

# Can muscle regeneration fail in chronic inflammation: a weakness in inflammatory myopathies?

■ I. Loell & I. E. Lundberg

From the Rheumatology Unit, Department of Medicine, Karolinska University Hospital, Solna, Stockholm, Sweden

**Abstract.** Loell I, Lundberg IE (Karolinska University Hospital, Solna, Stockholm, Sweden) Can muscle regeneration fail in chronic inflammation: a weakness in inflammatory myopathies? (Review) *J Intern Med* 2010; doi: 10.1111/j.1365-2796.2010.02334.x.

Idiopathic inflammatory myopathies (IIMs), collectively termed myositis, include three major subgroups: polymyositis, dermatomyositis and inclusion body myositis. IIMs are characterized clinically by muscle weakness and reduced muscle endurance preferentially affecting the proximal skeletal muscle. In typical cases, inflammatory cell infiltrates and proinflammatory cytokines, alarmins and eicosanoids are present in muscle tissue. Treatment with glucocorticoids and other immunosuppressants results in improved performance, but complete recovery is rarely seen. The mechanisms that cause muscle weakness and reduced muscle endurance are multi-factorial, and different mechanisms predominate in different phases of disease. It is likely that a combination of immune-mediated and nonimmune-mediated mechanisms contributes to clinical muscle symptoms. Immune-

mediated mechanisms include immune cell-mediated muscle fibre necrosis as well as direct effects of various cytokines on muscle fibre contractility. Among the nonimmune-mediated mechanisms, an acquired metabolic myopathy and so-called endoplasmic reticulum stress may be important. There is also a possibility of defective repair mechanisms, with an influence of both disease-related factors and glucocorticoid treatment. Several proinflammatory molecules observed in muscle tissue of myositis patients, including interleukin (IL)-1, IL-15, tumour necrosis factor, high-mobility group box-1 and eicosanoids, have a role in muscle fibre regeneration, and blocking these molecule may impair muscle repair and recovery. The delicate balance between immunosuppressive treatment to downregulate proinflammatory molecules and an inhibitory effect on muscle fibre regeneration needs to be further understood. This would also be relevant for other chronic inflammatory diseases.

**Keywords:** cytokines, eicosanoids, inflammation, inflammatory myopathies, myositis, pathogenesis, physical exercise, regeneration.

## Background

Idiopathic inflammatory myopathies (IIMs), collectively known as myositis, are characterized clinically by muscle weakness and a low level of muscle endurance preferentially affecting the proximal skeletal muscle. Initial symptoms include difficulty in climbing stairs and rising from a chair. Muscle weakness may develop over weeks to months into severe weakness, and occasionally, patients may become wheelchair dependent. Difficulty in swallowing or breathing may also occur owing to involvement of the pharyngeal or thoracic muscles. Pain is a less common problem and is usually reported as delayed onset muscle soreness after exercise. A typical finding from muscle biopsies is inflammatory cell infiltrates composed mainly of T

cells, macrophages and dendritic cells [1, 2]. Based on various clinical and histopathological phenotypes, three major subgroups of IIMs have been identified: polymyositis, dermatomyositis and inclusion body myositis (IBM) (Tables 1 and 2) (Fig. 1a–c) [3]. Other organs are frequently involved, such as skin in dermatomyositis and lung in both polymyositis and dermatomyositis. Less often, the joints, heart and gastrointestinal tract are affected. Autoantibodies are commonly found in IIMs, and positive antinuclear autoantibodies are found in up to 80% of cases of polymyositis and dermatomyositis but less frequently in IBM (20%) (Table 1) [4]. Treatment is based on glucocorticoids in high doses over weeks to months, often in combination with other immunosuppressants such as azathioprine or methotrexate [5].

**Table 1** Presentation of clinical symptoms and autoantibodies in subsets of myositis

Clinical symptoms	Polymyositis	Dermatomyositis	Inclusion body myositis (IBM)
Proximal muscle weakness	++	++	++ (quadriceps)
Distal muscle weakness	+	+	++ (finger flexors)
Low muscle endurance	++	++	+
Skin rash	+ (mechanic's hands)	++ (heliotrope rash, Gottron's papules or sign)	–
Interstitial lung disease (ILD)	+	+	–
Nonerosive arthritis	+	+	–
Heart involvement (myocarditis)	+	+	–
Autoantibodies	80%	80%	20%

++: pronounced symptoms present in many patients.

+: present less often or mild symptoms.

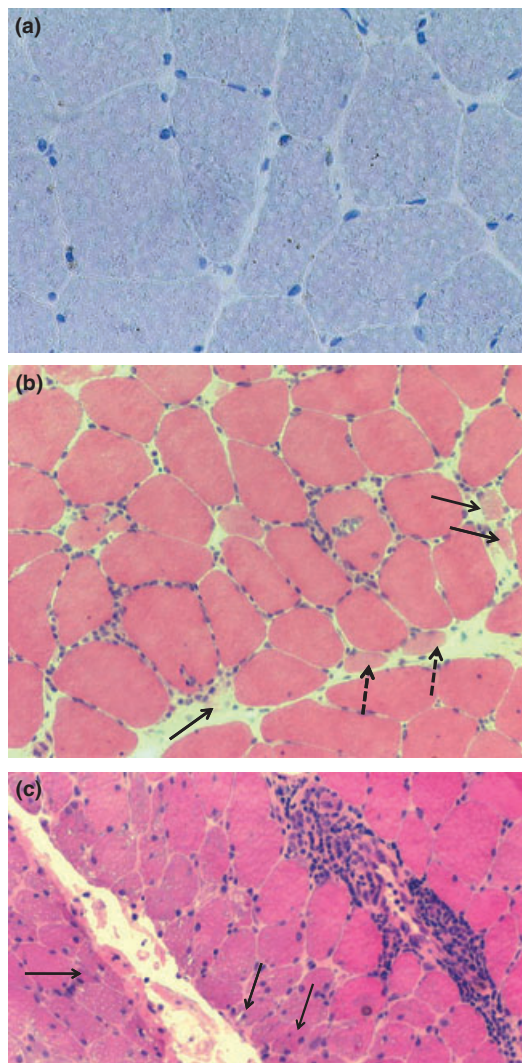
**Table 2** Muscle biopsy characteristics in subsets of myositis

