and treatment responses [6]. The clinical features develop slowly over time, resulting in weakness mainly affecting proximal muscles, shoulder and pelvic girdle muscle being most severely affected. Weakness in neck muscles is quite common whereas muscle pain is rarely experienced [3]. Extra muscular organ manifestations are evident as lung [7, 8] and heart involvement [9, 10] and cancer association [11-13]. Skin rash is one of the diagnostic criteria in dermatomyositis . The cutaneous features in dermatomyositis appear as rashes over the knuckles called Gottron's papules/signs, heliotrope rash on the upper eyelids and 'mechanics hands' (thick, cracked skin at the finger tips) [14].

With muscle being the target organ of this systemic inflammation, the pathological signs within the muscle tissue in the patients include expression of inflammatory molecules involved in antigen presentation, immune cells, and cytokines but also signs of muscle degeneration and regeneration. The inflammation and tissue remodeling is ongoing in both the affected muscle groups as well as in the non-symptomatic muscles [15]. There are patients with little or no signs of tissue inflammation even with a pronounced muscle weakness, indicating that different mechanisms can be the cause of the disabled function [16]. The muscle fiber often express major histocompatibility complex (MHC) class I [17] and therefore have the potential to present antigens to infiltrating T cells. MHC class I may have the ability to mediate muscle damage [18] and mice overexpressing MHC class I in muscle fibers have less capacity of force generation compared to normal mice [19]. Abnormalities in the vascularity are displayed as activated endothelial cells and thickening vessel walls [20, 21]. Interspersed adipose tissue within the muscle fascicles can also be seen.

Aside from a triggered immune response, during more recent years hypotheses of non-immune mechanisms have arisen to explain the pathophysiology leading to muscle weakness. Among the theories investigated is endoplasmic reticulum (ER) stress [18, 22] that may be induced by MHC class I molecules expression on the muscle fibers and hypoxia [23-26].

The first-line drug treatment in myositis is high dose glucocorticoids, slowly tapered over time [27, 28]. Although most patients improve to some extent in response to immunosuppressive treatment, many patients experience persistent weakness and some

patients do not respond at all. Furthermore, many patients suffer from the steroid side-effects of weight gain, water retention, hyperglycemia and reduced muscle and bone mass [29]. Although some pathways have been discovered from the myositis research, there are still gaps in our understanding of what causes muscle weakness in polymyositis and dermatomyositis and whether it is the same cause triggering the weakness in an early state of disease as in the chronic, treated phase.
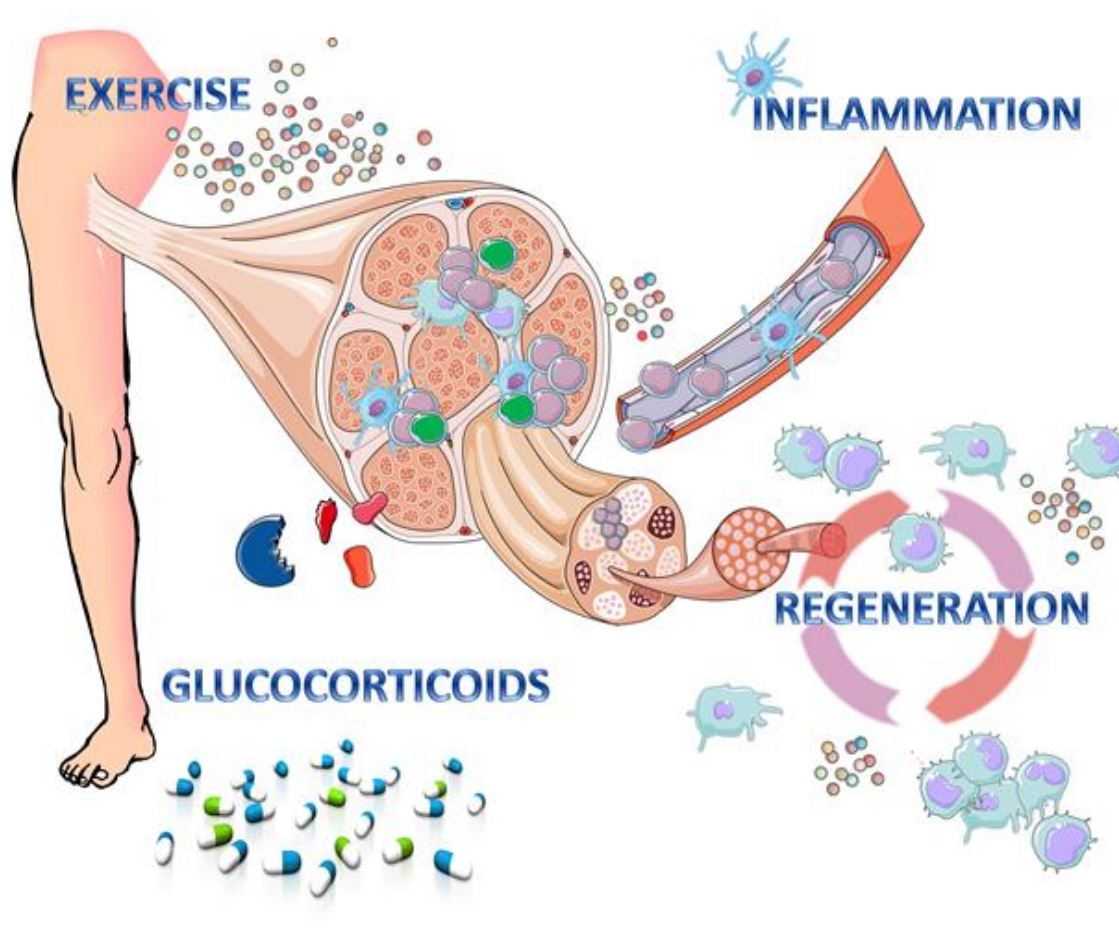

Figure 1. Different influences on skeletal muscle of patients with Polymyositis and dermatomyositis that will be discussed in this thesis. Parts of the picture are provided by courtesy of Servier.

4   INFLAMMATION

The immune response is composed of the innate and adaptive immune system, serving to defend the body against pathogens. In brief, the innate response is a rapid, non-specific defense where pathogen associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) exposed on the invading pathogen are recognized and phagocytized by macrophages, dendritic cells (DCs) or neutrophils. The adaptive immune response provides a specific, immunological memory, through the recruitment of the effector cells; T and B lymphocytes with the DCs as the major link between innate and the adaptive immunity. In the adaptive immune response, antigen presenting cells expose non-self epitopes/antigens via MHC molecules to the T cell receptor (TCR). There are two major types of T cells, the CD8^{+} cytotoxic T cells that recognize antigens presented on MHC class I molecules and CD4^{+} T helper cells that through antigen recognition mediated by MHC class II molecules are able to recruit myeloid cells to sites of inflammation, produce cytokines and chemokines, induce macrophage activity and antibody production by B cells. Another T cell subgroup is the regulatory T cells (Treg) that maintain immune tolerance to self and prevents the development of autoimmune diseases. Under normal conditions the resolution of inflammation is actively initiated and failure to do so results in chronic inflammation.

4.1   NUCLEAR FACTOR KAPPA B

The transcription factor nuclear factor kappa B (NFκB) is a regulator involved in a number of transcriptional programs important to the immune system, epithelium and skeletal muscle. NFκB regulates cell survival, proliferation and differentiation for maintenance but also in pathological conditions [30]. NFκB exists in the cytoplasm in a complex with inhibitory IκB proteins. Upon activation, IκB protein is degraded and NFκB is translocated into the cell nucleus where it can modulate gene expression. NFκB is largely responsible for the transcription of pro-inflammatory cytokines and chemokines. Early NFκB activation of tumor necrosis factor alpha (TNFα) cytokine production then leads to further NFκB induction. Another early target is activation of vascular endothelial cells regulating the transmigration of immune cells into the tissue [31]. Furthermore, NFκB is involved in T cell survival and cytokine production and promotes the proliferation, differentiation and maturation of B cells. NFκB is also important for the signaling and induction of prostaglandins [32, 33] and leukotrienes [34, 35]. Finally, NFκB is also active in the resolution of inflammation [31]. In skeletal muscle NFκB plays a role in regulating differentiation of cultured myoblasts by several mechanisms and in adult muscle NFκB is a part of adaptations such as muscle hypertrophy and atrophy [36].

In polymyositis and dermatomyositis, NFκB subunits have been extensively investigated. Enhanced expression has been documented in endothelial cells of blood vessels, atrophic and CD8^{+} invaded non-atrophic muscle fibers and regenerating fibers. The expression in fiber nuclei is similar in both myositis patients and healthy individuals. In the inflammatory cells infiltrating the muscle tissue, NFκB expression has been recorded in both CD4^{+} and CD8^{+} T cells as well as in B cells [37].

4.2 IMMUNE CELLS

4.2.1 T lymphocytes

Infiltrating T cells are often present in the muscle tissue of polymyositis and dermatomyositis patients. The immune cell infiltration follows two different patterns. First, the endomysial infiltration of invading CD8^{+} T cells surrounding non-necrotic, MHC I expressing muscle fibers as well as CD4^{+} T cells, DCs and macrophages can be seen, preferentially in polymyositis patients. CD8^{+} T cells express granzyme B and perforin and is hypothesized to cause muscle fiber injury. Conversely, perivascular infiltration comprises CD4^{+} T cells, DCs, macrophages and occasional B cells and is predominantly seen in patients with dermatomyositis [38]. T helper 17 (Th17) cells have also been detected in muscle tissue in patients with myositis. These cells produce IL-17, a pro-inflammatory cytokine capable of inducing MHC class I expression and IL-6 production in cultured myoblasts and could play a role in the pathogenesis of myositis [39]. Another subtype of T cells lack the CD28 molecule and CD28[null] accumulation in both CD4^{+} and CD8^{+} T cell populations has been reported in myositis tissue [40]. These cell populations show treatment resistance and are potentially toxic (unpublished data). Tregs are not so extensively present in the muscle tissue and the proportion seems to decrease after immunosuppressive treatment in patients with polymyositis or dermatomyositis (unpublished data). The interplay between subsets of T cells as well as between T cells and other cells in myositis is still not completely understood.

4.2.2 Macrophages

Macrophages play a role in both the innate as well as in the adaptive immune response. In the innate response macrophages acts as phagocytes and scavengers, ingesting pathogens and removing dead cell material. Antigen presentation and the activation of B and T lymphocytes by secreting cytokines are additional roles for macrophages [41]. In skeletal muscle post tissue injury and during the following regeneration resident macrophages are present in the connective tissue of muscle and participate in the regeneration process. The pro-inflammatory response upon injury starts with invasion of classically activated M1 macrophages [42] followed by a decline and transition to M2 macrophages. M2 macrophages are known as alternatively avtivated and are able to release IL-10 and IL-1 receptor antagonist (IL-1ra) [42]. M2 macrophages are present during the regenerative phase after tissue damage [43, 44]. The mechanisms underlying macrophage subset involvement in tissue repair are not known but macrophage depletion in muscle injury models leads to disturbances in muscle differentiation, growth and regeneration [45, 46]. Both CD68 and CD163 expressing macrophages have been reported to be overexpressed in polymyositis and dermatomyositis muscle tissues compared to in healthy individuals [47].

Muscle tissue infiltrates in myositis are predominantly comprised of T cells and macrophages. Whereas the expression of T cells, or at least subgroups of T cells, seem persistent to immunosuppressive treatment the numbers of macrophages declines in response to drug therapy [48].

4.2.3   Others
DCs, both plasmacytoid dendritic cells (pDCs) which are the major producers of type I interferons (IFNs), and the potent antigen presenting myeloid DCs have been reported in myositis and are inferred to be involved in the pathogenesis [49]. Other immune cells less frequently present in the muscle tissue of polymyositis and dermatomyositis patients are B cells [50], natural killer (NK) cells [51, 52], mast cells and neutrophils (unpublished data).

4.3   CYTOKINES, CHEMOKINES AND ADHESION MOLECULES
Cytokines and chemokines are soluble molecules important in the immune cell and tissue communication. Adhesion molecules are major contributors in immune cell transmigration and response. All three types of molecules are generally induced/produced by immune cells but muscle fibers could also contribute to the production [53, 54].

4.3.1   Cytokines
Many cytokines are expressed both in the circulation and in the muscle tissue of patients with myositis. The most common of these are the pro-inflammatory interleukin **(IL)-1α** and **IL-1β**. Whereas IL-1β is solely produced by macrophages and DCs, IL-1□ is additionally expressed on endothelial cells in capillaries, an expression that seems resistant to glucocorticoid treatment [17, 55]. IL-1 promotes proliferation of T and B cells as well as augmenting adhesion molecule expression on endothelial cells in response to induction by other cytokines such as TNF-□ and IFNs [56-58]. **IL-18**, or IFN-y inducing factor, belongs to the same family as IL-1 and is produced by macrophages, DCs, endothelial cells and smooth muscle cells. It is regulated by IL-18 binding protein (IL-18BP) and enhances T cell maturation, regulates B cell activation and antibody production along with the induction of cytokines (TNF-α, chemokines and adhesion molecules such as intracellular adhesion molecule (ICAM)-1and vascular cell adhesion molecule (VCAM)-1 [59-61]. IL-18 is over-expressed in myositis tissue [62, 63] and immunosuppressive treatment leads to diminished IL-18 expression in inflammatory cells with a seemingly unaffected expression in tissue capillaries (unpublished data).
TNF-α can activate T cells, B cells and macrophages, induce cytokines and adhesion molecule expression. In myositis the expression has been established in both T cells and macrophages infiltrating the tissue but also in endothelial cells in dermatomyositis. TNF-α can induce the MHC class I expression and might have a direct role in affecting muscle weakness [64, 65]. Thus TNF-α is considered to play an important role in the pathogenesis of myositis. However, blocking of TNF-α in this patient group has shown inconsistent responses arguing against a major role of TNF□ in the pathogenesis of polymyositis and dermatomyositis [66-68].
IFNs play a major role in the innate immune response and have been extensively studied in myositis. Both type I; **IFN-α/β** and type II; **IFN-γ**, exhibit a broad spectrum of pro-inflammatory capacity. Data on the presence of IFN-γ in myositis is somewhat inconsistent [69-71] whereas the type I IFNs seem to be highly involved in the pathogenesis, particularly in dermatomyositis [72]. Reports from gene expression analyses present data on overexpression of type I IFN inducible genes and proteins in muscle tissue from patients with polymyositis and dermatomyositis [72, 73]. It has also

been suggested that a particular type I IFN gene signature in blood highly correlate with disease activity in polymyositis and dermatomyositis patients [74, 75].

High mobility group box-1 (**HMGB-1)** is a nuclear protein that can influence transcription and also act as an extracellular alarmin upon tissue damage [76]. In myositis muscle HMGB-1 has been located to mononuclear cells, endothelial cells and the myofibers themselves [77]. HMGB-1 can be released upon proinflammatory stimulation from monocytes and macrophages but also from necrotic or damaged cells in a passive manner. Released HMGB-1 is able to induce cytokine production, cell proliferation, differentiation and chemotaxis [78]. Immunosuppressive treatment in myositis patients has been shown to decrease the expression of HMGB-1 along with a diminished number of immune cells, but the expression in muscle fibers and in endothelial cells of capillaries remained unaffected. This might indicate that HMGB-1 expression is differently regulated in different cell types and a role for HMGB-1 in maintaining MHC class I expression has been implied [77].

IL-6 is a pleiotropic cytokine produced by T cells, macrophages and endothelial cells and is involved in a numerous processes within the immune response [79-82]. In myositis, only sparse IL-6 protein expression has been observed in the muscle tissue.

IL-15, expressed in macrophages and endothelial cells is a cytokine with the ability to activate T cells, B cells and NK cells. In addition to the IL-15 receptor alpha (IL-15Rα), the biological function of IL-15 can be mediated through the IL-2 receptor, possibly causing the functional similarity to IL-2 in T cell activation [83, 84]. Interestingly, IL-15 has been shown to down-regulate the expression of CD28 on both $CD4^+$ [85] and $CD8^+$ T [86] cells. In the generated $CD28^{null}$ phenotype, IL-15 is able to continue to stimulate the growth and augment the cytotoxic properties of these particular T cells [87, 88]. High serum levels of IL-15 have been reported in patients with myositis [89] and data on IL-15 protein expression in myofibers and myoblasts has previously been published [90, 91], indicating that muscle fibers could be a potential source of IL-15.

Several of the pro-inflammatory cytokines have dual effects and besides the involvement in the immune response they may also influence skeletal muscle metabolism, regeneration and function. This adds complexity in whether biologic blockade would be favorable in patients having muscles as the primary organ of inflammation.

In recent years it has been postulated that IL-6 is a so called myokine as it can be secreted from muscle fibers upon contraction [54] and it has been suggested that exercise induced IL-6 has anti-inflammatory properties and is involved in energy metabolism [92].

TNF-α plays a role in degeneration upon muscle injury and activates satellite cells, promoting their proliferation and differentiation [93].

