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THÈSE

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Rôle de la mitochondrie et du stress oxydant au cours des myopathies inflammatoires

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1. IFN-β-induced reactive oxygen species and mitochondrial damage contribute to muscle impairment and inflammation maintenance in dermatomyositis.

   Meyer A, Laverny G, Allenbach Y, Grelet E, Ueberschlag V, Echaniz-Laguna A, Lannes B, Alsaleh G, Charles AL, Singh F, Zoll J, Lonsdorfer E, Maurier F, Boyer O, Gottenberg JE, Nicot AS, Laporte J, Benveniste O, Metzger D, Sibilia J, Geny B.

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RÉSUMÉ

Les myopathies inflammatoires sont des maladies auto-immunes rares dont le dénominateur commun est la faiblesse musculaire et l'inflammation. Cependant, elles couvrent un large spectre de pathologies présentant des caractéristiques cliniques hétérogènes, mettant en lumière des différences physiopathologiques qui doivent être mieux caractérisées. Les traitements actuels reposent sur l'utilisation empirique de corticoïdes et de traitements immunomodulateurs. Ces médicaments induisent une récupération partielle, exposent à de nombreux effets indésirables, et les rechutes de la maladie sont fréquentes. Il est donc nécessaire d'améliorer la compréhension et le traitement des myopathies inflammatoires.

Les études épidémiologiques des maladies rares sont utiles pour mettre en évidence les disparités géographiques, démographiques, les clusters et les tendances temporelles afin d'identifier de possibles déterminants de ces maladies. Nous avons donc revu de façon systématique et méta-analysé les données concernant l'incidence et la prévalence des myopathies inflammatoires. L'incidence globale a été estimée à 7,98 (IC 95 %: 7,38-8,66) cas par million et par an entre 1951 et 2010.

Bien qu'une hétérogénéité méthodologique limitait les comparaisons, certaines tendances épidémiologiques ont été mises en évidence. L'incidence relative des dermatomyosites (DM) suivait un gradient latitudinal dans l'hémisphère nord qui était concordant avec une action immunomodulatrice des rayonnements ultraviolets. La prévalence de la myosite à inclusions sporadique était corrélée avec la fréquence de HLA-DR3. L'incidence saisonnière et temporelle des myosites juvéniles était non aléatoire et compatible avec un rôle des maladies infectieuses, bien que d'autres facteurs environnementaux puissent être impliqués. Ces données sont un préliminaire à une étude en cours visant à enregistrer l'incidence et la prévalence des myopathies inflammatoires en Alsace.

D'un point de vue mécanistique, nous avons concentré nos efforts sur les DM qui sont définies par une histopathologie musculaire particulière associée à une signature interféron (IFN) de type I (dans le sang et le muscle) dont l'origine et la conséquence sont inconnues.

Dans le muscle des patients atteints de DM récentes, non traitées, le premier groupe fonctionnel de gènes dont l'expression était diminuée était relié à la mitochondrie. Les études histochimiques, de microscopie électronique et l'oxygraphie *in situ* ont mis en évidence des dysfonctions mitochondriales musculaires, avec une augmentation de la production de radicaux libres dérivés de l'oxygène (RLO), une diminution de la respiration mitochondriale corrélée aux faibles capacités d'exercice et à la signature d'IFN de type I. Dans des myotubes humains, la production de RLO était induite par l'IFN-β et contribuait aux dysfonctionnements mitochondriaux. De façon importante, dans un modèle de souris, un inhibiteur des RLO (la N-acétylcystéine) réduisait les dysfonctionnements mitochondriaux musculaires, les taux des transcrits musculaires stimulés par IFN de type I, l'infiltrat de cellules inflammatoires et la faiblesse musculaire.

Nous avons également analysé le rôle de TLR7, un récepteur de l'immunité innée hyperexprimé dans les leucocytes des patients atteints de DM. Nous avons étudié les leucocytes d'une patiente qui a développé une DM lors d'un traitement par imiquimod, un puissant agoniste de TLR7 utilisé pour le traitement des carcinomes basocellulaires cutanés et pour lequel un effet inhibiteur sur le complexe 1 de la chaine respiratoire mitochondriale, associé à une induction de RLO, a été démontré récemment. L'analyse des leucocytes a révélé une augmentation de la sécrétion d'IFN-β induite par l'imiquimod chez notre patiente alors que la sécrétion des cytokines pro-inflammatoires induite par TLR4 ne différait pas des témoins sains.

Ces données mettent en évidence un rôle central des mitochondries et des RLO dans les DM. Les dysfonctionnements mitochondriaux, médiés par les RLO induits par l'IFN-β, contribuent à la diminution des capacités d'exercice. De plus, les dysfonctionnements mitochondriaux augmentent la production de RLO, qui entraîne l'expression des gènes induits par l'IFN de type I et l'inflammation musculaire, ce qui peut auto-entretenir la maladie. Comme les facteurs étiologiques impliqués dans les DM (tels que les rayons ultraviolets, certains médicaments, les virus et les cancers) induisent des dysfonctions mitochondriales, ces résultats indiquent un lien mécaniste possible entre ces facteurs et la DM. Enfin, comme

les traitements actuels des DM ont une efficacité partielle et exposent à des effets indésirables graves (dont une toxicité musculaire), ces résultats ouvrent de nouvelles pistes thérapeutiques pour les DM.

INTRODUCTION

ARTICLE 1 : LES NOUVELLES MYOPATHIES INFLAMMATOIRES

La meilleure description clinique, les avancées de l'histologie musculaire et de l'immunologie ont fait apparaitre que les myopathies inflammatoires (MI) sont, à l'image des rhumatismes inflammatoires, un groupe de pathologies très hétérogènes.

- Il existe une importante variabilité dans la topographie, la sévérité et la rapidité d'installation de la myopathie, dont la sémiologie histologique inclut au moins cinq profils distincts qui pourraient témoigner de processus physiopathologiques différents.
- La plupart des MI sont des connectivites affectant de nombreux organes, le plus fréquemment la peau, les articulations et le poumon. Ces manifestations peuvent précéder la maladie musculaire, et le clinicien doit savoir évoquer le diagnostic de MI devant ces atteintes même en l'absence de myopathie évidente.
- Une vingtaine d'auto-anticorps ont été décrits au cours des MI. Certains sont mutuellement exclusifs et reliés à une association originale de manifestations cliniques. Sur le modèle du syndrome des antisynthétases, on peut définir aujourd'hui une dizaine de syndromes associés aux auto-anticorps spécifiques des MI.

Ainsi, l'entité historique de « polymyosite » tend à disparaitre, et de nouveaux cadres nosologiques émergent. Même s'il n'existe pas actuellement de classification unanime des MI, la clinique, les auto-anticorps et l'histologie musculaire permettent d'identifier des groupes de patients dont les complications, la réponse aux traitements et le pronostic sont relativement homogènes.

Ces différences cliniques suggèrent une hétérogénéité physiopathologique. De façon concordante, les groupes actuellement individualisés sur ces éléments phénotypiques ont aussi des facteurs génétiques et d'environnement distincts les uns des autres. Cependant, l’origine exacte des myopathies inflammatoires est aujourd’hui inconnue.

Pourtant, la meilleure compréhension des mécanismes en cause est indispensable à une meilleure prise en charge. Les traitements actuels reposent sur des données empiriques, ont

une efficacité limitée et exposent à des effets secondaires graves. Ces médicaments induisent une récupération partielle, exposent à de nombreux effets indésirables, et les rechutes de la maladie sont fréquentes. Il est donc nécessaire d'améliorer la compréhension et le traitement des myopathies inflammatoires.

Dans ce travail, nous nous sommes attachés à étudier les déterminants et les mécanismes en cause dans les myopathies inflammatoires en utilisant des outils épidémiologiques et translationnels.

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Review

Inflammatory myopathies: A new landscape

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ABSTRACT

Greater accuracy in clinical descriptions combined with advances in muscle histology and immunology have established that inflammatory myopathies (IMs), similarly to inflammatory rheumatic diseases, constitute a highly heterogeneous group of conditions. The topographic distribution, severity, and tempo of onset of the myopathy vary widely, and the histological findings distinguish at least five different profiles, which may reflect different pathophysiological processes. Most IMs are connective tissue diseases that can affect multiple organs, among which the most common targets are the skin, joints, and lungs. The extramuscular manifestations may antedate the muscular involvement and should therefore suggest a diagnosis of IM even in the absence of obvious muscle disease. About 20 different autoantibodies have been identified in patients with IM. Some are mutually exclusive and associated with specific combinations of clinical manifestations. Following the model of antisynthetase syndrome, about 10 syndromes associated with autoantibodies specific of IM have been identified. Thus, polymyositis is now emerging as a rare entity that is often mistaken for more recently described patterns of IM. No consensus exists to date about the classification of IMs. Nevertheless, the clinical manifestations, autoantibody profile, and muscle histology can be used to distinguish patient subgroups with fairly homogeneous patterns of complications, treatment responses, and outcomes. These subgroups are also characterized by specific genetic and environmental factors. The advances made in the nosology of IMs have benefited the diagnosis, personalization of treatment strategies, and understanding of pathophysiological mechanisms. They can be expected to assist in the development of specific treatments.

1. The skeletal muscle: a common denominator

Involvement of the skeletal muscle is the historical defining criterion for IM [1]. Typically, myalgia and/or muscle weakness in a symmetrical proximal distribution develop over several weeks. Muscle wasting may occur. Muscle enzyme levels [creatine kinase (CK), aldolase, and transaminases] in blood are usually elevated. Creatine kinase elevation has good sensitivity and specificity for the diagnosis of muscle disease [2]. Transaminase elevation may mistakenly suggest liver involvement, which is rare in IM. The electromyogram (EMG) shows the typical myopathic triad of polyphasic, low amplitude, and short duration motor-unit action potentials, as well as spontaneous fibrillation potentials and positive sharp waves. Magnetic resonance imaging (MRI) usually discloses edema and/or fatty infiltration of the skeletal muscles, which may provide information on inflammatory activity and muscle damage [3]. MRI may be useful for guiding the muscle biopsy [4]. The histological features consist of an inflammatory infiltrate and/or muscle fiber necrosis. However, none of these findings is specific of IM. The diagnosis relies on the combination of muscle

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Table 1
Differential diagnosis of inflammatory muscle disease.

Lesions	Differential diagnoses
MRI	
Muscle edema (high signal on T2 images)	Trauma: fissure in an adjacent bone, muscle injury, strenuous physical activity Denervation: radiculopathy, neuropathy, motor neuron disease Amyloid myopathy Genetic muscle diseases: acid maltase deficiency (Pompe disease), the etiologies of inflammatory lymphoplasmacytic infiltrate listed below
Muscle biopsy	
Inflammatory lymphoplasmacytic infiltrate	Infectious muscle diseases: HIV, toxocariasis, trichinosis, borreliosis Hematological malignancies: secondary invasion by malignant cells, graft-versus-host disease after bone marrow transplantation Genetic muscle diseases: dysferlinopathy, dystrophin muscular dystrophy (Becker's myopathy), facioscapulohumeral muscular dystrophy, mutation in the fukutin-related protein gene *FKRP*, laminopathy, titinopathy (Udd myopathy), autoinflammatory syndrome (interferonopathy)
Necrosis/regeneration with no inflammatory infiltrate	Toxic myopathy: alcohol, statin, fibrate Dysthyroidism Genetic muscle disease: dystrophy

abnormalities, extramuscular manifestations, and immunological alterations (Tables 1 and 2).

2. Distinguishing separate inflammatory muscle diseases: why and how

IM was long viewed as a single entity called "dermatopolymyositis". As with inflammatory joint disease, greater accuracy in clinical descriptions combined with advances in histology and immunology have established that IMs constitute a heterogeneous group of conditions whose clinical manifestations, etiological factors, treatment responses, and outcomes vary widely.

2.1. Distinctions based on the clinical and histological muscle involvement

The topographic distribution, severity, and tempo of onset of the myopathy vary across IMs (Table 2). Necrotizing autoimmune myopathies (NAIM, see histological definition below) induces muscle disease at the worst end of the severity spectrum, which translate into severe muscle weakness (< 3/5) in about half the patients [5], with a quarter of patients being unable to walk [6]. Muscle wasting is common and the serum CK level often approximates 10,000 IU/L [5], indicating intense muscle fiber necrosis. The full-blown clinical picture develops within 6 months in most patients. The slower onset in the remaining 20% to 30% of patients may mistakenly suggest a genetic muscle disease [7,8]. This difference in development kinetics remains unexplained. The muscle involvement is mild or absent in other IMs such as amyopathic dermatomyositis. The diagnostic criteria for sporadic inclusion body myositis (sIBM) include a very slow onset and preferential involvement of the quadriceps and flexors of the wrists and fingers [9]. Demonstration by MRI of this distinctive pattern of distribution may assist in the diagnosis [10].

Table 2
Features that distinguish inflammatory muscle diseases (other than inclusion body myositis) from genetic muscle diseases.

	Features suggesting inflammatory muscle disease (other than sIBM)	Features suggesting genetic muscle disease
Family history	Autoimmune diseases	Consanguinity, cardiomyopathy, neuromuscular diseases
Age at onset	Any age > 2 years	Childhood or early adulthood
Tempo of disease onset	Rapid but may be gradual in NAIM	Slow but may accelerate intermittently
Topographic distribution of affected muscles	Proximal; symmetric, spares the face	Variable; may be asymmetric; may involve the facial, oculomotor, or distal muscles
Muscle trophicity	Generally normal except during NAIM	Selective hypertrophy or amyotrophy
Muscle tone	Normal	Myotony in myotonic dystrophy syndromes
Involvement of the myocardium and/or respiratory muscles	Rare except for myocarditis in scleromyositis	Common
Extramuscular manifestations	Skin, ILD, IR, acrosyndrome	Dysmorphism, central and/or peripheral nervous system, endocrine glands
Creatine kinase level	Usually < 5000 IU/L except in NAIM	Often > 5000 IU/L
EMG	Fibrillation potentials and positive sharp waves	Myotony (myotonic dystrophy) or concomitant neuropathy
Abnormal MRI signal from muscle	High signal on T2 images	High T1 signal (high T2 signal may be present)
Autoantibodies	Yes	No
Inflammatory infiltrate within muscles	Perimysial and/or perivascular, absent or moderate in NAIM	Absent or endomysial, without invasion of non-necrotic fibers
HLA class 1	Diffuse expression except in NAIM	Expression absent or focal
C5b-9	Deposits on capillaries, but deposits may occur on muscle fibers in NAIM and ASS	Deposits on fibers
Immunolabeling and/or Western blot for proteins involved in muscular dystrophies	Normal	Diminished
Response to immunomodulating treatment	Usually good, but corticoid resistance may develop	Usually absent, but a moderate improvement is possible

sIBM: sporadic inclusion body myositis; NAIM: necrotizing autoimmune myositis; ILD: interstitial lung disease; IR: inflammatory rheumatism; EMG: electromyogram; MRI: magnetic resonance imaging.

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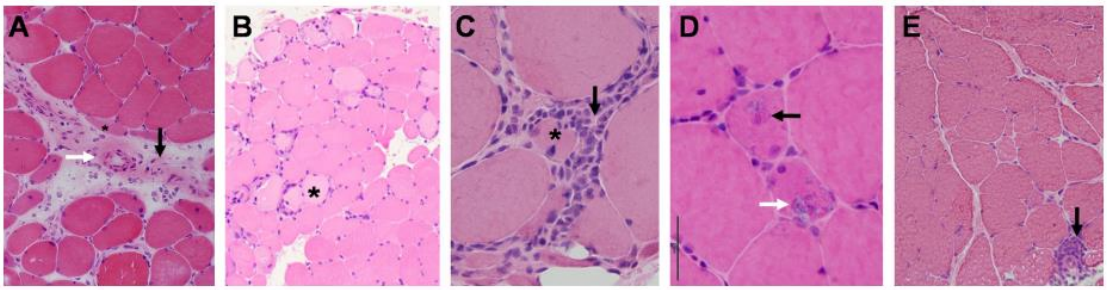

Fig. 1. Five histological patterns of inflammatory muscle disease (IM). A. Perifascicular IM: the fibers in the perifascicular area are atrophic (asterisk, dermatomyositis) and/or necrotic (AS), express HLA class 1 (dermatomyositis) or 2 (AS), and carry C5b9 deposits (AS). The scant capillaries are dilated and carry C5b9 deposits (which are more common in dermatomyositis). A lymphocytic infiltrate is visible in the perimysium (black arrow) and surrounding the vessels (white arrow). B. NAIM: muscle fiber necrosis is the predominant feature (asterisk), and there is no inflammatory infiltrate. Immunolabeling may demonstrate HLA-I expression and C5b9 deposits on vessels and muscle fibers. C. IM with cytotoxicity: the inflammatory infiltrate is in the endomysial area (arrow) and invades nonnecrotic muscle fibers (asterisk) demonstrating diffuse HLA-I expression. D. Histological inclusion body myositis: the lesions are identical to those described above. In addition, the cytoplasm contains rimmed vacuoles (white arrow), eosinophilic inclusions (black arrow), and protein aggregates indicating impaired autophagy, which can be demonstrated by immunolabeling for SMI-31, TDP-43, phosphorylated tau, and ubiquitin. E. Nonspecific myositis: the inflammatory infiltrate is perimysial and/or perivascular (arrow) and the features of the above-described patterns are lacking.

The histological alterations seen in IMs have been described in far greater detail than in the past. Abnormalities of the muscle fibers and capillaries, together with the features of the inflammatory infiltrate, are now used to identify distinct histological patterns, which may reflect different pathophysiological processes (Fig. 1).

2.1.1. Perifascicular IM

Historically, pathologic changes in the perifascicular region were associated with dermatomyositis [11]. They have since then been found also in antisynthetase syndrome (ASS, see the clinical description below), whose histological features differ somewhat from those of dermatomyositis [12–14]. In dermatomyositis, the perifascicular fibers are atrophic but rarely necrotic. They express HLA-I and only rarely HLA-II. Deposits of C5b-9 membrane attack complex are found on the capillaries. In ASS, in contrast, the perifascicular fibers are necrotic, express both HLA-I and HLA-II, and carry C5b-9 deposits. Capillary deposits of C5b-9 are less common. In 81% of patients, electron microscopy visualizes actin aggregation in the muscle fiber nuclei, a finding that may be highly specific of ASS [13]. The pathophysiological significance of these abnormalities has not been elucidated. The underlying mechanism may involve direct cytotoxic effects of type 1 interferon and/or effects of vasculopathy-induced ischemia on the perifascicular region [15].

2.1.2. Necrotizing autoimmune myopathies (NAIM)

The predominant abnormality is muscle fiber necrosis and invasion by macrophages [11]. The inflammatory infiltrate is meager or absent. These features may be mistaken for a noninflammatory muscle disease due, for instance, to a toxic agent, endocrine disorder, or genetic abnormality. Immunolabeling studies may show HLA-I expression and C5b-9 deposits on the vessels and muscle fibers [5]. The mechanism causing necrosis may consist in complement activation by antibodies to the muscle fibers [16].

2.1.3. IM with cytotoxicity

The inflammatory infiltrate is located in the endomysial spaces and invades nonnecrotic muscle fibers that express HLA-I. This histological pattern, previously believed to indicate polymyositis [11], probably indicates early sIBM [17].

2.1.4. Histological inclusion body myosotis (IBM)

In addition to the histological abnormalities described in the previous paragraph, rimmed vacuoles and protein aggregates in the cytoplasm indicate defective autophagy, which can be confirmed using immunolabeling to detect SMI-31, TDP-43, phosphorylated tau protein, and ubiquitin [9]. These abnormalities strongly support a role for abnormal protein degradation in sIBM [18].

2.1.5. Nonspecific inflammatory myositis (IM)

The inflammatory infiltrate exhibits a perimysial and perivascular distribution. None of the features in the above-described patterns is present. These nonspecific abnormalities are common in overlap syndromes, most notably scleromyositis, which combines manifestations of IM and systemic sclerosis [19,20]. They probably constitute a histological subgroup whose members remain to be identified.

2.2. Distinctions based on the systemic manifestations

The skin lesions of dermatomyositis were the first extramuscular manifestations described in IMs. Since then, IMs have emerged as systemic conditions that can involve numerous organs. As shown in Table 3 and Fig. 2, the abundance of extramuscular manifestations is usually in inverse proportion to the severity of the muscle involvement. Thus, extramuscular manifestations are generally prominent in dermatomyositis with anti-MDA-5 autoantibodies but absent in NAIM. The extramuscular manifestations may antedate the muscle involvement by several years. They should therefore suggest the diagnosis of IM even in the absence of obvious muscle disease.

2.2.1. Joint involvement

The joints are among the most common targets of IM (about 50% of patients). Joint involvement occurs in up to 90% of patients with ASS or anti-MDA-5-positive dermatomyositis [21]. The rheumatologist may therefore be the first physician to see patients with IM and should consider the diagnosis, particularly as an explanation to seronegative peripheral inflammatory rheumatism [22]. Although arthralgia of the small joints is the most common presentation, polyarthritis is not uncommon. Radiographs were classically believed to remain normal, but osteoarticular abnormalities have now been described and may occur in over one-third of patients with ASS [23,24]. Anti-citrullinated peptide antibodies (ACPA) may be detected, suggesting overlap with rheumatoid arthritis. This fact warrants careful attention to the risk of structural damage, whereas the extramuscular manifestations may be less severe [24]. Establishing the diagnosis of IM is particularly important given that TNFα

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Table 3
Suggested classification for inflammatory muscle diseases in adults.

	Muscle histology	Autoantibodies targeting	Muscle involvement	Risk and features of extramuscular manifestations				
				Cutaneous involvement	Thoracic involvement	Joint involvement	Other involvements	Excess risk of cancer
Dermatomyositis	Perifascicular IM (except anti-MDA-5-positive IM)	Mi-2	No specificity	DM	Rare	Rare	–	No
		MDA-5	Moderate to absent	DM, MH, papules on the palms, ulcers	ILD, pneumo-mediastinum	Polyarthralgia/polyarthritis	Fever	No
		NXP-2	Severe	DM, calcinosis	Controversial	Rare	–	Yes
		TIF-1γ	No specificity	DM, extensive, psoriasis-like, red-on-white, lesions	Rare	Rare	–	Yes
Necrotizing autoimmune myositis	Muscle fiber necrosis	SAE SRP HMGCR Seronegative	Dysphagia Severe, amyotrophy, dysphagia (SRP > HMGCR)	DM Absent	ILD (Japanese) Usually absent	Rare Rare	– –	Possibly HMGCR and seronegative
Antisynthetase syndrome	Perifascicular IM	Jo-1, PL7, PL12, EJ	Severe, amyotrophy, dysphagia (Jo1 > PL7 or PL12)	Mechanic's hands, sclerodactyly	PID (Jo1 < PL7 or PL12), PAH	Polyarthralgia/polyarthritis (Jo1 > PL7 or PL12)	Fever, renal (rare)	No
Scleromyositis	Variable Inflammation inconsistently present	Scl70 PM/Scl Ku RNApolIII RNP	Dysphagia No specificity	Scleroderma, fingertip ulcers	ILD (Scl70, Ku), myocarditis (Scl70), PAH (RNP)	Polyarthralgia/polyarthritis	Renal	RNApolIII
Mixed connective tissue disease	Perifascicular or nonspecific IM	RNP	No specificity		ILD, PAH	Polyarthralgia/polyarthritis	Neurological	No
Inclusion body myositis	IM with cytotoxicity, rimmed vacuoles, and deficient autophagy	Mup44	Slow, asymmetrical, amyotrophy, knee extensors and finger flexors, dysphagia	Absent	Absent	Absent	Associated autoimmune diseases	No

DM: dermatomyositis; ILD: interstitial lung disease; MH: mechanic's hand; PAH: pulmonary arterial hypertension; IR: inflammatory rheumatism; IM: inflammatory myopathies.

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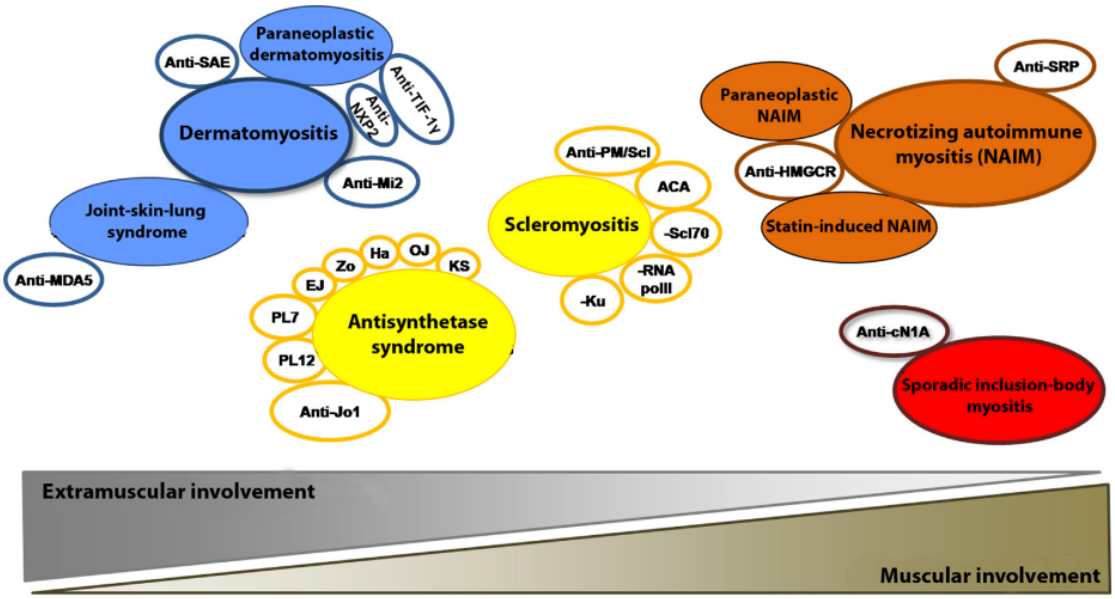

Fig. 2. Clinical and serological diversity of inflammatory myopathies (IM). The prominence of the extramuscular manifestations is usually in inverse proportion to the severity of the muscle involvement. The autoantibody profile can be used to identify patient subgroups with globally homogeneous clinical features and outcomes.

antagonist therapy may prompt the development or exacerbation of systemic involvement [24].

2.2.2. Thoracic involvement

Most thoracic abnormalities related to IM have been associated with an increase in mortality. Risk factors have been identified.

Interstitial lung disease (ILD) is present in 20% to 78% of patients with IM [25]. In most cases, the ILD lesions are non-specific interstitial pneumonia. However, the other types of ILD (usual interstitial pneumonia, bronchiolitis obliterans organizing pneumonia, and diffuse alveolar damage) or mixed patterns on computed tomography (CT) are not rare [26]. ILD occurs chiefly in ASS, scleromyositis, and anti-MDA-5-positive dermatomyositis, but is uncommon in paraneoplastic IM, MNAI and sIBM. ILD is associated with an increase in overall mortality [27]. Nevertheless, the severity spectrum ranges from asymptomatic disease to acute respiratory distress. Factors known to be associated with adverse outcomes are CT evidence of diffuse alveolar damage [28]; low vital capacity or transfer factor for carbon monoxide [29]; and presence of anti-PL7, anti-PL12 [30], or anti-MDA-5 [31].

Pneumomediastinum is a severe complication associated with skin ulcers, amyopathic dermatomyositis, ILD, and/or presence of anti-MDA-5 antibodies [32,33].

Pulmonary arterial hypertension has been reported in patients with ASS [34], scleromyositis [19], and mixed connective tissue disease [35] and can occur in anti-MDA-5-positive dermatomyositis. Mortality is increased in patients with pulmonary arterial hypertension [34].

The respiratory muscles may be involved. However, ventilatory failure is rare [36] and should suggest another diagnosis such as Pompe disease [37].

Symptomatic cardiac involvement occurs in fewer than 10% of patients with IM [38]. In contrast, abnormalities in the electrocardiogram or cardiac imaging studies (echocardiography, MRI, scintigraphy) are very common (up to 85% of patients) [39,40]. Although the prognostic significance of asymptomatic cardiac alterations is unclear in IM, some of these abnormalities (QTc prolongation, left ventricular diastolic dysfunction) have been associated with increased cardiovascular morbidity and mortality in the general population. Risk factors may include older age, longer disease duration, and presence of autoantibodies [39]. Troponin T is expressed not only by the myocardium, but also by the skeletal muscle. Thus, serum troponin T is elevated in IM in correlation with the degree of myolysis and independently from any cardiac involvement [41]. Troponin I is specifically expressed by the myocardium. However, whether serum troponin I assays are helpful in IM is unclear, as levels are not significantly different between patients with IM and healthy volunteers [39]. In myocarditis due to other causes, the troponin I assay is specific (89%) but lacks sensitivity (34%) [42].

2.2.3. Skin involvement

Recent work has highlighted the heterogeneity of the skin lesions seen in dermatomyositis, which have been associated with syndromes of widely diverging prognosis (as detailed in the section on dermatomyositis).

Hyperkeratosis with cracks over the fingertips and, most prominently, the medial aspect of the thumb, lateral aspects of the forefinger and middle finger (mechanic's hands) was described first in AS and subsequently in anti-MDA-5-positive dermatomyositis [43] and anti-PM/Scl scleromyositis [44].

Sclerodactyly is common in AS [30]. Marked sclerosis should suggest overlap with systemic sclerosis, particularly in patients with other skin manifestations of this condition (fingertip ulcers and telangiectasia).

Raynaud's phenomenon is present in about 25% of patients with IM [45]. This proportion is higher (about 50%) in ASS, scleromyositis, and anti-MDA-5-positive dermatomyositis, during which it may be complicated by fingertip ulcers and/or necrosis of the extremities [43,46].

2.2.4. Calcinosis

Calcinosis occurs in about 10% of adults with IM but is more common in children [47]. Hydroxyapatite crystals deposit in the skin,

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subcutaneous tissue, fascia, tendons, or skeletal muscle. The lesions may fistulize or cause joint stiffness. Related abnormalities include fingertip ulcers, prolonged disease activity, and the presence of anti-MDA-5, anti-NXP2 [48], or anti-PM/Scl [49] antibodies.

2.2.5. Oropharyngeal and gastrointestinal involvement

Swallowing impairments and dysphagia occur in 20% of cases overall and are more common in sIBM [50], scleromyositis [51], paraneoplastic dermatomyositis [52], and NAIM [53]. Except in the case of sIBM, dysphagia responds to immunomodulators but may require intravenous immunoglobulin therapy [54]. Intestinal pseudoobstruction has been reported [55].

Gastrointestinal vasculitis is rare in adults. An association with primary biliary cirrhosis has been reported in patients with antimitochondrial antibodies [56].

2.3. Distinctions based on the autoantibody profile

Autoantibodies are found in over 80% of patients with IM [57]. About 20 different autoantibodies have been identified, and most are highly specific of IM. Detection kits are commercially available for all these autoantibodies except anti-cN1A (for which a kit is being developed). Nevertheless, testing must often be performed in a specialized medical laboratory.

These specific autoantibodies are mutually exclusive. Each has been associated with a distinctive clinical profile (Table 3). Thus, following the ASS model, about ten syndromes associated with autoantibodies specific of IM have been described. Detecting these autoantibodies may not only assist in the diagnosis of IM, but also improve the personalisation of treatment and follow-up strategies. Furthermore, patient follow-up may benefit from the identification of associations linking disease activity to the titers of some autoantibodies (anti-SRP [58], anti-HMGCR [59], anti-Jo1 [60], anti-Mi2, anti-Tif1 gamma [61], and anti-MDA-5 [62]).

2.4. Distinctions based on etiologies

The etiologies of IM are unknown. Nevertheless, several constitutional and genetic etiological factors are linked to specific clinical and serological subgroups of IM.

2.4.1. Immunogenetics

Polymorphisms in *PTN22*, *UBE2L3*, *CD28*, *TRAF6*, and *STAT4* are associated with IMs of any type, indicating a role for a common genetic background [63]. Furthermore, distinct HLA haplotypes are associated with each specific autoantibody [64–68], suggesting that immunogenetic factors may contribute to the heterogeneity of IM. However, the low twin concordance rates [69], high prevalence of the same HLA haplotypes in the general population, and low incidence of IM support an important influence of environmental factors.

2.4.2. Cancer, dermatomyositis, and necrotizing autoimmune myopathies (NAIM)

About 20% of cases of IM in adults are related to malignancies [70]. Factors positively associated with cancer are older age, male gender, and greater severity of the skin lesions. On the other hand, cancer is less common in patients with joint and/or lung involvement [70].

In dermatomyositis, anti-TIF-1 autoantibodies are associated with presence of a tumor [65], although the association may be weaker when a highly sensitive assay is used [71]. Anti-NXP-2 [71] and anti-SAE [72] antibodies may also be associated with cancer. In keeping with these findings, these three autoantibodies target proteins involved in cell cycle regulation.

In patients with NAIM, the absence of any of the known autoantibodies and, to a lesser degree, the presence of anti-HMGCR are associated with paraneoplastic IM [73].

2.4.3. Ultraviolet radiation and dermatomyositis

A systematic review suggests that the relative incidence of anti-Mi-2-positive dermatomyositis may be related to ultraviolet radiation exposure [74]. Consistent with this possibility, the Mi-2 antigen is involved in repairing DNA breaks, of which one cause is ultraviolet radiation exposure, which induces Mi-2 overexpression [75].

2.4.4. Antiviral immunity and dermatomyositis

One argument suggesting a role for environmental factors in dermatomyositis is that many patients report a viral infection within a few months before the onset of the disease [76]. Further evidence for a viral factor consists in the presence of a type I interferon signature [77] and overexpression of several receptors involved in innate antiviral immune responses [endosomal toll-like receptors (TLRs), TLR-4, MDA-5, RIG-1] in muscle tissue and/or leukocytes from patients with dermatomyositis [78]. Finally, the dermatomyositis-specific anti-MDA-5 antibodies recognize an antigen involved as a receptor in innate antiviral immune response. The prevalence of anti-MDA-5 autoantibodies is associated with the epidemiology of coxsackie virus infections [79].

2.4.5. Statins and necrotizing autoimmune myopathy (NAIM)

A 2010 report describes a subgroup of patients with NAIM characterized by the presence of autoantibodies to the enzyme HMGCoA reductase. A history of statin therapy was found in two-thirds of these patients, a significantly higher proportion than in patients with other types of IM [6]. In keeping with a causal role for statins, the *HMGCR* gene is expressed by regenerating muscle fibers from affected patients [80] and anti-HMGCoA reductase autoantibodies are not found in statin-exposed patients without evidence of IM [81].

2.4.6. Smoking and antisynthetase syndrome (AS)

Smoking is associated with the presence of anti-Jo-1 autoantibodies [82], which target histidyl-tRNA synthetase. This enzyme is found in the lung alveoli in a form that is highly sensitive to cleavage by granzyme B [83], whose expression in the lung alveoli is increased by smoking [84]. Smoking may therefore increase Jo-1 cleavage in the lungs, thereby promoting loss of self-tolerance in immunogenetically susceptible patients.

2.4.7. Muscle aging, impaired autophagy, autoimmunity, and sporadic inclusion body myositis (sIBM)

The most common IM in individuals older than 50 years is sIBM. Several lines of evidence suggest a role in sIBM of impaired autophagy, which in turn is associated with aging [85]. In immunolabeling studies, the protein aggregates found in muscle fibers in sIBM are recognized by antibodies to SMI-31, TDP-43, phosphorylated tau, and ubiquitin, whose presence is a strong diagnostic criterion for sIBM [9]. These proteins are normally degraded by lysosomal enzymes. Similarly, mutations in the autophagy-regulating gene *VCP* produce a muscular phenotype akin to sIBM, and polymorphisms in this gene are associated with sIBM [86]. Thus, the autoimmune response in sIBM may result from chronic antigen stimulation by nondegraded proteins in patients at risk due to their immunogenetic background [87] and age [88].

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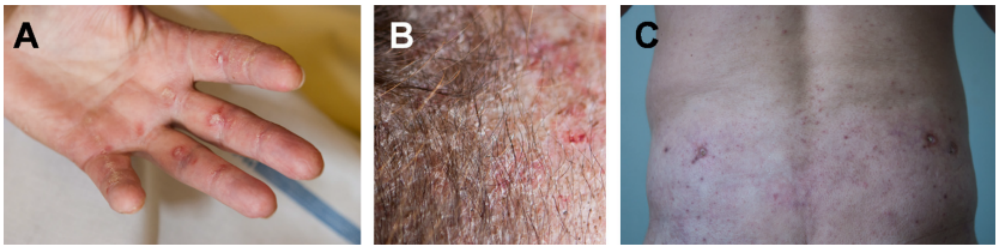

Fig. 3. A. Papules with an ivory-colored center at the palm and hyperkeratosis with cracks at the fingers in a female patient with anti-MDA-5-positive dermatomyositis. B. Erythematous, scaly, psoriasis-like lesions of the scalp in a female patient with anti-TIF1γ-positive dermatomyositis. C. Calcinosis of the lumbar region with fistulisation to the skin in a female patient with anti-NXP-2-positive dermatomyositis.

2.5. Distinctions based on therapeutic response - optimal personalization of patient care

Treatment responses to immunomodulating agents vary widely in patients with IM. Several clinical, serological, and/or histological features may help to predict the response, thereby improving treatment personalization from the outset.

Anti-Mi-2-positive dermatomyositis and anti-Jo-1 AS respond well to immunotherapy [30,57,89]. In these diseases, a simple regimen such as a glucocorticoid combined with a disease-modifying antirheumatic drug may be appropriate.

NAIM with anti-SRP or anti-HMGCR antibodies are less sensitive to immunomodulation and may therefore require rescue treatments such as intravenous immunoglobulins (about 50% of patients) or rituximab [5,8,53]. Early treatment is associated with better muscle function recovery [7].

Recent findings in anti-MDA-5-positive dermatomyositis suggest that first-line intensive treatment (combined prednisone, cyclosporine, and cyclophosphamide) may improve survival compared to first-line conventional treatment [90].