Muscle biopsy feature	Polymyositis	Dermatomyositis	Inclusion body myositis (IBM)
Inflammatory cell infiltrates with preferentially endomysial distribution	++	+	++
Inflammatory cell infiltrates with preferentially a perimysial distribution	+	++	+
CD8+ T cells in infiltrates	++	–	++
CD4+ T cells in infiltrates	++	++	++
Macrophages	++	++	++
B cells	–	+	–
Plasmacytoid dendritic cells (pDCs)	+	++	+
Major histocompatibility complex (MHC) class I expression on muscle fibres	+	+	+
Capillary loss	+	++	?
Perifascicular 'atrophy' of muscle fibres	+	++	?

The molecular mechanisms that cause muscle weakness and reduced muscle endurance in patients with myositis have not been fully clarified. These mechanisms may vary between patients and in different phases of the disease. It has been suggested that the cause of muscle weakness is loss of muscle fibres because of fibre degeneration and necrosis as a result of direct cytotoxic effects of T cells [6]. However, there is a dissociation between the degree of histopathological changes and the degree of muscle weakness, which suggests that other mechanisms may also lead to low muscle performance. One possibility is an effect of cytokines, released from the infiltrating inflammatory cells, on muscle contractile properties including effects on Ca<sup>2+</sup> release from the sarcoplasmic reticulum as has been demonstrated for tumour necrosis factor (TNF) and more recently also for the

alarmin high-mobility group box (HMGB1) [7, 8]. Both TNF and HMGB1 have been detected in muscle tissue of myositis patients (Table 3) [9]. Another mechanism that may contribute to the low muscle performance is loss of capillaries in muscle tissue and, as a consequence, tissue hypoxia which could have a negative effect on muscle performance [10, 11]. There are also signs of nonimmune mechanisms in muscle tissue of myositis patients, including endoplasmic reticulum (ER) stress, that could affect muscle performance [12].

Most patients have a partial clinical improvement with immunosuppressive treatment, but few recover their former muscle performance. The reason for this is unclear, but may be owing to persistent inflammation or merely inflammatory molecules that have a



**Fig. 1** (a–c) Characteristics of healthy muscle and of muscle from patients with polymyositis and dermatomyositis. (a) Cross-sectional muscle biopsy tissue from a healthy subject (haematoxylin and eosin staining). Image courtesy of Dr Cecilia Grundtman. (b) Cross-sectional muscle biopsy sample from a patient with polymyositis; image shows fibre size variation, several fibres with central nuclei, mononuclear inflammatory cells with an endomysial distribution and surrounding muscle fibres (haematoxylin and eosin staining), degenerating fibres (thick arrows) and regenerating fibres (broken arrows). Image courtesy of Dr Inger Nennesmo. (c) Cross-sectional muscle biopsy sample from a patient with dermatomyositis; image shows a perimysial and perivascular inflammatory cell infiltrate and perifascicular distribution of small fibres (arrows) so-called perifascicular muscle atrophy). Often, these smaller fibres are regenerating fibres. Image courtesy of Dr Inger Nennesmo.

negative effect on muscle contractility, or a persistent atrophy because of disuse or defective muscle regeneration. Another explanation could be replacement of muscle tissue by fat which is often seen in patients with IBM, regardless of immunosuppressive treatment. Possible molecular mechanisms that could contribute to the persistent chronic muscle weakness in polymyositis and dermatomyositis will be further discussed below.

### Muscle weakness in myositis

#### *Loss of muscle fibres owing to myocytotoxic effect of immune cells*

The most often advocated mechanism of muscle weakness in myositis is an immune-mediated loss of muscle fibres through a myocytotoxic effect of infiltrating inflammatory cells [6]. Muscle tissue in polymyositis, dermatomyositis and IBM is typically characterized by infiltration of inflammatory cells; in particular, of T cells, macrophages, dendritic cells and occasionally B cells [2, 13]. The T-cell infiltrates are composed of CD4<sup>+</sup> and CD8<sup>+</sup> T cells [2]. A direct cytotoxic effect through an interaction between CD8<sup>+</sup> T cells and major histocompatibility complex (MHC) class I on muscle fibers has been suggested as a mechanism for muscle fiber damage in polymyositis and inclusion body myositis. However, the costimulatory molecules required for the T cell-mediated cytotoxic effects have not been convincingly demonstrated in muscle tissue of myositis patients. In the light of this, the recently demonstrated high prevalence of CD28<sup>null</sup> T cells in muscle tissue of myositis patients is of particular interest as these T cells have a phenotype that resembles natural killer (NK) cells and can exert a cytotoxic effect without CD28 and its ligands [14]. It is also interesting that the NK cell properties are observed for both CD4<sup>+</sup> and CD8<sup>+</sup> CD28<sup>null</sup> T cells. Therefore, T cell-mediated myocytotoxicity and loss of muscle fibres could be induced by both CD4<sup>+</sup> and CD8<sup>+</sup> CD28<sup>null</sup> T cells (Fig. 2), but whether CD28<sup>null</sup> T cells really have a myocytotoxic effect remains to be determined.