An additional role for HMGB-1 has been reported in skeletal muscle tissue where it may influence muscle fiber regeneration [94-96]. IL-15 is also referred to as a myokine secreted from contracting muscle but there are inconsistencies among the reports [97-99]. Interestingly, IL-15Rα can, without interacting with IL-15, be able to define fast skeletal muscle oxidative capacity and fatigue resistance [100].

4.3.2   Chemokines

Chemokines are cytokines operating the leukocyte migration into tissues by chemotaxis and can be divided into two major groups; α-chemokines (C-X-C motifs) and β-chemokines (C-C motifs). The α-chemokines are INF-γ inducible and CXCL9 and

CXCL10 have been localized to both T cells and to various degree in MHC class 1 expressing myofibers [101]. Myofibers themselves are also a possible source of CXCL9 Apart from controlling leukocyte migration; the β-chemokines can also be involved in tissue repair and skeletal muscle regeneration, evident by the proliferation of myoblast in response to CCL2, CCL3 and CCL4. Muscle fibers themselves might be a source of both CCL5 and CCL2 as human myoblasts have shown constitutive expression as well as expression upon pro-inflammatory stimulation [53]. A wide number of different chemokines have been detected in polymyositis and dermatomyositis muscle tissue and may possibly be involved in the pathophysiology of these diseases. Some of the chemokines found up-regulated are the α-chemokines CXCL9 and CXCL10 [101] and CCL2, CCL3, CCL4 and CCL5 among the β-chemokines [101, 102]. The expression is often corresponding with the infiltrating cell pattern and shows an endomysial or perivascular localization in the tissue [103]. However, the interpretation of chemokine expression needs to be cautious as discordances between mRNA and protein expression have been reported [103].

4.3.3 Adhesion molecules

A characteristic feature of muscle tissue in patients with polymyositis and dermatomyositis is presence of infiltrating immune cells. Leukocytes can either originate from the blood stream or proliferate locally. The homing into the tissue from the blood is mediated by the adhesion molecules on the leukocytes as well as on the endothelial cells [104]. VCAM-1 and ICAM-1 are both expressed on the vascular endothelium and ICAM-1 also on immune cells. Both ICAM-1 and VCAM-1 have been reported to be overexpressed in muscle tissue of patients with myositis, especially in an early phase of the disease [105]. The expression in the muscle tissue most likely plays a central role in leukocyte infiltration in the disease, an expression that is only partly affected by immunosuppressive treatment [106]. The vascular expression, which is unresponsive to conventional treatment, could be a potential drug target.

4.4 EICOSANOIDS

Eicosanoids are short-lived, lipid molecules derived from the 20-carbon unit polyunsaturated fatty acid arachidonic acid (20:4, ω6). Besides modulating the immune response, eicosanoids are also able to alter cardiovascular, pulmonary, reproductive and secretory functions in a variety of cells typically via G-coupled receptors. Esterified arachidonic acid is stored in cell membrane phospholipids and metabolized along the cyclooxygenase pathway to form prostaglandins (PG), the lipoxygenase pathway for leukotrienes (LT) production [107] or the cytochrome P450 pathway for epoxyeicosatrienoic acid (EET) synthesis [108]. The eicosanoid production progresses by the release of arachidonic acid from membrane phospholipids catalyzed by cytosolic phospholipase A_2 (cPLA$_2$). cPLA$_2$, activated by increases in intracellular Ca^{2+} concentrations in response to mitogens or cytokines, translocates from the cytosol into the nuclear membrane [109]. Here it regulates the release of arachidonic acid and thus controls the eicosanoid production.
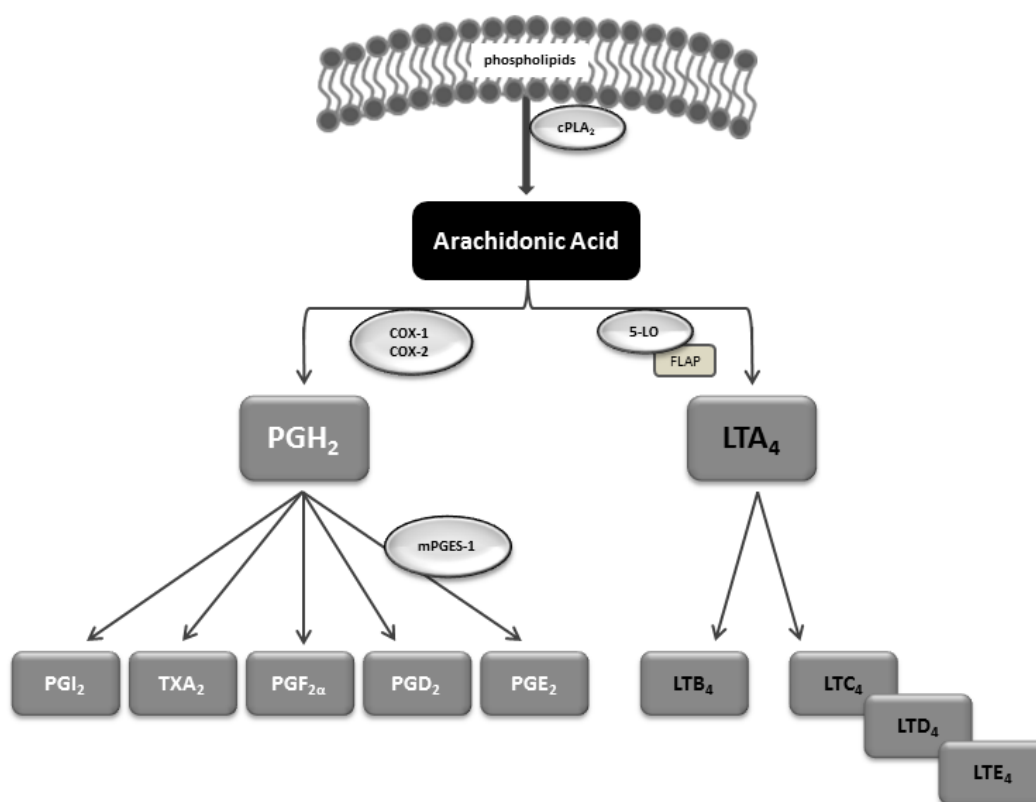
Figure 2. Schematic illustration of prostaglandin and leukotriene biosynthesis. Arachidonic acid is released from nuclear membrane phospholipids and synthesized down the COX- or 5-LO pathway.

4.4.1 Prostaglandins

The released arachidonic acid is converted into the unstable intermediate PGH_2 by the cyclooxygenase (COX) enzymes; COX-1 and COX-2. Under most conditions the COX-1 isotype is constitutively expressed in most cells to maintain housekeeping functions of the prostaglandins. Conversely, COX-2 is inducible under pro-inflammatory conditions. However, the opposed functions for both enzymes can occur as well [110]. PGH_2 is converted into different prostanoids by their respective terminal synthase; PGE_2 with pro- and anti-inflammatory properties, PGD_2 causes vasodilation and triggers asthmatic responses, [111] $PGF_{2\alpha}$ controlling contraction/relaxation and modulation of inflammation, and the anti-thrombotic PGI_2 (prostacyclin) and pro-thrombotic tromboxane counterbalancing cardiovascular functions [112, 113]. The different prostaglandins act in autocrine and paracrine fashions and operate in inflammatory responses and vascular stability. Non-steroidal anti-inflammatory drugs (NSAIDs), the most common over-the-counter drug inhibit COX enzymes. NSAIDs reduce pain, inflammation and fever due to non-selective COX inhibition. As an unwanted consequence this may result in gastrointestinal toxicity by the obstruction of the PGE_2 synthesis produced by COX-1 [114]. To circumvent these adverse effects the selective COX-2 inhibitors (coxibs) were developed but they lead to cardiovascular complications as a result of a disruption in the balance between PGI_2 and TXA_2 [115].

4.4.2 PGE_2

The conversion of PGH_2 into PGE_2 is catalyzed by PGE synthases (PGES). Cytosolic PGES-1 (cPGES-1), functionally linked to COX-1 for PGE_2 synthesis, is constitutively

expressed in a wide variety of cells [110]. Microsomal PGES-1 (mPGES-1) is inducible and acts in concert mainly with COX-2 as well as with COX-1 in some conditions [116]. The third PGES; mPGES-2 is a constitutively expressed enzyme and functionally coupled to both COX-enzymes [117]. PGE_2 is the most abundant as well as the most pleiotropic prostaglandin and has been extensively studied under numerous different conditions. PGE_2 is involved in several biologic events, such as gastrointestinal protection, renal blood flow, airway homeostasis, hypertension, fertility and in immune responses. During inflammation PGE_2 controls vascular permeability, macrophage and neutrophil infiltration, production of inflammatory mediators but also anti-inflammatory functions such as Treg activity and IL-10 induction [118]. PGE_2 acts through four different receptors, EP1-EP4 that are expressed on a wide variety of cells, indicating ubiquitous roles for PGE_2. EP3 and EP4 are high affinity receptors for PGE_2 whereas EP1 and EP2 have lower affinity and require higher concentrations of PGE_2. EP3 is the only PGE_2 receptor with several isoforms due to alternative splicing of the C-terminal. The signal transduction pathways of the different EP subtypes are mediated through regulation of Ca^{2+} concentration, changes in cyclic adenosine monophosphate (cAMP)-levels or a phosphatidylinositol (PI) response, however, the EP coupling to respective pathway is not exclusive [119-122]. The different sensitivities and accessibilities to the receptors add to the complexity of the PGE_2 response. In skeletal muscle, PGE_2 production can be stimulated by IL-1 and TNF [123, 124], both of these being markedly expressed in myositis [17, 82]. Involvement of PGE_2 has been reported in protein turnover and myogenesis, but also in mediating inflammation and muscular pain [125-127]. Expression of COX-1 and COX-2 mRNA has been reported in polymyositis and dermatomyositis [128], suggesting a role for PGE_2 pathway in myositis. The downstream mPGES-1 could be a possible drug target in order to reduce inflammation without causing side gastric ulcers or cardiovascular side-effects typical for traditional NSAIDs or COX-2 inhibitors [129].

4.4.3 Leukotrienes

As for the prostaglandin production, leukotriene synthesis starts from the $cPLA_2$-mediated release of arachidonic acid. 5-lipoxygenase (5-LO) catalyzes the two first steps in the leukotriene biosynthesis, first the oxygenation of arachidonic acid to 5-HPETE followed by the dehydration converting 5-HPETE to the unstable intermediate leukotriene A_4 (LTA_4) [130]. 5-LO is expressed on a variety of leukocytes, neutrophils, monocytes/macrophages, DCs, and B cells. In a resting state, 5-LO resides in the cytosol but upon stimuli it translocates into the nuclear membrane where it actively converts arachidonic acid to LTA_4 in two reactions [131]. The leukotriene synthesis also requires 5-LO activating protein (FLAP), a non-enzymatic scaffolding protein. FLAP presents arachidonic acid to 5-LO but can also increase the efficiency of the conversion of 5-HPETE into LTA_4 thus FLAP and 5-LO are co-localized in activated cells. Although their transcriptional regulation differs, 5-LO and FLAP often exhibit similar expression [131, 132]. The unstable intermediate LTA_4 is transformed into either LTB_4 or to cysteinyl leukotriene LTC_4 which might be further converted into LTD_4 and LTE_4. FLAP inhibitors acts as broad-spectrum leukotriene modifier drugs by blocking the formation of leukotriene B_4 as well as the cysteinyl leukotrienes; LTC_4, LTD_4 and LTE_4.

4.4.4 LTB_4

LTB_4 is a potent chemoattractant for T cells and neutrophils and can, in addition, promote smooth muscle cell proliferation, leukocyte adhesion and augment vascular permeability. LTA_4 hydrolase (LTA_4H) is a broadly expressed enzyme and converts LTA_4 into LTB_4 [133]. The effects of LTB_4 are mediated by the high affinity receptor BLT1, primarily expressed on leukocytes, but also on endothelial cells and smooth muscle cells. The low affinity BLT2 receptor binds other eicosanoids in addition to LTB_4 and is ubiquitously expressed [134]. In the context of polymyositis and dermatomyositis, LTB_4 is interesting as this molecule may be produced in skeletal muscle as previously demonstrated in patients with fibromyalgia using microdialysis technique [135, 136]. Enhanced expression of 5-LO mRNA has also been demonstrated in muscle tissue from patients with polymyositis or dermatomyositis indicating a role of the LTB_4 pathway role in the pathogenesis of these diseases [137]. In addition to the pro-inflammatory effects, LTB_4 contributes to muscle regeneration by promoting the proliferation and differentiation of satellite cells and differentiation of myoblasts through the BLT1 receptor [138]. Thus LTB_4 might play an important role in different pathogenic processes in inflammatory myositis. However, detailed characterization of the LTB_4 biosynthetic pathway in muscle tissue from patients with polymyositis and dermatomyositis is lacking.

5 MUSCLE STRUCTURE

Skeletal muscle, the largest organ in the body, is highly adaptable, and responds well to physiological as well as pathological challenges. To understand the skeletal muscle weakness and reduced muscle endurance in patients with polymyositis and dermatomyositis not only the effects of the immune responses, but also mechanisms in normal muscle physiology need to be clairified.

5.1 MUSCLE FIBER TYPES

Muscle fibers are generally divided into fast and slow twitch fibers depending on their contractility. Whereas the slow twitch, oxidative type I fibers mainly are used for aerobic endurance work the fast twitch, glycolytic type II fibers are predominately made for more explosive, shorter pulses of high-intensity muscle work. Type II fibers can be further divided into subtypes; type IIA contain a mix of oxidative and glycolytic features, type IIB is highly glycolytic and type IIC is a developmental, transient type that could differentiate to exhibit either oxidative or glycolytic characteristics [139, 140]. Different muscle groups are constituted of somewhat different proportions of the type I and type II fibers respectively, depending on the distinctive demand on that specific muscle. One of the quadriceps in thigh muscle; *m.vastus lateralis* is generally composed by an approximately equal number of slow and fast twitch fibers [141].

5.2 MUSCLE CONTRACTION

A muscle contraction is initiated by the nervous system communicating with skeletal muscle fibers by the release of the neurotransmitter acetylcholine at the neuromuscular junction, thereby producing an action potential that spreads along the whole muscle fiber. Released Ca^{2+} ions from the sarcoplasmic reticulum (SR) bind to troponin, which induces a conformational modulation of tropomyosin uncovering the myosin binding sites. This allows an interaction between myosin and actin and by utilizing stored ATP, a cross-bridge cycle is created and the muscle contracts. Muscle contraction therefore consumes a vast amount of ATP and resting muscle fibers only contain enough ATP and creatine phosphate (CP) reserves to sustain a contraction until new ATP has been generated. Energy release starts with the breakdown of dietary macronutrients and stored glycogen during glycolysis, where pyruvate is generated from glucose. In the absence of oxygen, glycolysis itself can generate a small amount of ATP used for anaerobic muscle contraction but the majority of ATP released comes from the citric acid cycle and electron transport chain in the mitochondria [142, 143].

When the muscle no longer can continue to perform contractions, it is said to be fatigued. Causes underlying muscle fatigue could for example be pulmonary, circulatory or microvascular insufficiency, sarcoplasmic dysfunction or mitochondrial enzyme insufficiency that may affect the energy process in the mitochondria [142, 144, 145].

5.3 MUSCLE FIBER PLASTICITY

Muscle fibers exhibit plasticity, in the sense that they are highly adaptive and can alter upon different physiological or pathological demands. Steroid myopathy, ageing, disuse, hypoxia and physical exercise are some examples of conditions where fast-to-slow or slow-to-fast transitions have been described [140]. Adaptations could be seen as an increased proportion of type IIC fibers, the intermediate fiber type between type I and type IIA [146]. Suggested mechanisms for the switch from slow-to-predominantly-fast twitch muscle fibers are through transcription factor FOXO1 induction and suppression of muscle oxidative capacity by inhibiting calcineuring pathway [147]. Another transcription factor that has been implied to be involved is chREBP, regulated by glucose [148]. At present there is only one report of high levels of glucose promoting a slow-to-fast shift in muscle fibers via chREPB. In patients with chronic myositis a characteristic feature of muscle tissue is low content of slow contractile, oxidative fibers in *m.vastus lateralis*, suspected to play a role in the impaired muscle endurance in polymyositis and dermatomyositis [149].

The fiber cross-sectional area (CSA) determines muscle force and correlates with muscle strength in healthy individuals. Atrophied muscle fibers, i.e. a reduced CSA, can be a consequence of several pathological conditions and the type II fibers in particular, seem to be sensitive [140]. In myositis fiber atrophy has been reported in text books and articles and is seen as either perifascicular atrophy or scattered throughout the muscle tissue [3]. In more recent years, these atrophic fibers have been considered being of a more regenerative type that could explain differences in muscle fiber size. Through the literature there has not been a standardized method to investigate cross-sectional area. Thus results from the earlier studies are difficult to compare with each other [150, 151]. In general, the cross sectional area of the different muscle fiber types in *m.vastus lateralis* is larger for men than for women. Type II fibers in women tend to be smaller than the type I fibers while the opposite relationship is seen in men [141].