In scleromyositis, the response of the muscle diseases varies with the histological pattern. Inflammation predicts a good outcome, whereas other abnormalities are associated with unresponsiveness to treatment [91]. This fact probably deserves consideration when assessing the risk/benefit ratio of glucocorticoid therapy, which is a risk factor for renal crisis in systemic sclerosis [92].

In sIBM, immunomodulating agents have little or no effect and may even increase the risk of death [93].

3. Newly identified subtypes of inflammatory myopathies (IM)

The historical entity polymyositis is increasingly being broken up into newly described subtypes of IM. No consensus about the classification of IM exists to date. Nevertheless, clinical features, autoantibody profiles, and muscle biopsy findings can be used to identify patient subgroups characterized by fairly homogeneous patterns of complications, treatment responses, and outcomes.

3.1. Dermatomyositis

All subtypes share the skin lesions typical for dermatomyositis: photosensitivity; facial rash, which may be accompanied with poikiloderma; more or less linear and scaly macules and/or papules (Gottron's) over the extensor surfaces of the hand joints, elbows, and knees; and periungual hyperemia and megacapillaries with cuticle necrosis. Nevertheless, both the detailed features of the skin lesions and the extracutaneous manifestations vary considerably. As described below, several of the autoantibodies specific of dermatomyositis are associated with distinctive clinical patterns and may therefore assist in developing a classification of these diseases.

3.1.1. Anti-Mi-2-positive dermatomyositis

Anti-Mi-2-positive dermatomyositis is associated with a rash over light-exposed areas, a low risk of other extramuscular manifestations, a good treatment response, and a good prognosis.

3.1.2. Anti-MDA-5-positive dermatomyositis

Anti-MDA-5-positive dermatomyositis is characterized by erythematous, scaly skin lesions (described as the typical dermatomyositis rash combined with mechanic's hands) and by papules with an ivory-colored center and ulcers on the palms of the hands [43] (Fig. 3A). There is little or no muscle involvement. In contrast, patients are at risk for rapidly progressive ILD and pneumomediastinum [32], which increase the mortality rate [94]. A first-line high-intensity treatment strategy may improve the outcome [90]. Patients may also be at risk for calcinosis [48]. Joint involvement is common and may be inaugural, leading to a mistaken diagnosis of rheumatoid arthritis [21].

3.1.3. Anti-TIF-1-positive dermatomyositis

Anti-TIF-1-positive dermatomyositis is associated with the classical picture of dermatomyositis. In particular, features include Gottron's papules; a rash, which may be extensive, localized to light-exposed areas, and pruriginous; scaly papules over the palms; psoriasis-like lesions (Fig. 3B); telangiectatic macules; and punctate follicular erythematous lesions over a leukodermic background (red-on-white lesions). The last two manifestations resemble those seen in subacute cutaneous lupus [95]. Involvement of the joints and lungs is rare. A concomitant malignancy is common, with the risk being highest in patients with both anti-TIF-1γ and TIF-1α autoantibodies [96].

3.1.4. Anti-NXP-2-positive dermatomyositis

Anti-NXP-2-positive dermatomyositis is associated with a well-defined pediatric syndrome of calcinosis (Fig. 3C), marked muscle weakness, and a risk of persistent disease activity after 2 years [97]. ILD is rare. In adults, the clinical relevance of anti-NXP-2 autoantibodies is less well understood. In several studies [48,98] and in our experience, the phenotype resembles that seen in children. In other studies, however, no patients had calcinosis [99], ILD was common [100], and/or the risk of a concomitant malignancy was high [71,99]. The reasons for these discrepancies are unclear.

3.1.5. Anti-SAE-positive dermatomyositis

Anti-SAE-positive dermatomyositis may differ from other dermatomyositis subtypes by an amyopathic disease onset and the presence of dysphagia [101]. In Asian populations, anti-SAE autoantibodies are associated with ILD [102] and cancer [72].

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3.2. Necrotizing autoimmune myopathies (NAIM)

The predominant histological finding by far is muscle fiber necrosis. The inflammatory infiltrate is moderate to absent. The severe muscle involvement contrasts with the low incidence and lesser prominence of extramuscular manifestations. The differential diagnosis may be difficult, most notably with drug-induced necrotizing myositis and muscular dystrophy, particularly when the symptoms set in slowly [7,8] and/or the response to first-line treatment is inadequate, as occurs in many cases [5,53,73].

Three subtypes of NAIM have been identified based on the serological profile.

3.2.1. Anti-SRP-positive NAIM

Anti-SRP-positive NAIM may be the subtype with the most severe skeletal muscle involvement. Thus, the motor weakness is more severe, muscle wasting and/or dysphagia more common, and the need for aggressive treatments greater than in other NAIM subtypes. Diffuse ILD is present in 20% of patients [5,53,73]. The risk of cardiac involvement suggested by early studies was not confirmed in subsequent work.

3.2.2. Anti-HMGCR-positive NAIM

Anti-HMGCR-positive NAIM is associated with a history of statin therapy in about two-thirds of cases [6,8]. A higher risk of cancer of borderline significance compared to the expected incidence was reported recently [73].

3.2.3. Seronegative NAIM

Seronegative NAIM is characterized by an incidence of cancer 8-fold higher than expected [73].

3.3. Overlap syndromes

All overlap syndromes are characterized by prominent extramuscular manifestations appart from the rash of dermatomyositis which may occur in some patients, though remaining discrete in our experience. Overlap syndromes may account for a large proportion of IMs [89].

3.3.1. Antisynthetase syndrome (AS)

AS is characterized by variable combinations of constitutional symptoms (fever, fatigue, and weight loss), Raynaud's phenomenon, inflammatory joint disease, IM, ILD, and skin lesions (mechanic's hands and sclerodactyly) [30]. The picture is often incomplete early in the disease, when there is often a single manifestation such as inflammatory joint disease (1 in 4 patients) or either IM or ILD (1 in 8 patients). The other features nearly always develop during follow-up. Thus, after a median follow-up of 80 months, only 1 in 10 patients had involvement of a single organ [103].

AS is related to the presence of autoantibodies against aminoacyl-tRNA synthetases, of which the most common target the histidyl-tRNA synthetases (Jo-1) (about 20% of patients with IM). The other specificities, which are far less common (1% to 5%), target the alanyl (PL-12), threonyl (PL-7), glycyl (EJ), isoleucyl (OJ), asparginyl (KS), tyrosyl-AS (Ha), and phenylalanyl (Zo) tRNA synthetases. The specificity of antisynthetase autoantibodies may influence the frequency of clinical features and the outcome. Thus, outcomes may be poorer in patients with antibodies to PL7 or PL12, which are associated with a higher frequency of ILD [30].

3.3.2. Scleromyositis

The muscles may be involved in up to 69% of patients with systemic sclerosis [91], and 29% of patients with IM may have systemic sclerosis [89].

Compared to other patients with systemic sclerosis, those with scleromyositis are more often male and have greater global disease severity with a younger age at onset (44–52 years) and higher frequencies of diffuse skin lesions (40%–63%), lung disease (24%–62%), and cardiac involvement (29%), which translate into a higher mortality rate [104–107].

Nevertheless, the serological and histological features should probably also be considered when assessing the prognosis. Among the autoantibodies associated with muscle involvement, anti-Scl70 are among the most common [108] and are associated with diffuse skin lesions, involvement of the heart and lungs, and a higher risk of death [109]. Anti-PM/Scl autoantibodies are associated with less severe disease, a better treatment response, and better survival [49,110]. Patients with anti-RNP autoantibodies are at risk for pulmonary arterial hypertension, although the prognosis of this condition is better than in other patients [111]. Anti-Ku autoantibodies are associated with lung disease. Their potential link to the prognosis is unclear [112,113]. Anti-RNA Pol III autoantibodies are associated with a higher risk of malignancy [114].

Muscle biopsy features vary widely in systemic sclerosis. No convincing correlations have been reported between serological and histological features [19]. Muscle inflammation may predict a good treatment response [91], whereas fibrosis may indicate a risk of renal crisis [19], particularly in patients taking glucocorticoid therapy [115].

3.3.3. Mixed connective tissue disease

By definition, mixed connective tissue disease is associated with anti-RNP autoantibodies. The diagnostic criteria for systemic sclerosis and/or systemic lupus erythematosus may be met [116]. IM is present in 20% to 25% of cases and may be associated with a higher frequency of pulmonary arterial hypertension, neurological involvement, and renal involvement [35,117].

3.4. Sporadic inclusion body myositis (sIBM)

Correctly diagnosing sIBM is of the utmost importance, as immunomodulating agents are not effective and may even increase the risk of death [93]. The diagnosis may be challenging, particularly as autoimmunity is common in sIBM and may manifest as the production of autoantibodies associated with IM [9]. Autoantibodies to 5′-nucleotidase 1A (cN1A) were found in larger proportions of patients with sIBM (33% to 73%) than of patients with other IMs. However, anti-cN1A autoantibodies also occur in lupus (14%–20%) and Sjögren's syndrome (23%–36%) [118].

3.5. Does polymyositis exist?

Since it was first described, no associations linking a clinical feature, autoantibody, or histological finding to polymyositis has withstood the test of time. On the contrary, the number of conditions mistakenly diagnosed as polymyositis has increased steadily. It has therefore been suggested that polymyositis is not a disease entity [119] but simply an inflammatory or noninflammatory muscle disease that has not yet been correctly diagnosed. The clinical, MRI, or histological features of many genetic muscle diseases can mimic those of IM [37] (Tables 1 and 2). The response to glucocorticoid therapy is not a strong diagnostic criterion, as significant benefits of glucocorticoids have been reported in patients with Duchenne muscle dystrophy [120] and IM can be steroid-resistant.

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4. Conclusion

Although there is no consensus about the classification of IMs, the available data allow the identification in everyday practice of patient subgroups characterized by fairly homogeneous clinical features, muscle biopsy alterations, autoantibody profiles, etiological factors, and outcomes. As with inflammatory rheumatic diseases, the improved characterization of IM will benefit the personalization of patient care and may promote the emergence of specific treatments.

Disclosure of interest

The authors have not supplied their declaration of competing interest.

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PATIENTS, MATÉRIEL, MÉTHODES

Les myopathies inflammatoires sont des maladies auto-immunes rares dont le dénominateur commun est la faiblesse musculaire et l'inflammation. Cependant, elles couvrent un large spectre de pathologies présentant des caractéristiques cliniques hétérogènes, mettant en lumière des différences physiopathologiques qui doivent être mieux caractérisées. Les traitements actuels reposent sur l'utilisation empirique de corticoïdes et de traitements immunomodulateurs. Ces médicaments induisent une récupération partielle, exposent à de nombreux effets indésirables, et les rechutes de la maladie sont fréquentes. Il est donc nécessaire d'améliorer la compréhension et le traitement des myopathies inflammatoires.

Le détail des méthodes utilisées est décrit dans la section « *Patients, matériel et méthodes »* de chacun des articles relatifs aux travaux rapportés.

Nous avons étudié du matériel épidémiologique, des patients, un modèle animal et un système cellulaire.

Nous avons notamment utilisé des méthodes d'exploration physiologiques, fonctionnelles, morphologiques et moléculaires.

Matériel épidémiologique

Les études épidémiologiques des maladies rares sont utiles pour mettre en évidence les disparités géographiques, démographiques, les clusters et les tendances temporelles afin d'identifier de possibles déterminants de ces maladies. Pour cela, nous avons travaillé en collaboration avec le Laboratoire d'Épidémiologie de Strasbourg. Nous avons revu de façon systématique et méta-analysé les données concernant l'incidence et la prévalence des myopathies inflammatoires. Ce travail est un préliminaire à une étude de la prévalence et de l'incidence des myopathies inflammatoires en Alsace (PHRC interrégional 2011).

Patients

Les myopathies inflammatoires sont des pathologies rares. Le recrutement des patients de cette étude s'est appuyé sur le Centre de référence des maladies auto-immunes, le Centre de référence des maladies neuromusculaires, et le réseau MyositEst, un réseau interdisciplinaire pour la prise en charge et la recherche dans le champ des myopathies inflammatoires dans le Nord-Est que nous avons créé il y a 5 ans. Avec l'accord des patients, les données cliniques, le sérum et de l'ADN des patients atteints de myopathies inflammatoires sont collectés de façon prospective. Quarante-six patients ont été inclus dans ce travail : seize patients atteints de dermatomyosite (dont une patiente ayant une dermatomyosite aggravée par l'imiquimod, observation rapportée dans l'article 6), 14 patients atteints d'autres myopathies inflammatoires (dans la classification de l'ENMC : myopathie nécrosante auto-immune, myosite non spécifique, polymyosite, myosite à inclusions sporadique) et 16 patients atteints de myalgies sans signe de myopathie (examen clinique musculaire, taux de CPK, électromyogramme et biopsie musculaire normaux).

Animaux

Des souris BALBc immunisées contre la myosine (myosite auto-immune expérimentale : MAE) développent une faiblesse musculaire et un infiltrat inflammatoire dans le muscle squelettique. Nous avons été formés à la réalisation de ce modèle par l'équipe Inserm, UMR 974, Myology research center (Sorbonne Universités UPMC Université Paris 06) qui a développé ce modèle. Nous avons utilisé 12 souris MAE et 12 souris contrôles.

Matériel cellulaire

Les études cellulaires ont été réalisées sur des myotubes humains grâce à la plateforme de culture cellulaire de l'Institut de génétique et de biologie moléculaire et cellulaire IGBMC - CNRS UMR 7104 - Inserm U 964.

Étude physiologique des capacités d'effort

En plus des données cliniques collectées dans le cadre de la cohorte MyosiEst, les patients participant aux travaux de recherche reportés dans ce manuscrit ont participé à une exploration de leurs capacités d'effort. Ceci a été réalisé grâce à l'unité Explorations Fonctionnelles de l'Effort (Service de physiologie, CHU de Strasbourg).

Études fonctionnelles mitochondriales

Les fonctions mitochondriales sont dépendantes de l'environnement intracellulaire et des relations avec les autres organelles cellulaires. Nous avons enregistré la respiration mitochondriale et la production de radicaux libres dérivés de l'oxygène *ex vivo* dans les muscles des patients et des animaux en utilisant un protocole qui maintient les mitochondries fonctionnelles dans leur position intracellulaire physiologique. Le laboratoire EA 3072 Stress oxydant protection musculaire a une expérience de plusieurs années dans la maitrise de cette technique.

Étude histologique

L'histologie musculaire est un outil diagnostique (Hoogendijk et al., 2004) et de recherche des myopathies inflammatoires. Le tissu musculaire des patients et des animaux a été étudié en microscopie optique et en microscopie électronique. Nous avons été formés à la lecture de l'histopathologie musculaire par le Département de Pathologie des Hôpitaux Universitaires de Strasbourg.

Étude moléculaire

L'approche transcriptomique est un outil performant pour la découverte des voies de signalisation impliquées dans des processus pathologiques et elle peut être facilement mise en œuvre sur du tissu musculaire (81). Cette technique permet d'étudier sans a priori l'expression différentielle d'un très grand nombre d'ARNm entre des échantillons. Le

regroupement hiérarchique des ARNm différentiellement exprimés selon leur niveau d'expression et leurs fonctions biologiques permet de révéler les mécanismes moléculaires à l'œuvre dans les tissus biologiques étudiés. Cette approche a été appliquée aux muscles des patients inclus dans cette étude, et les résultats ont été confirmés par RT-qPCR chez les patients et les animaux. Ces études ont été réalisées en collaboration avec l'équipe de Monsieur le Docteur Daniel METZGER, IGBMC, CNRS UMR S 964 1 - Inserm U 964.

RÉSULTATS

ARTICLE 1 : les nouvelles myopathies inflammatoires

Cet article est présenté dans la section INTRODUCTION.

ARTICLE 2 : incidence et prévalence des myopathies inflammatoires : une revue systématique de la littérature

Les études épidémiologiques des maladies rares sont utiles pour mettre en évidence les disparités géographiques, démographiques, les clusters et les tendances temporelles afin d'identifier de possibles déterminants de ces maladies. Nous avons donc revu de façon systématique et méta-analysé les données concernant l'incidence et la prévalence des myopathies inflammatoires. L'incidence globale a été estimée à 7,98 (IC 95 %: 7,38-8,66) cas par million et par an entre 1951 et 2010.

Même si une hétérogénéité méthodologique limitait les comparaisons, certaines tendances épidémiologiques ont été mises en évidence. L'incidence relative des dermatomyosites (DM) suivait un gradient latitudinal dans l'hémisphère nord qui était concordant avec une action immunomodulatrice des rayonnements ultraviolets. La prévalence de la myosite à inclusions sporadique était corrélée avec la fréquence de HLA-DR3. L'incidence saisonnière et temporelle des myosites juvéniles était non aléatoire et compatible avec un rôle des maladies infectieuses, bien que d'autres facteurs environnementaux puissent être impliqués. Ces données sont un préliminaire à une étude en cours visant à enregistrer l'incidence et la prévalence des myopathies inflammatoires en Alsace.

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Incidence and prevalence of inflammatory myopathies: a systematic review

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Abstract

Objectives. The aims of this study were to determine the incidence and prevalence of inflammatory myopathies (IMs), their epidemiological tendencies over time and their possible key determinants.

Methods. All original articles in English or French regarding the prevalence and/or incidence of IMs were searched. The methods of case ascertainment, epidemiological analysis and diagnostic criteria were systematically analysed.

Results. Forty-six articles published between 1966 and 2013 were found in which the incidence of IMs as a whole ranged from 1.16 to 19/million/year and their prevalence ranged from 2.4 to 33.8 per 100 000 inhabitants. Methodological heterogeneities limited comparisons, although certain epidemiological tendencies were highlighted. The relative incidence of DM may follow a latitudinal gradient in the northern hemisphere that may be explained by the immunomodulatory action of ultraviolet radiation. The prevalence of sporadic inclusion body myositis (sIBM) was correlated with the frequency of HLA-DR3. Juvenile myositis onset was non-random over seasons and/or time, consistent with a role of infectious diseases, although other environmental factors may be involved. Disparities according to sex, age and geographical origin were also found. The frequency of IM increased over time, which may reflect progress in diagnostic performance, although there is still a need to increase the level of awareness with regard to these diseases, especially sIBM, as attested by its considerably delayed diagnosis.

Conclusion. This first systematic literature review confirms the rarity of IM and may highlight certain genetic and environmental determinants of IM. There is a need for uniformity in diagnostic and classification criteria as well as more exhaustive case ascertainment to refine IM epidemiology.

Key words: inflammatory myopathies, polymyositis, dermatomyositis, inclusion body myositis, epidemiology, incidence, prevalence, meta-analysis.

Introduction

Inflammatory myopathies (IMs) have long been designated under the generic terms PM and DM [1, 2]. They now encompass a heterogeneous group of acquired diseases characterized by muscle inflammation and muscular weakness, which mainly includes PM, DM, juvenile myositis (JM), overlap myositis (OM), myositis associated with cancers (MC), immune-mediated necrotizing myopathies and sporadic inclusion body myositis (sIBM) [3].

Although these conditions are believed to be very rare, the epidemiological features of IMs have been poorly studied and synthesis of the existing data regarding incidence and prevalence are lacking. Epidemiological studies of rare diseases are essential to identify their geographical and population disparities as well as clusters and time trends and hence their possible key determinants. Such epidemiological patterns may provide useful clues towards improving our understanding of IMs. In this literature review we analysed the worldwide

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prevalence and incidence of IMs, their possible key determinants and their epidemiological tendencies over time.

Methods

A systematic analysis of the literature was performed. The inclusion criteria were original articles in English or French pertaining to the prevalence and/or incidence of IMs in adults and children. In these reports, the incidence and/or prevalence of IMs had to be directly mentioned or easily calculated from the available data (the total number of IM cases at the endpoint of the study period and/or the number of new cases diagnosed during the study period and the global size of the population studied).

A flowchart (see supplementary Fig. S1, available at *Rheumatology* Online) was first constructed from PubMed (1 December 2013) using the following keywords: myositis, inflammatory myopathy, inflammatory myopathies, polymyositis, dermatomyositis, inclusion body myositis, necrotizing myopathy, incidence, prevalence and epidemiology. Among the 1561 articles first selected, 1489 were excluded on the basis of the title because they were not written in English or French or did not address the subject. A further 40 publications were excluded after reading the full text because the incidence and prevalence were not mentioned or calculable. Seven articles were added by manual search (found in the bibliographies consulted). Seven articles were found on the Web of Knowledge database. Two abstracts were found upon review of the main international congresses (annual American Congress of Rheumatology, annual European Congress of Rheumatology, annual meeting of the American Academy of Neurology and annual meeting of the European Neurological Society). A total of 46 reports were finally included in the systematic literature review [4–49] (Tables 1–6). The methods of case ascertainment and epidemiological analysis as well as the diagnostic and classification criteria were systematically examined.

Incidence and prevalence in groups and subgroups were estimated based on meta-analysis, under the Bayesian paradigm. Minimally informative prior distributions were chosen [N(0;var = 100)]. Non-informative hyper-priors were also used. Prevalence and incidence estimates are based on a binomial parameter posterior distribution and are given with their expected mean values and 95% CI. Results were displayed in forest plots and heterogeneity was assessed through I^2 and τ^2 calculation with their associated frequentist Cochran *Q* test *P*-value. Analyses were run with R 3.0.0 (R Project for Statistical Computing, Vienna, Austria) and WinBUGS 1.4 (MRC Biostatistics Unit, Cambridge Institute of Public Health, Cambridge, UK). Plots were drawn with GraphPad (GraphPad Software, La Jolla, CA, USA).

Results

Meta-analysis

Overall IM incidence was estimated at 7.98 cases/million/year (95% CI 7.38, 8.66) between 1951 and 2010 from the results of 16 surveys [4–9, 11, 13–15, 18–20, 22–24] in which incidence ranged from 1.16 [4] to 19/million/year (95% CI 17, 21) [19]. Overall IM prevalence was estimated at 14.00 cases/100 000 inhabitants (95% CI 12.84, 15.46) between 1982 and 2010 from the results of 10 surveys [6, 10, 12, 13, 16, 17, 19–21, 24] in which prevalence ranged from 2.4 [10] to 33.8/100 000 [20] (Table 1). Estimation of the incidence and prevalence of each IM subtype (adult IM, juvenile IM and sIBM) is shown in Tables 2, 4 and 5). Forest plots of the meta-analysis and heterogeneity calculations are shown in supplementary Fig. S2, available at *Rheumatology* Online.

Geographical variation

Studies reporting the prevalence and incidence of IMs were performed in five continents. There were broad differences in the results, but no clear geographical disparities were found when taking into account methodological variations.

Overall IM epidemiology

Incidence. Four studies ascertained patients through hospital records. Two used personal diagnosis criteria [6, 7] while two others used the Bohan and Peter criteria and targeted almost the same period: in Allegheny County, PA, USA (1963–82), Oddis *et al.* [11] estimated the incidence of IMs at 5.5/million/year (95% CI 0.3, 10.7), while in Israel (1960–76), Benbassat *et al.* [8] recorded a lower incidence (2.18/million/year), which nevertheless remained within the wide CIs of the Oddis *et al.* data.

Four other surveys combined hospital records with one or more other sources for case ascertainment. Rose and Walton [5] used personal criteria. Weitoft *et al.* (Gävleborg County, Sweden, 1984–93) [14] and Patrick *et al.* (Victoria State, Australia, 1989–91) [15] both used Bohan and Peter criteria with exclusion of possible PM/DM and inclusion of sIBM. They obtained strikingly similar results [7.6 and 7.4/million/year (95% CI 6.0, 9.0), respectively]. Including possible PM/DM as defined by Bohan and Peter, Kusumi *et al.* [13] (Tottori Prefecture, Japan, 1988–92) estimated the annual incidence of IMs at 10.1/million/year (95% CI 4.50, 15.70).

Higher incidences of IMs were found in four studies that used solely health insurance administrative databases for case ascertainment and inclusion. Differences in case definitions limited comparison: in Japan, according to Tanimoto *et al.* [50], the incidence reached 13.4/million in 2008. In Taiwan (2000–08), using 710-3 (PM) and 710-4 (DM) diagnosis fields from the ninth revision of the International Classification of Diseases (ICD-9), the annual incidence was found to be between 11.5 [18] and 15/million (95% CI 12, 17) [21]. In England, Tran *et al.* [19] found 19 cases/million/year (95% CI 17, 21), although the diagnosis codes used for case definitions were not reported.

Prevalence. Using a single source for case ascertainment, three surveys reported IM prevalences between 2.4 (in Kumamoto Prefecture, Japan, 1983) [10] and

TABLE 1 Incidence and prevalence of overall IMs

Country	Reference	Year of publication	Period of study	Method of case ascertainment	Inclusion criteria	Size of study population	Incident cases, *n*	Incidence /million/ year (95% CI)	Prevalent cases, *n* (year)	Prevalence/ 100 000	SR, F/M
USA; California	Pearson [4]	1966	9-year period	Personal observations	Study specific	10 million	104	1.16	NR	NR	NR
North England	Rose and Walton [5]	1966	1954–64	Personal observations, enquiries sent to practitioners	Study specific	3 294 000	89	2.46	NR	NR	NR
USA; Minnesota (Rochester)	Kurland *et al.* [6]	1969	1951–67	Mayo Clinic records review	Study specific	~50 000	4	6.0	3 (NR)	6.3	3/1
USA; Tennessee (Shelby County)	Medsger *et al.* [7]	1970	1947–68	Records review of 19 hospitals	Study specific	0.4–0.7 million	61	5.0	NR	NR	1.5/1
Israel	Benbassat *et al.* [8]	1980	1960–76	All general hospitals records review	Bohan and Peter [1]	2.3 million	86	2.18	NR	NR	1.7/1
Libya; Benghazi	Radhakrishnan *et al.* [9]	1987	1983–85	Intensive search	DeVere and Bradley [72]	0.52 million	13	8.8	NR	NR	2.25/1
Japan; Kumamoto City	Araki *et al.* [10]	1987	1977–82	Enquiries sent to practitioners' offices and hospitals	Study specific	560 000	NR	NR	27 (1982)	5.0	4.4/1
Japan; Kumamoto Prefecture			1982–83	Enquiries sent to practitioners' offices and hospitals	Study specific	1.8 million	NR	NR	44 (1983)	2.4	
USA; Pennsylvania (Allegheny County)	Oddis *et al.* [11]	1990	1963–82	Records review of 35 hospitals	Bohan and Peter	1.3–1.6 million	177	5.5 (0.3, 10.7)	NR	NR	2.2/1
Sweden; Orebro County	Ahlström *et al.* [12]	1993	1988	Central register of institutional care, departments cards indexes, National Health Insurance register, insurance records, enquiries sent to general practitioners, outpatient departments records review	Myositis established by a rheumatologist or neurologist	269 341	NR	NR	29 (1988)	11	8/1
Japan; Tottori Prefecture	Kusumi *et al.* [13]	1995	1988–92	University Hospital Yonago City records review, written and telephone enquiries addressed to general hospitals	Bohan and Peter [1]	614 725	31	10.1 (4.50, 15.70)	61 (NR)	9.92	2.44/1
Sweden; Gävleborg County	Weitoft *et al.* [14]	1997	1984–93	Records review of hospitals and outpatient centres and NUUHL laboratory	Bohan and Peter (excluding posPM/DM and including sIBM)	307 018	21	7.6	NR	NR	1.1/1
Australia; Victoria	Patrick *et al.* [15]	1999	1989–91	Records review of 18 hospitals and SNSMU	Bohan and Peter (excluding posPM/DM and including sIBM)	4 420 373	94	7.4 (6.0, 9.0)	NR	NR	1.41/1

(continued)

TABLE 1 Continued

Country	Reference	Year of publication	Period of study	Method of case ascertainment	Inclusion criteria	Size of study population	Incident cases, *n*	Incidence /million/ year (95% CI)	Prevalent cases, *n* (year)	Prevalence/ 100 000	SR, F/M
Egypt; Assiut Governorat	El-Tallawy *et al.* [16]	2005	NR	Door-to-door survey	NR	52 203	NR	NR	6 (1997)	11.49	NR
Canada; province of Quebec	Bernatsky *et al.* [17]	2009	1989–2003	Physician billing and hospitalization databases review	ICD-9 (codes 710-3 and 710-4)	7.5 million	NR	NR	1613 (2003)	21.5 (19.4, 23.9)	NR
Taiwan	Kuo *et al.* [18]	2011	2003–07	National Health Insurance research database	ICD-9 (codes 710-3 and 710-4)	22.7 million	1303	11.5	NR	NR	2.30/1
England	Tran *et al.* [19]	2012	2000–09	General Practice Research Hospital Episode Statistics databases	Diagnosis code of myositis	1.72 million	326	19 (17, 21)	465(2009)	27.2 (24.8, 29.8)	1.6/1
Canada; province of Alberta	Barnabe *et al.* [20]	2012	1994–2007	Physician billing claims and hospitalization data	16 (ICD-9) and 25 (ICD-10) discharges diagnosis fields	3.7 million (First Nations 3.7%)	NR	NR	1239 (2007)	33.8 (28.9, 39.6) non-First Nations, 25.0 (13.4, 49.0) First Nations	NR
Taiwan	Yu *et al.* [21]	2013	2000–08	National Health Insurance research database	ICD-9 (codes 710-3 and 710-4)	1 million randomly sampled from 23 753 407	117	15 (12, 17)	28 (2000)	2.9 (0.8, 4.2)	1.9 (1.3, 2.8)/1
Hungary	Vincze *et al.* [22]	2013	1999–2010	Myositis Study Group records review	NR	9.82 million	1119	9.5	NR	NR	2.1/1
New Zealand; Counties Manukau	Gupta *et al.* [23]	2013	2004–08	Computer-generated data of both inpatients and outpatients	Bohan and Peter [1]	464 000	12	5.1	NR	NR	1/1
Japan	Ohta *et al.* [24]	2013	2003–10	Registration system on intractable diseases database	Tanimoto *et al.* [50]	127 million	11 521	6.8 (2003) to 13.4 (2008)	17 000 (2010)	13.2	
Meta-analysis	Present article	—	1951–2010	—	—	186 million	15 578	7.98 (7.38, 8.66)	20 488	14.00 (12.84, 15.46)	—

IM: inflammatory myopathy; EMG: electromyogram; F: female; M: male; NR: not reported; ICD-9: International Classification of Diseases, 9th revision; NUUHL: Neuromuscular Unit at the University Hospital in Linköping, Sweden; posPM/DM: possible PM or DM; SNSMU: State Neuropathology Service of Melbourne University; sIBM: sporadic inclusion body myositis; SR: sex ratio (when both incidence and prevalence were provided, incident cases were considered for sex ratio calculation).

TABLE 2 Incidence and prevalence of adult IM

Country	Reference	Year of publication	Period of study	Method of case ascertainment	Inclusion criteria	Size of study population	Incident cases, *n*	Incidence/ million/year (95% CI)	Prevalent cases, *n*	Prevalence/ 100 000	SR, F/M
Singapore	Koh *et al.* [25]	1993	1986–91	Records review of Tan Tock Seng Hospital and three main EMG laboratories	Bohan and Peter [1]	1.62 million	75	7.70 adjusted for ⩾16 years of age	NR	NR	1.86/1
Finland	Kaipiainen-Seppänen *et al.* [26]	1996	1990	Sickness Insurance Act data	Bohan and Peter [1]	~1 million	4	3.7 (1.1, 10.2) adjusted for ⩾16 years of age	NR	NR	NR
New Zealand, North Canterbury region	Lynn *et al.* [27]	2005	1989–2001	Christchurch hospital records review	Study specific	430 000	44	8.7	NR	NR	4/1
South Australia	Limaye *et al.* [28]	2007	1990–004	Biopsy reports from the Neuropathology Laboratory, Hanson Institute	Study-specific histological inclusion criteria	1 491 418	120	5.4	NR	NR	
Greece, prefecture of Magnesia	Anagnostopoulos *et al.* [29]	2010	2007–08	Enquiries sent to 3528 randomly selected individuals	NR	1705 responses among 176 433 inhabitants	NR	NR	1	58 (50, 180)	NR
USA	Furst *et al.* [30]	2012	2003–08	National managed care organization records review	ICD-9 (codes 710-3, 710-4 and 728.81)	Sample of ~35 million	1941	66 (62, 69) adjusted for ⩾18 years of age	681 (2003) to 1017(2008)	14.0 (12.9, 15.0) to 17.4 (16.3, 18.4) adjusted for ⩾18 years of age	1.85/1
USA	Smoyer-Tomic *et al.* [31]	2012	2004–08	MarketScan databases	ICD-9-CM (codes 710-3, 710-4 and 728.81)	~14 million	2990	42.7 (40.9, 44.4) adjusted for >18 years of age	7155	Range 15.35 to 32.74 adjusted for >18 years of age	2.05/1
South Australia	Tan *et al.* [32]	2013	1980–2009	Biopsy reports from the Neuropathology Laboratory, Hanson Institute	Study-specific histological inclusion criteria	1.47 million	352	8 (7.2, 8.9)	NR	NR	NR
Argentina, City of Buenos Aires	Rosa *et al.* [33]	2013	1999–2009	Records review of 28 hospitals and centres and biology laboratory	Bohan and Peter [1]	146 747	10	10.7 (5, 18.4) adjusted for >18 years of age	17 (2009)	17.4 (10.1, 27.8)	2.3
Meta-analysis	Present article	—	1986–2009	—	—	24 million	5416	19.97 (18.82, 21.34)	2351	29.97 (14.44, 70.23)	—

IM: inflammatory myopathy; EMG: electromyogram; F: female; M: male; NR: not reported; ICD-9-CM: International Classification of Diseases, 9th revision clinical modification; SR: sex ratio (when both incidence and prevalence were provided, incident cases were considered for sex ratio calculation).

TABLE 3 Incidence of adult IM subtypes according to the Bohan and Peter classification [1]

Country	Reference	Year of publication	Period of study	Method of case ascertainment	PM		DM		Myositis associated with cancer		OM	
					Incident cases, *n*	Relative incidence, %	Incident cases, *n*	Relative incidence, %	Incident cases, *n*	Relative incidence, %	Incident cases, *n*	Relative incidence, %
USA; Pennsylvania (Allegheny County)	Oddis *et al.* [8]	1990	1963–82	Records review of 35 hospitals	88	56.4	27	17.3	20	12.8	21	13.5
Singapore	Koh *et al.* [15]	1993	1986–91	Records review of Tan Tock Seng Hospital and three main EMG laboratories	22	29.3	21	28.0	17	22.7	15	20..0
Sweden; Gävleborg County	Weitoft *et al.* [10]	1997	1984–93	Records review of hospitals and outpatient centres and NUUHL laboratory	8	38.1	3	14.3	3	14.3	7	33.3
Australia; Victoria	Patrick *et al.* [11]	1999	1989–91	Records review of 18 hospitals and SNSMU	56	64.4	12	13.8	8	9.2	11	12.6

IM: inflammatory myopathy; OM: overlap myositis; EMG: electromyogram; NUUHL: Neuromuscular Unit at the University Hospital in Linköping, Sweden; SNSMU: State Neuropathology Service of Melbourne University, Australia.

11.49 cases/100 000 inhabitants (in Assiut Governorate, Egypt) [16]. The nature of the source was heterogeneous (hospital records [6], enquiries sent to practitioners [10], door-to-door ascertainment [16]), while diagnosis criteria were study specific or not reported and CIs were not reported.

Two surveys used more than one source for case identification. In Japan (Tottori Prefecture, Japan, 1988–92) the prevalence was 9.92/100 000 inhabitants [10], while in Sweden (Orebro County, 1988), Ahlström *et al.* [12] identified 11 prevalent cases/100 000 inhabitants. Diagnosis criteria used in this latter study were not reported.

Finally, surveys constructed solely from health system administrative databases [17, 19–21, 24] generally found the highest prevalence of IMs. Only Yu *et al.*'s [21] survey reported a surprisingly low prevalence in Taiwan [2.9/100 000 (95% CI 0.8, 4.2)] when compared with the high estimation of IM incidence in Taiwan. Since the case definition was the same as that used by Bernatsky *et al.* [17] (710-3 and 710-4 diagnosis fields of the ICD-9), one can speculate that the prevalence in Taiwan may be significantly lower than in the province of Quebec, Canada [21.5/100 000 (95% CI 19.4, 23.9)].

Adult IM epidemiology

Incidence. Three studies recorded IMs in adult populations according to the Bohan and Peter criteria but used different case ascertainment methods: Kaipiainen-Seppänen and Oho [26] (Finland, 1990) used Sickness Insurance Act data and estimated IM incidence at 3.7/million/year (95% CI 1.1, 10.2). Koh *et al.* [25] (Singapore, 1986–91) and Rosa *et al.* [33] (Buenos Aires, Argentina, 1999–2009) used clinical centre records combined with electromyography or biology laboratory information and found 7.70 and 10.7 cases/million/year (95% CI 5, 18.4), respectively.

Furst *et al.* [30] (2003–08) and Smoyer-Tomic *et al.* [31] (2004–08) recorded adult IM cases in all of the USA through administrative claims databases using ICD-9 for case definition and found much higher incidences of IM in adults ($\geqslant$18 years): 66 (95% CI 62, 69) and 42.7/million/year (95% CI 40.9, 44.4), respectively.

Four reports examined in greater detail the proportion of different adult IM subtypes according to the Bohan and Peter classification [11, 14, 15, 25]. The relative incidence of adult DM was higher in Singapore (28.0%) as compared with Allegheny County (17.3%), Victoria State (14.3%) and Gävleborg County (13.8%). Relative incidences of PM, OM and MC were also unequally distributed (Table 3).