### Cytokine-mediated muscle weakness

The presence of several cytokines and chemokines has been reported in muscle tissue of myositis patients. Cytokines have pleiotropic effects and may have pro- or anti-inflammatory properties and thereby serve as targets for therapy. Several different cytokines have effects on muscle fibres, both on muscle fibre contractility and remodelling; these effects will be the focus of the discussion in this review. The

**Table 3** Molecular elements (cytokines, chemokines and lipid mediators) of the immune response detected in muscle tissue of myositis patients

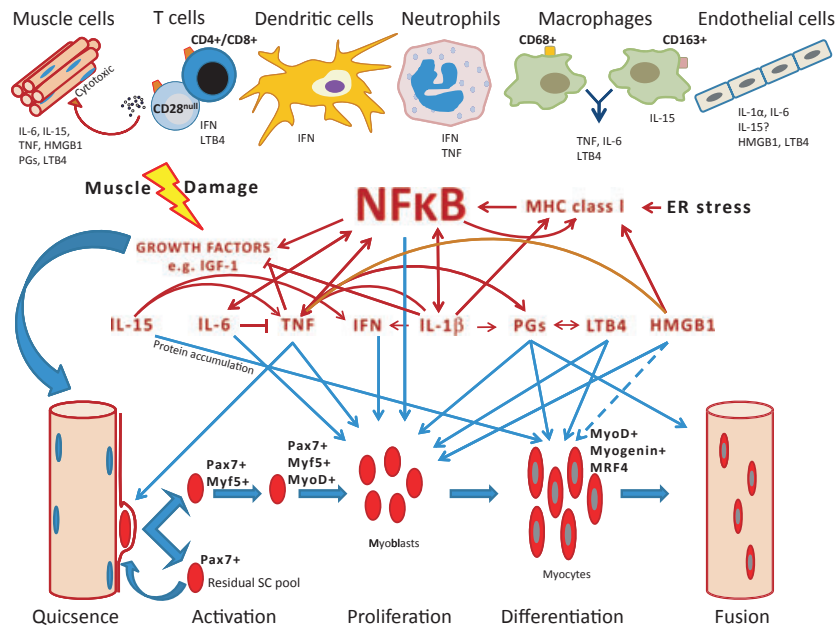
Cytokines	Origin
TNF	Scattered mononuclear cells, macrophage cytoplasm, degenerating and regenerating muscle fibre nuclei, endomysial and perimysial connective tissue
IFN- $\alpha$	Plasmacytoid dendritic cells
IFN- $\gamma$	T cells
IL-1	Inflammatory cells, endothelial cells, capillaries
IL-6	Sparse expression in scattered inflammatory cells
IL-15	Mononuclear inflammatory cells, predominantly macrophages
IL-18	Endomysial and perimysial macrophages and dendritic cells
HMGB-1	Macrophages, endothelial cells, muscle fibres
Chemokines	
CXCL9	Mononuclear inflammatory cells, some myofibres
CXCL10	T cells and macrophages
CCL2	Blood vessels, T cells
CCL3	Mononuclear inflammatory cells, myofibres
CCL19	Myofibres
Lipid mediators	
mPGES-1 (PGE <sub>2</sub> )	Macrophages
5-LO (LTB <sub>4</sub> )	Macrophages

TNF, tumour necrosis factor; IFN, interferon; IL, interleukin; HMGB-1, high-mobility group box-1; CXCL,  $\alpha$ -chemokine inducible by IFN- $\gamma$ ; CCL,  $\beta$ -chemokine effecting leucocyte activation and trafficking with role in tissue repair and skeletal muscle regeneration; mPGES-1, microsomal prostaglandin E synthase 1; 5-LO, 5 lipoxygenase; LTB<sub>4</sub>, leukotriene B<sub>4</sub>.

cellular source of cytokines in muscle tissue may be infiltrating inflammatory cells or other cellular sources such as endothelial cells or muscle fibres (Table 3) (Fig. 2). Chemokines have a role in attracting cells into the tissue of inflammation, in this case into muscle tissue, and are also possible targets of therapy, although their role in disease mechanisms of myositis has not been well documented and will not be further discussed in this review.

Tumour necrosis factor has a central role in several chronic inflammatory diseases including rheumatoid arthritis and Crohn's disease [15]. It is also clear that TNF has catabolic effects on muscle in inflammatory conditions [16]. TNF is secreted by macrophages, monocytes, T cells, NK cells and neutrophils [17] and can be released by damaged muscle fibres upon skeletal muscle injury (Fig. 2) in inflammatory myopathies and Duchenne muscular dystrophy [18]. TNF has several roles in inflammation including activation and chemotaxis of leucocytes, expression of adhesion molecules and regulation of the secretion of other proinflammatory cytokines [17].

Tumour necrosis factor appears to be important throughout the degenerative phase of postinjury muscle regeneration by several mechanisms [19, 20]. TNF expression is upregulated in injured muscle fibres during the repair process and returns to normal during the first days postinjury. [18]. TNF acts via promoting the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF $\kappa$ B) which causes proteolysis and can also promote the expression of atrogen-1, leading to muscle protein catabolism [19, 20]. TNF expression in damaged muscle fibres does not correlate with the level of inflammatory infiltrates in muscle tissue [21] thus suggesting that TNF in muscle fibres may have other functions besides the conventional proinflammatory properties. In primary myoblasts, it has been shown that TNF is able to activate quiescent satellite cells as well as enhance cell proliferation and promote differentiation (Fig. 2) [22]. This suggests that TNF has both degenerative and regenerative capacities within skeletal muscle. In addition, TNF has a direct negative effect on muscle fibre contractility through an inhibitory effect on Ca<sup>2+</sup> release



**Fig. 2** A schematic diagram illustrating the positive effects on muscle fibre regeneration and remodelling of molecules that might have a dual role in muscle tissue both promoting inflammation and affecting skeletal muscle fibre 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 fibre differentiation. Upon skeletal muscle fibre damage, an 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 fibres injured in various ways. Therefore, suppressing the immune system or blocking single cytokines or receptors might disturb skeletal muscle regeneration. SC, satellite cells.

from the sarcoplasmic reticulum thus causing muscle fatigue [7].