5.4 MUSCLE REGENERATION

Upon skeletal muscle injury, caused by trauma or micro tearing of muscle fibers caused by strenuous exercise the onset of the following inflammatory response follows a fairly consistent pattern [152, 153]. If the muscle repair mechanisms are inadequate, the consequence might be reduced muscle function and muscle wasting [152]. Signs of fiber degeneration and regeneration are classical histopathological features of myositis, but to what extent the inflammatory response in myositis is a feature that promotes muscle injury or muscle growth and repair is still not fully understood.

An initial step in muscle degeneration with fiber necrosis is the disruption of the sarcolemma followed by increased serum levels of proteins such as CK [154]. Initially, the injured muscle activates an inflammatory response driven by Th1 cytokines, such as IFNγ and TNF-α. Phagocytes such as neutrophils and M1 macrophages respond early in the process in order to remove cell debris. After the pro-inflammatory phase, quiescent muscle satellite cells are activated by different growth factors and starts proliferating and migrate to the site of injury. The muscle progenitor cells develop into myoblasts and promoted by IL-4, IL-10 and IL-13, the macrophage profile switches to M2 macrophages, present in wound-healing tissue

[45]. Following the proliferation stage the myoblasts become terminally differentiated myotubes and fuse with each-other to replace the damaged fibers [154]. At present, it is not possible to distinguish the features of the chronic inflammatory response in autoimmune myositis that causes damage from those that promote muscle regeneration and repair.
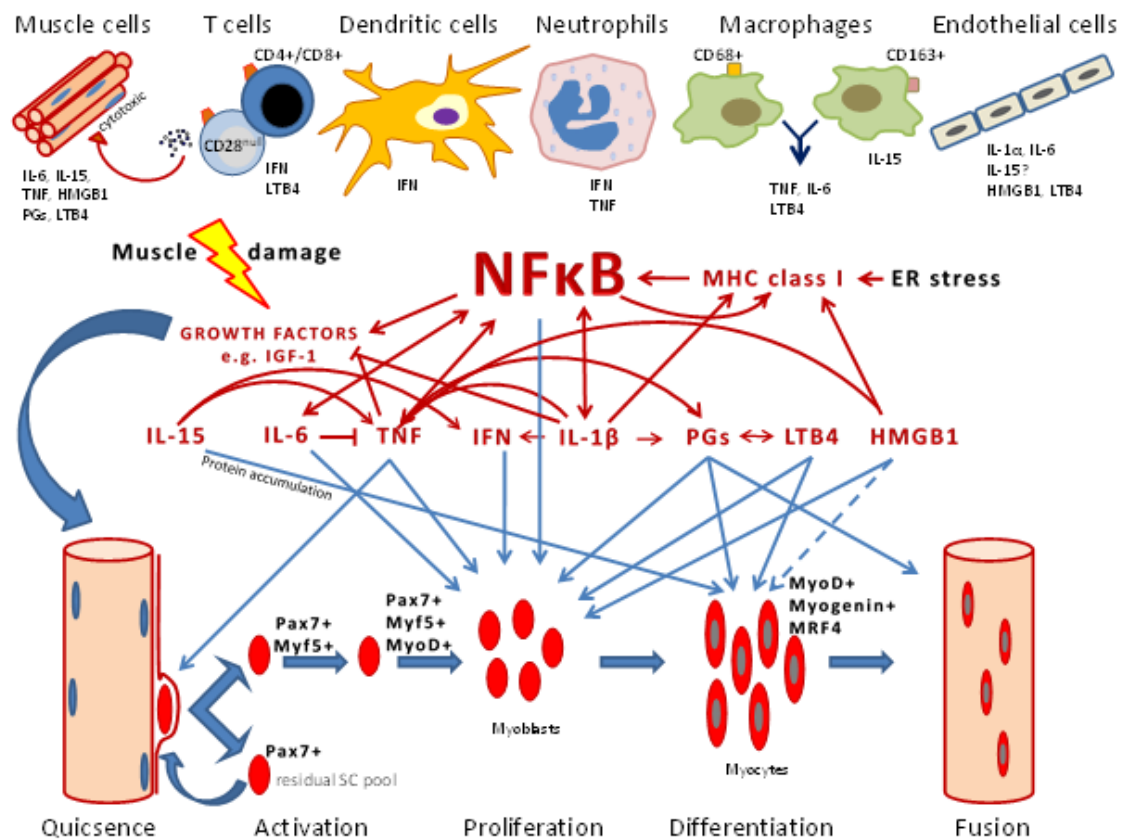
Figure 3. A schematic diagram illustrating the positive effects on muscle fiber regeneration and remodelling of molecules that might have a dual role inmuscle tissue both promoting inflammation and affecting skeletal muscle fiber regeneration and remodelling in myositis patients. The key molecules can influence each other (red arrows) as well as the different phases of skeletal muscle regeneration; the focus in this diagram is on the less frequently described positive effects (blue arrows) rather than the established negative effects. The broken blue arrow indicates inhibiting effects on muscle fiber differentiation. Upon skeletal muscle fiber damage, anti-inflammatory / immune response precedes myogenesis. The local immune response in muscle tissue has a direct effect on satellite cell activation, proliferation, differentiation or fusion. This local immune response might be disturbed (e.g. by immunosuppressive treatment), thus potentially influencing the functional restoration of fibers injured in various ways. Therefore, suppressing the immunesystem or blocking single cytokines or receptors might disturb skeletal muscle regeneration. Picture originally published in *J Intern Med 2011*. Reprinted with permission from John Wiley and Sons Inc.

6 TREATMENT

6.1 IMMUNOSUPPRESSIVE TREATMENT

The general concept of immunosuppressive treatment is to reduce the activity of the immune response. The suppression could involve specific target molecules or in a broader range, affecting several inflammatory pathways. Under optimal conditions the treatment only affects the intended mechanism but this is rarely the case and immunosuppressive drugs often pose a risk of side-effects. Below, the most common types of immunosuppressive treatments in polymyositis and dermatomyositis are presented.

6.2 GLUCOCORTICOIDS

Glucocorticoids are the first line of treatment in inflammatory and autoimmune conditions. These drugs have been extensively used since they were discovered in the late 1940s and the effectiveness is well proven although the responses vary largely between individuals. The physiological control of glucocorticoids involves regulation of carbohydrate, protein and fatty acid metabolism. The side-effects of glucocorticoid treatment are also well established and include Cushing syndrome, osteoporosis, diabetes mellitus and steroid myopathy, necessitating a demand for a tightly balanced administration in order to yield the most potent effect while keeping the adverse properties of the drug to an absolute minimum. To improve their efficacy and safety synthetic glucocorticoids should exhibit more glucocorticoid influence and less mineralocorticoid activity. More tailored methods of administration targeting the site of inflammation are also desirable.

The effects of glucocorticoids are dose-dependent and are determined by the rate of absorption, metabolism as well as the affinity for the glucocorticoid receptor. In myositis, the recommended initial dose is 0.75mg/kg bodyweight, which generally is around 60mg/day. This high dose is administrated during 2-4 weeks and tapered with approximately 2.5-5mg/month.

6.3 MECHANISMS OF GLUCOCORTICOID ACTION

As they are lipophilic in their structure, glucocorticoids can passively diffuse across plasma membranes and interact with cytosolic glucocorticoid receptors (GRs). The GR is a heteromeric complex that belongs to the steroid hormone receptor family and acts as a ligand-activated transcription factor. Ligand-free GR heteromers form complexes together with chaperones (e.g. heat shock proteins (Hsp's)) and co-chaperones (Hsp-binding or organizing proteins) [155]. The components in this receptor complex could act as modulators of affinity, nuclear translocation and also link steroid hormones to other signalling pathways. The glucocorticoid-GR association causes receptor activation and dissociation from its chaperones and modulators followed by a translocation into the cell nucleus, taking place within 10-30 min after glucocorticoid exposure.

In the nucleus the GR interacts with binding sites on the DNA called glucocorticoid-responsive elements (GRE) that are located in the promoter region of target genes [156]. The interaction could either be direct with GRE, or by protein-protein interaction

14

upon association with transcription factors on the DNA. Depending on what target gene the glucocorticoid-GR complex has bound the effects of either transactivation or transrepression can start, up to 120 minutes after entering the nucleus.

6.3.1 *Transactivation*
The GR complex enters the nucleus, binds to GRE sequences and activates the transcription of its target genes. Among the target genes are anti-inflammatory mediators such as IL-10, annexin 1 and inhibitors of NFκB. Additionally, transcription is initiated for regulator proteins important for metabolism and thus transactivation is considered to be the pathway that induces most side-effects of glucocorticoid treatment [157].

6.3.2 *Transrepression*
Transrepression is the main mechanism through which GR exerts its anti-inflammatory activities. Repression of a target gene could either be executed through binding to a GRE overlapping another transcription binding site thus blocking this gene, or by interacting with a transcription factor in an adjacent binding site and could in this way alternate the neighbouring gene transcription [157].

6.3.3 *Non-genomic effects*
Non-genomic effects are the actions upon glucocorticoids binding to GR but not depending on the subsequent binding to the glucocorticoid responsive elements (GREs). GR could regulate gene activation without binding directly to GRE segments on the DNA. Instead GR may physically interact with other transcription factors (e.g. AP-1 or NFκB) and thereby alter the gene expression [29, 158]. Through transrepression, GR can inhibit the genes that are under control of transcription factor NFκB; IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-18, COX-2, IFNs, TNF, CCL2 and VCAM [155, 157].

In addition, if high doses of glucocorticoids are given intravenously or intra-articularly, rapid responses that cannot origin from transcriptional alterations occur. These effects could be mediated by the proteins dissociating from the activated GR-complex [159]. Upon the detachment from the GR-complex, Src tyrosine kinase is able to mediate tyrosine phosphorylation of the epidermal growth factor receptor (EGFR), resulting in the inhibition of arachidonic acid release and eicosanoid synthesis [159]. Specific interactions with membrane-bound GRs, i.e. found on monocytes in RA [160], or non-specific interactions with plasma or mitochondrial membranes, may interfere with the membrane potentials, disrupting Ca^{2+} or Na^+ levels and thereby prevent signalling or ATP synthesis [158, 161].

6.3.4 *Induction of apoptosis in inflammatory cells*
In order for GR to induce apoptosis in a cell, the cell seems to require a certain level of endogenous GR protein and examples of immune cells susceptible to GR-induced programmed cell death are T cells and monocytes. Glucocorticoids are believed to induce apoptosis through both transrepression and transactivation mechanisms upon transcription factors and cytokines required for survival [155].
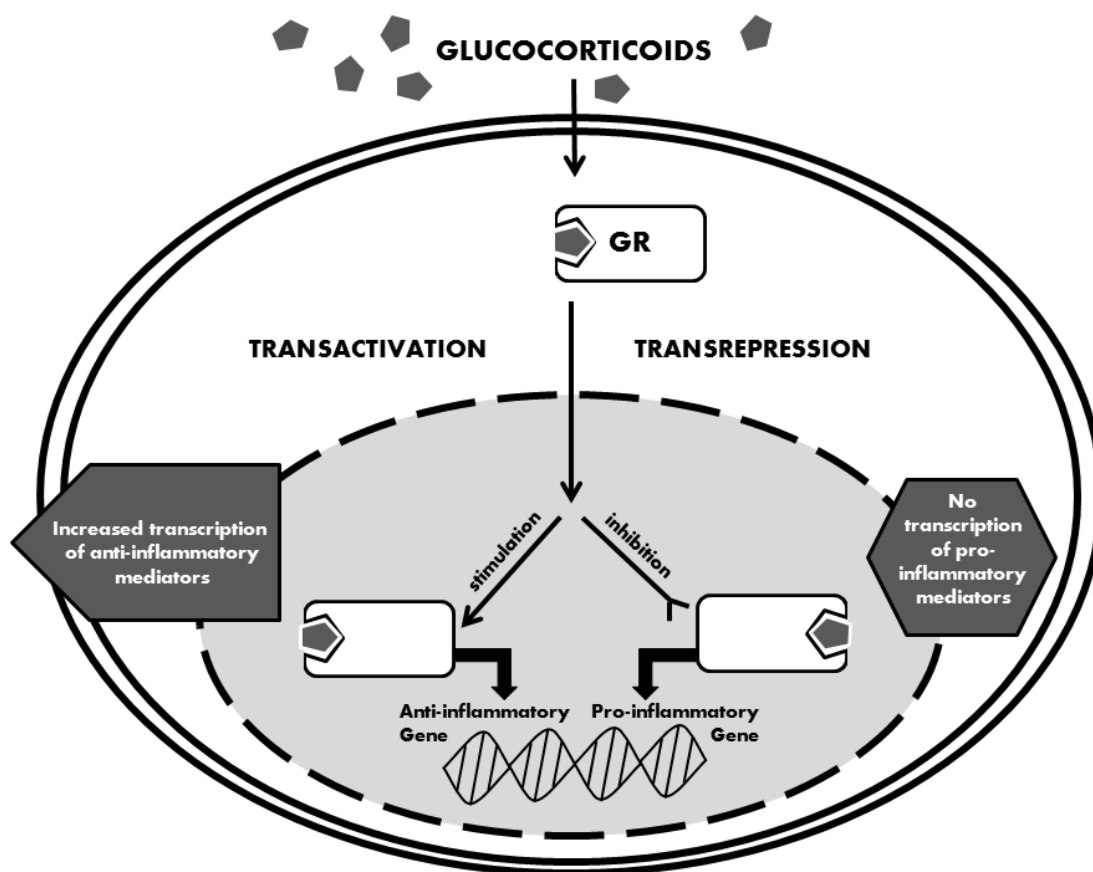
Figure 4. Schematic illustration of the immune modulation by glucocorticoids. Glucocorticoids bind to GR and translocates into the nucleus where it can modulate inflammation via both genomic and non-genomic mechanisms. GR=glucocorticoid receptor

6.4 SIDE-EFFECTS OF GLUCOCORTICOIDS

Diabetes or hyperglycemia is a very common side-effect in glucocorticoid treated patients. The primary role of glucocorticoids is to maintain blood glucose levels and the anti-insulin effect is accomplished by blocking glucose uptake in tissues. In addition, glucocorticoids up-regulate gluconeogenesis by increasing protein breakdown in order to release amino acids for glucose production .

Glucocorticoids induce bone loss and glucocorticoid-induced osteoporosis is often associated with fractures. The mechanism underlying the low bone turnover is the glucocorticoid stimulation of osteoclast differentiation and function along with inhibition of the same features in the osteoblasts [162].

Hypertension is a common side-effect in steroid treated patients. Approximately 20% of patients treated with exogenous glucocorticoids have been reported to have hypertension and research of the mechanisms involved is at present focused on oxidative stress and nitric acid interaction with eicosanoid synthesis [163].

The glucocorticoid effect on skeletal muscle proteins acts through inhibition of protein synthesis and induction of protein breakdown, mainly via the ubiquitin-proteasome pathway. Glucocorticoids can also induce the expression of myostatin, an important negative regulator of muscle growth [164].

16

6.5   STEROID MYOPATHY

Steroid myopathy is a non-inflammatory, toxic myopathy in response to high doses of glucocorticoids. The clinical manifestations remind a great deal of what patients with polymyositis and dermatomyositis experience from their disease i.e. proximal muscle weakness affecting daily life activities such as walking up stairs and rising from chairs. The mechanism underlying steroid myopathy is not known and there is no clear relationship between glucocorticoid dose or period of administration and the weakness [165]. In addition to the weakness and fatigue the muscle fibers display a shift from fast-to-slow twitch fiber predominance and selective type II atrophy [164]. The histopathological features tend to vary inter-individually due to disease and treatment duration, glucocorticoid dose, nutritional status and physical activity. Electromyography (EMG) looks normal within most patients and serum creatine kinase (s-CK) tends to stay within normal range [165].

6.6   DISEASE MODIFYING ANTI-RHEUMATIC DRUGS

Second-line disease-modifying anti-rheumatic drugs (DMARDs) can be used in order for patients to taper off the glucocorticoids more rapidly. There are several immunosuppressive agents used and they are selected based on clinical experience and some case series. Metotrexate (MTX) and azathioprine (AZA)are the most commonly used immunosuppressant added in myositis drug therapy and are well tolerated. Cyclophosphamide and cyclosporine A (CsA) are often used if the patient exhibit pulmonary involvement. Mycophenolate mofetil (MMF) is an immunosuppressive alternative to azathioprine, suppressing T cells and B cells [166, 167].

Intravenous immunoglobulin (IVIG) is composed by IgG antibodies from around 1000 blood donors and is able to modulate the immune response in the recipient. IVIG is sometimes the choice in refractory myositis [168].

6.7   BIOLOGIC AGENTS

Biological agents have been available for over a decade and the first agents available were TNF-blockers. The mechanisms behind the drug action are involved in the prevention of TNF-α receptor binding, thereby reducing inflammation. The efficacy of TNF-blockade in myositis has only been evaluated in case series with diverse outcomes and in a uncontrolled study including 13 patients with refractory myositis, where 16 weeks of TNF-blockade resulted in disease flares in some patients [66].