Prevalence. The aforementioned studies conducted by Furst *et al.* [30], Smoyer-Tomic *et al.* [31] and Rosa *et al.* [33] reported similar prevalence of IM [14.5 (95% CI 12.9, 15.0)–17.4 (95% CI 16.3, 18.4), 15.35–32.74 and 10.22 (95% CI 4.9, 18.8), respectively]. The unique DM cases identified in a small, randomly selected population sample (1705 inhabitants) in the prefecture of Magnesia (Greece) that reported directly any diagnosis of rheumatic

TABLE 4 Studies of the incidence and prevalence of JM

Country	Reference	Year of publication	Period of study	Methods of case ascertainment	Inclusion criteria	Size of study population	Incident cases, *n*	Incidence/million/year (95% CI)	Prevalent cases, *n*	Prevalence/100 000	SR, F/M
Finland	Pelkonen *et al.* [35]	1994	1983–86	Prospective hospital case collection, NHDR review	Bohan and Peter [1]	1 020 737	12	3.0 adjusted for <15 years of age	NR	NR	1.0/1
USA; Massachusetts	Denardo *et al.* [34]	1994	1987–91	Prospective multicentre patient registry	Bohan and Peter [1]	1.4 million	NR	4.0 (1.0, 7.0) adjusted for <18 years of age	NR	NR	2.0/1
UK and Ireland	Symmons *et al.* [36]	1995	1992–93	Enquiries sent to consultant members of the BPA and the FPRCPI	Bohan and Peter [1]	12 488 300	48	1.9 (1.4, 2.6) adjusted for <16 years of age	NR	NR	5.0/1
Japan	Fujikawa and Okuni [38]	1997	1984–93	Enquiries sent to the paediatric departments of hospitals	NR	29 910 000	320	1.6 adjusted for <16 years of age	NR	NR	1.7/1
Western Sweden	Darin and Tulinius [39]	2000	1979–94	Paediatric hospitals, child rehabilitation centres, orthopaedic hospitals, paediatric clinics, departments of pathology records review; NSBHW register of causes of death records review	Bohan and Peter [1]	359 676	13	2.4 adjusted for <16 years of age	9	2.5 adjusted for <16 years of age	1.4/1
USA	Mendez *et al.* [37]	2003	1995–98	NIAMSD and PRDR registry records review	Study specific	62.296 million	788 (714, 862)	3.2 (2.9, 3.4) adjusted for 2–17 years of age	NR	NR	2.3/1
Meta-analysis	Present article	—	1983–98	—	—	98 million	1209	2.69 (2.28, 3.17)	—	—	—

IM: inflammatory myopathy; BPA: British Paediatric Association; FPRCPI: Faculty of Paediatrics of the Royal College of Physicians of Ireland; F: female; M: male; NHDR: National Hospital Discharge Register; NIAMSD: National Institute of Arthritis and Musculoskeletal and Skin Disease; NR: not reported; NSBHW: National Swedish Board of Health and Welfare; PRDR: Pediatric Rheumatology Disease Registry; SR: sex ratio.

TABLE 5 Studies of the incidence and prevalence of sporadic inclusion body myositis

Country	Reference	Year of publication	Period of study	Method of case ascertainment	Inclusion criteria	Size of study population	Incident cases, *n*	Incidence/ million/year (95% CI)	Prevalent cases, *n*	Prevalence/ 100 000 (95% CI)	SR, F/M
Sweden; Göteborg city	Lindberg *et al.* [40]	1994	1983–92	18 consecutive patients with sIBM	Study specific	440 000	10	2.20	NR	NR	0.8/1
Finland	Kaipiainen-Seppänen *et al.* [26]	1996	1990	Sickness Insurance Act data	NR	~1 million	1	0.9 (0, 5.6) adjusted for ⩾16 years of age	NR	NR	NR
Netherlands	Badrising *et al.* [41]	2000	1982–99	Enquiries sent to neurological and rheumatological centres	ENMC (1997) [53]	15 654 192	NR	NR	76 (1999)	0.49 (1.6 adjusted for ⩾50 years of age)	0.5/1
Western Australia	Phillips *et al.* [42]	2000	1988–98	ANRI and pathology laboratory records review; enquiries sent to neurologists and rheumatologists	Griggs *et al.* [52]	1 831 399	NR	NR	17 (1998)	0.93 (3.53 adjusted for ⩾50 years of age)	0.7/1
USA; Connecticut	Felice and North [43]	2001	1992–2000	MDA Clinic records review	Griggs *et al.* [52]	3.2 million (1998)	NR	NR	35	1.07 (2.89 adjusted for ⩾45 years of age)	0.53/1
New Zealand; North Canterbury Region	Lynn *et al.* [27]	2005	1989–2001	Christchurch hospital records review	Mastaglia and Phillips[a] [51]	430 000	6	1.2	NR	NR	NR
USA; Minnesota (Olmsted County)	Wilson *et al.* [44]	2008	1981–2000	Mayo Clinic records review	Griggs *et al.* [52]	124 277	8	3.2 [7.9 (2.4, 13.5) adjusted for ⩾30 years of age]	6 (2000)	4.8 [7.06 (0.87, 13.24) adjusted for ⩾30 years of age)	2.0/1[b]
Western Australia	Needham *et al.* [45]	2008	1997–2007	ANRI and pathology laboratory records review; enquiries sent to neurologists and rheumatologists	Needham and Mastaglia[a] [54]	2 080 966	NR	NR	31 (2006)	1.49 (5.13 adjusted for ⩾50 years of age)	0.7/1
Turkey; Istanbul	Oflazer *et al.* [46]	2011	1993–2011	Muscle biopsies request forms and findings from the neurology laboratory of Istanbul University	Study specific	13 255 685	NR	NR	9 (2010)	0.0679 (0.3834 adjusted for ⩾50 years of age)	NR
New Zealand; Counties Manukau	Gupta *et al.* [23]	2013	2004–08	Computer-generated data of both inpatients and outpatients	NR	464 000	1	0.43	NR	NR	NR
South Australia	Tan *et al.* [32]	2013	1980–2009	Biopsy reports from the Neuropathology Laboratory, Hanson Institute	NR	1.47 million	126	2.9 (2.4, 3.4)	73 (2009)	5.05 (4.02, 6.27) [13.93 (11.01, 7.39) adjusted for ⩾50 years of age]	1.1/1[b]
Meta-analysis	Present article	—	1983–2010	—	—	38 million	160	1.85 (1.34, 2.52)	253	2.01 (1.51, 2.69)	—

ANRI: Australian Neuromuscular Research Institute; F: female; M: male; MDA: Muscular Dystrophy Association; UCHC: University of Connecticut Health Center; NR: not reported; sIBM: sporadic inclusion body myositis; SR: sex ratio. [a]These criteria are equivalent. [b]Given for the entire cohort.

TABLE 6 Studies of the incidence and prevalence of other IM subtypes

Country	Reference	Year of publication	Period of study	Methods of case ascertainment	Inclusion criteria	Size of study population	Incident cases, *n*	Incidence/ million/year (95% CI)	Prevalent cases, *n*	Prevalence/ 100 000 (95% CI)	SR, F/M
South Africa; Pretoria	Findlay *et al.* [47]	1969	NR	Personal observation	NR	~2.33 million	35	Not less than 2.1	NR	NR	NR
New Zealand	Couchman and Wigley [48]	1971	1950–66	Hospital records	ICD8 code for DM (710.0)	2.677 millions	85	2.0 (North Island) *vs* 2.3 (South Island) (NS)	NR	NR	NR
USA; Minnesota (Olmsted County)	Wilson *et al.* [44]	2008	1981–2000	Mayo Clinic records review	Bohan and Peter [1] (⩾20 years of age, PM only)	124 277	6	4.1 (0.8, 7.3) adjusted for ⩾20 years of age	3 (2000)	3.45 (0.00, 7.35) adjusted for ⩾20 years of age	5.0/1[a]
USA; Minnesota (Olmsted County)	Bendewald *et al.* [49]	2010	1976–2007	Rochester Epidemiology Project records review	Gerami *et al.* [73] (DM only, including CADM)	121 000	29	9.63 (6.09, 13.17)	26 (2007)	21.42 (13.07, 29.77)	1.32

IM: inflammatory myopathy; CADM: clinically amyopathic DM; F: female; M: male; NR: not reported; NS: non-significant; SR: sex ratio. [a]Given for the entire cohort.

disease yielded an unreliable estimation of adult IM in this area [58/100 000 (95% CI 50, 180)] [29].

JM epidemiology

Incidence. Three retrospective surveys used two or more sources of ascertainment. Two were based on Bohan and Peter criteria and targeted relatively the same period (1979–94 and 1992–93): one was conducted in western Sweden [39] and the other in the UK and Ireland [36]. Both found similar incidence of JM [2.4 and 1.9/million/year (95% CI 1.4, 2.6), respectively]. The 1995–98 national US survey conducted by Mendez *et al.* [37] used two independent registry records (one using Bohan and Peter criteria and the other using study-specific criteria) in which the incidence was corrected by a capture–recapture method and found a higher incidence of JM (2–17 years of age) of 3.2/million/year (95% CI 2.9, 3.4).

In two other surveys, a prospective multicentre recording of patients using Bohan and Peter criteria was used. In Massachusetts (USA) from 1987 to 1991, Denardo *et al.* [34] reported the highest incidence of JM (<18 years of age): 4.0/million/year (95% CI 1.0, 7.0), while the 3.0/million/year incidence of JM (<15 years of age) recorded by Pelkonen *et al.* [35] in Finland (1983–86) remained within the CI range of the Denardo *et al.* study.

Prevalence. Only Darin and Tulinius [39] assessed the prevalence of JM (<16 years of age), with a result of 2.5/100 000.

sIBM epidemiology

Incidence. Using a similar method for case ascertainment (review of the records from the referral medical centre of their studied area), Lynn *et al*. [27] recorded a more than 2-fold lower incidence (1.2/million/year in the North Canterbury region of New Zealand, 1989–2001) than Wilson *et al.* [44] (3.2/million/year in Olmsted County, MN, USA, 1981–2000). However, different diagnostic criteria were employed (Mastaglia and Phillips [51] and Griggs *et al.* [52], respectively) and the number of cases was small (six and eight cases, respectively).

The remaining surveys reported sIBM incidence and used various methods for case ascertainment (consecutive observations [40], Sickness Insurance Act records [26], computer-generated data [23] and muscle biopsy records [32]), diagnosis criteria were either study specific or not reported and CIs overlapped {ranging from 0.9 (95% CI 0, 5.6) [26] to 2.9/million/year (95% CI 2.4, 3.4) [32]}.

Prevalence. Four studies were based on data obtained from a unique referral centre in the studied area. Two studies used Griggs *et al.* [52] criteria for case definition and were conducted in the USA. In Connecticut (1992–2000), Felice and North [43] estimated the prevalence of sIBM at 2.89/100 000 inhabitants ⩾45 years of age, while in Olmsted County, Minnesota (1981–2000), Wilson *et al.* [44] found a prevalence slightly more than 3-fold higher (7.06/100 000 inhabitants ⩾30 years of age) but the 95% CI of their results (0.87, 13.24) included Felice and North estimation. Two other studies used muscle biopsy request forms and/or reports for both

case identification and inclusion, although the diagnosis criteria and assumed population size served by each centre differed. In Turkey (1993–2011), while assuming that all of the country's population (>13 million) was served by the Neurology Laboratory of Istanbul, and without the availability of EM or immunochemistry, Oflazer *et al.* [29] found the lowest prevalence of sIBM: 0.3834/100 000 inhabitants ⩾50 years of age. Conversely, when assuming the entire population of the State of South Australia (~1.5 million) and including EM and immunochemistry for diagnosis, Tan *et al*. [32] found the highest prevalence of sIBM: 13.93/100 000 (95% CI 11.01, 17.39) for inhabitants ⩾50 years of age in 2009.

In the Netherlands, Badrising *et al.* [41] contacted the larger neurological and rheumatological centres of the country by telephone and in writing in order to identify patients diagnosed with sIBM according to European Neuromuscular Centre (ENMC)> criteria [53] from 1982 to 1999. This nationwide survey resulted in the establishment of a prevalence of 1.6/100 000 for the population ⩾50 years of age.

In Western Australia, two consecutive studies used letters sent to all neurologists and rheumatologists and referral centre records. During the 1988–98 period, the prevalence of sIBM according to Griggs *et al.* [52] criteria was 3.53/100 000 for a population ⩾50 years of age, while increasing to 5.13/100 000 from 1997 to 2007 using Needham *et al.* [45] and Needham and Mastaglia [54] criteria.

Spatiotemporal clusters

An unusually large number of cases of IM were observed in some areas and/or during certain periods, suggesting possible spatiotemporal clustering.

Geographical clustering

Patrick *et al.* [15] demonstrated spatial clustering of overall IM in the State of Victoria, Australia, without being able to speculate on the reason for the observed clustering. Among four studies assessing the difference between rural and urban areas [16, 17, 20, 32], only Barnabe *et al.* [20] found a significant difference, with a higher incidence in older, rural women in the province of Alberta, Canada. In addition, some methods of case ascertainment were demonstrated to be more sensitive in one community *vs* others [17].

Time clustering

There was no seasonal [7] or time–space [15] clustering when IM cases were considered as a whole. However, with regard to JM, a seasonal excess in cases was reported in two studies. Medsger *et al.* [7] noted more frequent onset of JM in February–April (45% of the children), which reached significance when compared with adults when considering patients with an interval of ⩽1 year from the onset of symptoms to the time of diagnosis. In the UK and Ireland during the 1992–93 period, there tended to be a cluster of JM cases with onset in April and May [36]. Moreover, in the USA, the year-by-year incidence rates of JM were non-random (higher for 1996 and 1997 than 1995 and lower in 1996 than in 1998) [37].

Time trends

Studies reporting the prevalence and incidence of IM over a period of 64 years (from 1947 to 2010) were collected. All surveys assessing time trends reported an increase over time in the frequency of IM, including overall IM [7, 8, 11, 24], adult IM [30, 32], DM [49] and sIBM [32, 42, 45] in various geographical areas (USA [7, 11, 30, 49], Israel [8], Australia [32, 42, 45], Japan [24]). However, this trend was reported to be significant only in three US studies: from 1947 to 1968 in Shelby County, TN, the age-adjusted incidence rate of overall IM increased from the first 11-year period (2.6/million/year) to the second 11-year period (6.5/million/year) [7]. In Allegheny County, PA, the incidence of 8.9/million/year for the 1973–82 period was >3-fold higher than the 2.5/million/year incidence for the 1963–72 period [11]. In the whole USA, the incidence of adult IM recorded in 2008 [79/million/year (95% CI 68, 90)] was significantly higher than in 2003 [58/million/year (95% CI 49, 67)] [30].

Influence of age and sex

The age of IM patients as a whole varied from 44.2 years (range 7–74) at disease onset [10] to 55.1 years (S.D. 19.5) at disease diagnosis [15], with a first peak during childhood and a second during adulthood [7, 8, 11, 13]. A greater proportion of females was a fairly consistent finding (Table 1). Mean age at onset of sIBM ranged from 60.4 (range 36–70) [40] to 64.3 years (range 41–80) [43] and all surveys except two [32, 44] found a similar male excess (Table 5).

Delay between onset and diagnosis

The median time to diagnosis of overall IM varied between 3 (range 1–123) [11] and 6 months (range 3 months–8 years) [14]. A considerably delayed diagnosis was a constant feature of sIBM [27, 41, 42, 45]. The mean duration of symptoms before diagnosis varied between 4.1 [44] and 8 years [41]. Upon reviewing the initial diagnoses of a cohort of 57 sIBM cases, Needham *et al.* [45] found that the disease had been misdiagnosed in nearly 50% of cases, the most common misdiagnoses being arthritis, motor neurone disease, PM and old age.

Discussion

This first systematic review of IM epidemiology confirms the extreme rarity of these conditions for which incidence is estimated at 7.98/million/year (95% CI 7.38, 8.66) and prevalence at 14.00/100 000 (95% CI 12.84, 15.46).

Although there were variations in the reported results, the review did not demonstrate clear geographical disparities in worldwide IM epidemiology due to heterogeneity in case ascertainment, inclusion criteria and/or CI overlap. Despite these limitations, geographical analysis may highlight certain tendencies in IM epidemiology. The relative

incidence of DM appears to exhibit a latitudinal gradient in the northern hemisphere. Incidence was 28.0% in Singapore, 17.3% in Allegheny County and 13.8% in Gävleborg County (Table 3), with the latitudes of these areas being +1.36° (Singapore), +38.0° (Pittsburgh) and +58.41° (Linköping), respectively. This is consistent with previous results showing that the relative prevalence of DM increases significantly with geographical latitude in nine European countries [55]. Multivariate analyses demonstrated that among 13 geoclimatic variables that may modulate the disease and the relative proportion of DM, surface ultraviolet (UV) radiation intensity most strongly contributed to the relative proportion of DM [56]. Moreover, UV radiation intensity has been shown to predict the relative distribution of DM in women [57]. Hence the latitudinal gradient highlighted in this review could result from the immunomodulatory action of UV radiation.

The prevalence of sIBM was found to be higher in Olmsted County than in Western Australia, the Netherlands and Turkey (Table 5). Interestingly, these disparities were consistent with the frequency of HLA-DR3 (HLA-DRB1*03:01) in the populations studied, which were 0.137 in Olmsted County, 0.135 in Western Australia, 0.116 in the Netherlands and 0.092 in Turkey [58]. This finding corroborates previous data regarding the role of HLA-DR3 in sIBM [59, 60]. Whether this is true in South Australia, where the highest prevalence of sIBM was recorded, remains unknown, as HLA-DR3 frequency was not available for the population in this area.

Cohort-based studies have previously suggested that there is no seasonal variation of disease onset in myositis patients as a whole, although there are different seasonal patterns in some IM subgroups [61–63]. In this review of population-based studies, whereas no seasonal or space-time clustering was demonstrated in overall IM [7, 15], JM onset was found to be non-random over seasons and/or time [7, 36, 37]. Also, recently it has been demonstrated that more than half of patients with JM had a history of infection 3 months prior to the onset of the disease [64, 65]. Thus it is tempting to speculate that juvenile infectious diseases modulate the epidemiology of JM over time, although other environmental factors may also be implicated.

All surveys assessing time trends found an increase in the frequency of IMs [7, 8, 11, 42, 45, 49]. However, according to all authors, this trend was more likely to reflect improvements in medical record indexing, increased awareness of physicians with community experience over time and improved diagnostic tools. However, a considerably delayed diagnosis was a constant feature of sIBM [40–45]. This may contribute to the underestimation of the true prevalence of this condition and also emphasizes the need to increase the level of awareness in the medical community with regard to this disease.

Certain characteristics particular to IM render the epidemiological features of this disease difficult to study. The first difficulty is the significant evolution in the concept of IM since the first study was initially performed. Most studies were based on the Bohan and Peter criteria [1, 2], which have several limitations. According to these authors, DM differs from PM solely by skin changes. These criteria were also developed before the full distinction between sIBM and PM. Furthermore, the Bohan and Peter criteria do not take into account myositis-specific and myositis-associated autoantibodies, which notably differentiate OM from PM [66]. Finally, it has been shown that these criteria overdiagnose PM at the expense of DM, sIBM [67] and OM [68]. Advances in the comprehension of the pathogenesis of IM have contributed to a better recognition of IM subtypes and new sets of diagnostic criteria have been developed that take into account advances in histopathology [69] and serum autoantibody detection [68], although there still remains a strong need to revise and reclassify [70].

Secondly, IM is an extremely rare condition. The larger the study population, the greater the number of cases, which means both incidence and prevalence rates are more reliable. The cohort sizes in the surveys presented here were quite variable and targeted either large populations, such as the nationwide child population in the USA (>62 million children) [37], or small population samples, such as in the prefecture of Magnesia, Greece (1705 inhabitants) [29]. In several studies the CIs were either large or not reported, thus making comparisons difficult.

Finally, IM is a multisystemic disease. Given that it affects various organs, different specialists are necessarily involved (rheumatologists, neurologists, dermatologists, pulmonologists, paediatricians) and it is therefore difficult to achieve exhaustive case ascertainment since it is easy to miss some of these cases when myositis is not the initial or most severe symptom. On the other hand, surveys based solely on administrative claims benefit from large case ascertainment, although the high frequencies of IM that were systematically reported in such studies may be influenced by miscoding and misdiagnosis [17–21, 24, 30, 31]. In only one study [37] did the authors use a capture–recapture analysis of two independent sources to overcome potential incomplete case findings and correct the calculated prevalence rate. This procedure takes advantage of duplicate information derived from multiple, disaggregated sources of case ascertainment in order to provide an estimate of the number of cases missed by any one source and hence the true number of cases in a given area [71]. It would certainly be of great interest to use this methodology in future studies.

In summary, the present review underscores the difficult challenges in the epidemiological study of IM, but may nevertheless provide useful clues for the comprehension of IMs. Despite comparative limitations due to methodological heterogeneity, some epidemiological disparities between geographical areas and populations may highlight key genetic and environmental determinants of these conditions. Time trends demonstrate some progress in diagnostic performance, but there is still a need to increase the level of awareness of IM in the medical community, especially with regard to sIBM. Better case identification and classification will certainly yield

greater insight into its key determinants and evolution over time.

Rheumatology key messages

- Incidence of inflammatory myopathies ranges from 1.16 to 19/million/year and prevalence from 2.4 to 33.8/100 000.
- Although methodological heterogeneity limits comparisons, variations of prevalence and/or incidence may highlight notable determinants of inflammatory myopathies.

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Supplementary data

Supplementary data are available at *Rheumatology* Online.

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ARTICLE 3 : la cryopréservation avec le diméthylsulfoxyde altère l'exactitude de l'analyse de la respiration mitochondriale des fibres musculaires squelettiques perméabilisées

Dans cet article, nous détaillons la mesure de la respiration mitochondriale dans les cellules musculaires perméabilisées. L'intérêt de cette analyse fonctionnelle repose sur la préservation de l'intégrité de la mitochondrie et de ses interactions avec les structures intracellulaires, deux éléments clés dans la régulation des fonctions mitochondriales. Des méthodes de cryoconservation ont été développées pour permettre le stockage d'échantillons pour une analyse plus approfondie ou un transfert d'un centre dans lequel l'évaluation de la respiration n'est pas disponible pour un centre qui effectue cette méthode. Ceci est particulièrement intéressant dans l'analyse de maladies rares telles que les MI.

Cependant, nous avons montré, dans cet article, que la consommation d'oxygène avec les principaux substrats mitochondriaux dans les muscles squelettiques du rat était plus élevée dans les échantillons frais que dans les échantillons cryoconservés et que cette différence n'était pas fixe, mais augmentait avec les taux de respiration, avec de larges fluctuations autour de la différence de moyenne. En conséquence, les effets délétères de l'ischémie-reperfusion observée sur les échantillons frais n'étaient plus détectés après cryopréservation. Ces données nous ont conduits à étudier les fonctions mitochondriales avec cette technique uniquement de façon prospective extemporanée, malgré la rareté des MI.

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Research paper

Cryopreservation with dimethyl sulfoxide prevents accurate analysis of skinned skeletal muscle fibers mitochondrial respiration

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ABSTRACT

Impact of cryopreservation protocols on skeletal muscle mitochondrial respiration remains controversial. We showed that oxygen consumption with main mitochondrial substrates in rat skeletal muscles was higher in fresh samples than in cryopreserved samples and that this difference was not fixed but grow significantly with respiration rates with wide fluctuations around the mean difference. Very close results were observed whatever the muscle type and the substrate used. Importantly, the deleterious effects of ischemia-reperfusion observed on fresh samples vanished when cryopreserved samples were studied. These data demonstrate that this technic should probably be performed only extemporaneously.

1. Introduction

Oxygraphic measurement of mitochondrial respiration in permeabilized muscle fibers is a powerful tool allowing the precise determination of the mitochondrial respiratory chain complexes activities. Using this technique, proposed for the first time by Chance and Williams [1], basal oxygen consumption in state 4 (V_0), and thereafter maximal fiber respiration rates are measured consecutively in the absence and presence of saturating amounts of adenosine diphosphate (ADP). The relative contribution of respiratory chain complexes I, II, III and/or IV to the global mitochondrial respiratory rates can be determined using several substrates. When V_{ADP} is recorded, electron flow travels through complexes I, III and IV. When complex I is blocked with amytal and complex II stimulated with succinate, mitochondrial respiration determines complexes II, III and IV activities (V_{succ}). Upon *N*, *N*, *N*′, *N*′-tetramethyl-*p*-phenylenediaminedihydrochloride (TMPD) and ascorbate additions, complex IV activity is determined as an isolated step of the respiratory chain (V_{TMPD}) [2–5] (Supplemental Fig. 1).

Mitochondrial respiration in skeletal muscle determination is a potent tool for the diagnosis of primary respiratory chain defects in mitochondrial cytopathies [4]. It is also very useful for the assessment of acquired impairment of mitochondrial respiration that may occur in numerous situations, ranging from acute to chronic disease, such as septic shock [5], chronic obstructive pulmonary disease [6] and ischemia-reperfusion [7]. Interestingly, even subtle skeletal muscle ischemia contributes to remote organ injuries, contributing to immediate and to long-term morbidities [8–11]. Skeletal muscle mitochondrial respiration is very sensitive to physiological processes such as low physical activity [12] and aging [13] and oxygraphic measurement of mitochondrial respiration in permeabilized muscle fibers allows highlighting positive effects of therapeutic interventions such as drugs intake [7], diet [13] or exercise programs [12].

To guarantee mitochondrial intactness, mitochondrial respiration is recorded immediately after muscle biopsy, which is an important restriction for its wide use, both in experimental and

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clinical settings. Therefore, cryopreservation methods have been developed that may allow the storage of samples for further analysis, or shipment from a center in which respiration assessment is not available to a center which performs this method.

However, the effect of cryopreservation on mitochondrial respiration of skeletal muscle needs further assessment in view of the conflicting results previously reported. Kuznetsov et al. have proposed a protocol after which, in almost all cases, they recorded no significant decrease of V_{ADP} and V_{succ} in frozen human *vastus intermedius* and rat ventricule or *soleus* [14]. On the other hand, using very similar methods, Larsen et al. observed that cryopreservation did not protect mitochondrial respiration in human *vastus intermedius* and *deltoideus* from a significant drop of V_{ADP} and V_{succ} [15].

Furthermore, there is not published information about the agreement between the results obtained in cryopreserved and fresh samples, according to Bland and Altman analysis [16]. To further address this issue, besides investigating correlation coefficients, we tested the agreement of mitochondrial respiration measurements in fresh and cryopreserved samples on five skeletal muscles characterized by different oxidative capacities, using main substrates that allowed determination of the respiratory chain activity from complex I to IV, from complex II to IV, and of complex IV (Supplemental Fig. 1).

We also determined whether cryopreservation might prevent the diagnosis of mild but significant mitochondrial impairment in a rat model of lower limb ischemia.

Finally, to approach mechanisms potentially involved in cryopreservation deleterious effects, we investigated the specific effect of DMSO on mitochondrial respiration and assessed the integrity of the outer mitochondrial membrane after cryopreservation using cytochrome *c* test.

2. Materiel and methods

2.1. Animals and experimental design

Adult Wistar rats (male, 250–300 g) were used in all experiments. The investigation conformed to the Guide for the Care and Use of laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996).

First, we compared 120 paired results (fresh versus cryopreserved muscles) in six normal animals. Then, we investigated the effects of lower limb ischemia-reperfusion on mitochondrial respiratory chain complexes activities in six rats with experimental aortic cross-clamping and six controls. As described previously, ischemia-reperfusion group had 3 h ischemia induced by aortic clamping and collateral vessel ligation, followed by 2 h of reperfusion while control group underwent only anesthesia and a midline laparotomy [16]. Finally, we assessed the integrity of the outer mitochondrial membrane with cytochrome *c* test in 9 cryopreserved *gastrocnemius* samples and the effect of the cryopreservation solution on mitochondrial respiration by exposition of fresh samples either to ice-cold relaxing solution alone ($n = 9$) or additionally containing 30% DMSO ($n = 9$).

2.2. Preparation of muscle fibers and cryopreservation

Immediately after sacrifice, *rectus femoris* ($n = 6$), *gastrocnemius* ($n = 6$), *soleus* ($n = 6$), *plantaris* ($n = 6$) *and vastus intermedius* ($n = 6$) muscles were removed from animals and were carefully dissected in ice-cold relaxing solution containing CaK_2EGTA (2.77 mM), K_2EGTA (7.23 mM with 100 nM of free Ca^{2+}), $MgCl_2$ (6.56 mM with 1 mM of free Mg^{2+}), imidazole (20 mM), taurine (20 mM), dithiothreitol (0.5 mM), K-methane sulfonate (50 mM), Na_2ATP (5.7 mM) and PCr (15 mM). Dissection of each sample was performed using two pairs of extra sharp forceps. The samples were then split in two sections. One was analyzed immediately for mitochondrial respiration as described below, the other was cryopreserved.

Cryopreservation experiments were performed as described by Kuznetsov et al. [14], Briefly, carefully dissected fibers were immersed in 100 µl of relaxing solution (using 1.8 ml NUNC cryotubes) additionally containing 30% dimethyl sulfoxide (DMSO) concentrations and 10 mg/ml fatty acid-free BSA and equilibrated with cryopreservation solution for 5 s. These fibers were then immediately frozen in liquid nitrogen at uniform freezing rates and kept in −80 °C between a median time of 10 days (range 7–12 days).

For further analysis, tubes were placed in a water bath at 37 °C. When the cryopreservation medium was completely thawed, fibers were immediately transferred and washed in the medium for respiration measurement. Fibers were then permeabilized as described below and used for respiration analysis.

2.3. Muscle mitochondrial respiration measurements and coupling

The relative contribution of the respiratory chain complexes I, II III and IV to the global mitochondrial respiratory rate was studied on saponin-skinned fibers, as described previously [13,17]. Oxygen consumption was measured polarographically with a Clark-type electrode (Strathkelvin Instruments, Glasgow, UK). The chamber volume in the Clark-electrode was 3 ml and stirrer speed was 750 rpm. When maximal fiber respiration (V_{ADP}) was recorded, electron flow went through complexes I, III and IV, because of the presence of glutamate (5 mmol/l) and malate (2 mmol/l). Then, complex I was blocked with amytal (0.02 mmol/l) and complex II was stimulated with succinate (25 mmol/l). Mitochondrial respiration in these conditions determined complex II, III and IV activities (V_{succ}). To explore the complex IV activity, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediaminedihydrochloride (TMPD, 0.5 mmol/l) and ascorbate (0.5 mmol/l) were added as an artificial electron donor to complex IV (V_{TMPD}). Results were expressed in µmol O_2/min/g dry weight.

Mitochondrial coupling was inferred from the ratio (RCR) between state 3 respiratory capacities (V_{ADP}) and basal oxygen consumption in state 4 (V_0).

2.4. Cytochrome c test

To assess the integrity of the outer mitochondrial membrane, 10 µM cytochrome *c* was added during state 3 respiration with succinate in 9 cryopreserved *gastrocnemius* samples.

2.5. Effect of DMSO on muscle mitochondrial respiration

To determine whether DMSO might impair muscle mitochondrial respiration, we submitted dissected fresh *gastrocnemius* samples during 10 min to either the ice-cold relaxing solution alone ($n = 9$) or to the ice-cold relaxing solution additionally containing 30% DMSO ($n = 9$). Then the fibers were transferred and washed in the medium for respiration measurement as described above.

2.6. Statistical analysis

All data are expressed as means ± standard error of mean (except when other indication is provided, i.e. when standard deviation or 95% confidence interval (95% CI) are used). Pearson's tests were used to analyze correlations between the data after checking that the distribution of the values was normal. The Mann Whitney *U* test was performed to compare the results obtained in cryopreserved muscle samples with the one obtained in fresh muscle

samples. Analysis of variance (ANOVA) was performed using Kruskall Wallis test to determine whether mitochondrial respiration was differently affected by the five muscles and the four substrates used. Post hoc testing was performed using Dunn's multiple comparison tests. Values of $P < 0.05$ were considered significant.

To compare the mitochondrial respiration measurements and evaluate whether there was agreement or bias, we used the method of Bland and Altman [18], where the difference between each set of determination ($V_{cryopreserved} - V_{fresh}$) is plotted against the mean of the two measurements ($V_{cryopreserved} + V_{fresh}/2$). Limits of agreement were defined as mean ($V_{cryopreserved} - V_{fresh}$) ± (1.96 standard deviation (SD)).

Statistical analyses were performed using Prism data base (GraphPad Prism 5, Graph Pad Software, Inc., San Diego, CA, USA).

3. Results

3.1. Oxygen consumption of cryopreserved muscle samples significantly correlated with oxygen consumption of fresh muscle samples

When all paired results were considered together regardless the muscle type and the substrate ($n = 120$), oxygen consumption in the cryopreserved muscle samples significantly correlated with oxygen consumption in the fresh muscle samples ($r = 0.91$ [95%CI: 0.88, 0.94], $p < 0.0001$) (Fig. 1).

Correlations between measurements in cryopreserved and fresh samples were also found when muscle types were considered separately, ranging from $r = 0.89$ [95% CI: 0.76, 0.95] in *gastrocnemius* ($p < 0.0001$), $r = 0.90$ [95% CI: 0.79–0.96] in *plantaris* ($p < 0.0001$), $r = 0.92$ [95% CI: 0.83–0.97] in *vastus intermedius* and *rectus femoris* ($p < 0.0001$) to $r = 0.98$ [95% CI: 0.94, 0.99] in *soleus* ($p < 0.0001$).

When respiration results were analyzed according to each substrate, significant correlations were maintained in presence of saturating amount of ADP, ranging from $r = 0.79$ [95% CI: 0.60, 0.90] for V_{TMPD} ($p < 0.0001$), $r = 0.80$ [95% CI: 0.62–0.90] for V_{succ} ($p < 0.0001$) to $r = 0.83$ [95% CI: 0.66, 0.91] for V_{ADP} ($p < 0.0001$). However, V_0 in cryopreserved muscles did not correlate with V_0 in fresh muscles ($r = 0.28$ [95% CI: −0.10, 0.59], $p = 0.14$).

3.2. Cryopreservation decreased mitochondrial respiration rates

Oxygen consumption in cryopreserved samples was significantly decreased compared to fresh sample in all muscles, regardless the substrate (Fig. 2). The decrease ranged from −0.86 ± 0.30 μmol/min/g ($V_{0\ fresh}$ vs $V_{0\ cryopreserved}$ in the *soleus*, $p < 0.01$), to −6.91 ± 0.34 μmol/min/g ($V_{TMPD\ fresh}$ vs $V_{TMPD\ cryopreserved}$ in the *vastus intermedius*, $p < 0.01$). Expressed as percentage of the absolute value in the fresh sample, decrease of oxygen consumption ranged from −14.83 ± 2.36% ($V_{TMPD\ fresh}$ vs $V_{TMPD\ cryopreserved}$ in the *soleus*, $p < 0.05$) to −61.00 ± 7.78% ($V_{0\ fresh}$ vs $V_{0\ cryopreserved}$ in the *vastus intermedius*, $p < 0.05$).

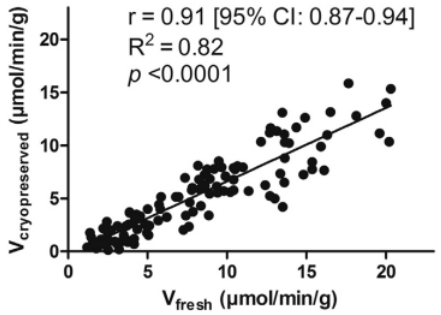

Fig. 1. Oxygen consumptions in cryopreserved and in fresh skeletal muscle samples are correlated. Oxygen consumption with four different substrates in fresh plotted versus paired cryopreserved samples of five different muscles from six Wistar rats (all substrates, all muscles, 120 paired results).

An ANOVA analysis demonstrated that the magnitude of the diminution in oxygen consumption after cryopreservation did differ depending of muscle type (Table 1) and was generally higher in *vastus intermedius* and *plantaris,* which were the muscles with the highest oxygen consumption. Some of these differences vanished when the drop of oxygen consumption after cryopreservation was normalized by oxygen consumption of the fresh sample.

When the muscle samples were pooled to analyze the effect of cryopreservation according to the substrates, a drop of oxygen consumption was found whatever the substrate, ranging from −1.61 ± 0.20 μmol/min/g (−47.67%) for $V_{0\ fresh}$ versus $V_{0\ cryopreserved}$ ($p < 0.001$), to −3.40 ± 0.46 μmol/min/g (−23.17%) for $V_{TMPD\ fresh}$ versus $V_{TMPD\ cryopreserved}$ ($p < 0.001$). The drop was significantly lower for V_0 compared to V_{ADP} (−3.40 ± 0.40 μmol/min/g, $p < 0.01$) and V_{TMPD} ($p < 0.05$) (Fig. 3F). However, these differences vanished when the drop of oxygen consumption after cryopreservation was normalized by oxygen consumption of the fresh sample. Taken as a whole, these results suggest that the higher the oxygen consumption capacity of the muscle, the higher the difference between fresh and cryopreserved samples.

Mitochondrial coupling, as inferred from the RCR, increased significantly ($p < 0.05$) in two muscles, the *rectus femoris* (+2.75 ± 1.39 μmol/min/g) and the *vastus intermedius* (+1.406 ± 0.29 μmol/min/g) (Fig. 3).

3.3. Cryopreservation disrupted the outer mitochondrial membrane

After cryopreservation, state 3 respiratory capacities with cytochrome *c* was significantly increased (+106 ± 31.69%, $p < 0.05$) as compared to corresponding controls. This supports that outer mitochondrial membrane was not entirely preserved in cryopreserved samples (Fig. 3).

3.4. DMSO exposition decreased V_0 and V_{ADP}

We observed a significant reduction in V_0 (2.59 ± 0.15 vs 1.78 ± 0.23, $p < 0.05$) and V_{ADP} (7.78 ± 0.35 vs 6.19 ± 0.51, $p < 0.05$) in fresh muscles samples with ice-cold relaxing solution containing 30% DMSO as compared to fresh muscles samples with ice-cold relaxing only (Fig. 4). RCR tended to increase although this was not significant.