Within muscle tissue from patients with myositis, TNF is mainly expressed by scattered mononuclear cells [23]. However, the role of TNF in the pathogenesis of myositis is still unclear, as treatment with TNF blockade has led to conflicting results and even caused worsening of inflammation [24].

Interleukin-1 $\alpha$  and -1 $\beta$  are among the most consistently expressed cytokines in muscle tissue of myositis patients, and IL-1 receptors are expressed on muscle fibres (Table 3) [25]. IL-1 may induce TNF, and both IL-1 $\beta$  and TNF may inhibit expression and activity of growth hormone and insulin-like growth factor 1 in muscle fibres (Fig. 2). In an open study using treatment with IL-1 blockade (anakinra), seven of 15 patients showed improvements in a number of clinical parameters including muscle performance, which might indicate a role of IL-1 in muscle performance in myositis (unpublished data).

Interleukin-6 is a pleiotropic cytokine that regulates immune response, inflammation and haematopoiesis. IL-6 is produced by macrophages, fibroblasts, endothelial cells and T cells and is rapidly induced by multiple stimuli such as viral infection, lipopolysaccharide and other cytokines [26]. There are a number of autoimmune and inflammatory diseases in which IL-6 is pathologically overproduced, and blockade of this cytokine signalling pathway is approved for treatment of rheumatoid arthritis [27]. However, data have shown that IL-6 may also exert inhibitory effects on TNF and IL-1 production (Fig. 2) [28]. Stimulation of IL-1 receptor antagonist (IL-1ra) and IL-10 has also been suggested as one of the anti-inflammatory effects of IL-6 [29].

Interleukin-6 also has a dual effect on skeletal muscle. Overexpression of IL-6 leads to skeletal muscle atrophy in mice accompanied by an increased expression of ubiquitins and cathepsins, which was blocked by treatment with a muscle IL-6 receptor antibody [30]. Local infusion of IL-6

decreased myofibrillar protein without entering the blood stream or the neighbouring muscle [31]. In humans, IL-6 has in recent years been identified as a 'myokine', a cytokine produced and secreted by skeletal muscle, and it has been demonstrated that plasma concentrations of IL-6 as well as IL-6 mRNA and protein expression in muscle tissue are elevated during physical exercise. These increased levels are related to exercise duration and intensity, the muscle mass involved as well as endurance capacity [32]. Exercise has also been found to increase the production of IL-6 receptors in human muscle thus suggesting a postexercise sensitizing mechanism [33].

The exact role of IL-6 in skeletal muscle in relation to exercise is still unclear, but recent work has identified this cytokine as an essential regulator of satellite cell-mediated overload-induced muscle hypertrophy suggesting a role in muscle remodeling [34]. In an experimental approach, IL-6<sup>-/-</sup> mice showed hypertrophy during overload but accompanied by a large increase in noncontractile tissue, implying that IL-6 is a critical factor in the regulation of the fibrotic response of skeletal muscle during overload-induced hypertrophy [35]. Blocking the effect of IL-6 produces unwanted effects on metabolic homeostasis such as an increase in whole body weight, total cholesterol and glucose intolerance, which might point towards a negative role in skeletal muscle [36].

In patients with dermatomyositis, elevated serum levels of IL-6 correlated with disease activity [37]; however, only low levels of *IL-6* gene expression were detected in muscle tissue from myositis patients, and IL-6 protein expression was only observed in a minority of the inflammatory cells in a fraction of patients (Table 3) [23]. Therefore, the role of IL-6 in the development of muscle weakness in myositis is unclear, and a potential negative effect on muscle metabolism and muscle fibre regeneration should be taken into account if treatment with IL-6 blockade is considered.

Interleukin-15 is another cytokine that has effects both on the immune system and on skeletal muscle fibres and that may have a role in the pathogenesis of myositis. This cytokine with proinflammatory properties is expressed in macrophages and endothelial cells inducing chemotaxis and proliferation of T cells (Table 3). IL-15 may also contribute to an increased production of other proinflammatory cytokines such as TNF, interferon (IFN)- $\gamma$  and IL-17 in T cells [38]. In

polymyositis and dermatomyositis, IL-15 and its receptor IL-15R $\alpha$  have been observed in muscle tissue, and, of interest, IL-15 was still expressed after more than 6 months of immunosuppressive treatment in patients with persistent muscle weakness [39] (Zong M, unpublished data).

Within skeletal muscle fibres, IL-15 can stimulate differentiated myocytes and muscle fibres to accumulate contractile proteins, and IL-15 can induce an accumulation of myosin heavy chain protein in differentiated myotubes in culture (Fig. 2) [40]. Another mechanism involved in the anabolic effects of IL-15 on skeletal muscle is a decrease in the rate of proteolysis [41], which is independent of changes in the levels of hormones such as insulin or glucocorticoids [42]. IL-15 mRNA expression in skeletal muscle has been shown to dominate in type II (glycolytic) muscle fibres [43]. IL-15 is also important in angiogenesis which might be the main reason for the changes in plasma levels of this cytokine after acute exercise [44]. The role of IL-15 in inflammation and the contradictory role in preventing muscle wasting raise the question of what therapeutic approach to take with IL-15 overexpression.