Rituximab, an anti-CD20 antibody causes B cell depletion and has been shown efficacy in case series in polymyositis and dermatomyositis [169-171].

IL-1 and IL-1R are both up-regulated in muscle tissue of myositis patients and the expression persists after conventional immunosuppressive treatment. Based on those data, therapy with IL-1 receptor could be a treatment prospect. To this date, only one case report on myositis has been published, and one open-label study undertaken at Karolinska University Hospital has shown some positive results [172].

6.8   EXERCISE

Until quite recently patients with polymyositis and dermatomyositis were advised against physical activity since it was believed to worsen the inflammation. However,

less obedient patients cleared the way for studies showing that not only was physical exercise safe to perform, but rather that it improved strength, oxygen capacity and quality of life [173-175] in patients with IIM. Thus exercise has evolved into an important part of treatment in myositis patients and the optimal training regimen now needs to be established. A 12-week muscle endurance program has been shown to restore the skewed fiber type distribution (lower proportion of oxidative fibers) in patients with chronic myositis [149]. Intensive resistance training seem to reduce expression of genes regulating inflammation and fibrosis as well as decrease the clinical disease activity in myositis patients [176]. In addition, creatine supplementation has been reported to enhance the improvements from exercise in patients with polymyositis and dermatomyositis, with no relevant adverse effects [177]. However, it is not recommended as part of standard care for patients with polymyositis or dermatomyositis with immunosuppressive treatment. Today, exercise research in adult myositis is focused on understanding mechanisms for muscle impairment and improved muscle function in relation to exercise.

Both chronic and acute exercise can modulate the immune response although the mechanisms are less clear. Studies primarily focus on circulating immune cells and cytokines that may not necessarily reflect the status in the tissue. Contracting muscle fibers have the ability to produce and release IL-6 [54, 92] and the myokine release (IL-6, IL-8 and MCP-1) from skeletal muscle during the inflammatory response after heavy resistance exercise seem to be under the transcriptional control of NFkB [178]. More studies on exercise in patients with polymyositis and dermatomyositis are needed.

7 AIMS OF THIS THESIS

The overall aim of my doctoral studies has been to do a broad-ranging investigation of changes in muscle molecules and phenotypes before and after immunosuppressive treatment in patients with polymyositis and dermatomyositis. By performing detailed studies of muscle tissue from the patients, I have investigated pathways and circumstances that might be involved in the pathogenesis of myositis and that could provide more information on possible characters involved in the muscle weakness and low muscle endurance in polymyositis and dermatomyositis.

The specific aims were;

- To determine the role of muscle tissue IL-15 and IL-15Rα expression in patients with polymyositis or dermatomyositis, the relation to clinical symptoms and the influence of immunosuppressive treatment.

- To examine the influence of PGE_2 pathway as well as the consequence of immunosuppressive treatment on the PGE_2 biosynthetic enzymes in patients with polymyositis or dermatomyositis.

- To investigate the significance of LTB_4 pathway in polymyositis and dermatomyositis and to study the impact of immunosuppressive treatment on the proteins involved.

- To confirm the presence of *in vivo* bioactive LTB_4 in patients with polymyositis and dermatomyositis in the relation to functional performance.

- To investigate if the low proportion oxidative muscle fibers seen in chronic polymyositis/dermatomyositis patients might have a role in the disease pathogenesis and low muscle endurance.

8 PATIENTS & METHODS

In this thesis, the core investigations have been performed on muscle tissue from patients with polymyositis and dermatomyositis at the time point of diagnosis and at follow-up after approximately six months of treatment. The time frame of six months was when practiced difficult to maintain and the second measure point could readily be delayed by several months. Newly diagnosed patients have also been compared to chronic, treatment responsive and non-responsive patients as well as different cohorts of healthy individuals.

I have personally performed or participated in every step in every method except culturing and staining myotubes. For this I have had the pleasure of benefit from the expertise from highly appreciated co-workers.

8.1 PATIENTS AND HEALTHY CONTROLS

Muscle biopsies from patients with polymyositis and dermatomyositis and healthy individuals formed the basis of my studies. For the different studies somewhat different patient groups were included depending on the research questions that were to be addressed.

Most patients included were recruited from the rheumatology unit at Karolinska University Hopital except for one patient **(papers I, II, III and IV)** who belonged to Malmö University Hospital. Additional patients were also included from Sahlgrenska hospital in Gothenburg and Akademiska hospital, Uppsala based on a multi-centre collaboration **(paper III)**

Patients who were followed longitudinally in the rheumatology clinic with structured clinical follow up and a repeated muscle biopsy performed after a median of 8 months of disease treated with conventional immunosuppressive therapy is the major cohort of my studies. Mucle biopsies from these patients are included in all studies **(paper I, II, III and IV).** The patients were treated according to the choice of the treating physician.

In addition:
In **paper II** we included nine patients, with new onset polymyositis or dermatomyositis without follow-up biopsies available and three treatment resistant patients.
In **paper III** we included microdialysis samples from patients with established polymyositis or dermatomyositis. The microdialysis was performed before an exercise intervention study.
In **paper IV** we also included muscle biopsies patients with established disease and treatment-refractory patients (non-responders).

Six muscle biopsies from healthy volunteers were made available as controls through collaboration with the Neurology clinic, Karolinska Hospital **(paper I, II, III and IV).** In **paper IV**, five additional healthy volunteers from the same neurology clinic was included.
For the exercise intervention study **(paper III)** healthy controls were selected on age and level of physical activity among relatives of the included patients and nursing staff.

8.2 MUSCLE BIOPSY

The percutaneous biopsy needle was introduced by Bergstrom in 1962 to be used for diagnostic purposes as well as in research. At the rheumatology unit at Karolinska University Hospital the Weil-Blakesley conchotome is the preferred tool for collecting muscle tissue from patients with myositis. Muscle biopsy is an important diagnostic tool used for histopathology, but can also be used in mRNA and protein analysis. For research purposes where repeated biopsies are taken, the percutaneous conchotome biopsy or semi-open biopsy [179, 180] can be used. This technique gives a good sample size, as opposed to the more widely used needle biopsy and minor discomfort for the patient. It is specific for detecting inflammation but since their patchy nature, infiltrates may be missed.

8.3 FIBER TYPING & CROSS-SECTIONAL AREA

To discriminate between the different the myosin isotypes in *m.vastus lateralis* the diverse sensitivity of their ATPase activity to alkali or acid preincubations is used and the human skeletal muscle fibers can be classified into slow-twitch, fast-twitch and intermediate variants of fibers. This histochemical method is carried out at different pHs of 4.3, 4.6 and 10.3. The resulting images needs to be compared to each-other in order to discriminate between the fiber types. Oxidative fiber type I, and the more glycolytic type IIa, IIb or the intermediate/developmental type IIc appear in black, white and shades of grey depending on the preincubation pH of the tissue. ATPase staining is widely used and has been so for over 40 years. Even though it is a solid method there are disadvantages such as the need for multiple, consecutive sections and the fact that the staining fades over time. Alternatives or as a complement, immunohistochemical double-staining with antibodies against different isoforms of myosin heavy chain (MyHC) could be used and would provide the same information in one tissue section and be more preserved against fading.

In order to analyze the cross-sectional area (CSA) of the muscle fibers, nicotinamide adenine dinucleotide (NADH) staining was used and approximately 50 fibers of type I and type II, respectively was measured. We used computerized software and a work tablet featuring a wireless, pressure sensitive pen for optimal precision as the circumference was measured. NADH staining is appropriate for measuring muscle fiber CSA as it does not provide any shrinkage to the tissue. Type I fibers appears as dark blue and type II as light blue but NADH staining is preferably cross-referenced with ATPase staining to determine the fiber type measured.

8.4 IMMUNOHISTOCHEMISTRY & IMMUNOFLUORESCENCE

In order to detect the presence and as well as to study any alterations on cellular and protein levels in muscle tissue, immunohistochemistry (IHC) was the predominantly method performed. To determine the cellular localization of some markers immunofluorescence was used. Cross-sectioned muscle tissue gives a very comprehensive picture of the local muscle milieu in the patients with myositis. Antibody-stained tissue sections not only render information about the protein

expression but also general information regarding muscle morphology, infiltration of inflammatory cells, muscle degeneration or regeneration. Critical for these types of analyses is the tissue preparation. The first important step is how to freeze the muscle specimen in order to avoid artefacts. In our department the biopsies are placed in isopentane pre-chilled with liquid N_2 within a time frame of approximately 3-15 minutes after the biopsy has been collected. This has been shown to reduce the freezing artefacts that could develop by placing the muscle tissue directly into liquid N_2. Placing some ice crystals in the tube before freezing the samples may also improve tissue histology despite freeze storage. Sectioning muscle tissue properly in a cryostat requires patience and experience and in our lab the usual protocol is to either freeze the sections for future acetone fixation directly before performing cell surface staining or to fix the samples in formaldehyde for intracellular staining.

Evaluation and analysis of IHC staining can be made using a conventional microscope either qualitatively describing and/or semi-quantitatively scoring the expression pattern. To reduce the subjectivity of the analysis computerized image analysis administers a more automated routine but still the settings of the computer programs needs to be generated by the evaluator. With the equipment and software used in this thesis it was not achievable to discriminate between the positive staining intensities in the sample.

8.5 PRIMARY MYOTUBE CULTURE

As it is challenging to perform experiments on skeletal muscle *in vivo,* culturing myoblasts from skeletal muscle satellite cells becomes a useful technique. The muscle cell culture is flexible in the way that one can stop and maintain at any stage according to the investigational agenda. From digested muscle biopsies, muscle/satellite?? cells are extracted and cultured into myoblasts and further differentiated into myotubes. Myoblasts are muscle precursor cells with reasonably similar metabolic characteristics to adult skeletal muscle. However, myoblasts are mononuclear cells that upon fusion become the multinucleated myotubes. Despite its convenience, an *in vitro* culture cannot represent the *in vivo* situation and therefore, the interpretation of the results must be done with cautiousness.

8.6 MICRODIALYSIS

Microdialysis is a way to collect molecules via a semi-permeable membrane from the interstitial space. The technique has been around for somewhat 30 years and has developed into a tool for monitoring small molecules in the biochemistry of peripheral tissues. Microdialysis is minimally invasive and allows chemical communication within an intact organ. The technique accumulates, with the exception of glucose and lactate, volumes ranging from micromolar to nanomolar that might put demands on the analytical methods used. Despite any challenge to analyse small volumes of dialysate, microdialysis is a method with good prospects for the future providing human *in vivo* study conditions where inflammation and muscle metabolism can be investigated.

8.7 ENZYME IMMUNOASSAY

For the analysis of microdialysis eluate we used a LTB_4 enzyme immunoassay (EIA) kit. The method is a competitive, heterogenous binding assay where the antigen in the microdialysis sample competes with labeled, LTB_4 Tracer to bind with antibodies. The amount of bound Tracer measured is inversely related to the concentration LTB_4. EIA is a common method to analyze lipid mediators in liquids and tissue extracts. The advantages are the detection limits, usually ranging within nanogram to pikogram and also the analysis of compounds can be done with little or no preparation and therefore circumvents the rapid degradation of LTB_4.

8.8 MICROARRAY

Gene expression profiling can provide an overview of the consequences in the expression of genes involved in inflammation and muscle tissue alterations in the same tissue section. This is a method that quickly delivers a large amount of data that can be further used to search for prognostic markers or create other hypothesis-driven studies. For the experiments performed and discussed in this thesis, Affymetrix array platform was used. The GeneChip® oligo array has been widely used but has, along with every platform manufacturer both advantages and disadvantages. The major obstacle when using microarray is rather in the variability that could origin from each stage in the experiment reaching from the study design to the analysis and interpretation of the large amount of data generated. Analysis of paired samples e.g. before and after an intervention, reduces the problem with inter-individual variations. The data generated reflects the mRNA and should preferably be combined with protein analysis to be biologically relevant. This method was not used in any of the papers but the results will be discussed in relation to the results generated in papers I-IV.

8.9 CLINICAL OUTCOME MEASURES

8.9.1 Cumulative dose of prednisolone

The starting dose of glucocorticoids administrated to patients with early myositis is approximately 40-60mg/day depending on the patient's body weight. The immediate clinical response may vary a great deal from patient to patient but normally the dose can be tapered within 4 weeks. If the patient does not experience any flares, the tapering continues until the medication can be stopped or a sufficient low maintenance dose has been attained. In order to detect any differences in clinical or molecular changes due to the total amount or average prednisone the patient has been exposed to the cumulative dose of prednisone up to the time of investigation was estimated. Every patient file chart was reviewed and every change of dosage and tapering plan recorded in order to get an as precise estimation as possible. In those cases where the patient belonged to a collaborating hospital, the responsible physician made the clinical information available thus all the estimations were made by the same person to limit errors.

8.9.2 Serum creatin kinase / creatine phosphokinase

One diagnostic criterium of myositis according to Bohan and Peter [4, 5] is elevation of muscle enzyme activities in serum and measuring the levels of s-CK in myositis

patients is routine practice at. S-CK is traditionally considered a very useful enzyme in the diagnostic workup of myositis patients as it is relatively specific for muscle and highly sensitive to muscle injury but it is not specific for myositis. Increased s-CK is a result of damaged muscle fibers and no statistical correlation between s-CK and myositis disease activity or muscle strength has been proven [181]. Normal reference values for s-CK varies since resting levels depend on the amount of muscle mass, thus men have higher s-CK levels compared to women. Dermatomyositis patients also tend to have a higher incidence of normal s-CK as some patients have a presence of skin rash, making it easier to set the diagnosis at an early time point [181, 182].

As an outcome measure, s-CK is somewhat blunt. Magnetic resonance spectroscopy may be a more sensitive measurement of disease activity but it is expensive and not available to do in routine practice.

8.9.3 Functional Index

Functional index (FI) of myositis was first developed in the 1990s and measured a total of 14 sets of different exercises involving grip strength, elbow flexion, shoulder flexion and abduction, hip flexion and abduction, step test, heel, toe and head lifts, and finally sit-ups. The index demonstrates disability in patients with myositis and has a maximal capacity score of 64 per body side [183]. The total score of the method creates a ceiling effect and in order to show improvement (20%) patients need to have a quite low start level which makes it a less optimal outcome measure. The original FI was revised in 2006 and did successfully remove the ceiling and floor effects [184], but still the measure contains an interval that could limit the practice.

8.9.4 Manual muscle test

Manual muscle test (MMT) is a commonly used method for documenting muscle impairment. The test involves proximal, distal and axial muscles. It was evaluated by the International Myositis Assessment and Clinical Studies group (IMACS) and is a tool to assess muscular outcome in patients with myositis in clinical practice and for longitudinal follow-up in therapeutic trials. In MMT8 used in this thesis, eight muscle groups are tested, including 1 axial, 5 proximal (two upper and three lower extremities) and two distal muscles (one upper and one lower extremity) with a maximum score of 80 [185]. As for FI, the maximum score of MMT gives a ceiling effect where actual improvements could get lost in the follow-up if the patient is not severely impaired from start. Still, the information obtained is valuable for correlating the molecular data with a clinical measurement.

9 RESULTS & DISCUSSION

Pro-inflammatory mediators are up-regulated in muscle tissues of polymyositis and dermatomyositis

Many markers of inflammation have previously been studied in myositis muscle tissue; MHC class I molecules expression by the muscle fibers, different subsets of T cells, macrophages, DCs along with cytokines and chemokine levels. In the present study we aimed to determine the involvement of the cytokine IL-15 and IL-15Rα in muscle tissues of polymyositis and dermatomyositis patients as IL-15 is a T cell activating cytokine and T cells are believed to have a role in the pathogenesis of myositis. We were able to confirm an over-expression of IL-15, localized the IL-15 expression to CD163$^+$ resident macrophages and for the first time demonstrated expression of IL-15Rα in myositis muscle (**paper I**). IL-15 expression in muscle tissue from polymyositis and dermatomyositis has previously been reported in a study of very few patients [90, 186]. Here we could establish that myoblasts but not differentiated myotubes expressed IL-15, which is in contrast to the previously published study. The expression of IL-15 in macrophages was co-localized with CD3$^+$ T cells in the muscle tissue inflammation possibly indicating a role for IL-15 in T cell activation.

Prior publications have reported that IL-15 could promote the loss of CD28 on T cell membranes and enhance the progression and cytotoxicity in the generated CD28^{null} subset [85, 86]. The involvement of IL-15 in the development of CD28^{null} T cells is interesting since our group previously reported a high frequency of CD28^{null} T cells in peripheral blood and in muscle tissue of patients with polymyositis or dermatomyositis [40]. T cell expression does not seem to respond to immunosuppressive treatment (**papers I, II & III**) and the proportion of CD28^{null} T cells is increased after treatment (unpublished data). Using a regression analysis we could conclude a statistically significant correlation between IL-15 and CD28^{null} expression in the muscle tissue of this cohort of patients (unpublished data). Thus one could speculate that the role of IL-15 in polymyositis and dermatomyositis is to promote the loss of the CD28 molecule and to contribute to the persistence of the local inflammation.