3.5. Bland and Altman analysis demonstrated negative bias, growing with magnitude of the measurements and large limits of agreement

To get more insight into the effect of cryopreservation on mitochondrial analysis, we compared the results obtained in cryopreserved and fresh muscle samples using the Bland and Altman method (Fig. 5 and Table 2).

Considering oxygen consumption in all paired results ($n = 120$, Fig. 4), the mean of the differences between cryopreserved and fresh samples was −2.79 μmol/min/g [95% CI: −3.18, +2.39 μmol/min/g], with wide limits of agreement between the two methods (−7.13, +1.56 μmol/min/g). Expressed in relative value, this mean difference between cryopreserved and fresh reached −51.45% [95% CI: −21.30, +4.15%] of the respiration rate, with (−127.78, +24.88%) limits of agreement. The higher the respiration rate, the higher the difference between cryopreserved and fresh respiration values

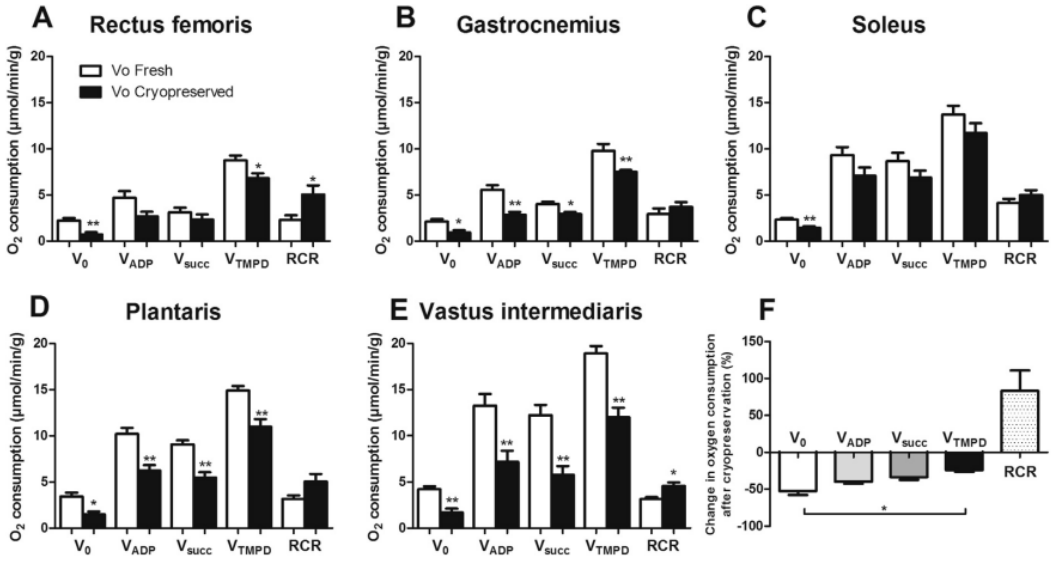

Fig. 2. Cryopreservation decreases oxygen consumption. V_0, V_{ADP}, V_{succ} V_{TMD} and RCR in fresh and cryopreserved *rectus femoris* ($n = 6$) (A), *gastrocnemius* ($n = 6$) (B), *soleus* ($n = 6$) (C), *plantaris* ($n = 6$) (D) and *vastus intermedius* ($n = 6$) (E) samples from Wistar rats. Mean decrease in V_0, V_{ADP}, V_{succ} and V_{TMD} (difference between results in cryopreserved minus results in fresh samples) when pooling results obtained in *rectus femoris*, *gastrocnemius*, *soleus*, *plantaris* and *vastus intermedius* (30 paired results for each substrate) (F). *: $p < 0.05$, **: $p < 0.01$.

($r = -0.54$ [95% CI: −0.66, −0.40], $p < 0.0001$). In addition, the scatter of the differences increased as the respiration rate increased. Of note, we do not find correlation between storage duration of cryopreserved samples and difference between respiration in fresh and cryopreserved samples.

Analyzing results according to either the substrate or the muscle provided similar results (Table 2). The mean differences between cryopreserved and fresh respiration results ranged from −1.55 [95% CI: −1.99, −1.11] (*rectus femoris*) to −5.49 µmol/min/g [95% CI: −6.46, −4.52] (*vastus intermedius*) and limits of agreements from (−3.76, +0.34) (*soleus*) to (−10.23, −0.75 µmol/min/g) (*vastus intermedius*). In most series, the differences between fresh and cryopreserved respiration results correlated with the respiration rates.

3.6. Cryopreservation prevented the detection of mitochondrial respiration defect induced by lower limb ischemia-reperfusion

To investigate whether cryopreservation might have consequences on the diagnosis of abnormal mitochondrial respiration, we used a rat model of lower limb ischemia characterized by lowered mitochondrial respiration. When studied on fresh samples V_{ADP} was significantly decreased in ischemic *plantaris* of the rats compared to control animals (9.63 ± 0.46 vs 8.04 ± 0.24 µmol/min/g, $p < 0.05$) (Fig. 6). This difference vanished when cryopreserved *plantaris* were studied. Indeed, after cryopreservation, V_{ADP} of *plantaris* was 6.82 ± 0.84 µmol/min/g in rats with chronic ischemia of inferior limb and 6.73 ± 0.59 µmol/min/g in controls ($p = 0.82$).

Table 1
Cryopreservation decreases oxygen consumption. Mean decrease in V_0, V_{ADP}, V_{succ} and V_{TMD} after cryopreservation (difference between result in fresh minus result in cryopreserved sample) in *rectus femoris* ($n = 6$), *gastrocnemius* ($n = 6$), *soleus* ($n = 6$), *plantaris* ($n = 6$) and *vastus intermedius* ($n = 6$).

	Rectus femoris	*Gastrocnemius*	*Soleus*	*Plantaris*	*Vastus intermedius*	ANOVA *p*
Decrease in V_0						
Absolute value (µmol/min/g)	−1.50 ± 0.50	−1.19 ± 0.24	−0.86 ± 0.30	−1.96 ± 0.57	−2.50 ± 0.31	**<0.05**
Relative value (%)	−57.17 ± 19.02	−57.00 ± 7.84	−33.83 ± 10.39	−52.50 ± 14.21	−61.00 ± 7.78	0.31
Decrease in V_{ADP}						
Absolute value (µmol/min/g)	−2.00 ± 0.52	−2.70 ± 0.56	−2.22 ± 0.50	−3.96 ± 0.72	−6.10 ± 2.16**	**<0.005**
Relative value (%)	−42.50 ± 6.98	−46.83 ± 5.93	−24.00 ± 4.67	−38.50 ± 5.88	−47.00 ± 6.16	0.074
Decrease in V_{succ}						
Absolute value (µmol/min/g)	−0.79 ± 0.33	−1.10 ± 0.31	−1.78 ± 0.47	−3.59 ± 0.46	−6.44 ± 0.76	**<0.001**
Relative value (%)	−28.17 ± 33.78	−26.33 ± 5.92	−19.83 ± 4.36	−40.00 ± 5.18	−53.50 ± 5.49	**<0.05**
Decrease in V_{TMPD}						
Absolute value (µmol/min/g)	−1.91 ± 0.34	−2.27 ± 0.84	−1.96 ± 0.25	−3.94 ± 1.039	−6.91 ± 0.34	**<0.01**
Relative value (%)	−21.67 ± 3.40	−21.00 ± 6.16	−14.83 ± 2.36	−25.83 ± 6.71	−36.70 ± 4.19	**<0.05**
Change in RCR						
Absolute value (µmol/min/g)	+0.77 ± 0.62	+0.87 ± 0.81	+1.86 ± 0.85	+2.75 ± 1.39	+1.41 ± 0.29	0.73
Relative value (%)	+42.50 ± 29.23	+31.83 ± 26.71	+65.50 ± 29.93	+231.7 ± 118.4	+45.00 ± 9.46	0.62

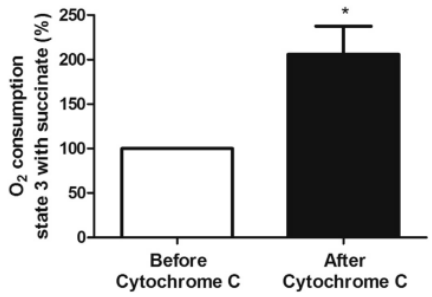

Fig. 3. Cryopreservation does not protect entirely mitochondrial outer membrane. Oxygen consumption in cryopreserved *gasctrocnemius* samples ($n = 9$) during state 3 respiration with succinate before and after adding 10 μM cytochrome *c*. *: $p < 0.05$.

4. Discussion

The main result of this study is to demonstrate that, although mitochondrial respiration of fresh samples strongly correlates to that of cryopreserved muscle samples, cryopreservation cannot generally be used instead of fresh samples when analyzing mitochondrial respiratory chain complexes I, II, III and/or IV activities. Indeed, respiration was systematically lower in cryopreserved samples, with fluctuations around the mean difference that exceeded the usually acceptable limits of agreements [19]. Accordingly, the deleterious effects of ischemia-reperfusion on mitochondrial respiration observed on fresh samples vanished when cryopreserved samples were studied.

Previous data concerning reliability of mitochondrial respiration analysis on muscle samples cryopreserved with DMSO are spare and controversial. Kuznetsov et al. have compared respiration in fresh versus frozen muscles samples with two substrates (V_{ADP} and V_{succ}) and using 3 different muscles (human *vastus intermedius*, rat ventricule and rat *soleus*). Larsen et al. have assessed respiration with the same substrates, in two human muscles (*vastus intermedius* and *deltoideus*). Statistical analysis compared the means in fresh and cryopreserved samples from each group supporting a need for further evaluations.

In this study, the use of four mitochondrial substrates and five skeletal muscles characterized by different oxidative capacities allowed a wide assessment of the cryopreservation effects on mitochondrial chain functioning in skeletal muscle. The sample size reached 120 paired results when all data were pooled, providing a good statistical power.

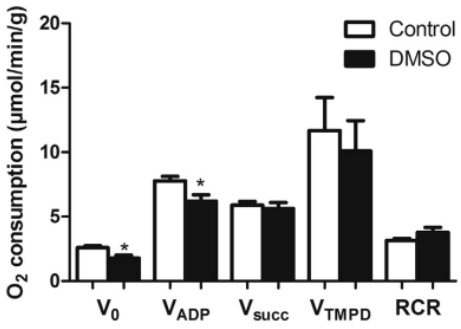

Fig. 4. DMSO exposition decreased V_0 and V_{ADP}. V_0, V_{ADP}, V_{succ} V_{TMD} and RCR in fresh *gastrocnemius* washed with ice-cold relaxing solution alone or additionally containing 30% DMSO ($n = 9$). *: $p < 0.05$.

The linear correlation of 0.91 between the two analysis for all the samples might implies that they are a good surrogate and therefore that they might be useful in certain context but, importantly, respiration was systematically lower in cryopreserved as compared to fresh samples strongly suggesting a deleterious effect of cryopreservation. On the other hand, the RCR increased in *rectus femoris* and *vastus intermedius*. Although the RCR was in the range reported in the literature [20–22] we cannot totally exclude that state 4 rates might have been overerestimated since it is strongly influenced by almost every functional aspect of oxidative phosphorylation [23]. However, bioenergetics dysfunction can alter V_{ADP} without affecting RCR [24] and the significant increase in RCR observed in two muscles in our study should probably not be viewed as a lack of cryopreservation deleterious effect. Rather a specific effect of DMSO might be involved since RCR tended to increase in fresh muscles exposed to DMSO. Thus, DMSO might decrease oxidative respiration without impairing mitochondrial coupling.

Further, extending previous work, we used the Bland and Altman test, where the differences between the two measurements for each paired samples are plotted against the mean of the two measurements, allowing a simple and pertinent assessment of agreement between two measurement methods: i) The mean difference evaluates the bias between the two measurements. ii) The limits of agreement (±1.96 SD of the differences) assess the random fluctuations around this mean. If the differences within limits of agreement are not important, the two methods may be used interchangeably. iii) Finally, the bias may be fixed or may show relationship with the measurements. If there is a fixed bias, it can be adjusted for by subtracting the mean difference from the new method. This is not possible when the methods do not agree equally through the range of measurements. Using the protocol of cryopreservation described by Kuznetsov et al., we found good correlation between fresh and cryopreserved muscle samples. However, correlation does not mean agreement and Bland Altman analysis clearly showed that results obtained in cryopreserved samples were lower than in fresh samples with fluctuations around the mean difference that exceeded the usually acceptable limits of agreements [19]. Moreover, the differences between respiration in cryopreserved and fresh samples was not fixed but growth with increasing respiration rates. Very close results were observed whatever the muscle type and whatever the substrate used.

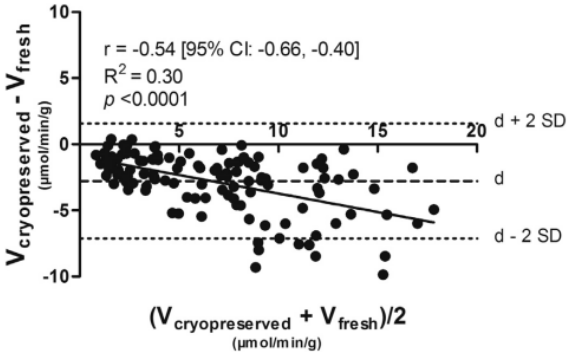

Fig. 5. Cryopreservation introduces negative bias, growing with magnitude of the measurements and large limits of agreement. Graphic presentation of the difference between the two measurements ($V_{cryopreserved} - V_{fresh}$) versus the mean of the two measurements ($V_{cryopreserved} + V_{fresh}/2$), according to Bland and Altman method (1986) (all substrates, all muscles, 120 paired results). Horizontal lines indicate the mean of difference or bias (d) and limits of agreement (d − 2 SD and d + 2SD).

Table 2
Cryopreservation introduces negative bias, growing with magnitude of the measurements and large limits of agreement regardless muscle type and substrates. Bland and Altman's analysis of agreement between oxygen consumption measured in fresh and cryopreserved samples, according to each substrate and each muscle.

	Difference ($V_{cryopreserved} - V_{fresh}$) (μmol/min/g)	Limit of agreements (bias ± 1.96SD) (μmol/min/g)	Correlation (Pearson's coefficient) between difference and average of the respiration measurements ($V_{cryopreserved} + V_{fresh}/2$) (μmol/min/g)
V_0	−1.61 [95% CI: −1.96, −1.25]	(−3.74, +0.53)	−0.36 [95% CI: −0.64, −0.0015], $p < 0.05$
V_{ADP}	−3.40 [95% CI: −4.16, −2.63]	(−7.59, +0.79)	−0.51 [95% CI:- 0.73, −0.18], $p < 0.005$
V_{succ}	−2.74 [95% CI: −3.60, −1.88]	(−7.44, +1.97)	−0.68 [95% CI: −0.82, −0.38], $p < 0.0001$
V_{TMPD}	−3.34 [95% CI: −4.31, −2.49]	(−8.37, +1.57)	−0.51 [95% CI: −0.73, −0.18], $p < 0.05$
Rectusfemoris	−1.55 [95% CI: −1.99, −1.11]	(−3.70, +0.60)	−0.26 [95% CI: −0.60, +0.16], $p = 0.21$
Gastrocnemius	−1.82 [95% CI: −2.39, −1.25]	(−4.60, +0.97)	−0.41 [95% CI: −0.70, −0.0028], $p < 0.05$
Soleus	−1.71 [95% CI: −2.13, −1.29]	(-3.76, +0.34)	−0.39 [95% CI: −0.69, +0.0028], $p = 0.057$
Plantaris	−3.36 [95% CI: −4.11, −2.62]	(−7.02, +0.29)	−0.33 [95% CI: −0.65, +0.081], $p = 0.11$
Vastus intermedius	−5.49 [95% CI: −6.46, −4.52]	(−10.23, +0.75)	−0.60 [95% CI: −0.81, −0.27], $p < 0.005$

Investigating an eventual pathophysiological consequence of such result, we observed that cryopreservation prevented the detection of the decrease of V_{ADP} in the muscles of rats submitted to ischemia-reperfusion. Taken as a whole, our results prevent the use of cryopreserved in place of fresh samples even if further studies will be useful to discriminate whether this finding is due to the reduction in the magnitude of the measurement, which would requires larger samples to attain statistically significant differences, or that cryopreservation may change the natural history of the damage progress induced by ischemia-reperfusion.

How does cryopreservation impact mitochondrial respiration? Several mechanisms deserve to be discussed.

Storage of the samples is not likely to play a role because time between cryopreservation and analyze of the samples was short in this study and we did not find correlation between storage duration and difference of respiration between fresh and cryopreserved samples.

DMSO may have a toxic effect on mitochondrial complexes. To minimize this potential effect, fibers were transferred to the respiration medium immediately on thawing, as it was done in previous studies [14,15]. However, we cannot totally eliminate a deleterious effect of DMSO on our fibers and accordingly, we observed that short duration exposure to DMSO significantly mildly reduced muscle mitochondrial respiration. This is consistent with previous data demonstrating that there is a significant reduction in activity in fresh mitochondria washed with DMSO compared to freshly isolated mitochondria of the brain Ref. [25].

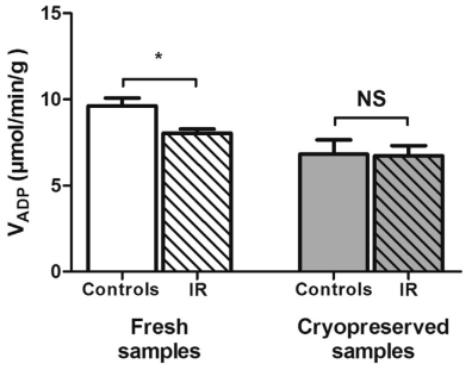

Fig. 6. Cryopreservation prevents the detection of mitochondrial respiration defect induced by lower limb ischemia-reperfusion. Comparison of the analysis of V_{max} in *plantaris* of rats with lower limb ischemia-reperfusion (IR) and theirs controls when performed on fresh or cryopreserved samples ($n = 6$). *: $p < 0.05$.

Freezing may also disrupt inner mitochondrial membrane. In Kusnetsov et al. found a trend toward an increase in oxygen consumption after cytochrome *c* addition in cryopreserved samples [14] while Larsen et al. found this increase to be significant [15]. In our cryopreserved samples, cytochrome *c* addition enhanced significantly mitochondrial respiration, supporting that the present protocol of cryopreservation does not perfectly preserve outer mitochondrial membrane in muscle samples.

5. Conclusion

This study demonstrates that available cryopreservation protocols of skeletal muscle samples do impact mitochondrial respiration analysis likely in relation with DMSO and outer mitochondrial membrane damage. This might have important implications for both physiological research and clinical investigations. Developments of new methods of cryopreservation allowing reliable analyze of mitochondrial respiration on this tissue are still needed.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2014.01.014.

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ARTICLE 4 et ARTICLE 5 : au cours des MI, les RLO sont au carrefour des mécanismes immuns et non immuns. Les RLO induits par l'IFN-β et les dégâts mitochondriaux contribuent à la faiblesse musculaire et à l'entretien de l'inflammation au cours de la DM

Dans ces articles mécanistiques, nous avons concentré nos efforts sur les DM qui sont définies par une histopathologie musculaire particulière associée à une signature interféron (IFN) de type I (dans le sang et le muscle) dont l'origine et la conséquence sont inconnues.

Dans le muscle des patients atteints de DM récentes, non traitées, le premier groupe fonctionnel de gènes dont l'expression était diminuée était relié à la mitochondrie. Les études histochimiques, de microscopie électronique et l'oxygraphie *in situ* ont mis en évidence des dysfonctions mitochondriales musculaires, avec une augmentation de la production de radicaux libres dérivés de l'oxygène (RLO), une diminution de la respiration mitochondriale corrélée aux faibles capacités d'exercice et à la signature d'IFN de type I. Dans des myotubes humains, la production de RLO était induite par l'IFN-β et contribuait aux dysfonctionnements mitochondriaux. De façon importante, dans un modèle de souris, un inhibiteur des RLO (la N-acétylcystéine) réduisait les dysfonctionnements mitochondriaux musculaires, les taux des transcrits musculaires stimulés par IFN de type I, l'infiltrat de cellules inflammatoires et la faiblesse musculaire.

Ces données mettent en évidence un rôle central des mitochondries et des RLO dans les DM. Les dysfonctionnements mitochondriaux, médiés par les RLO induits par l'IFN-β, contribuent à la diminution des capacités d'exercice. De plus, les dysfonctionnements mitochondriaux augmentent la production de RLO qui entraîne l'expression des gènes induits par l'IFN de type I et l'inflammation musculaire, ce qui peut auto-entretenir la maladie.

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In the idiopathic inflammatory myopathies, reactive oxygen species are at the crossroad between immune and non-immune cell-mediated mechanisms

Dear editor,

We read with great interest the article by Lightfoot *et al*[1] who suggested that in the idiopathic inflammatory myopathies (IIM), reactive oxygen species (ROS) contribute to muscle weakness. The authors opposed immune and non-immune cell-mediated mechanisms in IIM and reviewed ROS as part of the latter. Here, we would like to point out recent data that reconcile this dichotomy and stress the interplay between ROS and immune cell-mediated processes during IIM.

In accordance with the authors' hypothesis, we recently observed high ROS formation, along with mitochondrial respiratory chain dysfunctions, in muscle of patients with dermatomyositis.[2] Furthermore, in muscle cells cultured in the absence of immune cells, interferon-β-induced mitochondrial dysfunctions in a ROS-dependent manner. Finally, in an antigen-induced mouse model of IIM, ROS scavenging with N-acetyl cysteine prevented muscle weakness and mitochondrial dysfunctions and immunize cell infiltrate in muscle.[2]

Thus, ROS and immune cells are not independent actors in IIM. Indeed, ROS participate in immune cell infiltrate, which in turn can further increase ROS formation and mediated damages in the muscle.[3] This definitely places ROS as central actors and potential therapeutic targets in IIM.

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ORIGINAL PAPER

IFN-β-induced reactive oxygen species and mitochondrial damage contribute to muscle impairment and inflammation maintenance in dermatomyositis

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Abstract Dermatomyositis (DM) is an autoimmune disease associated with enhanced type I interferon (IFN) signalling in skeletal muscle, but the mechanisms underlying muscle dysfunction and inflammation perpetuation remain unknown. Transcriptomic analysis of early untreated DM muscles revealed that the main cluster of down-regulated genes was mitochondria-related. Histochemical, electron microscopy, and in situ oxygraphy analysis showed mitochondrial abnormalities, including increased reactive oxygen species (ROS) production and decreased respiration, which was correlated with low exercise capacities and a type I IFN signature. Moreover, IFN-β induced ROS production in human myotubes was found to contribute to mitochondrial malfunctions. Importantly, the ROS scavenger *N*-acetyl cysteine (NAC) prevented mitochondrial dysfunctions, type I IFN-stimulated transcript levels, inflammatory cell infiltrate, and muscle weakness in an experimental autoimmune myositis mouse model. Thus, these data highlight a central role of mitochondria and ROS in DM. Mitochondrial dysfunctions, mediated by IFN-β induced-ROS, contribute to poor exercise capacity. In addition, mitochondrial dysfunctions increase ROS production that drive type I IFN-inducible gene expression and muscle inflammation, and may thus self-sustain the disease. Given that current DM treatments only induce partial recovery and expose to serious adverse events (including muscular

Alain Meyer, Gilles Laverny, Daniel Metzger, Jean Sibilia, and Bernard Geny have contributed equally to this work.

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toxicity), protecting mitochondria from dysfunctions may open new therapeutic avenues for DM.

Introduction

DM is an autoimmune muscle disease defined by a characteristic myopathological pattern, including perimysial and perivascular inflammatory infiltrates along with changes in perifascicular myofibres and capillary injury [12, 22]. Several DM subtypes might exist. Approximately 20% of adults with DM are paraneoplastic, although the aetiology of the remaining is unknown. DM patients experience muscle weakness and low aerobic capacities [3, 48], which are associated with poor health status [2] and increased mortality [30]. The origin of this muscle impairment remains unknown. The severity of the inflammatory infiltrate in DM muscle is poorly correlated with muscle weakness [14], and although DM is an autoimmune disorder, adaptive immunity (notably auto-antibodies) does not appear to be directly involved in muscle alterations [12]. Moreover, while IFN-β, a major regulator of innate immunity, has been shown to play a pivotal role in both initiation [38] and maintenance [25, 40] of the disease, its implication in muscle dysfunction remains to be determined. Current therapies are based on empirical use of corticosteroids and immunosuppressive agents. However, they induce only partial recovery [2], expose to many adverse effects, and disease relapses are frequent [29]. Hence, there is a need to improve the current understanding and treatment of DM.

Methods

Patients, sera, and muscle biopsies

Fifteen consecutive early untreated DM patients, 14 patients with non-DM inflammatory myopathies (non-DM IM), and 16 gender- and age-matched sedentary controls were prospectively included according to the Helsinki declaration. Written informed consent was obtained from all participants. Control subjects suffered from myalgia, but presented no evidence of neuromuscular disease (no muscle weakness, normal neurological examination, normal blood creatine kinase level, normal electromyographic recording, and normal muscle biopsy). Diagnosis of DM and non-DM IM (polymyositis, inclusion body myositis, necrotizing autoimmune myopathy, and non-specific myositis) was based according to European Neuromuscular Centre criteria [22]. Patients with associated cancer and/or symptomatic lung disease were excluded from the study. Deltoid muscle biopsies were performed during the diagnostic procedure. Sera were harvested at the time of muscle biopsy and stored at −80 °C. The delay between inclusion and disease onset was ≤6 months, and the patients were not treated by immunomodulatory drugs at inclusion.

Muscle optical and electron microscopy studies

Haematoxylin & Eosin, NADH-tetrazolium reductase, SDH, COX, and Gomori trichrome staining, as well as ultrastructural analyses were performed on deltoid muscle biopsies as described [15]. COX staining, performed separately from SDH staining, was applied over night. Necrosis was defined by pale and/or hyalinised staining on Haematoxylin & Eosin and Gomori trichrome staining. Regenerating fibres were identified by increased basophilia. Atrophy was determined by fibre cross-sectional area on merosin laminin Alpha 2 Chain (clone MER3/22B2, Leica, that reacts with the 300 kD fragment of merosin) stained biopsies as described [16]. Cellular inflammation was quantified by the number of cells and/or clusters (10 cells or more) in a 20 × field, according to an international consensus: <4, score 0; >4 and/or 1 cluster, score 1; >20 and cluster >2, score 2 [45]. For each patient, 400–2000 deltoid fibres were analysed in optical studies. Twenty perifascicular deltoid fibres were analysed in electron microscopy study.

Serum cytokine level measurements

Serum cytokine levels were measured by ELISA kits purchased from PBL (IFN-β) and R&D systems (IL-6 and TNF-α) according to the manufacturer's instructions. Healthy subjects were used as controls.

Aerobic capacity measurements

DM patients who were able to cycle performed a cycloergometric incremental symptom-limited maximal exercise test, while measuring O_2 by means of an open circuit metabolic cart (Sensor Medics Vmax229, Yorba Linda). Peak VO_2 was considered as the VO_{2max} as described previously [50].

Experimental autoimmune myositis mice and *N*-acetyl cysteine treatment

Experimental procedures were approved by the local animal ethics committee *(CREMIA Strasbourg AL/71/78/02/13)*.

Experimental autoimmune myositis (EAM) mice were generated as previously described [5]. Briefly, 12-week-old female BALB/c mice (Charles River Laboratories) were immunised three times, at 1-week intervals, with 100-µl phosphate-buffered saline containing 1-mg myosin or vehicle emulsified with an equal volume of complete Freund's

adjuvant (CFA, Sigma-Aldrich). These preparations were injected on bilateral sides of the hind foot pads (first immunisation) and the tail base (second and third immunisations). Pertussis toxin (Sigma-Aldrich) was injected intraperitoneally (500 ng in 200-µl saline) during the first immunisation. The ROS scavenger N-acetyl cysteine [NAC (Sigma-Aldrich), 300 mg/kg/day) [31] was added in the drinking water, starting the day before the first injection. Two weeks after the last immunisation, animals were sacrificed by cervical dislocation. Gastrocnemius, soleus, as well as tibialis muscles were harvested.

Grip strength in mice

The day prior sacrifice, a grip strength meter (Bioseb, France) was used to measure limb grip forces [16]. The test was repeated three consecutive times within the same session, and the mean value was recorded as the maximal grip strength for each mouse.

Cell culture

Human LHCN-M2 myoblasts (gift from J. Laporte) were grown in Dulbecco's modified Eagle's medium (DMEM Low glucose, Milerium, VWR International) supplemented with 20% foetal calf serum and 1% antibiotics (100-U/ml penicillin and 100 µg/ml streptomycin; Gibco) at 37 °C under a humidified 5% CO_2 atmosphere. Differentiation was initiated at 80% confluence by replacing the growth medium with DMEM supplemented with 2% horse serum. At 5 days of differentiation, the myotubes were treated with IFN-β (PBL Assay Science, 100 UI/mL), TNF-α (R&D systems, 10 ng/ml), IL-6 (R&D systems, 100 ng/ml), and NAC (Sigma-Aldrich, 1 mM) for 72 h [37].

RNA extraction and RT-qPCR analysis

Total RNA was isolated from human biopsies and EAM tibialis using TRIzol reagent (Invitrogen). cDNA was synthesised from 2 µg of RNA by reverse transcription using random primers and SuperScript II reverse transcriptase (Invitrogen, Life Technologies), according to the manufacturer's protocol. Quantitative RT-PCR analysis was performed with gene-specific primers using the QuantiTectTM SYBR \Green PCR kit (Roche), according to the manufacturer's protocol. For each sample, the relative abundance of the transcript level of a given gene was calculated by normalisation to a housekeeping gene (human RPLP0 or mouse 18S). The results represent the mean of the relative abundance in each group [16]. The primer sequences are given in Supplementary Table 1 and 2.

Microarray analysis

Biotinylated single strand cDNA targets were prepared, starting from 150 ng of total RNA of deltoid muscle, using the Ambion WT Expression Kit (Cat # 4411974) and the Affymetrix GeneChip® WT Terminal Labeling Kit (Cat # 900671) according to Affymetrix recommendations. Following fragmentation and end-labelling, 3 µg of cDNAs were hybridised for 16 h at 45 °C on GeneChip® Human Gene 2.0 ST arrays (Affymetrix), interrogating over 400000 RefSeq transcripts and ~11000 LncRNAs represented by approximately 27 probes spread across the full length of the transcript. Chips were washed and stained in the GeneChip® Fluidics Station 450 (Affymetrix) and scanned with the GeneChip® Scanner 3000 7G (Affymetrix) at a resolution of 0.7 µm. Raw data (CEL Intensity files) were extracted from the scanned images using the Affymetrix GeneChip® Command Console (AGCC) version 4.0. CEL files were further processed with the Affymetrix Expression Console software version 1.3.1 to calculate probe set signal intensities using Robust Multi-array Average (RMA) algorithms with default settings. The fold change rank ordering statistics (FCROS) method was used to select statistical differentially expressed genes as described [13]. Briefly, the FCROS method proceeds as follows: using pair of samples from two biological conditions, fold changes (FC) are computed for all the genes. These FCs are sorted in increasing order and their ranks are associated with genes. This is repeated with all the pair of samples in the data set. An average rank is computed and used as statistic leading to associate probability values with genes. Threshold values used to select the significant up- and down-regulated genes were set to 0.975 and 0.025, respectively. The genes with FC level values >1.2 and <0.8 were considered. Data sets, available on GEO data sets, were uploaded on the Database for Annotation, Visualization and Integrated Discovery (DAVID) for gene ontology analyses.

Dihydroethidium staining

Ten-µm muscle cryosections were incubated for 30 min at 37 °C with 2.5-µM dihydroethidium (DHE) in phosphate-buffered saline, as described [11]. DHE produces a red fluorescence when oxidised to ethidium bromide by ROS. After staining, the sections were rinsed, air-dried, mounted in Vectashield (Vector Laboratories, Burlingame, CA), and slides examined under a fluorescence microscope. The emission signal was recorded using a Zeiss 573–637 nm filter.

Muscle sample preparation for mitochondrial respiration and ROS production recording

For functional analysis (mitochondrial respiration recording, H_2O_2 measurement, and detection of ROS unpaired electrons), immediately after patient biopsy and animal muscle harvesting, a portion of muscle tissue was inspected under a stereomicroscope microscope (Zeiss, Germany) at magnification ×6.5 and remaining conjunctive tissue (fascia, tendon, fat) was removed if present. After functional analysis, the samples were dried for 15 min at 150 °C.

Mitochondrial respiration recording

Mitochondrial oxygen consumption was analysed in situ in saponin-skinned fibres and saponin-permeabilised cells using a Clark electrode (Strathkelvin Instruments) in an oxygraphic cell (3 ml) at 22 °C with continuous stirring as described [23]. After addition of fibres or cells, basal oxygen consumption (V_0) due to proton leak in the presence of glutamate (5 mmol/L) and malate (2 mmol/L) was measured. Maximal fibre respiration (V_{max}) was recorded after addition of saturating amounts of ADP (2 mmol/L) leading to electron flow through complexes I, III, and IV and oxidative phosphorylation of ADP. To explore complex IV activity, *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediaminedihydrochloride (TMPD, 0.5 mmol/l) and ascorbate (0.5 mmol/l) were added as artificial electron donors to complex IV (V_{TMPD}).

H_2O_2 measurement

H_2O_2 production in saponin-skinned muscle fibres was determined with 5 mmol/L Amplex Red (Invitrogen), which reacts with H_2O_2 in a 1:1 stoichiometry catalysed by horseradish peroxidase (HRP; 0.5 U/ml, Invitrogen) to yield the fluorescent compound resorufin and a molar equivalent of O_2. Resorufin has excitation and emission wavelengths of 563 and 587 nm, respectively, and is very stable once formed. Fluorescence was measured continuously with a Fluoromax 3 (Jobin–Yvon) spectrofluorometer at 37 °C and magnetic stirring at baseline (reactants only) and after adding, in order, a permeabilised fibre bundle (8–10 mg), 10 mmol/L glutamate, 2 mmol/L malate, 5 mmol/L succinate, and 2 mmol/L ADP as substrates. Results are reported in pmol/min/mg dry weight.

Detection of ROS unpaired electrons

Electron Paramagnetic Resonance (EPR) was performed as described [7]. Minced muscle samples were washed twice with Krebs HEPES Buffer containing 25-µmol/L deferoxamine and 5-µmol/L diethyldithiocarbamate (DETC) to minimise CMH auto-oxidation. Samples were then incubated in a plate at 37 °C with the spin probe CMH (200 µM) for 30 min under 20 mmHg of oxygen partial pressure (mimicking physiological conditions) using a Gas-Controller (Noxygen Sciences Transfer). The reaction was stopped by placing the plate on ice. Supernatant (40 µl) was injected in a disposable capillary tube and placed in an E-SCAN benchtop EPR spectrometer (Bruker) at 15 °C. Detection of ROS unpaired electrons was conducted under the following EPR settings: centre field 3461.144 g, microwave power 21.85 mW, modulation amplitude 2.40 g, sweep time 5.24 s (10 scans), sweep width 60 g, and number of lag curve points 1. The amplitude of the signal was measured, and the concentration of CM radicals was determined by calibration with standard concentrations of the CM radicals and $O_2^{\cdot-}$. Production is expressed in nM per minute per milligram of dry weight (nM/min/mg).

Data analysis

All experiments and data analyses were performed blindly. Data are represented as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was used to compare more than two experimental conditions. Further analysis was conducted when differences were observed. Post hoc testing was performed using Newman–Keuls analysis. The Spearman test was used to analyse correlation between the data. The samples followed a normal distribution, and the variances were similar. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed using Prism 5 (Graph Pad Software Inc).

Results

Muscle mitochondrial dysfunctions are a hallmark of early untreated DM patients

To gain insight into muscle dysfunction in DM, 15 early untreated patients were analysed for which characteristics are shown in Table 1. Transcriptomic analyses of deltoid muscle biopsies from 3 DM patients and 4 age-matched sedentary controls, suffering from myalgia without evidence of neuromuscular disease, revealed that the transcript

Table 1 DM patient characteristics

Patient	Age/sex	Co-morbidities	Medication	Muscle symptoms (weeks)	CK (UI/L)	MRC grade	Antibodies	Asymptomatic ILD on HRTC and/or PFT
P1[a]	45/F	None	Ibuprofen	6	5608	4	Anti-Jo1	HRTC
P2[a]	52/F	None	None	12	2752	4	None	HRTC
P3	35/F[a]	None	None	12	600	4	Anti-RNP	No lung involvement
P4	62/F	None	Alprazolam	24	150	4+	Anti-MDA-5	HRTC
P5	52/M	None	Paracetamol	12	6813	3	None	No lung involvement
P6	27/F	None	None	24	1389	4+	None	No lung involvement
P7	51/F	None	Fluoxetine	24	1700	3	Anti-Mi2	No lung involvement
P8	42/F	None	Tramadol	24	2862	4	Anti-Mi2	No lung involvement
P9	70/F	None	None	8	1200	4	Anti-EJ	HRTC
P10	56/F	None	None	3	1340	4	Anti-NXP2	No lung involvement
P11	63/F	Gastro oesophageal reflux	Etodolac, omeprazole	6	7000	4	None	No lung involvement
P12	65/F	None	None	7	1420	4	None	No lung involvement
P13	49/F	None	None	8	2500	4	None	No lung involvement
P14	26/F	None	None	3	210	4	Anti-NXP2	No lung involvement
P15	76/F	AHT, hypothyroidism	L-thyroxine, atenolol	4	500	3+	Anti-TIF1γ	No lung involvement

Autoantibody test were performed by immunodot and included: anti-SSa, -SSb, -SM, -RNP, -Scl70, -centromere, -Mi2, -TIF1gamma, -SAE, -MDA5, -Jo1, -PL7, -PL12, -EJ, -OJ, -Zo, -SRP, and -HMGCR. All patients were screened by high-resolution computed tomography (HRTC) and performed pulmonary functional tests (PFT)

AHT arterial hypertension, *CK* creatine kinase blood level at the time of the muscle biopsy, *F* female, *ILD* interstitial lung disease, *M* male, *MRC* Medical Research Council

[a] Transcriptomic analysis

levels of 1537 genes were increased in DM patients, whereas those of 956 genes were decreased (fold change >1.2- or <0.8 relative to controls) (Fig. 1a, d, Supplementary Figure 1 and Supplementary Table 3).