High-mobility group box 1 is a highly conserved, ubiquitously expressed nonhistone DNA-binding protein that upon secretion serves as an alarmin, activating the immune system, but also causes muscle regeneration and repair. HMGB1 can be translocated from the nucleus of necrotic cells, but can also be actively released by monocytes and macrophages [45]. Extracellular expression of HMGB1 has been reported in muscle tissue of patients with polymyositis or dermatomyositis as well as extranuclear expression in infiltrating mononuclear cells, endothelial cells and skeletal muscle (Table 3) [46]. After treatment with high doses of glucocorticoids for more than 3 months, the total HMGB1 protein expression was downregulated, but cytoplasmic expression remained abnormal in muscle fibres and endothelial cells in patients with persistent muscle weakness suggesting a potential role in chronic weakness in myositis patients. HMGB1 has the potential to upregulate MHC class I expression (Fig. 2) in muscle fibres; this upregulation is not normally seen and is believed to be involved in IIM pathogenesis. In physiological experiments, HMGB1 exposure irreversibly impaired Ca<sup>2+</sup> release during repeated tetanic stimulation [8], which is accompanied by a reduced force production; thus, HMGB1 might contribute to the muscle fatigue that characterizes myositis.

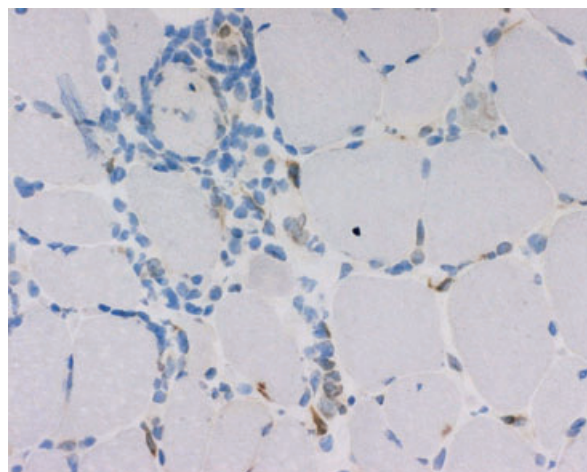
Results of recent studies have demonstrated that HMGB1 can modulate stem cell function and thereby tissue regeneration. HMGB1 has skeletal muscle regenerative capability as evidenced by enhanced myoblast recruitment to the site of injury and increased numbers of regenerating fibres as well as blood vessel formation in a mouse model of hindlimb ischaemia (Fig. 2) [47]. Another potential regeneration mechanism of HMGB1 was shown by promotion of differentiation of myogenic cells in a rat cell line [48, 49]. HMGB1 may therefore have both negative and positive effects on muscle fibres in myositis. It is a potential target for new therapies, but its effects on muscle performance still need to be clarified.

### Eicosanoids in myositis

Eicosanoids are signalling molecules generated by oxidation of 20-carbon fatty acids that exert complex control over many systems and processes including inflammation and immunity. There are different families of eicosanoids, but only prostaglandins (PGs) and leukotrienes will be discussed here.

Prostaglandins are a group of lipid mediators formed in response to various stimuli. They include  $\text{PGD}_2$ ,  $\text{PGE}_2$ ,  $\text{PGF}_{2\alpha}$  and  $\text{PGI}_2$  (prostacyclin) and are released outside cells immediately after synthesis via cyclooxygenase (COX) enzymes and terminal synthases. They exert their actions by binding to receptors on the target cells. PGs were initially considered to be proinflammatory, as they could reproduce the cardinal signs of inflammation; however, it is now clear that they also possess anti-inflammatory activity [50].

Human skeletal muscles have a considerable capacity to produce  $\text{PGE}_2$ ,  $\text{PGD}_2$ ,  $\text{PGF}_{2\alpha}$  and  $\text{PGI}_2$  [51]. During muscle regeneration, myofibres as well as infiltrating leucocytes secrete PGs that may influence myogenesis (Fig. 2) [52].  $\text{PGE}_2$  appears to be involved in a number of biological processes, including protein turnover and myogenesis, and is a potent mediator of muscular pain and inflammation [53–57].  $\text{IL-1}\beta$  and  $\text{TNF}$ , which are highly expressed in myositis muscle tissue, stimulate  $\text{PGE}_2$  production in skeletal muscles [9, 58–60]. This production might be the result of the enhanced expression of the terminal synthase microsomal PGE synthase-1 (mPGES-1) in muscle tissue of patients with myositis (Table 3) (Fig. 3). Moreover, this expression was not affected by conventional immunosuppressive treatment.  $\text{PGF}_{2\alpha}$  is produced by myoblasts and can prevent apoptosis as well as promote cell fusion (Fig. 2) [55]. Similarly, in mice,  $\text{PGI}_2$  signalling controls myoblast



**Fig. 3** Infiltrating inflammatory cells in muscle tissue of an untreated patient with myositis; brown staining indicates expression of microsomal prostaglandin synthase-1.

motility and enhances cell fusion [61], but whether  $\text{PGF}_{2\alpha}$  and  $\text{PGI}_2$  are produced in muscle of myositis patients is not known. In mice, muscle injury induces secretion of  $\text{PGE}_2$ ,  $\text{PGI}_2$  and  $\text{PGF}_{2\alpha}$  by activated myoblasts [62]. Simultaneously, invading leucocytes are capable of producing  $\text{PGE}_2$ , yet little is known about the effect on myogenesis of PGs released from leucocytes.