IL-15Rα was detected in mononuclear cells within the same infiltrates as the IL-15 expressing cells and a positive correlation between IL-15 and the receptor was shown. IL-15Rα was also frequently seen in both endothelial cells of larger vessels and in capillaries similarly to the healthy individuals.

Additional roles for IL-15 and IL-15Rα seem to be to influence muscle phenotype in different ways. Loss of IL- IL-15Rα gives a more fatigue resistant muscle in mice due to an adaptive increase of oxidative fibers and mitochondrial content [100]. The IL-15 and IL-15Rα expression in capillaries and vessels was similar in both patients and healthy individuals and immunosuppressive treatment did not have any effect on their expression. This may indicate a role for IL-15 and its receptor in muscle metabolism. To investigate this, biopsies from the same muscle in all included patients need to be compared.

The involvement of eicosanoids in myositis has not been much investigated. PGE_2 and LTB_4 are potent mediators of inflammation, produced in skeletal muscle and the different arachidonic acid pathways show prospects as probable drug targets. Thus the pathways of PGE_2 **(paper II)** and LTB_4 **(paper III)** were investigated in patients with polymyositis and dermatomyositis.

In the PGE_2 pathway we could determine an overexpression of COX-1, COX-2 and mPGES-1 in patients compared to healthy individuals and mPGES-1 was predominantly found in infiltrating macrophages. Overexpression of IL-1 and TNF in the muscle tissue of patients with myositis could play a role in the induction of mPGES-1 in macrophages [123, 124, 187] thus contributing to PGE_2 production in polymyositis and dermatomyositis patients.

In the LTB_4 pathway, we could record an up-regulation in cells expressing 5-LO and the BLT1 expression in capillaries in comparison to healthy individuals. In patients, BLT1 expression in cells or larger vessels did not differ from healthy controls. The total FLAP expression did not differ from healthy controls but here we could identify a distinct sub group of patients (n=6/16) that expressed a significantly higher level of FLAP in comparison to the remaining patients as well as the controls. The lack of significance in the expression of cellular BLT1 between patients and healthy individuals was somewhat unexpected due to the almost total absence of immune cells in healthy muscle tissue. Thus we investigated whether skeletal muscle satellite cells might be the source of the BLT1 expression by staining consecutive tissue sections for BLT1 and PAX7 but we could not confirm any co-expression (unpublished data).

Pro-inflammatory mediators are only partly affected by immunosuppressive treatment.

Patients with polymyositis and dermatomyositis vary in their response to immunosuppressive treatment and the persistence of inflammatory markers in the muscle tissue of patients undergoing treatment has previously been reported.

In the first study **(paper I)**, IL-15 expression in muscle tissue was decreased after treatment in the whole group of patients. However, when the patients were divided into two subgroups based on the levels of IL-15 after immunosuppressive treatment almost half of the group still had elevated IL-15 compared with the healthy controls.

The group with remaining high IL-15 expression had a poorer functional outcome both after the 8 months (median value) of treatment and also in the 5-year follow up. The outcome measures assessed, FI in the study and MMT in the long term follow-up, are not directly comparable but still give an indication of a potential distinction in recovery of the patients with persistent IL-15 expression. The CD28^{null} T frequency in muscle tissues of polymyositis and dermatomyositis patients is increased after immunosuppressive treatment (unpublished data), suggesting that IL-15 might contribute to the maintained dominance of CD28^{null} cytotoxic T cells in myositis muscle tissue. IL-15 might thus be a predictor of disease progression.

The heterogeneity in disease mechanisms in myositis may be demonstrated by the importance of different pathways driving the inflammation. We identified another distinct subgroup through the expression of FLAP **(paper III)**, a major regulator in the

LTB$_4$ biosynthetic pathway. At the group level FLAP expression was down-regulated by treatment but after further analysis it was apparent that the decrease was only significant for patients with initially high FLAP levels. However, the treatment did not reduce the FLAP expression in parity to the levels observed in the subgroup of patients with low FLAP levels or healthy individuals. Whereas 5-LO and BLT1 expression in mononuclear cells were decreased after drug therapy, BLT1 expression in capillaries remained unaffected. Interestingly, the effect of immunosuppressive treatment on 5-LO and FLAP was different. The existing literature describes differential regulation of 5-LO and FLAP and moreover of leukotriene synthesis, the latter being suggested to be linked to the tight regulation of FLAP by endogenous PGE$_2$ [188].

In the PGE$_2$ pathway **(paper II)** immunosuppressive treatment did have an effect on COX-2 as previously described [128] but was not able to down-regulate the expression of COX-1 or mPGES-1. The decreased COX-2 expression could be a result of the previously described anti-inflammatory effects of glucocorticoids or as an indirect effect of a reduced number of CD68$^+$ macrophages. Targeting COX-2 through immunosuppressive treatment may interfere with the production of other prostaglandins involved in skeletal muscle regeneration. There are reports of mPGES-1 using COX-1-generated PGH$_2$ for PGE$_2$ synthesis [189, 190]. The persisting expression of mPGES-1 and COX-1 following immunosuppressive treatment in patients with polymyositis and dermatomyositis may maintain PGE$_2$ production and contribute to the chronicity in these patients. However, the functional coupling between COX-1 and mPGES-1 on total PGE$_2$ production in muscle tissue needs further investigation.

Microsomal PGES-1 has received a lot of attention as a potential drug target in inflammatory diseases. There are selective inhibitors of either mPGES-1 expression or its enzyme activity [191-193]; however, the main issues that still need to be evaluated are whether the selective inhibition of mPGES-1 will lead to isolated PGE$_2$ suppression or will interfere with the production of other prostaglandins.

The COX and 5-LO pathways are highly interconnected and blocking different steps of one pathway can shunt the arachidonic acid metabolism to another pathway. Long-term COX inhibition can switch the eicosanoid production over to the 5-LO pathway [194] and LTB$_4$ production and BLT1 signalling may in turn regulate the levels and stability of COX-2 mRNA [195]. There are some FLAP inhibitors in clinical trials for treating respiratory and cardiovascular disorders. By targeting FLAP in the upper part of the leukotriene pathway, the total leukotriene production will be affected. To avoid an undesired reduction in all downstream leukotrienes, inhibition of LTA$_4$ hydrolase or the BLT-1 receptor could provide an even more specific target.

Whether FLAP inhibitors, IL-15 or mPGES-1 blockade would work without major side-effects damaging the already afflicted skeletal muscle is yet to be explored.

Detection of *in vivo* LTB$_4$ levels and the consequence of a bout of acute exercise
Based on the evidence of the up-regulated expression of the markers of LTB$_4$ pathway in muscle tissues we wanted to compare the levels of LTB$_4$ in thigh muscles in patients with polymyositis or dermatomyositis as well as in healthy controls. We detected LTB$_4$

and could also reflect that a single bout of acute exercise elevated LTB_4 levels in the patient group but not in the healthy controls. A further study with a larger cohort is needed to confirm this finding. However, the results may also indicate the existence of potential subgroups within these patients, similarly to the subgroups with different FLAP-expression. It is an appealing idea that the patients with the high *in vivo* LTB_4 levels also have elevated expression of FLAP and/or 5-LO, but this remains to be investigated.

Fiber type transition and muscle atrophy in relation to muscle weakness & low endurance

Even though patients with polymyositis or dermatomyositis respond to some extent to the immunosuppressive treatment, the majority still develop sustained disability [196] and have a reduced quality of life [197]. In patients with established disease and persisting muscle weakness it is a problematic assignment to discriminate between which aspects of muscle tissue morphology belongs to the myositis pathogenesis and which are likely to be related to a steroid myopathy.

Previous studies from our research group have demonstrated a low proportion of type I fibers in the quadriceps muscle in patients with persisting weakness without signs of inflammation. When comparing patients in different state of disease and also treatment response in the chronic patients we determined that newly diagnosed patients with polymyositis or dermatomyositis exhibited a fiber type composition similar to that of healthy individuals. Thus the reduced endurance at disease onset is not a result of a lower proportion of oxidative fibers. Nonetheless, the predominance of glycolytic, less fatigue resistant type II fibers in the chronic phase might have an impact on muscle function.

Low endurance or fatigue is the inability to maintain a certain force of power output in the muscle and even if this recorded shift in fiber types is recognized, the question is whether the change has an assessable function. Comparisons of fiber type distribution **(paper IV)** were made in different groups without the further strength that paired comparisons from individual follow-up data might have provided. In the cohort of patients selected for the microarray experiment (see below), we had routine ATPase staining performed by a pathologist. An analysis of the type I and type II compositions before and after immunosuppressive treatment in these patients revealed a significant change towards a more pronounced glycolytic fiber phenotype (unpublished data) confirming the data in paper IV.

A second finding in **paper IV** was that no atrophy was evident in polymyositis and dermatomyositis patients after analysing fiber cross-sectional area in the different groups. These results are contradictory to previous publications that have suggested selective type II atrophy in patients with myositis. In addition, we found female myositis patients have a 20% smaller type II cross-sectional area compared to men, a feature mirroring muscle fiber composition in the healthy population. In order to avoid errors in incorporating measurements from the perifascicular areas where atrophic fibers are often seen, at least in dermatomyositis, we excluded those parts completely, but the results could have been strengthened further by measuring a higher number of

28

fibers. Our interpretation of these results is that there is a gender difference in muscle fiber CSA, particularly for type II fibers, in patients with polymyositis or dermatomyositis similar to that of the healthy population. This information could be valuable when interpreting muscle tissue sections in aspects of atrophy or muscle force-generating questions in patients with myositis.

Gene expression and tissue proteins manifestation after immunosuppressive treatment

To investigate markers of inflammation and muscle tissue structure in a wider sense we studied gene expression in muscle tissue from newly diagnosed, untreated patients during the disease and treatment progress. In this experiment we included six patients (2 polymyositis, 4 dermatomyositis) with biopsies from *m.vastus lateralis* before and after a median of 10 (8-16) months of immunosuppressive treatment (unpublished data).

Alterations in a number of genes coding for inflammatory responses and muscle tissue remodelling were observed. These results are not yet validated but might still give some indication on the different pathways of particular interest for our research group. Among the genes that were down-regulated following immunosuppressive treatment there was a high representation of HLA-genes encoding MHC I and II. The co-stimulatory proteins CD80 and CD86 works together to prime T cells along with CD28, thus promoting activation and survival in the T cells. Among the cytokines we noted a partial down-regulation of IL-18 and the IL-18BP as well as IL-15. Genes involved in type I and type II IFN signaling were to a large extent affected by immunosuppressive treatment. A variety of chemokine ligands and receptors were also down-regulated, both α-chemokines such as CXCL10 and CXCL11 and the β-chemokines CCL2, CCL5 and CCR2. ICAM-1 was also partially down-regulated after immunosuppressive treatment. In the genes related to the leukotriene pathway there was no noteworthy alteration in prostaglandin reductase 1, which can inactivate LTB_4. Prostaglandin E_2 receptors EP3 and EP4 were up- and down-regulated respectively.

This data provides indications that the gene transcription of a number of pro-inflammatory genes in skeletal muscle tissue of patients with polymyositis and dermatomyositis goes down during immunosuppressive treatment. Our interpretation of these results is that immunosuppressive treatment may have an impact on skeletal muscle inflammation but that the local milieu could be accountable for the preserved expression of inflammatory cells and mediators in polymyositis and dermatomyositis.

When we analyzed the genes involved in muscle tissue structure we noted that the fast-twitch muscle protein α-actinin 3 and vinculin were both up-regulated. The negative regulator of muscle growth myostatin was up-regulated while the myostatin regulating protease bone morphologic protein 1 (BMP1) was down after treatment. Ras associated with diabetes (RRAD) is involved early in skeletal muscle regeneration [198] and was found to be down-regulated after the drug therapy. The GR co-chaperone protein FK506 binding protein 5 (FKBP5), recently suggested to be involved in fiber type transition [199], was up regulated in response to treatment. Further, myosin heavy chain 4 (MYH4) coding for fast-twitch fibers was up-regulated. Myosin binding protein H (MYBPH) was strongly down-regulated. MYBPH is principally associated with fast

twitch fibers but according to others it may also represent an early sign of regeneration [200]. These transcriptional alterations in muscle tissue from patients with polymyositis and dermatomyositis could be interpreted as ongoing muscle tissue remodeling.

These results need to be further validated.

9.1 METHODOLOGICAL CONSIDERATIONS

Handling small number of patients for a rare disease raises questions of representativeness and makes the selection criteria quite broad. This can be rather sensitive to changes in time points for the longitudinal follow-up and when an outcome measure is lacking the material diminishes further. In addition, the patient material was collected over a long period of time (1998-2004) and this result in a discrepancy in the clinical data available. The IMACS outcome measures were not taken in use until 2003.

10 CONCLUSIONS

In this thesis, some novel molecular features in polymyositis and dermatomyositis have been investigated. The pro-inflammatory cytokine IL-15 and its receptor, markers in both the PGE_2 and LTB_4 pathways, as well as the composition of oxidative and glycolytic muscle fibers in patients at diagnosis and in a later phase of disease have all been examined and reported. Whereas IL-15, COX-1, mPGES-1 and the LTB_4 pathway might be involved as early markers of myositis chronicity, the low proportion of oxidative muscle fibers is not a marker of disease in early myositis but instead this feature appears to develop over time.

.
- IL-15 and IL-15Rα were overexpressed in polymyositis and dermatomyositis compared to healthy individuals and there was a negative correlation between baseline IL-15 and muscle function improvement. A defined subgroup did not respond to immunosuppressive treatment accompanied with a lesser functional outcome.

- The PGE_2 pathway was up-regulated in patients compared to healthy individuals and immunosuppressive treatment only gave partial down-regulation of the pathway.

- Our results revealed an elevated LTB_4 pathway in polymyositis and dermatomyositis. Drug therapy was only able to partially suppress LTB_4 production.

- We could detect *in vitro* LTB_4 in myositis patients and a bout of cycling significantly increased the levels.

- A low proportion of oxidative muscle fibers was not present in untreated patients at the time point of diagnosis. A skewed fiber type composition was thus excluded as a contributing factor to the low muscle endurance and pathogenesis in early disease.

11 FUTURE PERSPECTIVES & CONCLUDING REMARKS

It would be valuable to be able to separate potential glucocorticoid effects on myositis skeletal muscle from the inflammatory myopathy. Therefore a robust animal model in which a straightforward comparison between a glucocorticoid-treated IIM condition and an untreated group could be performed would be of interest.

Muscle biopsies are a gold mine of information and much data can be retrieved from multiple repeated biopsies, both to see very early alterations and to cover the prolonged disease process.

The microdialysis thechnique, and thereby an *in vivo* method that works in patients with polymyositis or dermatomyositis provides possibilities to investigate both physiologically and pathologically processes in the skeletal muscle. Here more studies evaluating inflammatory or metabolic responses could be performed either under resting conditions or under exercise induced stress in myositis patients.

With these muscle biopsies and dialysis perfusates available, methods providing large scale data could be assessed and this information could serve as a library in which new possible pathways important in polymyositis and dermatomyositis could be explored.

To me, the major challenge when investigating polymyositis and dermatomyositis patients has been to figure out a way to distinguish between which clinical and molecular features are caused by the disease *per se*, or by on-going normal or failed muscle regeneration or profound glucocorticoid treatment provocation. In my opinion, the complexity of the polymyositis and dermatomyositis diseases arises from an inflammatory target organ that grows and regenerates with inflammation. The Janus face of each immune cell and the dual features of every cytokine, known or just not discovered yet, are hurdles in the search for an optimal drug treatment.

Investigating differential expressions will not say anything about the actual function of the end protein being synthesized, but will give essential pointers of where to look and what to further investigate.

12 POPULÄRVETENSKAPLIG SAMMANFATTNING

Polymyosit och dermatomyosit (myosit) är sjukdomar som innefattar kronisk inflammation i skelettmuskulaturen. Patienterna upplever gradvis muskelsvaghet och sämre uthållighet som inverkar på det dagliga livet. Förutom muskelinflammationen löper patienterna även risk att utveckla lungsjukdom, hjärtproblem och cancer. Patienter med dermatomyosit har dessutom alltid karaktäristiska hudutslag. Myositpatienter behandlas inledningsvis med höga doser kortison som gradvis trappas ned och kombineras med ytterligare immundämpande medicin. Trots aggressiv behandling blir patienterna ofta inte helt återställda utan känner av muskelsvaghet även om inflammationen i musklerna minskat.

I min avhandling har jag undersökt muskelvävnad från patienter med polymyosit eller dermatomyosit. Muskeln är tagen med biopsi vid diagnostillfället då patienterna ännu inte fått någon immundämpande behandling samt vid ett uppföljningstillfälle efter ungefär 8 månaders medicinering. Förutom muskelbiopsier har vi samlat vätska inifrån muskeln på patienterna med microdialys, där man via en kateter sköljer ut molekyler från området mellan muskelfiberrna (=avlånga muskelceller). Vi har även tagit biopsier och gjort microdialys på frivilliga friska personer för att jämföra med våra patienter.