The main up-regulated gene cluster, identified using Cluster 3 and DAVID software, encoded proteins involved in inflammatory responses (Fig. 1b, Supplementary Figure 1). RT-qPCR analyses revealed that the transcript levels of numerous type I IFN-stimulated genes, including *IRF7*, *RIG1*, *MXA*, *IFI44L*, *IFI27, RSAD2, IFIT3,* and *ISG15,* were 3- to 79- fold higher in biopsy of DM patients than of controls and of non-DM inflammatory myopathies (IM) patients (Fig. 1c), confirming the previous reports [20, 34] that muscle type I IFN response is a major feature of DM.

Strikingly, the main cluster of down-regulated genes encoded proteins involved in mitochondrial integrity and function (Fig. 1e, Supplementary Figure 1). To determine whether muscle mitochondrial integrity and function are selectively affected in DM, molecular, morphological, and functional analyses of this organelle were performed in deltoid biopsies from DM, non-DM IM, and control patients. Transcript levels of genes encoding proteins of the electron transport chain complexes (e.g., *NDFAB11*, *COX8a,* and *ATP5D*) and those involved in mitochondrial biogenesis (*TFAM* and *PPARGC1A*) were decreased by 1.2- to 1.5-fold in muscle biopsies from DM patients compared to controls, while they were unaffected in non-DM IM (Fig. 1f). Histochemical staining for NADH dehydrogenase (mitochondrial respiratory complex I) and succinate dehydrogenase (SDH, complex II) activities in deltoid muscle biopsies showed irregular staining in DM patients, especially in the perimysial area (Fig. 2a). In addition, the number of cytochrome c oxidase (COX, complex IV) pale fibres was 30-fold higher in muscle of DM patients than of controls

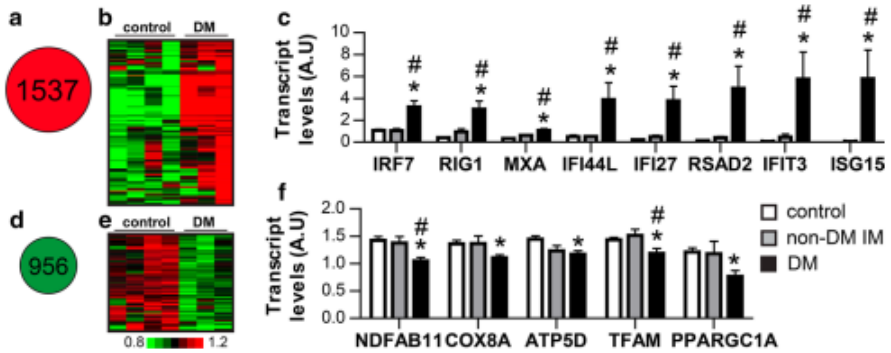

Fig. 1 Transcriptomic analysis highlights mitochondrial impairment in the early untreated DM muscle. Pie chart representation of the number of up-regulated genes (**a**) and down-regulated genes (**d**) in deltoid muscle of DM patients compared to controls. Heat map representation of the cluster of genes, the expressions of which are up-regulated (**b**) and down-regulated (**e**) in DM deltoid muscles compared to controls. **e** Transcript levels of *IRF7, RIG1, MXA, IFI44L, IFI27, RSAD2, IFIT3, and ISG15* encoding proteins involved in type I interferon immune response in deltoid biopsies of DM ($n = 9$, *black bars*), non-DM ($n = 9$, *grey bars*), and control ($n = 9$, *white bars*) patients. **f** Transcript levels of *NDHUB11*, *COX8A*, *ATP5D*, *TFAM*, and *PPARGC1A* encoding proteins involved in mitochondria respiration and biogenesis in deltoid biopsies of DM ($n = 9$, *black bars*), non-DM IM ($n = 9$, *grey bars*), and control ($n = 9$, *white bars*) patients. $^*p < 0.05$ vs. controls, $\#p < 0.05$ vs. non-DM IM, by one-way ANOVA and post hoc Newman–Keuls test

(Fig. 2a, b). In contrast, NADH dehydrogenase and SDH activities were abnormal only in few endomysial fibres of non-DM IM patients (Fig. 2a), and the number of COX-pale fibres in non-DM IM was fivefold lower than in DM patients (Fig. 2a, b). Mitochondrial respiration from complexes I to IV (V_{max}) and IV (V_{TMPD}) were, respectively, 2.0- and 1.8-fold lower in permeabilised deltoid fibres of DM patients than in controls, while not significantly decreased in non-DM IM patients (Fig. 2c, d). The respiratory control ratio (RCR = V_{max}/V_0, V_0 representing mitochondrial respiration in the absence of ADP) was decreased by 1.4-fold in skeletal myofibres from DM patients compared to controls, but was similar in controls and non-DM-IM patients (Fig. 2e), showing a reduced mitochondrial coupling between respiration and oxidative phosphorylation [8, 23] in DM muscles. Electron microscopy analysis revealed abnormal mitochondrial morphology in 80% of fibres from DM biopsies, including mitochondrial swelling, condensation, and/or abnormal cristae shape throughout the fibres (Fig. 2f). In contrast to mitochondrial dysfunctions, we could not observe a significant difference in muscle inflammation, necrosis, and atrophy between DM and non-DM IM patients (Supplementary Figure 2a–c). Furthermore, transcript levels of genes encoding proteins involved in muscle atrophy (i.e., FOXO1, FOXO3, REDD1, *MURF-1*, and *ATROGIN)* were similar in controls, non-DM IM, and DM patients (Supplementary Figure 2d). Thus, mitochondrial functional defects are a hallmark of DM.

Given that mitochondria are vulnerable to oxidative damages and are an important source of ROS, especially when malfunctioning [43], ROS production in deltoid muscles was determined. DHE-positive pixel counts were fivefold higher in deltoid muscle of DM patients than in controls (Fig. 2g), and were present in both myofibres and inflammatory cells (Fig. 2h). H_2O_2 production in the absence of mitochondrial substrates in skinned muscle fibres of DM patients was sevenfold higher than of controls and of non-DM IM patients (Fig. 2i). Moreover, under maximal respiration conditions, H_2O_2 production in DM patients was 17-fold higher than in controls, and threefold higher than in non-DM IM patients (Fig. 2i). Thus, ROS levels are high in muscle of DM patients, with mitochondrial dysfunctions contributing to their production.

Mitochondrial dysfunctions are correlated with poor aerobic capacities in DM patients

Maximal exercise capacity, a general indicator of survival in healthy subjects [30], is reduced [3], and is correlated with health status [2] in the early DM patients. Since mitochondrial function is a critical factor for maximal exercise capacities [51], we determined whether reduced aerobic capacity might be related to mitochondrial dysfunction in DM patients. Maximal aerobic capacity, determined on a cycloergometer, was correlated with maximal mitochondrial respiration in DM muscle (Fig. 2j). Accordingly, the proportion of COX-pale fibres was inversely correlated with maximal aerobic capacity (Fig. 2k). In contrast to mitochondrial dysfunctions, the intensity of other muscle lesions (e.g., inflammation, necrosis, and atrophy) did not correlate with maximal aerobic capacities (Supplementary Figure 2e–g). Taken together, these data indicate that impaired myofibre mitochondrial function contributes to poor aerobic capacity of DM patients.

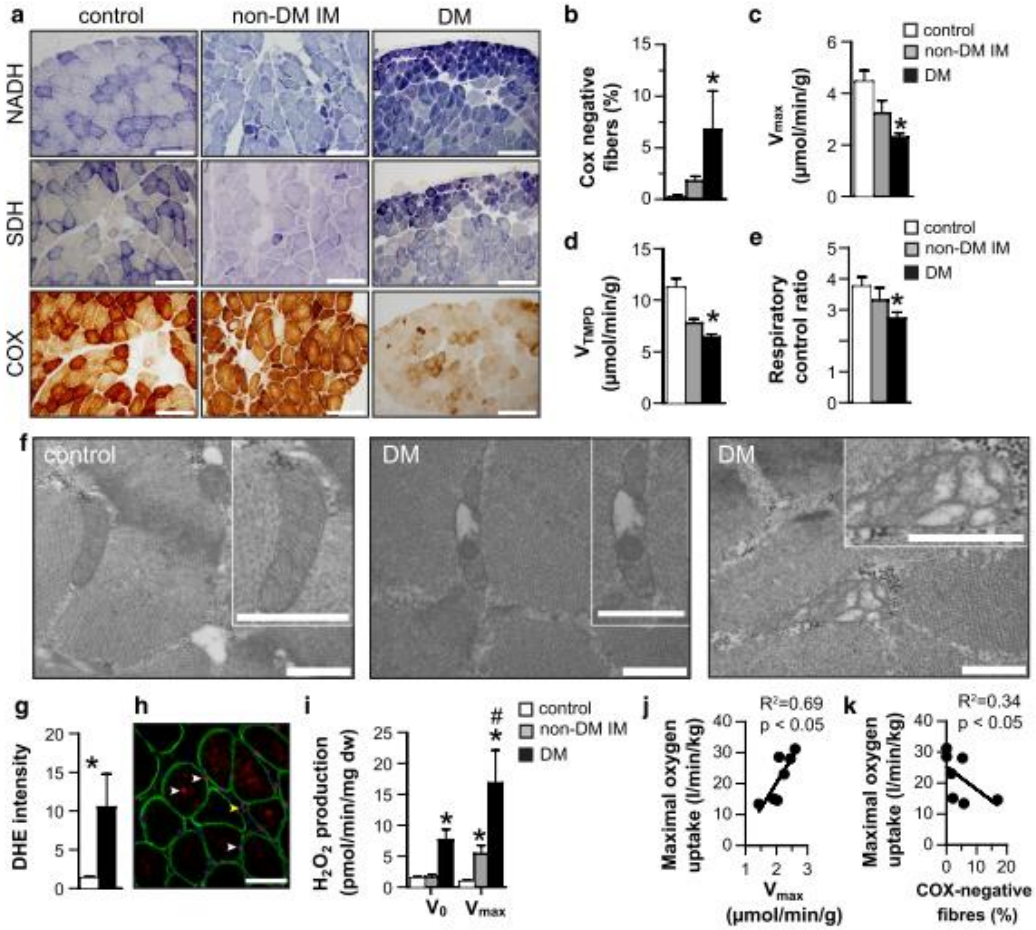

Fig. 2 Muscle mitochondrial dysfunctions with high reactive oxygen species production contribute to poor aerobic capacities in the early untreated DM. **a** Representative histological staining of deltoid muscle biopsies from DM patients ($n = 15$) and gender- and age-matched non-DM IM ($n = 14$) and control ($n = 16$) patients for NADH, SDH, and COX activity. *Scale bars* 100 µm. **b** Number of COX-pale fibres in deltoid muscle biopsies from DM patients ($n = 15$, *black bars*) and gender- and age-matched non-DM IM ($n = 14$, *grey bars*) and control ($n = 16$, *white bars*) patients. Mitochondria respiration rates in the presence of glutamate and malate and saturating amounts of ADP (V_{max}) (**c**), TMPD + ascorbate (V_{TMPD}) (**d**), and respiratory control ratio (RCR; V_{max}/V_0, V_0 representing respiration rates in the presence of glutamate and malate without ADP) (**e**) determined on deltoid fibres of DM ($n = 15$, *black bars*), non-DM IM ($n = 14$, *grey bars*), and control ($n = 16$, *white bars*) patients. **f** Representative electron micrographs of deltoid muscle biopsies from DM patients (*left panel* swelling; *right panel* condensation) and from gender- and age-matched controls ($n = 3$). *Scale bars* 100 nm. **g** DHE staining intensity quantification in deltoid fibres of DM patients ($n = 15$, *black bars*) and controls ($n = 16$, *white bars*). **h** Representative DHE (*red*), merosin (*green*), and DAPI (*blue*) co-staining of deltoid muscle sections from DM patients. Nuclei stained with DHE + DAPI (*purple*) due to reactive oxygen species in the cell are present both inside muscle fibres (*white arrow*) and outside muscle fibres (inflammatory infiltrate, *yellow arrow*). *Scale bar* 50 µm. **i** H_2O_2 production in the presence of glutamate and malate (V_0) and saturating amounts of ADP (V_{max}) in deltoid fibres of DM ($n = 15$, *black bars*), non-DM IM ($n = 14$, *grey bars*), and control ($n = 16$) patients. Correlation between aerobic capacities determined on a cycloergometer and V_{max} (**j**) and COX-pale fibre proportion in DM patients (**k**) ($n = 7$, $p < 0.05$ by Spearman test). *$p < 0.05$ vs. controls, #$p < 0.05$ vs. non-DM IM, by one-way ANOVA and post hoc Newman–Keuls test

IFN-β-induced ROS impair myofibre mitochondrial respiration

Given the evidence of crosstalk between immunity, mitochondrial functions, and ROS production in other settings [35, 36, 47], we investigated the relationship between muscle inflammation, impaired mitochondrial respiration, and high ROS production in DM.

To explore the inflammatory pathways involved in DM, the serum level of pro-inflammatory cytokines previously linked with disease activity [25, 32] was recorded. In agreement with the previous findings [18, 21] and with the type

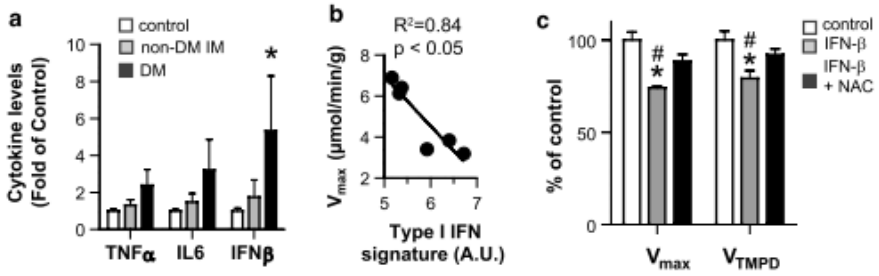

Fig. 3 IFN-β-induced ROS mediate mitochondrial respiration impairment. **a** Serum levels of TNF-α, IL-6, and IFN-β in DM ($n = 7$, *black bars*), non-DM IM ($n = 5$, *grey bars*), and healthy controls ($n = 12$, *white bars*). **b** Correlation between mitochondrial maximal respiration (V_{max}) and type I IFN signature in DM muscles ($p < 0.05$). **c** Maximal mitochondrial respiration (V_{max}) and mitochondrial respiration with TMPD and ascorbate (V_{TMPD}) in LHCN-M2 human myotubes exposed to IFN-β (100 UI/ml) for 3 days with (*black bars*) or without (*grey bars*) the ROS scavenger *N*-acetyl cysteine (NAC 1 mM). Untreated LHCN-M2 human myotubes (*white bars*) ($n = 6$). *$p < 0.05$ vs. control, #$p < 0.05$ vs. NAC, by one-way ANOVA and post hoc Newman–Keuls test

1 IFN signature of DM muscle (Fig. 1d), IFN-β serum levels were threefold higher in DM patients comparatively to non-DM IM patients, while TNF-α and IL-6 serum levels were similar in these two groups (Fig. 3a).

We, therefore, determined whether high IFN-β blood levels lead to muscle mitochondrial dysfunction. In DM muscle, the type I IFN score (median fold change in expression of the seven type I IFN-stimulated genes, see [33]) was negatively correlated with V_{max} (Fig. 3b). Moreover, 3-day treatment of human myotubes with IFN-β (100 UI/ml) decreased maximal mitochondrial respiration (V_{max}) and V_{TMPD} by 25% (Fig. 3c).

To investigate the link between high blood IFN-β, muscle mitochondrial dysfunctions, and high ROS production in DM, IFN-β-treated human myotubes were cultured in the presence of the ROS scavenger *N*-Acetyl-Cystein (NAC), and we showed that it prevents mitochondrial respiration impairment (Fig. 3c). In contrast, 3-day treatment of human myotubes with the pro-inflammatory cytokine IL-6 (100 ng/ml) did not affect V_{max} and V_{TMPD}. Note that high levels of TNF-α (10 ng/ml) decreased V_{max} in a ROS-dependent manner, although had no effect on V_{TMPD} (Supplementary Figure 3).

Taken together, these results show that IFN-β, the level of which is selectively increased in DM, induces mitochondrial impairment in myotubes in a ROS-dependent manner.

Mitochondrial impairment, muscle weakness, and inflammation are prevented in experimental autoimmune myositis by ROS scavenging

There is currently no animal model allowing to entirely mimic DM. However, immunisation of mice with myosin has been shown to lead to an experimental autoimmune myositis (EAM) exhibiting muscle impairment and inflammation [5]. Herein, grip strength in EAM mice was 30% lower 2 weeks after the last immunisation (Fig. 4a), and histological analyses revealed intense muscular inflammatory infiltrates (Fig. 4b). Although no perifascicular myofibre injury, typical of DM [22], was detected, the transcript levels of type I IFN-stimulated genes (e.g., *Ifit3, Mxa,* and *Isg15*) were twofold higher in the muscles of EAM mice than in controls (Fig. 4c). While the transcript levels of *Sdhb* and *Uqcr11,* encoding proteins involved in complex II and III, respectively, were slightly reduced in the muscles of EAM mice, those of genes encoding *Ndfub* (Complex 1), *Cox4i,* and *Cox5B* (complex IV), as well as *Atp5o* (complex V) were decreased by 1.4-fold in EAM mice compared to non-immunised mice (Fig. 4d). Moreover, mitochondrial respiration and mitochondria coupling measured in individual fibres were twofold lower in mixed-gastrocnemius and oxidative-soleus muscle from EAM mice than in controls (Fig. 4e–g). In addition, ROS levels, determined by electron paramagnetic resonance, were 2.2- and 1.8-fold higher in gastrocnemius and quadriceps of EAM mice than of controls, respectively (Fig. 4h). Thus, EAM mice allow determining the link between muscle ROS, mitochondrial damages, and type I IFN signalling in vivo.

To explore the effect of ROS on mitochondrial damages and type I IFN signalling, EAM mice were treated with NAC (300 mg/kg/day) [31]. As expected, NAC reduced ROS levels in hindlimb muscles (Fig. 4h), and prevented muscle weakness (Fig. 4a). Moreover, NAC treatment improved myofibre V_{max} and RCR in immunised mice, and prevented the decrease in muscle transcripts involved in mitochondrial respiration (Fig. 4d–g). Furthermore, NAC prevented the increase in type I IFN-stimulated transcripts and the inflammatory infiltrate in muscles (Fig. 4b, c). Taken together, these results show that ROS play a central role in the induction of muscle weakness, mitochondria dysfunction, type I IFN signature, and inflammation in EAM mice.

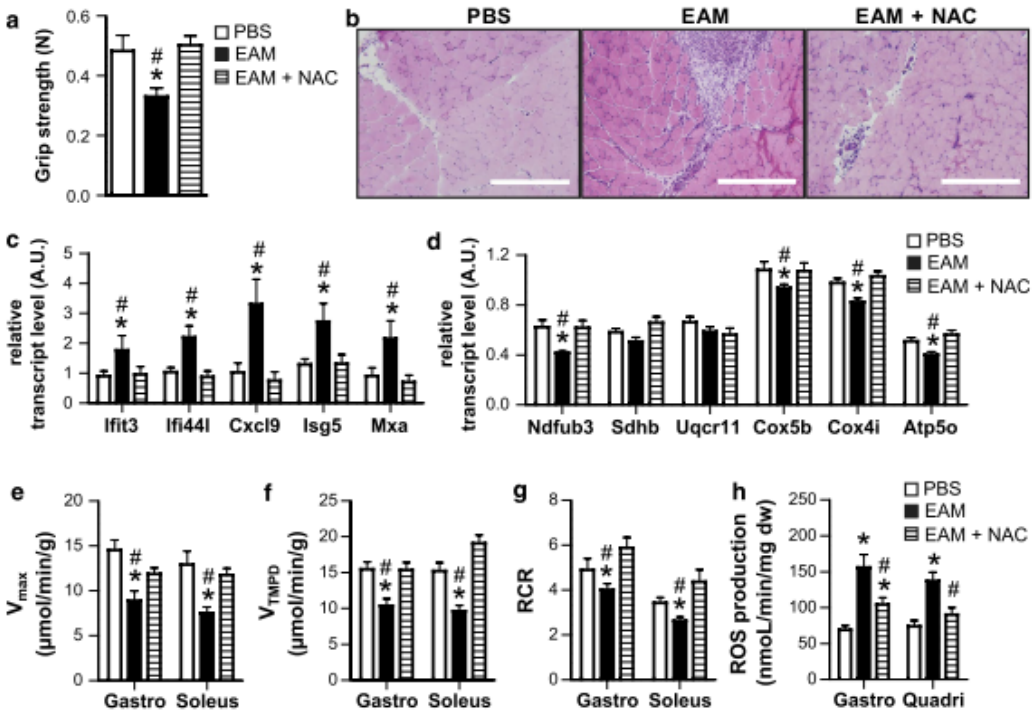

Fig. 4 ROS induce mitochondrial dysfunctions, muscle weakness, and inflammation in experimental autoimmune myositis (EAM). **a** Grip strength measurement of control (*white bar*) and EAM mice, treated (*dashed bar*) or not (*black bar*) with NAC ($n = 12$ per group). **b** Representative Haematoxylin & Eosin staining of gastrocnemius cryosections from control and EAM mice, treated or not with NAC ($n = 6$ per group). *Scale bar* 100 µm. Transcript levels of the interferon-stimulated genes *Ifit3, Ifi44 l, Isg15, Cxcl9,* and *Mxa* (**c**) and of the nuclear genes encoding mitochondrial proteins [*Ndhub* (complex I), *Sdhb* (complex II), *Uqcr11* (complex III), *Cox5b & Cox4A* (complex IV), and *Atp50* (complex V)] (**d**) in the tibialis muscle of control (*white bars*) and EAM mice treated (*dashed bars*) or not (*black bar*) with NAC ($n = 6$ per group). Mitochondria respiration rates in the presence of glutamate, malate, and saturating amounts of ADP (V_{max}) (**e**), TMPD + ascorbate (V_{TMPD}) (**f**), and respiratory control ratio (V_{max}/V_0, V_0 representing respiration rates in the presence of glutamate and malate without ADP) (**g**) in gastrocnemius and soleus muscle of control (*white bars*) and EAM mice treated (*dashed bars*) or not (*black bar*) with NAC ($n = 12$ per group). **h** Reactive oxygen species levels in gastrocnemius and quadriceps muscles. $^*p < 0.05$ vs. controls, $\#p < 0.05$ vs. NAC-treated EAM, by one-way ANOVA and post hoc Newman–Keuls range test

Discussion

The mechanisms underlying muscle dysfunctions and inflammation perpetuation in DM currently remain unknown. We show herein that muscle mitochondrial malfunctions, mediated by IFN-β-induced ROS, contribute to the early poor aerobic capacity and furthermore drive type I IFN-inducible gene expression and muscle inflammation, likely contributing to disease maintenance.

In addition, our findings using histological, functional, and molecular analyses reveal that mitochondrial dysfunctions of perifascicular muscle fibres are a hallmark of early untreated DM. This extends previously reported histological data showing mitochondrial abnormality in perifascicular fibres of DM patients as compared with healthy controls [49] and age-matched patients with non-DM IM [10, 44] or neurogenic atrophy [4]. However, the only previously reported functional analysis did not find decreased respiration in mitochondria isolated from DM muscle [9, 27]. Contrary to the aforementioned method, the functional protocol used herein allowed the preservation of mitochondrial integrity and interactions with other intracellular structures which are critical for mitochondrial function [23]. The present method confirmed that DM muscle exhibits severe mitochondrial respiratory defects. Finally, while only a recent study demonstrated lipid peroxidation in DM muscle, an indirect sign of high ROS production that was not compared to other diseases of skeletal muscle [17], our observations reveal, through the use of two different methods, that ROS production is higher in DM compared to non-IM DM patients with mitochondrial dysfunctions contributing to this increase.

The histological muscle abnormalities of the perifascicular area (including muscle fibre atrophy, necrosis, and inflammatory infiltrate), which are the classical hallmark of DM, are poorly correlated with muscle symptoms. These features can remain unchanged despite clinical improvement [6], and conversely, muscle impairment can persist even after these lesions have improved [26]. In contrast, mitochondrial dysfunctions demonstrated herein were strongly correlated with aerobic capacity, a critical factor of exercise performance [51], and a powerful indicator of survival in healthy subjects [30], as well as of health status in DM patients [2]. The present findings thus provide new insights into poor exercise capacities of DM patients.

High blood IFN-β has been correlated with DM activity, although the mechanisms underlying muscle impairment were unknown [19]. The present study shows that type I IFN signature is correlated with mitochondrial dysfunction and that IFN-β induces mitochondrial dysfunction in a ROS-dependent manner in muscle cells. This is consistent with the location of both mitochondrial dysfunctions [44] and type I IFN-stimulated genes expression [34] within perifascicular myofibres in DM, as well as with the effects of IFN-β reported in other cell types [35, 36]. The irregular staining of NADH and SDH, along with pale COX staining in DM muscle, may point to a decrease in complex IV function. Accordingly, in DM and in IFN-β-treated myotubes, both V_{max} and V_{TMPD} were decreased. Complex IV is believed to be the pacesetter for mitochondrial oxidative metabolism, and is highly regulated at the transcriptional, translational, posttranslational, and assembly levels [39]. However, the transcripts levels of genes encoding complexes I and V were also decreased in the muscle of DM patients suggesting that these complexes play a role in mitochondrial dysfunctions of these patients. Interestingly, while the expression of genes encoding mitochondrial-related proteins are mildly, but significantly, down-regulated in the muscle of DM patients, this decrease might be sufficient to induce mitochondrial dysfunctions, as unravelled by histological and functional analyses. However, other mechanisms, such as post transcriptional modifications of mitochondrial proteins, might also contribute to mitochondrial dysfunctions in DM muscle. Moreover, in muscles, additional mechanisms linking high IFN-β and mitochondrial damages are possible. In particular, IFN-β has been shown to reduce muscle arteriogenesis [42]. It may thus participate in microvascular unit depletion observed in DM muscle, which drives ischemia/reperfusion injuries [17], a cause of mitochondrial dysfunctions [24].

The source of self-sustained type I IFN signature in DM muscle has yet to be resolved. Overexpression of innate immune receptors in DM myofibres, such as RIG-1, may participate in IFN-β production [40], although the cause of their activation remains unknown. Mitochondria and ROS have recently emerged as critical components of innate immunity [47]. Notably, RIG-1 has been reported to act through mitochondrial antiviral signalling (MAVS) which positively regulates the RIG-1 pathway in response to mitochondrial dysfunctions and ROS production [41]. Moreover, mitochondrial dysfunction has been shown to induce the release of mitochondrial DNA in the cytoplasm where it engages innate immune receptors leading to the expression of type I IFN-stimulated genes [1, 46]. We demonstrate that mitochondrial dysfunctions increase ROS production in muscles of DM patients, and that ROS scavenging prevents IFN-β-induced mitochondrial dysfunctions in human myotubes. Moreover, even though autoimmune mechanism directed against myosin does not induce perifascicular atrophy in EAM mice, ROS scavenging prevents mitochondrial dysfunctions and type I IFN signature. Thus, these data strongly suggest that ROS-induced mitochondrial damage can self-sustain the innate immune response in DM muscle. Thus, our data strongly suggest that ROS-induced mitochondrial damage can self-sustain the innate immune response in DM muscle.

Our findings may also explain the partial efficacy of corticosteroids, the current cornerstone of the conventional DM management, since these potent anti-inflammatory drugs are known to induce muscle mitochondrial dysfunction and oxidative stress [28]. The present data highlighting a central role of mitochondria and ROS in DM open new therapeutic avenues for DM.

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ARTICLE 6 : une poussée de DM lors d'un traitement par l'imiquimod indiquant un rôle de TLR7 dans cette maladie

Dans cet article, nous rapportons une patiente qui a développé une exacerbation sévère de DM anti-NXP2 positive suite à un traitement par imiquimod, un agoniste TLR7 utilisé pour le traitement des carcinomes basocellulaires. L'analyse des cellules mononuclées du sang périphérique a révélé une augmentation de la sécrétion d'IFN-β en réponse à la stimulation de TLR7, alors que la sécrétion de cytokines pro-inflammatoires induite par TLR4 ne différait pas des témoins appariés. Ces résultats indiquent non seulement que TLR7 participe à la DM, mais signalent également que la peau est un organe principal permettant aux agonistes de TLR7 d'induire les poussées de DM. Le lien avec les anomalies mitochondriales est discuté dans la partie discussion.

Connective tissue diseases

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CLINICAL CASE

Dermatomyositis flare on imiquimod therapy highlights a crucial role of aberrant TLR7 signalling

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Dermatomyositis (DM) is a chronic systemic disease that primarily affects skin and/or muscles and is associated with cancer in about 20% of cases. Although DM is an autoimmune disorder, there is evidence that innate immunity plays a crucial role in the disease. In particular, it has been associated with elevated interferon (IFN)-β in blood[1] which is critical in the initiation[2] and perpetuation[3] of the disease. However, the origin of elevated IFN-β remains elusive. It has been speculated that it may result from the engagement of endosomal toll-like receptor (TLR) signalling due to the increased expressions of TLR7 and TLR9 in peripheral blood leucocytes[4] of patients with DM, but direct evidence of endosomal TLR involvement in DM is lacking.

We report herein a patient who developed severe exacerbation of anti-NXP2-positive DM on imiquimod therapy, a potent TLR7 agonist approved for the treatment of cutaneous basocellular carcinoma. Peripheral blood mononuclear cells (PBMC) analysis revealed an increase in IFN-β secretion on TLR7 stimulation, whereas TLR4-induced pro-inflammatory cytokines secretion did not differ from healthy matched controls. This report not only provides evidence that endosomal TLR7 participates in human DM but also pointed to the skin as a primary organ allowing TLR7 agonists to induce DM flare.

A woman aged 47 years presented with fever, arthralgia, myalgia and a facial DM rash (figure 1). She declared that a slight facial rash suggesting of DM had been present for over 1 year and that its exacerbation and extracutaneous signs onset appeared 1 month after starting topic imiquimod (5%, once daily) for a cutaneous basocellular carcinoma of the anterior chest wall (diagnosed 6 months after DM rash onset). Temperature was 38°C, and limb girdle muscles were painful but with no signs of weakness. Joints of the hand were tender without arthritis. C reactive protein level was 1.5 mg/dL (normal <0.4) while creatine kinase level was normal. She tested positive

Key messages

What is already known about this subject?

- It has been speculated that elevated interferon (IFN)-β in blood characterising dermatomyositis patients results from the engagement of endosomal toll-like receptor (TLR) signalling due to the increased expressions of TLR7 and TLR9 in peripheral blood leucocytes.

What does this study add?

- We provide direct evidence that supports this hypothesis by reporting herein a patient who developed severe exacerbation of anti-NXP2-positive dermatomyositis (DM) on imiquimod therapy, a potent TLR7 agonist and whose peripheral blood mononuclear cells secreted high level of IFN-β upon TLR7 stimulation as compared with healthy donors.

How might this impact on clinical practice?

- These data indicate that aberrant TLR7 signalling may represent a therapeutic target in DM.

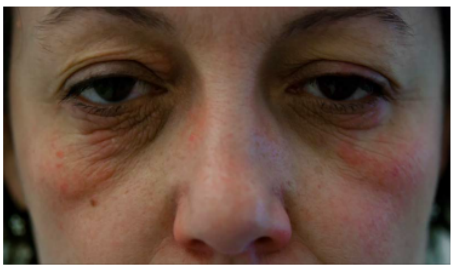

Figure 1 Eyelid erythema and oedema after imiquimod intake.

for anti-NXP2 antibodies (DTek and Euroimmun) and negative for anti-Mi2, anti-SAE, anti-TIF1γ and anti-MDA5. Electromyographic recordings were normal. Hand radiographs demonstrated no damage. CT and spirometry demonstrated no evidence of interstitial lung disease. Cutaneous basocellular carcinoma had hardly disappeared when she was diagnosed with DM and no other cancer was found on ^{18}F-FDG PET/CT, gastroscopy and colonoscopy. Eyelids skin rash, polyarthralgia, fever and anti-NXP2 demonstrated by two immunoassay test kits indicate that our patient did suffer from amyopathic DM. Accordingly, she was successfully treated with prednisone, topical tacrolimus for the DM rash and imiquimod discontinuation (after 6 weeks treatment and complete clinical regression of the basocellular carcinoma).

In vitro, TLR7 stimulation of the patient's PBMC (sampled 12 months after imiquimod discontinuation) led to an increase in several pro-inflammatory cytokines involved in DM,[5] including IFN-β. Imiquimod also lead to an increase in TLR7 expression in the PBMC of our

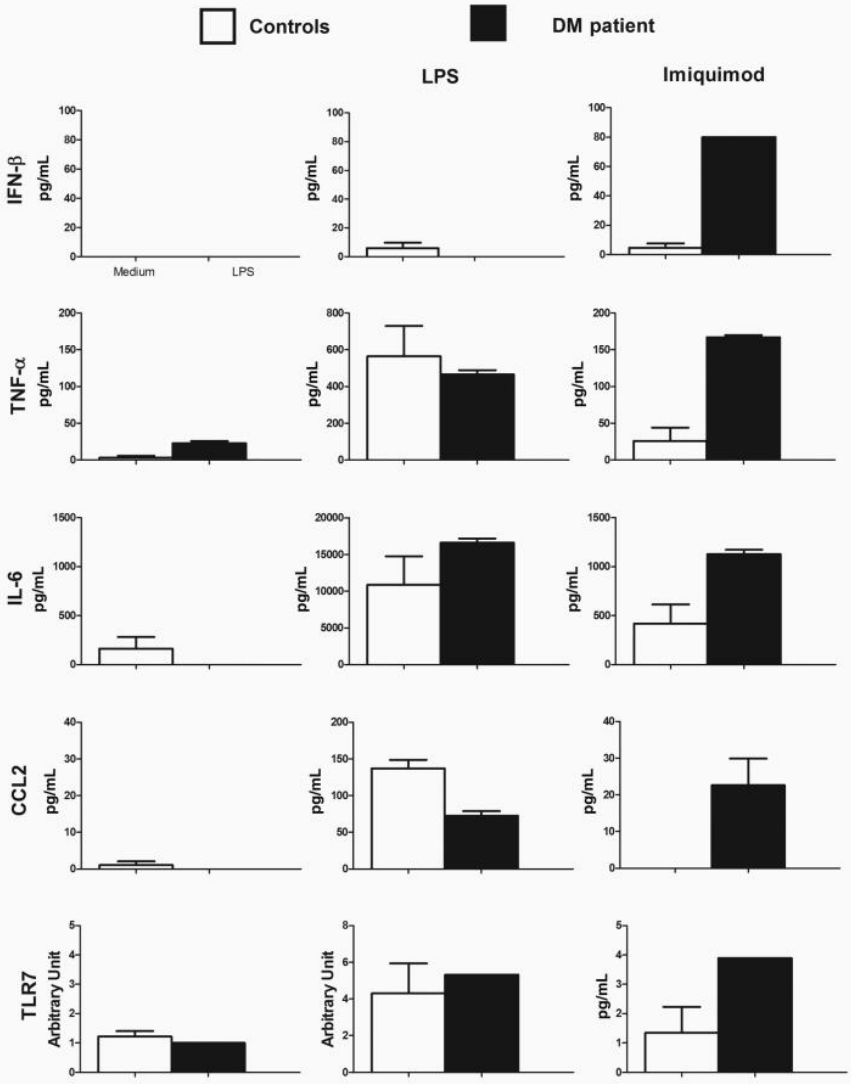

Figure 2 IFN-β, IL-6, TNF-α and CCL2 release were determined by ELISA in culture supernatants of PBMC stimulated with LPS from *Salmonella abortusequi* (1 µg/mL Sigma–Aldrich (Saint-Quentin-Fallavier, France)) or imiquimod (5 µg/mL, Sigma–Aldrich (Saint-Quentin-Fallavier, France)) for 3 hours. TLR7 expression was determined by RT-qPCR. Results were normalised to Gapdh and expressed as fold change compared with samples from cells incubated in medium alone. PBMC was isolated from the DM patient and three age-matched healthy controls. The patient had discontinued imiquimod 1-year topical tacrolimus 2 weeks before PBMC were sampled. CCL2, chemokine ligand 2; DM, dermatomyositis; IL-6, interleukin-6; INF-β, interferon-β; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cells; TLR7, toll-like receptor-7; TNF-α, tumour necrosis factor-α.

patient but not in controls. Pro-inflammatory cytokine secretion on TLR4 stimulation, which expression has been reported to be unchanged in DM PBMC,[4] did not differ from age-matched female controls (figure 2).

The present report suggests that aberrant endosomal TLR signalling, including high IFN-β secretion by PBMC and TLR7 signal auto-amplification, participates in DM. Given the presence of a feed-forward loop between IFN-β and TLR7 signalling,[6] the latter is likely to participate in disease initiation and maintenance. This extends the previous description of abnormal endosomal TLR expression in PBMC of patients with DM.[4] Natural ligands of TLR7 in DM patients probably include microbial RNA since a clinical history consistent with an infection is frequently reported prior to disease onset,[7] but endogenous RNAs are also likely to be involved notably during cancer and UV radiation damages, two frequent local conditions involving TLR7-mediated response[8 9] and triggering DM. Consistently with this view, skin TLR7 activation in mice[10] and human[11] has been previously reported to trigger lupus, an autoimmune disease that is also characterised by a type on interferon signature in blood. These data indicate that TLR7 may represent a therapeutic target in DM.

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Competing interests None declared.

Patient consent Obtained.