Leukotrienes are lipid mediators derived from membrane-released arachidonic acid.  $\text{LTB}_4$  is a powerful chemoattractant to direct myeloid leucocytes and activated T cells into inflamed tissue (Table 3) [63].  $\text{LTB}_4$  also shows involvement in the differentiation of naïve T cells [64] as well as the ability to augment the cytokine production by activated T cells [65].  $\text{LTB}_4$  is formed by the 5-lipoxygenase (5-LO) pathway and exerts its actions mainly through the inducible, high-affinity leukotriene  $\text{B}_4$  receptor (BLT1) that is expressed on neutrophils, eosinophils, macrophages and, to a lesser extent, on T lymphocytes [66–70]. BLT1 expression has been demonstrated in human endothelial cells and smooth muscle cells [71]. In addition,  $\text{LTB}_4$  contributes to muscle regeneration by promoting proliferation and differentiation of satellite cells (Fig. 2) and differentiation of rat myoblasts through BLT1 receptors [64].  $\text{LTB}_4$  production by human skeletal muscle has been determined by microdialysis and is upregulated in patients with fibromyalgia [56, 72]. In addition, enhanced expression of 5-LO mRNA has been demonstrated in muscle tissue from patients with polymyositis and

dermatomyositis, suggesting a role of 5-LO in the pathogenesis of these diseases [73].

#### The transcription factor NF $\kappa$ B in muscle repair

When highlighting molecules that share their effects between the immune system and muscle fibre regeneration and muscle remodelling, the well-established transcription factor NF $\kappa$ B is of particular interest. NF $\kappa$ B is critical in immune reactions but also has a role in myogenesis, muscle regeneration and muscle atrophy. Several of the cytokines discussed above act through NF $\kappa$ B. This transcription factor is ubiquitously expressed, and its signalling pathway regulates cellular functions such as proliferation, differentiation, survival, apoptosis and the immune response [74]. NF $\kappa$ B is composed of different subunits responsible for the so-called classical or alternative NF $\kappa$ B signalling pathways [75]. It has been reported that activation of NF $\kappa$ B family members is associated with regulating myogenesis and muscle regeneration, and this involvement may be subunit specific and occurs via both the classical and alternative pathways. In degenerative muscle diseases, muscle fibres undergo a programme of regeneration which is the same as after muscle injury, and the NF $\kappa$ B pathway has been found to modulate this regenerative process [76]. The effects of NF $\kappa$ B seem to be a balance between those of the classical and alternative pathways. The classical signalling maintains myoblasts in the proliferative stage, preventing their premature differentiation, and once myogenic differentiation is initiated, this pathway is shut down. The alternative pathway is then activated, promoting mitochondrial biogenesis, possibly to provide the forming myotubes with the required energy (Fig. 2 [77]). Myogenic differentiation can be inhibited by NF $\kappa$ B through stimulation of cyclin D1 accumulation and cell cycle progression [78]. Another mechanism through which NF $\kappa$ B could inhibit myogenesis is thought to be through the increased expression of the transcription factor Yin Yang1 which directly represses the synthesis of the differentiation genes  $\alpha$ -actin, muscle creatine kinase and myosin heavy chain IIb [79].

Nuclear factor kappa B regulates the expression of proinflammatory cytokines, including IL-1 $\beta$ , IL-6, IL-15 and TNF, in systemic inflammatory disorders such as Duchenne muscular dystrophy, IIMs and rheumatoid arthritis [80, 81]. These cytokines are also potent activators of NF $\kappa$ B, creating a positive feedback loop [82]. IL-1 $\beta$  and TNF may both block the differentiation of cultured myoblasts into myotubes

through the activation of NF $\kappa$ B [83]. Another mechanism of myogenic inhibition is through the reduction of cellular levels of MyoD protein by post-transcriptional modification. On NF $\kappa$ B activation, proinflammatory cytokines can destabilize MyoD mRNA in skeletal muscle cells and thereby inhibit the transition from proliferation to differentiation [84].

In inflammatory myopathies, NF $\kappa$ B activation occurs both in infiltrating inflammatory cells and in muscle fibres within the muscle tissue. Patients with inflammatory myopathies have an overexpression of MHC class I in muscle cells which is thought to induce ER stress, which in turn can activate NF $\kappa$ B. Upon activation, NF $\kappa$ B regulates the expression of genes inducing MHC class I causing a positive feedback for the progression of inflammatory myopathies [12, 23].

Nuclear factor kappa B cells can also induce expression of proteins of the ubiquitin–proteasome system involved in skeletal muscle protein degradation, and this partly works through increased expression of muscle RING finger protein 1 (MuRF1). Increased expression of MuRF1 has been reported in animal models of muscle atrophy induced by immobilization, IL-1 and glucocorticoids [20].

Targeting NF $\kappa$ B for pharmacological therapy is an attractive option for chronic inflammatory diseases such as IIM. The NF $\kappa$ B pharmacological models available today involve curcumin [85], NBD peptide (NBD = NEMO binding domain where NEMO is included in a NF $\kappa$ B subunit essential for classic signalling) [86, 87] and L-arginine [88], [89] which protect the muscle and stimulate muscle regeneration. A systemic administration of NBD peptide would probably affect the homeostatic functions of NF $\kappa$ B in immune system. Targeting NF $\kappa$ B in immune cells directly as well as the muscle cells might give a more specific effect. Another approach would be to target muscle wasting-related alternatives downstream of NF $\kappa$ B, e.g. MuRF1, in order to inhibit muscle protein breakdown.

#### Muscle weakness: an acquired metabolic myopathy

The main clinical symptom experienced by patients with polymyositis and dermatomyositis is impaired endurance. Muscle endurance is dependent on oxidative type I fibres and requires an oxygen supply. Loss of capillaries in muscle tissue is a hallmark of dermatomyositis. A reduced number of capillaries compared with the number in healthy individuals has been demonstrated in both the early and late



phases of polymyositis without detectable inflammatory infiltrates suggesting an impaired microcirculation in muscle in both polymyositis and dermatomyositis [10, 11]. Notably, several of the cytokines reported to have aberrant expression in muscle tissue can be induced by hypoxia, including TNF, IL-1 and the alarmin HMGB1. Further support for the role of hypoxia in skeletal muscle weakness is the low levels of adenosine triphosphate and phosphocreatine recorded by magnetic resonance spectroscopy [90], as well as a decreased proportion of oxidative, type I muscle fibres in patients with established polymyositis or dermatomyositis and persistent reduced muscle performance [91]. These observations all support the notion of an acquired metabolic myopathy causing muscle fatigue.