I dessa prover har jag sedan undersökt molekyler i olika inflammatoriska signalvägar som kan tänkas ligga bakom muskelsvagheten. Dessutom har jag försökt fastställa om sammansättningen av snabba och långsamma muskelfiber är en bidragande orsak. För dessa undersökningar färgas muskelns protein, oftast med antikroppar som binder specifikt till den molekyl som eftersöks. I avhandlingen lyckades vi först fastställa att myositpatienterna hade höga värden av signalmolekylen IL-15 och dess receptor. IL-15 kan aktivera de immunceller som kallas T celler och 'elda på' inflammationen samt göra T cellerna väldigt giftiga för muskelfiberrna. Den immundämpande behandlingen hade ingen verkan på IL-15 i 1/3 av patienterna. Sedan kunde vi även påvisa att proteiner som tillverkar prostaglandin E_2 (PGE$_2$) och leukotrien B_4 (LTB$_4$), som båda är signalmolekyler av fettsyror, i högre grad finns i myositpatienters muskler jämfört med friska personer. PGE$_2$ och LTB$_4$ upprätthåller inflammation genom att de attraherar immunceller som cirkulerar i blodet att ta sig ut till musklerna. Även dessa signalvägar visade sig bara delvis nedregleras av medicineringen. Mikrodialysproverna visade att patienterna hade lika mycket LTB$_4$ som friska under vila men efter ett cykelprov hade patienterna högre nivåer LTB$_4$ vilket kan bero på att de har en större andel av proteinerna som behövs under tillverkningen. Tidigare har man sett att myositpatienter har en låg andel långsamma, uthålliga muskelfiber och trott att det möjligen bidrog till muskelsvagheten. Då vi jämförde obehandlade patienter med patienter som under lång tid fått behandling kunde vi se att de obehandlade patienterna inte skiljde sig från friska. Därmed avskrev vi fiberfördelningen som orsak till muskelsvaghet, i alla fall i ett tidigt sjukdomsstadie.

Slutsatserna jag dragit av resultaten är att dagens immundämpande behandling inte är tillräcklig för att hämma muskelinflammationen hos många som har myosit och att det finns undergrupper ibland patienterna där de olika signalvägarna har olika stor betydelse för sjukdomsbilden. Kanske kan man behandla de olika signalvägarna individuellt men IL-15, PGE$_2$ och LTB$_4$ har samtliga egenskaper som behövs vid vanlig muskelförnyelse vilket gör behandlingssituationen mer komplex.

13 ACKNOWLEDGEMENTS

Walking the crooked line of life has taken me on a journey I would never have imagined. The people who joined me for the ride, some for parts of it and others all the way are acknowledged below.

To my supervisor **Ingrid**, I consider myself very lucky to have you as my supervisor. The things you have taught me about research, teamwork, patients and most important about myself are absolutely invaluable. Thank you for taking me on board and believing in me. You are the ultimate role model for women in science – you are *in persona* a highly skilled professor, medical doctor, woman, friend and human being at the same time. Never stop being a scientific rock star!

My co-supervisor **Marina**, not only have you taught me every practical step I've taken during my education, you have also been my biggest security knowing that you always give me your true opinion no matter what the question is. I've always trusted your honesty and felt very safe knowing that you look after my interest. Our friendship has made my road less rocky and your view on life and what's really important will always inspire me.

These two fantastic women have made marks both in science and in my life and I am forever thankful for them chaperoning me through this new world.

Lars, thank you for providing such an open and encouraging work environment. For always being so genuinely interested, forthcoming and up to date on everything going on. I am very proud to have been given the opportunity to belong to your department.

Ralf, thank you for accepting the mentorship, I knew you were a top choice! I have appreciated all our talks, lunches, high and low, inspirational science discussions and personal logistics. You've always been just an email away, always positive and ready to help me make sense of it all.

Mona, lovely Mona – a colourful explosion of exploration. Such genuine interest and curiosity to understand everything and everyone around you. You have become a very dear friend and I am happy that we've shared our journeys. I will never let you go.

Eva dearest, there has been absolutely no time when you haven't been there for me, work wise and personally. I can never reciprocate what you have done for me but I do try to be less sloppy in order to make your life easier. And since I obviously don't always succeed, I occasionally bake cookies for you.

Past and current members of the myositis group; **Cissi,** a special thanks to you, the one who got me into this place. **Sevim,** Thanks for all your wisdom and pep talks. You are true fighter! Thank you **Maryam** for your endless generosity and for sharing your lovely family. **Li, Helene and Malin**; wonderful ladies and wonderful friends. Thanks for all great laughs, scientific and personal discussions. **Mei,** thanks for being such an important addition to our group. Your warm personality and hunger for knowledge is

inspiring. **Jayesh;** I got such a dear friend when you joined our group. **Christina**, to my knowledge the best nurse in the world. I am so touched by your big, warm heart embracing all your patients and coworkers. **Lara**, **Inka & Andreas,** always with a smile and a kind word. **Paulius**, let's travel some more! **John**, I enjoy having you at my shoulder. **Anna**, my friend, let's keep it that way forever.

VIP persons who carried me through these years; Kära **Marianne**, nu får vi mer tid att prata igen. **Gunnel**, tack för att du alltid reder ut allt och aldrig förebrår mig. **Susanne,** trollar med knäna och alltid med ett skratt. **Veronica**, ser fram emot mer party med dig. **Uffe**, troget hjälper du mig när jag gång på gång behöver inloggningshjälp – det är alltid trevligt att sitta hos dig.

The eicosanoid people; **Per-Johan**, you are such a blast of ideas one can hardly defend oneself. A very fun and challenging addition of testosterone to my work. **Sven**, I will never forget that you let my 1-year old taste nutella for breakfast. Fortunately you have many other lovable features. **Karina**, even though we've mostly been pregnant we've still found times to work together and become friends. **Helena, Elena, Karin & Hanna**; I'm clueless of your work but you're such great gal's and there's so much of other fun stuff we can talk about. **Patrick**, I cannot thank you enough for all the support you provided. Thanks for all nice chats over the bench. Ever so lovely **Joan**, thank you for all the help, support and great times over gels and membranes. I had so much fun this last months doing experiments side by side with you.

For being wonderful female role models and just exeptionally kind people; Vivi, Marie WH, Helena H, Anca and Eva Jansson.

And not to forget the male role models; Leonid, all you have to do is smile and it feels comforting & Bob; thank you for always being available with kind advice and practical help.

Shankar – you are pure sunshine and provide balance to my life. I hope that all good things in life will come to you. **Lasse** for all your help and wise input – and for always blaming the hardware/software/IT department etc. instead of me. Viole, Heidi, Hanna, Hulda, Peter, Therese Ö, Lotta, Karine, Eva J, Vilja, Sukanya, Mona, Kristina, Akilan, Vivek, Srini, Marie Westman, Eva Melin, Dimitrios, Olga, Therese W, Emeli, Marcus, Aurelie, Alex, Omri, Nånnis, Jessi, Magda, Mikael...oh, oh, oh, I know I am forgetting some. I've been around since 2005 so I have to sum it up;
thanks to everyone in the Rheumatology and Neuroimmunology groups.

Thanks to all my new friends at K1; Thomas, Christina, Elisabeth, Birgitta & Susanne. Ni får mig att trivas I 'köket' och er forskning fascinerar mig.

Gustavo, my great teacher and friend both while working in DC and now here at CMM. I will always be grateful for you making me feel less alone over there. My other dear DC friends; Yetrib, Javad, Bill and Andrea. **Raju**; I am happy that I got the opportunity to work with you and I am honored that you are my friend. Eric Hoffmann, thank you for open up your lab for me to visit. It was a great adventure.

Alla mina nuttisar som såg till att jag klarade tentorna och hade roligt under tiden;
Ann, Burre, Sofia, Sevim, Susanne, Lisa, Sofie, Caroline, Carolina, Per & William

My in-law family; Ingrid, Veronica, Bengt, Jonas and Mattias with families – min förlängda familj. Jag är så glad att jag fick er på köpet och sätter stort värde på att ha er i mitt liv.

My dearest and oldest friend **Jenny**. Who would have known that the two of us starting to play soccer together at age 6 would end up PhDs with our children playing together on the opposite side of the country? I love you for never letting go and for bringing Sergio, Felicia and Marta into my life.

Friends I carry around in my heart always; Niklas, Martin, Maria, Linda, Stefan, Dick, Mats & Christian.

Meine **Oma**, words are not enough to express the impact this incredible woman has had on my life. I know how proud she would have been if she'd still been around. I miss you so much and I try to cherish the memory of you in everything that I do.

Mamma **Maria** och pappa **Arne**, allra käraste syster **Anne** och storebror **Peter** för att ni gjort mig till den jag är.

Min **Henrik**. Tack för ditt oerhörda stöd och din tro på mig. Your love makes me wanna be the best person I can, and allows me to be the person I usually end up being. Tack för ditt ändlösa tålamod och för våra älskade bus-loppor, **Alma and Inez**, who helps me to put things in perspective and teaches me more in one afternoon than 4 years of postgraduate studies. My love for you is eternal.

14 REFERENCES

1.   Mastaglia FL, Phillips BA. Idiopathic inflammatory myopathies: epidemiology, classification, and diagnostic criteria. *Rheum Dis Clin N Am*. 2002 Nov; 28(4):723-+.
2.   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*. 1991 Nov; 70(6):360-374.
3.   Dalakas M. Polymyositis, dermatomyositis and inclusion-body myositis. *N Engl J Med*. 1991; 325(21):1487-1498.
4.   Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). *New England Journal of Medicine*. 1975; 292(8):403-407.
5.   Bohan A, Peter JB. Polymyositis and dermatomyositis (first of two parts). *New England Journal of Medicine*. 1975; 292(7):344-347.
6.   Plotz PH, Rider LG, Targoff IN, et al. NIH conference. Myositis: immunologic contributions to understanding cause, pathogenesis, and therapy. *Annals of internal medicine*. 1995 May 1; 122(9):715-724.
7.   Marie I, Hatron PY, Hachulla E, et al. Pulmonary involvement in polymyositis and in dermatomyositis. *The Journal of rheumatology*. 1998 Jul; 25(7):1336-1343.
8.   Fathi M, Lundberg IE. Interstitial lung disease in polymyositis and dermatomyositis. *Current opinion in rheumatology*. 2005 Nov; 17(6):701-706.
9.   Haupt HM, Hutchins GM. The heart and cardiac conduction system in polymyositis-dermatomyositis: a clinicopathologic study of 16 autopsied patients. *Am J Cardiol*. 1982 Nov; 50(5):998-1006.
10.   Gonzalez-Lopez L, Gamez-Nava JI, Sanchez L, et al. Cardiac manifestations in dermato-polymyositis. *Clinical and experimental rheumatology*. 1996 Jul-Aug; 14(4):373-379.
11.   Zahr ZA, Baer AN. Malignancy in myositis. *Current rheumatology reports*. 2011 Jun; 13(3):208-215.
12.   Aggarwal R, Oddis CV. Paraneoplastic Myalgias and Myositis. *Rheum Dis Clin N Am*. 2011 Nov; 37(4):607-+.
13.   Spiera R, Kagen L. Extramuscular manifestations in idiopathic inflammatory myopathies. *Current opinion in rheumatology*. 1998 Nov; 10(6):556-561.
14.   Santmyire-Rosenberger B, Dugan EM. Skin involvement in dermatomyositis. *Current Opinion in Rheumatology*. 2003 Nov; 15(6):714-722.
15.   Dorph C, Englund P, Nennesmo I, et al. Signs of inflammation in both symptomatic and asymptomatic muscles from patients with polymyositis and dermatomyositis. *Annals of the Rheumatic Diseases*. 2006 Dec; 65(12):1565-1571.
16.   Coley W, Rayavarapu S, Nagaraju K. Role of non-immune mechanisms of muscle damage in idiopathic inflammatory myopathies. *Arthritis research & therapy*. 2012 Apr 27; 14(2):209.
17.   Nyberg P, Wikman AL, Nennesmo I, et al. Increased expression of interleukin 1alpha and MHC class I in muscle tissue of patients with chronic, inactive polymyositis and dermatomyositis. *The Journal of rheumatology*. 2000 Apr; 27(4):940-948.
18.   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 and Rheumatism*. 2005 Jun; 52(6):1824-1835.

19.	Salomonsson S, Grundtman C, Zhang SJ, et al. Upregulation of Mhc Class I in Transgenic Mice Results in Reduced Force-Generating Capacity in Slow-Twitch Muscle. *Muscle & Nerve*. 2009 May; 39(5):674-682.

20.	Grundtman C, Lundberg IE. Vascular involvement in the pathogenesis of idiopathic inflammatory myopathies. *Autoimmunity*. 2009; 42(7):615-626.

21.	Cid MC, Grau JM, Casademont J, et al. Leucocyte/endothelial cell adhesion receptors in muscle biopsies from patients with idiopathic inflammatory myopathies (IIM). *Clin Exp Immunol*. 1996 Jun; 104(3):467-473.

22.	Alger HM, Rayavarapu S, Nagaraju K. Measurement of Activation of the Endoplasmic Reticulum Stress Response in Autoimmune Myositis. *Method Enzymol*. 2011; 489:207-225.

23.	Estruch R, Grau JM, Fernandez-Sola J, et al. Microvascular changes in skeletal muscle in idiopathic inflammatory myopathy. *Human pathology*. 1992 Aug; 23(8):888-895.

24.	Grundtman C, Tham E, Ulfgren AK, et al. Vascular Endothelial Growth Factor Is Highly Expressed in Muscle Tissue of Patients With Polymyositis and Patients With Dermatomyositis. *Arthritis Rheum*. 2008 Oct; 58(10):3224-3238.

25.	Probst-Cousin S, Neundorfer B, Heuss D. Microvasculopathic neuromuscular diseases: Lessons from hypoxia-inducible factors. *Neuromuscular Disord*. 2010 Mar; 20(3):192-197.

26.	Emsliesmith AM, Engel AG. Microvascular Changes in Early and Advanced Dermatomyositis - a Quantitative Study. *Ann Neurol*. 1990 Apr; 27(4):343-356.

27.	Mastaglia FL, Phillips BA, Zilko P. Treatment of inflammatory myopathies. *Muscle & nerve*. 1997 Jun; 20(6):651-664.

28.	Oddis CV. Therapy of inflammatory myopathy. *Rheumatic diseases clinics of North America*. 1994 Nov; 20(4):899-918.

29.	Revollo JR, Cidlowski JA. Mechanisms generating diversity in glucocorticoid receptor signaling. *Annals of the New York Academy of Sciences*. 2009 Oct; 1179:167-178.

30.	Hayden MS, Ghosh S. NF-kappa B, the first quarter-century: remarkable progress and outstanding questions. *Gene Dev*. 2012 Feb 1; 26(3):203-234.

31.	Hayden MS, Ghosh S. NF-kappa B in immunobiology. *Cell Res*. 2011 Feb; 21(2):223-244.

32.	Herrmann O, Baumann B, de Lorenzi R, et al. IKK mediates ischemia-induced neuronal death. *Nature medicine*. 2005 Dec; 11(12):1322-1329.

33.	Barakat W, Herrmann O, Baumann B, et al. NF-kappa B induces PGE(2)-synthesizing enzymes in neurons. *N-S Arch Pharmacol*. 2009 Aug; 380(2):153-160.

34.	Sanchez-Galan E, Gomez-Hernandez A, Vidal C, et al. Leukotriene B4 enhances the activity of nuclear factor-kappa B pathway through BLT1 and BLT2 receptors in atherosclerosis. *Cardiovasc Res*. 2009 Jan 1; 81(1):216-225.

35.	Lee SJ, Kim CE, Seo KW, et al. HNE-induced 5-LO expression is regulated by NF-kappa B/ERK and Sp1/p38 MAPK pathways via EGF receptor in murine macrophages. *Cardiovasc Res*. 2010 Nov; 88(2):352-359.

36.	Peterson JM, Bakkar N, Guttridge DC. NF-kappa B SIGNALING IN SKELETAL MUSCLE HEALTH AND DISEASE. *Curr Top Dev Biol*. 2011; 96:85-119.

37.	Creus KK, De Paepe B, De Bleecker JL. Idiopathic inflammatory myopathies and the classical NF-kappaB complex: current insights and implications for therapy. *Autoimmunity reviews*. 2009 Jun; 8(7):627-631.

38.	Engel AG, Arahata K. Mononuclear cells in myopathies: quantitation of functionally distinct subsets, recognition of antigen-specific cell-mediated cytotoxicity in some diseases, and implications for the pathogenesis of the different inflammatory myopathies. *Human pathology*. 1986 Jul; 17(7):704-721.

39.	Tournadre A, Miossec P. Interleukin-17 in inflammatory myopathies. *Curr Rheumatol Rep*. 2012 Jun; 14(3):252-256.

40.	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 Oct 1; 183(7):4792-4799.

41.	Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nature reviews Immunology*. 2008 Dec; 8(12):958-969.