Ethics approval Human cells were obtained after informed consent was obtained from donors. Ethical approval was granted by the institutional ethics committee, Hopitaux Universitaires de Strasbourg, France.

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DISCUSSION

Par l'approche épidémiologique, nous avons montré que l'étude de l'incidence er de la prévalence est un outil utile pour mettre en évidence des déterminants des myopathies inflammatoires. Une meilleure identification et une meilleure classification des patients atteints de ces maladies sont cependant nécessaires pour préciser l'épidémiologie des myopathies inflammatoires.

Par l'approche translationnelle, nous avons montré que, par rapport aux autres myopathies inflammatoires, des dysfonctions mitochondriales périfasciculaires sont une caractéristique des dermatomyosites, qui jouent un rôle dans l'intolérance à l'effort et le maintien de l'inflammation. Nos résultats pourraient avoir des implications au-delà des myopathies inflammatoires. Les mitochondries sont des régulateurs de l'immunité qui jouent un rôle dans d'autres maladies auto-immunes. Les mitochondries sont régulées par l'inflammation et pourraient jouer un rôle dans les capacités d'exercice au cours d'autres pathologies inflammatoires chroniques non primitivement musculaires, comme les spondylarthropathies.

1- L'étude de l'épidémiologie des myopathies inflammatoires

Une estimation précise de l'épidémiologie des MI est difficile. En raison de la rareté de ces maladies, la prévalence et l'incidence sont fiables dans une grande population d'étude aboutissant à un nombre suffisant de cas pour que l'intervalle de confiance du résultat soit étroit. La validation des cas est délicate. Les enquêtes basées uniquement sur les données administratives bénéficient d'un recrutement large, mais les fréquences élevées généralement enregistrées dans ces études ont pu être influencées par des erreurs de codes et des diagnostics erronés. À l'inverse, la revue des dossiers médicaux bénéficie d'un diagnostic précis. Mais il est difficile d'obtenir un recrutement exhaustif. Différents spécialistes sont impliqués (rhumatologues, neurologues, dermatologues, pneumologues, pédiatres). Des cas peuvent facilement être manqués lorsque la myopathie n'est pas au premier plan. Une seule étude (Mendez et al., 2003) a utilisé une analyse en capture-

recapture pour estimer le nombre de cas manqués par une seule source et pour corriger le taux de prévalence.

L'Alsace, région de l'est de la France, peut offrir l'opportunité d'évaluer avec précision l'épidémiologie des MI. La densité médicale et l'accès aux soins de santé sont élevés, indépendamment des facteurs géographiques pour les 2 millions d'habitants de cette région relativement petite, puisque 100 % de la population est à moins de dix minutes de trajet d'un médecin (Callewaert et al., 2013). La région est couverte par un Centre de référence des maladies auto-immunes, et un Centre de référence des maladies neuromusculaires. Enfin, l'Alsace est encadrée par le Rhin à l'est, le massif des Vosges à l'ouest, l'Allemagne au nord et la Suisse au sud qui jouent le rôle de barrières géographiques pour rechercher des soins à l'extérieur de la région.

Nous avons débuté une étude financée par le PHRC interrégional 2011 pour déterminer la prévalence et l'incidence des MI en Alsace au 1er janvier 2011 en utilisant une méthode capture-recapture (Meyer et al., 2016; Wittes et al., 1974), croisant les données obtenues par les sources de recrutement suivantes :

- Déclaration des médecins généralistes et spécialistes concernés ;
- Revue des résultats des biopsies musculaires du centre d'histologie musculaire du CHU de Strasbourg ;
- Déclaration des laboratoires de biologie publics et privés. Ces laboratoires ont été invités à déclarer les patients qui étaient positifs pour au moins un anticorps associé aux myopathies inflammatoires.
- Déclaration des PMSI des établissements de santé publics et privés. Ces établissements ont été invités à déclarer les hospitalisations enregistrées sous les codes : M330, M331, M332, M339 M608, M609, G718, G719, G724, G728, G729, G737.

Les résultats de cette étude constitueront les premières données obtenues par une méthode capture-recapture de plus de deux sources.

2- Les limites de la définition de la dermatomyosite : y a-t-il un DM classique ?

Dans l'article 3, nous avons montré que des dysfonctions mitochondriales périfasciculaires sont une caractéristique des DM par rapport aux autres MI.

Nous avons basé le diagnostic de DM sur les critères de l'ENMC (Hoogendijk et al., 2004). Selon ces critères, le diagnostic de DM repose sur : i) une éruption cutanée de DM et/ou ii) des lésions des fibres périfasciculaires (atrophie ou expression de l'HLA de classe I) ; iii) des lésions capillaires (dépôts de C5b9 sur les capillaires ou réduction de la densité capillaire ou inclusions tubulo-réticulaires dans les cellules endothéliales).

Depuis le début de ce travail, il a été montré qu'au sein de cette entité histologique, des groupes de patients ayant des particularités lésionnelles peuvent être reconnus sur la base de leurs sérologies.

Les patients atteints de DM avec anticorps antisynthétases (Aouizerate et al., 2014; Mescam-Mancini et al., 2015; Stenzel et al., 2015) sont caractérisés par des fibres périfasciculaires plus souvent en nécrose (79 % vs 35 %), moins souvent en atrophie (44-59 vs 53-85%), exprimant moins souvent l'HLA II+ (83 vs 28 %). Ces fibres présentent aussi, dans 81 % des cas, des agrégats nucléaires d'actine qui ne sont jamais observés au cours des autres DM. La vasculopathie est plus modérée si l'on en juge les dépôts capillaires de C5b9 moins denses et les lésions ischémiques (infarctus et myofibrinolyse) qui sont rares (7 % vs 40 et 33 % respectivement). Il a été proposé que ces patients soient envisagés comme étant atteints d'une maladie à part au sein des DM en raison de ces particularités histologiques et sérologiques associées à un syndrome clinique spécifique : le syndrome des antisynthétases.

Les données récentes indiquent que la plus ou moins grande fréquence de certaines lésions histologiques musculaires en fonction du statut sérologique existe aussi pour au moins trois autres groupes de DM.

Les patients atteints de DM anti-TIF1γ ont une histologie musculaire marquée par des dépôts denses de C5b9 sur les capillaires (82.4 % vs 8.2 %, $p < 0.001$) et des fibres

vacuolisées (myofibrinolyse : 73.5 % vs 11.2 %, p < 0.001). Comme les patients avec anticorps antisynthétases, ces patients ont un syndrome clinique spécifique.

Les patients atteints de DM anti-MDA5 ne présentent pas les caractéristiques classiques de l'atrophie de la fibre périfasciculaire ni de l'expression du complexe majeur d'histocompatibilité de classe I. Ils ont peu de perte capillaire ; les formations tubulo-réticulaires dans l'endothélium sont observées moins fréquemment. L'inflammation périfasciculaire est focale et moins intense. Comme les patients avec anticorps antisynthétases, ces patients ont un syndrome clinique spécifique et un risque plus grand de cancer.

L'histologie musculaire fine de la DM à anti-Mi2, -NXP2 et -SAE n'a jusqu'alors pas été rapportée, mais des éléments indiquent que des différences existent : l'infiltrat inflammatoire serait plus intense chez les patients avec anti-Mi2, et plus faible (malgré un pronostic fonctionnel plus sombre) chez les patients avec anti-NXP2 (Albayda et al., 2017).
Ces différences au sein des DM pourraient reposer sur une variabilité physiopathologique. Même si les effectifs étaient faibles pour étudier ces sous-groupes, dans notre expérience clinique, l'atteinte mitochondriale histologique pourrait être la plus sévère au cours des DM à anti-NXP2 et anti-TIF1γ.

Une perspective de ce travail est de déterminer s'il y a des différences dans l'importance de l'atteinte mitochondriale entre les différents sous-groupes de DM.

3- Les dermatomyosites : des myopathies mitochondriales périfasciculaires ischémiques ou médiées par une toxicité de l'IFN-I ?

Dans le travail présenté en article 3, nous avons montré que l'IFN-β, dont le taux est spécifiquement augmenté au cours des DM par rapport aux autres MI, participe aux dysfonctions mitochondriales des fibres périfasciculaires des patients atteints de DM. En effet, l'expression des gènes interféron-induits dans le muscle et les anomalies mitochondriales sont toutes deux localisées au niveau des fibres périfasciculaires (Suárez-

Calvet et al., 2017). La signature interféron de type I dans le muscle est corrélée à l'importance des anomalies mitochondriales. Et *in vitro*, l'IFN- β induit des dysfonctions mitochondriales dans les myotubes humains.

Cependant, d'autres facteurs pourraient aussi contribuer à ces dysfonctions mitochondriales, en particulier l'ischémie-reperfusion. Au cours de la DM, il existe une vasculopathie qui débute en amont de la perte capillaire des faisceaux, au niveau des artères périmysiales autour desquelles les infiltrats inflammatoires prédominent, et qui est responsable de phénomènes d'ischémie-reperfusion (Gitiaux et al., 2013). Les fibres périfasciculaires qui présentent les anomalies mitochondriales sont aussi celles qui sont soumises à l'ischémie et expriment HIF-1α, un gène majeur de réponse à l'hypoxie (De Luna et al., 2017). Les anomalies mitochondriales musculaires (mesurées par le nombre de fibres COX négatives) sont corrélées à la déplétion capillaire (Chariot et al., 1996). Des travaux du laboratoire ont montré que l'ischémie-reperfusion induit des dysfonctions mitochondriales, chez l'homme, dans des modèles murins et cellulaires (Lejay et al., 2014).

Les effets de l'IFN-β et de l'hypoxie sur le fonctionnement mitochondrial des fibres périfasciculaires des patients atteints de DM ne sont pas mutuellement exclusifs et pourraient au contraire s'auto-entretenir :

L'IFN-β pourrait contribuer à la vasculopathie. En effet, l'IFN-β diminue l'artériogénèse des muscles cardiaques et squelettiques chez l'homme et l'animal (Cochain and Zernecke, 2015; Schirmer et al., 2008). D'autre part, chez la souris, le KO pour le récepteur de l'INF-α/β (IFNAR) est protégé contre les lésions d'ischémie-reperfusion rénale et hépatique (Freitas et al., 2011; Zhai et al., 2008). Il est donc possible que l'IFN-β favorise la vasculopathie et l'ischémie des fibres périfasciculaires.

Très récemment, il a été montré que l'ischémie des fibres périfasciculaires contribue à leur production d'IFN-β (De Luna et al., 2017). RIG1, un récepteur de l'immunité innée dont l'expression périfasciculaire est spécifique de la DM, (Suárez-Calvet et al., 2017) conduit à l'expression d'IFN-β. L'expression de cette protéine est sous la dépendance de l'IFN-I, mais

aussi de HIF-1α par l'intermédiaire d'éléments de réponse à l'hypoxie localisés dans le promoteur de *RIG-1*.

Les anomalies mitochondriales sont donc à la convergence de l'effet de l'IFN-β et de l'ischémie, deux phénomènes qui vraisemblablement s'auto-entretiennent.

4- Quelle est l'origine de la DM ?

Dans l'article 3, nous montrons que les anomalies mitochondriales périfasciculaires participent vraisemblablement au maintien de la signature IFN-I dans les fibres périfasciculaires. En effet, les dysfonctionnements mitochondriaux augmentent la production de RLO qui entraîne l'expression des gènes induits par l'IFN de type I et l'inflammation musculaire, ce qui peut auto-entretenir la maladie.

Comme les facteurs étiologiques impliqués dans les DM (tels que les rayons ultraviolets, certains médicaments, les virus et les cancers) induisent des dysfonctions mitochondriales, ces résultats indiquent un lien mécaniste possible entre ces facteurs et la DM.

Mitochondries, RLO, DM et cancer : Environ 20 % des MI de l'adulte sont associées à un cancer (Wang et al., 2013). La présence d'un cancer a été reliée à un âge plus avancé, au sexe masculin, à une atteinte cutanée plus sévère alors que l'atteinte articulaire et/ou pulmonaire a négativement été associée au cancer (Wang et al., 2013).

Au cours des DM, les anticorps anti-TIF1 ont aussi été reliés à la présence d'une tumeur (Trallero-Araguás et al., 2012), même si cette association pourrait être moins forte lorsque ces anticorps sont détectés par des kits de grande sensibilité (Fiorentino et al., 2013). La présence des anticorps anti-NXP2 (Fiorentino et al., 2013) et anti-SAE (Muro et al., 2015) pourrait aussi être un facteur de risque. De façon concordante, les cibles de ces trois auto-anticorps sont des protéines impliquées dans la régulation du cycle cellulaire.

Les cellules cancéreuses ont une signature métabolique particulière, mise en évidence il y a presque 100 ans, caractérisée par une production de lactate en présence d'oxygène

(Wallace, 2012), témoin de dysfonctions mitochondriales. Des mutations somatiques dans l'ADNmt et dans l'ADN nucléaire affectent le fonctionnement de la chaine de respiration mitochondriale et ont été associées à une plus grande tumorigénèse. De façon concordante, plusieurs variants de gènes codés par ADNmt ont été associées à un risque de cancer. Pourtant, les mitochondries sont essentielles à la tumorigénèse : la surpression de l'ADNmt dans diverses cellules cancéreuses par le bromure d'éthidium résulte en un taux de croissance et des capacités de formation de cancers chez les souris nude diminués (réf. 9 à 15 de Wallace). Ceci implique que les dysfonctions mitochondriales constituent un signal nécessaire à la tumorigénèse. Ce signal est actuellement partiellement compris : il a été montré que les dysfonctions mitochondriales qui surviennent dans les tumeurs exercent un effet sur le métabolisme, la sécrétion de RLO et la méthylation de l'ADN des cellules tumorales, mais aussi sur le métabolisme et la mitophagie des cellules stromales non tumorales.

Il est possible que ces anomalies mitochondriales tumorales soient aussi, dans le cas des DM paranéoplasiques, à l'origine de l'initiation de la réponse interférongénique qui se pérennise ensuite au niveau musculaire par les mécanismes que nous avons décrits.

Mitochondries, RLO, DM et UV : Nous avons montré que l'incidence relative des DM à anti-Mi2 a été reliée au rayonnement ultraviolet (UV) (Meyer et al., 2015). De façon concordante, l'antigène Mi2 est une molécule impliquée dans la réparation des cassures de l'ADN, provoquées notamment par les radiations UV, qui induit l'hyper-expression de Mi2 (Burd et al., 2008).

L'ADNmt a un taux de mutation élevé, ~ 10 - 20 – fois supérieur au taux de l'ADN nucléaire. Ceci est dû au fait que l'ADNmt n'est pas protégé par les histones et que les mécanismes de réparation sont relativement faibles. L'ADNmt est une cible privilégiée des rayonnements ultraviolets et les dégâts induits par les UV, tels que les dimères de pyrimidine, ne peuvent pas être réparés dans l'ADNmt. Il a même été indiqué que les mutations dans l'ADNmt pourraient constituer un biomarqueur de la photo-exposition et personnaliser la surveillance

de risque de cancer (Birch-Machin et al., 2013). Cependant, actuellement, l'on ne sait pas si les mutations de l'ADNmt identifiées dans des mélanomes sont le résultat de la photo-exposition ou un d'un processus lié à la tumorigénèse. Dans le contexte des DM, il est possible que les mutations photo-induites soient à l'origine de l'initiation de la réponse interférongénique, qui se pérennise ensuite au niveau musculaire par les mécanismes que nous avons décrits.

Mitochondries, RLO, DM et virus : Le rôle de l'environnement viral au cours des DM est suggéré par le fait qu'une infection virale est souvent mise en évidence dans les mois ayant précédé le début de la maladie (Manlhiot et al., 2008). La présence d'une signature interféron de type 1 (Greenberg et al., 2005) et l'hyperexpression de plusieurs récepteurs de l'immunité innée antivirale (TLR endosomaux, TLR4, MDA5, RIG 1) dans le muscle et/ou les leucocytes des patients atteints de DM (Li et al., 2015) suggèrent aussi l'implication de cette voie dans ce sous-groupe. Enfin, l'antigène reconnu par les anticorps anti-MDA5, spécifiques des DM, est un ligand de l'immunité innée antivirale. La fréquence de ces auto-anticorps a été reliée à l'épidémiologie des infections à coxsackie (Muro et al., 2011).

La mitochondrie est un acteur de la réponse interféron en réponse aux infections virales, par au moins deux mécanismes. Il a été montré que les herpèsvirus induisent la libération d'ADNmt dans le cytoplasme, qui est reconnu par le récepteur antiviral cGAS, ce qui est à l'origine d'une potentialisation de la réponse interféron de type I (West et al., 2015). La cellule se sert ainsi de l'ADNmt pour booster la réponse antivirale. Il a aussi été montré que des dysfonctions mitochondriales et l'induction de RLO mitochondriaux étaient capables d'augmenter la réponse interféron antivirale en augmentant le signal véhiculé par la MAVS (Castanier et al., 2010; Nobre et al., 2015; Zhao et al., 2012), une molécule en aval de la cascade de signalisation provoquée par l'engament des récepteurs antiviraux RIG1 et MDA5, et dont l'activation nécessite sa localisation à la mitochondrie. Ces mécanismes sont détaillés dans le chapitre « *Le rôle de la mitochondrie dans la régulation de l'immunité* ».

Dans le contexte de la DM, il est possible que la réponse interféron médiée par l'infection virale dépasse son objectif physiologique parce que l'amplification du signal au niveau de la mitochondrie est trop importante et se pérennise au niveau musculaire par les mécanismes que nous rapportons.

Mitochondries, RLO, DM et médicaments : Plusieurs médicaments ont été mis en cause dans la survenue de DM.

Le traitement par l'IFN-β dans le cadre de la sclérose en plaques peut se compliquer de DM (Somani et al., 2008). Nous rapportons ici que l'IFN-β induit des dysfonctions mitochondriales.

Nous rapportons une patiente qui a développé une DM dans les suites d'un traitement par l'imiquimod. Après la publication de notre observation, il a été rapporté que l'imiquimod provoque une inhibition du complexe I, à l'origine d'une production de RLO mitochondriaux qui induit l'activation de NLRP3 (Groß et al., 2016). Ainsi, il est possible que l'effet pro-inflammatoire de cette molécule que nous avons enregistré chez notre patiente repose aussi sur cette dysfonction mitochondriale.

5- Le rôle de la mitochondrie dans la régulation de l'immunité

Plusieurs récepteurs de l'immunité innée (TLR3, TLR9, RIG1) sont surexprimés dans le muscle des patients atteints de DM, et ceci pourrait contribuer au maintien de l'inflammation musculaire (Li et al., 2015). Parmi ceux-ci, RIG1 a été spécifiquement relié à la DM (Suárez-Calvet et al., 2017). Cependant, les mécanismes conduisant à l'activation de ces récepteurs pas été expliqué. Nos données suggèrent que les dysfonctions mitochondriales des fibres périfasciculaires pourraient être impliquées dans ce phénomène. Ceci est en accord avec une littérature récente indiquant que la mitochondrie est un régulateur de l'immunité.

5.1 Le rôle de la mitochondrie dans la réponse immunitaire innée

La mitochondrie est une plateforme essentielle pour la transduction du signal de plusieurs récepteurs de l'immunité innée

Les récepteurs de l'immunité innée sont des protéines membranaires ou cytoplasmiques qui sont capables de reconnaitre des motifs moléculaires exogènes, portés par des virus ou des bactéries (*pathogen associated molecular patterns : PAMPs*), mais aussi des motifs moléculaires endogènes qui se trouvent à leur proximité dans les situations d'agression cellulaire (*danger associated molecular patterns : DAMPs*). L'engagement de ces récepteurs conduit à l'inflammation.

Plusieurs voies de signalisation de ces récepteurs interagissent avec la mitochondrie (Monlun et al., 2017) (figure 1).

Les récepteurs TLR 1, 2, 4 augmentent l'activité bactéricide des macrophages par production de radicaux libres dérivés de l'oxygène (RLO). Cette réponse implique la translocation de TRAF6, une protéine impliquée dans la transduction du signal de ces TLRs, vers la mitochondrie, conduisant à l'ubiquitination et à la dégradation de la **protéine mitochondriale ECSIT**, une protéine impliquée dans l'assemblage de la chaîne respiratoire mitochondriale, ainsi qu'à une augmentation de la production de RLO mitochondriaux. Les macrophages déplétés en ECSIT ont un niveau de production de RLO induit par les TLR qui est diminué et un pouvoir bactéricide altéré (West et al., 2011).

La signalisation par TLR7 est régulée positivement par la protéine de la membrane externe mitochondriale **MARCH5**. En réponse à l'engagement de TLR7, MARCH5 catalyse la poly-ubiquitination et la dégradation de TANK, un répresseur de la voie de signalisation des TLRs. Le KO de MARCH5 altère l'activation de NF-kB induite par TLR7 (Shi et al., 2011a).

La cascade de signalisation des RIG like receptors (RLRs: RIG-1 and MDA-5) implique **la MAVS**, une protéine dont l'association à la membrane mitochondriale externe est nécessaire pour une transduction du signal par ces récepteurs (Seth et al., 2005).

L'activation de NLRP3 requiert également son recrutement vers la mitochondrie et son interaction avec la MAVS pour aboutir à la production d'interleukine 1 (Subramanian et al., 2013).

Le fonctionnement mitochondrial module les voies de signalisation qui convergent à la mitochondrie

Les dysfonctions mitochondriales de la chaine de respiration induites par le blocage du complexe I (Nobre et al., 2015; Tal et al., 2009) ou la surexpression de la sous-unité COX5B du complexe IV (Zhao et al., 2012) augmentent la formation de RLO mitochondriaux et accroissent le signal des RLRs par l'intermédiaire de la MAVS.

L'inhibition du découplage mitochondrial par le KO pour la protéine uncoupling protein 2 (*UCP2*) augmente la formation de RLO mitochondriaux et le signal de TLR4 (Emre et al., 2007), ce qui confère, chez la souris, une résistance complète à l'infection à T. Gondii (Arsenijevic et al., 2000).

Un défaut de mitophagie conduit à une accumulation de mitochondries défectueuses et de RLO mitochondriaux qui provoquent une augmentation du signal par les RLRs (Tal et al., 2009) et par l'inflammasomme (Zhou et al., 2011).

La fusion et la fission mitochondriales expérimentales conduisent respectivement à l'augmentation et à la diminution de la réponse inflammatoire conduite par la MAVS (Castanier et al., 2010).

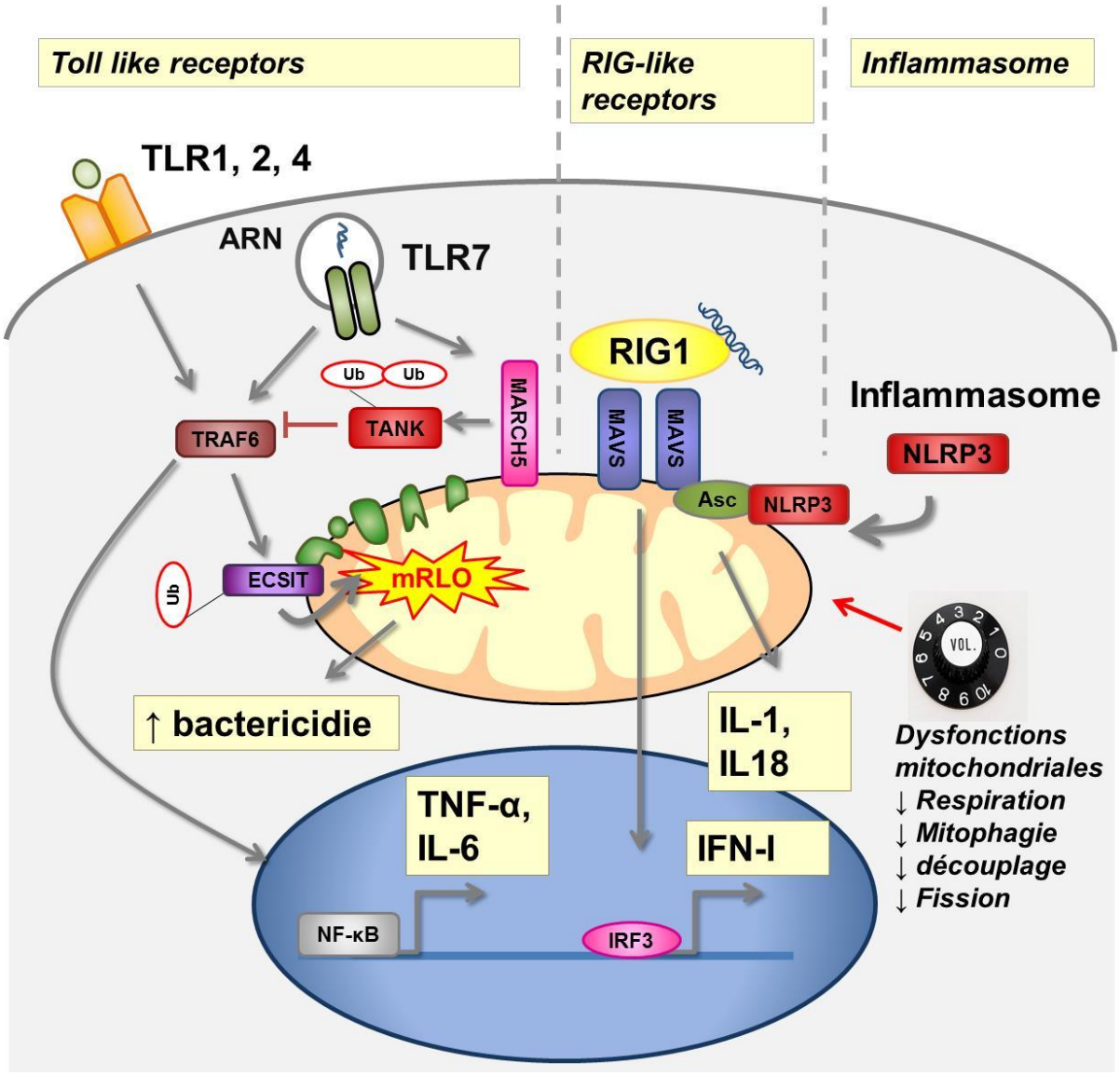

Figure 1 : Les voies de signalisation de TLR1, 2, 4, 7, RIG1 et NLRP3 impliquent des protéines mitochondriales pour aboutir à l'inflammation. Des dysfonctions mitochondriales telles qu'une inhibition de la respiration, de la mitophagie, du couplage ou de la fission aboutissent à une augmentation du signal de ces voies pro-inflammatoires.

Le relargage de fragments mitochondriaux active les récepteurs de l'immunité innée

En plus de leur capacité de moduler la signalisation des récepteurs de l'immunité innée, les mitochondries contiennent des ligands des récepteurs de l'immunité innée. Dans des conditions pathologiques (infection, traumatisme, maladies auto-immunes), ces ligands

peuvent sortir de la mitochondrie dans le cytoplasme et/ou le milieu extra cellulaire. Ils peuvent alors agir comme des DAMPs en liant leurs récepteurs et en activant l'inflammation (Figure 2).

Le récepteur cGAS est un senseur de l'ADN étranger dans le cytosol des cellules mammifères et déclenche une réponse interféron de type I. Il a été montré que, lors d'une infection virale (West et al., 2015) et de la mort cellulaire non apoptotique (Rongvaux et al., 2014), **l'ADN mitochondrial** est relâché dans le cytosol et est capable d'activer ce récepteur et la synthèse d'interféron de type I. Au cours du lupus, un relargage d'ADN mitochondrial oxydé contribue à la sécrétion d'interféron de type I par l'engagement de ce récepteur (Lood et al., 2016).

TLR9 est un TLR endosomal qui déclenche une réponse interféron de type I en réponse à sa liaison avec des fragments d'ADN portant des motifs CpG. Ces motifs sont caractéristiques de l'ADN bactérien. **L'ADN mitochondrial** a conservé ces motifs CpG au cours de son évolution et est capable d'activer TLR9 notamment au cours du syndrome de réponse inflammatoire systémique (Zhang et al., 2010) .

Le formyl peptide receptor-1 (FPR-1) reconnait les motifs formyl peptides qui sont portés par les protéines bactériennes. Les protéines mitochondriales ont conservé ces motifs au cours de leur évolution et sont capables d'activer FPR-1 (Zhang et al., 2010).

L'activation de NLRP3 et de l'inflammasome en réponse aux dommages cellulaires est déclenchée en partie par **l'ATP qui est libérée des mitochondries** (Iyer et al., 2009).

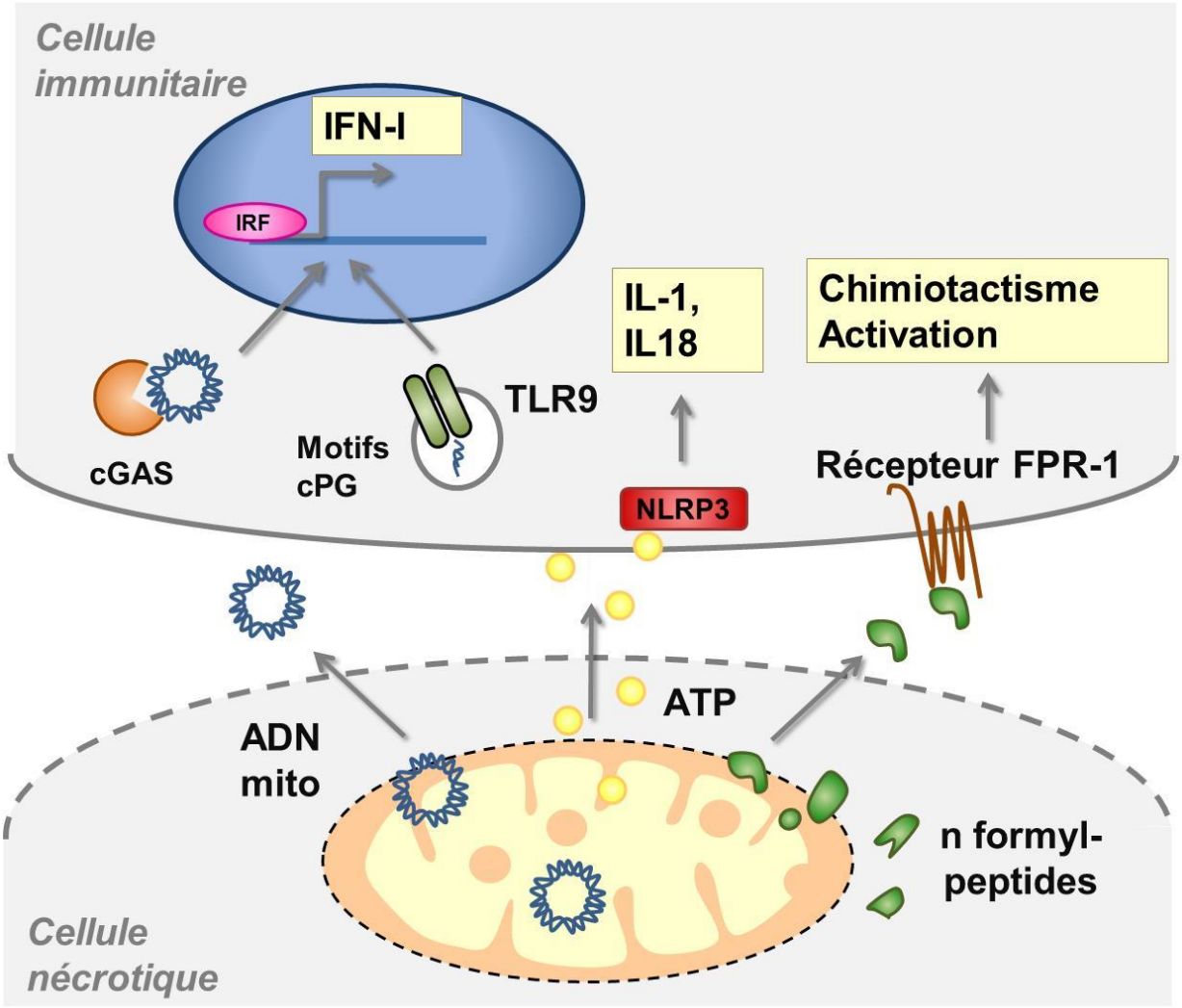

Figure 2 : Lors de situations pathologiques conduisant à la nécrose cellulaire, la mitochondrie libère des composés mitochondriaux qui agissent comme des DAMPs et active l'immunité innée.

5.2 Le rôle de la mitochondrie dans la réponse immunitaire adaptative

Ces cellules de l'immunité adaptative régulent leur activation et leur différenciation par l'intermédiaire de leurs mitochondries

L'activation et la différenciation des lymphocytes B et T reconnaissant des antigènes constituent la réponse immunitaire adaptative. Ces cellules de l'immunité adaptative régulent leur activation et leur différenciation par l'intermédiaire de leurs mitochondries.

Il a été démontré que **l'activation des lymphocytes T** requiert une augmentation de la consommation du glucose et de la glutamine mettant en jeu la mitochondrie (Carr et al., 2010; Sinclair et al., 2013), une translocation mitochondriale vers la synapse immunologique (Quintana et al., 2007) et la formation de RLO mitochondriaux (Sena et al., 2013).

Une fois activés, **les lymphocytes T se différencient** en lymphocytes pro-inflammatoires (notamment Th1, Th2, Th17, Th22) ou en lymphocytes anti-inflammatoires (Treg). Les Th17 ont un métabolisme glycolytique plus élevé et un niveau de phosphorisation oxydative plus bas que les Th17 (Michalek et al., 2011). *In vitro*, l'inhibition de l'oxydation des acides gras par l'etomoxir diminue la différenciation des Treg, mais n'affecte pas la différenciation des lymphocytes pro-inflammatoires (Berod et al., 2014). À l'inverse, l'inhibition pharmacologique du métabolisme du glucose par l'administration de désoxyglucose diminue le développement des Th17 et protège de l'encéphalomyélite auto-immune expérimentale, un modèle murin de pathologie Th17-dépendante (Shi et al., 2011b)

L'activation des lymphocytes B requiert aussi une augmentation de la consommation du glucose et de la glutamine (Le et al., 2012) ainsi qu'une augmentation de la production de RLO (Capasso et al., 2010) d'origine mitochondriale (Wheeler and Defranco, 2012).

Les cellules présentatrices d'antigènes modulent leur maturation et leurs signaux activateurs des lymphocytes T par l'intermédiaire des mitochondries

Les cellules dendritiques sont des cellules spécialisées dans la présentation des antigènes aux lymphocytes T. Lors de l'exposition à un antigène, les cellules dendritiques entrent en maturation, en augmentant leur capacité phagocytaire, leur expression du complexe majeur d'histocompatibilité et migrent vers les organes immunitaires secondaires riches en lymphocytes T.

L'activation de ce programme de maturation des cellules dendritiques entraîne une diminution de la respiration mitochondriale et une augmentation du métabolisme

glycolytique dans les cellules dendritiques (Everts et al., 2012) qui est nécessaire pour répondre aux exigences bioénergétiques et biosynthétiques accrues d'un DC activé (Everts et al., 2014). De façon schématique, les cellules dendritiques peuvent exercer une activité immunogène ou une activité tolérogène. Les cellules dendritiques immunogènes présentent une forte activité phagocytaire, une expression forte du complexe majeur d'histocompatibilité après activation, et induisent l'activation de lymphocytes T effecteurs. Au contraire, les cellules dendritiques tolérogènes se caractérisent par une résistance à la maturation ainsi que par l'expression de facteurs immunomodulateurs, ce qui induit une augmentation de la réponse des lymphocytes T régulateurs (Treg). Il a été montré que les cellules dendritiques tolérogènes ont un fort niveau d'oxydation des acides gras et de faibles niveaux de glycolyse (Cook et al., 2012; Ferreira et al., 2012; Szanto et al., 2010). Des cellules dendritiques qui n'ont pas de PPAR-γ, un gène favorisant la biogenèse mitochondriale, ont une plus grande immunogénicité et une moindre capacité à induire les mécanismes de tolérance immunitaire (Klotz et al., 2007).

Certains néo-peptides mitochondriaux (émergeant du taux de mutation élevé de l'ADN mitochondrial : ~ 10 - 20 - fois le taux de l'ADN nucléaire) sont reconnus par le système immunitaire adaptatif et peuvent contribuer à la rupture de tolérance (Chen et al., 2014; Duvvuri et al., 2014). De façon intéressante, la plupart des aminoacyl-ARNt synthétases (qui sont ciblées par des auto-anticorps au cours du syndrome des antisynthétases, une maladie auto-immune touchant les muscles squelettiques, la peau et les poumons) sont synthétisées à la fois par le génome nucléaire et le génome mitochondrial.

Génétique mitochondriale et maladies auto-immunes ou inflammatoires

De façon concordante avec un rôle de la mitochondrie dans la modulation de l'immunité innée et adaptative, des polymorphismes et/ou des mutations dans des codant des protéines impliquées dans la structure et/ou la fonction mitochondriale ont été liés à une prédisposition

à développer des maladies auto-immunes et/ou inflammatoires (Chen et al., 2014; Duvvuri et al., 2014).

Les patients atteints de cytopathies mitochondriales (mutation monogénique provoquant un défaut dans la chaîne respiratoire mitochondriale) développent des pathologies inflammatoires telles que des myosites (Marotta et al., 2009), la sclérose en plaques (Degos et al., 2014; Echaniz-Laguna et al., 2010) et des polyendocrinopathies auto-immunes (Bortot et al., 2009).

Des polymorphismes de l'ADN mitochondrial ont été reliés au lupus et à la sclérose en plaques chez l'homme (Jönsen et al., 2009; Vyshkina et al., 2008) et à la susceptibilité à des pathologies auto-immunes expérimentales (arthrite au collagène, pancréatite auto-immune, glomérulonéphrite) chez la souris (Yu et al., 2009).

Des polymorphismes de gènes nucléaires codant des protéines mitochondriales ont aussi été impliqués dans l'émergence de pathologies inflammatoires telles que celles dans le gène *UCP2* qui ont été reliées à la susceptibilité à la sclérose en plaques chez l'homme (Vogler et al., 2005) et à l'encéphalite auto-immune expérimentale chez la souris (Vogler et al., 2006).