#### Nonimmune mechanisms

##### *MHC class I upregulation*

An obvious finding in muscle biopsies from patients with inflammatory myopathies is MHC class I expression in muscle fibres, which is not observed in healthy individuals. This may be the only sign of pathology in muscle tissue and may be present without adjacent inflammatory cell infiltrates. MHC class I can be induced by proinflammatory cytokines (e.g. IFNs, IL-1 and HMGB1) that have been detected in myositis tissue (Fig. 2). It is interesting that genetically modified mice with muscle-specific upregulation of MHC class I developed muscle weakness before inflammatory infiltrates could be detected in muscle tissue, supporting the notion that this phenotypic modification of muscle fibres could affect muscle contractility [92]. MHC class I expression could also be induced and sustained by the ER stress response (Fig. 2). This is a protective mechanism in cells subjected to stress. Signs of ER stress have been observed in muscle tissue from both MHC class I transgenic mice and from patients with myositis [12].

Major histocompatibility complex class I molecules are localized in the ER and could thereby affect protein synthesis of muscle fibres and muscle fibre contractility. In single fibres from MHC class I transgenic mice, force production was reduced in slow twitch type I fibres owing to an as yet undetermined intrinsic effect [93]. A differential effect on fibre types resembles the clinical effect in myositis, as patients more often complain of reduced muscle endurance, which mainly depends on oxidative type I muscle fibres, rather than impaired single high-force movements [94]. These data support the hypothesis that MHC

class I expression in muscle fibres could affect muscle performance, even in the absence of muscle fibre necrosis or inflammatory cell infiltrates, and contribute to chronic muscle fatigue in myositis.

#### Skeletal muscle regeneration

Another possible cause of the chronic persistent muscle weakness observed in the three types of myositis could be a defective repair mechanism after the loss of muscle fibres. The regeneration of skeletal muscle depends on the balance between pro- and anti-inflammatory factors that determine whether the damage will be repaired with muscle fibre replacement and functional contractility or with scar tissue formation [95]. Muscle tissue has an inborn repair mechanism whereby muscle fibres can regenerate from satellite cells. Skeletal muscle regeneration includes three distinct stages, degeneration, muscle repair and remodelling, and follows a fairly consistent pattern irrespective of the underlying cause of injury (e.g. direct trauma, tissue ischaemia, old age or genetic defects) [96, 97]. If the muscle repair mechanisms are inadequate, the consequence might be reduced muscle function and muscle wasting [97]. Adult skeletal muscle is a stable tissue with limited nuclear turnover [98], but it has the ability to rapidly regenerate in response to damage to maintain well-innervated, vascularized and contractile muscle. The well-orchestrated course of satellite cell activation and differentiation is largely similar to the gene expression during embryonic development of muscle while the main difference is the presence of immune cells during muscle regeneration in myositis patients [99]. Signs of fibre 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 inadequately understood.

The initial event in the degeneration of muscle, necrosis of muscle fibres, is triggered by disruption of the sarcolemma followed by increased serum levels of proteins such as creatine kinase and myoglobin [76]. In the early phase of muscle damage, the injured muscle activates an inflammatory response driven by T helper (Th)1 cytokines, such as IFN $\gamma$  and TNF. Neutrophils are rapid responders within the first hours, followed by CD68-expressing M1 phenotype macrophages during the first 24 h. These two cell types contribute to further muscle membrane lysis by production of free radicals but also clear the cellular debris by phagocytic removal [100]. Whether this

elimination of debris is of importance for further regeneration is not fully understood.

After the proinflammatory phase, quiescent Pax7-expressing muscle satellite cells are exposed to signals such as IGF-1, fibroblast growth factor 2, and hepatocyte growth factor promoting activation, proliferation and migration to the site of injury (Fig. 1) [101]. During this stage, the cells become MyoD- and Myf5-expressing myoblasts. Throughout the shift from the proliferative stage to the differentiation phase, the M1 macrophages are replaced by CD163<sup>+</sup> M2 macrophages, activated by Th2 cytokines such as IL-4, IL-10 and IL-13. The M2 macrophages display a more anti-inflammatory phenotype and are found during the healing phase of acute inflammation, in wound-healing tissue and in chronic inflammation, but are rarely observed in myositis [100]. Following the proliferation stage, expression of myogenin and myogenic regulatory factor 4 is upregulated, and the myoblasts become terminally differentiated and exit the cell cycle. These muscle progenitor cells then fuse together or with the existing fibres to replace the damaged muscle cells [76]. At present, it is not possible to distinguish the features of the chronic inflammatory response in autoimmune myositis that cause injury from those that promote muscle regeneration and repair.