42.	Gordon S. Alternative activation of macrophages. *Nature Reviews Immunology*. 2003 Jan; 3(1):23-35.

43.	Moyer AL, Wagner KR. Regeneration versus fibrosis in skeletal muscle. *Current opinion in rheumatology*. 2011 Nov; 23(6):568-573.

44.	Brunelli S, Rovere-Querini P. The immune system and the repair of skeletal muscle. *Pharmacological research : the official journal of the Italian Pharmacological Society*. 2008 Aug; 58(2):117-121.

45.	Tidball JG, Villalta SA. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am J Physiol-Reg I*. 2010 May; 298(5):R1173-R1187.

46.	Zhang L, Dong Y, Cheng J, et al. Role of integrin-beta3 protein in macrophage polarization and regeneration of injured muscle. *J Biol Chem*. 2012 Feb 24; 287(9):6177-6186.

47.	Englund P, Nennesmo I, Klareskog L, et al. Interleukin-1alpha expression in capillaries and major histocompatibility complex class I expression in type II muscle fibers from polymyositis and dermatomyositis patients: important pathogenic features independent of inflammatory cell clusters in muscle tissue. *Arthritis Rheum*. 2002 Apr; 46(4):1044-1055.

48.	Korotkova M, Helmers SB, Loell I, et al. Effects of immunosuppressive treatment on microsomal prostaglandin E synthase 1 and cyclooxygenases expression in muscle tissue of patients with polymyositis or dermatomyositis. *Ann Rheum Dis*. 2008 Nov; 67(11):1596-1602.

49.	de Padilla CM, Reed AM. Dendritic cells and the immunopathogenesis of idiopathic inflammatory myopathies. *Curr Opin Rheumatol*. 2008 Nov; 20(6):669-674.

50.	Grundtman C, Malmstrom V, Lundberg IE. Immune mechanisms in the pathogenesis of idiopathic inflammatory myopathies. *Arthritis research & therapy*. 2007; 9(2):208.

51.	Pedrol E, Grau JM, Casademont J, et al. Idiopathic Inflammatory Myopathies - Immunohistochemical Analysis of the Major Histocompatibility Complex Antigen Expression, Inflammatory Infiltrate Phenotype and Activation Cell Markers. *Clin Neuropathol*. 1995 May-Jun; 14(3):179-184.

52.	Arahata K, Engel AG. Monoclonal antibody analysis of mononuclear cells in myopathies. IV: Cell-mediated cytotoxicity and muscle fiber necrosis. *Ann Neurol*. 1988 Feb; 23(2):168-173.

53.	De Rossi M, Bernasconi P, Baggi F, et al. Cytokines and chemokines are both expressed by human myoblasts: possible relevance for the immune pathogenesis of muscle inflammation. *Int Immunol*. 2000 Sep; 12(9):1329-1335.

54.	Pedersen BK, Febbraio MA. Muscles, exercise and obesity: skeletal muscle as a secretory organ. *Nature reviews Endocrinology*. 2012 Apr 3.

55.	Lundberg I KA, Alexanderson H, Patarroyo M. Decreased expression of interleukin-1alpha, interleukin-1beta, and cell adhesion molecules in muscle tissue

following corticosteroid treatment in patients with polymyositis and dermatomyositis. *Arthritis & Rheumatism*. 2000; 43(2):336-348.

56.	Chevrel G, Granet C, Miossec P. Contribution of tumour necrosis factor alpha and interleukin (IL) 1 beta to IL6 production, NF-kappa B nuclear translocation, and class I MHC expression in muscle cells: in vitro regulation with specific cytokine inhibitors. *Annals of the Rheumatic Diseases*. 2005 Sep; 64(9):1257-1262.

57.	Mantovani A, Bussolino F, Dejana E. Cytokine Regulation of Endothelial-Cell Function. *Faseb J*. 1992 May; 6(8):2591-2599.

58.	Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood*. 1996 Mar 15; 87(6):2095-2147.

59.	Morel JCM, Park CC, Zhu K, et al. Signal transduction pathways involved in rheumatoid arthritis synovial fibroblast interleukin-18-induced vascular cell adhesion molecule-1 expression. *J Biol Chem*. 2002 Sep 20; 277(38):34679-34691.

60.	Dai SM, Matsuno H, Nakamura H, et al. Interleukin-18 enhances monocyte tumor necrosis factor alpha and interleukin-1 beta production induced by direct contact with T lymphocytes - Implications in rheumatoid arthritis. *Arthritis and Rheumatism*. 2004 Feb; 50(2):432-443.

61.	Dinarello CA. Interleukin-18, a proinflammatory cytokine. *Eur Cytokine Netw*. 2000 Sep; 11(3):483-486.

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

63.	Tucci M, Quatraro C, Dammacco F, et al. Increased IL-18 production by dendritic cells in active inflammatory myopathies. *Ann Ny Acad Sci*. 2007; 1107:184-192.

64.	Goetz FW, Planas JV, MacKenzie S. Tumor necrosis factors. *Dev Comp Immunol*. 2004 May 3; 28(5):487-497.

65.	Nagaraju K, Raben N, Merritt G, et al. A variety of cytokines and immunologically relevant surface molecules are expressed by normal human skeletal muscle cells under proinflammatory stimuli. *Clin Exp Immunol*. 1998 Sep; 113(3):407-414.

66.	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. *Annals of the Rheumatic Diseases*. 2008 Dec; 67(12):1670-1677.

67.	Dold S, Justiniano ME, Marquez J, et al. Treatment of early and refractory dermatomyositis with infliximab: a report of two cases. *Clinical Rheumatology*. 2007 Jul; 26(7):1186-1188.

68.	Anandacoomarasamy A, Howe G, Manolios N. Advanced refractory polymyositis responding to infliximab. *Rheumatology*. 2005 Apr; 44(4):562-563.

69.	Tezak Z, Hoffman EP, Lutz JL, et al. Gene expression profiling in DQA1*0501(+) children with untreated dermatomyositis: A novel model of pathogenesis. *Journal of Immunology*. 2002 Apr 15; 168(8):4154-4163.

70.	Schmidt J, Barthel K, Wrede A, et al. Interrelation of inflammation and APP in sIBM: IL-1 beta induces accumulation of beta-amyloid in skeletal muscle. *Brain*. 2008 May; 131:1228-1240.

71.	Baird GS, Montine TJ. Multiplex immunoassay analysis of cytokines in idiopathic inflammatory myopathy. *Arch Pathol Lab Med*. 2008 Feb; 132(2):232-238.

72.	Greenberg SA, Pinkus JL, Pinkus GS, et al. Interferon-alpha/beta-mediated innate immune mechanisms in dermatomyositis. *Ann Neurol*. 2005 May; 57(5):664-678.

73.   Greenberg. Molecular profiles of inflammatory myopathies (vol 59, pg 1170, 2002). *Neurology*. 2004 Feb 24; 62(4):678-678.

74.   Greenberg SA, Higgs BW, Morehouse C, et al. Relationship between disease activity and type 1 interferon- and other cytokine-inducible gene expression in blood in dermatomyositis and polymyositis. *Genes and immunity*. 2012 Apr; 13(3):207-213.

75.   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 Nov; 56(11):3784-3792.

76.   Harris HE, Andersson U, Pisetsky DS. HMGB1: a multifunctional alarmin driving autoimmune and inflammatory disease. *Nature reviews Rheumatology*. 2012 Apr; 8(4):195-202.

77.   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 and Rheumatism*. 2004 May; 50(5):1586-1594.

78.   Harris HE, Andersson U, Pisetsky DS. HMGB1: A multifunctional alarmin driving autoimmune and inflammatory disease. *Nat Rev Rheumatol*. 2012 Apr; 8(4):195-202.

79.   Sehgal PB, Wang L, Rayanade R, et al. Interleukin-6-type cytokines. *Interleukin-6 Type Cytokines*. 1995; 762:1-14.

80.   Hirano T. The Biology of Interleukin-6. *Chem Immunol*. 1992; 51:153-180.

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

82.   Lundberg I, Ulfgren AK, Nyberg P, et al. Cytokine production in muscle tissue of patients with idiopathic inflammatory myopathies. *Arthritis and Rheumatism*. 1997 May; 40(5):865-874.

83.   Carson WE, Giri JG, Lindemann MJ, et al. Interleukin (Il)-15 Is a Novel Cytokine That Activates Human Natural-Killer-Cells Via Components of the Il-2 Receptor. *Journal of Experimental Medicine*. 1994 Oct 1; 180(4):1395-1403.

84.   Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. *Blood*. 2001 Jan 1; 97(1):14-32.

85.   Alonso-Arias R, Moro-Garcia MA, Vidal-Castineira JR, et al. IL-15 preferentially enhances functional properties and antigen-specific responses of CD4+CD28(null) compared to CD4+CD28+ T cells. *Aging cell*. 2011 Oct; 10(5):844-852.

86.   Chiu WK, Fann M, Weng NP. Generation and growth of CD28nullCD8+ memory T cells mediated by IL-15 and its induced cytokines. *J Immunol*. 2006 Dec 1; 177(11):7802-7810.

87.   Alonso-Arias R, Moro-Garcia MA, Vidal-Castineira JR, et al. IL-15 preferentially enhances functional properties and antigen-specific responses of CD4+CD28(null) compared to CD4+CD28+T cells. *Aging Cell*. 2011 Oct; 10(5):844-852.

88.   Chiu WK, Fann M, Weng NP. Generation and growth of CD28(null)CD8(+) memory T cells mediated by IL-15 and its induced cytokines. *Journal of Immunology*. 2006 Dec 1; 177(11):7802-7810.

89.   Suzuki J, Morimoto S, Amano H, et al. Serum levels of interleukin 15 in patients with rheumatic diseases. *Journal of Rheumatology*. 2001 Nov; 28(11):2389-2391.

90. Sugiura T, Harigai M, Kawaguchi Y, et al. Increased IL-15 production of muscle cells in polymyositis and dermatomyositis. *Int Immunol*. 2002 Aug; 14(8):917-924.

91. Sugiura T, Kawaguchi Y, Harigai M, et al. Increased CD40 expression on muscle cells of polymyositis and dermatomyositis: Role of CD40-CD40 ligand interaction in IL-6, IL-8, IL-15, and monocyte chemoattractant protein-1 production. *Journal of Immunology*. 2000 Jun 15; 164(12):6593-6600.

92. Pedersen BK. Muscles and their myokines. *J Exp Biol*. 2011 Jan; 214(2):337-346.

93. Warren GL, Hulderman T, Jensen N, et al. Physiological role of tumor necrosis factor alpha in traumatic muscle injury. *Faseb J*. 2002 Oct; 16(12):1630-1632.

94. De Mori R, Straino S, Di Carlo A, et al. Multiple effects of high mobility group box protein 1 in skeletal muscle regeneration. *Arterioscl Throm Vas*. 2007 Nov; 27(11):2377-2383.

95. Sorci G, Riuzzi F, Arcuri C, et al. Amphoterin stimulates myogenesis and counteracts the antimyogenic factors basic fibroblast growth factor and S100B via RAGE binding. *Molecular and cellular biology*. 2004 Jun; 24(11):4880-4894.

96. Riuzzi F, Sorci G, Donato R. The amphoterin (HMGB1)/receptor for advanced glycation end products (RAGE) pair modulates myoblast proliferation, apoptosis, adhesiveness, migration, and invasiveness. Functional inactivation of RAGE in L6 myoblasts results in tumor formation in vivo. *J Biol Chem*. 2006 Mar 24; 281(12):8242-8253.

97. Gray SR, Kamolrat T. The effect of exercise induced cytokines on insulin stimulated glucose transport in C2C12 cells. *Cytokine*. 2011 Aug; 55(2):221-228.

98. Zanchi NE, Lira FS, de Siqueira Filho MA, et al. Chronic low frequency/low volume resistance training reduces pro-inflammatory cytokine protein levels and TLR4 mRNA in rat skeletal muscle. *European journal of applied physiology*. 2010 Aug; 109(6):1095-1102.

99. Tamura Y, Watanabe K, Kantani T, et al. Upregulation of circulating IL-15 by treadmill running in healthy individuals: is IL-15 an endocrine mediator of the beneficial effects of endurance exercise? *Endocrine journal*. 2011; 58(3):211-215.

100. Pistilli EE, Bogdanovich S, Garton F, et al. Loss of IL-15 receptor alpha alters the endurance, fatigability, and metabolic characteristics of mouse fast skeletal muscles. *The Journal of clinical investigation*. 2011 Aug; 121(8):3120-3132.

101. De Paepe B, Creus KK, De Bleecker JL. Role of cytokines and chemokines in idiopathic inflammatory myopathies. *Current opinion in rheumatology*. 2009 Nov; 21(6):610-616.

102. Civatte M, Bartoli C, Schleinitz N, et al. Expression of the beta chemokines CCL3, CCL4, CCL5 and their receptors in idiopathic inflammatory myopathies. *Neuropathology and applied neurobiology*. 2005 Feb; 31(1):70-79.

103. Figarella-Branger D, Civatte M, Bartoli C, et al. Cytokines, chemokines, and cell adhesion molecules in inflammatory myopathies. *Muscle & nerve*. 2003 Dec; 28(6):659-682.

104. Liu Y, Shaw SK, Ma S, et al. Regulation of leukocyte transmigration: cell surface interactions and signaling events. *J Immunol*. 2004 Jan 1; 172(1):7-13.

105. Jain A, Sharma MC, Sarkar C, et al. Increased expression of cell adhesion molecules in inflammatory myopathies: diagnostic utility and pathogenetic insights. *Folia neuropathologica / Association of Polish Neuropathologists and Medical Research Centre, Polish Academy of Sciences*. 2009; 47(1):33-42.

106. Lundberg I, Kratz AK, Alexanderson H, et al. Decreased expression of interleukin-1alpha, interleukin-1beta, and cell adhesion molecules in muscle tissue

following corticosteroid treatment in patients with polymyositis and dermatomyositis. *Arthritis Rheum*. 2000 Feb; 43(2):336-348.

107.   Khanapure SP, Garvey DS, Janero DR, et al. Eicosanoids in inflammation: biosynthesis, pharmacology, and therapeutic frontiers. *Current topics in medicinal chemistry*. 2007; 7(3):311-340.

108.   Spector AA. Arachidonic acid cytochrome P450 epoxygenase pathway. *Journal of lipid research*. 2009 Apr; 50 Suppl:S52-56.

109.   Kudo I, Murakami M. Phospholipase A2 enzymes. *Prostaglandins & other lipid mediators*. 2002 Aug; 68-69:3-58.

110.   Murakami M, Kudo I. Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. *Progress in lipid research*. 2004 Jan; 43(1):3-35.

111.   Sandig H, Pease JE, Sabroe I. Contrary prostaglandins: the opposing roles of PGD2 and its metabolites in leukocyte function. *Journal of leukocyte biology*. 2007 Feb; 81(2):372-382.

112.   Ricciotti E, FitzGerald GA. Prostaglandins and inflammation. *Arteriosclerosis, thrombosis, and vascular biology*. 2011 May; 31(5):986-1000.

113.   Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *The Journal of clinical investigation*. 2001 Jul; 108(1):15-23.

114.   Vane JR, Botting RM. Mechanism of action of nonsteroidal anti-inflammatory drugs. *Am J Med*. 1998 Mar 30; 104(3A):2S-8S; discussion 21S-22S.

115.   Sellers RS, Radi ZA, Khan NK. Pathophysiology of cyclooxygenases in cardiovascular homeostasis. *Veterinary pathology*. 2010 Jul; 47(4):601-613.

116.   Murakami M, Naraba H, Tanioka T, et al. Regulation of prostaglandin E2 biosynthesis by inducible membrane-associated prostaglandin E2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem*. 2000 Oct 20; 275(42):32783-32792.

117.   Hara S, Kamei D, Sasaki Y, et al. Prostaglandin E synthases: Understanding their pathophysiological roles through mouse genetic models. *Biochimie*. 2010 Jun; 92(6):651-659.

118.   Kalinski P. Regulation of immune responses by prostaglandin E2. *J Immunol*. 2012 Jan 1; 188(1):21-28.

119.   Coleman RA, Smith WL, Narumiya S. International Union of Pharmacology Classification of Prostanoid Receptors - Properties, Distribution, and Structure of the Receptors and Their Subtypes. *Pharmacol Rev*. 1994 Jun; 46(2):205-229.

120.   Narumiya S, Sugimoto Y, Ushikubi F. Prostanoid receptors: Structures, properties, and functions. *Physiol Rev*. 1999 Oct; 79(4):1193-1226.

121.   Matsuoka T, Narumiya S. Prostaglandin receptor signaling in disease. *Thescientificworldjo*. 2007; 7:1329-1347.

122.   Sugimoto Y, Narumiya S. Prostaglandin E receptors. *J Biol Chem*. 2007 Apr 20; 282(16):11613-11617.

123.   Baracos V, Rodemann HP, Dinarello CA, et al. Stimulation of muscle protein degradation and prostaglandin E2 release by leukocytic pyrogen (interleukin-1). A mechanism for the increased degradation of muscle proteins during fever. *The New England journal of medicine*. 1983 Mar 10; 308(10):553-558.