Des souris KO pour Nrf2, un gène régulant la réponse antioxydante, développent une glomérulonéphrite qui ressemble au lupus (Yoh et al., 2001).

6- L'implication de ces résultats au-delà des dermatomyosites

Le rôle des dysfonctions mitochondriales et des RLO que nous avons mis en évidence au cours des DM pourrait avoir des implications plus générales.

- D'une part, des mécanismes similaires pourraient être à l'œuvre dans d'autres types cellulaires, expliquant la pérennisation des connectivites telles que la polyarthrite rhumatoïde, le lupus, la sclérodermie systémique et le Sjögren ;
- D'autre part, nos résultats pourraient mettre en lumière un mécanisme général liant l'inflammation et les diminutions de capacité d'exercice survenant de façon caricaturale au cours des DM et de façon modérée, mais significative au cours de

nombreux états pathologiques caractérisés par une inflammation systémique et une intolérance à l'effort.

6.1 Le rôle de la mitochondrie au cours d'autres maladies auto-immunes

6.1.1 La polyarthrite rhumatoïde

La polyarthrite rhumatoïde (PR) est une maladie auto-immune qui se caractérise par une prolifération du tissu synovial (pannus synovial), responsable de douleurs, du gonflement et de la destruction des articulations. Plusieurs études ont montré que les synoviocytes de la membrane synoviale des patients atteints de PR ont des dysfonctions mitochondriales caractérisées, notamment par une diminution de la respiration mitochondriale accompagnée d'une augmentation de la production des RLO ; et que ces modifications participent à la pathogénie de la PR (Fearon et al., 2016). Les dysfonctions mitochondriales induites par l'oligomycine (un inhibiteur de l'ATP synthase) dans des synoviocytes sains induisent la production de cytokines impliquées dans la PR (COX-2, PGE2, et IL8) de façon RLO dépendante, et amplifient la synthèse de ces cytokines en réponse à l'IL-1β (Onodera et al., 2015; Valcárcel-Ares et al., 2014). Les RLO stimulent aussi la production de metalloprotéases, inhibent la synthèse des protéoglycanes du cartilage (Henrotin et al., 2003) et accélèrent la résorption osseuse (Tsay et al., 2010). Des débris d'ADNmt sont présents dans le liquide synovial des patients atteints de PR, et leur concentration est corrélée à l'activité de la maladie. Ces débris sont capables d'activer l'expression de RANKL par les PNN (Contis et al., 2017).

Les anomalies mitochondriales constatées dans les synoviocytes rhumatoïdes pourraient être multifactorielles, mettant en jeu des facteurs induits et constitutionnels. Une hypoxie a été enregistrée dans le pannus rhumatoïde dont l'importance est corrélée à l'épaisseur du pannus et est associée aux taux de cytokines pro-inflammatoires dans le pannus (Ng et al., 2010). Dans les synoviocytes, rhumatoïde, l'induction de la réponse à l'hypoxie entraine une

diminution des capacités de respiration mitochondriale, de la synthèse de l'ATP, des anomalies de morphologie mitochondriale et une augmentation de la production des RLO (Biniecka et al., 2016). Par ailleurs, l'IL17 augmente les anomalies mitochondriales des synoviocytes rhumatoïdes (Kim et al., 2017). Enfin, un polymorphisme dans le gène de la catalase (C262T), une enzyme antioxydante a été associée à une plus forte activité de la PR (Bohanec Grabar et al., 2009), et des anticorps dirigés contre cette enzyme ont été mis en évidence chez les patients atteints de PR (Mansour et al., 2008).

6.1.2 Le lupus érythémateux systémique

Le lupus érythémateux systémique (LES) est une maladie auto-immune qui touche de nombreux organes. Elle se caractérise notamment par une activation aberrante des lymphocytes B et T, une sécrétion de cytokine pro-inflammatoire (dont les IFN de type I) et d'auto-anticorps (différents de ceux des myopathies inflammatoires, dirigés contre les acides nucléiques). Des données, dont les plus récentes sont concomitantes de nos résultats, indiquent que la mitochondrie et les RLO sont impliqués dans le LES, selon des mécanismes qui ressemblent en partie à ceux que nous avons décrits au cours de la DM.

Une augmentation de la production de RLO, une hyperpolarisation de la membrane mitochondriale, une diminution de la phosphorisation oxydative et une diminution de la synthèse d'ATP ont été constatées dans les leucocytes des patients atteints de LES, de façon prédominante dans les lymphocytes T. Ces anomalies lymphocytaires ont été associées à une augmentation de l'apoptose, une diminution de la capacité à sécréter l'IL10 et une augmentation de la sécrétion d'IFN-I (Buskiewicz et al., 2016; Gergely et al., 2002a, 2002b). De façon similaire au modèle que nous proposons pour expliquer le maintien de l'inflammation et des dégâts musculaires de la DM, il a été proposé récemment que le phénotype des lymphocytes lupiques était probablement induit par une boucle d'auto-entretien impliquant une oligomérisation de la MAVS, induite par les RLO et une augmentation de la sécrétion d'IFN-I. En effet, l'oligomérisation de la MAVS dans les

lymphocytes lupiques était corrélé à la sécrétion d'IFN-I et à la production de RLO mitochondriaux, dont l'inhibition supprimait l'oligomérisation de la MAVS et la sécrétion d'IFN-I. (Buskiewicz et al., 2016).

De façon similaire, il a été rapporté encore plus récemment que les cellules souches mésenchymateuses dérivées de la moelle osseuse des patients atteints de LES ont aussi une forte production augmentée de RLO, associée à un taux de prolifération réduit, une augmentation des cytokines pro-inflammatoires, dont l'IFN-β, et une faible sécrétion de cytokines anti-inflammatoires. Le même mécanisme d'auto-entretien a été proposé pour expliquer ce phénotype. En effet, le *silencing* de la MAVS supprimait la sécrétion d'IFN-β et le phénotype particulier de ces cellules (Gao et al., 2017).

Les PNN sont une source importante de la stimulation de la sécrétion d'IFN-I au cours du LES. Lorsque des PNN de patients lupiques (ou des PNN de patients sains traités à l'IFN-α) sont exposés à des anticorps anti-Sm/RNP, ils meurent par NETose en libérant des fragments d'ADN qui sont très interferongéniques (Garcia-Romo et al., 2011). Il a été récemment montré que ces fragments sont reconnus par le récepteur antiviral de l'immunité cGAS (Lood et al., 2016) et que le pouvoir interferongénique de ces fragments d'ADN est lié à leur origine mitochondriale ainsi qu'au fait qu'ils soient oxydés (Lood et al., 2016). Ceci est dû à un défaut de dégradation lysosomale de l'ADNmt oxydé, induit par l'IFN-I (Caielli et al., 2016) et dépendant des RLO (Lood et al., 2016).

Comme au cours de la PR, une partie de ces anomalies pourrait être immuno-médiée : des anticorps anticatalase, anti-SOD (Kurien and Scofield, 2003; Mansour et al., 2008) et anti-ADNmt oxydés (Caielli et al., 2016) ont été mis en évidence chez les patients atteints de LES, mais leur rôle pathogène n'a pas été étudié. Plusieurs polymorphismes dans des gènes impliqués dans la biogenèse des RLO et/ou la mitochondrie ont été associés au risque et/ou à la sévérité du LES tels que l'allèle NCF1-339T qui code pour une sous-unité de NOX2 (Olsson et al., 2017), SOD2-Ala16Val (codant la SOD mitochondriale) (Jevtovic Stoimenov et al., 2017), Nrf2-653G (codant un facteur majeur de la régulation de la réponse antioxydante) (Córdova et al., 2010), ND2-mt4917G (codant une sous-unité du complexe I),

ATP6-mt9055A (codant une sous-unité du complexe V) (Vyshkina et al., 2008) et mt16189 T (présent dans une région non codante qui pourrait être impliquée dans la régulation de la réplication et la transcription) (Jönsen et al., 2009). Un polymorphisme de la MAVS a été associé à une activité moindre du LES (Buskiewicz et al., 2016).

6.1.3 La sclérodermie systémique

La sclérodermie systémique (SSc) est une connectivite caractérisée par une fibrose progressive de nombreux organes. L'atteinte cutanée est la caractéristique de la SSc, mais la mortalité est en rapport avec l'atteinte viscérale. Cette fibrose repose sur des mécanismes auto-immuns et sur une vasculopathie. Plusieurs équipes, dont la nôtre, ont mis en évidence un rôle des RLO au cours de la SSc.

Différents marqueurs de stress oxydatif sont élevés dans le plasma des patients atteints de SSc (Cruz-Domínguez et al., 2013). Les niveaux de RLO sont aussi plus élevés dans la peau (Bourji et al., 2015) et dans les fibroblastes dermiques des patients atteints de SSc (Sambo et al., 2001; Tsou et al., 2012). Le statut antioxydant est altéré chez les patients atteints de SSc. La glutathion transférase des érythrocytes (eGST) (Fabrini et al., 2013) et de l'augmentation du pouvoir antioxydant total (TAP) (Ogawa et al., 2011) dans le sang sont augmentés. À l'inverse, les systèmes antioxydants non enzymatiques tels que l'acide ascorbique (vitamine C), l'α-tocophérol, le β-carotène et le sélénium sont diminués dans le sang des patients atteints de SSc (Bourji et al., 2015; Herrick and Matucci Cerinic, 2001; Lundberg et al., 1992).

Plusieurs études ont montré une association de ces marqueurs avec des facteurs de mauvais pronostic de la maladie. Les niveaux de RLO dans la peau fibrotique (Bourji et al., 2015) et l'augmentation des marqueurs de stress oxydatif dans le sang (Ogawa et al., 2006) des patients atteints de SSc sont inversement corrélés avec la capacité vitale. Les marqueurs de stress oxydatif sont plus élevés chez les patients SSc atteints d'HTAP (Hoshikawa et al., 2001; Reis et al., 2013). Un stress oxydatif (Cruz-Domínguez et al., 2013),

un niveau de défense antioxydante totale (Ogawa et al., 2011) dans le sang et les niveaux de RLO dans la peau fibrotique (Bourji et al., 2015) des patients atteints de SSc sont associés à un syndrome inflammatoire (augmentation de la VS et/ou de la CRP). Les marqueurs de stress oxydant dans le sang sont plus élevés chez les patients porteurs d'anticorps anti-Scl70 (Cruz-Domínguez et al., 2013).

Un rôle de l'auto-immunité dans cette dérégulation a été proposé. Des anticorps anti-Prx I (peroxirédoxine I) ont été mis en évidence chez des patients atteints de SSc limitées ou diffuses (mais aussi au cours d'autres maladies auto-immunes) (Iwata et al., 2007). Le même groupe a montré que des anticorps anti-MSRA (Methionine Sulfoxide Réductase A) sont présents chez les patients atteints de SSc et que leur taux est positivement corrélé à la gravité de la maladie (Ogawa et al., 2010).

À l'image du rôle des RLO dans la maintenance de l'inflammation et des dégâts sur les fibres musculaires que nous rapportons dans la DM, il a été montré que ces molécules participent au phénotype pro-inflammatoire et fibrosant des fibroblastes sclérodermiques. Le traitement de ces fibroblastes avec la NAC inhibait la production de RLO et s'accompagnait d'une diminution de la prolifération et de la production de collagène a1 (I) et a2 (I) par ces cellules (Sambo et al., 2001). Ceci repose sur une activation aberrante du récepteur au PDGF (PDGFR) dont la déphosphorylation inactivatrice par la protéine tyrosine phosphatase (PTP) 1 b est bloquée en raison de l'oxydation de cette dernière enzyme (Tsou et al., 2012). Le rôle de la mitochondrie dans ces anomalies n'a pas été étudié. Nous projetons de caractériser, chez les patients atteints de SSc, les fonctions mitochondriales des PBMC, leurs liens avec la production de RLO, la métabolomique de la peau, la dysfonction endothéliale et les caractéristiques conventionnelles de la maladie (PHRC interrégional 2014-A00518-39 « Scléromics »).

6.1.4 Le syndrome de Sjögren

Le syndrome de Sjögren est une maladie auto-immune des glandes exocrines, qui s'accompagne parfois de manifestations viscérales**.** Une augmentation des RLO et/ou des marqueurs de stress oxydant ont été mis en évidence dans le sang (Norheim et al., 2012), la salive (Shimizu et al., 2008), l'épithélium conjonctival (Cejková et al., 2008) et les larmes (Li et al., 2014; Wakamatsu et al., 2013) des patients atteints de SS. L'origine de ces anomalies n'a pas encore été déterminée.

6.2 Un retentissement musculaire de l'inflammation joue-t-il un rôle dans la fatigue des rhumatismes inflammatoires ? (PHRC interégional 2016 « FaMuSpa »)

La spondylarthropathie axiale (SpA) est l'un des rhumatismes inflammatoires les plus fréquents (≈1 % de la population générale) (REVEILLE et al., 2012). Pourtant, cette pathologie serait encore sous-diagnostiquée, et un quart des patients de 20-45 ans évalués en médecine générale pour des rachialgies chroniques aurait en fait une SpA méconnue (van Hoeven et al., 2014).

La SpA a été définie en 2009 par les critères de l'Assessment of Spondylo Arthritis international Society (ASAS) (Rudwaleit et al., 2009) comme la présence de rachialgies inflammatoires chroniques (>3 mois) ayant débuté avant l'âge de 45 ans et associées à :

- Soit la présence d'une sacro-iliite (démontrée par des radiographies ou une IRM) accompagnée d'un autre élément clinico-biologique caractéristique de spondylarthropathie.
- Soit la présence de l'HLAB27 associé à deux autres éléments clinico-biologiques caractéristiques de la spondylarthropathie.

Les autres éléments clinico-biologiques caractéristiques de spondylarthropathie étant la présence d'arthrite(s), d'enthésite(s) d'uvéite(s), de dactylite(s), d'un psoriasis, d'une

maladie inflammatoire chronique de l'intestin, une histoire familiale de spondylarthropathie, l'HLAB27 et/ou la bonne réponse de la rachialgie aux AINS.

Les mécanismes physiopathologiques sont une inflammation des enthèses (zone de transition des ligaments et des tendons à l'os) qui, probablement par contigüité, conduit à une inflammation de la synoviale et/ou de l'os (Kehl et al., 2016). Cette inflammation se caractérise notamment par une expression importante de TNFα (Brandt et al., 2000), d'interleukine 12/23 (Poddubnyy et al., 2014) et d'IL-17 (Baeten et al., 2015) dans les enthèses et dans le sang dont l'antagonisation améliore la maladie.

Les facteurs étiologiques sont génétiques et environnementaux. L'allèle HLA B27 (un haplotype de l'HLA de classe 1 codé par le locus B du complexe majeur d'histocompatibilité) est présent chez environ ¾ des patients atteints de SpA. Sa présence expose à un risque de 2-10 % de développer une SpA (Taurog et al., 2016). Une trentaine d'autres gènes augmentant le risque de SpA a été identifiée, codant généralement des protéines impliquées dans la régulation de l'inflammation (Robinson and Brown, 2014).

Pourtant, la concordance entre jumeaux est d'environ 50 % (Lambert, 2016), indiquant que des facteurs de l'environnement sont impliqués. Ceux-ci sont notamment représentés par des agents microbiens (Costello et al., 2014) et des facteurs traumatiques locaux (Jacques et al., 2014).

Les conséquences individuelles et médico-économiques de la SpA sont importantes. Les patients atteints de SpA souffrent de handicaps (Dagfinrud et al., 2004) dont l'importance est similaire à celle enregistrée au cours des cancers, de l'insuffisance cardiaque, du diabète ou de la dépression (Braun et al., 2007). Ils présentent une réduction de la qualité de vie (Webers et al., 2016), et environ un quart des patients sont en incapacité de travail complète (Castillo-Ortiz et al., 2016). La SpA réduit aussi l'espérance de vie (Exarchou et al., 2016), principalement du fait d'une augmentation du risque d'événements cardiovasculaires

secondaires à la sédentarité et/ou l'inflammation (Bakland et al., 2011; Haroon et al., 2015). Ces éléments sont associés à l'importance de l'activité de la maladie.

La fatigue est une caractéristique majeure de la spondylarthrite. Elle peut être définie comme une sensation de réduction des capacités musculaires, un manque d'énergie et d'épuisement (Bedaiwi et al., 2015). Elle forme avec la douleur et la raideur l'un des trois composants du Bath AS Disease Activity Index (BASDAI) qui est largement utilisé pour évaluer l'activité de la maladie SpA (Garrett et al., 1994). Un score ≥ 5 à la première question de ce score (« Où situeriez-vous votre degré global de fatigue ? » entre 0 et 10) est généralement considéré comme correspondant à une fatigue sévère, (Dernis-Labous et al., 2003; Gossec et al., 2016; van Tubergen et al., 2002). La fatigue peut être aussi évaluée par des scores dédiés comme le *fatigue severity scale* (FSS). Un seuil ≥ 4, est retenu comme correspondant à une fatigue anormalement haute (appelée « fatigue sévère » par la suite dans ce paragraphe) (Bedaiwi et al., 2015; Fava et al., 2005; Hadjimichael et al., 2008; Krupp et al., 1989). Selon ces deux définitions, la fatigue atteint un niveau sévère chez plus de deux tiers des patients atteints de spondylarthropathie (Aissaoui et al., 2012; Bedaiwi et al., 2015; Calin et al., 1993; Haywood et al., 2014; Missaoui and Revel, 2006). Elle a été associée à un handicap plus important, un état de santé et une qualité de vie moins bons (Bedaiwi et al., 2015; Bianchi et al., 2014; Stebbings et al., 2014; van Tubergen et al., 2002).

Un retentissement musculaire squelettique est présent au cours des SpA. Il se caractérise par une diminution des capacités d'exercice indépendamment des douleurs et de l'ankylose, (Carter et al., 1999; Hebestreit et al., 1998; Mengshoel et al., 2004; Sahin et al., 2011a) mais associée à une diminution de la force (Carter et al., 1999; Marcora et al., 2006; Mengshoel et al., 2004; Sahin et al., 2004, 2011a, 2011b) et de la masse musculaire (Marcora et al., 2006; Plasqui et al., 2012; El Maghraoui et al., 2016; Toussirot et al., 2001) dont l'importance est variable suivant les études.

L'origine de cette atteinte musculaire est vraisemblablement multifactorielle. Le degré de sédentarité est corrélé à la diminution de la masse musculaire (Plasqui et al., 2012). Les cytokines pro-inflammatoires pourraient aussi jouer un rôle indépendamment de cette sédentarité. La perte de la masse musculaire est corrélée à l'intensité du syndrome inflammatoire (Plasqui et al., 2012). Le TNFα, dont le taux plasmatique est augmenté au cours des SpA, conduit à une atrophie des cellules musculaires *in vitro* (De Larichaudy et al., 2012) et à une diminution de la force chez l'animal (Reid et al., 2002). De façon concordante, même si les données sont limitées, chez l'animal (Buck et al., 1996) et chez les patients atteints de SpA (Briot et al., 2005), les anti TNFα augmenteraient la masse musculaire. La masse musculaire serait plus importante chez les patients qui prennent ce traitement (El Maghraoui et al., 2016).

L'origine et les facteurs de risques de la fatigue au cours des SpA sont inconnus

- Un rôle de l'inflammation a été suggéré par le fait que la fatigue est corrélée à l'activité inflammatoire clinique et/ou biologique (Bedaiwi et al., 2015; Gossec et al., 2016; Ibn Yacoub et al., 2010; Stebbings et al., 2014; van Tubergen et al., 2002), mais ceci n'a pas été mis en évidence dans toutes les études (Dagfinrud et al., 2004; Stebbings et al., 2014). L'effet positif des anti-TNFα (Bedaiwi et al., 2015; Brophy et al., 2013) suggère aussi qu'une partie de la fatigue au moins réside sur l'inflammation, mais cet effet est partiel, et 80 % des patients garderaient un niveau de fatigue sévère malgré ce traitement, ce qui suggère que d'autres facteurs sont en jeu (Bedaiwi et al., 2015).
- Une plus forte prévalence chez la femme (Bianchi et al., 2014; Missaoui and Revel, 2006) pourrait indiquer que des facteurs génétiques, environnementaux et/ou culturels pourraient aussi être en cause. Ce résultat n'a cependant pas été reproduit par toutes les études ou seulement avec un effet marginal (Bedaiwi et al., 2015; Gossec et al., 2016). D'autre part, une plus grande fréquence de la fatigue chez la femme est aussi présente dans la population générale (Dagfinrud et al., 2005).

- Une association avec certaines caractéristiques de la maladie comme la présence d'une atteinte digestive (Stebbings et al., 2014) et d'enthésites (Bedaiwi et al., 2015) suggère que des facteurs propres au processus pathologique sont en jeu. Cependant, ces associations n'ont pas été enregistrées par toutes les études (Bedaiwi et al., 2015).
- Une plus grande sédentarité déclarée (Bianchi et al., 2014; van Tubergen et al., 2002) a été aussi enregistrée chez les patients les plus fatigués, qui pourrait aussi bien refléter un effet qu'une cause de la fatigue au cours des SpA.
- Au contraire de la polyarthrite rhumatoïde (Munsterman et al., 2013), les facteurs psychologiques tels que la dépression et l'anxiété auraient un effet limité sur la fatigue au cours des SpA (Aissaoui et al., 2012; Brophy et al., 2013).

L'implication du retentissement musculaire squelettique dans la fatigue n'a pas été étudiée au cours des spondylartropathies.

- Le muscle squelettique représente 40 % du poids du corps. Il assure la locomotion, mais exerce aussi des effets systémiques, notamment endocrino-métaboliques (Karsenty and Olson, 2016), immunologiques (Alexanderson and Lundberg, 2012) et neuropsychologiques (Agudelo et al., 2014) .
- Un meilleur statut musculaire a été relié à une meilleure qualité de vie, un moindre risque d'hospitalisation et une moindre mortalité de façon indépendante chez les sujets sains (Myers et al., 2002; Rantanen et al., 2000), au cours de nombreux états pathologiques (Mak et al., 2011; Meyer et al., 2013; Middlekauff, 2010; Zhou et al., 2010) et de l'âge (Gale et al., 2007; Malafarina et al., 2012).
- Une diminution de la force et de la masse musculaire a aussi été reliée à la fatigue. Pourtant, la fatigue (sensation subjective) n'est pas toujours reliée à une diminution objective des capacités d'exercice (Strasser, 2008), et cette relation pourrait dépendre du processus en cause. Une relation entre fatigue et capacités d'exercice a été mise en évidence notamment au cours des cancers (Banzer et al., 2014) et de l'âge (Mänty et al., 2012), deux pathologies qui se caractérisent aussi par une inflammation et une

sédentarité. En revanche, au cours de la polyarthrite rhumatoïde, la fatigue ne reposerait par sur une altération objective des capacités aérobies, mais sur les répercussions psychologiques de la maladie, comme la dépression et l'anxiété (Munsterman et al., 2013).

Il n'y a actuellement pas de traitement spécifique de la fatigue et du retentissement musculaire au cours des SpA.

La prise en charge des SpA vise actuellement à réduire les symptômes pour limiter le handicap, améliorer la qualité de vie et permettre l'insertion socioprofessionnelle. Il n'y a pas de traitement curatif (HAS; Taurog et al., 2016).

- La première ligne de traitement repose sur les anti-inflammatoires stéroïdiens.
- Lorsque ces traitements sont insuffisamment efficaces et/ou mal tolérés, une biothérapie est recommandée. Au cours des trois premières années de la maladie, environ un tiers des patients atteints de SpA ont recours à une biothérapie (Harvard et al., 2016). Actuellement, elle repose presque exclusivement sur l'utilisation de médicaments bloquant, spécifiquement le TNFα (anti-TNFα), dont 5 molécules sont disponibles (adalimumab, infliximab, golimumab, certolizumab, etanercept). Toutes ces molécules ont montré une efficacité sur le contrôle de l'activité de la maladie. Les effets secondaires sont majoritairement représentés par les infections qui sont plus fréquentes et plus sévères. Le surcoût engagé par ces traitements est important, dépassant les 10 000 euros par an et par patient, comparativement aux patients qui ne sont pas traités par anti-TNFα (Harvard et al., 2016)
- Des données récentes indiquent que des médicaments bloquant l'IL17 (secukimumab) (Baeten et al., 2015) et l'IL23 (ustékinumab) (Yeremenko et al., 2014) seraient efficaces. Il n'y a pas de recommandations concernant leur usage actuellement.

L'effet des anti-TNFα sur la fatigue, symptôme fréquent invalidant et qui représente un sous-paramètre essentiel de l'activité, a cependant été peu étudié (Bedaiwi et al., 2015; Dougados et al., 2015; Sieper et al., 2015). Une amélioration a été montrée, (Bedaiwi et al., 2015;

Braun et al., 2007; Brophy et al., 2013; Heiberg et al., 2005; Revicki et al., 2008; Sieper et al., 2015) mais qui n'atteint pas toujours la significativité (Dougados et al., 2015; Wanders et al., 2004), et malgré cette amélioration, 80 % des patients gardent une fatigue sévère (Bedaiwi et al., 2015). La fatigue serait aussi un facteur d'arrêt précoce de traitement (Glintborg et al., 2010).

Les données sur l'effet des anti-TNFα sur le retentissement musculaire au cours de la SpA sont très limitées : les anti-TNFα augmenteraient la masse musculaire (Briot et al., 2005), et la masse musculaire serait plus importante chez les patients qui prennent ce traitement (El Maghraoui et al., 2016). La signification clinique de cet effet n'a pas été étudiée.

Une meilleure caractérisation de la réponse de ces paramètres aux traitements par antiTNFα et l'identification des facteurs de prédiction de réponse à ces traitements sont donc nécessaires.

Dans le cadre du projet « FaMuSpa » (PHRC interégional 2016), nous projetons d'étudier, chez des patients atteints de SpA axiale sévère, le rôle du retentissement musculaire squelettique dans la fatigue, son lien avec les caractéristiques des patients et de la maladie, les fonctions mitochondriales et la production de RLO dans le muscle, les cytokines pro-inflammatoires dans le sang, ainsi que la réponse de ces paramètres aux traitements anti-TNFα.

CONCLUSIONS ET PERSPECTIVES

Nos résultats ont mis en lumière la rareté et la diversité des MI. L'étude PREMIA (PHRC 2011 Numéro HUS 4963) permettra de mieux caractériser l'épidémiologie de ces maladies et d'en révéler les déterminants.

Nous avons aussi montré que des anomalies mitochondriales périfasciculaires sont caractéristiques de la DM, participent à l'intolérance à l'effort des patients et au maintien de la signature interféron de type 1 dans le muscle. Nous souhaitons maintenant :

- Mieux caractériser le lien entre les anomalies mitochondriales périfasciculaires et la capacité d'exercice (aérobie, force musculaire, marche de 6 minutes), l'activité de la maladie au moment de la biopsie et à trois ans d'évolution, le handicap et la qualité de vie au moment de la biopsie musculaire et à trois ans d'évolution (projet APJC 2016 numéro HUS 6431).
- Mieux caractériser les voies moléculaires impliquées dans les fibres périfasciculaires des patients atteints de DM (étude du génome, transcriptome et protéome dans les biopsies musculaires) et déterminer comment la mitochondrie participe à la signature IFN-1 dans le muscle (en culture cellulaire).

Nos résultats ont aussi des implications pour d'autres maladies inflammatoires que nous projetons d'étudier de la façon suivante :

Chez les patients atteints de SSc, nous projetons de caractériser les fonctions mitochondriales des PBMC, leurs liens avec la production de RLO, la métabolomique de la peau, la dysfonction endothéliale et les caractéristiques conventionnelles de la maladie (PHRC interrégional 2014-A00518-39 « Scléromics »).

Chez des patients atteints de SpA axiale sévère, nous projetons d'étudier le rôle du retentissement musculaire squelettique dans la fatigue, son lien avec les caractéristiques des patients et de la maladie, les fonctions mitochondriales et la production de RLO dans le muscle, les cytokines pro-inflammatoires dans le sang, ainsi que la réponse de ces

paramètres aux traitements anti-TNFα (projet « FaMuSpa », PHRC interégional 2016 HUS 6639).

ANNEXES

1-LA MITOCHONDRIE

La mitochondrie est un organite descendant des alpha-protéobactéries, dont la différenciation phylogénique s'est initiée il y a des billions d'années par une endosymbiose (Gray, 2011). La mitochondrie a gardé de son ancêtre bactérien sa structure en double membrane (similaire aux bactéries à Gram négatif). Elle comporte aussi plusieurs exemplaires de son propre ADN (mtADN), distinct de l'ADN nucléaire. Cependant, l'ADN mitochondrial humain encode seulement 13 protéines mitochondriales (<90 % du protéome mitochondrial), et les autres protéines mitochondriales sont encodées dans le noyau puis transportées dans la mitochondrie. Ainsi, la mitochondrie n'a pas d'indépendance génétique, ce qui pérennise sa « mise au service » de la cellule eucaryote.

Les fonctions de cet organite sont multiples. En plus d'assurer la majorité de l'approvisionnement cellulaire en énergie sous forme d'ATP, il participe à l'homéostasie calcique, à la régulation du pH intracellulaire, à la synthèse d'hormones stéroïdes et des hèmes, à la régulation de la thermogenèse, à la mort cellulaire, et joue un rôle régulateur de l'immunité innée et adaptative (voir chapitre dédié). Il constitue aussi l'un des principaux sites de production des radicaux libres qui sont impliqués dans de nombreuses voies de signalisation intracellulaire.

2.1. Structure générale

Les muscles squelettiques possèdent un contenu variable en mitochondries. Elles peuvent occuper 1 % du volume cellulaire dans les muscles glycolytiques et jusqu'à 6 à 30 % dans les muscles oxydatifs. Ce stock est aussi régulé par les mécanismes de la biogenèse mitochondriale et de la mitophagie, deux mécanismes qui sont sous l'influence de facteurs physiologiques et pathologiques (Green et al., 2011).

Morphologiquement, les mitochondries ont longtemps été considérées comme des organelles ellipsoïdes, physiquement indépendantes les unes des autres. Il s'agit en fait d'un

réseau dont la forme est influencée par de nombreux stimuli et qui modifie les fonctions mitochondriales (Youle and van der Bliek, 2012). La mobilité de ce réseau est un moyen de régulation constante des fonctions mitochondriales aux besoins de la cellule. Cette dynamique mitochondriale est rendue possible par une interaction avec le cytosquelette de la cellule réalisant des phénomènes de fission ou de fusion de ce réseau. L'organisation globale du réseau est dessinée par deux membranes mitochondriales délimitant un espace intermembranaire et une matrice (figures S1 et S2).

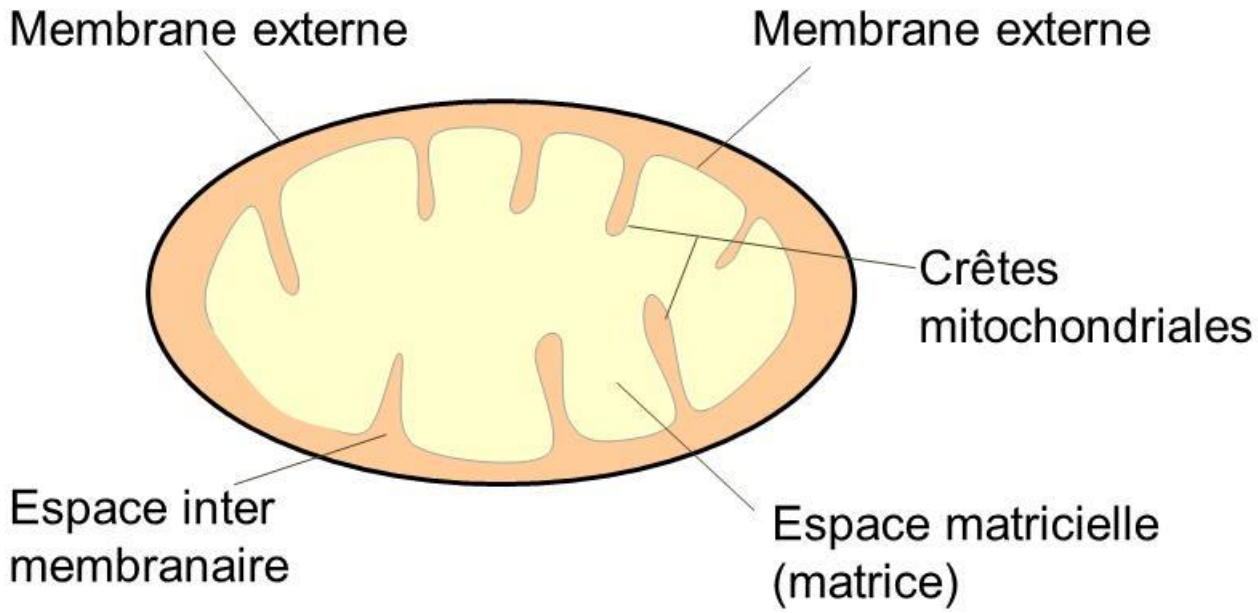

Figure S1 Représentation schématique de l'organisation générale mitochondriale

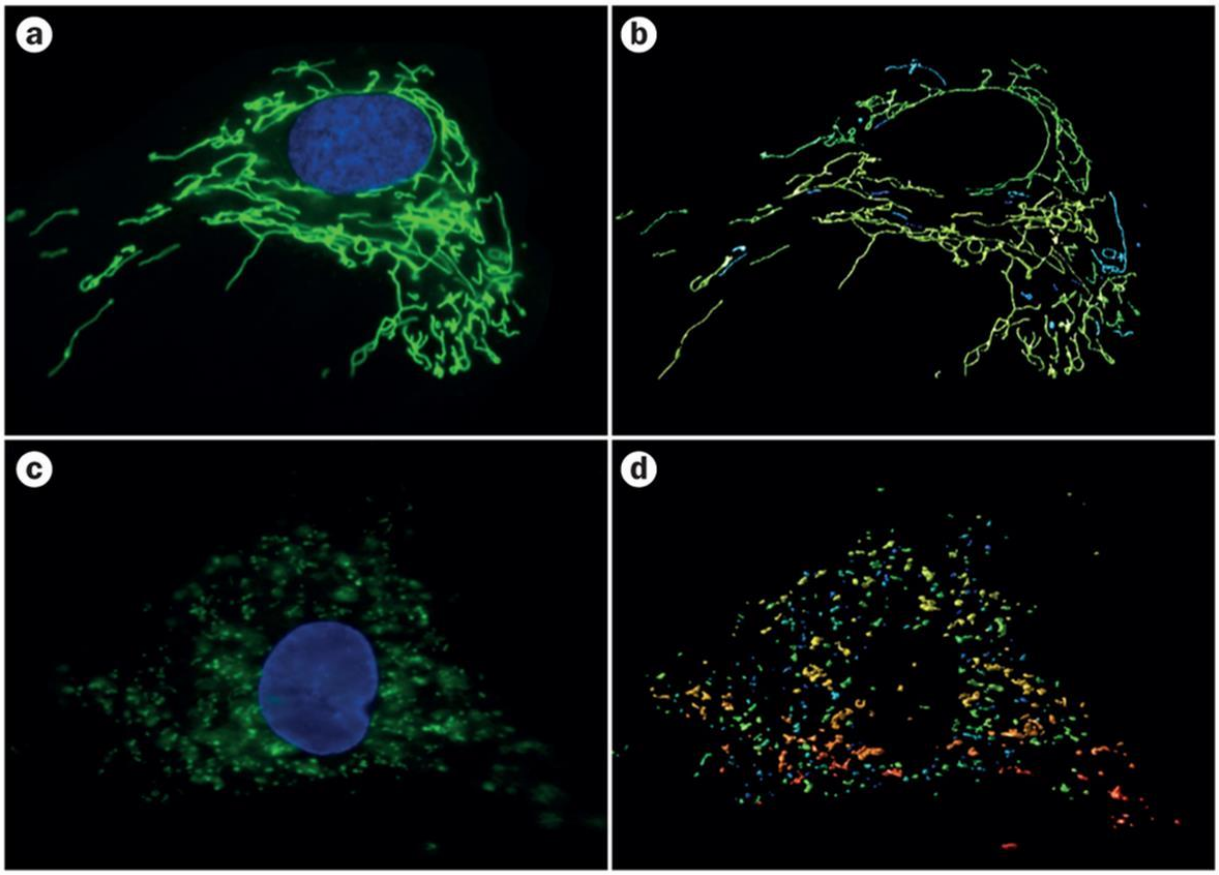

Figure S2 : Réseau mitochondrial (en vert, marqué par MitoTracker TM) dans les fibroblastes. Organisation normale dans un fibroblaste témoin (A : avant et B après déconvolution de l'image). Fragmentation du réseau dans un fibroblaste mutant OPA1, une protéine impliquée dans la fusion mitochondriale (C avant et D après déconvolution de l'image). Le noyau apparait en bleu (marqué par Hoechst 33342) (Burté et al., 2015).

2.1.1. La membrane externe

C'est une membrane rigide qui contient de nombreuses protéines formant des pores régulant les échanges entre le cytosol et l'espace intermembranaire de la mitochondrie.

Le voltage dépendant anion channel (VDAC) est la principale voie par laquelle transitent les ions et les métabolites mitochondriaux tels que l'ATP, l'ADP, le pyruvate, le succinate, et le phosphate inorganique. Les trois isoformes dénombrées chez les mammifères (VDAC1,

VDAC2, VDAC3) présentent des différences de perméabilité qui joueraient un rôle dans la régulation des fonctions mitochondriales (Noskov et al., 2016).

Le transport de molécules plus volumineuses est réalisé par le complexe TOM. Ce complexe reconnait, par sa sous-unité TOM20, les protéines portant une séquence d'adressage mitochondrial et assure leur transfert vers l'espace intermembranaire (Endo and Yamano, 2010).

La membrane externe porte aussi le transporteur pour les acides gras à longue chaine CPT1. Les acides gras à courte et moyenne chaine diffusent passivement, à travers la membrane externe de la mitochondrie.