#### Effect of anti-inflammatory agents on muscle regeneration

Glucocorticoids in high doses still form the basis for treatment of polymyositis and dermatomyositis although catabolic effects and steroid-induced muscle atrophy are well-known side effects. The degree of these side effects in polymyositis and dermatomyositis is uncertain, as the fibre atrophy has recently been questioned [102–104]. Glucocorticoid-induced atrophy is characterized by a decrease in muscle fibre cross-sectional area in fast twitch, type II muscle fibres with an accompanied decrease in muscle strength [105]. The actions of glucocorticoids on skeletal muscle include both an inhibitory effect on protein synthesis and a stimulatory effect on muscle proteolysis. The anti-anabolic outcome is a consequence of both the inhibition of amino acid transport into the muscle [106] and the inhibition of IGF-1, a growth factor that increases myogenesis but also decreases proteolysis and apoptosis [107]. Glucocorticoids also activate different systems responsible for protein degradation (ubiquitin–proteasome system, cathepsins and calpains) and stimulate muscle production of myostatin, a growth factor that downregulates satellite cell proliferation and differentiation [108]. Muscle

wasting is illustrated by decreased levels of the transcription factor for muscle differentiation, MyoD, and it appears that glucocorticoids can stimulate the degradation of MyoD, at least in the nucleus. The levels of another myogenic transcription factor, myogenin, also seem to be reduced in response to dexamethasone administration in murine C2C12 muscle cells [109]. Glucocorticoids also have an effect on the COX-dependent PG synthetic pathway, downregulating cytosolic phospholipase A2, and therefore might impair the role of PGE<sub>2</sub> in muscle regeneration [110, 111]. Thus, glucocorticoids may act as a double-edged sword in the recovery of strength in myositis patients by reducing inflammation but at the same time inhibiting muscle regeneration. This supports the need for more targeted therapies in these conditions.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are popular over the counter medications for treating muscle injury. They mainly inhibit COX enzymes in the PG biosynthetic pathway [112]. It has been shown that NSAIDs attenuate the exercise-induced increase in satellite cell number, supporting a role for PGs in muscle regeneration [113]. Furthermore, selective COX-2 inhibition results in decreased proliferation of satellite cells, whereas nonselective COX inhibition may decrease satellite cell differentiation and fusion [114]. Not only can COX inhibitors affect the satellite cell response to exercise, it appears that the COX-2 pathway regulates muscle protein synthesis and growth via several mechanisms that can be attenuated by COX-2 inhibition [115]. Traditional NSAIDs such as aspirin and ibuprofen are able to reduce the effects of PGs on skeletal muscle regeneration.

#### Effects of exercise in inflammatory myopathies

Persistent muscle weakness and reduced muscle endurance could also be a consequence of a low level of physical activity, as, until recently, myositis patients were advised to refrain from exercise owing to fear of worsening muscle inflammation. However, several studies have demonstrated that physical exercise, in combination with pharmacotherapy, is safe and improves muscle strength and performance, oxygen capacity and quality of life [91, 116–120]. It is interesting that physical exercise was found to induce a shift in fibre type composition towards a normal distribution, suggesting that exercise might have normalized oxygen tension within the muscle tissue [91]. Furthermore, exercise with creatine supplementation led to a higher functional performance compared

with exercise alone [121]. Of note, 7 weeks of resistance exercise induced downregulation of genes that regulate inflammation in muscle tissue suggesting that exercise might reduce inflammation [122]. An anti-inflammatory effect of training has also been seen in healthy individuals measured as lower serum levels of IL-6 and C-reactive protein [123].

Anti-inflammatory properties of physical exercise could arise from the muscle cells themselves via secretion of contraction-induced 'myokines', and, as mentioned above, both IL-6 and IL-15 appear to have beneficial roles when released from muscle fibres. IL-6 is the first cytokine to be released into the circulation upon initiation of exercise followed by a rise in circulating levels of IL-1ra, IL-10 and soluble tumour necrosis factor-receptor (sTNF-R), resulting in an anti-inflammatory environment [124]. The anti-inflammatory properties of exercise-induced IL-6 seem to have mainly systemic metabolic effects. Lower levels of TNF have been demonstrated in muscle tissue after physical exercise in patients with chronic heart failure [125] and exercise resulted in decreased levels of TNF and increased IL-10 within skeletal muscle with a fibre type specific pattern in rats [126]. Exercise and muscle contractions might stimulate NF $\kappa$ B activation through several pathways. The functions of exercise-stimulated NF $\kappa$ B are currently unknown, but it is possible that NF $\kappa$ B may counteract oxidative stress, induce a brief proinflammatory response critical for muscle regeneration and lead to changes in postexercise muscle glucose transport, glycogen repletion and lipid oxidation [127].

In response to muscle overload, satellite cells are activated and progress either to contribute to myotube fusion or to the pool of self-renewing satellite cells. Upon physical exercise, the release of inflammatory substances and/or growth factors signal to the satellite cells thus regulating their activation and proliferation. Aerobic exercise can induce satellite cell proliferation, and exercise-induced hypoxia may trigger systemic stem cell mobilization, which in muscle tissue would be the satellite cell, that could be recruited in muscle regeneration. Aerobic exercise may also stimulate vascular endothelial growth factor secretion contributing to satellite cell proliferation and migration. Whether these mechanisms are applicable to myositis patients still needs to be determined. Exercise has the potential to counteract the catabolic effect of glucocorticoids on muscles, and this provides further support for prescribing exercise as part of treatment for myositis patients.

## Conclusions

The interplay between the immune system and muscle is complex and involves several mechanisms that may contribute to muscle weakness and that need to be taken into consideration when treating patients with myositis. Different mechanisms may predominate in different phases of disease (e.g. in early or established disease), and careful phenotyping of patients is essential in future studies. Several of the cytokines, alarmins and eicosanoids that predominate in muscle tissue may act both as catabolic and anabolic factors. The dual effect of these molecules – proinflammatory and anabolic/catabolic – may also be relevant for other chronic inflammatory diseases and should be the focus of future research.

## Conflict of interest statement

No conflict of interest was declared.

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*Correspondence:* Ingrid E. Lundberg MD, PhD, Rheumatology Unit, Karolinska University Hospital, Solna, SE-171 76 Stockholm, Sweden.  
(fax: +46 8 5177 3080; e-mail: Ingrid.Lundberg@ki.se) ■