124.   Schafers M, Sorkin LS, Sommer C. Intramuscular injection of tumor necrosis factor-alpha induces muscle hyperalgesia in rats. *Pain*. 2003 Aug; 104(3):579-588.

125.   Rodemann HP, Goldberg AL. Arachidonic acid, prostaglandin E2 and F2 alpha influence rates of protein turnover in skeletal and cardiac muscle. *J Biol Chem*. 1982 Feb 25; 257(4):1632-1638.

126.   Zalin RJ. The role of hormones and prostanoids in the in vitro proliferation and differentiation of human myoblasts. *Experimental cell research*. 1987 Oct; 172(2):265-281.

127.   Otis JS, Burkholder TJ, Pavlath GK. Stretch-induced myoblast proliferation is dependent on the COX2 pathway. *Experimental cell research*. 2005 Nov 1; 310(2):417-425.

128.   Studynkova JT, Kuchen S, Jeisy E, et al. The expression of cyclooxygenase-1, cyclooxygenase-2 and 5-lipoxygenase in inflammatory muscle tissue of patients with polymyositis and dermatomyositis. *Clinical and experimental rheumatology*. 2004 Jul-Aug; 22(4):395-402.

129.   Samuelsson B, Morgenstern R, Jakobsson PJ. Membrane prostaglandin E synthase-1: a novel therapeutic target. *Pharmacological reviews*. 2007 Sep; 59(3):207-224.

130.   Samuelsson B, Haeggstrom JZ, Wetterholm A. Leukotriene Biosynthesis. *Annals of the New York Academy of Sciences*. 1991 Jul 31; 629:89-99.

131.   Peters-Golden M, Brock TG. 5-lipoxygenase and FLAP. *Prostag Leukotr Ess*. 2003 Aug-Sep; 69(2-3):99-109.

132.   Bell RL, Harris RR. The enzymology and pharmacology of 5-lipoxygenase and 5-lipoxygenase activating protein. *Clin Rev Allerg Immu*. 1999 Spr-Sum; 17(1-2):91-109.

133.   Borgeat P, Naccache PH. Biosynthesis and Biological-Activity of Leukotriene-B4. *Clin Biochem*. 1990 Oct; 23(5):459-468.

134.   Tager AM, Luster AD. BLT1 and BLT2: the leukotriene B-4 receptors. *Prostag Leukotr Ess*. 2003 Aug-Sep; 69(2-3):123-134.

135.   Hedenberg-Magnusson B, Ernberg M, Alstergren P, et al. Pain mediation by prostaglandin E2 and leukotriene B4 in the human masseter muscle. *Acta Odontol Scand*. 2001 Dec; 59(6):348-355.

136.   Hedenberg-Magnusson B, Ernberg M, Alstergren P, et al. Effect on prostaglandin E2 and leukotriene B4 levels by local administration of glucocorticoid in human masseter muscle myalgia. *Acta Odontol Scand*. 2002 Jan; 60(1):29-36.

137.   Studynkova JT, Kuchen S, Jeisy E, et al. The expression of cyclooxygenase-1, cyclooxygenase-2 and 5-lipoxygenase in inflammatory muscle tissue of patients with polymyositis and dermatomyositis. *Clinical & Experimental Rheumatology*. 2004; 22(4):395-402.

138.   Sun R, Ba X, Cui L, et al. Leukotriene B4 regulates proliferation and differentiation of cultured rat myoblasts via the BLT1 pathway. *Molecules and Cells*. 2009; 27(4):403-408.

139.   Pette D, Staron RS. Molecular basis of the phenotypic characteristics of mammalian muscle fibres. *Ciba Foundation Sypmosium*. 1988; 188:22-34.

140.   Bassel-Duby R, Olson EN. Signaling pathways in skeletal muscle remodeling. *Annual Review of Biochemistry*. 2006(75):19-37.

141.   Staron RS, Hagerman FC, Hikida RS, et al. Fiber type composition of the vastus lateralis muscle of young men and women. *Journal of Histochemistry & Cytochemistry*. 2000 May; 48(5):623-629.

142.   Martini F. Fundamentals of Anatomy & Physiology. 6th ed. San Fransisco: Pearson Education Inc, 2004.

143.   McArdle W. Exercise Physiology, Energy, Nutrition and Human Performance. 5th ed. Baltimore: Lippincott WIlliams & Wilkins, 2001.

144.   Allen DG, Lamb GD, Westerblad H. Skeletal muscle fatigue: Cellular mechanisms. *Physiol Rev*. 2008 Jan; 88(1):287-332.

145.   Keyser RE. Peripheral fatigue: high-energy phosphates and hydrogen ions. *PM & R : the journal of injury, function, and rehabilitation*. 2010 May; 2(5):347-358.

146.   Pette D, Staron RS. Cellular and molecular diversities of mammalian skeletal muscle fibers. *Reviews of physiology, biochemistry and pharmacology*. 1990; 116:1-76.

147.   Yuan Y, Shi XE, Liu YG, et al. FoxO1 regulates muscle fiber-type specification and inhibits calcineurin signaling during C2C12 myoblast differentiation. *Molecular and cellular biochemistry*. 2011 Feb; 348(1-2):77-87.

148.   Towle HC. Glucose as a regulator of eukaryotic gene transcription. *Trends Endocrin Met*. 2005 Dec; 16(10):489-494.

149.   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. *Arthrit Rheum-Arthr*. 2007 Oct 15; 57(7):1303-1310.

150.   Scola RH, Werneck LC, Prevedello DM, et al. Diagnosis of dermatomyositis and polymyositis: a study of 102 cases. *Arq Neuropsiquiatr*. 2000; 58(3B):789-799.

151.   Hilário MO, Yamashita H, Lutt iD, et al. Juvenile idiopathic inflammatory myopathies: the value of magnetic resonance imaging in the detection of muscle involvement. *Sao Paulo Med J*. 2000; 2(118):35-40.

152.   Bodinefowler S. Skeletal-Muscle Regeneration after Injury - an Overview. *J Voice*. 1994 Mar; 8(1):53-62.

153.   Grounds MD. Age-associated changes in the response of skeletal muscle cells to exercise and regeneration. *Towards Prolongation of the Healthy Life Span*. 1998; 854:78-91.

154.   Charge SBP, Rudnicki MA. Cellular and molecular regulation of muscle regeneration. *Physiol Rev*. 2004 Jan; 84(1):209-238.

155.   Necela BM, Cidlowski JA. Mechanisms of glucocorticoid receptor action in noninflammatory and inflammatory cells. *Proceedings of the American Thoracic Society*. 2004; 1(3):239-246.

156.   Hayashi R, Wada H, Ito K, et al. Effects of glucocorticoids on gene transcription. *Eur J Pharmacol*. 2004 Oct 1; 500(1-3):51-62.

157.   Newton R, Holden NS. Separating transrepression and transactivation: A distressing divorce for the glucocorticoid receptor? *Mol Pharmacol*. 2007 Oct; 72(4):799-809.

158.   Strehl C, Spies CM, Buttgereit F. Pharmacodynamics of glucocorticoids. *Clinical and experimental rheumatology*. 2011 Sep-Oct; 29(5 Suppl 68):S13-18.

159.   Croxtall JD, Choudhary Q, Flower RJ. Glucocorticoids act within minutes to inhibit recruitment of signalling factors to activated EGF receptors through a receptor-dependent, transcription-independent mechanism. *Brit J Pharmacol*. 2000 May; 130(2):289-298.

160.   Bartholome B, Spies CM, Gaber T, et al. Membrane glucocorticoid receptors (mGCR) are expressed in normal human peripheral blood mononuclear cells and up-regulated after in vitro stimulation and in patients with rheumatoid arthritis. *Faseb J*. 2004 Jan; 18(1):70-80.

161.   Stahn C, Buttgereit F. Genomic and nongenomic effects of glucocorticoids. *Nat Clin Pract Rheum*. 2008 Oct; 4(10):525-533.

162.   Carpinteri R, Porcelli T, Mejia C, et al. Glucocorticoid-induced osteoporosis and parathyroid hormone. *Journal of endocrinological investigation*. 2010; 33(7 Suppl):16-21.

163.   Ong SLH, Whitworth JA. How Do Glucocorticoids Cause Hypertension: Role of Nitric Oxide Deficiency, Oxidative Stress, and Eicosanoids. *Endocrin Metab Clin*. 2011 Jun; 40(2):393-+.

164.   Schakman O, Gilson H, Thissen JP. Mechanisms of glucocorticoid-induced myopathy. *J Endocrinol*. 2008 Apr; 197(1):1-10.

165.   Minetto MA, Lanfranco F, Motta G, et al. Steroid myopathy: Some unresolved issues. *Journal of endocrinological investigation*. 2011 May; 34(5):370-375.

166.   Bandelier C, Guerne PA, Genevay S, et al. Clinical experience with mycophenolate mofetil in systemic autoimmune conditions refractory to common immunosuppressive therapies. *Swiss medical weekly*. 2009 Jan 24; 139(3-4):41-46.

167.   Morganroth PA, Kreider ME, Werth VP. Mycophenolate mofetil for interstitial lung disease in dermatomyositis. *Arthritis care & research*. 2010 Oct; 62(10):1496-1501.

168.   Helmers SB, Dastmalchi M, Alexanderson H, et al. Limited effects of high-dose intravenous immunoglobulin (IVIG) treatment on molecular expression in muscle tissue of patients with inflammatory myopathies. *Ann Rheum Dis*. 2007 Oct; 66(10):1276-1283.

169.   Fernandez RR, Rubio JLC, Cano DS, et al. Rituximab in the treatment of dermatomyositis and other inflammatory myopathies. A report of 4 cases and review of the literature. *Clinical and experimental rheumatology*. 2009 Nov-Dec; 27(6):1009-1016.

170.   Cambridge G, Leandro MJ, Edwards JCW, et al. Serologic changes following B lymphocyte depletion therapy for rheumatoid arthritis. *Arthritis Rheum*. 2003 Aug; 48(8):2146-2154.

171.   Valiyil R, Casciola-Rosen L, Hong G, et al. Rituximab Therapy for Myopathy Associated With Anti-Signal Recognition Particle Antibodies: A Case Series. *Arthritis care & research*. 2010 Sep; 62(9):1328-1334.

172.   Zong M MV, Lundberg IE. Anakinra Effects on T Cells in Patients with Rrefractory Idiopathic Inflammatory Myopathies *Annals Rheum Dis*. 2011; 70(2).

173.   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 Oct 15; 57(7):1303-1310.

174.   Alexanderson H, Stenstrom CH, Jenner G, et al. 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.

175.   Alexanderson H, Dastmalchi M, Esbjornsson-Liljedahl M, et al. Benefits of intensive resistance training in patients with chronic polymyositis or dermatomyositis. *Arthritis Rheum*. 2007 Jun 15; 57(5):768-777.

176.   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 Nov-Dec; 16(11-12):455-464.

177.   Chung YL, Alexanderson H, Pipitone N, et al. Creatine supplements in patients with idiopathic inflammatory myopathies who are clinically weak after conventional pharmacologic treatment: Six-month, double-blind, randomized, placebo-controlled trial. *Arthritis Rheum*. 2007 May 15; 57(4):694-702.

178.   Vella L, Caldow MK, Larsen AE, et al. Resistance exercise increases NF-kappaB activity in human skeletal muscle. *American journal of physiology Regulatory, integrative and comparative physiology*. 2012 Mar; 302(6):R667-673.

179.	Dorph C, Nennesmo I, Lundberg IE. Percutaneous conchotome muscle biopsy. A useful diagnostic and assessment tool. *The Journal of rheumatology*. 2001 Jul; 28(7):1591-1599.

180.	Dietrichson P, Coakley J, Smith PE, et al. Conchotome and needle percutaneous biopsy of skeletal muscle. *Journal of neurology, neurosurgery, and psychiatry*. 1987 Nov; 50(11):1461-1467.

181.	Targoff IN. Laboratory testing in the diagnosis and management of idiopathic inflammatory myopathies. *Rheumatic diseases clinics of North America*. 2002 Nov; 28(4):859-890, viii.

182.	Rider LG, Miller FW. Laboratory evaluation of the inflammatory myopathies. *Clinical and diagnostic laboratory immunology*. 1995 Jan; 2(1):1-9.

183.	Josefson A, Romanus E, Carlsson J. A functional index in myositis. *The Journal of rheumatology*. 1996 Aug; 23(8):1380-1384.

184.	Alexanderson H, Broman L, Tollback A, et al. Functional index-2: Validity and reliability of a disease-specific measure of impairment in patients with polymyositis and dermatomyositis. *Arthritis Rheum*. 2006 Feb 15; 55(1):114-122.

185.	Rider LG, Koziol D, Giannini EH, et al. Validation of manual muscle testing and a subset of eight muscles for adult and juvenile idiopathic inflammatory myopathies. *Arthritis care & research*. 2010 Apr; 62(4):465-472.

186.	Sugiura T, Kawaguchi Y, Harigai M, et al. Increased IL-15 production in muscle cells of polymyositis/dermatomyositis. *Arthritis and Rheumatism*. 1999 Sep; 42(9):S158-S158.

187.	Khan KMF, Kothari P, Du BH, et al. Matrix Metalloproteinase-Dependent Microsomal Prostaglandin E Synthase-1 Expression in Macrophages: Role of TNF-alpha and the EP4 Prostanoid Receptor. *Journal of Immunology*. 2012 Feb 15; 188(4):1970-1980.

188.	Maxis K, Delalandre A, Martel-Pelletier J, et al. The shunt from the cyclooxygenase to lipoxygenase pathway in human osteoarthritic subchondral osteoblasts is linked with a variable expression of the 5-lipoxygenase-activating protein. *Arthritis research & therapy*. 2006; 8(6):R181.

189.	Boulet L, Ouellet M, Bateman KP, et al. Deletion of microsomal prostaglandin E-2 (PGE(2)) synthase-1 reduces inducible and basal PGE(2) production and alters the gastric prostanoid profile. *J Biol Chem*. 2004 May 28; 279(22):23229-23237.

190.	Dieter P, Scheibe R, Jakobsson PJ, et al. Functional coupling of cyclooxygenase 1 and 2 to discrete prostanoid synthases in liver macrophages. *Biochemical and biophysical research communications*. 2000 Sep 24; 276(2):488-492.

191.	Guerrero MD, Aquino M, Bruno I, et al. Synthesis and pharmacological evaluation of a selected library of new potential anti-inflammatory agents bearing the gamma-hydroxybutenolide scaffold: a new class of inhibitors of prostanoid production through the selective modulation of microsomal prostaglandin E synthase-1 expression. *J Med Chem*. 2007 May 3; 50(9):2176-2184.

192.	Bruno A, Di Francesco L, Coletta I, et al. Effects of AF3442 [N-(9-ethyl-9H-carbazol-3-yl)-2-(trifluoromethyl)benzamide], a novel inhibitor of human microsomal prostaglandin E synthase-1, on prostanoid biosynthesis in human monocytes in vitro. *Biochem Pharmacol*. 2010 Apr 1; 79(7):974-981.

193.	Mbalaviele G, Pauley AM, Shaffer AF, et al. Distinction of microsomal prostaglandin E synthase-1 (mPGES-1) inhibition from cyclooxygenase-2 inhibition in cells using a novel, selective mPGES-1 inhibitor. *Biochem Pharmacol*. 2010 May 15; 79(10):1445-1454.

194.	Harizi H, Juzan M, Moreau JF, et al. Prostaglandins inhibit 5-lipoxygenase-activating protein expression and leukotriene B-4 production from

dendritic cells via an IL-10-dependent mechanism. *Journal of Immunology*. 2003 Jan 1; 170(1):139-146.

195. Zhai BB, Yang HQ, Mancini A, et al. Leukotriene B-4 BLT Receptor Signaling Regulates the Level and Stability of Cyclooxygenase-2 (COX-2) mRNA through Restricted Activation of Ras/Raf/ERK/p42 AUF1 Pathway. *J Biol Chem*. 2010 Jul 30; 285(31):23568-23580.

196. Alexanderson H. Exercise effects in patients with adult idiopathic inflammatory myopathies. *Current opinion in rheumatology*. 2009 Mar; 21(2):158-163.

197. Sultan SM, Ioannou Y, Moss K, et al. Outcome in patients with idiopathic inflammatory myositis: morbidity and mortality. *Rheumatology (Oxford)*. 2002 Jan; 41(1):22-26.

198. Hawke TJ, Kanatous SB, Martin CM, et al. Rad is temporally regulated within myogenic progenitor cells during skeletal muscle regeneration. *Am J Physiol-Cell Ph*. 2006 Feb; 290(2):C379-C387.

199. Fisher I, Abraham D, Bouri K, et al. Prednisolone-induced changes in dystrophic skeletal muscle. *Faseb J*. 2005 May; 19(7):834-836.

200. Norman H, Nordquist J, Andersson P, et al. Impact of post-synaptic block of neuromuscular transmission, muscle unloading and mechanical ventilation on skeletal muscle protein and mRNA expression. *Pflug Arch Eur J Phy*. 2006 Oct; 453(1):53-66.