Enfin, la membrane externe est aussi une plateforme de localisation de plusieurs protéines de signalisation cytosolique. Cette localisation à la membrane externe est nécessaire au bon fonctionnement de ces protéines, et des phénomènes mitochondriaux (fusion, fission, respiration mitochondriale, formation de radicaux libres mitochondriaux…) influencent le signal conduit par ces protéines. C'est le cas notamment de la MAVS (voir chapitre dédié à la régulation de l'immunité innée par la mitochondrie).

2.1.2. L'espace intermembranaire

L'espace intermembranique joue un rôle central dans la coordination des fonctions mitochondriales avec les autres processus cellulaires. Ces fonctions comprennent l'échange de protéines, de lipides ou d'ions métalliques entre la matrice et le cytosol, la coordination des cascades apoptotiques, la régulation de la respiration et les fonctions métaboliques, la protection contre les RLO produits par la chaîne respiratoire et le contrôle de la morphogenèse mitochondriale. L'espace intermembranaire s'invagine au niveau des crêtes mitochondriales. Ces invaginations ont longtemps été interprétées comme un moyen d'augmenter la surface de la membrane interne. En fait, ces crêtes ont la forme de tubules ronds ou ovales de 12 à 40 nm de diamètre et d'environ 50 nm de longueur dont la jonction avec le reste de la membrane interne forme un anneau qui contient des complexes de

prohibitins (181), la protéine mitofilin (74, 147) et la protéine OPA1 associée à la dynamique mitochondriale (21, 49). La fonction de ces annaux n'est pas connue, mais il est possible qu'ils permettent d'utiliser l'espace intermembranaire contenu dans les crêtes comme un réservoir permettant de mieux réguler les flux de protons, de RLO, de cytochrome C entre la mitochondrie et le cytosol (Herrmann and Riemer, 2010).

2.1.3. La membrane interne

La membrane interne présente une perméabilité réduite et sélective, elle forme la barrière la plus distincte entre le cytosol et la matrice mitochondriale. Des transporteurs permettent les échanges entre les compartiments qui permettent de maintenir un gradient de concentration pour des protéines, des ions et des métabolites tels que les protons et l'ATP qui participent au bon fonctionnement de la mitochondrie et de la cellule.

- Les complexes de la chaîne respiratoire sont représentés par 5 complexes protéiques, dont l'ATP synthase, ancrés dans la membrane interne. Ils assurent le maintien du gradient de proton et la formation d'ATP.

- L'ANT est constitué de deux sous-unités de 32 kDa et d'un site unique de liaison à l'ATP ou l'ADP. Selon sa conformation, il fait face, alternativement, à la matrice ou à l'espace intermembranaire. Ceci permet un échange de l'ATP mitochondrial par l'ADP cytosolique dans un rapport 1:1. L'ANT est exprimé par trois isoformes : ANT1, ANT2 et ANT3. Chez l'homme et le rat, ANT1 est l'isoforme exprimée de manière prédominante dans le muscle cardiaque et squelettique, ANT2 est ubiquitaire, exprimé dans tous les tissus en quantité variable en fonction de l'activité respiratoire du tissu. ANT3 a un faible niveau d'expression dans le cerveau, le foie, le rein, le cœur et les muscles squelettiques.

- Les protéines découplantes (UCPs) sont enchâssées dans la membrane interne et permettent le passage de protons de la matrice à l'espace intermembranaire qui court-circuite le passage par l'ATP synthase. Ce phénomène est responsable d'un couplage incomplet entre la respiration mitochondriale et la phosphorylation, appelé respiration

mitochondriale découplée. Une partie de l'énergie est ainsi perdue sous forme de chaleur. Concernant le rôle physiologique de ce découplage, il se pourrait que cette fuite des protons permette de diminuer la production des radicaux libres par une augmentation de l'oxydation de l'ubiquinone, et donc minimise le stress oxydant et les dégâts inhérents au niveau de l'ADN (Brand, 2000). Les UCPs joueraient donc un rôle de protection contre le stress oxydant (Echtay et al., 2002).

2.1.4. La matrice mitochondriale

La matrice est le compartiment interne de la mitochondrie. Délimité par la membrane mitochondriale interne, il contient des centaines d'enzymes nécessaires au cycle de Krebs et à la β-oxydation des acides gras, ainsi que l'ADN mitochondrial codant pour des protéines composant certaines sous-unités des complexes I, III, IV et V de la chaîne respiratoire, des ribosomes mitochondriaux, des tRNA, des rRNA et des enzymes nécessaires à l'expression de l'ADN.

2.2. Distribution des mitochondries dans la cellule musculaire

Ces analyses ont permis de regrouper les mitochondries en deux groupes : les mitochondries interfibrillaires et subsarcolemmales. Ces deux populations présentent des propriétés biochimiques et des niveaux d'activité enzymatique différents et semblent répondre différemment au stress métabolique (Lesnefsky & Hoppel, 2003). Les mitochondries sont ancrées dans la cellule grâce à des protéines du cytosquelette et peuvent se déplacer grâce à des protéines dites motrices telles que les myosines, la dynéine ou la kinésine. Dans les cellules musculaires oxydatives, les mitochondries intermyofibrillaires ont un arrangement cytoarchitectural assimilable à un cristal permettant d'optimiser le transfert d'énergie (Vendelin et al., 2005).

2.3. La phosphorisation oxydative

Les éléments clés constituant la mitochondrie sont, d'une part, les réactions enzymatiques d'oxydation des substrats énergétiques et, d'autre part, la chaîne de transport des électrons ou chaîne respiratoire. L'oxydation des substrats par le cycle de Krebs ou la β-oxydation entraînent la réduction du NAD+ en NADH et du FAD2+ en FADH 2. Ces intermédiaires sont appelés équivalents réducteurs et fournissent des électrons à la chaîne respiratoire. Cette dernière est composée de cinq complexes :

- le complexe I ou NADH déshydrogénase
- le complexe II ou succinate déshydrogénase
- le complexe III ou ubiquinol cytochrome c réductase
- le complexe IV ou cytochrome c oxydase
- le complexe V ou ATP synthase.

Tous sont composés de plusieurs sous-unités protéiques. Seul le complexe II est entièrement codé dans le noyau tandis que les autres complexes résultent de l'association de protéines codées par les ADN nucléaire et mitochondrial (Kirby et al., 2004).

Les électrons sont transportés par le NADH et le FADH 2, respectivement aux complexes I et II qui transfèrent l'électron à l'ubiquinone, cette dernière l'acheminant au complexe III.

L'arrivée de l'électron au complexe IV constitue la fin de la chaîne d'oxydoréduction et entraîne la réduction de l'oxygène moléculaire en eau. Les réactions associées au passage de l'électron au niveau des complexes I, III et IV entraînent le passage d'un proton vers l'espace intermembranaire à travers les complexes. Ce passage établit un gradient électrochimique de protons qui crée une force proton-motrice utilisée par le complexe V pour phosphoryler des molécules d'ADP en ATP, assurant le couplage de la chaîne des oxydoréductions avec la production d'ATP (Figure S3) (Stock et al., 1999).

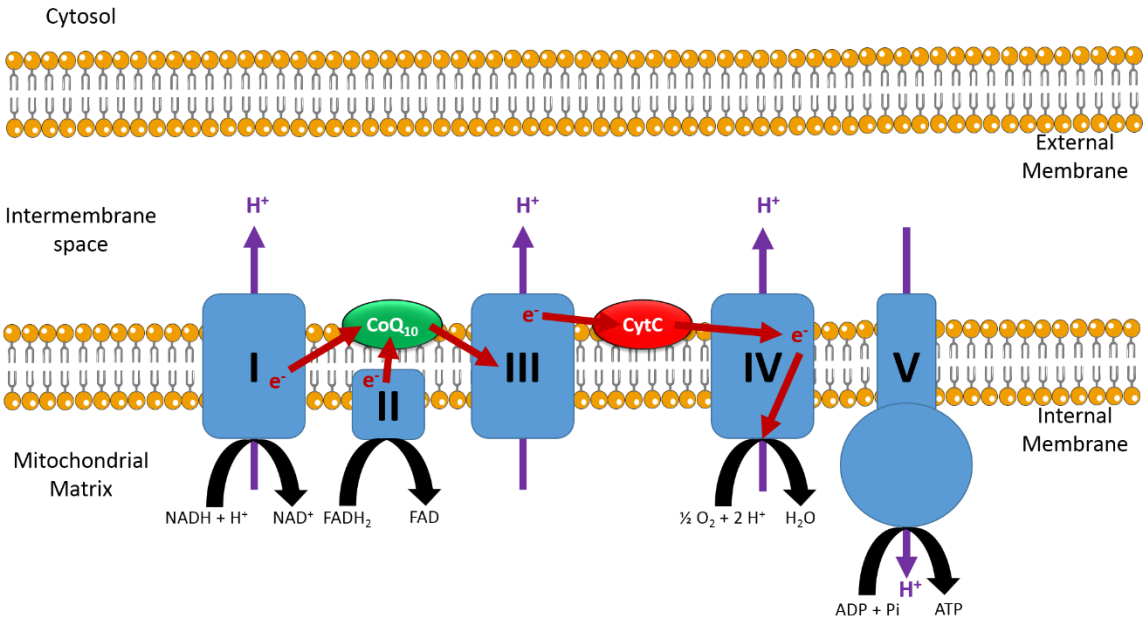

Figure S3 : Chaîne de transport des électrons. Les électrons sont apportés au niveau de la chaîne respiratoire par les équivalents réducteurs : NADH (complexe I) et FADH 2 (complexe II).
L'électron est ensuite amené au niveau du complexe IV où il est utilisé pour former de l'eau. Le passage de l'électron au niveau des complexes I, III et IV entraîne le passage de protons (H +) dans l'espace intermembranaire et crée un gradient protonique qui va permettre la formation ATP lors du passage des protons au niveau de l'ATP synthase.

a. Complexe I : NADH déshydrogénase

C'est la première enzyme de la chaîne respiratoire. Elle catalyse le transfert de deux électrons du NADH (oxydé en NAD+) à l'ubiquinone (réduit en ubiquinol), couplé à la translocation de quatre protons au travers de la membrane, ce qui participe à la force proton-motrice (Scheffler, 2007; Wirth et al., 2016).

NADH ↔ NAD+ + H+ + 2 e-

NADH + Q + 5 H+ ↔ NAD+ + QH2 + 4 H+

Avec son poids moléculaire de 980 kDa environ, elle constitue l'un des plus gros complexes protéiques membranaires. Cette enzyme est constituée de 45 sous-unités (44 protéines différentes, dont une présente en double exemplaire : NDUFAB1). La majorité de ces protéines (n=37) sont codées par le génome nucléaire, tandis que les 7 autres sont codées par le génome mitochondrial. De façon schématique, ce complexe contient un domaine

hydrophile, matriciel, qui porte le site de liaison au NADH. Le système de pompage des protons est localisé dans la partie membranaire du complexe (Kao et al., 2003). Le potentiel réducteur du complexe I est le plus faible de l'ensemble de la chaine respiratoire. C'est pourquoi il s'agit d'un site de fuite d'électrons vers des molécules non spécifiques, différentes des protéines formant la chaine de respiration mitochondriale. Lorsque ces électrons réduisent l'oxygène de cette façon, non contrôlés par l'ensemble de la chaine de respiration mitochondriale, ils conduisent à la formation de radicaux libres.

b. Complexe II : Succinate déshydrogénase

Ce complexe oxyde le succinate en fumarate et réduit l'ubiquinone en ubiquinol.

Succinate + Q ↔ Fumarate + QH2

Contrairement aux complexes I, III et IV, la réaction que catalyse le complexe II ne conduit pas à l'extrusion de protons de la matrice vers l'espace intermembranaire. Le complexe II fait à la fois partie de la chaîne respiratoire et du cycle de Krebs. Il s'agit du complexe le plus petit de la chaine respiratoire, composé de quatre sous-unités (A à D). Les sous-unités A et B portent le domaine hydrophile de l'enzyme dépassant dans la matrice. Elles constituent la partie enzymatique du complexe II (succinate deshydrogénase). Les sous-unités C et D constituent le domaine d'ancrage du complexe dans la membrane interne mitochondriale. Les quatre gènes codant pour ces quatre sous-unités font partie du génome nucléaire. Lorsque le complexe II délivre aux ubiquinones une quantité d'électrons supérieure à ce que le cytochrome c peut transporter, un flux réverse d'électrons au travers du complexe I peut être généré (Favier et al., 2005), entraînant une réduction plus importante de ce complexe, ce qui favorise la production de radicaux libres dérivés de l'oxygène.

c. Ubiquinone (Coenzyme Q_{10}, Co Q_{10})

Il s'agit d'une molécule liposoluble et mobile localisée dans la membrane interne de la mitochondrie, qui transfère les électrons depuis les complexes I et II vers le complexe III. Elle est codée par le génome mitochondrial (Crane, 2007). Elle existe en trois états redox : i)

l'ubiquinone est la forme complètement oxydée ; ii) l'ubisemiquinone porte un électron ; iii) l'ubiquinol porte 2 électrons (Figure S4).

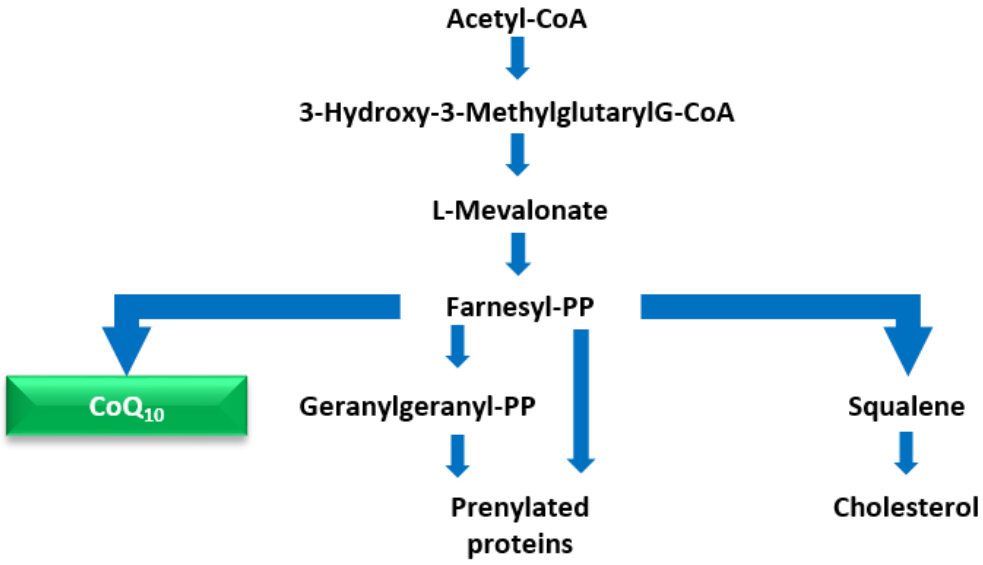

Figure S4 : Représentation schématique de la biosynthèse du CoQ_{10}

d. Complexe III : Complexe b-c1 ou ubiquinone-cytochrome c réductase

Ce complexe catalyse le transfert de deux électrons de l'ubiquinol (oxydé) vers le cytochrome C (réduit). Cette réaction est associée au transfert de 4 protons de la matrice vers l'espace intermembranaire.

$$QH_2 + 2\ \text{cyt}\ c^{3+} + 2\ H^+ \leftrightarrow Q + 2\text{cyt}\ c^{2+} + 4\ H^+$$

Il contient quatre groupes prosthétiques redox actifs : 2 cytochromes b (bL et bH), le cytochrome c1 et un centre Fer/Soufre. Ce complexe est composé de 11 sous-unités protéiques, parmi lesquelles les sous-unités III, IV et V constituent les groupes redox, les 8 autres sous-unités ne comportant pas de groupement prosthétique. Seule la sous-unité comprenant les cytochromes b est codée par le génome mitochondrial (Borisov, 2002). Ainsi, des électrons dérivant de l'oxydation de l'ubiquinol sont recyclés par le site ubiquinol réductase de cette enzyme, ce qui permet le pompage des protons.

e. Cytochrome c

Le cytochrome c est une petite (12.3 kDa) hémoprotéine hydrophile localisée au niveau de la membrane interne de la mitochondrie. Elle transfère les électrons du complexe III vers le complexe IV. Elle est codée par le génome nucléaire (Cai et al., 1998). Elle joue aussi un rôle dans la mort cellulaire : son relargage dans le cytosol conduit à l'apoptose de la cellule.

d. Complexe IV : cytochrome c oxydase

Ce complexe catalyse l'oxydation du cytochrome par l'oxygène (accepteur final des électrons). Quatre protons sont expulsés vers l'espace intermembranaire pendant cette réaction.

$$4\ \text{cyt c2+} + 8\ \text{H+in} + \text{O2} \rightarrow 4\ \text{cyt c3+} + 4\text{H+out} + 2\ \text{H2O}$$

Il appartient à la superfamille des oxydases à hème-cuivre. Son isoforme humaine est composée de 13 sous-unités dont trois (Cox I, Cox II et Cox III) sont codées par le génome mitochondrial (Borisov, 2002). Les électrons cédés par le cytochrome c entrent dans ce complexe protéique par le centre cuivre CuA puis sont transférés successivement à l'hème a, puis au site actif binucléaire où s'effectue la liaison de l'oxygène et sa réduction en eau. Ce complexe pourrait être la locomotive de la chaine de respiration mitochondriale. Son activité est régulée sur les plans transcriptionnel, traductionnel, post-traductionnel et au niveau de son assemblage (Srinivasan and Avadhani, 2012).

e. ATP synthase

Elle est composée de deux sous-complexes. La partie F 0 est insérée dans la membrane interne mitochondriale et conduit les protons depuis l'espace intermembranaire vers la matrice. Cette partie, chez les mammifères, est composée de 5 sous-unités (A6, b, c, d et Oligomycin Sensitivity Conferral Protein). Le segment F 1 est matriciel, au contact de la membrane interne, et utilise le gradient de protons pour convertir l'ADP en ATP et vice versa.

Il est composé de 5 sous-unités (α, β, γ, δ, ε dans un ratio 3, 3, 1, 1, 1). Des protéines accessoires (e, f, g et F6) sont également associées à ce complexe. Le fonctionnement de

ce dernier repose sur les sous-unités c (au nombre de 10) de la partie F 0 qui, connectées à la sous-unité γ de F 1, agissent comme un rotor qui utilise le passage des protons à travers le stator (sous-unités A6, b, d et OSCP) pour fonctionner. La sous-unité γ cause, quant à elle, un changement conformationnel dans le trimère circulaire des sous-unités α et β de la partie F 1 et permet ainsi successivement la liaison de l'ADP, puis la réaction avec le phosphate inorganique pour générer l'ATP et, enfin, le relargage de l'ATP.

3-LES RADICAUX LIBRES DÉRIVÉS DE L'OXYGÈNE

Un radical libre est une espèce chimique, atome ou molécule, contenant un électron non apparié. Extrêmement instable, ce composé a une forte propension à réagir avec les molécules les plus stables pour apparier son électron. Il peut soit arracher un électron (se comportant comme un oxydant), soit en céder un (agissant alors comme un réducteur). Cette première réaction conduit généralement à la formation en chaîne de nouveaux radicaux.

L'oxygène comporte un électron non apparié sur sa couche électronique. Sous forme de dioxygène (O2), les électrons non appariés de chaque atome d'oxygène s'apparient entre eux et se stabilisent ainsi partiellement. Ils restent susceptibles de capter 1 ou 2 électrons. L'O2 est alors réduit en anion superoxyde (O_2 + 1 e^- → O_2•-) ou en ion peroxyde (O_2 + 2 e^- →$O_2$2•-). Ces ions s'associent aux protons pour donner respectivement le radical hydroxyle (OH•) et le peroxyde d'hydrogène (H2O2).

3.1. Rôles des RLO dans le muscle squelettique

Les RLO sont impliqués dans la signalisation cellulaire et l'homéostasie physiologique comme le transport du glucose vers le muscle (Sandström et al., 2006) et la réponse physiologique à l'entrainement (Ristow et al., 2009).

Cependant, lorsqu'ils sont produits en excès, les RLO endommagent diverses macromolécules telles que des protéines, des lipides membranaires et/ou de l'ADN, ce qui peut avoir un impact sur de nombreux aspects du fonctionnement cellulaire (en particulier

l'appareil contractile, le réticulum sarcoplasmique, la mitochondrie) et parfois conduire à la mort cellulaire (Lejay et al., 2014; Westerblad and Allen, 2011).

3.2. Sources des radicaux libres dérivés de l'oxygène

3.1.1. La xanthine oxydase

Dans les cellules saines, la xanthine déshydrogénase transforme la xanthine ou l'hypoxanthine en acide urique en transférant les électrons au NADH. Lors de l'ischémie-reperfusion, cette enzyme peut être à l'origine d'une production de RLO. Pendant l'ischémie, la xanthine déshydrogénase peut être convertie en xanthine oxydase par l'action de protéases calcium dépendantes. Par ailleurs, l'ADP accumulé à mesure de l'épuisement de l'ATP est métabolisé en hypoxanthine. Lorsque le tissu est réoxygéné, l'hypoxanthine accumulée est oxydée par la xanthine oxydase, ayant pour conséquence la génération de superoxyde et de peroxyde d'hydrogène (Paradis et al., 2016).

3.1.2. Les NADPH Oxydases

Les NADPH oxydases sont des enzymes génératrices de superoxyde (O2 • -) qui sont d'abord identifiées dans les phagocytes : elles sont essentielles à l'activité bactéricide de ces cellules. Il a ensuite été montré que ces enzymes sont aussi impliquées dans la production d'O2 • - dans les cellules non phagocytaires. Au cours de la dernière décennie, plusieurs oxydases NADPH non phagocytaires ont été identifiées. La sous-unité catalytique de ces oxydases, NOX, définit la famille NOX, dont 5 homologues ont été identifiés (NOX1 à NOX5). Il existe deux enzymes apparentées, DUOX1 et DUOX2.

Le muscle squelettique exprime trois isoformes de NADPH oxydases (Nox1, Nox2 et Nox4) qui ont été identifiées comme des modulateurs de l'homéostasie redox. Nox2 est la

principale source de ROS du muscle squelettique pendant la contraction musculaire, ce qui participe à la signalisation de l'insuline et au transport du glucose ainsi qu'à la médiation de la réponse des myocytes à l'exercice. Les Nox2 et Nox4 sont notamment impliqués dans les anomalies musculaires squelettiques observées au cours de la myopathie de Duchenne, la sclérose latérale amyotrophique, mais aussi de l'obésité. NOX1 est la NADPH la moins exprimée dans le muscle squelettique, et il ne lui a pas actuellement été attribué, à notre connaissance, de rôle physiologique dans cet organe (Ferreira and Laitano, 2016; Katsuyama et al., 2012).

3.1.3. Le réticulum endoplasmique

Le réticulum endoplasmique (ER) est spécialisé dans le pliage et le trafic de protéines. Ces phénomènes sont très sensibles aux changements de l'homéostasie intracellulaire et aux stimuli extracellulaires. Une altération de ce processus peut provoquer une accumulation de protéines mal repliées dans l'ER, un phénomène appelé « stress du réticulum endoplasmique ». Le stress du réticulum endoplasmique affecte de nombreux phénomènes cellulaires, notamment l'équilibre redox, la production d'énergie, l'inflammation, la différenciation et l'apoptose. La réponse aux protéines mal pliées (*unfolded protein response: UPR*) est un ensemble de voies de signalisation adaptative dont la finalité est de résoudre la mauvaise conformation protéique et de restaurer un pliage efficace des protéines dans le réticulum endoplasmique (Cao and Kaufman, 2014). Cette réponse met en jeu : i) une augmentation du pliage des protéines ; ii) une diminution de la traduction des ARN messagers ; iii) une autophagie des protéines qui sont mal pliées.

Le stress du réticulum endoplasmique et l'UPR peuvent conduire, lorsqu'ils sont intenses et/ou prolongés, à la formation de RLO par plusieurs mécanismes:

- L'UPR, en réponse au stress du réticulum endoplasmique, peut être accompagnée d'une production d'H2O2 lors de la réalisation d'un « pliage oxydatif » qui consiste en la

formation de ponts disulfures par l'oxydation des résidus –SH. Ces oxydations sont réalisées par l'ER oxydoréductase 1 (ERO1) et la protéine disulfide isomérase (PDI).

- D'autre part, le tampon redox déterminé par le ratio glutathion (GSH) / glutathion disulfide (GSSG) est plus oxydant (1:1 à 3:1) dans le réticulum endoplasmique que celui enregistré dans le cytosol (30;1 - 100;1) (figure S5).
- Enfin ERO1 peut aussi induire un relargage calcique depuis l'ER par IP3R. Ceci induit l'activation des kinases sensibles au calcium (CaMKII) qui provoquent notamment l'activation de NOX2.
- Le relargage calcique dans le cytosol est pris en charge par la mitochondrie. La mitochondrie est en interaction physique avec l'ER par l'intermédiaire des mitochondria-associated ER membranes (MAMs). Ces protéines pourraient servir de canaux, co-formés par les IP3R et les canaux anioniques mitochondriaux sensibles au voltage pour faciliter le transport de calcium entre les deux organelles (Szabadkai et al., 2006). L'afflux de calcium vers la mitochondrie induit une ouverture du pore de transition mitochondrial, une perte du cytochrome C conduisant à une dysfonction de la chaine de respiration de la mitochondrie et à une augmentation de la production de RLO mitochondriaux.

Le stress du réticulum endoplasmique et l'UPR induisent aussi des réponses capables de prendre en charge l'augmentation des RLO. La voie PERK de l'UPR induit ATF4 et NRF2, deux facteurs de transcription qui induisent la mitohormèse et l'augmentation des défenses antioxydantes telles que les SODs, hème oxygenase-1, la glutathione transferase, et UCP1 (Santos et al., 2009).

Le stress de l'ER et l'UPR sont eux-mêmes sous l'influence des RLO. En effet, l'UPR induite par le 7-ketocholesterol est inhibée par la N-acetylcysteine. La localisation des RLO dans la cellule pourrait influencer l'intensité du stress de l'ER. En effet, l'H_20_2 ne stimule que certains composants de l'UPR et son effet reste modéré (Santos et al., 2009). En revanche, les RLO mitochondriaux ouvrent les canaux IP3R et RYR du réticulum sarcoplasmique et induisent

un important stress de celui-ci, qui augmente encore les dysfonctions mitochondriales (Brand, 2010; St-Pierre et al., 2002).

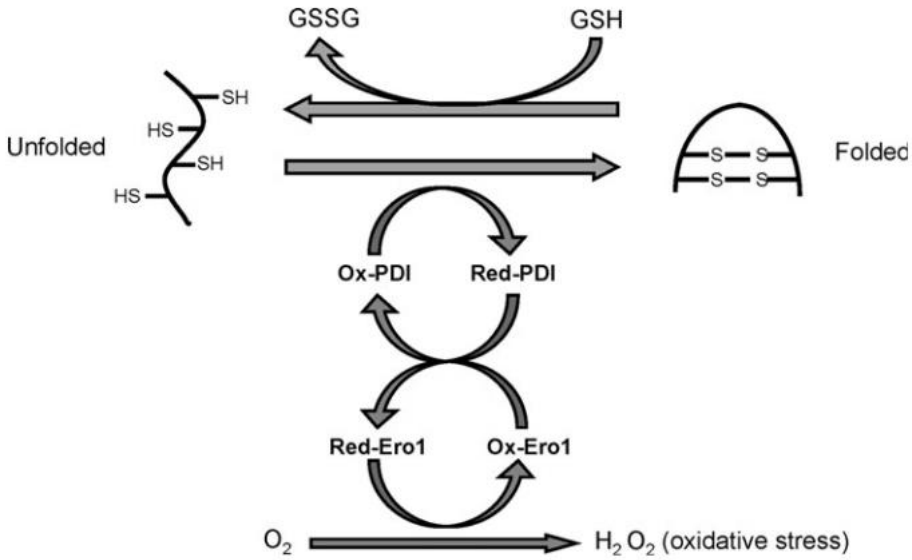

Figure S5 : Production des RLO par le stress du réticulum sarcoplasmique (Cao and Kaufman, 2014).

3.1.4. Les peroxysomes

Chez les mammifères, les peroxysomes jouent un rôle indispensable dans différentes voies biochimiques, incluant la synthèse des phospholipidiques, l'oxydation des acides gras, des acides biliaires, le catabolisme des acides aminés et la synthèse de RLO (Fransen et al., 2012; Van Veldhoven, 2010).

Les peroxysomes contiennent de multiples oxydoréductases contenant du FAD qui produisent de l'H2O2 dans le cadre de leur activité catalytique. Selon l'organisme, le tissu et le type de cellule, les peroxysomes de mammifères peuvent également contenir de la xanthine oxydoréductase (XDH) et la nitric oxyde synthase inductible (NOS2), deux sources potentielles d'anion superoxyde (O2• -). Pour prévenir l'accumulation de ROS et les dommages oxydatifs, les organelles possèdent la catalase, la peroxiredoxine-5 (PRDX5), la

superoxyde dismutase 1 (SOD1)) et des systèmes antioxydants non enzymatiques (par exemple, l'acide ascorbique et le glutathion) (Antonenkov et al., 2010; Fransen et al., 2017). De façon intéressante, les peroxysomes et les mitochondries coopèrent dans des procédés tels que la bêta-oxydation ou la réponse interféron. Ces deux organelles ont une régulation en partie commune de leur nombre et de leurs morphologies, par des activateurs communs de leur biogenèse, de leur dégradation et de leurs dynamiques. De plus, une communication existe entre les deux organelles, qui repose notamment sur des contacts directs de leurs membranes, l'envoi de vésicules dérivées de la mitochondrie (Fransen et al., 2017).

3.1.5. La chaine de respiration mitochondriale

La mitochondrie produirait 90 % des RLO cellulaires (Balaban et al., 2005). Une faible partie des électrons transportés par la chaine de respiration mitochondriale (0,15 à 0,8 %) quittent la chaine avant le complexe IV et conduisent à une réduction de l'oxygène sous forme de RLO (Murphy, 2009).

Les complexes I et III de la chaine de respiration mitochondriale sont les sites de production essentiels des RLO mitochondriaux (Figure S6).

Le complexe I isolé est capable de réduire l'O_2 en $O_2^{\bullet -}$ à partir d'électrons transférés du NADH vers l'O_2 par la flavine mononucléotide (FMN) lorsque le rapport NADH/$NAD^+ + H^+$ est élevé. Dans la chaine de respiration mitochondriale, le transfert d'électrons du NADH vers l'O_2 par la FMN, réduisant précocement l'O_2 en $O_2^{\bullet -}$, se produit quand le complexe I est inhibé alors que le rapport NADH/NAD+ + H+ est élevé (Murphy, 2009).

Le complexe I est aussi capable de former de l'$O2^{\bullet -}$ à partir d'électrons du succinate, par un flux inverse des électrons venant du complexe II. Le complexe II peut en effet délivrer à l'ubiquinone une plus grande quantité d'électrons que le complexe III ne peut en transporter. L'hyper-réduction de l'ubiquinone peut conduire à un flux inverse d'électrons vers le complexe I (Favier et al., 2005) capable de réduire l'O_2 en $O_2^{\bullet -}$. L'implication du complexe I dans ce phénomène est démontrée par le fait que cette production d'$O_2^{\bullet -}$, qui s'enregistre

lorsque la mitochondrie fonctionne en présence de succinate, est abolie quand le complexe est inhibé.

Au niveau du complexe III, les RLO sont générés par la semi-ubiquinone qui correspond à la forme partiellement réduite de l'ubiquinone. La semi-uniquinone est instable et peut, dans certaines conditions, réduire l'O2 en O2 • - (Turrens et al., 1985). Ceci a lieu au site Qo, localisé sur la face intermembranaire du complexe III, alors que le site Qi localisé à la face matricielle ne participe pas à la formation O2 • -. En effet, la production de RLO est augmentée en présence d'antimycine A, un inhibiteur du site Qi (Bleier and Dröse, 2013) alors qu'elle est abolie en présence de stigmatellin, un inhibiteur du site Qo. Ainsi, la participation du complexe III pourrait être moins importante que celle du complexe I.

Des enzymes interconnectées soit au pool de NADH (comme α -ketoglutarate déshydrogénase) soit au CoQ (comme l'α –glycérophosphate déshydrogénase ou la protéine trifonctionnelle ETF) sont aussi impliquées dans la génération de RLO.

Une dysfonction de la chaine respiratoire mitochondriale, qui conduit à un transfert moins efficient des électrons à travers l'ensemble de la chaine mitochondriale jusqu'au complexe IV, provoque une augmentation de la réduction précoce de l'O_2 sous forme de RLO. Si ces RLO sont produits en grande quantité et/ou de façon prolongée, ils peuvent aggraver les dysfonctions mitochondriales en particulier par : i) des dégâts oxydatifs des complexes, perturbant leur fonctionnement ; ii) des dégâts oxydatifs de la membrane lipidique interne de la mitochondrie, modifiant le fonctionnement de l'ensemble de la chaine respiratoire ; iii) des dégâts oxydatifs de l'ADN mitochondrial, perturbant la synthèse des protéines mitochondriales.

La cellule déploie deux régulations de défense contre ce phénomène :

- Une augmentation de la mitophagie : l'augmentation de la production de RLO est un signal qui active la mitophagie du pool de mitochondries qui dysfonctionnent (Youle and van der Bliek, 2012).
- Une augmentation de la production de nouvelles mitochondries (biogenèse mitochondriale) : l'augmentation des RLO conduit à l'expression de facteurs de

transcription tels que SIRT, PGC1α et β qui sont de puissants stimulants de la biogenèse mitochondriale (Lejay et al., 2014).

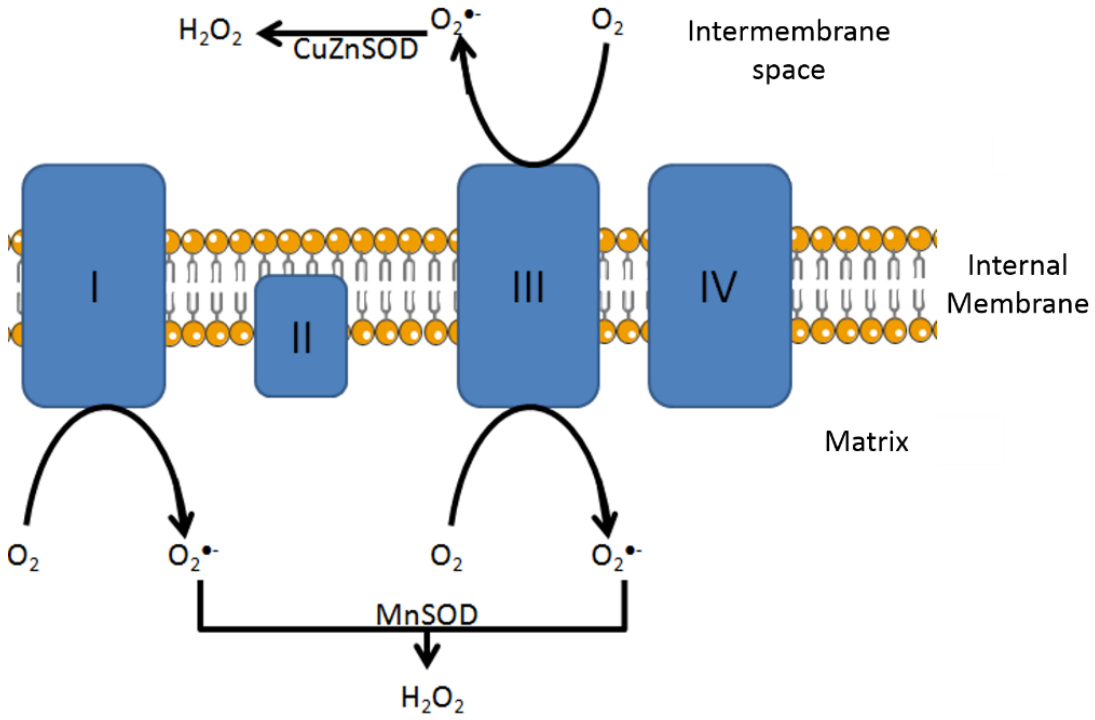

Figure S6 : Production des RLO dans la mitochondrie.

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Alain MEYER

Rôle de la mitochondrie et du stress oxydant au cours des myopathies inflammatoires

Résumé

Les myopathies inflammatoires sont des maladies auto-immunes rares dont le dénominateur commun est la faiblesse musculaire et l'inflammation. Leur origine n'est pas connue et les traitements sont conventionnels partiellement efficaces.

Par une approche épidémiologique, nous avons montré que l'étude de l'incidence er de la prévalence est un outil utile pour mettre en évidence des déterminants des myopathies inflammatoires. Une meilleure identification et une meilleure classification des patients atteints de ces maladies sont cependant nécessaires pour préciser l'épidémiologie des myopathies inflammatoires.

Par une approche translationnelle, nous avons montré que, par rapport aux autres myopathies inflammatoires, des dysfonctions mitochondriales périfasciculaires sont une caractéristique des dermatomyosites, qui jouent un rôle dans l'intolérance à l'effort et le maintien de l'inflammation.

Ces résultats ouvrent des nouvelles voies pour mieux comprendre et traités les myopathies inflammatoires.

Abstract

Inflammatory myopathies are rare autoimmune diseases whose common denominator is muscle weakness and inflammation. Their origin is not known and conventional treatments are partially effective.

Using an epidemiological approach, we have shown that the study of incidence and prevalence is an useful tool for identifying determinants of inflammatory myopathies. However, better identification and classification of patients is mandatory to refine the epidemiology of inflammatory myopathies.

Using a translational approach, we have shown that, compared with other inflammatory myopathies, perifascicular mitochondrial dysfunctions are a characteristic of dermatomyositis, which play a role in exercise intolerance and in the maintenance of inflammation.

These results open up new avenues to better understand and treat inflammatory myopathies.